FEDERAL UNIVERSITY OF SANTA CATARINA
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The functional and technological potential of guabiroba fruit (*Campomanesia xanthocarpa* O.Berg) and its application in dairy products and innovative foods using by-products with a focus on the use of emerging technologies

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THE FUNCTIONAL AND TECHNOLOGICAL POTENTIAL OF GUABIROBA FRUIT (*Campomanesia xanthocarpa* O.Berg) AND ITS APPLICATION IN DAIRY PRODUCTS AND INNOVATIVE FOODS USING BY-PRODUCTS WITH A FOCUS ON THE USE OF EMERGING TECHNOLOGIES

O presente trabalho em nível de doutorado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

Profª. Drª. Elane Schwinden Prudencio - Presidente - UFSC

Drª. Cristhiane Stecanella de Oliveira Cattani - Membro Externo - MAPA- Ministério da Agricultura e Pecuária

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Profª. Drª. Elane Schwinden Prudêncio
Orientador

Florianópolis, 2023.

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RESUMO

A guabiroba (*Campomanesia xhantocarpa* O. Berg) é uma fruta nativa brasileira com uma composição nutricional em destaque para os altos teores de compostos bioativos, como vitamina C, carotenoides e compostos fenólicos. No entanto, por se tratar de uma fruta regional, sua aplicação é muito limitada a pequenos produtores rurais e não explorada pelo setor industrial. Como a adição de frutas em produtos lácteos possui um bom histórico de aceitação sensorial e o mercado consumidor atual está sempre em busca de alimentos funcionais, este trabalho teve como objetivo adicionar polpa e suco de guabiroba em produtos lácteos fermentados e desenvolver novos produtos a partir dos resíduos de processo e aplicação de tecnologias emergentes. A adição de 10% polpa de guabiroba em iogurtes probióticos adicionados de cepas de *Bifidobacterium animalis* ssp. lactis-BB12, promoveu um aumento da contagem das células probióticas (8 - 9 log UFC g^{-1}) em etapas do cólon intestinal, em uma simulação gastrointestinal *in vitro*. Essa alta contagem de células viáveis, somada a altos teores de compostos fenólicos totais e atividade antioxidante durante toda a etapa gástrica, pode caracterizar os compostos bioativos da guabiroba como potenciais agentes prebióticos e protetores para as bactérias lácteas. Uma bebida láctea fermentada probiótica também foi produzida a partir do soro de leite, um subproduto da indústria de lácteos, concentrado pelo processo de crioconcentração em blocos. Esta tecnologia emergente, com provada eficiência no setor de laticínios, permite obter produtos líquidos concentrados com a utilização de baixas temperaturas e mantém os compostos naturais altamente termolábeis, sendo uma alternativa viável aos processos tradicionais de concentração. Com a adição de 10% de polpa de guabiroba em soro lácteo concentrado (do segundo estágio da crioconcentração) adicionado de culturas termofílicas (*Lactobacillus acidophilus* LA-5, *Bifidobacterium* sp. BB-12 e *Streptococcus thermophilus*), houve contagens de células viáveis > 8 log UFC g^{-1}, caracterizando um produto inédito com potencial probiótico. A adição da polpa de guabiroba também promoveu um aumento de 164% para teores de carotenoides e 1,61 vezes mais compostos fenólicos comparada a uma bebida controle. O processo de crioconcentração também foi eficiente para concentrar os teores de compostos bioativos de suco de guabiroba obtido por prensagem a frio. Esta técnica torna-se muito eficaz para a extração do suco sem adição de calor, o que preserva todos os compostos nutricionais e sensoriais originais da fruta. Com uma eficiência de processo elevada para o primeiro estágio da crioconcentração em blocos (75,73%), o suco concentrado obteve um aumento de 173% para compostos fenólicos totais e 561% para teores de carotenoides. Assim, ao adicionar na proporção de 15% em iogurtes naturais, o suco crioconcentrado promoveu um aumento de 4556% para fenóis totais, assim como aumento em, aproximadamente, 226% para os teores de carotenoides, 2991% para vitamina C e perfil mineral (Ca, K, Mg e Na) de 140 – 225%, o que também contribuiu para um aumento significativo da atividade antioxidante de 440 – 883%. A adição do suco concentrado de guabiroba em formulações de iogurte potencializa a propriedade funcional do alimento com a preservação da maioria dos compostos bioativos durante a prensagem a frio associada com a crioconcentração. Subprodutos de processos também podem ser aplicados em outros produtos alimentícios, com seu reaproveitamento e aumento do valor agregado. Durante os estágios de crioconcentração, a fração de gelo não é usualmente aplicada em produtos. No entanto, ao crioconcentrar o suco de guabiroba, significantes teores de compostos bioativos ficaram retidos nas frações de gelo. Assim, uma bebida carbonatada foi produzida com a adição da fração de gelo residual com elevados teores de compostos funcionais (151,3% a mais de compostos

fenólicos do que a bebida controle,295,8% a mais em atividade antioxidante,168% a mais nos teores de carotenoides e 159% superior em vitamina C), contribuindo para o reaproveitamento dos subprodutos de um processo e agregando valor funcional ao produto. Portanto, este trabalho de potencial inovação e ineditismo visou expandir o conhecimento sobre a guabiroba para os setores acadêmico e industrial, tanto no desenvolvimento de produtos quanto na concentração de seus compostos bioativos. Assim, futuramente, a produção e comercialização do fruto da guabiroba pode expandir para escala industrial, sendo possível incentivar a aplicação de tecnologias emergentes não térmicas no processamento dos frutos, mantendo as propriedades naturais e melhorando a composição dos futuros produtos desenvolvidos.

Palavras-chave: família *Myrtaceae*; leites fermentados; alimento funcional; agente prebiótico; tecnologias emergentes; concentração; crioconcentração em blocos.

ABSTRACT

Guabiroba *(Campomanesia xhantocarpa* O. Berg) is a native Brazilian fruit with a nutritional composition highlighted by the high levels of bioactive compounds, such as vitamin C, carotenoids, and phenolic compounds. However, as it is a regional fruit, its application is very limited to small rural producers and has not been explored by the industrial sector. As the addition of fruits in dairy products has a good history of sensory acceptance and the current consumer market is always looking for functional foods, this work aimed to add guabiroba pulp and juice in fermented dairy products, with the application of emerging technologies and reuse of process by-products. The addition of 10% guabiroba pulp in probiotic yogurts added with strains of *Bifidobacterium animalis* ssp. lactis-BB12, promoted an increase in probiotic cell count (8 - 9 log CFU g^{-1}) in gut steps in an *in vitro* gastrointestinal simulation. This high viable cell count, added to the high levels of total phenolic compounds and antioxidant activity throughout the gastric steps, may characterize the bioactive compounds of guabiroba as potential prebiotic and protective agents for lactic bacteria. A probiotic fermented lactic beverage was also produced from whey, a by-product of the dairy industry, concentrated by the block freeze concentration process. With proven efficiency in the dairy sector, this emerging technology allows obtaining concentrated liquid products at low temperatures. It keeps natural compounds highly thermolabile, a viable alternative to traditional concentration processes. With the addition of 10% guabiroba pulp in whey concentrate (from the second stage of freeze concentration) added with thermophilic cultures (*Lactobacillus acidophilus* LA-5, *Bifidobacterium* sp. BB-12 and *Streptococcus thermophilus*), there were viable cell counts > 8 log CFU g^{-1}, featuring an unprecedented product with probiotic potential. Adding guabiroba pulp also promoted a 164% increase in carotenoid contents and 1.61 times more phenolic compounds than a control beverage. The freeze concentration process also efficiently concentrated the levels of bioactive compounds in guabiroba juice obtained by cold pressing. This technique becomes very effective for extracting juice without adding heat, which preserves all the fruit's original nutritional and sensory compounds. With a high process efficiency for the first stage of block freeze concentration (75.73%), the concentrated juice increased by 173% for total phenolic compounds and 561% for carotenoid contents. Thus, when adding 15% to yogurts, the concentrated juice promoted an increase of 4,556% for total phenols, as well as an increase of approximately 226% for carotenoid contents, 2,991% for vitamin C and profile mineral (Ca, K, Mg, and Na) of 140 – 225%, which also contributed to a significant increase in antioxidant activity of 440 – 883%. The addition of concentrated guabiroba juice in yogurt formulations enhances the functional property of the food by preserving most of the bioactive compounds during cold pressing associated with freeze concentration. Process by-products can also be applied to other food formulations, with their reuse and increased added value. The ice fraction is not usually applied to products during the freeze concentration stages. However, in the freeze concentration of guabiroba juice, significant amounts of bioactive compounds were retained in the ice fractions. Thus, a carbonated beverage was produced with the residual ice fraction with high levels of functional compounds (151.3% more phenolic compounds than the control beverage, 295.8% more antioxidant activity, 168% more carotenoid content, and 159% higher for vitamin C), contributing to the reuse of by-products from a process and adding functional value to the product. Therefore, this potential innovation and unique work aimed to expand knowledge about guabiroba to the academic and industrial sectors, both in product development and in the concentration of its bioactive compounds. Thus, in the future, the production and commercialization of the guabiroba fruit can expand to an industrial scale, making it possible to encourage the application of emerging non-thermal technologies in the

processing of the fruits, maintaining the natural properties and improving the composition of the future products developed.

Keywords: *Myrtaceae* family; fermented milk; functional food; prebiotic agent; emerging technologies, concentration; block freeze concentration.

RESUMO EXPANDIDO
Conforme a Resolução Normativa nº 154/2021/CUN

Introdução

A guabiroba (*Campomanesia xhantocarpa* O. Berg) também conhecida como "guavirova", "guabiroba-miúda", "guabirobeira-do-mato", "gavira" e "guabiroba-do-campo" é uma fruta da árvore frutífera da família *Myrtaceae* pertencente a uma das 3600 espécies distribuídas em mais de 100 gêneros desta família botânica. Essa fruta é nativa do nordeste, centro-oeste (regiões do cerrado) e sul do Brasil, mas também pode ser encontrada em países da América do Sul, como Paraguai, Argentina, Bolívia e Uruguai. O fruto da guabiroba é uma baga achatada, amarelada, globosa, comestível, com 5 a 6 pequenas sementes coriáceas. A fruta *in natura* pode ser utilizada na forma de sucos, doces e sorvetes. Quanto ao valor nutricional, apresenta baixo teor energético, devido à reduzida concentração de macronutrientes, principalmente lipídios. Além disso, a fruta ainda é fonte de cálcio, zinco, ferro e uma quantidade razoável de fibras. Em relação às suas características funcionais, a guabiroba possui altos teores de vitamina C, carotenóides e compostos fenólicos, o que potencializa os benefícios para o consumo humano, uma vez que esses compostos bioativos estão relacionados a uma alta atividade antioxidante e antimicrobiana e podem reduzir a incidência de doenças crônicas não transmissíveis, quando introduzida em uma rotina alimentar. Porém, por ser uma fruta nativa, seu conhecimento ainda não é difundido no meio acadêmico e industrial, havendo uma pequena produção voltada apenas para pequenos produtores agrícolas das regiões brasileiras. A funcionalidade de seus compostos bioativos torna-se interessante de ser explorada aplicada em produtos lácteos, uma vez que a adição de frutas nestes produtos específicos possui um bom histórico de aceitação sensorial pelos consumidores, além do fato da constante busca por alimentos com apelo saudável. Os compostos bioativos das frutas também podem ser concentrados com a utilização de tecnologias emergentes não térmicas, uma vez que são altamente termolábeis e podem ser reduzidos e/ou eliminados da matriz com a utilização de processos de concentração tradicionais que empregam calor. A crioconcentração é uma tecnologia baseada no congelamento prévio de produtos líquidos, seguido de uma separação da fração concentrada e da fração de água congelada. É uma técnica elucidada no setor de laticínios, principalmente pelo processo de crioconcentração gravitacional em blocos. Assim, os compostos bioativos são preservados e concentrados para aplicações futuras em alimentos. O desenvolvimento de pesquisas com o fruto da guabiroba torna possível sua aplicação na ciência e engenharia de alimentos, podendo difundir seu conhecimento além do meio acadêmico, potencializando sua aplicação em escala industrial.

Objetivos

O seguinte trabalho de tese teve como objetivos aplicar a polpa e suco de guabiroba em produtos lácteos e aprimorar a sua funcionalidade e valor nutricional, assim como associar a tecnologias emergentes não térmicas, como a crioconcentração em blocos. Desenvolver produtos inéditos com subprodutos de processos e incentivar, por meio de resultados promissores, a produção, comercialização e processamento do fruto da guabiroba em escala industrial, com a disseminação do conhecimento sobre esta fruta regional.

Metodologia

Iogurtes probióticos contendo a cepa *Bifidobacterium* sp. BB-12 adicionados de 10% de polpa de guabiroba foram submetidos a uma simulação gastrointestinal *in vitro* de acordo com Verruck et al. (2020) e, para todas as etapas gástricas, a contagem de células viáveis foi

realizada bem como a análise de compostos fenólicos totais (SINGLETON; ROSSI, 1965) e atividade antioxidante (BRAND-WILLIAMS; CUVELIER; BERSET, 1995; BENZIE; STRAIN, 1996). Bebidas lácteas probióticas fermentadas foram produzidas com soro de leite concentrado pela técnica de crioconcentração em blocos (CANELLA et al., 2018) com adição de 10% de polpa de guabiroba e culturas lácteas termofílicas (*Lactobacillus acidophilus* LA-5, *Bifidobacterium* sp. BB-12 e *Streptococcus thermophilus*). A contagem de células viáveis foi realizada (VINDEROLA; REINHEIMER, 1999) assim como a análise dos compostos bioativos :carotenoides (RODRIGUEZ-AMAYA, 2001), compostos fenólicos totais (SINGLETON; ROSSI, 1965) e atividade antioxidante (BRAND-WILLIAMS; CUVELIER; BERSET, 1995; RE et al., 1999). A crioconcentração gravitacional em blocos também foi realizada para a concentração de suco de guabiroba prensado a frio e do seu resíduo, a fração de gelo, foi elaborada uma bebida carbonatada com uma proposta funcional. A partir da melhor performance de processo, o suco concentrado foi adicionado na proporção de 0, 10 e 15% em iogurtes. Para todas as frações de concentrado, gelo, iogurtes e bebidas carbonatadas foram realizadas análises físico-químicas (umidade, sólidos totais, proteína, lipídios, cinzas, acidez total titulável) (AOAC,2019), perfil mineral, e análises de compostos bioativos: carotenoides (RODRIGUEZ-AMAYA, 2001), compostos fenólicos totais (SINGLETON; ROSSI, 1965) e atividade antioxidante (BRAND-WILLIAMS; CUVELIER; BERSET, 1995; RE et al., 1999).

Resultados e Discussão

O iogurte probiótico adicionado de 10% de polpa de guabiroba apresentou elevadas contagens de células viáveis mesmo em condições extremas de pH estomacal na simulação *in vitro*, com contagens entre 8-9 log UFC g^{-1} em etapas do cólon intestinal, local ideal para desenvolvimento de células probióticas. Com elevados teores de compostos fenólicos e atividade antioxidante também para estas etapas finais da digestão simulada, atribui-se aos compostos bioativos da guabiroba uma potencial atividade prebiótica e protetiva às bactérias lácticas. Para a bebida láctea fermentada probiótica com soro de leite concentrado (proveniente do segundo estágio de crioconcentração, o qual apresentou maior eficiência de processo; 95,4%), houve contagens de células viáveis > 8 log UFC g^{-1}, caracterizando um produto inédito com potencial probiótico. A adição da polpa de guabiroba também promoveu um aumento de 164% para teores de carotenoides e 1,61 vezes mais compostos fenólicos comparada a uma bebida controle. Para o suco de guabiroba prensado a frio e crioconcentrado, o melhor estágio de processo foi o primeiro, com eficiência de 75,73%. Em relação aos compostos bioativos, o suco concentrado obteve um aumento de 173% para compostos fenólicos totais e 561% para teores de carotenoides comparado com o suco original. Assim, ao adicionar o concentrado na proporção de 15% em iogurtes naturais, houve um aumento de 4556% para fenóis totais, assim como aumento em, aproximadamente, 226% para os teores de carotenoides, 2991% para vitamina C e perfil mineral (Ca, K, Mg e Na) de 140 – 225%, o que também contribuiu para um aumento significativo da atividade antioxidante de 440 – 883%. A elevada concentração de carotenoides presentes na composição da guabiroba modificou a cor do iogurte com 15% de suco (diferença total de cor $\Delta E > 3,0$), podendo ser observado a olho nu a mudança de cor com uma tonalidade laranja. A adição do suco concentrado de guabiroba em formulações de iogurte potencializa a propriedade funcional do alimento com a preservação da maioria dos compostos bioativos durante a prensagem a frio associada com a crioconcentração. A elevada concentração dos compostos bioativos da guabiroba também se torna evidente em frações da fruta processada, como o gelo residual do processo de crioconcentração que, até os dias de hoje, não possui estudos acadêmicos ou aplicações industriais. Assim, a bebida carbonatada produzida com a fração de gelo residual do segundo estágio do processo possuiu elevados teores de compostos fenólicos (151,3% a mais do que a bebida controle), 295,8% a mais em atividade antioxidante,168% a mais nos teores de carotenoides e 159% superior em vitamina C, quando

comparada com a bebida contendo apenas o suco de guabiroba. Além disso, as análises de diferença total de cor mostraram que não há diferença visível de cor ou tonalidade a olho nu, evidenciando a cor laranja marcante do fruto nos produtos obtidos.

Considerações finais

Os teores de compostos bioativos em abundância presentes na composição da guabiroba caracterizam a mesma como um alimento funcional. Pesquisas sobre a sua incorporação em formulações de alimentos podem intensificar o interesse industrial no desenvolvimento de novos produtos, em especial para o setor de laticínios, o qual possui elevada aceitação do mercado consumidor na fusão de polpas e sucos de frutas com produtos lácteos. A aplicação de tecnologias emergentes não térmicas viáveis em lácteos e em sucos de frutas, como a crioconcentração em blocos, pode concentrar os compostos bioativos relacionados a atividades funcionais e aspectos sensoriais sem alterar os seus teores pelo processo ocorrer em baixas temperaturas. A provada ação antioxidante dos compostos bioativos da guabiroba pode substituir conservantes de origem sintética na formulação de alimentos, assim como os corantes, uma vez que a coloração do fruto da guabiroba possui uma tonalidade laranja intensa devido aos altos teores de carotenoides em sua composição. Essa possível substituição na indústria de alimentos é de extrema importância devido a quadros comuns de alergia alimentar em parte da população. O incentivo de estudos e pesquisas com a guabiroba, bem como sua aplicação e resultados promissores em alimentos, pode ampliar o conhecimento desta fruta regional para o meio acadêmico e para o setor industrial. A divulgação da rica composição desta fruta nativa pode aumentar a produção, comercialização e processamento do fruto em escala industrial.

Palavras-chave: família *Myrtaceae*; leites fermentados; alimento funcional; agente prebiótico; tecnologias emergentes; concentração; crioconcentração em blocos.

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SUMMARY

INTRODUCTION

The guabiroba (*Campomanesia xhantocarpa* O. Berg) also known as “guavirova”, “guabiroba-miúda”, “guabirobeira-do-mato”, “gavira”, and “guabiroba-do-campo” is a fruit of the guabirobeira, a fruit-bearing tree from the *Myrtaceae* family belonging to one of the 3,600 species distributed in more than 100 genera of this botanical family. This fruit is native to the northeast, central west (Cerrado regions), and south of Brazil; however, it can also be found in South American countries such as Paraguay, Argentina, Bolivia, and Uruguay. The nutritional composition of this fruit is highly rich, with emphasis on the levels of Vitamin C (17.8 – 233.0 mg $100g^{-1}$: corresponding to up to six times the orange content), carotenoids (12.3-3400 mg $100g^{-1}$), and polyphenols (9033.2 mg CAE $100g^{-1}$). In addition, the guabiroba is a source of fibers, carbohydrates, and potassium (DE PAULO FARIAS et al., 2020; PRESTES et al., 2022; SANTOS et al., 2009; SCHMIDT et al., 2019). With these functional properties, the guabiroba has been studied recently in the development of new products, including liqueurs, jams, dairy products, and the production of biodegradable films (LEONARSKI et al., 2020, 2021; MALHERBI et al., 2019; PRESTES et al., 2021; RAMOS MESSIAS et al., 2021). In dairy products, for example, the high consumer acceptability combined with the nutritional properties of fruit and milk can increase the functional appeal of the products.

In recent years, the consumption of functional foods has been increasing due to the healthy appeal sought by the consumer market. In 2019, the global market for fermented dairy products, including probiotic/symbiotic products, was approximately US$ 64 billion and is expected to reach 6% CAGR (Compound Annual Growth Rate) by 2024 (RESEARCH AND MARKETS, 2019; SAKANDAR; ZHANG, 2021). With the development of new functional products by the dairy industries, the potential market for probiotics will grow even more globally, emphasizing China, which is expected to reach US$ 20 billion by the end of 2022 (SAKANDAR; ZHANG, 2021). There are several benefits of including probiotic/symbiotic fermented dairy products in a dietary routine, going beyond gastrointestinal health, as well as improved immunity, reduced cholesterol levels, cognitive growth, and potential anticancer, antioxidant, and antimutagenic activities (ABDOLLAHZADEH et al., 2018; AYYASH et al., 2018; CHEN et al., 2010; FAZILAH et al., 2018; MALLAPPA et al., 2021; PRESTES et al., 2021). Furthermore, fermented dairy products are highly recommended for consumption by

individuals with certain lactose intolerance since digestibility is easier when compared to unfermented dairy products (EZZATPANAH, 2020; MALLAPPA et al., 2021).

The development of new fermented dairy products is wide, with emphasis on the addition of seeds, extracts, and mainly fruits, which are very sensorially accepted and have bioactive compounds that improve the nutritional and functional composition of fermented milk, such as vitamins, phenolic compounds, carotenoids, fatty acids, and fibers. These compounds, in addition to contributing to the consumer's health, tend to potentiate the development of probiotic microorganisms in the formulation, with prebiotic properties (ABDOLLAHZADEH et al., 2018; BALTHAZAR et al., 2019; FLORENCE et al., 2016; LAMOTHE et al., 2014; PRESTES et al., 2021; RAMIREZ-SANTIAGO et al., 2010). Studies with the addition of guabiroba fruit and its fractions in these dairy products lead to the dissemination of knowledge about this native fruit, with a rich nutritional composition, however little valued in large-scale productions, with only production aimed at small agricultural producers in Brazilian regions (PRESTES et al., 2022a, 2021).

Incorporating guabiroba fruit fractions into dairy products highlights the importance of technological innovation in applicability on an industrial scale. According to normative instruction IN 30/2017 of the Ministry of Agriculture, Livestock and Supply (MAPA, 2017) in Brazil, the description of processes, methodologies, equipment, and new ingredients becomes essential to validate and implement new procedures at any stage of product manufacturing. The publication of scientific articles on the importance of adding guabiroba pulp or juice to enhance the functional characteristics of dairy products is an interesting support for implementing industrial technological innovation. This procedure must be adapted to the specific installation and production characteristics of the establishment that wishes to apply it. During the years of work on this Ph.D thesis, there was an important research and extension partnership between the federal university of Santa Catarina- Brazil and EMBRAPA Florestas (Brazilian agricultural research company) to encourage Brazilian agriculture and family farming. In Paraná, Brazil, EMBRAPA Florestas has been working with guabiroba in the most different ways. Guabiroba pulp was one of the products developed by EMBRAPA together with a group of farmers from the *Pinho De Baixo* community, located in the interior of the Irati city– PR (25° 28' 3" South, 50° 39' 4" West). Between 2021/2022, three tons of this fruit were collected, which resulted after pulping. This group of farmers also includes milk producers. The development of dairy products with the incorporation of this fruit becomes interesting to generate an extra source of income for producers. Thus, it resulted in an extension project with producers in the Irati city-

PR in developing new guabiroba pulp dairy products (Figures 1 and 2, Annex B). This pioneering project aims to expand knowledge of guabiroba, and its technological approach, encouraging other fruit producing regions to expand technological knowledge about the products that can be made from guabiroba fractions.

Figure 1- Extension project with farmers in Irati city-PR, Brazil.

Figure 2- Training rural producers of Irati city (SC, Brazil) with the development of products with guabiroba pulp

The bioactive compounds of guabiroba, including the high levels of carotenoids and phenolic compounds, are also targets for the application of emerging technologies for the extraction and concentration of these compounds (CAPELETTO et al., 2016; CZAIKOSKI et al., 2015; DIAS et al., 2020). In food engineering, the concentration of compounds is very important for future application in food. Regarding the bioactive compounds of guabiroba, which are sensitive to high temperatures, this concentration process must be carried out using non-thermal emerging technologies. Among these techniques, cold pressing is a smart strategy for obtaining whole fruit juices at a controlled temperature, preserving their nutritional properties as much as possible and ensuring the functional appeal of the product (MARTINS et al., 2020; PRESTES et al., 2023). Furthermore, the fusion of this process with non-thermal concentration technologies can enhance the maintenance of the fruit's original compounds. The freeze concentration is when a gravitational process previously freezes the liquid product, and pure water is separated into ice fractions. The liquid product is concentrated at approximately 50%, a simple, cheap, and eco-friendly technology without using solvents and external energy (DANTAS et al., 2021; PRESTES et al., 2022b). The combination of the cold pressing process with the subsequent freeze concentration technology of guabiroba juice could be a strategy to increase the content of vitamin C, phenolic compounds, minerals, and carotenoids in the final products, being able to enhance the functionality of dairy products by adding them to formulations. The original intense yellow/orange color of guabiroba fruits is also related to the high levels of functional compounds, specifically the levels of carotenoids (PRESTES et al., 2022a). Incorporating guabiroba and its fractions in dairy products may also be a potential strategy for replacing synthetic food colorings, considered allergenic for a significant portion of the world's population.

With the aim of waste management and the resulting circular economy, the ice fractions resultant from each stage of freeze concentration still do not have industrial application, and many academic studies are considered a processing residue. However, due to the fractions of bioactive compounds that tend to be retained in the ice fractions during the process, it would be interesting to reuse the residue in developing new products, contributing to increasing its added value. The same logic can be applied to reuse by-products from the dairy industries. Whey, from the manufacture of cheeses, has a very high pollutant load and, nowadays, is reused in the preparation of various food products (ARRANZ et al., 2019; BARROS et al., 2021; CANELLA et al., 2018; NZEKOUE et al., 2021). The combination of fractions of the guabiroba fruit with co-products from the dairy industry in the development of

new products tends to increase the nutritional and functional value of the food, contribute to the sensory appeal, and present new product possibilities to the consumer market, encompassing knowledge of this Brazilian fruit.

The objective of this Ph.D thesis was to enhance the nutritional, functional and technological properties of the guabiroba fruit in the elaboration of new products, reuse of by-products, and application of emerging technologies to maintain the fruit's bioactive compounds as much as possible, with promising results to be applied in the future on industrial-scale production. The dissemination of knowledge about guabiroba internationally can promote large-scale production of this fruit and contribute economically to family farming. The rich composition of guabiroba and its fractions enhances the functional properties of new products, especially in the dairy sector. Furthermore, the intelligent and unprecedented strategy of developing products made from process by-products enhances the circular economy by reusing waste, potentially promoting industrial economic interest.

WORK STRUCTURE

This work was organized into 1 (one) section and 11 (eleven) chapters, following a logical sequence based on its execution steps. The bibliographic review is divided into 7 (seven) chapters in the format of scientific articles or book chapters, with different and fundamental theoretical subjects addressed during the execution of this work. Thus, it is structured as follows:

Section 1- Introduction.

In this initial section, there is a general approach regarding consuming fermented milks and improving their nutritional and functional composition by adding fruit pulps to the formulation. The presentation of the native Brazilian fruit guabiroba is also found in this introduction, with its main nutritional composition highlights. The emerging technique of freeze concentration was also addressed in this topic, which aims to add value to liquid foods and increase the final quality. The main objectives of this project are also described in this section.

Chapter 1 – "Potential properties of guabiroba (*Campomanesia xanthocarpa* O.Berg) processing: a native Brazilian fruit."

This first chapter of the bibliographic review approaches the properties of guabiroba (planting characteristics, fruit structure, and nutritional composition). It also presents its potential characteristics for the industrial sector and the different studies on the development of new products with this regional fruit.

Studies on emerging technologies of extraction/concentration that have already been carried out with guabiroba were also addressed, as well as prospects for the industrial exploitation of the fruit and possible techniques to be applied in the extraction/concentration of its bioactive compounds.

This work was published in *Advances in Food Technology and Nutritional Sciences.* DOI: **https://dx.doi.org/10.17140/AFTNSOJ-8-174.**

Chapter 2- "Sensory Profile of Yoghurt and Related Products"

This chapter addresses the concepts and types of processing of the main yoghurts known and commercialized worldwide. How the fermentation of each product occurs, and the main strains of microorganisms used in each fermented milk are explained. The main techniques used in sensory analysis for these specific products are also addressed in this chapter.

This work is a chapter published in the book *Sensory profiling of dairy products* – Wiley Blackwell, 2023. **ISBN: 9781119619215**.

Chapter 3- "The Improvement of the Functional Potential of Dairy Products Using Fruits and Plant Extracts".

This chapter presents the main benefits found in recent studies of the addition of bioactive compounds to dairy products (cheese, fermented milk, dairy beverages, butter, ice cream) from plant extracts, fruit juices, and pulps, seeds, and roots to expand the benefits to consumer health, as well as a natural preservative action and increasing the shelf life of products, with an innovative characteristic.

This work is a chapter published in the book Dairy Products and Health; Tatiana C. Pimentel; Marciane Magnani; Elane S. Prudencio (Eds.), Nova Science Publishers, 2023. DOI: **https://doi.org/10.52305/XXQT1047; ISBN: 979-8-88697-661-8.**

Chapter 4- "How to improve the functionality, nutritional value and health properties of fermented milks added of fruits bioactive compounds: a review."

This chapter briefly presents the main fermented milks and their production methods and the main microbiological cultures used in the processes. The main bioactive compounds present in the composition of fruits (fatty acids, phenolic compounds, carotenoids, vitamins and fibers) are emphasized in recent studies applied to dairy products. Benefits to consumer health were also addressed, as well as the improvement of the development of probiotic strains present in dairy products when added with different bioactive compounds.

This work was published in the journal *Food Science and Technology*. DOI: **https://doi.org/10.1590/fst.17721.**

Chapter 5- "Conventional and alternative concentration processes in milk manufacturing: a comparative study on dairy properties."

This chapter presents the main and traditional thermal technologies used in the concentration of dairy products and their operating methods. In parallel, there is the exposition of emerging non-thermal technologies used to concentrate food products while maintaining their nutritional, functional, and sensory properties. The incentive to replace traditional thermal techniques is presented during the writing with the presentation of their advantages and disadvantages. Among the products concentrated by these emerging technologies, those obtained from milk manufacturing are included, with recent studies addressing the improvements obtained by applying non-thermal techniques.

This work was published in the journal *Food Science and Technology*. **DOI: https://doi.org/10.1590/fst.08822**

Chapter 6- "Freeze concentration techniques as alternative methods to thermal processing in dairy manufacturing: A review."

This chapter discusses the concepts and methods of the emerging freeze concentration technology. The different techniques are presented and compared for their economic, processing, and performance advantages and disadvantages. Recent studies on dairy products applying each technique are presented with their main physicochemical, structural, nutritional, and microbiological improvements.

This work was published in the *Journal of Food Science*. DOI: **https://doi.org/10.1111/1750-3841.16027.**

Chapter 7- “The use of cold pressing technique associated with emerging non-thermal technologies in the preservation of bioactive compounds in tropical fruit juices: an overview”.

This last chapter in the bibliographical review describes the cold pressing process as a viable alternative to the traditional processes of extracting fruit juices. Through this method, it is possible to obtain high sensory and nutritional juices, characterizing them as premium juices. The association of cold-pressed juices with emerging non-thermal technologies can improve product conservation characteristics and fruit juice extraction.

This work was published in the *Current Opinion in Food Science*. DOI: **10.1016/J.COFS.2023.101005.**

Chapter 8- “Influence of guabiroba pulp (*Campomanesia xanthocarpa* O. Berg) added to fermented milk on probiotic survival under in vitro simulated gastrointestinal conditions”.

In this research chapter of this project, guabiroba pulp was added to probiotic fermented milks with the strain *Bifidobacterium BB-12*. These dairy products were submitted to an in vitro gastrointestinal simulation. Total phenolic compounds and antioxidant activity were analyzed, with results obtained in all gastric steps.

This work was published in the journal *Food Research International*. DOI: **https://doi.org/10.1016/j.foodres.2021.110135.**

Chapter 9- “Whey block freeze concentration aiming a functional fermented lactic beverage with the addition of probiotic and guabiroba pulp (*Campomanesia xanthocarpa* O. Berg), a native Brazilian fruit”.

In this chapter, whey, considered a by-product of the dairy industry, was reused through the freeze concentration process, and added to create a functional dairy beverage with probiotic bacteria and guabiroba pulp. The bioactive compounds of the fruit and the concentrated solids of the whey, combined with the benefits of probiotic cells, increase this unprecedented product's functional and antioxidant properties.

This work was published in the journal Food science and Technology. DOI: **https://doi.org/10.5327/fst.26923.**

Chapter 10 – “Production of functional yogurt with cold-pressed guabiroba juice (*campomanesia xanthocarpa* o. Berg) concentrated by freeze concentration process”

In this chapter, the guabiroba juice, obtained by a cold-pressed, was subjected to the gravitational block freeze concentration followed by the evaluation of its proximate composition, antioxidant activity, phenolic and mineral profile, in addition to the colorimetric characteristics of the concentrated pulp and the ice fraction. The concentrated juice was applied in fermented milks, and the products were evaluated in terms of their physicochemical composition, phenolic and mineral profile, antioxidant activity, and colorimetric analysis.

This work was submitted to the *Journal of Dairy Research*.

Chapter 11- "The use of ice fraction from the freeze concentration process of cold-pressed guabiroba juice aims the elaboration a functional carbonated beverage: waste management and circular economy".

In this chapter, the residual ice fraction from the freeze concentration of the guabiroba juice was used to prepare an innovative carbonated beverage without added sugar and with potential functionality. The abundant bioactive compounds from the guabiroba fruit remain in their fractions, increasing the antioxidant activity of the residue, which currently does not have industrial application or academic studies.

This work was submitted to the journal *International Journal of Gastronomy and Food Science*

Articles published in scientific journals and works presented at events in can be found in Annex A and Annex B.

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CHAPTER 1

Potential properties of guabiroba (*Campomanesia xanthocarpa O.Berg*) processing: a native Brazilian fruit

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Potential properties of guabiroba (*Campomanesia xanthocarpa O.Berg*) processing: a native Brazilian fruit

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ABSTRACT

Guabiroba (*Campomanesia xanthocarpa* O.Berg) is a native Brazilian fruit with an important nutritional value and a great economic potential for processing. This fruit is a source of fibers, carbohydrates, potassium, and bioactive compounds, such as polyphenols, carotenoids, and Vitamin C. The phytochemicals of guabiroba are elucidated regarding their high antioxidant activity, which is related to human health benefits when introduced into a dietary routine. In addition, the antioxidant property of this native fruit can act as a natural preservative against oxidative and enzymatic reactions, and microbiological spoilage, extending the shelf-life of food. Thus, the addition of guabiroba in the development of new products, in addition to improving the functionality of the food, can reduce the use of chemical additives. Studies related to encouraging the use of guabiroba in food formulation, as well as the use of emerging technologies in the processing of this native fruit, become the basis of this review that aims to expand the knowledge of this Brazilian fruit and enhance its application in the food industry.

Keywords: *Myrtaceae* family; gavirova; new products; emerging technologies; technological approach.

1 INTRODUCTION

The guabiroba (*Campomanesia xanthocarpa* O.Berg) also known as “guavirova”, “guabiroba-miúda”, “guabirobeira-do-mato”, “gavira”, and “guabiroba-do-campo” is a fruit of the guabirobeira, a fruit-bearing tree from the *Myrtaceae* family belonging to one of the 3,600 species distributed in more than 100 genera of this botanical family (ALVES et al., 2013; BARBIERI et al., 2018; DE PAULO FARIAS et al., 2020; SANTOS et al., 2013; VALLILO et al., 2008). This fruit is native to the northeast, central west (cerrado regions) and south of Brazil, however, it can also be found in South American countries such as Paraguay, Argentina, Bolivia, and Uruguay (BARBIERI et al., 2018; DE OLIVEIRA et al., 2018; DE OLIVEIRA RAPHAELLI et al., 2021; JACOMINO et al., 2018; SANTOS et al., 2009; VALLILO et al.,

2008). The name “guabiroba” refers to bitter fruit, in the Tupi-Guarani language spoken by specific indigenous groups in Brazilian regions (DE OLIVEIRA RAPHAELLI et al., 2021).

The fruits can be harvested at different stages of ripeness, which enhances fresh consumption or after processing as sweets, ice creams, fermented milks, homemade liqueurs, and jams (BARBIERI et al., 2018; DE PAULO FARIAS et al., 2020; PRESTES et al., 2021; SANTOS et al., 2009). In addition, bioactive compounds from guabiroba fruits, leaves, and seeds have received attention in promising studies on the development of functional products, food packaging, and medicines due to their potential antioxidant, antithrombotic, antiproliferative, trypanocidal, and prebiotic activities (DE OLIVEIRA RAPHAELLI et al., 2021; KLAFKE et al., 2012; MALHERBI et al., 2019; PRESTES et al., 2021; SALMAZZO et al., 2021; SILVA-RODRIGUES et al., 2020; VIECILI et al., 2014).

The consumption and processing of native fruits have received encouragement lately due not only to the technological potential but also to the diversification of fruit production for processing in a specific region and the high functional and nutritional significance for human health (SANTOS, 2012; SCHMIDT et al., 2019). For guabiroba, although it has a high potential for processing on an industrial scale, crop data are still scarce for the commercial use of the fruit (BARBIERI et al., 2018; SANTOS, 2012). The knowledge and studies about this native Brazilian fruit, as well as its by-products, contribute to adding value and enhancing the commercial and industrial application (MENDES; PINTO; SOARES, 2018).

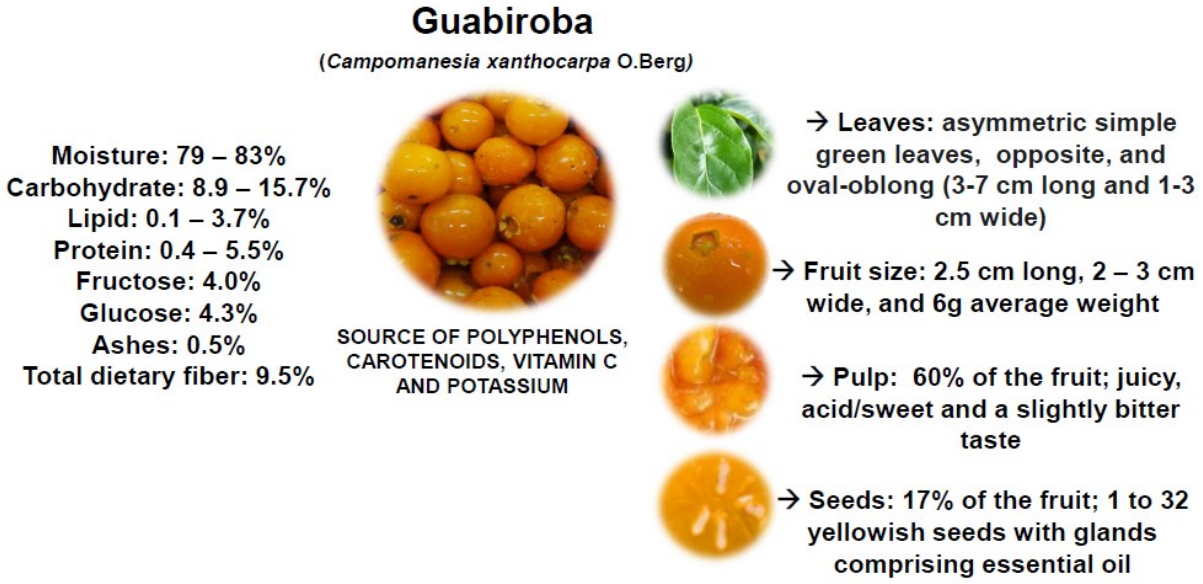
This review aims to present the technological properties of guabiroba, bringing studies addressing the application of the fruit and by-products in the development of new products, the potential application of emerging technologies as well as expanding knowledge about this Brazilian fruit for an increase in its consumption and future industrial applications.

2 COMPOSITION AND PROPERTIES OF GUABIROBA

The *Campomanesia xanthocarpa O.Berg* species (with botanical synonyms *C. crenata, C. dusenii, C. littoralis, C. malifolia,* and *C. rhombea*) is a shrub or tree-shaped, 10 to 20 meters high, 30 to 70 cm in diameter, with asymmetric simple green leaves, opposite, and oval-oblong (3-7 cm long and 1-3 cm wide) (LISBÔA; KINUPP; DE BARROS, 2011; VALLILO et al., 2008). The fruits are classified as small glabrous berries (2.5 cm long, 2 – 3 cm wide, and 6g average weight), with a green epicarp when young and yellow-orange, thin and smooth when ripe (DE PAULO FARIAS et al., 2020; LISBÔA; KINUPP; DE BARROS,

2011; VALLILO et al., 2008). The endocarp is juicy, sweet, acid, with a slightly bitter taste, aromatic, and contains 1 to 32 yellowish seeds with glands comprising essential oil (DE PAULO FARIAS et al., 2020; LISBÔA; KINUPP; DE BARROS, 2011; SANTOS et al., 2009) (Figure 1). In general, the fruit composition is 55% mesocarp, 18% epicarp, 13% seeds, 10% endocarp, and 3.9% chalice(DE OLIVEIRA RAPHAELLI et al., 2021; SANTOS et al., 2009). It is a botanical species with good adaptability, being able to develop in dry, compact, and low fertility soils (LISBÔA; KINUPP; DE BARROS, 2011).

Figure 3- Campomanesia xanthocarpa *O.Berg properties.*



Source: Data according to Vallilo et al. (2008), De Paulo Farias et al. (2020), Santos (2012), Schmidt et al. (2019), Lisbôa, Kinupp, de Barros (2011), and Pereira et al. (2012).

Concerning the nutritional composition, the guabiroba fruit is a good source of macronutrients, vitamins, and minerals, with variable contents according to the climatic conditions, season, agronomic factors, soil conditions, management, variety, plant nutrition, and ripening stage (Table 1) (DE OLIVEIRA RAPHAELLI et al., 2021; SANTOS, 2012; PEREIRA et al., 2012; SCHMIDT et al., 2019; VALLILO et al., 2008). The combination of these intrinsic and extrinsic factors influences the phytochemical metabolism of the plant, diversifying the content of bioactive compounds and vegetable composition (SCHMIDT et al., 2019). However, even if growing conditions vary, the guabiroba fruits usually contain high moisture (79-84%), which characterizes the juiciness of the pulp, and low caloric value (57.3 kcal $100g^{-1}$) due to the low concentration of carbohydrates, lipids, and proteins in the endocarp,

mesocarp, and seeds (DE OLIVEIRA RAPHAELLI et al., 2021; SANTOS et al., 2009, 2013; VALLILO et al., 2008).

Table 1- Nutritional, physicochemical composition and antioxidant properties of *Campomanesia xanthocarpa* O.Berg fruits.

Parameter	Content (unit per 100g)	References
Moisture	79.0 - 83.0 g [b]	(VALLILO et al., 2008; DE PAULO FARIAS et al., 2020; DE OLIVEIRA RAPHAELLI et al., 2021; SANTOS et al., 2009; SCHMIDT et al. 2019)
Carbohydrate	8.9 - 15.7 g [a]	(VALLILO et al., 2008; SCHMIDT et al. 2019)
Lipid	0.1 - 3.7 g [a]	(SANTOS et al., 2009; VALLILO et al., 2008; SCHMIDT et al. 2019)
Protein	0.4 – 5.5 g [a]	(DE OLIVEIRA RAPHAELLI et al., 2021; PEREIRA et al. 2012; SCHMIDT et al. 2019)
Total Sugar	34.4 g [a]	(DE PAULO FARIAS et al., 2020; SCHMIDT et al. 2019)
Reducing Sugar	8.3-34.1 g [a]	(DE PAULO FARIAS et al., 2020; SANTOS et al., 2009; SCHMIDT et al. 2019)
Fructose	4.0 g [b]	(SANTOS et al., 2009)
Glucose	4.3 g [b]	(SANTOS et al., 2009)
Ashes	0.5 g [b]	(SCHMIDT et al. 2019)
Total dietary fiber	6.3-9.7 g [a]	(VALLILO et al., 2008; SCHMIDT et al. 2019)
Insoluble dietary fiber	9.5 g [a]	(SCHMIDT et al. 2019)
pH	3.9 – 4.58 [b]	(DE PAULO FARIAS et al., 2020; SANTOS et al., 2009)
Total Titratable acidity	0.3-0.5g [a]	(SANTOS et al., 2009; SCHMIDT et al. 2019)
Total soluble solids	12.0-15.3 [b]	(SANTOS et al., 2009; SCHMIDT et al. 2019)
Vitamin A (Retinol)	0.2-0.9 µg RE [a]	(VALLILO et al., 2008; SCHMIDT et al. 2019)
Vitamin C (Ascorbic Acid)	17.8-233.0 mg [a]	(VALLILO et al., 2008; SANTOS et al., 2009; SCHMIDT et al. 2019)
Vitamin B1 (Thiamine)	$3x10^{-3}$ µg [a]	(SCHMIDT et al. 2019)
Vitamin B2 (Riboflavin)	0.1-1.5 mg [a]	(VALLILO et al., 2008; DE PAULO FARIAS et al., 2020; SCHMIDT et al. 2019)
Vitamin B5 (Pantothenic Acid)	0.3µg [a]	(SCHMIDT et al. 2019)
Vitamin B6 (Pyridoxin)	0.1µg [a]	(SCHMIDT et al. 2019)
Vitamin B7 (Biotin)	0.3µg [a]	(SCHMIDT et al. 2019)
Essential oil	0.2g [b]	(VALLILO et al., 2008)
Total Carotenoids	20.7-3.10^{7} mg [b]	(SCHMIDT et al. 2019; SANTOS, 2012)
Total Polyphenols	9033.2 mg CAE [a]	(SCHMIDT et al. 2019)
Total Flavonoids	68.0 mg QE [b]	(DE PAULO FARIAS et al., 2020; SANTOS, 2012)
Total Anthocyanins	3.2-11.7 mg [b]	(DE PAULO FARIAS et al., 2020)
ABTS radical scavenging capacity	50.7 mmol TE [a]	(SCHMIDT et al. 2019)
K	208.4 mg [b]	(VALLILO et al., 2008)
Na	2.6 mg [b]	(VALLILO et al., 2008)
Ca	28.4 mg [b]	(VALLILO et al., 2008)
Mg	13.5 mg [b]	(VALLILO et al., 2008)
P	14.9 mg [b]	(VALLILO et al., 2008)

Zn	0.4 mg [b]	(VALLILO et al., 2008)
Fe	0.6 mg [b]	(VALLILO et al., 2008)
Cu	0.3 mg [b]	(VALLILO et al., 2008)
Mn	0.12 mg [b]	(VALLILO et al., 2008)
Se	0.12 mg [b]	(VALLILO et al., 2008)
Al	0.32 mg [b]	(VALLILO et al., 2008)
Ba	0.14 mg [b]	(VALLILO et al., 2008)
Pb	0.13 mg [b]	(VALLILO et al., 2008)
As	0.09 mg [b]	(VALLILO et al., 2008)
Ni	0.12 mg [b]	(VALLILO et al., 2008)

Note: RE- retinol equivalent; CAE- Chlorogenic acid equivalent; GAE- Gallic acid equivalent; QE- Quercetin equivalent; [a] Values expressed based on dry weight; [b] Values expressed based on fresh weight.

In general, the guabiroba presents high nutritional properties and can be considered a functional food, with higher carbohydrate content when compared to protein and lipids (Table 1), which is characteristic in fruits belonging to the *Myrtaceae* family (SANTOS, 2012; SANTOS et al., 2007, 2009). The fruits contain high levels of vitamin C (17.8 – 233.0 mg $100g^{-1}$), which corresponds to up to six times the orange content, offering a potential benefit to human health due to the antioxidant activity, which acts on the mechanism of scavenging free radicals, related to aging processes and degenerative diseases (SANTOS et al., 2009; SCHMIDT et al., 2019; VALLILO et al., 2008). Guabiroba also presents considerable amounts of vitamin A (20-90 µg. g^{-1} RE, Table 1), essential in the physiology of the retina, bone remodeling, epithelial tissue maintenance, and the reproductive system (DE OLIVEIRA RAPHAELLI et al., 2021; ENGELKING, 2015; VALLILO et al., 2008). It is estimated that the consumption of 10 fruits contributes approximately 5.4% fiber, 1.6% vitamin B2, and 8.5% vitamin C in the daily diet of adult individuals, when based on the recommended values by the World Health Organization (OMS, 2003; VALLILO et al., 2008).

Among the mineral's composition, guabiroba is rich in potassium, calcium, sodium, phosphorus, iron, manganese, and zinc (Table 1). Sodium and potassium contents influence the texture of the fruit, with an increase in hardness by reducing the electrostatic repulsion of carboxyls present in the composition of plant cells (DE OLIVEIRA RAPHAELLI et al., 2021; SANTOS et al., 2009). For iron contents, guabiroba presents higher levels (0.6 mg 100 g^{-1}) than commonly consumed fruits, such as banana (0.4 mg $100g^{-1}$) and apple (0.1 mg 100 g^{-1}) (DE OLIVEIRA RAPHAELLI et al., 2021; SANTOS, 2012).

Phenolic compounds and carotenoids are non-nutritive compounds present in fruits and vegetables related to important benefits for health due to antioxidant, antimicrobial, anti-obesity, antihypertensive, antihyperglycemic activities, and neuroprotective effects (DONADO-PESTANA et al., 2018; FIDELIS et al., 2018, 2020). The important antioxidant

potential, in addition, may increase industrial interest in food formulations to replace preservatives, additives, and even artificial colorants, since carotenoids are fat-soluble pigments responsible for the orange, yellow, and red coloration (DE PAULO FARIAS et al., 2020; PEREIRA et al., 2012; RODRIGUEZ-CONCEPCION et al., 2018). For *Campomanesia xanthocarpa* O.Berg composition, there is an interesting source of carotenoids (mainly β-carotene, lutein, cryptoxanthin, and zeaxanthin), with higher amounts of β- carotene (12.3-3400 mg $100g^{-1}$, Table 2), considered the one with the greatest vitamin A potential, when compared to other fruits, such as papaya (0.04 mg $100g^{-1}$), watermelon (0.36 mg $100g^{-1}$), and orange (0.09 mg $100g^{-1}$) (DE PAULO FARIAS et al., 2020; SCHMIDT et al., 2019). The fruits also present high content of cryptoxanthin (9.31 mg $100g^{-1}$), the main carotenoid that characterizes the orange-colored pulp in several fruits, standing out from other fruits such as nectarine (0.8 mg $100g^{-1}$), papaya (0.5 mg $100g^{-1}$), and apricot (0.6 mg $100g^{-1}$) (PEREIRA et al., 2012).

Table 2- Individual phenolic compounds and carotenoids of *Campomanesia xanthocarpa* O.Berg fruits.

	Compounds	Content (unit per g)	References
Phenolic compounds	Gallic acid	3050.8 µg [b]	(DE PAULO FARIAS et al., 2020; DE OLIVEIRA RAPHAELLI et al, 2021; SANTOS et al.; 2012; PEREIRA et al. 2012)
	Elagic acid	123.6 µg [b]	
	Ferulic acid	22.9 µg [b]	
	ρ-coumaric acid	15.5 µg [b]	
	Epicatechin	5760.4 µg [b]	
Carotenoids	β- carotene	123.5 – 3.4 x 10^4 µg [b]	
	α- carotene	55.5 -1.7 x10^4 µg [b]	
	lutein	14.9 µg [a]	
	zeaxanthin	3.2 µg [a]	
	Lycopene	0.9 µg [b]	
	β-carotene 5,6-epoxide	0.8 µg [a]	
	cryptoxanthin	12.1 µg [a]	
	β- cryptoxanthin	93.1 µg [b]	
	13-cis-β-carotene	0.6 µg [a]	
	9-cis-β-carotene	0.5 µg [a]	
	violaxanthin	2.8 µg [b]	

Note: [a] Values expressed based on dry weight; [b] Values expressed based on fresh weight.

For total phenolic compounds, the guabiroba fruit presents higher amounts (9033.2 mg CAE $100g^{-1}$, Table 1) when compared to conventional fruits, such as apples (150-350 mg GAE $100g^{-1}$), grapes (720-1232 mg GAE $100g^{-1}$), and some fruits also from *Myrtaceae* family such as yellow guava (*Psidium cattleianum* Sabine*;* 3713.2 mg CAE $100g^{-1}$) and uvaia (*Eugenia pyriformis* Cambess; 3482.0 mg CAE $100g^{-1}$), relating this native fruit to high antioxidant activity, bringing health benefits when introduced in a dietary routine and may contribute to the reduction of chronic non-transmissible diseases (CORONA-LEO; MEZA-MÁRQUEZ; HERNÁNDEZ-MARTÍNEZ, 2021; DE PAULO FARIAS et al., 2020; PANTELIĆ et al., 2016; PEREIRA et al., 2012). An individual composition, guabiroba has high contents of gallic acid (3050.8 $\mu g\ g^{-1}$) and epicatechin (5760.4 $\mu g\ g^{-1}$; Table 2), in which phenolic acids and flavonoids are reported to reduce oxidative stress and inflammation, heart diseases, the incidence of type-2 diabetes mellitus, in addition to antibacterial, antiproliferative, antioxidant, and anticarcinogenic activities (CAPELETTO et al., 2016; DE OLIVEIRA RAPHAELLI et al., 2021; DE PAULO FARIAS et al., 2020; SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018).

From a technological point of view, guabiroba has composition properties that contribute to fruit processing. The moisture (79 - 83%), the total soluble solids content in the ripe pulp (12 - 15%), and the total titratable acidity (0.3 - 0.5%) are in the range recommended for fruits destined for processing, contributing to a natural flavor for the product and reducing the addition of sugars, acidulants, and artificial flavors. In addition, the process can be more economical due to the high product yield, a short time in evaporation steps, and less energy expenditures (DE PAULO FARIAS et al., 2020; PEREIRA et al., 2012; VALLILO et al., 2008).

The high fibers content (6.3 – 9.7 g $100g^{-1}$; Table 1), including insoluble and soluble fractions (mainly pectin), are also an important characteristic that can favor the guabiroba processing by the food industries due to the gelling and stabilizing properties, very important for the texture of fruit-based jellies or candies (DE OLIVEIRA RAPHAELLI et al., 2021; SANTOS et al., 2009). Dietary fibers are also related to health benefits when routinely consumed, as hypoglycemic, antioxidant, anti-tumor, and anti-inflammatory properties (BARBIERI et al., 2019; MINZANOVA et al., 2018).

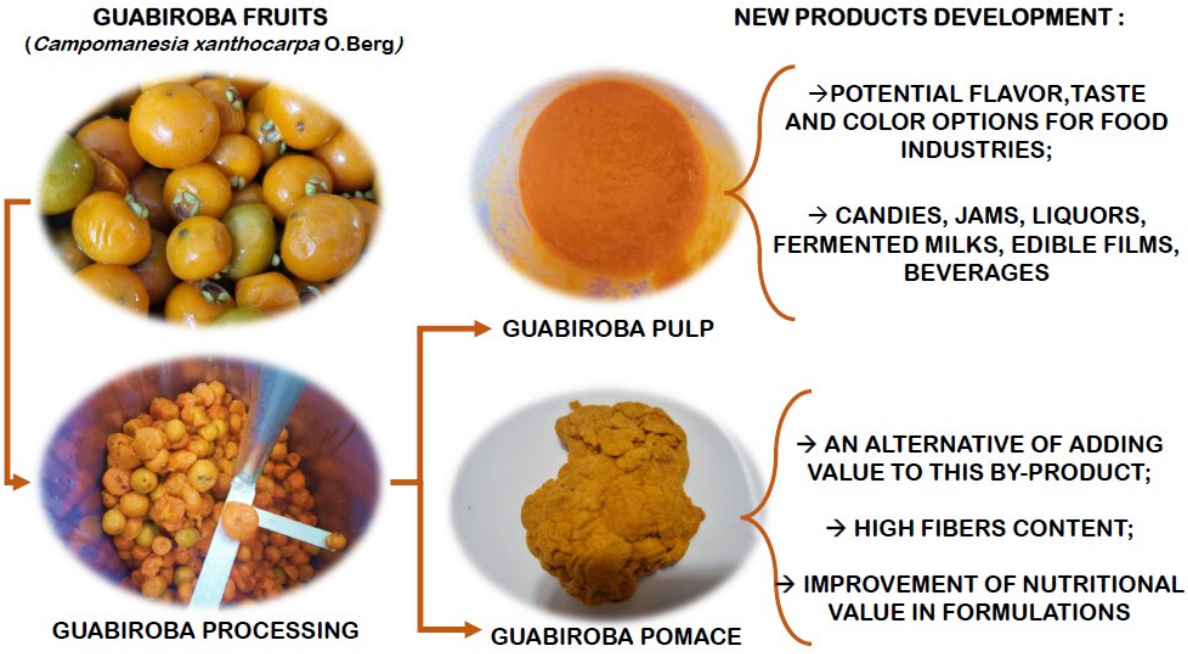
The essential oil present in the guabiroba pulp and seeds has a citric flavor and light-yellow color. When compared to other fruits from the same genus, the oil content (0.2 %) exceeds 3 times those obtained for *Campomanesia adamantium* (0.06%) and by 10 times for *Campomanesia phaea* (<0.02%) (VALLILO et al., 2005, 2006, 2008). In the composition, there are monoterpene hydrocarbons (limonene, α- pinene, o-cimene, β- pinene), most of which are non-toxic to mammals, and can be widely used in artificial flavorings and pharmaceutical formulations due to safety recognition by the United States Food and Drug Administration. The presence of these compounds in the essential oil also contributes to the use as a flavoring and in alcoholic distillates, ice cream, and sweets (CHAGAS et al., 2002; VALLILO et al., 2008).

3 NEW PRODUCTS DEVELOPMENT WITH GUABIROBA

The guabiroba fresh fruit is highly perishable and its original characteristics can be preserved for a maximum of 6 days in refrigerated storage (CAMPOS et al., 2012; DE OLIVEIRA RAPHAELLI et al., 2021). The encouragement and application of technologies in fruit processing can increase the demand and commercialization of fresh guabiroba and its products, enable the sustainable development of small rural producers, add value to native fruits (until then unexplored industrially), in addition to enhancing the consumption of new products with potential nutritional and functional value (CAMPOS et al., 2012).

New products development is essential for industrial businesses and interesting for the consumer market. The processing of guabiroba fruits represents new and potential aroma, flavor, and color options for food industries. The constant search of consumers for new products and lack of interest in traditional products makes the market increasingly competitive, which leads the food industries to search for prominence with the development of new products (SANTOS et al., 2013) (Figure 2).

Figure 4- The processing of guabiroba fruit to develop new products.



Source: The authors (2022).

The use of guabiroba fruits as raw material to produce candies and jams can be an income alternative for rural producers. From the physicochemical properties, which characterize the guabiroba with high moisture, pectin contents, and soluble solids suitable for fruit processing (SANTOS, 2012; SANTOS et al., 2013; VALLILO et al., 2008). Santos et al. (2013) developed formulations of guabiroba jam (Table 3). The original acidity of the fruits was ideal for conventional jam production (1.2 – 1.3 g $100g^{-1}$ citric acid), without the need to add acidulants to the formulation. In the traditional jam production (guabiroba pulp, sucrose, and pectin), with a long processing time, the vitamin C loss is higher when compared to the jam without added sugar (Vitamin C= 97.4% for traditional jams and 113.4 – 123.7 % for diet jams). High temperatures can also improve the extraction of phenolic compounds in a short exposure time, releasing free phenolic groups in the middle (TOOR; SAVAGE, 2006). This explains the higher total phenolic content in guabiroba jams without added sugar (68.9 - 72.5 mg GA $100g^{-1}$) where the processing time was lower compared to conventional jams (32.2 – 33.2 mg GA $100g^{-1}$). High antioxidant activities (approximately 45 – 53 TEAC μMol mL^{-1}, for DPPH radical scavenging capacity and 23 – 29 TEAC μMol mL^{-1}, for ABTS radical scavenging capacity), besides being related to phenolic compounds content, they are also associated with total carotenoids, which showed good retention in the formulations (74.8 – 87.7 β -carotene μg g^{-1}). Guabiroba jams were also produced by Leonarski et al. (2020), who added fructooligosaccharides (FOS), with a prebiotic property (Table 3). Even after thermal processing, the jams showed at least 35% of original bioactive compounds from guabiroba fruit (phenolic compounds = 466.7 – 512.0 mg GAE 100 g^{-1}; carotenoids = 43.7- 51.3 μg β-carotene g^{-1}; and vitamin C= 200.3-212.6 mg AA $100g^{-1}$), which enhances this product in the preservation of some functional characteristics, bringing benefits to the consumer's health. The presence of FOS in the jam formulation, besides contributing to the development of probiotic microorganisms present in the human gut, can modify the jam texture and water retention, since this prebiotic presents OH groups available for bonding with water molecules, which reduces the rate of water evaporation and forms a thick gel.

Table 3- Studies about new products development with guabiroba (*Campomanesia xanthocarpa* O.Berg

Product	Conclusions	Authors
Guabiroba jam	Original acidity ideal for jam formulations; high total phenolic content in jams without added sugar (68.9 - 72.5 mg GA 100g-1); good retention of total carotenoids (74.8 – 87.7 β -carotene μg g^{-1}) with high antioxidant activities (approximately 45 – 53 TEAC μMol mL^{-1}, for DPPH and 23 – 29 TEAC μMol mL^{-1} for ABTS).	(SANTOS et al., 2013)
Guabiroba jam with prebiotic	The presence of FOS (fructo-oligosaccharides) can modify the texture and jam water retention. Even with a heat treatment, the product retained at least 35% of guabiroba bioactive compounds.	(LEONARSKI et al., 2020)
Probiotic fermented milk with guabiroba pulp	High probiotic counts (8-9 CFU g^{-1}) during the entire simulated gastrointestinal steps, classifying it as a probiotic product. The addition of 10 g $100g^{-1}$ of guabiroba pulp presented higher total phenolic content and antioxidant activity in the gut steps and even in stomach region.	(PRESTES et al., 2021)
Petit Suisse cheese with guabiroba pulp	High energy content (92.1 kcal $40g^{-1}$) when compared to commercial products; the original color of the guabiroba pulp influenced the final aspect of the product, with a yellow color. This can help the consumer to associate the fresh fruit with the final product.	(RAMOS MESSIAS et al., 2021)
Gluten-free edible film reinforced with guabiroba pulp	Guabiroba pulp (10, 15, and 20%) provided films with high resistance to tearing, high thickness and increased biodegradability, with 100% of the films degraded in 45 days.	(SILVA-RODRIGUES et al., 2020)
Edible film with guabiroba pulp for olive oil packaging	Guabiroba pulp (20%) provided films with higher water vapor permeability and solubility. The orange color was predominant in the packaging due to the original color of the fruit. In 15 days, olive oil presented peroxide and acidity index below the maximum content allowed by local legislation.	(MALHERBI et al., 2019)
Guabiroba liquor	The liqueurs with guabiroba fruit presented low acidity (0.08-0.09 g acid citric 100 mL^{-1}), high pH (4.78-5.28) and high phenolic compounds content (31.62- 34.91 mg GAE $100g^{-1}$). The acceptance and purchase intention tests showed a preference for sweet liqueurs (344 gL^{-1}).	(LEONARSKI et al., 2021)
Nile tilapia burger with guabiroba peel	The addition of 5% guabiroba peel increased the moisture (67.82%), carbohydrate (2.71%), lipid (7.15%), and fibers content (4.43%) of the fish burgers. The results of TBARS showed a potential natural antioxidant activity from guabiroba peel (TBARS ≈ 1.5 mg MDA kg^{-1}, and 1.2 mg MDA kg^{-1}) after 300 days of storage.	(CRISTOFEL et al., 2021)

Probiotic fermented milks were developed with the addition of guabiroba pulp by Prestes et al. (2021), and an in vitro gastrointestinal simulation was performed to evaluate the influence of bioactive compounds from this fruit on the development and survival of probiotic cells throughout the gastrointestinal tract. Before and during the gastric steps, the

Bifidobacterium BB-12 count was 8–9 log CFU g^{-1}, which enhanced the product in its probiotic characteristics, with a count above the recommended for a probiotic property (HILL et al., 2014). The addition of 10 g $100g^{-1}$ of guabiroba pulp in the fermented milks showed the highest total phenolic content (TPC) (535.2 mg GAE L^{-1}; 346.0 mg GAE L^{-1} for the control sample without guabiroba pulp) and antioxidant activities (DPPH= 1232.0 μmol.L^{-1}, and 516.0 μmol.L^{-1} for control sample; FRAP= 4504.5 μmol.L^{-1}, and 1686.3 μmol.L^{-1} for control sample) in the gut steps, which is the ideal region for the development of probiotic cells, and even in the extreme pH regions of the stomach (TPC= 162.2 mg GAE L^{-1} and 154.6 mg GAE L^{-1} for control sample; antioxidant activity: FRAP= 1206.4 μmol.L^{-1}, and 358.5 μmol.L^{-1} for control sample; DPPH= 342.0 μmol.L^{-1} and 82.0 μmol.L^{-1} for control sample). Phenolic acids and flavonoids from fruits of the *Myrtaceae* family have a high molecular weight in a glycosylated form (FIDELIS et al., 2020). These compounds, in an appropriate concentration, may act as prebiotic and/or protective agents on the bifidobacteria development. In addition, the metabolism of probiotic cells can hydrolyze phenolic compounds in simpler forms for microbial absorption through enzymatic activities (OU; GU, 2014). Thus, the bioactive compounds present in the guabiroba fruit, linked to fermented milk, potentiated this food both in the growth of probiotic cells and in providing a new product with a functional appeal to the consumer market.

Petit Suisse cheeses with this native fruit were produced by Ramos Messias et al. (2021). The guabiroba pulp was added to the formulation at a concentration of 20g $100g^{-1}$ and presented a significant influence on the physicochemical properties of the products. Color parameters characterized the Petit Suisse cheese with a yellow color (L= 71.6; a*= -0.9; b*=31.5; C*= 31.5) due to the original color of the pulp, being determinant in the coloring properties of this dairy product. This effect may benefit the consumer to associate the color of the products with the presence of fresh fruit. Concerning the nutritional value, the petit Suisse cheese presented high energy content (92.1 kcal 40 g^{-1}) due to the high carbohydrate (11.5 g per 40 g), total fat (4.0 g 40 g^{-1}), and protein content (2.7g 40 g^{-1}). In determining the shelf-life of this product, microbiological stability was achieved during 28 days at 10°C, which is considerable time for production, transport, commercial storage, and final consumption of this dairy product. The use of this native fruit to develop a new Petit Suisse cheese flavor becomes relevant for new dairy options with a healthy and innovative appeal.

The application of guabiroba fruit can also be an innovative alternative in the development of active biodegradable packaging since there is a constant incentive to reduce the use of materials from non-renewable sources related to environmental problems. Bioactive

compounds and antioxidant properties of guabiroba fruit in the packaging material can act under storage conditions, reduce oxidation reactions, improve the safety and sensory properties of the product, and extend its shelf-life. Malherbi et al. (2019) produced a biodegradable active film with guabiroba pulp, corn starch, and gelatin for application as a package for extra-virgin oil. The addition of 10% and 20% guabiroba pulp in the polymer matrix presented an orange color, due to the original carotenoid content in the fruit composition, and a granular texture related to insoluble natural fibers (13.3 g $100g^{-1}$) that did not solubilize in the film-forming solution. Fibers content also can be related, in addition to carbohydrates and protein, to the highest thickness of the films with the addition of 20% guabiroba pulp (0.1243 mm, and 0.0895mm for the control blend film). These major compounds have a high molecular mass and increased the total solids content in the film solution, which may have contributed to the increase of thickness. However, the presence of original compounds from fruits, such as sucrose, glucose, maltose, and cellulose can significantly influence the physical barrier properties of the film, due to their high hydrophilic characteristics, increasing the solubility (36.92% with 20% pulp, 28.84% with 10% pulp, and 19.78% for control film), and the water vapor permeability (12.95 g mm m^{-2} d^{-1} kPa^{-1} with 20% pulp, 6.75 g mm m^{-2} d^{-1} kPa^{-1} with 10% pulp, and 3.88 g mm m^{-2} d^{-1} kPa^{-1} for control film). In a 15-day storage period of olive oils in polymeric sachets with 10% guabiroba pulp, the peroxide index increased from 6.14 to 8.21 meq kg^{-1} (for control film and the film with 10% pulp), due to traces of oxygen present at the time of packaging production, while the acidity index remained below 0.1%. These olive oil quality control parameters were below the maximum content allowed by local legislation (20 meq kg^{-1} and 0.8% oleic acid for peroxide and acidity index, respectively) (MALHERBI et al., 2019), relating the potential antioxidant activity of guabiroba fruits in preserving foods in active packaging. Similar behavior was obtained by Silva-Rodrigues et al. (2020), who also applied guabiroba pulp in the development of gluten-free edible, and functional films (Table 3). The films presented a compact form, without cracks and with excellent mechanical properties due to the high concentration of fibers (6.62%), polysaccharides (8.15% reducing sugars), and other polymeric compounds that can provide a cohesive polymer and result in a film with better rupture stress performance. The natural fibers of guabiroba also are susceptible to degradability, providing films 100% degraded in 45 days.

The original acid/sweet flavor, slight bitterness, and pleasant taste of the guabiroba fruit, in addition to its natural color, become interesting characteristics in the development of beverage blends, which can provide a unique flavor to the product. For alcoholic beverages, for

example, the consumer market is diversified and can provide an important point in the processing of native fruits with new products of high quality and innovative flavor. Leonarski et al. (2021) developed liqueurs from Brazilian native fruits, including the guabiroba (Table 3). Guabiroba blends (2:1; alcohol: fruit) provided liqueurs with 19.0% alcohol content (80 gL^{-1}, dry liqueur) and 21.5% (344 gL^{-1}, sweet liqueur),with values allowed by the Brazilian legislation (15 to 54% alcohol content by volume at 20°C) (MAARA, 2009), low acidity (0.08-0.09 g acid citric 100 mL^{-1}) and high pH (4.78-5.28). These specific physicochemical properties can provide beverages with highlighted sweetness, pungency and are related to the ripening stage, season, and fruit cultivation. Due to the high contents of phenolic compounds in the natural guabiroba composition (Table 2), the liqueurs presented 31.62- 34.91 mg GAE $100g^{-1}$, which can also influence the flavor of the beverages due to pungency and bitter taste, characteristic of some phenolic groups. In addition, antioxidant activities from phenolic compounds can exert antimicrobial properties, reducing the incidence of the proliferation of spoilage microorganisms and increasing the shelf-life of products. The use of native fruits in the development of liqueurs can improve the income of small rural producers, with simple processing, regional fruits, and an increase in added value (LEONARSKI et al., 2021).

Antioxidant and functional properties of guabiroba fruit can improve the shelf-life and the nutritional value of foods with high lipid content, which is susceptible to oxidation reactions. The use of fruit peels in food formulations, in addition, to containing high amounts of bioactive compounds, may represent an alternative of adding value to this raw material. Cristofel et al. (2021) developed a functional Nile tilapia burger with guabiroba peel, amaranth, and quinoa, to reduce the high lipid oxidation of this fish and increase its shelf-life (Table 3). The addition of 5g $100g^{-1}$ guabiroba peel provided fish burgers with a lower luminosity (45, and 50 for the control burger) and a higher a* parameter (approximately 80, and 78 for the control burger), with a tendency to yellow color due to the guabiroba original pigment. The concentration of guabiroba did not significantly affect the water activity (Aw = 0.97), and pH (6.4). However, the fruit composition increased the carbohydrate (2.71%, and 1.40% for the control raw burger), lipid (7.15%, and 6.14% for the control burger, and fibers content (4.43% and 3.22% for the control burger) of the products. Fibers from guabiroba peel also increased the moisture of the product (67.82%, and 67.03% for control raw burger) but did not substantially affect its physical characteristics. The results of TBARS showed a potential natural antioxidant activity from guabiroba peel (TBARS ≈ 1.5 mg MDA kg^{-1}, and 1.2 mg MDA kg^{-1} after 300th day of storage),

which can prevent lipid oxidation in foods with high lipid content and improve their nutritional value.

Bioactive compounds and the nutritional value from guabiroba pulp, peel, seed, or leaves can exert benefits in food formulation due to the high natural antioxidant properties that can improve the taste, texture, and reduce oxidative reactions, and microbiological spoilage of the product. These properties can also become potential natural preservatives to reduce, in the future, the use of chemical additives in the development of new food products.

4 TECHNOLOGIES ALREADY EMPLOYED IN GUABIROBA PROCESSING

Functional aspects of the guabiroba fruit added to its flavor and color characteristics are the aim of recent studies for the extraction and encapsulation of compounds through emerging technologies (CZAIKOSKI et al., 2015; DIAS et al., 2020; PEREIRA et al., 2015). Native fruits become an important base for studies due to the innovative and low-cost possibilities of obtaining pigments, or bioactive compounds that can be used as natural antioxidants and antimicrobials, colorants, and flavoring in the food and pharmaceutical industries.

The extraction of bioactive and thermolabile compounds from fruits can be achieved by non-thermal and environmentally friendly emerging technologies. These processes can extract compounds at mild temperatures and/or use safe, available, and low-cost solvents (CZAIKOSKI et al., 2015; DIAS et al., 2020; ZIELINSKI et al., 2021). Czaikoski et al. (2015) obtained natural guabiroba extracts from supercritical CO_2 extraction ($scCO_2$), an emerging technique that produces GRAS extracts (generally recognized as safe) in which a high compressible fluid is used as a solvent at low or middle temperature. The extraction performed at 313.15K (40°C) and 25 MPa obtained the maximum yield (3.90 wt %; extraction percent: 57.44%), corresponding to the highest pressure and lowest temperature evaluated. The extracts presented orange color and chemical composition rich in monoterpene hydrocarbons (α-eudesmol, β-eudesmol, γ-eudesmol, caryophyllene (E), α-sabinene, β-sabinene, germacrene B, δ-cadinene, humulene and selina-3,7(11)-diene). These compounds from guabiroba oil and extracts contribute to the use of these native fruits as a flavoring in beverages, candies, and alcoholic distillates. For antioxidant properties, the extraction at 353.15K and 25 MPa promoted the highest phenolic content (39.12 mg $GAEg^{-1}$ of extract), and at the same temperature and 15 MPa, it was obtained the highest antimicrobial activity against *Staphylococcus aureus*.

Bioactive compounds from guabiroba seeds were also extracted by $ssCO_2$ and compressed n-butane by Capeletto et al. (2016).The extracts were obtained at 40°C and 250 bar for $ssCO_2$, while for n-butane 35 °C and 10 bar. As a solvent, n-butane is cheaper, plenty available, and can be applied at much lower pressures compared to CO_2. The extraction yield using $ssCO_2$ was 8.02 wt%, whereas with compressed n-butane 24.71 wt%. Nonpolar solvents, such as the alkanes, are stronger solvents and show faster properties than $ssCO_2$ during the extraction. In the chemical composition, guabiroba seed extracts presented levels of terpenoids, flavonoids, and alkaloids. A higher total phenolic (TPC) and flavonoids (TFC) content were obtained for the extract from compressed n-butane (TPC= 68.58 mg g^{-1}, and 17.18 mg g^{-1} for $ssCO_2$; TFC= 8.10 mg g^{-1}, and 2.31 mg g^{-1} for $ssCO_2$). Consequently, extracts from compressed n-butane showed higher antioxidant activity (≈ 59% and 50% inhibition of DPPH for ssCO2). Extracts from guabiroba (whole fruit, pulp, or seeds) can be an important natural source of bio compounds with a great interest for food or pharmaceutical industry applications, mainly using emerging technologies with an environmental appeal.

Emerging environment-friendly technologies can also be alternative methods to extract pectin from fruits and replace traditional processes, which require high-temperature processing, large amounts of raw material, and toxic/corrosive solvents, such as nitric, sulfuric, and hydrochloric acids (DIAS et al., 2020; EINHORN-STOLL; KUNZEK, 2009). The industrial production of pectin is an alternative for adding value to solid resides and can potentiate this by-product into an important functional ingredient such as a thickener, gelling agent, texturizer, and emulsifier (CHAN et al., 2017; JAMSAZZADEH KERMANI et al., 2015). Thus, pressurized hot water extraction (PHWE) is also an efficient “green technology” studied to extract macromolecules from vegetables and fruits (DIAS et al., 2020; PLAZA; TURNER, 2015). In this process, the water is maintained under pressure with a temperature between normal boiling point (100°C) and critical point (374°C) to keep the water in the liquid state. This procedure makes the extraction advantageous since this specific water state provides an effective mass transfer, higher solubility of hydrophilic compounds, enhances the diffusion, vapor pressure, and shows low viscosity and surface tension (ADETUNJI et al., 2017; ZAKARIA; KAMAL, 2016). In this context and considering the important structural properties of guabiroba composition, Dias et al. (2020) performed a PHWE of pectin from guabiroba fruits at different process conditions. The maximum pectin yield (5.70 wt%, and 5.05 wt% compared to a conventional extraction) was achieved at optimal extraction conditions:120°C, a pressure of 150 bar, and a flow rate of 1.5 mL min^{-1}. The pectin yield is related to the increase in

temperature and pressure: the thermodynamic properties that can maintain the water in the liquid state and enhance the solubility and diffusion. These physical conditions may facilitate the solvent's permeability through the cell membrane and improve the polysaccharides extraction that is more adhered to cell walls. The guabiroba pectin from PHWE presented a varied chemical composition (arabinose = 44.3 – 59.7%; galactose = 8.9 - 18.7%; rhamnose = 0.6-1.5%; xylose = 0.3-1.3%; mannose = 0.5-2.8%; glucose = 0.5-1.6%; fucose = 0.1- 0.3%) and an increase of 10.3% in galacturonic acid content compared to traditional hot water extraction (34.8%; traditional extraction= 25.7%). This emerging technology is promising to obtain pectin from guabiroba fruits with great characterization and potential to be applied in the food and/or pharmaceutical industries.

On large-scale production, fruit bio compounds have their application limited due to their recurring instability during processing and storage conditions such as pH, temperature, light, interaction with formulation components, oxygen exposure, and during consumer's digestion (stomach pH, digestive enzymes, inappropriate surrounding, and interaction with other digested nutrients) (FANG; BHANDARI, 2010). Encapsulation by nanotechnology processes can exert a protective effect on these compounds and improve their solubility, enhancing the functionality and maintaining their bioactivity during processing and even in the digestion steps (PEREIRA et al., 2015, 2018). Synthetic polymers, such as polylactic-co-glycolic acid (PLGA) are advantageous due to their reproducibility over natural polymers, higher purity, and safe to be ingested (USKOKOVIC; STEVANOVIC, 2009). With these promising properties, PLGA nanoparticles were synthesized for delivery of phenolic extracts from guabiroba fruit by Pereira et al. (2018). A PLGA 50:50 (lactic acid: glycolic acid) obtained higher antioxidant activity (378.3 gg^{-1} for DPPH assay and 229 $\mu molL^{-1}$ TEg^{-1} for ORAC assay) compared to free guabiroba phenolic extract (GPE) (254.5 gg^{-1} for DPPH assay and 174.7 $\mu molL^{-1}$ TEg^{-1} for ORAC assay), with nanoencapsulated extracts related to better protection of guabiroba phenolic compounds during storage. In addition, concentrations around 10 times lower for PLGA (24 $\mu g\ mL^{-1}$) than free GPE (202 $\mu g\ mL^{-1}$) were required to reduce ROS (reactive oxygen species) generation (approximately 100% for PLGA, and 94% for GPE), which is related to be a crucial event in the initiation of cancer cells (SCHUMACKER, 2006). For antimicrobial activity, a concentration for PLGA (2.67 µg mL-1) around 3 times lower than free GPE (8.11 $\mu g\ mL^{-1}$), showed an improved action against *Listeria innocua*. Nanoparticles of guabiroba phenolic extracts proved to be an effective method in preserving bioactive extracts until its application and for a prolonged storage.

5 FUTURE PERSPECTIVES FOR GUABIROBA PROCESSING IN THE FOOD INDUSTRY

The importance of guabiroba biocompounds and their functionality has been the target of studies related to the improvement of antioxidant and antimicrobial activities in both the food and pharmaceutical sectors (AMARAL et al., 2019; CAPELETTO et al., 2016; PRESTES et al., 2021; SALMAZZO et al., 2021). New technologies may be a potential in the addition of guabiroba (pulp, seed, leaves, peel, and pomace) in formulations to improve the bioactivity of the raw material and enhance the nutritional, functional, and sensory value in the development of new products. High temperatures applied in traditional food processing such as concentration, drying, extraction, pasteurization, or sterilization can affect the bioactivity of fruit phytochemicals, reducing or inactivating their natural benefits in addition to generating unwanted sensory changes with the appearance of off-flavors.

Emerging non-thermal technologies can be applied in guabiroba processing to improve their safety, functionality, and sensory aspects. For food preservation, innovative technologies can be used with promising results. The pulsed electric field is one of the alternatives that have a direct action on microbial cells by applying electrical pulses to the target product, with the achievement of microbiologically safe food and maintaining nutritional and sensory characteristics (HERNÁNDEZ-HERNÁNDEZ; MORENO-VILET; VILLANUEVA RODRÍGUEZ,2019). In fruit and juices, this emerging process is related to improving polyphenols content, vitamins, and ensuring microbiological stability (DZIADEK et al., 2019; EL KANTAR et al., 2018). Guabiroba products also can be conserved with the use of pulsed light technology, a non-thermal process used for microbial decontamination of surfaces by short-time pulses of an intense spectrum with UV-C light, which proved to be efficient against mesophilic aerobic cells, *Escherichia coli*, and *Pichia fermentans* in fruit juices (GÓMEZ-LÓPEZ et al., 2007; MUÑOZ et al., 2011; PALGAN et al., 2011).

In the food and pharmaceutical industries, synthetic colorant additives are largely used, however, there are concerns about the addition of these chemical pigments due to adverse health effects. With these facts, natural pigments are encouraged to replace these synthetic ingredients. The yellow/orange color of the guabiroba fruits is a highlight due to its high carotenoids content and this pigment may be a promising replacement in food and pharmaceutical products (PEREIRA et al., 2012; SCHMIDT et al., 2019; VALLILO et al., 2006). High-pressure fluid

technologies (HPFT) are consolidated as environment-friendly processes and can be applied from compounds extraction until product formulation (ZIELINSKI et al., 2021). Methods including supercritical water extraction, pressurized fluid extraction, and supercritical/subcritical CO_2 extraction obtained potential results in the extraction of carotenoids from persimmons, and mango peels (SÁNCHEZ-CAMARGO et al., 2019; ZAGHDOUDI et al., 2016). This phytochemical can also be extracted by ultrasound techniques. The ultrasound-assisted extraction (UAE) is a technology that allows the release of high amounts of carotenoids and other bio compounds due to the rupture of cell walls by the phenomenon of cavitation. This technology proved to be an efficient method to extract carotenoids from mango with a decrease of wastewater, faster release, and extraction of this phytochemical with reducing operating temperatures (MERCADO-MERCADO et al., 2018).

The concentration of carotenoids and other phytochemicals from guabiroba fruits can also potentially be performed by techniques that employ the use of low temperatures, preserving most of the original compounds, such as the freeze concentration, an unconventional concentration process in which liquid foods are concentrated over a pre-freezing step followed by the separation of pure ice crystals, proved to be an efficient technique to concentrate juices from different fruits with high amounts of bioactive compounds (MORISON; HARTEL, 2018; SÁNCHEZ et al., 2009, 2010; ZIELINSKI et al., 2019).

For future applications in high demand in the food industry, studies on the guabiroba fruit must be constantly encouraged to expand knowledge about the properties of this Brazilian fruit to different parts of the globe. The valorization of the guabiroba associated with emerging technologies can reduce the loss of functional properties of this fruit, generate several opportunities for rural producers, new choices for the industrial sector, and new functional/nutritive products for the consumer market.

CONCLUSION

Guabiroba (*Campomanesia xanthocarpa* O.Berg) is a native fruit that is consolidated by several recent studies about its high fiber and carbohydrates content, polyphenols, carotenoids, and vitamin C. These nutritional and functional properties enhance this fruit for application in the food and pharmaceutical sectors. However, due to its regional and little widespread knowledge, guabiroba is not a fruit intended for processing on a large scale, only with homemade jams, candies, and liqueurs by rural producers. Promising results of recent

researches increase the benefits of guabiroba inserted in the formulation of new products, in the barrier properties of biodegradable packaging, and its potency as a prebiotic agent. Furthermore, emerging non-thermal technologies are constantly being improved to increase their effectiveness in extracting fruit compounds and applying a new product, being able to associate environmentally friendly processes with increased quality and retention of most bioactive compounds. The potential technological approach of guabiroba showed in this review can boost the development of effective processes for the extraction and processing of fruits and by-products, enriching the formulation of new products and increasing the added value of this native Brazilian fruit, hitherto unexplored by large industries.

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CHAPTER 2

Sensory Profile of Yoghurt and Related Products

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Sensory Profile of Yoghurt and Related Products

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1 INTRODUCTION

Dairy products, as fermented milks, have their appreciation and manufacturing knowledge since antiquity. Historically, fermentation is a millennial technique to preserve foods, and with this method, it was contested that sour milk was more stable than fresh, improving the quality, sensory attributes and, mainly, the shelf - life. Fermented milks production was developed and improved over the centuries with an evolution of a home manufacture to an industrial large – scale with specific strains, protocols and equipment.

The milk fermentation process occurs by the LAB (Lactic Acid Bacteria) action, in a metabolic pathway without any involvement of oxygen or its agents. Specific strains can hydrolyze lactose molecules, by an enzymatic activity, in glucose and galactose monosaccharides resulting, in the end of the process, in a lactic acid synthesis. The acidic property can reduce the pH of the milk until reaching the casein isoelectric point around pH 4.6, forming gel-like products with a protein precipitation and binding with calcium and phosphorus. This phenomenon is responsible to change the physicochemical and organoleptic characteristics; in addition, the presence of LAB and an acidic surrounding is important to inhibit the development of deteriorating microorganisms, enhancing the fermented milk shelf-life.

In different parts of the world there are several types of fermented milks. Around 400 names are related to handmade and industrial process, which are different according to the manufacture, dairy matrix, specific strains, fermentation time, and physicochemical

composition, resulting in products with diverse aspects and tastes. Considering that fermented milks are very appreciated around the world and their manufacture is constantly studied and improved, this chapter aims to show the main fermented milks and their production principle, as well as the fermentation process and particular strains in each product, being an essential theory background to produce these fermented dairy products.

2 YOGURT

The yogurt is one of the most produced and consumed fermented milks in several countries. According to the International Dairy Federation (IDF), the yogurt global consumption has increased in the last five years with a highlight to exporting countries as United States, Germany, Belgium and United Kingdom (COMTRADE, 2020). This increase of the consumption may be related to functional and healthy properties which were attributed to fermented milks, since the population has looking for a healthy diet and lifestyle. In addition, the increase of yogurts acceptance is due to the better digestibility related to a previous LAB action, which transform proteins in bioactive peptides that are better assimilated by the human organism (NGUYEN et al., 2020).

With an old origin in the Middle East, the yogurt discovery is reported to the first Neolithic civilizations who observed a change in the raw milk aspect when it was stored in a few days. At the beginning, this change was accidentally occurred by LAB present in the milk surrounding, however, this type of sour milk was tasty and could be consumed during several days. The word "yogurt" is derived from a Turkish verb "*jugurt*" around the 8th century, which means "coagulated" or "curdled" and its consumption was related to stories about a long and prosperous life (GAWAI; MUDGAL; PRAJAPATI, 2017; TAMIME; ROBINSON, 1999). At that time, most of the population was composed by nomads, and their traditional yogurt was manufactured and stored inside of animal leather bags. After the raw milk coagulation, animal skin pores were important to drain the whey, making the yogurt consistent and thick, with high lactic acid and solid content (KILARA; CHANDAN, 2013). Nowadays, yogurts manufacture had several modifications and are very important for the dairy country's economy. Produced in a large-scale, yogurts have strict protocols and good manufacturing practices to be accomplished.

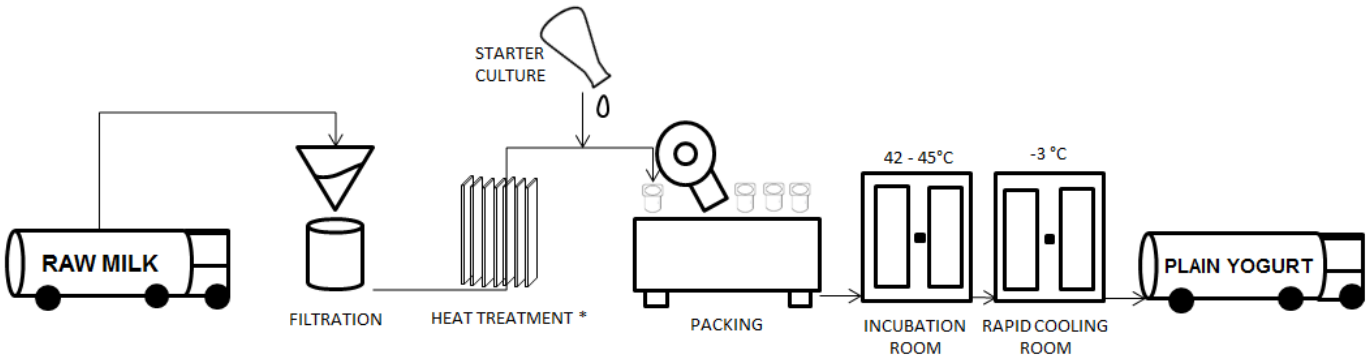
According to the Codex Alimentarius (FAO, 2003), yogurt is derived from the milk fermentation, with or without composition modifications, by a symbiotic action of two specific

thermophilic strains: *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* which must be abundant (minimum of 10^7 $CFUg^{-1}$ - Colony-Forming Units), active and viable in the product. If the product is heat treated after the fermentation, is not applying the requirement for viable cells. In some countries as Portugal, Spain, USA, France, Belgium or Sweden, there is a particular legislation that allows the choice and the use of *L. bulgaricus* or *S. thermophilus*. According to the types of yogurt formulation, is allowed to add pulp or fruit juices, powered milk, other symbiotic LAB, potable water, thickeners, stabilizers, sweeteners, emulsifiers, colorants or flavor enhancers.

The dairy matrix for yogurt production, as well as all the fermented milks, is usually from cow's milk, although, in diverse countries is used milks from other mammalian animals as buffalos, sheep, goats, mares and camels, changing sensory attributes and the solid fraction. In yogurts, according to the legislation, it must have a minimum 2.7% milk proteins (m/m), less than 15% milk fat, and minimum 0.6% lactic acid (FAO, 2003). In several parts of the world is permitted to produce dairy products from raw milk, however, in some countries it is only allowed to produce and sell all the fermented milks with a previous milk heat treatment as pasteurization or sterilization (MAPA, 2007).

There are diverse types of yogurts, changing some additives or unit operations. The plain yogurt, which contains only milk, starter cultures and no sugar or sweeteners, is summarized in Figure 3.

Figure 5- Plain yogurt process



Source: Prestes et al. (2021).

Note: * In the legislation of some countries, a pre-heat treatment is required.

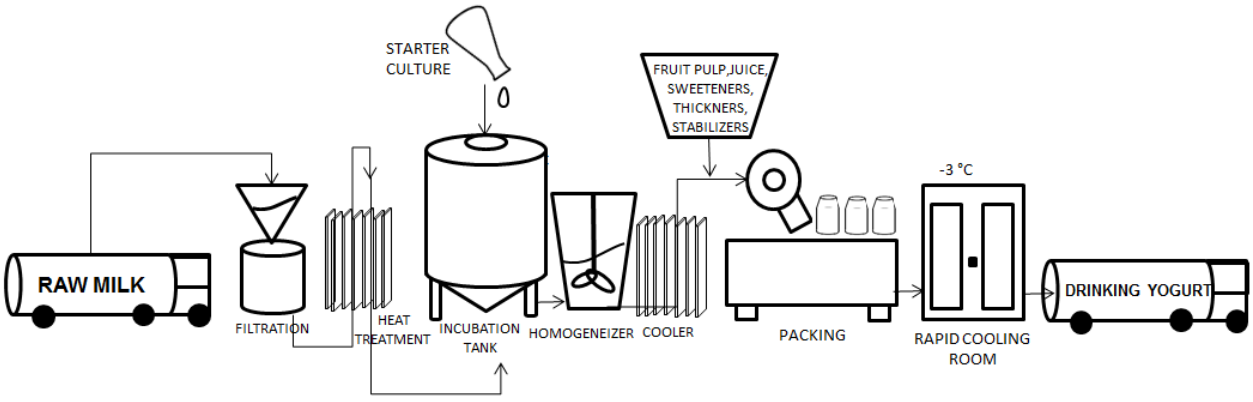
In the pre-treated milk, after a filtration to remove solid particles or heat treatment (often involved to enhance the yogurt's shelf-life), there is the addition of thermophilic starter cultures preferably at a 1:1 ratio at 2 - 5%. Inside of individual packing occurs an incubation at 42 to 45°C for 3 - 6 hours until titratable total acidity and pH reach 0.9 - 1.2 % and 4.4, respectively (SURONO; HOSONO, 2011). At the end of the fermentation, plain yogurts are stored in a cooled room, at -3°C and are transported and sold at refrigerated temperature. This fermented milk has a firm and consistency gel-like structure, white color, slight flavor and low acid taste.

3 DRINKING YOGURT

One of the most popular yogurts, that has become a trend nowadays, is the drinking type. This fermented milk formulation combines functional properties, new flavor and healthy appeal, with fruits, juices, fibers, probiotics, vitamins or minerals adding. Consumers very much accepted this type of dairy product due to the facility of eating a healthy dairy product anywhere, mainly if this product is easy to consume.

The drinking yogurt process is similar to that of plain yogurt (Figure 4), however the fermentation occurs inside large incubation tanks and not inside the packaging. In addition, it is performed the breaking of the coagulum after the fermentation by a high speed of agitation inside of a homogenizer. With this operation, the yogurt aspect has a liquid form, what makes it easy to drink. However, in this product manufacture and storage, the separation and release of the whey is a challenge, being necessary to include stabilizers, as carboxymethylcellulose or gelatin.

Figure 6- Drinking yogurt process



Source: Prestes et al. (2021)

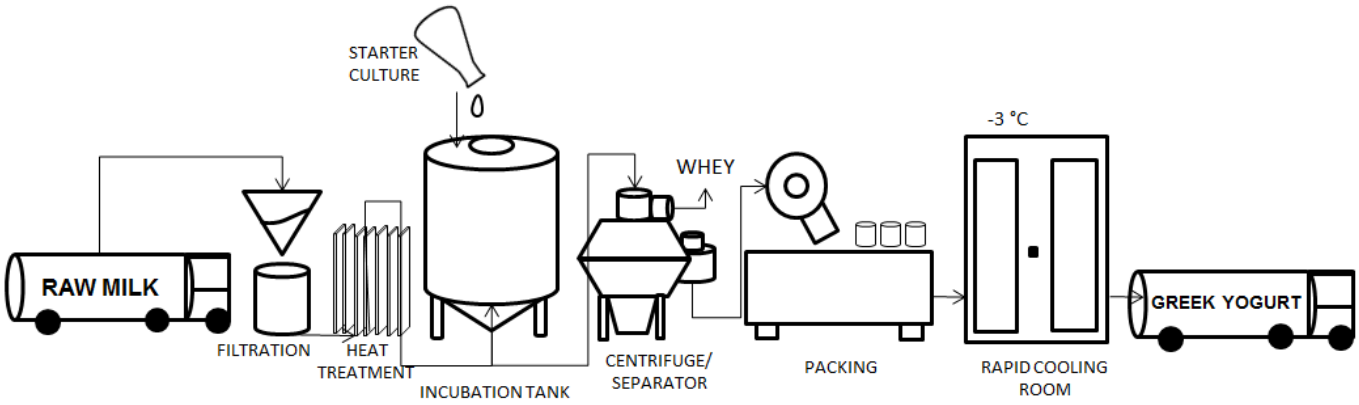
The drinking yogurt physicochemical composition usually consist of 9 % milk nonfat solids (proteins, carbohydrates, minerals), 1.5 % fat, 8% sugar, 0.5% stabilizers and 5 to 15% fruit pulp, juice or syrup (GAWAI; MUDGAL; PRAJAPATI, 2017). The final aspect of the drinking-type yogurt is characterized as a liquid texture with flavor and taste modified by the addition of sugar, flavor or fruits.

4 GREEK YOGURT

The Greek yogurt, also called strained or concentrated yogurt, is one of the most consumed yogurts due to the healthy appeal of its advertisements. The name "Greek yogurt" become world famous in 90's when the United Stated started selling a concentrated yogurt from a Greek industry. However, the origin of this dairy product was in the Balkan peninsula (from Bulgaria to Turkey). The strained yogurt is a typical product in the Middle East with diverse names as *labneh*, *labaneh* or *lebneh* (in some Arab regions), *mast/mastou* in Iraq, *laben zeer* and *tan/than* in Egypt and Armenia regions, respectively (TAMIME, 2003). In America and Western Europe, the "Greek" word on the packing is what enhances the sales because many consumers associate it with a Mediterranean diet and healthy habits (LITOPOULOU-TZANETAKI; TZANETAKIS, 2014; PIMENTEL et al., 2017)

The Codex Alimentarius defines concentrated fermented milks when the protein has been increased to minimum 5.6% after the fermentation process, about 2 to 2.5 times that the traditional yogurt (FAO, 2003). In a traditional manufacture, after the fermentation step, the yogurt concentration occurs by straining through cheesecloth overnight at 4°C. This is a slow process only with the action of gravity. In dairy industries, with a large demand, after a complete fermentation in a tank with controlled temperature and mild agitation, a portion of the whey is removed in a centrifuge/separator. In this equipment, a very fast rotation causes the phase separation, concentrating the densest portion at the bottom of the centrifuge and the whey, with the lowest density, is removed on top of the equipment (Figure 5). This released whey may be used in some food formulations, as dairy beverages or supplements. The concentration also can be performed by membrane filtration as microfiltration or ultrafiltration. In this process, small pores can separate the liquid fraction (whey) than solid fraction. Powdered milk also can be added to enrich the solid fraction in concentrated yogurts manufacturing.

Figure 7- Greek yogurt process.



Source: The authors (2020).

The concentration step causes an increase of total solid content from 14% to 21-23% with approximately 10% fat, also resulting in changes in the physicochemical structure with a strained, creamy smooth and viscous texture. Even though Greek yogurt has a higher fat content, dairy industries have offered reduced-fat versions to healthy consumer appeal (CHANDAN; O'RELL, 2006; ROBINSON; TAMIME; WSZOLEK, 2002). However, with a part of the whey removed and with the addition of sweeteners, powdered milk, flavoring or colorants, occurs physicochemical modifications, resulting in the change of the LAB development due to the surrounding stress. With all these composition changes, cells may synthesize unwanted metabolites as exopolysaccharides, modifying the final aspect. Therefore, in the Greek yogurt protocol it is necessary to fit the dosage and the type of the culture. Besides, the fermentation time and culture conditions must be adapted to obtain an ideal dairy product at the end of the process (PIMENTEL et al., 2017).

5 FROZEN YOGURT

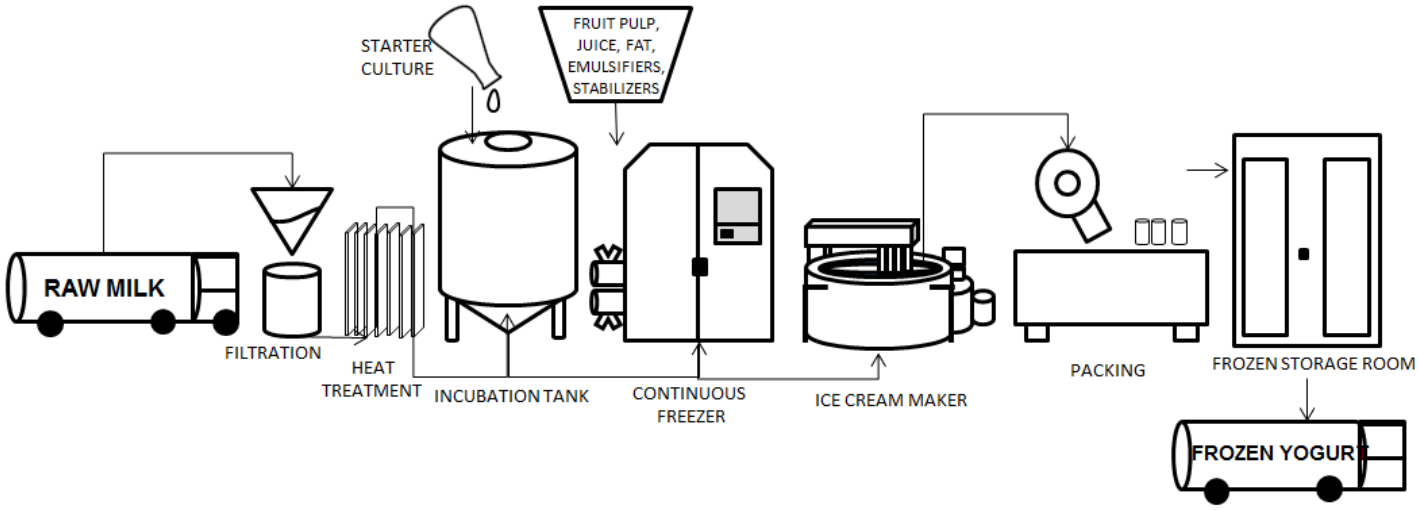
Frozen yogurt, as well as all frozen dairy desserts, is categorized by containing milk or milk solids and must be consumed in the frozen state. It is generally associated with frozen milk with a typical yogurt flavor (CHANDAN; GANDHI; SHAH, 2017; GOFF, 2011). According to the legislation, frozen yogurts are edible ices and must have been treated by freezing. This dairy product may be obtained by an emulsion of edible fat and protein in addition of permitted additives as sugar, emulsifiers, colorants, stabilizers, fruits, pulps or juices. In the Codex classification, fermented milks with the term "frozen" is applied when the fermented

dairy product is submitted to freezing operation and the specific starter cultures can be reactivated in considerable counts by thawing (FAO, 2003).

Frozen fermented dairy desserts are relatively recent and were development in the 70's (SHAH, 2003). At the beginning, this "new ice cream" with the similarity to the yogurt led to the improvement of formulations since consumers were starting to look for healthy foods. The healthy appeal was the best dairy industries advertisement in the past and, nowadays, is also a marketing investment. Depending on the formulation and with a restriction of sugar and fat, the *frozen* yogurt contains few calories when compared to a common ice cream and approximately 70% less fat.

The manufacture of frozen yogurt is shown in the Figure 6. The fermentation is similar to the plain yogurt production, with an addition of the two LAB (*S. thermophilus* and *L. bulgaricus*) which may be added in a different proportion (commonly 10-20%). After the fermentation step and with 0.3% of total titratable acidity, other additives are included in the yogurt, as emulsifiers, fat or stabilizers and are essential to improve the final texture (GOFF, 2011). The mixture is pumped into a continuous freezer at -6 to -2°C to aid in the hardening and air incorporation steps. The mixture is quickly stirred to incorporate air (overrun) and the volume is increased. The agitation accelerates the overrun and there is a decrease of the viscosity due to the partial rupture of fat globules and the protein coagulum. In some minutes, the mixture is frozen, the required overrun is achieved about 50% and the texture is similar to an ice cream. After the packing, the frozen yogurt is stored, transported, and commercialized at freezing temperature.

Figure 8- Frozen yogurt production.



Source: The authors (2020).

The frozen yogurt composition varies according to the manufacture or between countries. The mean composition of this dairy dessert is 1.8 – 6.0% fat, 3.5 – 3.8% protein, 28.8 - 34.0% total solids and pH 6.4 - 7.1 (TAMIME, 2003).

6 DRIED YOGURT

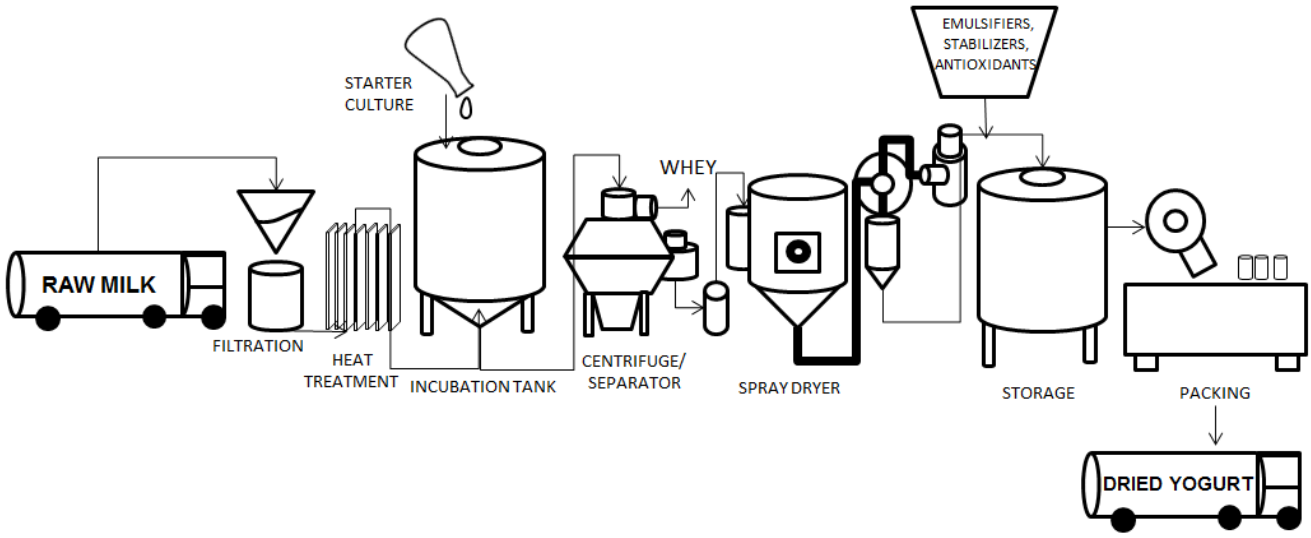
Dried yogurt, yogurt powder or instant yogurt is also a type of concentrated fermented milk with the aim of preserving quality and maintaining shelf-life in a powdered form without a need of refrigeration. In addition, due to the reduction of bulk, the dried yogurt requires less packing, decreasing the storage costs (KUMAR; MISHRA, 2004). Diverse applications of yogurt powder are made in food industries due to its unique flavor in food formulations, as beverages, candies, dips and replacing fresh yogurts in baby food manufactures and baking industries (TAMIME, 2003; WEERATHILAKE et al., 2014).

In regions of Turkey, where the first yogurts were developed, is common a millennial practice to salt concentrated yogurts and after sun-dried. Yogurt balls were made and stored in glass jars containing olive oil or, in other regions, the dried yogurt (also known as *kishk*) is covered with wheat flour to maintain the quality during a long period of time. In India, Nepal and Bangladesh, the practice of yogurt concentration is made in earthenware pots. With the water evaporation through the pores, the dairy product is concentrated and can be consumed in several days. However, the habitual use of refrigerators nowadays decreases these traditional methods of preserving dairy products (CHANDAN; GANDHI; SHAH, 2017; SHAH, 2003).

According to the Codex, milk powder or dried dairy products are obtained by the partial removal of water or cream from the matrix. The milk solids, as fat or protein, may have been adjusted using additives (stabilizers, emulsifiers, firming agents, acidity regulators and/or antioxidants) or removal of milk constituents, however, cannot be changed the whey protein to casein ratio of the dairy matrix (FAO, 1999).

In large-scale production, as shown in Figure 7, after the fermentation step of non-fat milk, which is similar in all the yogurt types, generally the yogurt is concentrated before drying to benefit the increase of total solids and improve the efficiency of drying process. Unit operations for concentration without heat application (straining, pressing, centrifugation or ultrafiltration) are normally used to preserve the maximum of flavor, color, nutrients and LAB counts (TAMIME, 2003).

Figure 9- Dried yogurt production.



Source: The authors (2020).

The concentration may be done by water evaporation under vacuum; however, the mechanic separation of the whey permits to apply it in other dairy products formulations or sell it. In sequence, the residual of water from the concentrated/strained yogurt may be removed by several methods such as spray-drying, freeze-drying, microwave-drying or vacuum drying.

In large production, is very common a highly viscous yogurt (25 – 36 g 100g -1 of total solids) to be pumped into a spray-dryer. It consists in equipment with a large chamber where yogurt is sprayed against a high temperature air flow (175 – 190°C). In this step, small drops of yogurt are instantly powdered due the intense heat transfer. However, studies report that the most flavor compounds and rheological characteristics are lost during the process. In addition, the survival rate of *S. thermophilus* and *L. bulgaricus* was only among 0.7 to 3% when a plain yogurt was submitted to a high temperature in a spray-drying process (KIM; BHOWMIK, 1990; KUMAR; MISHRA, 2004; PEREZ SILVA; CERVANTES; GALINDO, 1997; TAMIME, 2003).

An alternative to preserve flavors and LAB is dry the dairy product by the freeze-drying process, which consist of drying the frozen yogurt under vacuum and remove the water by a sublimation process. However beneficial it may be in relation to physicochemical, microbiological and sensory characteristics, the freeze-drying process is costlier than spray-drying and due to this financial issue, some industries choose a cheaper and more efficient process.

Nowadays, several big industries choose to use a recent separation technology, which preserve all the product flavors, the ultrafiltration. This method consists of separating solids by membranes with high selective permeability, employing pressure gradient that allows retaining big molecules and passing small molecules. A limiting factor of this technique is, in the dried yogurt production, a high viscosity of the concentrated yogurt through the small pores, taking a very long time to filter. Therefore, in this case, industries normally use spray-drying or freeze-drying methods. In the end of the process, the yogurt powder is transferred by pneumatic pumps to storage silos and is packing after being releasing by the quality control. The final mean concentration is shown in Table 4.

Table 4- Mean composition of dried yogurts.

Component	$g.100g^{-1}$
Protein	33.0 – 36.0
Fat	1.2 – 2.0
Lactose	50.0 – 51.5
Ash	7.0 – 8.0
Moisture	3.0 – 5.0

Source: Krasaekoopt and Bhatia (2012), Weerathilake, Rasika, Ruwanmali and Munasinghe (2014).

Dried yogurts can be stored during 1 to 2 years, under dry and cool conditions for spray-dried yogurt or at 4°C for freeze-dried yogurt. LAB active counts are guaranteed during one year of storage (KUMAR; MISHRA, 2004).

7 KEFIR

Kefir, also called *kefyr*, *kephir*, *kefer*, *kiaphur*, *knapon*, *kepi* or *kippi*, is a fermented milk characterized by a mixed lactic acid and ethanol fermented beverage. With a singular yeast-like flavor, low alcoholic, and carbonated taste, is very consumed in Eastern Europe and some Asian countries. The origin of the word "kefir" is from the Turkish "*kef*", which means "pleasant taste" and this old lactic-yeast fermentation product was originated between mountainous regions of Caucasus and Mongolia, in Central Asia. Due its studied functional proprieties, kefir became important in the human diet, being consumed in several parts of the world including South Asia, America, Middle East and North Africa (SHAH, 2014; SINGH; SHAH, 2017).

Kefir is produced by adding kefir grains into milk, which consist in a complex mixture of LAB and yeasts in symbiosis into a matrix of proteins, lipids and polysaccharides. These

grains are white, elastic, irregular, with 0.3 to 2.0 cm in size and have a similar aspect to a cauliflower in color and form (Figure 8) (SINGH; SHAH, 2017; WSZOLEK et al., 2006). When kefir grains are added into the milk, there is a multiplication in mass and, if the maintenance is careful, this culture preserve its activity for years (RATTRAY; O'CONNELL, 2011).

The microbiological composition of the grains is complex with approximately 83- 90% of LAB and 10 - 17% of yeasts. The milk fermentation and final aspect of kefir is attributed to a diversity of species and genera as the *Lentilactobacillus kefiri* (one of the species which was belonged to the *Lactobacillus* genera and was recently reclassified into 25 new distinct genera according to the Microbiology Society (ZHENG et al., 2020). In addition, the kefir culture also contains species of *Leuconostoc*, *Lactococcus* and *Acetobacter* genera, yeasts which perform lactose fermentation (*Kluyveromyces marxianus*) and non-lactose-fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae* and *Saccharomyces exiguus*).These last produce ethanol and carbon dioxide molecules from glucose fermentation, which attributes the mild alcoholic and frizzy taste in the final product (FAO, 2003; RATTRAY; O'CONNELL, 2011; WSZOLEK et al., 2006).

Figure 10- Kefir grains

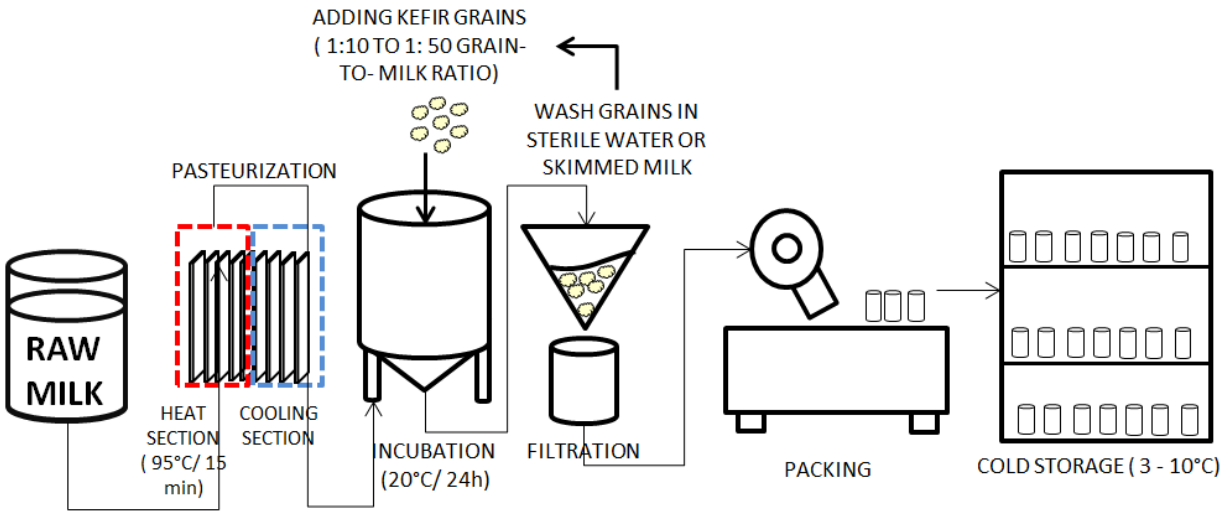
Source: The authors (2020).

Around the world, different dairy matrixes are used in kefir manufacturing beyond the bovine's milk, including those from goats, buffalos and sheep. In the handmade manufacturing raw milk is generally used, however, this fresh matrix contains diverse species of microorganisms, including deteriorating ones which compete for substrate for their development. This competition may decrease or extinguish the kefir culture, with the possibility

that the final consistency not being required. In addition, fermented milks produced with raw milks have a reduced shelf-life when compared to a dairy product from milk with previous heat treatment.

The traditional kefir production is performed with kefir grains, which is shown in the Figure 9. After the milk pasteurization, kefir grains are inoculated into the milk in a specific proportion (1:10 to 1:50 grain-to-milk ratio) and, after the incubation during 24h at a controlled temperature, kefir is filtered, grains are washed in sterile water or skimmed milk and are reutilized in a next fermentation process (RATTRAY; O'CONNELL, 2011). As all fermented milk, kefir must be stored, transported and sold at a refrigeration temperature (4 – 10°C) to maintain is properties and shelf-life.

Figure 11- Traditional kefir production.

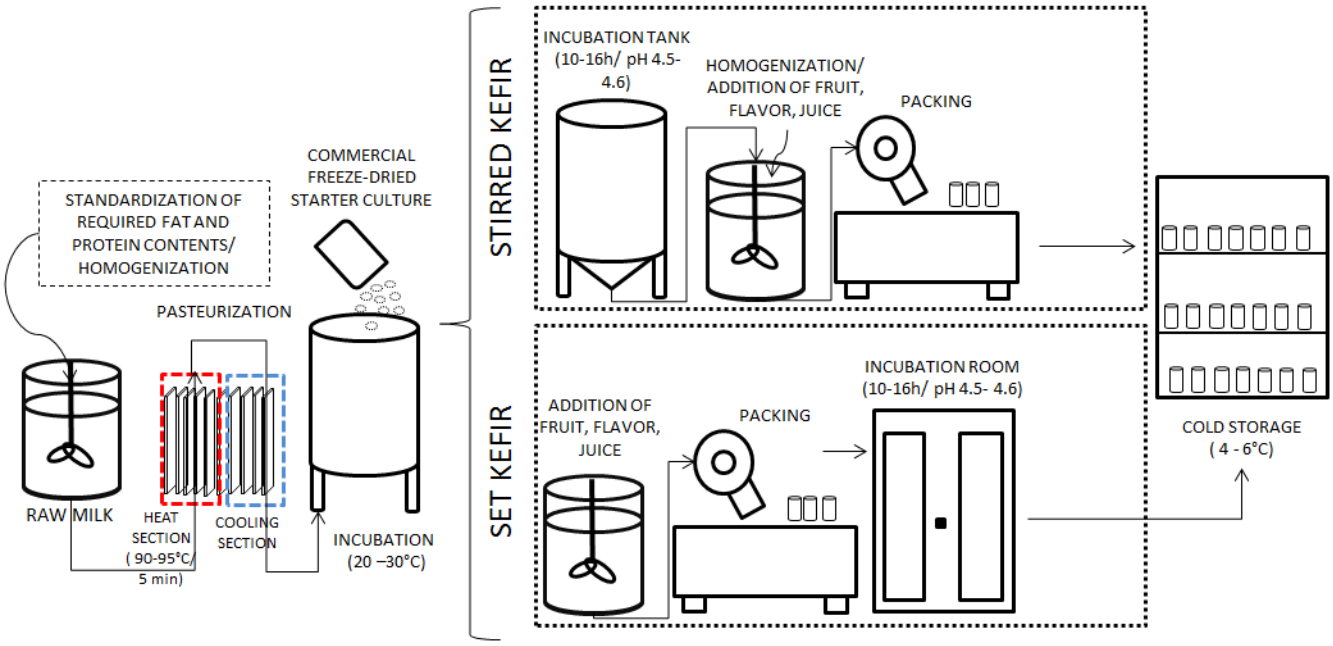


Source: The authors (2020).

On a large - scale production, it is viable to produce kefir with its commercial culture in a freeze-dried form. With this alternative, the volume of the starter culture is reduced and there is no need for grains wash step, which makes the process faster. In addition, commercial manufacturing allows obtaining a standard product with always the same quality, since the starter culture was meticulously select in biotechnology laboratories. The Figure 10 shows a large-scale for commercial kefir. Before all the procedures, the milk is standardized in relation to fat and protein proportion to maintain the same physicochemical and sensory attributes. After the heat treatment, the commercial freeze-dried culture is added into the milk to start the fermentation. Industries can use two fermentation steps in a kefir processing: with the fermentation inside large incubation tanks (stirred kefir) or inside the final packaging, which is

incubated in a large room with a controlled temperature (set kefir). In both processes, it is optional to add flavors, juices or fruits in the formulation. However, in the set kefir, these additions occur before packing and fermentation. When the pH reaches 4.5-4.6, the fermentation step is finished and kefir is stored at cooling temperature.

Figure 12- Commercial kefir production



Source: The authors (2020).

According to the Codex, the final composition of kefir must have minimum 2.7% milk, less than 10% fat milk and minimum 0.6% lactic acid. In the microbiological count, the sum of starter culture must have minimum 107 $CFUg^{-1}$ (Colony-forming units per gram) and yeasts with minimum 104 $UFCg^{-1}$ (FAO, 2003).

The shelf-life of commercial kefir is approximately 28 days at a cooling temperature and it is higher when compared to traditional kefir, within 12 days. Kefir grains contain a wide diversity of bacteria, yeast and many of them have a probiotic property that may bring several functional benefits to the consumer's health. This healthy appeal of kefir is what spread its consumption and production around the world.

8 KOUMISS

Koumiss (kumys or kumiss) is a traditional fermented milk very appreciated in Central and Western Asia countries as Kazakhstan, Mongolia, and Russia. Beyond sensory attributes,

some regions of Russia include koumiss in the children's nutrition due to its functional, healthy, and nutritive properties. Characterized by a liquid without curd, milky-grey color, effervescent, striking acid and alcoholic taste, koumiss is generally produced with mare's milk in a spontaneous fermentation and formation of lactic acid and ethanol (SINGH; SHAH, 2017; UNIACKE- LOWE, 2011).

The word "koumiss" is from the name of the Kumanese tribe, a civilization who survived until 1237 in central Asian regions and used to consume this fermented dairy beverage in social relationships and religious rituals. The handmade production crossed the centuries, and this manufacturing is *still* present in remote Asian regions, however, the large demand led to produce this fermented milk in large scale with standardized protocols. In addition, the large production is directed to therapeutic uses due to nutritional properties of mare's milk being similar to human milk. The consumption may be included in treatment of anemia, nephritis, gastritis, diarrhea, or cardiovascular diseases (LI et al., 2019; UNIACKE- LOWE, 2011).

Similar to kefir, the koumiss fermentation occurs by a mixture of starter cultures as LAB and yeasts. In studies, it was detected a diverse microbial population in koumiss starter culture including *Lactobacillus bulgaricus*, *Lacticaseibacillus casei* (basonym *Lactobacillus casei*), *Lactobacillus helveticus*, *Lactobacillus kefiranofaciens*, *Lentilactobacillus kefiri* (basonym *Lactobacillus kefiri*), *Streptococcus parauberis*, *Lactococcus lactis* spp. *lactis*, *Kluyveromyces fragilis* and *Saccharomyces unisporus* (CAGNO et al., 2004; MONTANARI et al., 1996; ZHENG et al., 2020).

In the traditional manufacturing, which is still performed in remote regions of Mongolia, the raw mare's milk (at ambient temperature) is added into bags made of smoked horse's leather, with a capacity of 25-30 L called *tursucks* or *burducks*. Microorganisms adhering to the leather are used as starter cultures. After incubation during 3 to 8 hours, the product is removed from the bags and a part of this fermented milk is used as inoculum in a new bath of fresh mare's milk. However, this production is not controlled, and an excess of acidification or yeast often occurs, resulting in an unpleasant taste (ROBINSON; TAMIME; WSZOLEK, 2002; UNIACKE- LOWE, 2011).

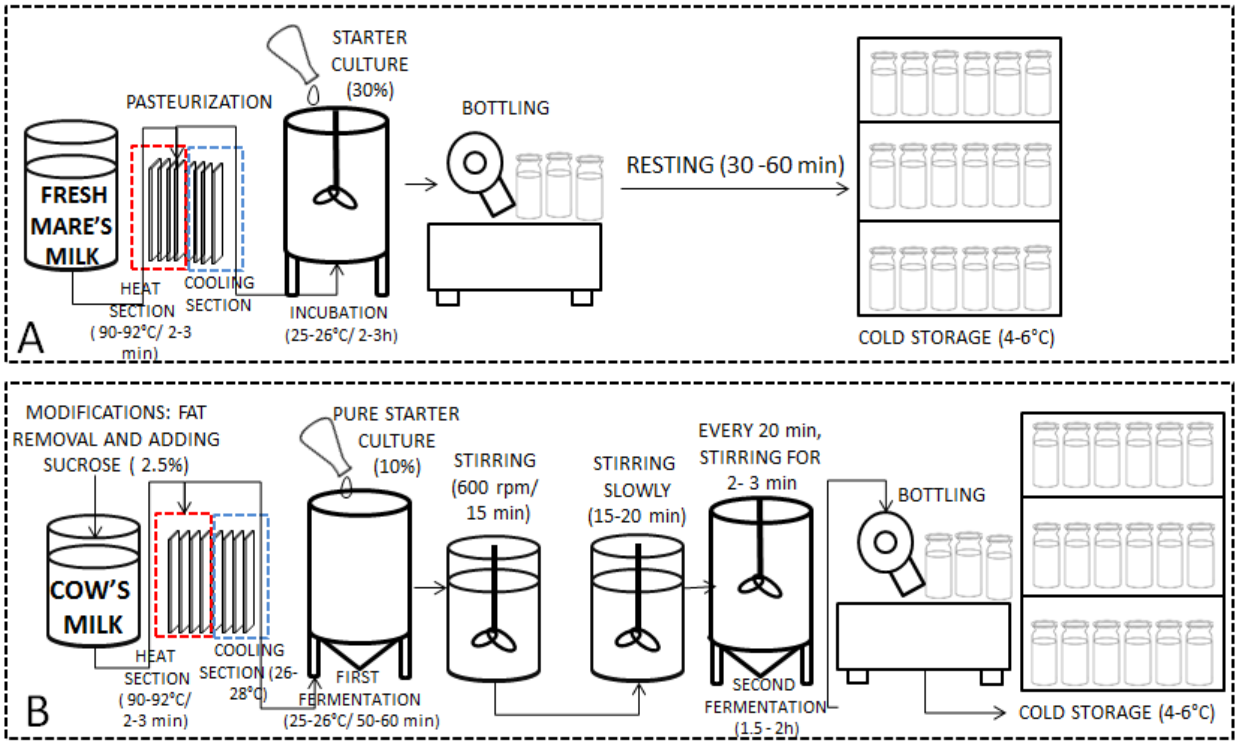
Nowadays, a large - scale manufacturing of koumiss became a potential for equine's milk. With standardized protocols and processes, this fermented milk can be distributed to many countries, stimulating its consumption. However, in the last decades, several adaptations of koumiss manufacturing were applied in the commercial process, including the dairy matrix. The popularity of koumiss and a restricted equine flock led the industries to also produce this

fermented milk with bovine's milk. However, the composition of cow's milk is different from mare's milk and some process modifications are performed to maintain the same standard and quality of products. In the koumiss manufacture from mare's milk there is no curd formation, since this matrix has low casein content, and this same characteristic must be present in a commercial koumiss from bovine's milk. The ratio of protein and fat is higher in cow's milk and, to overcome this difference in composition, skimmed milk is diluted in water to reduce the casein content and concentrated protein or whey is added to increase the protein content. Emerging technologies as microfiltration, ultrafiltration or nanofiltration can be also used in the protein separation of cow's milk. To balance the carbohydrate content and also to improve the yeasts development, generally is included sucrose, glucose or lactose hydrolyzed into the cow's milk (SINGH; SHAH, 2017).

The commercial koumiss production from mare's milk is shown in Figure 11A. To produce the starter culture, specific microorganisms are added into mare's milk and incubated during 4 days at 28°C. This liquid starter culture is able to use when the total acidity reaches 1.4%. After a previous heat treatment, 30% of this starter culture is added into the mare's milk inside an incubation tank to start the fermentation during 2- 3 hours at 25-26°C. In this step, a continuous agitation is important for aeration of the mixture and enhances the culture growth. In sequence, koumiss is bottled and, after 60 minutes of rest, is stored at 4-6°C (SINGH; SHAH, 2017).

The Figure 11B shows a large – scale production of koumiss from cow's milk. Modifications in the dairy matrix are performed as fat removal, change in protein content and addition of 2.5% of sucrose. After the pasteurization of this skimmed milk, 10% pure starter culture is added into the milk inside of large incubation tanks. The first fermentation occurs during 60 minutes in a controlled temperature at 25-26°C. In sequence, two stirring steps are included in the process to aerate the mixture and promote an increase of yeasts and LAB in the fermented milk. The mixture is pumped in another incubation tank for the second fermentation for 2 hours more until total acidity reaches 0.9%. After bottling, koumiss is storage at cooling temperature.

Figure 13 - Commercial koumiss production. A: Koumiss from mare's milk; B: Koumiss from cow's milk



Source: The authors (2020).

In the end of fermentation, koumiss usually contains approximately 90% water, 1-1.3% fat, 2-2.5% protein (including 1.2% casein and 0.9% whey proteins) and 4.5 – 5.5% lactose (UNIACKE- LOWE, 2011). According to the Codex, koumiss must contain minimum of 0.5% ethanol, minimum of 107 CFU.g-1 in the sum of microorganisms from the starter culture and minimum of 104 CFU.g-1 of yeasts (FAO,2003).

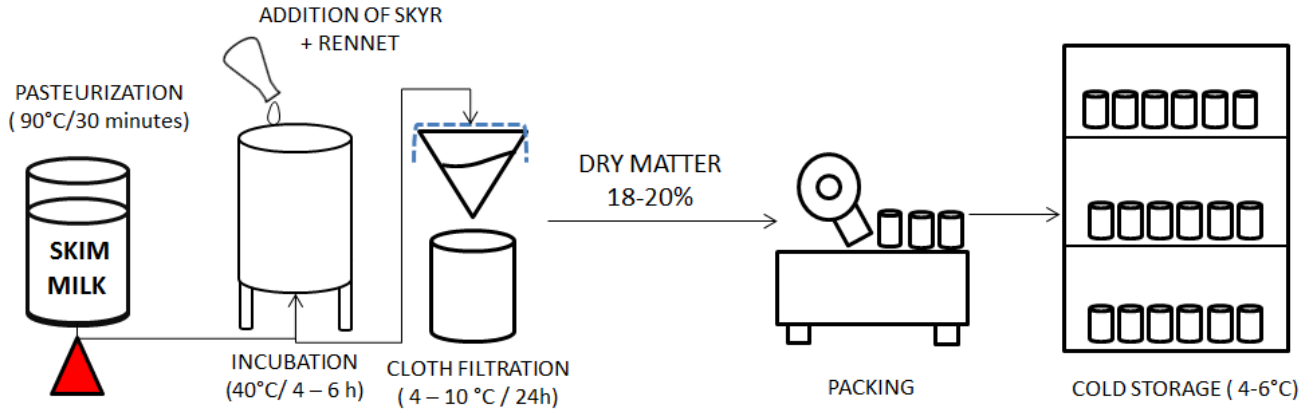
9 SKYR AND OTHER RELATED PRODUCTS

Skyr is a popular fermented milk also obtained by the concentration process and can be classified as variation of yogurt in its strained form or a fresh acid-curd cheese from skim milk. With the knowledge of its process since the 10th century, skyr (which means "curdled milk") was already produced in Scandinavia before the Iceland colonization, where was the place of origin of this dairy product. This region was inhabited by Vikings who used the yogurt whey to preserve meats and vegetables, including extra protein in the diet to survive on extremely cold days. With the management of the first cattle herds in Iceland, skyr was included

in the basic diet of the population and, over the centuries, its manufacturing was introduced into Denmark in the mid-1900s (VEDAMUTHU, 2006).

The skyr process is very similar to the yogurt procedure, with the use of thermophilic LAB as Lactobacillus bulgaricus, Streptococcus thermophilus and *Lacticaseibacillus casei* (basonym *Lactobacillus casei*) in a commercial starter culture. In the process that is used only LAB cultures to curdle the milk, it is denominated "auto-coagulated skyr" and the coagulum is formed gradually by lactic-acid fermentation. In the "coagulated skyr", a small fraction of rennet is also included into the milk to enhance the skyr process and this procedure is applied in several industries, mainly when commercial rennet became available. In the traditional "coagulated skyr" process (Figure 12), the skim milk is pasteurized during 30 minutes at 90°C, cooled to 40°C to add a previous skyr produced, to serve as a starter culture, and the rennet. In sequence, the milk is incubated during approximately 4 – 5 hours until pH reaches 4.6 and, after this time, there is a cloth filtration at 4-10°C during 24 hours. With dry matter of 18-20%, skyr is packed and stored at cooling temperature (GUDMUNDSSON; KRISTBERGSSON, 2016; VEDAMUTHU, 2006).

Figure 14- Traditional skyr production

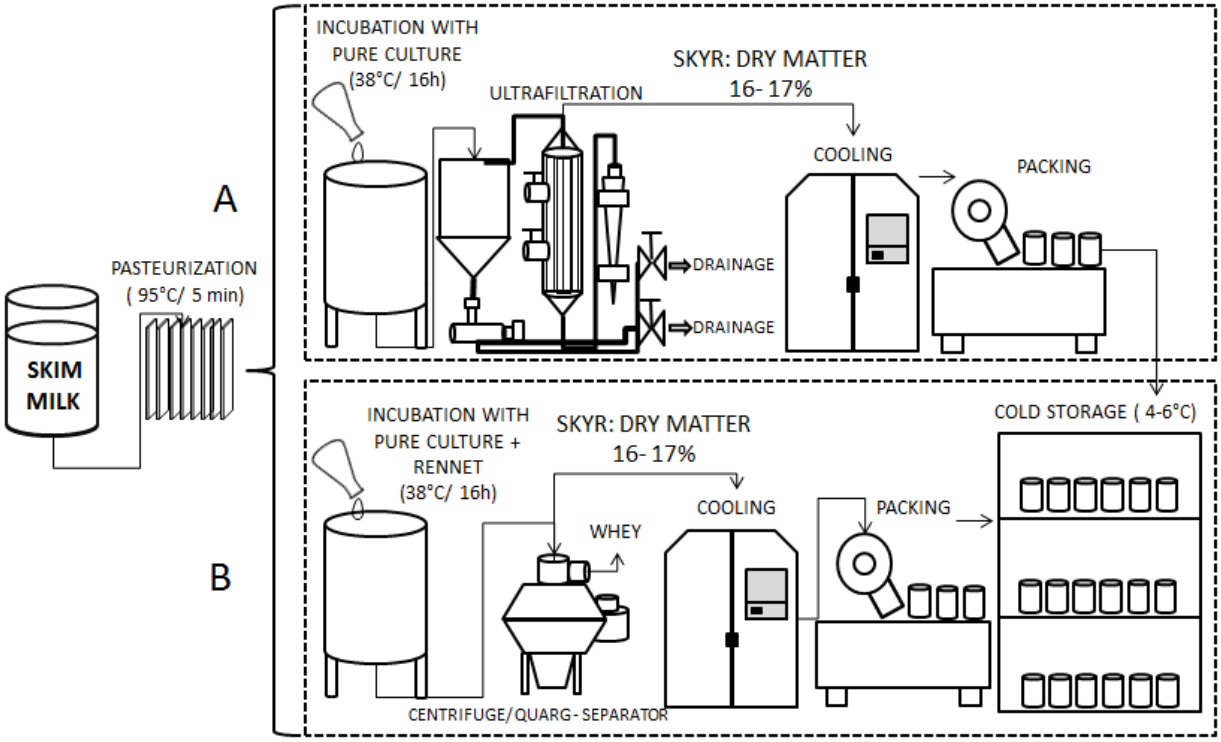


Source: The authors (2020).

The skyr manufacturing has changed in the last 80 years. Nowadays, the main differences in the commercial production is the standardized process, the use of pure starter culture and the separation of whey. In the large-scale production, the concentration of this fermented milk can be performed by centrifugation or Quarg-separators, however, nowadays industries invest in ultrafiltration (Figure 11 A, B). This technique allows obtaining a higher protein yield, minimizing casein loss and due to the small pores also retain whey protein

adhered the curds. The protein composition, due to this higher retention, is different from traditional skyr production with cloth filtration. In the production line (Figure 13A), after the skim milk is pasteurized, a pure starter is added into an incubation tank to the fermentation step during 16 h at 38°C. When the pH reaches 4.6, the fermented milk is pumped to an ultrafiltration, which will separate the solid fraction from de liquid fraction (drained whey) (GUDMUNDSSON; KRISTBERGSSON, 2016). At cooling temperature, the product with 16-17% dry matter is packing and stored. In the process with a Quarg-separator (Figure 13B), a small quantity of rennet is also used with a variation in the quantities among different process, generally 0.01- 1.5% starter culture and 50-100 ppm rennet. After the incubation step, the fermented milk is centrifuged, cooled, packed and stored at 4-6 °C. The final product contains approximately 13% protein, white color, mild acid flavor and thick texture.

Figure 15- Commercial skyr production; A: production line with ultrafiltration; B: production line with centrifugation or Quarg- separator.



Source: The authors (2020).

In other countries, there are also concentrated fermented milks with different tastes, textures and procedures. In Denmark, one of these dairy products is produced since 1930 from an ultrafiltered coagulum and is called Ymer. In the manufacturing, skim milk is fermented during almost 20 hours by a culture which contains *Lactococcus* lactis subsp. *lactics*,

Lactococcus cremoris and *Leuconostoc mesenteroides* subsp. *Cremoris*. In the traditional concentration step, the curd is broken and the whey is removed. Today, a Quarg-separator may be used to enhance the whey removal. The final product contains approximately 15% total solids, including 5-6% milk protein and must be stored and sold at 5°C.

A typical fermented milk from Sweden is the Lactofil, with the concentration similar to Ymer. This consistent dairy product is fermented by *Lactococcus lactis* subsp. *Lactics* and *Leuconostoc mesenteroides* (the same starter culture to produce the traditional fermented milks Filmjölk and Karnmjölk from Nordic countries). Other popular concentrated fermented milk from Nordic countries is the Kokkeli in Finland, produced by a spontaneous fermentation and the sour milk is warmed to facilitate the whey removal (ROGINSKI, 2011).

Each fermented milk has its taste, consistence, and flavor, depending on specific cultures and procedures. To summarize, all these fermented milks discussed in this chapter are shown in Table 5 with their specific starter cultures and main sensory aspects.

Table 5- Starter cultures and main sensory aspects of fermented milks.

Fermented Milk	Starter cultures	Sensory aspects
Plain Yogurt	*L. bulgaricus* and *S. thermophilus**	Clean, firm and consistency gel-like, slight flavor and low acid taste.
Drinking Yogurt	*L. bulgaricus* and *S. thermophilus**	Liquid texture, variation of flavor, taste and color according to additives.
Greek Yogurt	*L. bulgaricus* and *S. thermophilus**	Creamy texture, higher content of milk solids (fat and protein).
Frozen Yogurt	*L. bulgaricus* and *S. thermophilus**	Similar to an ice cream texture with a yogurt flavor.
Dried Yogurt	*L. bulgaricus* and *S. thermophilus**	Dry consistence and characteristic flavor of fresh yogurt (depending on the dry process).
Kefir	*Leuconostoc spp.*, *Lactococcus spp. Acetobacter spp., K. marxianus, S. unisporus, C. cerevisiae, S. exiguus*	Yeast-like flavor, mild alcoholic and frizzy taste. White color without curd formation.
Koumiss	*L. bulgaricus, L. casei, L. helveticus, L. kefiranofaciens, L. kefiri, St. parauberis, L. lactis, K. fragilis, S. unisporus*	Liquid aspect without curd, milky - grey color, sharp acid and alcoholic taste.
Skyr	*L. bulgaricus, L. casei, S. thermophilus*	White color, mild acidic flavor and thick texture

Note: * In some countries, the legislation allows to choose only one of these two specific LAB.

10 SENSORY PROFILE OF YOGHURT AND RELATED PRODUCTS

The sensory analysis of yoghurt and its related products has to take into account the wide range of products within the category, e.g. natural yoghurt, set yoghurt, stirred yoghurt, concentrated yoghurt, flavoured yoghurt, high-protein yoghurt, frozen yoghurt, among others. Thus, the analyst must keep in mind that the choice of sensory techniques will depend on the objective to be investigated and the type of product to be evaluated. The combination of sensory and consumer study techniques seems to assume greater importance in the yoghurt market than for other dairy product categories. It appears that product segmentation in the yoghurt market is matched by a corresponding segmentation among yoghurt consumers, creating marketing associations:

•• Natural yoghurts without additives for individuals concerned about food purity;
•• Organic yoghurts aimed at individuals with ecological concerns;
•• Sweetened and flavoured yoghurt presented in playful packaging for children;
•• Yoghurts enriched with whey protein aimed at the athletic and fitness segment.

Milk, as the fundamental ingredient in yoghurt, can vary widely in composition (species, whole milk, semi-skimmed, skim milk, lactose-free, organic) and quality.

Regardless of the type of milk, flavour attributes such as softness, sweetness, acidity and the absence of extraneous flavours are fundamental to the sensory profile of yoghurt. These central sensory attributes are not adequate to address the particular sensory characteristics of the wide range of yoghurt categories and products. The sensory team must also be aware of consumer trends and changes in presentation or form of consumption of yoghurt products (portioned pots for individual consumption with a spoon, multi-serving pots, beverage bottles, etc.). The primary sensory characteristics for the main yoghurt categories will be discussed according to the specifics and market considerations of each category.

10.1 SENSORY PROFILE OF NATURAL YOGHURT (SET AND STIRRED TYPE)

Natural yoghurt is generally marketed as ‘set yoghurt’ or ‘stirred yoghurt’. The first type is traditionally fermented in the pot it is sold in, resulting in a firmer texture than stirred yoghurt but also the potential for texture variation from pot to pot. In stirred type, there is batch standardization, so inter-pot variation can be minimized.

Natural yoghurt is characterised by its soft and viscous structure and slightly acidic, delicate flavour with nutty notes (KOSIKOWSKI, 1982). Such characteristics are attributable to the symbiotic relationship developed by colonies of *S. salivarius* ssp. *Thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, and also by the acid concentration in the matrix. In his study, Crawford (1962) postulated that titratable acidity values between 0.85% and 0.90% (expressed as lactic acid) make the final ready-to- eat product most desirable. Acetic acid, diacetyl and acetaldehyde contribute to yoghurt's mild, slightly sour taste, and the balance between them is critical (CHENG,2010). Any increase in acidity in the supply chain post-production, due to secondary fermentation, is considered objectionable and is recognized as a shelf-life limiting factor.

Sensory evaluation of the yoghurt and other milk fermentation products began from the perspective of identifying sensory defects and off-flavours. In this way, it was already known since the beginning of the industrialization of milk processing that the use of low-quality ingredients and technical errors made during processing would be reflected in several sensory defects in the final product. Regulatory agencies such as the United States Department of Agriculture (USDA) developed standardized tools for judging the quality of milk and milk products based on the detection of the important defects found in the final product and correlation with deviations in the quality of the raw milk and/or processing procedures (SCHIANO et al., 2017).Trout et al. (1939) published the document Official Flavor Criticisms of Dairy Products Judged in the National Contest, which became one of the landmarks for the standardized evaluation of milk, butter, cheese and ice cream and was also used as a basis for the evaluation of yoghurt. Although the dairy products score card was also generally applicable to yoghurts, it was not included in the first version of the guidelines. Instead, the USDA published in 2001 a specification for yoghurt, non-fat yoghurt and low-fat yoghurt, which presented critical sensory attributes for these products. The USDA (2001) specified that yoghurt should have pleasant clean acid flavour. In addition, yoghurt must not have a bitter, rancid, oxidized, stale, yeasty or unclean taste. In relation to texture, the USDA (2001) recommended that yoghurt should be full-bodied and firm and, like cream, should be smooth and homogeneous (i.e. without grains). Yoghurt should have a uniform colour with a smooth and velvety appearance. The default colour of natural yoghurt should range from a glossy white to a duller off-white. A visual homogeneity is also desired, with only minor separation of the serum acceptable. Mould growth or surface discoloration/fading were ground for rejection.

In recent decades, descriptive and quantitative descriptive analysis (QDA)® (STONE, 1974) has been widely used for yoghurts and related products. So, it is possible to observe the refinement and sophistication of the evaluated attributes, especially when seeking to meet the increasing demands of consumers. The following are examples of studies that have applied descriptive analysis to yoghurts over the last five decades.

Modler et al. (1983) used descriptive analysis to evaluate the effect of adding milk proteins to yoghurt. In the study, the authors analyzed only the attributes 'softness', 'firmness', 'acidity' and appearance. Harper et al. (1991) used a team of 10 trained panelists to evaluate the intensity of 17 commercial natural yoghurts and 153 consumers to evaluate the acceptability of the same yoghurts. The authors correlated descriptive data with consumer responses and analytical measurements. For this, they chose the attributes 'sour', 'astringent', 'sally', 'sweet', 'cooked milk', 'buttery', 'bitter', 'yeasty', 'fruity', 'acetaldehyde', 'cheesy', 'milky' and 'caramel'. Isleten and Karagul-Yuceer (2006) compared the physical and sensory properties fat-free yoghurts containing added powdered milk. In the study, the authors profiled yoghurts through the attributes: free whey, lumpiness, thickness, chalkiness, aftertaste, cooked, whey, creamy, cereal, animal-like, cardboard, fermented, sour, salty, sweet, astringent. Grygorczyk et al. (2013) studied the texture preferences of yoghurt consumers by comparing preferred attribute elicitation methods and conventional descriptive analysis. The attributes used in the descriptive analysis were 'stringiness', 'sour aroma', 'sweet', 'sour', 'dairy', 'thickness', 'chalkiness', 'aftertaste intensity' and 'colour'. Kycia et al.(2020) used descriptive analysis to investigate the effect of adding pullulan (a natural extracellular polysaccharide) on the sensory properties of low-fat yoghurt. The attributes used for sensory profiling were fermented milk aroma, heated milk aroma, sweet aroma, irritating aroma, different aroma, colour, thickness, smoothness, viscosity and for the flavour, fermented milk taste, sterilisation taste, acid taste, sweet taste, salty taste, bitter taste, chalky taste, impure taste and unnatural/different taste. Thus, it can be seen that some attributes are central and common to the sensory evaluation of yoghurt and related products (Table 6) and related to the basic fundamentals of the yoghurt fermentation process.

Table 6- Key attributes for yoghurt profiling and unflavored or modified counterparts

Attributes	Definition
Appearance	
Whiteness	White colour intensity.
Brightness	Ability of the surface to reflect light.
Homogenous	Product uniformity.
Thick	The ability of the product to flow from the spoon.
Aroma	
Sweet aroma	Specific aroma of products sweetened with sucrose or glucose.
Acid aroma	Aroma that refers to products with low pH.
Milky	Characteristic aroma of high-quality fresh milk.
Cooked	Suggestive of overheated milk, slightly sulphureous, and caramelised.
Flavour	
Acid taste	Classical definition of the taste promoted by substances with acidic pH.
Sweet taste	Specific taste of sucrose or glucose.
Bitter taste	Taste related to quinine or caffeine.
Creamy	Sour cream flavour, slightly greasy.
Cooked	Suggestive of boiled milk. Refers to sulphur compounds.
Astringent	Sensation by the shrinkage of the mucous membranes of the palate and tongue.
Milky	Features of high-quality fresh milk.
Yoghurt	Characteristics of unflavoured full-fat yoghurt.
Mouthfeel	
Thick	Ability to spread in the mouth with consistency.
Graininess	Presence of granular solids.
Creamy	Ability to fill the mouth pleasantly.
Firm	Ability to resist mouth movements.
Smoothness	Ability to demonstrate lightness when in the mouth.
Aftertaste	
Milky	Persistent milky flavour.
Sour milk	Persistent fermented taste after swallowing.
Astringent	Mouthfeeling tied after swallowing.
Bitter	Persistent bitter taste after swallowing.

Source: The authors.

There is also the possibility of generating sensory profiling of yoghurts with more agile methodologies that use untrained consumers to the detriment of highly trained panelists, e.g. check-all-that-apply(CATA) (CRUZ et al, 2013) and rate-all-that-apply (RATA) (TAN et al., 2020). The CATA technique consists of a structured questionnaire that asks consumers to select, from a closed list, the attributes that best describe the sample. In this test, the consumer describes the samples based only on perceived attributes. Unlike the classic descriptive analysis, the consumer does not need to select, and, therefore, evaluate, attributes that they do not recognise in the product. Therefore, CATA has been considered more intuitive and consumer-friendly, minimising cognitive processing (NG et al. 2013). RATA follows the same logic as

CATA, but differs by asking consumers to indicate the intensity of the attribute recognised in the sample. Consumer-based methods are also valuable for investigating the commercial perception of yoghurts. Cruz et al. (2013) evaluated the perception of yoghurt consumers with the techniques of projective mapping, CATA questionnaire and intensity scale. In the 1990s, Risvik et al. (1994) proposed the projective map, a technique that asks participants to position products in a two-dimensional space, bringing together those of more significant similarity. Esmerino et al. (2017a) tested pivot profile, projective mapping and CATA methods on Greek yoghurt samples. The idea of pivot is to obtain the relative meaning of the description of the products, collecting the free description of the consumers when placed to compare a sample tested against another considered pivot. The study highlighted the ability of all the three methods to describe Greek yoghurt samples in detail. Acceptance tests using the hedonic scales are highly recommended as an essential part of any sensory analysis strategy for the analysis of yoghurts and related products. The hedonic classification represents a natural characteristic of the human response to food and its stimuli, involving the likes and dislikes of a person and relating different attitudes, such as preference between two or more products, acceptability of products, frequency of consumption and purchase intention.

The term ‘all-natural’ on food labels, including yoghurt, is an essential factor to be explored in consumer tests, where market development is the objective. It is necessary to consider, a priori, the absence of precise regulation involving the terminology ‘natural’ on food labels in general. The Food and Drug Administration (FDA) in the United States considers as natural a product that does not contain any added artificial substance: including colouring, texturisers and flavourings and does not associate ‘natural’ with any nutritional or other health benefits (FDA 2018).

The appeal of ‘natural’ on the label of food products can refer to a positive feeling related to the idea of health and well-being. Consumers can sometimes associate products with ‘all-natural’ labels with characteristics of higher sensory and nutritional quality, which can be reflected in a greater willingness to pay a higher price for these products (UMBERGER et al., 2009). However, generalizations like this can be misleading and it is necessary to verify the importance of the natural appeal for specific products and markets. In yoghurt, for example, when studied in a population of primarily young women, the ‘all-natural’ designation received significantly lower acceptance scores compared to ‘high-protein’, ‘low-fat ‘or ‘made with stevia’ designations on the label. Thus, it is essential to include techniques in the sensory methodology that address consumer motivations.

The Food Choice Questionnaire (FCQ) is an instrument designed to assess the level of importance given by individuals to nine factors related to food choices: health, mood, convenience, sensory appeal, natural content, price, weight control, familiarity, and ethical concerns. The FCQ has been successfully used a few times to understand the motivations of yoghurt consumers (POHJANHEIMO; SANDELL, 2009)

10.2 SENSORY PROFILE OF SWEETENED AND FLAVOURED YOGHURT PRODUCTS

The USDA general recommendations are still relevant for the quality assurance of commercial yoghurt products. However, the intensification of competition in the dairy market has driven the search for more diversified products to different consumer niches. At the beginning of large-scale industrial production, yoghurt was generally sold in its natural form, i.e. without the addition of sweeteners, flavourings or colouring agents. Over time, yoghurts flavoured with fruits were successfully introduced to market and now represent more than two-thirds of yoghurt sales. In 2007, the top 10 flavours utilised in the production of flavoured yoghurt products were: vanilla, strawberry, mixed berries, blueberry, peach, raspberry, strawberry/banana, cherry, lemon, and lime (TRIBBY, 2009). Glanbia Nutritionals have recently reported that among recently launched flavoured yoghurt products, 32% were berry flavoured, 6% were citrus and 32% were other fruits such as: pineapple, mango and banana (GLANBIA NUTRITIONALS, 2021).

The addition of jams and fruits to yoghurt led to the need to expand the sensory attributes analyzed to encompass all the attributes of flavoured yoghurt in the early 1980's. Bodyfelt (1981) published a study questioning whether the American Dairy Science Association (ADSA) dairy scorecard was consistent with contemporary sensory assessment principles and proposed a revised version of the ADSA scorecard (Table 7) suitable for the assessment of flavoured yoghurts. At the end of the decade, the same author (BODYFELT et al., 1988) published an updated and expanded version of the list of attributes to be adopted in the scoring of flavoured yoghurts, keeping the focus of the sensory analysis on the presence or absence of specifically defined defects. Later, the USDA specified that in the case of flavoured and coloured yoghurts the distribution of colouring or flavouring must be homogeneous to achieve a natural appearance (USDA, 2001).

Table 7- Revised ADSA scorecard for flavoured yoghurts.

Appearance	Defects
Foamy (nonhomogeneous)	Shrunken
Free whey	Surface growth
Colour or hue	
Atypical colour	Too light
Colour "bleeding"	Too dark
Fruit frequency	
Excess fruit	Lacks fruit
Flavour defects	
Unnatural	Lacks flavouring
Lacks fine flavour	
Acidity level and microbiological characteristics	
High acid	Low acid
Green	Fruity/fermented
Sweetened	
Lacks sweetness	Too sweet
Dairy ingredients	
Cooked	Oxidised
Lacks freshness	Rancid
Old ingredient	Unclean
Other	
Bitter	Stabiliser

Source: Adapted from Bodyfelt (1981).

There are a range of aspects to be considered when formulating sweetened and flavoured yoghurt products, in particular:

•• The choice of natural or synthetic flavourings;

•• The choice of sweetener (natural or synthetic);

•• The choice of a light, diet or conventional formulations;

•• Whether to add fibre or other probiotic ingredients.

Yoghurt flavouring began with the addition of fruit jams or jellies. However, advances in the flavours industry made the flavouring process more reproducible, and most yoghurt producers soon adopted the use of concentrated flavour extracts or synthetic flavours. From a sensory point of view, attention must be paid to the different flavouring designations used on

yoghurt labelling. For example, Janiaski et al. (2016) analyzed artificially flavoured strawberry yoghurts, so the list of evaluated attributes contained the terms ‘Artificial strawberry odour’, ‘Particles (related to the colouring agent)’ and ‘Artificial strawberry flavour’.Sweetness is a critical attribute in flavoured yoghurts. De Souza et al. (2021) established, via an acceptance test with a hedonic scale, the levels of sucrose that could be added in the production of strawberry yoghurt without compromising product acceptability (compromised acceptance threshold) or risk of sensory rejection (hedonic rejection threshold). On the other hand, increasing consumers concerns about the intake of sugars and the need to serve the diabetic public motivated the expansion of versions of flavoured yoghurt products sweetened with sugar alternatives. To match the intensity of the sweet taste between the standard sample (usually, sucrose) and the sugar-free sample can be challenging, due to the difference in potency between the sweeteners used. Even minor variations in the concentration of high-potency sweeteners can impact consumer acceptability.

CONCLUSION

Fermented milks, with diverse methods of manufacturing, tastes, textures, and starter cultures are very appreciated around the world. This constant demand led industries to produce these products in large- scale, investing in new technologies and equipment. In dairy industries, several alternatives and changes are included as concentration, ultrafiltration, drying or freeze. The development of new products from emergent technologies, in addition to standardized processes and formulations, are constantly studied to enhance the production and guarantee the same quality of these popular dairy products. In this chapter, specific methods of production were showed and explained, being an important theory background to put them into practice.

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CHAPTER 3

The Improvement of the Functional Potential of Dairy Products Using Fruits and Plant Extracts

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THE IMPROVEMENT OF THE FUNCTIONAL POTENTIAL OF DAIRY PRODUCTS USING FRUITS AND PLANT EXTRACTS

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1. INTRODUCTION

Dairy products are widely consumed around the world with several origins, raw material, manufacturing types, textures, and flavor. The large-scale production of dairy products means that the production process always has to deal with strategies that extend the shelf-life of the product, such as the addition of preservatives and changes in distribution logistics. However, artificial additives can generate allergic conditions in a portion of the population, in addition, the current consumer market has been looking for functional products, with nutritional appeal and with natural additives in their composition.

In the last years, the addition of plant extracts and fruit pulps in different dairy products has been widely studied with the function of being additives with a potential preservative action, due to the great antioxidant and antimicrobial properties, being able to prolong the shelf-life of the dairy product without the need for artificial preservatives. In the scenario of the addition of fruit pulp, there is wide global acceptance by consumers in addition to the improvement of the formulation with an increase in the nutritional value and enhancement of the color of the products naturally, due to the presence of biocompounds responsible for the original color of the fruits and vegetables.

The functionality of dairy products can be enhanced by the incorporation of vegetables, natural extracts, or fruits. Recent studies, which will be addressed in this chapter, link bioactive compounds with functional and preservative potential, since the composition of fruits and extracts may have a prebiotic activity, improving the development of probiotic microorganisms in the formulation. Furthermore, the incorporation of functional dairy products into a healthy dietary routine can enhance the consumer's health by decreasing the incidence of

chronic non-communicable diseases such as diabetes, hypertension, obesity, heart disease, and cancer.

The following book chapter aims to list the main dairy products consumed across the globe, with emphasis on recent studies on increasing the functionality of products added with different plant extracts, fruits, or seeds, increasingly stimulating the development of new products and the reduction of artificial additives in industrial production.

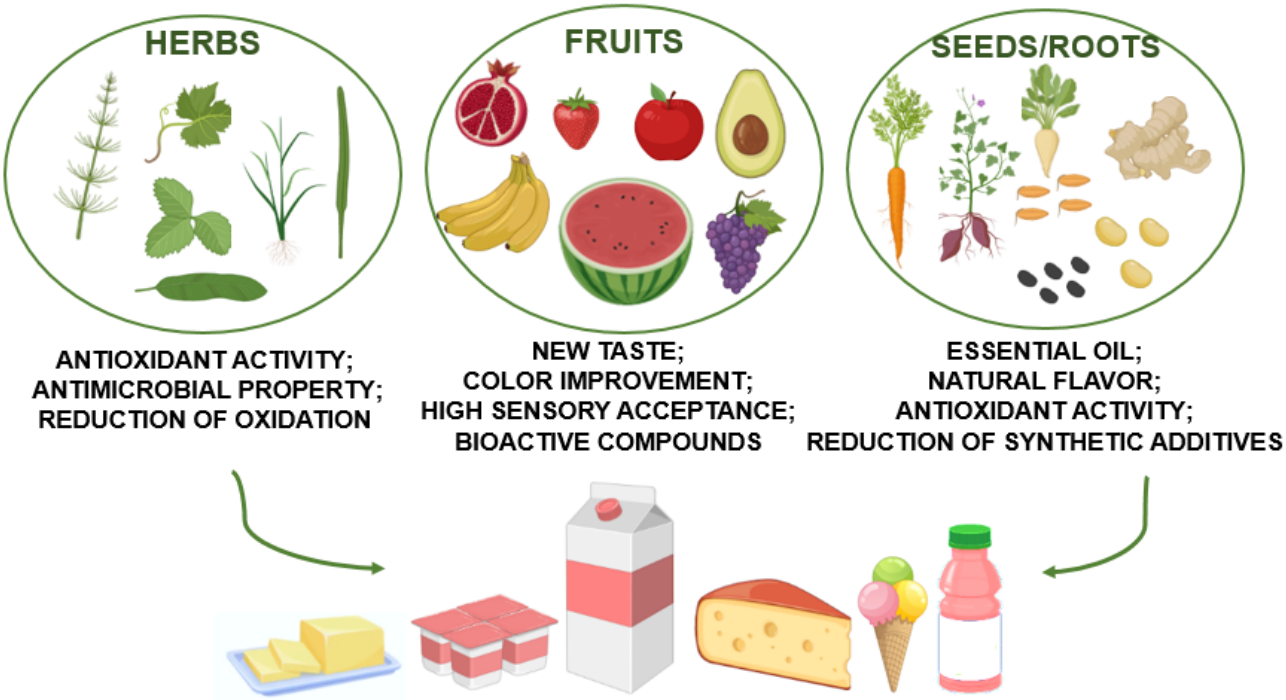
2. ADDITION OF PLANT EXTRACTS AND FRUITS IN DAIRY PRODUCTS

In recent decades, the demand for consumption of products with nutritional and functional appeal has increased. This current consumption scenario is strongly linked to socio-economic changes, to the desire for a longer life expectancy with quality and to the several studies that are important for the development of new products with healthy characteristics that are present in the market today (BIMBO et al., 2017) . Currently, researches estimate that the increase of the health food market has an average growth rate of 8.5% per year, causing the food industries to invest hard in the production of healthy, new nutritional modified, and functional foods (RESEARCH AND MARKETS, 2014; SANAULLAH KHAN et al., 2014) .

Functional dairy products and with enrichment of their nutritional composition are one of the largest segments of the market, corresponding to approximately 43% of world sales, in addition, dairy products are elucidated in the literature as one of the largest carriers of functional ingredients and very accepted by consumers (ABDOLLAHZADEH et al., 2018; BALTHAZAR et al., 2019; BARROS et al., 2022; BIMBO et al., 2017; CAMELO-SILVA et al., 2021; DE LIZ et al., 2020; MUÑOZ et al., 2018; SAKANDAR; ZHANG, 2021; VERRUCK et al., 2015, 2020).

The addition of extracts and fruit pulp to dairy product formulations, in addition to contributing to the development of a new flavor, can improve the properties of texture, water retention, reduction of lipid oxidation and microbiological proliferation, and contribution to the final color, through its original characteristics. The natural composition of vegetables, herbs, and fruits contributes to the development of a product with natural final characteristics, without the addition or as little as possible of synthetic additives, which is one of the greatest challenges of the 21st century in food industries (CHRISTAKI et al., 2021) (Figure 16).

Figure 16- Functional properties of plant extracts and fruits added in dairy products



Source: The authors (2022).

Fruits, vegetables, seeds, and herbs synthesize, during their metabolism, chemical compounds that can generate several benefits both for the formulation of dairy products and for the consumer's health. These biocompounds, called "phytochemicals", are produced by plant defense systems and act as insecticidal, antioxidant, antibacterial and antifungal agents (DOMÍNGUEZ DÍAZ; FERNÁNDEZ-RUIZ; CÁMARA, 2020; YAHIA; GARCÍA-SOLÍS; CELIS, 2019). Depending on each plant species, bioactive compounds present different chemical conformations and concentrations. Among these compounds classified as "non-nutrients", and with potential beneficial action to the human body, are phenolic compounds, carotenoids, fatty acids, phytosterols, vitamins, and fibers (PRESTES et al., 2021b; SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018; YAHIA; GARCÍA-SOLÍS; CELIS, 2019).

The effectiveness of the application of extracts and fruit pulps in the formulation of dairy products varies according to the process of obtaining, concentration used, forms of application and storage temperatures (MISHRA et al., 2020). The method of application (by immersion, embedded in micro/nano capsules, powders, or spray), and the concentration used are the primary factors for the effectiveness of the action of bioactive compounds of plant origin. The sensory impact of the final product must also be considered when determining the concentration of the natural additive in the formulation, combining the conservative potential

of the biocompound with the minimization of undesirable changes in sensory properties (GUTIERREZ; BARRY-RYAN; BOURKE, 2008). For different types of dairy products, in addition to improving the final physicochemical characteristics, phytochemicals can enrich the formulation with compounds with potential antioxidant, antimicrobial, antidiabetic, and antiproliferative action.

2.1- CHEESES

Since ancient times, different types of cheese have been developed and are present in people's dietary routine, with manufacturing techniques perfected over time. The production of cheeses can be specified according to the raw material (with milk from different mammals), the coagulating agent used, time and conditions of maturation and storage, in addition to different thermal treatments (HAO et al., 2021; KAMATH; BASAK; GOKHALE, 2022). In the development of new dairy products, including cheeses, milk fat, and protein substitutes, natural additives are studied and applied with the aim of reducing product expenditures, as well as improving functionality, nutritional value and reducing food allergy and lactose intolerance.

In cheese manufacturing, the processed cheese sector is innovative, always aiming to extend the product's shelf-life, recycle defective cheeses and develop new textures, flavors, and functional properties (ALY et al., 2016). To increase the nutritional value and functional property of a type of processed cheese (made with a mixture of Egyptian Ras cheese, white cheese and cheddar cheese), El-Sayed, Elaaser, and El-Sayed (2021) developed microcapsules with mustard seed extract and probiotic strains of *Bifidobacterium bifidum* to be added in cheese formulations in the proportion of 1, 2 and 3% (Table 8). Mustard seeds are a good source of omega-3, fatty acids, vegetable protein, phenolic compounds, minerals, and have potential antioxidant, antimicrobial activities (DUBIE et al., 2013). These properties probably contributed to a high probiotic viability in the cheese formulation (above 10^8 CFU.g^{-1} with 1 and 2% microcapsules and 10^9 CFU.g^{-1} for 3% considered, then, a probiotic product due to the high cell count), indicating mustard seeds as possible prebiotic agents. In addition, the microencapsulation efficiency was above 97% for *B. bifidum* and 90% for the extract. For sensorial acceptance, the flavor of the cheeses enhanced considerably and the ratios of 1% and 2% showed more acceptable flavor among all the produced cheeses. Probiotic cheeses were also produced by Diniz-Silva et al. (2020) who added oregano and rosemary essential oils as potential protective agents for *Lactobacillus acidophilus* and against *Escherichia coli* in Minas

Frescal cheese. During a gastrointestinal simulation, terpenes in the composition of oregano and rosemary oils presented a protective effect for probiotic cells during all the gastric steps, in addition, essential oils controlled the development of *E. coli* in cheeses after 15-21 days of storage (with a decrease of 2.3 and 2.9 log $CFU.g^{-1}$ in cheese with oregano oil and rosemary oil, respectively), providing a promising natural strategy for cheese preservation. The use of herbs (fresh and extracts) and spices promote excellent sensory properties of the different types of cheeses. In addition to contributing to the final flavor, specific extracts, including rosemary, have potential preservative activities, as already reported. According to Youssef and El-Sayed (2018), dry rosemary presents high antioxidant and antibacterial properties due to the high contents of caffeic and rosmarinic acids, flavones, and phenolics. The addition of this plant extract significantly reduced numbers of foodborne pathogens, as well as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus* (EL-SAYED; YOUSSEF, 2019; YOUSSEF; EL-SAYED, 2018). In active packaging applications for soft cheese, the bioactive compounds of rosemary allowed the storage of this dairy product for 60 days at 4°C without spoilage, characterizing this specific herb as a good natural antioxidant and preservative additive (KONTOGIANNI et al., 2022).

Foodborne pathogens can also have the development rate reduced by the presence of bioactive compounds in fruits (pulp, peel, pomace, or seeds), which may act on the cell walls of microorganisms or interfere with the cell center, interrupting microbiological growth. Based on this, Elafify et al. (2022) studied the addition of pomegranate peel extract, which is rich in tannins, with the aim of reducing *Salmonella enteritidis* count in Egyptian soft cheeses. The concentration 0.5 and 1% pomegranate peel extract showed, respectively, 0.4, and 1.1 log $CFUg^{-1}$ reduction in *S. enteritidis* counts for 4 weeks storage, at 4 °C. This reduction is related to the presence of phenolic acids and flavonoids in the pomegranate composition. Peel phenolic compounds can react with cell membrane and cause bacterial cell lysis and/ or can react with protein sulfhydryl groups, generating phenolic toxicity and make the bacterial cells unavailable for growth (AKHTAR et al., 2015). Generally, the same bioactive compounds that have antimicrobial action are also related to functional and beneficial properties for the consumer's health. In a development of coalho cheese, a traditional Brazilian cheese, Costa et al. (2020) evaluated the potential biopreservative and functional properties of passion fruit in the formulation (Table 8). Aromatic metabolites, phenolic compounds, carotenoids, and fibers are phytochemicals present in this fruit with antimicrobial and antioxidant potential, related to reduce inflammatory and nociceptive events in studies *in vivo* (DE LAVOR et al., 2018; LEAL

et al., 2020). The aqueous extract of passion fruit presented high inhibitory activity against *Staphylococcus aureus* and low inhibitory activity against lactic acid bacteria (LAB). In the coalho cheese produced with ground passion fruit, the development of *Staph. aureus* was inhibited (approximately 10^6 $CFUg^{-1}$ and 10^4 $CFUg^{-1}$ in the first and seventh day of storage, respectively), attributing this behavior to bioactive compounds of the dried fruit, which were slowly released in the middle. In relation to the LAB count, the passion fruit did not interfere in the cell development with high counts (approximately 10^8-10^9 $CFUg^{-1}$ in all the 7 days of storage)(COSTA et al., 2020). This becomes relevant for the development of specific LAB during the production of dairy products, including strains with probiotic properties, leading to an increase in the functional potential of the product.

The addition of fruit juices, extracts or pulp is very accepted when incorporated in soft cheeses formulations (RAMOS MESSIAS et al. 2021; SILVA et al. 2021; PASINI DEOLINDO et al. 2019). *Petit Suisse* is a fresh and smooth cheese, with a texture similar to a thick yogurt. This dairy product is considered the soft cheese with the highest sensory acceptance by adults and children (BERMUDEZ-BELTRÁN et al., 2020). The addition of phenolic compounds from Bordeaux grape peel and seeds were added in a *Petit Suisse* formulation by Pasini Deolindo et al. (2019) to evaluate the functional and technological potential of this new dairy product (Table 8). Grape seed extract (antioxidant activity : 3637 mg AAE $100\ g^{-1}$) inhibited approximately 80% of ACE (angiotensin- converting enzyme activity, which the inhibition is related to the reduction of the risk of hypertension and associated diseases) and grape skin flour (extract with 1033 ± 10 mg AAE$100\ g^{-1}$ antioxidant activity) inhibited 38% of ACE activity. This behavior is related to high amounts of phenolic compounds in grape berries, which is estimated that 60% of fruit's polyphenols are found in the seeds (BARBA et al., 2016). These bioactive compounds did not interfere with the sensory acceptance of *Petit Suisse*, with an approximate global acceptance of 73%, a factor that is also of extreme importance for the food industry when developing a new product to market.

Potential functional properties were also obtained in the development of several cheeses with the addition of natural extracts and fruits, relating high functionality with high levels of bioactive compounds, mainly phenolic compounds. Among the natural additives, several studies continue to be developed with potential extracts and fruits in cheese production, such as the addition of *Arbutus unedo L*. extract (the strawberry tree, with small spherical fruits, rough, and red when fully ripe) in soft cheeses (MASMOUDI et al., 2020); cape gooseberry (*Physalis peruviana* L.) (BERMUDEZ-BELTRÁN et al., 2020); *Moringa oleifera* in goat soft

cheese (ABDEEN; IBRAHIM; KHOLIF, 2021); *ora-pro-nóbis* (*Pereskia aculeata* Miller) in freeze dried *Petit Suisse* (SILVA et al., 2021); Pine needles (*Cedrus deodara* (Roxb.) Loud.) extract in *Kalari* cheese (a hard dry cheese type of India); star anise, guava tree, and turmeric extracts in cheeses matured for 28 days (SHORI; YONG; BABA, 2022); turmeric, sage, and marjoram extracts in *Kariesh* cheese (an Egyptian soft cheese type) (HASNEEN et al., 2020).

Table 8- Recent studies on the addition of plant extracts and fruit pulp in the manufacture of dairy products.

Dairy product	Country	Natural additive	Conclusions	Authors
Processed cheese	Egypt	Mustard seed extract	High probiotic cells count in cheese formulations (above 10^8 CFU.g^{-1}); microencapsulation efficiency was above 90% for all the treatments; High sensorial acceptance.	(EL-SAYED; ELAASER; EL-SAYED, 2021)
Minas Frescal cheese	Brazil	Oregano and rosemary essential oils	Bioactive compounds from the herbs composition had a protective effect in probiotic survival during a gastrointestinal simulation; These bioactive compound also reduced the cells count of foodborne pathogens.	(DINIZ-SILVA et al., 2020)
Soft cheese	Egypt	Pomegranate peel	The concentration 0.5 and 1% pomegranate peel extract (with high phenolic content) showed, respectively, 0.4, and 1.1 log CFUg^{-1} reduction of foodborne pathogens counts for 4 weeks storage, at 4 °C.	(ELAFIFY et al., 2022)
Coalho cheese	Brazil	Passion fruit extracts	The aqueous extract presented high inhibitory activity against foodborne pathogens and low inhibitory activity against lactic acid bacteria (LAB).	(COSTA et al., 2020)
Petit Suisse	Brazil	Grape peel and seed extracts	Grape seed and peel extracts inhibited approximately 80% and 38% of ACE ACE activity, respectively; Almost 73% of sensory acceptance; Improvement of the nutritional value.	(PASINI DEOLINDO et al., 2019)
Petit Suisse	Colombia	Cape gooseberry (*Physalis peruviana* L.) and moringa (*Moringa oleifera*) leaf powder	High nutritional value of the developed product (higher levels of fiber, minerals, vitamins,) when compared to the control sample; Main foodborne pathogens, such as *L. monocytogenes* and *Salmonella* spp. were absent in the product.	(BERMUDEZ-BELTRÁN et al., 2020)

Yogurt	Brazil	Guabiroba pulp (*Campomanesia xhantocarpa* O. Berg)	10% of guabiroba pulp provided high probiotic development in all the gastric steps, characterizing its bioactive compounds as potential prebiotic agents.	(PRESTES et al., 2021a)
Yogurt	Brazil	Camu-camu (*Myrciaria dubia*) seed extract	The seed extract presented high antioxidant activity and inhibited the carcinogenic cell proliferation; High in vitro activity of activity of α-amylase, α-glucosidase, and angiotensin-converting enzyme.	(FIDELIS et al., 2020)
Skimmed milk yogurt and *Kariesh* cheese	Egypt	Herbal extract (turmeric, sage, and marjoram)	High phenolic content in the extracts; 1% of herbal extracts did not interfere in organoleptic properties.	(HASNEEN et al., 2020)
Fermented milk	Brazil	Passion fruit by-product	Improvement in the development of probiotic cells during 2 weeks; bacteria related to high intestinal dysbiosis had reduced growth with the presence of high counts of probiotic strains.	(NEVES CASAROTTI et al., 2020)
Buttermilk yogurt	Australia	Curcuminoids extract from turmeric	After an in vitro gastrointestinal simulation, the addition of powdered curcuminoids into buttermilk, before the yoghurt fermentation, had 33% total potential bioavailability.	(FU et al., 2019)
Ice cream	Brazil	Grape juice resides	High phenolic content and antioxidant activity in the developed samples. The predominant purple color is related to high amounts of anthocyanins.	(VITAL et al., 2018a)
***Kulfi* (a dairy product similar to ice cream)**	India	*Aloe vera* extract	15% A. vera extract promoted high phenolic content (9.10^3mg GAE$100g^{-1}$) with high antioxidant and antimicrobial properties against *Escherichia coli.*	(MAHAJAN et al., 2022)
Ice cream	India	Ginger juice, powder, and candy	Increase in total solids content with addition of ginger powder and candy. The addition of different ginger forms in ice cream formulations improved the total phenolic content in all the samples.	(GABBI; BAJWA; GORAYA, 2018)
Ice cream	India	Black carrot concentrate	7.5% was the best concentration of black carrot concentrated sensorially accepted in dairy products formulation. The extract promoted an increase in magnesium and iron content and improved the antioxidant property.	(PANDEY et al., 2021)

Dairy beverage	Spain	Blend of fruit juices (orange, kiwi, pineapple, and mango juices)	The blend of fruit juices, after an *in vitro* gastrointestinal simulation, improved the total phenolic content and lipophilic bioactive compounds.	(RODRÍGUEZ-ROQUE et al., 2014)
Dairy beverage	Spain	Orange juice	The fermentative process by *L. plantarum* and *L. brevis* with the addition of orange juice increased levels of carotenoids, total phenolic compounds, and antioxidant activity.	(DE LA FUENTE et al., 2021)
Butter	Sri Lanka	Cinnamon extract	Antioxidant and antimicrobial properties from cinnamon reduced the peroxide index, formation of free fatty acids, and the microbiological deterioration of the butter for 9 weeks.	(VIDANAGAMAGE; PATHIRAJE; PERERA, 2016)
Goat's milk butter	Brazil	Turmeric	Rats on a diet rich in turmeric butter had reduced levels of LDL and triglycerides in their blood. Compared to a control sample, the turmeric butter presented higher antioxidant activity and levels of polyphenols and carotenoids.	(COSTA et al., 2021)

2.2- FERMENTED MILKS

Fermented milks are dairy products widely consumed by the global population, with diversity in manufacturing, and fermentation processes by specific strains. In the food industry, the ease and practicality of consuming fermented milks has become an opportunity to add different natural additives (with high sensory acceptance) with functional appeal in the formulation of these dairy products, including fruit pulp and juice, grains, seeds ,and plant extracts. Because it is an easy-to-produce product, there are many studies with the addition of fruits and plant extracts in different types of fermented milks (PRESTES et al., 2021b).

The risk of developing chronic diseases can be reduced with the presence of a balanced diet by individuals, also containing dairy products, fruits, and vegetables. Among the current recommendations in the diet is the inclusion of prebiotic fibers combined with the consumption of probiotic/symbiotic products (BIANCHI et al., 2019; NEVES CASAROTTI et al., 2020). To evaluate the impact on the diet and health of obese individuals with the incorporation of probiotics and prebiotics in dairy products, Neves Casarotti et al. (2020) developed a probiotic low-fat fermented goat milk with passion fruit by-product (Table 8). Through a Simulator of Human Intestinal Microbial Ecosystem (SHIME) over a two-week period, there was an

improvement in the development of probiotic cells (*Lactobacillus* and *Bifidobacterium*), which are characteristic of the intestinal colon, due to the presence of fibers and other bioactive compounds in the passion fruit composition. On the other hand, bacteria related to high intestinal dysbiosis in obese individuals (*Prevotella*, *Megamonas* and *Succinivibrio*) had reduced growth with the presence of high counts of probiotic strains in this period. Passion fruit and buriti (*Mauritia flexuosa* L.) pulp, also a tropical fruit, were also the target of the study by Borgonovi, Casarotti, and Penna (2021), who developed a potential probiotic fermented milk with bioactive compounds from these fruits. The acidity of passion fruit (pH 3.0-3.8) may have contributed to better probiotic cell (*Lacticaseibacillus casei;* >11 log $CFUmL^{-1}$) development in the middle and the bioactive compounds from this fruit (high amounts of vanillic acid, quercetin, trans-cinnamic acid, p-cumaric acid, and others in lower amount; total phenolic content = 220.99 EAG $100g^{-1}$) also promoted a potential prebiotic effect. However, buriti pulp presented higher phenolic content (561.62 EAG $100g^{-1}$; high amounts of protocatechuic acid, chlorogenic acid, (−)-epicatechin, luteolin, and (+)-catechin), and some of these compounds, such as chlorogenic acid and quercetin, have immunomodulatory and antimicrobial effects, which can reduce the LAB development. *Lacticaseibacillus casei*, on the other hand, showed low reduction in the presence of buriti pulp. Some microorganisms can use bioactive compounds as an energy source through reduction, hydrolysis, and cleavage of these compounds (BORGONOVI; CASAROTTI; PENNA, 2021).

A gastrointestinal simulation was also used in the study of Prestes, Verruck, et al. (2021) with the addition of guabiroba pulp (*Campomanesia xhantocarpa* O. Berg) in probiotic yogurt (Table 8). The addition of 10% of guabiroba pulp provided a high development of *Bifidobacterium* BB-12 throughout the gastrointestinal tract (8-9 log $CFUg^{-1}$), alleging to bioactive compounds of the fruit a protective and prebiotic characteristic. A fruit from the same genetic family as guabiroba (*Myrtaceae*), the camu-camu (*Myrciaria dubia*), had the seed extract added to yogurt to evaluate its potential functional characteristics (FIDELIS et al., 2020) (Table 8). Camu-camu seeds are a rich source of phenolic compounds (vescalagin, castalagin, gallic acid, and procyanidin A2). These compounds enhanced the antioxidant activity in fermented milks, in addition the antiproliferative and cytotoxic effects in cancerous cell lines.

The addition of herbal extracts in fermented milks is also present in recent studies, focusing on its antioxidant and antimicrobial action, combined with a concentration that is sensorially accepted. Hasneen et al. (2020) developed dairy products, including a skimmed milk yogurt, with an addition of aqueous extracts of turmeric, sage, and marjoram (Table 8). 1% of

the aqueous extracts did not interfere in the sensorial acceptance. Above this concentration, in addition to the change in flavor, there may be changes in color, aroma, texture and growth inhibition of starter cultures due to the high concentrations of phenolic compounds, with potential antimicrobial characteristics (total phenolic content: 8.3, 3.4, and 2.9 mg GA.g^{-1} for turmeric, sage, and marjoram, respectively). A buttermilk yogurt was also fortified with curcuminoids from turmeric by Fu et al. (2019) who also performed an *in vitro* gastrointestinal simulation (Table 8). Fecal bacteria were capable to convert the bioactive compounds in the gut and the addition of powdered curcuminoids into buttermilk, before yoghurt fermentation, had 33% total potential bioavailability, transforming curcuminoids as potential functional additives in the production of dairy products.

The addition of natural bioactive extracts is an alternative to decrease the use of synthetic preservatives, pigments, antioxidants, and antimicrobial agents in fermented milks. Current studies are always under development to provide the research sector all possible promising results to be applied, in the future, in large-scale production (PRESTES et al., 2021b). Among these studies with fermented milks, there is an addition of mango peel in kefir development (VICENSSUTO; DE CASTRO, 2020); probiotic yogurt sheep's milk with strawberry juice (BALTHAZAR et al., 2019), apple pomace in a stirred-type yogurt (WANG; KRISTO; LAPOINTE, 2020), date extract in a probiotic yogurt (ABDOLLAHZADEH et al., 2018), pomegranate juice in probiotic fermented milk (PENA et al., 2021), black carrot extract added in yogurt and buttermilk (PANDEY et al., 2021). The constant search for new products with functional appeal makes the fermented milk sector very conducive to innovating by adding different fruits and extracts, with potential benefits addressed in studies such as these.

2.3- ICE CREAMS

Ice cream is one of the best consumed dairy products around the world and mostly eaten as a dessert. However, the original formulation of this product is functionally poor, lacking phenolic compounds, fibers, prebiotic agents, probiotics, and antioxidants. Therefore, researches are developed to discover the possibility of improving functional and nutritional values of ice creams with natural additives with health benefits (EL-SAYED; YOUSSEF, 2019).

Fruit juices and pulp are the most used ingredients in the development of new ice cream flavors, with great sensory acceptance by consumers. Vital et al. (2018) produced ice creams

with grape juice resides (2.5, 5, and 10%) and evaluated its functional potential (Table 8). The total phenolic content in the ice cream formulation (0.46, 0.71 and 1.17 mg GAE g^{-1} for 2.5, 5, and 10% of grape juice reside, respectively) is related to high antioxidant property and the purple color of the products is influenced by the presence of types of anthocyanins. In addition, the authors agreed that the addition of natural by-products as functional and colorant agents, the products do not require the addition of synthetic flavorings or pigments. Anthocyanins as pigment agents were also found in black carrot, which also was added in dairy products formulations including ice creams (PANDEY et al., 2021). The addition of black carrot concentrates into ice creams promoted a significant increase in magnesium (18.23 and 13.81 mg $100g^{-1}$ for samples with black carrot extract and control, respectively) and iron profile (1.38 and 1.01 mg $100g^{-1}$). In addition, the extract promoted a significant increase in total phenolic content (513.63 and 14. 32 mg GAE 100g $^{-1}$ for samples with black carrot extract and control, respectively) and antioxidant activity (ABTS: 24.64 and 3.01μmolTEg^{-1}), due to high amounts of anthocyanins in the black carrot concentrate (1682.7 mg $100g^{-1}$). 7.5% showed to be the best concentration of black carrot extract into ice creams with high acceptability, which potentiate this natural additive to enhance the nutraceuticals properties of food.

Different parts of plants can also be an innovative natural additive into ice cream formulations. Ginger rhizomes, for example, which have great antioxidant, antimicrobial, and anti-inflammatory activities, were processed into pulp, juice, powder, and candy to be added into ice creams mixture during freezing steps (GABBI; BAJWA; GORAYA, 2018). Ginger juice decreased the total solids (33.44%; control: 37.80%) due to its high water content, while ginger powder and candy increased the total solids content (37.98 and 40.73%, respectively). The antioxidant activity (AA) and total phenolic compounds (TPC) increased significantly on addition of ginger in different forms (control: TPC = nondetectable and 4.2% for AA; ginger juice: TPC = 1.2 mg$100g^{-1}$ and 11.0 - 31.9% for AA; ginger candy: TPC= 1.3 – 4.9 mg$100g^{-1}$ and 14.5 – 51.9% for AA; ginger powder: TPC= 0.5- 1.8 mg$100g^{-1}$ and 8.8 – 22.8% for AA).

Mahajan et al. (2022) developed an edible film with *Aloe vera* to be added into a frozen dairy product, the *Kulfi*, which is popular in many south Asian countries and the production is similar to ice cream, with higher total solids content with little or no whipping (Table 8). The edible film was developed for improved microbial and lipid oxidative stability. 15% *Aloe vera* showed highest antioxidant and antimicrobial potential against *Escherichia coli* strains (± 18 and 10 mm of inhibition halos, for 15%extract and control sample, respectively) due to the high content of carotenoids, phenolic compounds (9.10^3 mg GAE $100g^{-1}$), vitamins C and E, proved

to be potential antioxidant agents. In addition, this plant extract showed significantly low values for free fatty acids (± 0.5 and 0.9 % oleic acid for 15% extract and control sample, respectively) and TBARS (0.6 and 1.0 mg malondialdehyde kg^{-1} 15% extract and control sample, respectively) compared to control samples during six-month storage at −18 °C. Another frozen dairy dessert very accepted by consumers is frozen yogurt (a combination of yogurt and ice cream) , which has an enhanced functional characteristic when added with extracts, fruits and/or probiotic microorganisms. Terpou et al. (2019) produced frozen yogurt fortified with sea buckthorn berries (*Hippophae rhamnoides* L.), which were used as an immobilization carrier of probiotic strains *L. casei*. The immobilization increased survival rates of probiotic cells during freezing storage (9.40 log CFU g^{-1} in 90 days; control with free probiotic cells = 6.97 log CFU g^{-1} in 90 days). The sea buckthorn berries had a protective effect on probiotic cells against harsh and acidic conditions during production and storage of the frozen yogurt, promoting high cells count and attributing to this product a probiotic property.

2.4- DAIRY BEVERAGES

Milk-based beverages are products that have dominated the consumer market in recent decades, mainly due to the constant development of new products and flavors. Natural additives from fruits, cereals or extracts can combine pleasant taste with functional properties in the development of dairy beverages. A beverage formulation with milk and a blended fruit juice (orange, kiwi, pineapple, and mango juices) was obtained by Rodríguez-Roque et al. (2014) with the proposal to evaluate the bioaccessibility of the bioactive compounds from fruits (Table 8). After an *in vitro* digestibility, total phenolic compounds were improved during the simulated gastric steps (10.56 $mg100mL^{-1}$, for non-digested, to 14.9 $mg100mL^{-1}$, for digested sample). Many phenolic compounds also can be released from macronutrients of the dairy matrices, such as proteins or carbohydrates, due to the acid pH and action of digestive enzymes, increasing the concentration. In addition, the bioaccessibility of lipophilic compounds (carotenoids) was increased around 27% in the dairy beverage due to improved solubilization of these compounds in the milk fat fraction. Dairy beverages with fruit juices are also a good medium for the development of lactic acid bacteria (LAB), with emphasis on cells with probiotic properties. An orange juice-milk based beverage was developed by de la Fuente et al. (2021) for evaluation of probiotic fermentation (*L. brevis* and *L. plantarum*) and antioxidant properties (Table 8). After the fermentation period (72h), when compared to control samples (non-fermented

beverage), total phenolic content (control ≅ 200 mgGAE100mL^{-1}; beverage with *L. brevis* ≅ 210 mgGAE100mL^{-1}; beverage with *L. plantarum* ≅ 230 mgGAE100mL^{-1}) total carotenoids (control ≅ 0.5 mg β-carotene100mL^{-1}; beverage with *L. brevis* ≅ 2.1 mg β-carotene100mL^{-1}; beverage with *L. plantarum* ≅ 0.75 mg β-carotene100mL^{-1}) and antioxidant activities (control= 152 μM TE; beverage with *L. brevis* = 217 μM TE; beverage with *L. plantarum*= 285 μM TE) were increased. During the fermentation process, different bioactive compounds from orange juice and milk can undergo different transformations, resulting in more active compounds with several antioxidant properties.

Bioactive compounds from fruit juices, plant extracts or seeds can naturally prevent oxidation reactions in dairy products due to the high antioxidant activity in their composition. A dairy beverage from milk protein fortified with linseed oil was produced by Rotta et al. (2020) and, to prevent the lipid oxidation, polyphenol extracts from passion fruit seeds (250 mg.GAE g^{-1}) were added to the formulation. The addition of the phenolic extract (0,1 and 1g.kg^{-1}) combined with a heat treatment of pasteurization or sterilization, provided to the dairy beverage a reduction in the formation of aldehydes (control ≅ 300 mmol.kg^{-1} fat; 0,1g.kg^{-1} ≅ 200 mmol.kg^{-1}; 1g.kg^{-1} ≅ 50 mmol.kg^{-1} during 9 days), which are formed by the oxidation of polyunsaturated fatty acids and have a negative effect on flavor and can induce the formation of toxic chemical compounds. Other natural sources of polyphenols, such as mushroom (VITAL et al., 2017) and okara (the reside from soy processing) (VITAL, CROGE, et al.,2018) were also effective at preventing oxidative reactions in milk and dairy beverages, due to the phenolic capacity to break free radical chains by donating hydrogen molecules from the hydroxyl groups, interrupting free radical chain reactions, resulting in stable products (ROTTA et al., 2020).

2.5- BUTTER

The most popular mode of consumption of the milk fat fraction is butter, a dairy product made by churning fresh or fermented cream or milk, with approximately 80% lipids. The incorporation of natural additives can potentiate its functional and sensory properties and increase its intake. On the other hand, natural bioactive compounds have antioxidant and antimicrobial properties, which can reduce enzymatic and non-enzymatic oxidative reactions and increase the shelf-life of the product, also reducing the rate of microbiological deterioration. A butter formulation was developed with an addition of cinnamon extract to improve its quality

by Vidanagamage, Pathiraje, and Perera (2016). The functional property of cinnamon derived mainly from polyphenols and present an important antimicrobial activity against many bacterial and fungal strains. In the study, a butter formulation (82.12% fat) with up to 3% cinnamon extract resulted in lower peroxide content (control ≅ 3.25 $mEq.kg^{-1}$; butter with extract ≅ 2.25) of butter during a storage time (approximately 9 weeks). The peroxide index increases with time due to the oxidative process of rancidity of the fat, which also releases free fatty acid to the medium. In addition, the antioxidant activity from cinnamon reduced about 50% of free fatty acids (control ≅ 0.5%; butter with extract ≅ 0.25%). Cinnamon butter (CB) has taken low microbial count when compared to the control butter (control = $2.00x10^2$ $CFUg^{-1}$; CB = $1.63x10^2$ $CFUg^{-1}$) due to the antimicrobial activity of this spice. Another natural additive with a potential antimicrobial, anti-inflammatory, and antioxidant properties is turmeric. When added to clarified goat's milk butter, Costa et al. (2021) observed a reduction in triglycerides (control= 65 $mgdL^{-1}$; butter with turmeric= 60$mgdL^{-1}$) and LDL (control = 75$mgdL^{-1}$; butter with turmeric = 50 $mgdL^{-1}$) in rats treated with 2000 $mg.kg^{-1}$ of butter with turmeric. In addition, this butter showed high phenolic compounds content (control = 24.28 $mgGAE.100g^{-1}$; butter with turmeric = 401.03 mg $GAE.100g^{-1}$), antioxidant activity (control = 0.1$\mu molTEAC.g^{-1}$; butter with turmeric = 1.22$\mu molTEAC.g^{-1}$) and levels of carotenoids (control = non-detectable ; butter with turmeric = 4.60$mg.100g^{-1}$).

Herbal extracts are also capable of promoting health benefits when ingested on a long-term basis or introduced into a healthy diet. Plant extracts contain active chemicals with also high antioxidant properties which play an important role to prevent degenerative diseases. Parmar and Khamrui (2017) developed a ghee manufactured with buffalo butter (99.92% fat) supplemented with 7% *arjuna* (*Terminalia arjuna*) extract, a medicinal tree native to Indian subcontinent, reported to be a good source of phytosterols, phenolic acids and flavonoids. 7% of alcoholic *arjuna* extract presented a creamery butter (yield = 88.18%) with higher phytosterols content (0.39$mg.g^{-1}$; control = 0$mg.g^{-1}$). In addition, studies reported that the addition of *arjuna* extract can increase the shelf-life of ghee, due to the presence of bioactive compounds which reduce oxidation reactions due to the presence of antioxidant compounds(PANKAJ et al., 2013). Other herbal extracts also promoted promising results in increasing the quality of butters and improving the contents of bioactives, such as the addition of sage, rosemary, and oregano extracts (AYAR et al., 2001), shatavari (*Asparagus racemosus*), an Indian medicinal plant with phytoestrogenic properties (PAWAR et al., 2012), and coriander extracts (PATEL et al., 2013).

During the production of dairy products enriched with natural additives, there are specific challenges in relation to the best formulation that performs functional activities with sensory acceptance, which becomes a very important factor, because in addition to generating health benefits, the product must have a pleasant taste, flavor, and color for the consumer market. From satisfactory sensory characteristics, the development of a new product can be destined, in the future, to a large-scale production. For this reason, it is essential for the development sector to have prior knowledge of the nature of all ingredients and additives for the development of a new dairy product with functional properties.

3- CONCLUSIONS

Dairy products enjoy great appreciation across the global market. The incorporation of synthetic food additives has become a worrying issue in the current food scenario due to the presence of allergic conditions in a significant part of the world population. The replacement of synthetic preservatives by natural ones is one of the most current topics in a product development, with the growth and encouragement of research around the world to obtain natural sources that can replace chemical additives, providing the same or similar antioxidant and antimicrobial properties, increasing the shelf-life of the products. On the other hand, bioactive compounds of natural origin, included by the addition of pulp, fruit juice and plant extracts, in addition to the great sensory acceptance, can improve the nutritional and functional properties of the dairy product. Studies reported in this chapter related the most abundant bioactive compounds in fruits and plant extracts as the main phytochemicals associated to a better development of probiotic cells in the dairy matrix, significant reduction of pathogenic microorganisms, and considerable increase in antioxidant and anticarcinogenic activities. The fusion of considerable sensory acceptance with potential preservative action by the addition of fruit or plant extracts to dairy formulations becomes the constant and current challenge for food science and technology. It is through promising results of research that the food industry seeks to apply it in its large-scale production, combining high quality products with low production costs.

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CHAPTER 4

How to improve the functionality, nutritional value and health properties of fermented milks added of fruits bioactive compounds: a review

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How to improve the functionality, nutritional value and health properties of fermented milks added of fruits bioactive compounds: a review

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ABSTRACT

Fermented milks, with diverse manufacturing, fermentations, and specific strains, have been consumed around the world, with a millennial knowledge of their production. These dairy products have a potential nutritional value, taking food industries to invest, nowadays, in dairy products with a functional and healthy appeal due to the changes in the habits and diet of the population. The addition of natural ingredients from vegetables and fruits into fermented milks is a tendency nowadays. The inclusion of natural additives may change the texture, composition, sensory attributes and increase of the shelf life since some compounds are related to have a high antioxidant activity, which decreases the development of deteriorating microorganisms. These called bioactive compounds are synthesized by plants and also may contribute to the fermented milk formulation, in special from fruits, which increase the sensory acceptance. Several classes of fruits bioactive compounds are associated to several health benefits and are a base of many studies about functional fermented milks, reported in this review. This theory background becomes essential for future studies and dairy products development.

Practical Application: Potential functional properties of fermented milks added of fruit bioactive compounds.
Keywords: Dairy products; functional food; natural additives; antioxidant activity; prebiotics.

1 INTRODUCTION

The dairy products manufacturing is known since antiquity, with the fermentation process as a traditional approach to food preservation. Nowadays, dairy products are substantial in most dairying countries where produce, in large demand, fermented products including butter, cheeses and fermented milks (SURONO; HOSONO, 2011). Due their nutritional value and taste, fermented milks are considered a product with high potential for the development of new dairy products, being explored by dairy industries (EL HATMI et al., 2018).

Fermented milks, with an old knowledge and appreciation around the world, have an acidic property due to the specific microorganism's development. The low pH of the product

prolongs the shelf-life of the milk and the fermentative process generates physicochemical and organoleptic changes due to synthesized metabolites by inoculated strains, contributing to sensory characteristics pleasant to the taste. Beyond sensory attributes, fermented milks, mainly yogurts, are very appreciated by consumers due to practicality and better digestibility compared to the milk. This dairy matrix contains all the necessary components to cell growth, becoming an ideal vehicle for the development of a probiotic product with a functional appeal (GRANATO et al., 2018; VERRUCK; DANTAS; PRUDENCIO, 2019).

Foods with a functional appeal are those that, besides to promote basic nutrients, when consumed in a routine, produce benefic effects on the organism (DA COSTA, 2017). The search for healthy food, with nutritive functions and health benefits, is quite common nowadays, once a bad diet induces an increase of cardiac diseases, diabetes, and obesity. Changes in the diet are increasingly present in everyday life and food industries invest in the development of products with functional appeal. In these products, there is a tendency to add natural ingredients in the formulation, including those that are in the fruits and vegetables composition; the bioactive compounds (DOMÍNGUEZ DÍAZ; FERNÁNDEZ-RUIZ; CÁMARA, 2020; FAZILAH et al., 2018; YASSIN et al., 2018).

Bioactive compounds are synthesized by vegetables, including leaves, fruits, seeds, or roots which promote health benefits when consumed regularly. The consumption of these natural compounds is related to a decrease in the incidence of noncommunicable diseases, diabetes, cardiovascular diseases, and the reduction of carcinogenic cells (CUTRIM; CORTEZ, 2018; YASSIN et al., 2018). Among the bioactive metabolized by plants, there are unsaturated and polyunsaturated fatty acids, several classes of polyphenols, carotenoids, vitamins, phytosterols, and dietary fibers (SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018). Emerging studies about adding bioactive compounds from fruits, juices or extracts in dairy matrices have interesting and promising results, once that milk is a good vehicle for the incorporation of these natural compounds and may decrease the use of synthetic additives (DE CARVALHO et al., 2019; GRANATO et al., 2018; JAIMEZ-ORDAZ et al., 2019). In fermented milks, the addition of fruit bioactive compounds is a potential to enrich the dairy product structure, besides being very accepted by consumers (BALTHAZAR et al., 2019; CASAROTTI et al., 2018; FIDELIS et al., 2020; JAIMEZ-ORDAZ et al., 2019).

Taking into consideration that the use of fruit bioactive compounds as an additive is an emerging area in studies and dairy industries, this review aims to show the main classes of bioactive compounds presents in fruits and also their influence in fermented milk properties,

once that this is an essential theory to study and develop a new dairy product with a functional appeal.

2 TYPES OF FERMENTED MILKS

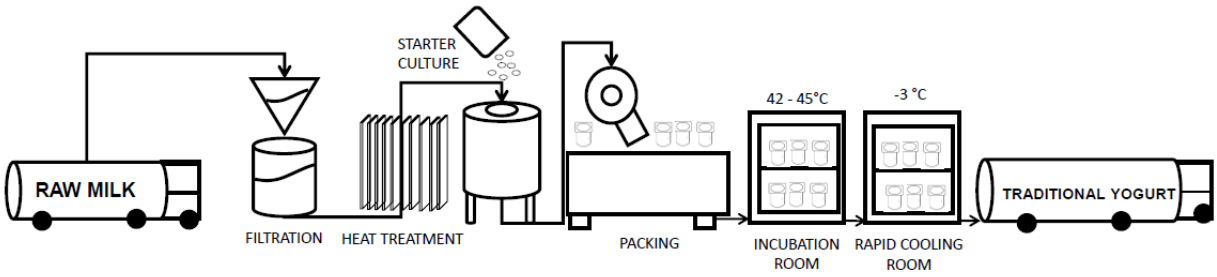
The fermented milk is formed by the ability of specific lactic acid bacteria (LAB) to hydrolyze lactose molecules in a fermentation process, synthesizing lactic acid to the surrounding and reducing the milk pH with or not coagulation. This acidity from lactic acid is responsible to form gel-like products by the casein precipitation due to reach the isoelectric point at pH 4.6 (SURONO; HOSONO, 2011). With the acidic surrounding, specifics LAB can inhibit the development of spoilage microorganisms, increasing the product shelf-life for several days. The starter culture must be viable, active, with a high count in the product and, if the fermented milk is submitted to a heat treatment at the end of the process, the requirement for the viability of the cells is not applied (FAO, 2003). According to this Codex Alimentarius, the dairy matrix may be enriched with powdered milk, flavorings, sweeteners, thickeners, and/or fruits pulp to obtain consistency and desirable flavor in the final product. Fermented milks comprise a diversity of products and all of them must meet all the prerequisites of standard and quality to guarantee a safe consumption.

Around the world, fermented milks are manufactured using dairy matrix from diverse species according to environmental characteristics to raise a flock. In some European and Asian countries, it is common to produce this dairy product from buffalo, donkey and mare's milk, while in African countries is also common to consume fermented milk from camel's milk (ASPRI et al., 2018; EL HATMI et al., 2018; MIAO et al., 2020; RASIKA et al., 2020). These dairy products are also produced from small animal milks, as sheep and goats; however, the yield is lower than those dairy products from large mammals (BALTHAZAR et al., 2019; RANADHEERA; NAUMOVSKI; AJLOUNI, 2018; VERRUCK; DANTAS; PRUDENCIO, 2019).

The most consumed fermented milk is the yogurt, which contains several textures, fat contents, and flavors. With an old Turkish origin, the word yogurt is derived from a Turkish verb (*jogurt*), which means "coagulated or curdled". Traditionally, the product is obtained by lactic fermentation, with a controlled inoculum or in a spontaneous and primitive process, since in the natural milk microbiota there are specific strains that synthesize lactic acid in metabolic pathways. However, this process is slower due to competition from other microorganisms for

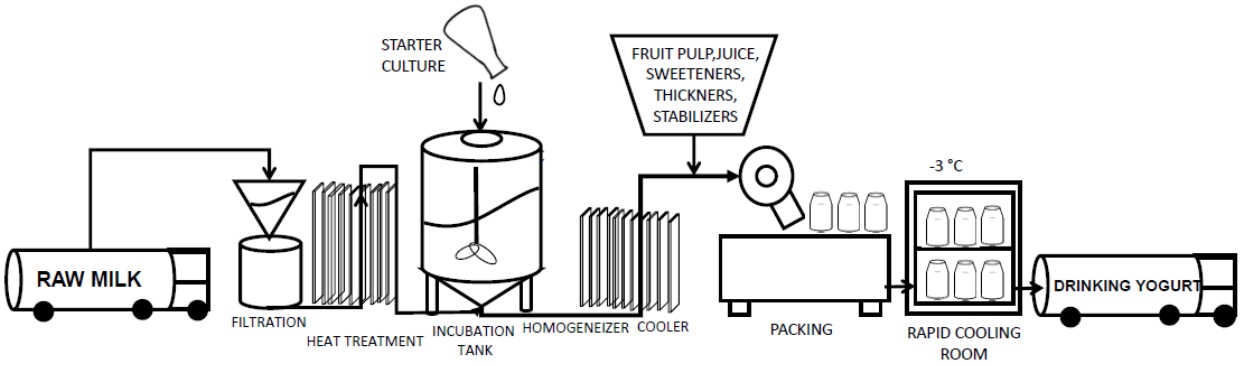
the same substrate (PIMENTEL et al., 2017; SURONO; HOSONO, 2011). Symbiotic thermophilic cultures of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (without a name modification according to the new reclassification of the *Lactobacillus* genera in the Microbiology Society by Zheng et al. (2020)) are normally used in yogurts manufacture. These strains may be also in symbiosis with other acid-lactic bacteria which can contribute to the particularity of the final product. Approximately 2 to 5% thermophilic culture is added, and the milk is incubated at 42-45°C for 3-6 hours until the total acidity reaches 0.9-1.2% and pH 4.4, characterizing the product with a smooth aspect, viscous gel and acidic flavor (PIMENTEL et al., 2017; SURONO; HOSONO, 2011). Yogurts are commercialized by the traditional method, which the fermentation occurs inside packing (Figure 14) or bottle after the fermentation incubated in tanks, classified as stirred (Figure 15). These products also may be produced as a drinking type, which is similar to stirred type, however, the coagulum is broken down to a liquid form before being packed (Figure 16).

Figure 17 - Traditional yogurt process.



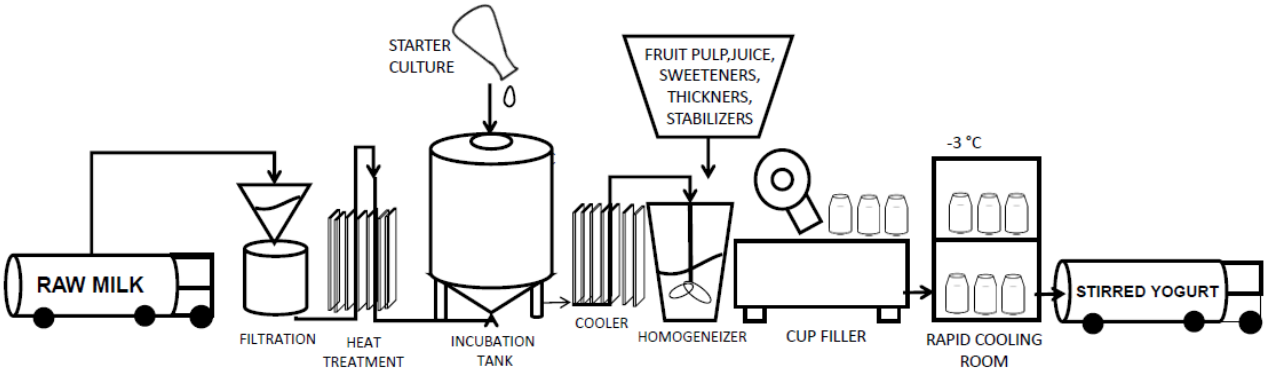
Source: The authors (2021).

Figure 18- Stirred yogurt process.



Source: The authors (2021).

Figure 19- Drinking yogurt process



Source: The authors (2021).

The acidophilus milk is manufactured with yogurt-like features, adding starter culture until the product reaches a maximum acidity of 1.5 to 2%, however, the fermentation must be made exclusively by *Lactobacillus acidophilus* strains (this species also without a name modification according to Zheng et al. (2020)). The cells development occurs slowly into the milk and it is indispensable to keep the inoculum activity with daily addition of starter culture. With milk coagulation in 18 to 24h, the product is cooled and homogenized to pack and stored at 4°C (SURONO; HOSONO, 2011).

Kefir, with an alcoholic and acidity characteristic, is a fermented milk in which the process occurs from kefir granules, composed by polysaccharides associated with bacteria and yeasts that are an initial culture to lactic fermentation. The process may result in a little gas formation, acidic flavor, creamy texture, and a mild aroma of fresh yeast, with low alcohol content among 0.08- 2% and 0.9-1.1% of lactic acid (FIORDA et al., 2017). In this product, the fermentative process comes from the metabolism of *Lentilactobacillus kefiri* (basonym *Lactobacillus kefir*, according to the recent reclassification , who regrouped 26 species of *Lactobacillus* in new genera according to specific genes and phenotypic characteristics) (ZHENG et al., 2020), *Lactobacillus casei* (without reclassified name), *Bifidobacterium* spp., *Streptococcus thermophilus*, some species from *Leuconostoc*, *Lactococcus* and *Acetobacter* genera, and the yeast *Kluyveromyces marxianus*. This one produces lactic acid, ethanol, and carbon dioxide. Some yeasts do not hydrolyze lactose (*Saccharomyces omnisporus*, *Saccharomyces cerevisiae*, and *Saccharomyces exiguous*), however, these strains synthesize ethanol and carbon dioxide from glucose molecules, which are previously released from LAB enzymatic hydrolysis (FIORDA et al., 2017; SURONO; HOSONO, 2011).

The Kumys (koumiss or kumiss) is another type of fermented milk with lactic acid and alcohol content in this composition. With a liquid aspect, there is effervescence, greyish-white

color, and shows an acidic and alcoholic taste. Traditionally, it is manufactured from mare's milk, however, in East Asian countries, the matrix of this dairy product is the camel's milk (AKUZAWA; MIURA; SURONO, 2011). The fermentation occurs by *Lactobacillus delbrüeckii* subsp. *bulgaricus*, *Saccharomyces lactis*, and *Kluyverromyces marxianus* with carbon dioxide metabolized by cells which confers gasification for the product, contributing to the final taste. The traditional kumys manufacturing is performed with raw equine's milk, quite common in Central Asian. The fermentation for 3 to 8 hours occurs inside of a leather sack, (denominated "*turdusk*") and the inoculum added is a part of the milk with a mixture of bacteria and yeasts of a pre-fermentation from previous days. The nontraditional manufacture is performed with standard protocols and it is important to expand the marketing and consumption of equine's milk to other countries. In the pasteurized mare's milk, pure cultures of Lactobacillus bulgaricus are used for kumys manufacturing and yeasts as Saccharomyces lactis are the best for alcoholic fermentation. The acidity ranges from 0.6-1.2% and alcoholic content from 0.7-2.5%, classifying this product, according to lactic acid and ethanol content, in three categories: mild, medium, and strong (SINGH; SHAH, 2017).

Another fermented milk appreciated in North America and Europe is the buttermilk, with its definition very flexible depending on the country. It may be associated with sour milk, cultured buttermilk, cultured skim milk or cultured milk. The buttermilk is the aqueous phase released in the manufacture of butter and, in the traditional fermentative type, may be produced by churning a cream with relatively low-fat content for the mesophilic fermentation. It also may be used skim milk for lactic acid fermentation; however, the appropriate denomination for this product is cultured or fermented buttermilk. The acidity must be not less than 0.60% in lactic acid, even though this product composition changes according to the butter-making technology and the season of the year. The fermentative process occurs with an addition of mesophilic cultures as *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *diacetylactis*, *Leuconostoc mesenteroides* and its subspecies (CHANDAN, 2007; SURONO; HOSONO, 2011).

In all fermented milks, the manufacture is performed with the use of desirable LAB. Several bacteria and yeasts genera are classified as probiotic, contributing to the functional and economic value of a probiotic dairy product. Probiotics are living microorganisms that confer innumerable health benefits to host's health when are regularly administrated in suitable amounts, must belong to the intestinal microbiota from humans or warm-blooded animals (HILL et al. 2014). Among the health benefits are included regularity of gut microbiota,

protection against pathogens in intestinal cells, anti-carcinogenic properties, antimicrobial and antidiabetic potential and improvements to the digestive process (RANADHEERA; NAUMOVSKI; AJLOUNI, 2018; RASIKA et al., 2020; SHAFI et al., 2019). Recent studies also highlight specific probiotic strains in potential protective effects on bones and in the treatment of osteoporosis (LEE; LEE; KIM, 2020). Species of Lactobacillus and Bifidobacterium genera are usually added in dairy products to confer probiotic effects since they are studied in the entire human gastrointestinal tract (BALTHAZAR et al., 2019; CASAROTTI et al., 2018; PENA et al., 2021; RASIKA et al., 2020; SHAFI et al., 2019). However, the probiotic effectiveness depends on the viable cells count per gram of food, in the moment of consumption. Only is a probiotic food if the count is at least 10^6 CFU (Colony-Forming Units) per gram, with a recommended daily dose of 108 to109 $CFUg^{-1}$ (BOYLSTON et al., 2004).

Dairy products contain a range of nutrients that are essential to microorganism's development, becoming the most vehicles for probiotic cells addition, in special to fermented milks. In addition to nutritional properties, dairy products may contribute to probiotic survival through the gastrointestinal due to the buffer effect and the fat globules, which protect the benefic bacteria against the stomach acid (BALTHAZAR et al., 2019; CASAROTTI et al., 2018; VERRUCK et al., 2020). The versatility of fermented milks and their high acceptance by consumers become these products a good daily source of probiotics.

3 FRUIT BIOACTIVE COMPOUNDS AND THEIR INFLUENCE IN FERMENTED MILKS

The large consumption of a variability of foods is particularly important not only for nutritional value but also for biological activity. Fruits and vegetables synthesize chemical compounds, classified as phytochemicals, which may generate several benefits to human health (DOMÍNGUEZ DÍAZ; FERNÁNDEZ-RUIZ; CÁMARA, 2020; YAHIA; GARCÍA-SOLÍS; CELIS, 2019). These "non-nutrients" are capable to act as modulators of metabolic processes in the organism, decreasing the early incidence of degenerative diseases (SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018).

The plant's evolutionary process made possible the synthesis of compounds related to their defense system. The biotic and abiotic stress influences the development of compounds in vegetable cells with insecticide, antifungal, and antibacterial functions. The oxidative stress

originates free radicals and plants also synthesize antioxidants capable to decrease chain reactions and reactive species of oxygen (ROS) content which, in excess, are toxic to cells (CHOUDHARY; KUMAR; KAUR, 2020). Bioactive compounds may act as antioxidants in the reducing action, hydrogen donation, inhibition of singlet oxygen or incapacity of chelate metals. The synthesis of these compounds is causally related to the environment of vegetable growth since the naturally cultivated plants have higher bioactive content. Depending on each species, native fruits, for example, have higher vitamins and phenolic compounds content, being the basis of several studies due to their promising health benefits (YAHIA; GARCÍA-SOLÍS; CELIS, 2019). In addition to the knowledge about the beneficial action of bioactive compounds in the human body, consumers have their criteria of acceptance, once that they choose food concerning its flavor and appearance. The addition of pulps, juices, extracts, or fruits pomace in fermented milks may enrich the taste and the quality of the dairy product, improving its nutritional, physicochemical and sensory properties (SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018).

3.1 FATTY ACIDS

A regular consumption of fatty acids, mainly polyunsaturated from omega 3, 6, and 9 families are related to health benefits as the reduction of LDL cholesterol levels, anti-inflammatory action, decrease in the incidence of carcinogenic and cardiovascular diseases, arteriosclerosis and Alzheimer. Avocados, coconuts and Amazonian fruits as açaí (*Euterpe oleracea*), tucumã (*Astrocaryum aculeatum*) and buriti (*Mauritia flexuosa*) are examples of fruits which the composition is rich in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (ESPÍRITO SANTO et al., 2010; SERRA et al., 2019).

From Omega 6 family, an isomer of the linoleic acid is the conjugated linoleic acid (CLA); related to several metabolism benefits due to its regular consumption, including anti-obesity effects, a decrease of LDL cholesterol and anticancer effects. Through the hydrogenation of the polyunsaturated acid, this isomer is formed usually in the digestive tract. In the human large intestine, some probiotic genera of bacteria (*Lactobacillus*, *Propionibacterium*, *Bifidobacterium* and some strains of *Clostridium* and *Leuconostoc*) are capable to metabolize linoleic acid in CLA by an enzymatic process after digestion of food that

contains polyunsaturated acids in their composition, as fruits with higher fat content, fermented meat, milk, and dairy products, including fermented milks (CSAPÓ; VARGA-VISI, 2015).

Dairy products provide only 70% of the total CLA daily intake, and due to achieving the recommended dose, several technological alternatives are being used to increase the CLA content in milk and dairy products as the addition of specific cultures that metabolize this fatty acid or enrich the dairy product with CLA from seeds, fruits or extracts to improve their nutritional value. Borges et al. (2019) evaluated the effect of *Leuconostoc mesenteroides* and *Lactococcus lactis* on the fatty acids profile and on the inhibition of *Listeria monocytogens* in fermented cream. This pathogenic bacteria count was reduced by the development and competition of the *Ln. mesenteroides* and *Lactococcus lactis* cells, besides, the fatty acids profile was changed with a decrease of MUFA and PUFA, as well as an increase of SFA and 60% of CLA, highlighting the potential of this functional property. The addition of açai pulp in yogurts was performed by Espírito Santo et al. (2010), who evaluated the effects of the fruit in probiotic bacteria development (*Lactobacillus acidophilus*, *Bifidobacterium animalis* ssp. *lactis* BI04 and *Bifidobacterium longum* BI05) and in the fatty acid profile (Table 6). The açaí pulp addition increased the probiotic cells count and monounsaturated and polyunsaturated fatty acids contents. Besides, this fruit improves the production of α-linoleic and its conjugated linoleic acid during the fermentation of the dairy product.

Table 9- Studies about fruits bioactive compounds in fermented milks.

Fermented Milk	Country	Fruit	Bioactive compound	Conclusion	Author
Probiotic Yogurt	Brazil	Açaí pulp (*Euterpe oleracea*)	Fatty acids (mainly oleic and palmitic acid)	The açaí pulp increased the CLA formation and improved the development of the probiotic cell.	(ESPÍRITO SANTO et al., 2010)
Organic yogurt	Brazil	Grape juice (GJ) and grape peel flour (GPF)	Phenolic compounds and soluble fibers	GJ increased phenolic content and antioxidant activity. GPF increased ash, fibers, and texture properties. 79% of sensory acceptance.	(KARNOPP et al., 2017)
Probiotic sheep's milk yogurt	Brazil	Strawberry juice	Phenolic compounds and prebiotic fibers	Increase of phenolic content and improvement of probiotic cells viability	(BALTHAZAR et al., 2019)
Yogurt	Malaysia	Red pitaya	Betacyanin (from anthocyanins group of phenolic compounds)	The higher stability of betalains from red pitaya pulp during the storage. Decrease of the syneresis and improvement of	(GENGATHARAN; DYKES; CHOO, 2016)

				thermophilic culture development.	
Yogurt	Brazil	Goji berry	Carotenoid (Zeaxanthin)	Incorporated into nanoparticles before adding in the yogurt, this technique enhances the carotenoid retention at the end of the storage and had a protecting effect in an *in vitro* gastrointestinal process.	(DE CAMPO et al., 2019)
Yogurt	Brazil	Cantaloupe melon	Carotenoid (β- carotene)	The encapsulation of carotenoid extract was added into yogurt formulation, providing a yellow color stabilized during 60 days of storage and being a potential substitute for synthetic colorants.	(MEDEIROS et al., 2019)
Kefir	Brazil	Mango peel	Phenolic compounds	Higher phenolic compounds content and antioxidant activity when compared to a control sample. Phenolic compounds improved the probiotic development, with a potential prebiotic action.	(VICENSSUTO; DE CASTRO, 2020)
Probiotic yogurt	Iran	Date extract	Phenolic compounds, dietary fibers	The date extract had a high influence on probiotic development, an increase of the total soluble solids, and antioxidant activity. The fermented milk was sensorially accepted.	(ABDOLLAHZADEH et al., 2018)
Probiotic yogurt	Italy	Passion fruit	Dietary fiber	The addition of fibers increases the viscosity and firmness of the yogurts, which were sensorially accepted.	(ESPÍRITO-SANTO et al., 2013)
Probiotic goat's milk yogurt	Brazil	Guava, orange and passion fruit	Dietary fiber, phenolic compounds, and carotenoids	Increase of phenolic compounds, β-carotene, and lycopene. Increase in the survival rate of probiotic cells. Fibers with a prebiotic effect.	(CASAROTTI et al., 2018)
Yogurt	Brazil	Camu-camu (*Myrciaria dúbia)*	Phenolic compounds, ascorbic acid (vitamin C)	Increase of antioxidant and anti-proliferative activities. High sensory acceptance.	(FIDELIS et al., 2020)
Stirred-type yogurt	Canada	Apple pomace	Phenolic compounds, soluble and not soluble fibers	The fibers increase the color, total phenolic content, viscosity and decrease the syneresis, which is typical in a stirred yogurt	(WANG; KRISTO; LAPOINTE, 2020)

Probiotic Fermented milk	Brazil	Pomegranate juice	Phenolic compounds	After an in vitro gastrointestinal simulation, the polyphenol content increased, as did the survival rate and probiotic cell count	(PENA et al., 2021)

3.2 PHENOLIC COMPOUNDS

Phenolic compounds are secondary metabolites synthesized by plants with a defense mechanism against insects, pathogenic microorganisms, ultraviolet radiation (UV), and are also related to fruit pigmentation. These compounds are responsible for astringent flavor, aroma, and oxidative stability of vegetables and fruits. According to the chemical structure, phenolic compounds show innumerable constituents, and all these compounds exhibit hydroxyls (OH) in molecules, which confer antioxidant activities. With one or more aromatic ring linked to at least one hydroxyl radical, phenolic compounds are classified according to the number of phenolic rings and their chemical structures, with the main groups: phenolic acids, flavonoids and non-flavonoids (LI et al., 2014). In the daily diet, vegetables and fruits are the main sources of phenolic compounds such as flavonoids, phenolic acids, stilbenes, and lignans. Several studies *are* related to the influence of regular consumption of fruits phenolic compounds with health benefits, as the reduction in the incidence of non-communicable diseases, diabetes, and cancer (ALENISAN et al., 2017; FIDELIS et al., 2020; YASSIN et al., 2018). According to the fruit's composition, phenolic classes or subclasses are abundant in specific species as grapes, apples, cherries, oranges, berries, and pomegranates (CASAROTTI et al., 2018; GULLON et al., 2016; PANTELIĆ et al., 2016; ZIELINSKI et al., 2019). Nowadays, several studies about native Brazilian fruits are on the rise according to their composition rich in phenolic compounds, highlighting fruits from *Myrtaceae* family as the camu-camu (*Myrciaria dubia*), guava (*Psidium guajava*) araçá (*Psidium cattleianum*) and guabiroba (*Campomanesia xanthocarpa* O. Berg) (FIDELIS et al., 2020; SILVA-RODRIGUES et al., 2020). Considered a functional food, the guabiroba fruit contains around 131.90 $mg100g^{-1}$ of phenolic compounds, besides, this fruit has a sweet and fresh pup with a potential application in future food formulations (SILVA-RODRIGUES et al., 2020).

When phenolic compounds from fruits, pulps, juices, pomace, or extracts are added in dairy products, changes in physicochemical, sensory, and microbiological properties are possible. In studies about the addition of juice and grape peel flour into yogurts, (KARNOPP

et al., 2017) measured changes in phenolic content, antioxidant activity, sensory, textural, and physicochemical properties of these dairy products (Table 6). The grape juice increased the phenolic content, antioxidant activity, and viscosity of yogurts while the grape peel flour increased the total fiber and ash content, hardness, and consistency. The grape peel contains fibers that act as stabilizers and change the yogurt texture and, besides, to give functional and textures properties to the yogurt, the use of fruits peel or pomace can contribute to the reduction of resides and add value to a fruit by-product.

In a research about the functional characterization of a yogurt with sheep's milk added of strawberry juice, inulin, and potato starch, Balthazar et al. (2019) measured the viability of a probiotic strain added to the formulation (*L. plantarum*) in *in vitro* simulated gastrointestinal conditions (Table 6). After the simulated digestion, cells showed high viability. Fruit juices, inulin and the potato starch have a prebiotic activity, influencing positively in the development of the probiotic cell. The strawberry juice increased the phenolic compounds and antioxidant activity of the fermented milk; besides, flavonoids and phenolic acids can inhibit the lipid oxidation, which is responsible for chemical compounds that form off-flavors. This antioxidant activity is related to electrons donation to neutralize the chain reaction by free radicals and may prolong the yogurt shelf-life. Also, the low pH (4.6) becomes fermented milks good carriers of phenolic compounds due to their interaction is maximum in an acid surrounding.

Phenolic compounds can also contribute to the color of fruits, which is the most important parameter in foods to influence the consumer's choice, and, nowadays, people prefer to consume colorful food with natural colorants from vegetables, roots, and fruits. Into the flavonoids class, anthocyanins are responsible for the pigmentation of fruits, ranging from red to dark blue and are included in grapes, berries, apple peels, and plums composition. The more intense the color is, the higher the anthocyanins content and this red/purple color can be added into the fermented milk formulation, improving the visual acceptance of the product. Betacyaninins included in the betalain class, from anthocyanin's group, were studied as a natural colorant in yogurts added of red pitaya pulp (Table 6) (GENGATHARAN; DYKES; CHOO, 2016). Compared to a commercial colorant, the natural colorant from the red pitaya showed more stability during the storage. Into the anthocyanin's composition from this fruit, there are phyllocactin and hylocerenin, which are more resistant pigments. Besides, yogurt with red pitaya pulp showed higher antioxidant activity and lower syneresis due to the ability of some phenolic compounds, as the vanillin, to stabilize milk peptides protein by hydrogen bonding and electrostatic interaction. These betalains also increased the viability of

Streptococcus thermophilus and *Lactobacillus bulgaricus*, the traditional thermophilic culture. In a research about kefir enhanced with mango peel, probiotic strains also have an increase in the viability and count. In this work, the fermented milk showed higher phenolic compounds content and antioxidant activity, when compared to a control sample (Table 6). During the fermentation, probiotic cells can synthesize enzymes that release phenolic compounds in the matrix (VICENSSUTO; DE CASTRO, 2020). Therefore, phenolic compounds in the fruit's peels, pulps, or seeds can have a positive effect on the antioxidant properties of fermented milk.

3.3 CAROTENOIDS

Carotenoids comprise the biggest group of pigmented compounds in nature, with approximately 600 isolated and characterized structures. These compounds are natural pigments with a diversity of colors, ranging from yellow to red color in fruits, flowers, leaves, and roots. In the same way, carotenoids are included in the coloring of some birds, fishes, and crustaceans can be also synthesized by some fungus, photosynthetic bacteria, and seaweeds (MAIANI et al., 2009; RODRIGUEZ-AMAYA; KIMURA, 2004). With a hydrophobic structure, in plants, these compounds are stored in lipid membranes and, in vegetable cells, carotenoids have photoprotection activity, with a light and oxygen reactive species capture, in addition to a membrane structural and antioxidant activities (RODRIGUEZ-AMAYA; KIMURA, 2004). These compounds are related to the provitamin A, a precursor of Vitamin A (retinol) in the metabolism, however, of all the 600 characterized structures, only 50 can be Vitamin A precursors. Retinoids (pre-vitamin A) from dairy products, meats, and eggs ingestion, in contact to the provitamin A in gut cells are converted by an enzymatic activity into retinol molecules, which are absorbed in epithelial cells. The main sources of carotenoids in fruits, vegetables, and roots are those with yellow, orange, or red colors which provide high β-carotene, α-carotene, β - kryptoxanthin, lycopene, lutein, and zeaxanthin contents, which are the principal carotenoids in foods (RODRIGUEZ-CONCEPCION et al., 2018). In fruits, the sources of these compounds are peach, nectarine, papaya, mango, orange, watermelon, and plum (Table 7).

Table 10- Vitamins in fruits

Vitamin	Fruits
Pro-vitamin A (Carotenoids)	Pineapple, mango, melon, watermelon, orange, papaya, peach, nectarine, persimmon, acerola, guava (*Psidium guajava*), camu-camu (*Myrciaria dubia*) and araçá (*Psidium cattleianum*) Amazonian fruits: tucumã (*Astrocaryum aculeatum*) and umari (*Poraqueiba sericeia* Tul)
Vitamin C (Ascorbic acid)	*Citrus* spp., strawberry, pineapple, acerola, blackcurrant, kiwifruit, apple, guabiroba (*Campomanesia xanthocarpa*), guava (*Psidium guajava*), camu-camu (*Myrciaria dubia*) and araçá (*Psidium cattleianum*)
Vitamin E (Tocopherol)	Avocado, melon, papaya, persimmon, mango, kiwifruit
Complex B	Grape (Vit. B1), Melon (Vit. B6, Vit. B9), Orange (Vit. B1, Vit. B9), banana (Vit. B6, Vit. B7, Vit. B9), avocado (Vit. B1, Vit. B3, Vit. B5, Vit.B6, Vit. B7), watermelon (Vit. B1, Vit. B6), plum (Vit. B6), mango (Vit. B1, Vit. B9), strawberry (Vit. B5), papaya (Vit. B7, Vit. B9), orange (Vit. B9), kiwifruit (Vit. B9)
Vitamin K	Avocado, banana, berries, grape, melon, kiwifruit, pomegranate, plum

Source: Medeiros et al. (2019), Mellidou et al. (2019), Rodriguez-Amaya & Kimura (2004), and Silva-Rodrigues et al. (2020).

The carotenoid content in fruits ranges according to the cultivar, environment, cultivation climate, and maturation. During the fruit ripening, in addition to the flavor and texture changes, there is the chlorophyll degradation and synthesis of carotenoids, increasing the content of this compound (MAIANI et al., 2009; RODRIGUEZ-CONCEPCION et al., 2018). Concerning health benefits, the daily inclusion of carotenoids in the diet can contribute to an increase of anti-carcinogenic and antioxidant activities, decrease in the incidence of cardiovascular diseases, and reduction in the degeneration of eyes cells, since there is a prevention of blood clots. Due to these benefits, there is an incentive for adding carotenoids in functional foods (RODRIGUEZ-CONCEPCION et al., 2018).

Besides aggregating functional properties to a food formulation, the natural color of carotenoids can substitute synthetic colorings, which are allergenic for several people. In a recent study about the incorporation of zeaxanthin from goji berry in yogurts, de Campo et al. (2019) added this carotenoid inside of nanoparticles before putting in the yogurt formulation (Table 6). In a food processing and storage, carotenoids are very susceptible to chemical degradation, related to its lipophilic nature and high sensitivity to the presence of oxygen, heat,

and light. Therefore, the nanoencapsulation of zeaxanthin was interesting to provide more functional properties to yogurts, with high carotenoids retention at the end of the storage. In addition to the nanoparticles being sensorially imperceptible, this technique provided to have a protective effect on carotenoids in an in vitro gastrointestinal simulation. In a study also about carotenoid encapsulation in yogurts, Medeiros et al. (2019) extracted carotenoids from cantaloupe melon which was incorporated into a yogurt formulation through the nanoparticles (Table 6). In the extract, the total carotenoid content was 46.2 μg g^{-1}, and according to previous studies, food matrices that contain more than 20 μg g^{-1} are a very important source of carotenoids, providing health benefits (FLESHMAN et al., 2011). With high β- carotene content, the extract added to yogurt gave a yellow color and this property was stabilized during 60 days of storage, is a potential substitute for synthetic colorants.

3.4 PHYTOSTEROLS

Phytosterols, also called plant sterols, are classified as steroid groups present in the plant composition and effect important functions in the vegetable metabolism. These compounds are related, mainly, to the ability to fluidize cell membranes and to increase the rigidity and reorganize the cytoskeleton (FERGUSON et al., 2016). Phytosterols are nonnutritive metabolites which are chemically similar to cholesterol; however, this is a sterol from mammalian cells, while phytosterols are exclusively derived from vegetable cells (DEMONTY et al., 2009).

In nature, more than 200 different phytosterols have been isolated and classified, however, a limited number of these compounds are found in the human diet with sitosterol, campesterol and stigmasterol being, approximately, 98% of the total phytosterols in the diet. Vegetable oils, nuts, seeds, and fruits are the main sources of these compounds. In fruits, it is common to find in avocados, berries, apples, and olives compositions (FERGUSON et al., 2016). The inclusion of phytosterols in the daily diet is related to the reduction of blood cholesterol levels and the circulating low-density lipoproteins (LDL), which in excess are an important risk factor to cardiovascular diseases. In studies, it was estimated that occidental populations consume approximately 150 – 450 mg of phytosterols in their daily diet; it is 50% less than the oriental population, who have a balanced diet that includes several phytosterols sources. In some situations, the intake of nutraceuticals with phytosterols is recommended to

reach the threshold daily dose of two grams (BRUFAU; CANELA; RAFECAS, 2008; FERGUSON et al., 2016).

With a growing global market for functional foods, there are promising opportunities for the development of enriching foods with phytosterols alleging health benefits. With a hydrophobic characteristic, these bioactive compounds have more homogeneity when esterified with fatty acids in food manufacture as dairy products, vegetable oils, margarine, orange juices, cakes, bread, chips, and mayonnaises. In fermented milks, the addition of phytosterols has been studied and used since the early 2000s. These dairy products contain fat globules in the composition, which are good phytosterol carriers, very accepted, and practical. In previous studies, the matrix food form can affect the efficiency of the cholesterol-lowering in the metabolism (DEMONTY et al., 2009). According to these authors, solid foods with the addition of phytosterols may be related to higher effect to decrease the LDL cholesterol (approximately 5.2%) when compared to liquid foods, probably due to the passage through the gastrointestinal tract in a longer period. With these evidence and future studies, yogurts with a rigid and viscous aspect can be potential dairy products enriched with phytosterols when compared to liquid foods, as milk or juices. Researches with the yogurt used as a food matrix for phytosterols, provided significant results of decrease LDL cholesterol levels. With daily intakes of 1.6 - 4.0 grams of phytosterols in fermented milk, the total cholesterol lowered in a median of 11% and LDL cholesterol by 15% in individual exams (SALO; KUUSISTO, 2016). According to a protocol of consumption during some weeks, fermented milk with an addition of phytosterols can provide health benefits since the properties are included in a healthy and balanced diet.

3.5 DIETARY FIBERS

Dietary fibers are compounds with a high molecular mass which include vegetable carbohydrates polymers, oligosaccharides and polysaccharides as cellulose, hemicellulose, lignin, pectin, and resistant starches that may be associated with some compounds, as polyphenols, waxes, proteins or phytates (SEMBRIES et al., 2003). During the digestion, dietary fibers are not digested by enzymatic action or absorbed in the small intestine. When there is the consumption, these compounds have some properties as the stimulation of gut colony fermentation, a decrease of intestinal constipation, reduction of total and LDL cholesterol levels, and decrease of the post-prandial blood glucose and insulin levels. Besides, the regular consumption of fibers is related to the importance of preventing obesity, heart

diseases, diabetes, atherosclerosis, and gut cancer (ESPÍRITO-SANTO et al., 2013; NAWIRSKA; KWAŚNIEWSKA, 2005).

Dietary fibers are present in most of the whole grains, roots, vegetables, and fruits, highlighting avocados, bananas, apples, guavas, passion fruits, citruses, papaya, and plums. Dietary fibers are classified into two groups: during the digestion and in contact with water, the soluble fibers tend to form gels, increase the viscosity of partially digested foods in the stomach and can be fermented by the gut microbiota. On the other hand, the insoluble ones remain intact during the entire gastrointestinal tract, improving the intestinal motility (SEMBRIES et al., 2003).

New dairy products are often developed with the addition of fibers from several sources, as different fruits, and their by-products, contributing to a new flavor and improvement of physicochemical properties. Besides, the use of peels or pomaces as ingredients may reduce the impacts of industrial waste (CASAROTTI et al., 2018; WANG; KRISTO; LAPOINTE, 2020). In the fermented milk manufacturing, fruits, vegetables, and by-products fibers are used as a potential stabilizer due to capacities of water binding, gelling, thickening, lipid retention, and improvement of textural properties (WANG; KRISTO; LAPOINTE, 2020). The addition of fruits fiber also can increase the volume and the production yield, and with this, it may reduce the caloric content.

Several researches evaluated the effect of fruits dietary fibers on dairy products and fermented milks quality. In a study by Espírito-Santo et al. (2013), it was manufactured probiotic yogurts enriched with passion fruit fiber, with an evaluation of the rheology, microstructure, and sensorial characteristics (Table 6). Yogurts added with the fruit fiber had an increase of viscosity, reducing the amount of whey release. The pectin included in the fiber composition has a water-holding capacity, constituted by anionic hydrocolloids which adsorb polysaccharides and interact with the casein, increasing the firmness of the product (EVERETT; MCLEOD, 2005). These yogurts were accepted in sensory analysis and the authors confirmed that passion fruit fiber is a neutral ingredient to provide high value-added fermented milk. The same behavior was evaluated by Wang et al. (2020), who added apple pomace into stirred-type yogurt (Table 6). During 14 days of cold storage, the soluble and not soluble fiber particles increased the viscosity and reduced the syneresis, which is typical in a stirred yogurt. Through an electrostatic linking, pectin can stabilize casein aggregates, resulting in casein-pectin complexes that maintain the yogurt gel. In addition, apple is the source of phenolic compounds,

which increased the total phenolic content in the stirred yogurt, becoming the apple pomace a potential natural stabilizer and a functional additive.

Prebiotic characteristics of fruit fibers are in a constant study due to their health benefits and the improvement of the dairy products' quality. In research by Casarotti et al. (2018), it was evaluated the survival rate of probiotic cells in fermented goat's milk in a gastrointestinal simulation, added with guava, orange, and passion fruit pomace flour (Table 6). Among the results, the total fiber content was high, as well as for total phenolic content and antioxidant activity due to a significant fraction of flavonoids be retained in the pomace after the juice extraction. With no interference in the fermentation time, the pomace addition increases the resistance of probiotics even in extreme digestion surroundings. This protective effect can be attributed to the buffer capacity of fibers and their nutrients for the development of the cell. These effects were also confirmed by Abdollahzadeh et al. (2018), who evaluated fermented milks enriched with date extract and the influence in probiotic development of *Lactobacillus acidophilus* strains (Table 6). Dates are rich in sugar (glucose and fructose), fibers (cellulose and pectin) in addition to phenolic compounds that confer a high antioxidant activity. These properties provided high cells count until 14 days due to the prebiotic properties of dietary fibers. In the fermented milk composition also had an increase of total solid content and antioxidant activity. The dairy product, among the results, was sensorially accepted, with the date extract a potential functional additive to promote healthy qualities in probiotic dairy products.

3.6 VITAMINS

Vitamins are organic molecules classified as micronutrients due to requiring low or traces quantity for a normal metabolism development (SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018). These compounds, without energetic value, are not synthesized in enough amounts by the human body and are supplied from the diet, especially from vegetables and fruits. According to the species, cultivar, or ripening, there are important variations of vitamins in plants. Nowadays there are recognized thirteen vitamins with diverse biological activities, solubility, and biochemistry reaction (DOMÍNGUEZ DÍAZ; FERNÁNDEZ-RUIZ; CÁMARA, 2020; MELLIDOU et al., 2019). The polarity can be one of the factors to classify vitamins. According to the solubility in water, there are vitamin C (ascorbic acid) and complex B, although vitamins A (retinol), D, E (tocopherol), and K are fat-

soluble. The main fruit sources of these vitamins are included in Table 7, except vitamin B12 and D which do not occur in fruits or vegetables (DISMORE et al., 2003; RODRIGUEZ-AMAYA; KIMURA, 2004). Daily consumption of diverse vitamins associated with other bioactive compounds can improve endothelial functions, anti-inflammatory and anti-carcinogenic capacities, antioxidant, and antitumor activities and, also, these compounds are related to the occurrence decrease of chronic non-communicable diseases (MELLIDOU et al., 2019; YAHIA; GARCÍA-SOLÍS; CELIS, 2019).

Fermented milks can be a source of several nutrients, including vitamin A, B-complex, and compared to the milk, the fermentation can enhance the nutritional value of these dairy products. Lactic acid bacteria (LAB) improve not only the physicochemical and organoleptic properties but also the nutritional value with vitamins synthesis in their metabolism, as the vitamin B12 (CAPOZZI et al., 2012). The addition of fruits in fermented milk can also increase the consumption of several vitamins and makes this dairy product a complete functional food. In a recent study about a characterization of yogurt added with a South American fruit seed extract, the camu-camu (*Myrciaria dubia*), the antioxidant and anti-proliferative activities were higher when compared to a control sample, without extract. Besides, this functional dairy product was very sensorially accepted (Table 6) (FIDELIS et al., 2020). The camu-camu fruit is studied by the high phenolic content and vitamin C (ascorbic acid) and is related to an intense antioxidant activity due to its high reducing power, protecting against decontrolled cell oxidation. High antioxidant activity was also evident in yogurts added with açaí juice (*Euterpe oleracea*) and strawberry preparations, relating health-promoting effects to natural classes of antioxidant compounds as phenolic compounds, vitamin C, vitamin E (tocopherol) and pro-vitamin A included in the composition of the fruit (COÏSSON et al., 2005).

Vitamins with antioxidant properties as retinol, ascorbic acid, and tocopherol also can stimulate the viability of probiotic cells in dairy products. In a recent study about the addition of ascorbic acid and tocopherol in fermented milks, the fermentation was faster when compared to a control sample, providing higher probiotic counts. The ascorbic acid and tocopherol, as reducing agents, promote the increase of H^+ and K^+ around the cells membrane and influence the acid lactic synthesis (SOTO et al., 2019). According to the Codex Alimentarius (FAO, 1995), the maximum allowed concentration of tocopherol in dairy desserts, including yogurts, is 500 $mg.L^{-1}$. However, this study showed a high activity in low concentrations of 5, 7.5, and 15 $mg.L^{-1}$. Therefore, natural sources of these bioactive compounds, as fruits and juices, also may improve the functionality and activity of dairy products.

CONCLUSION

Fruits bioactive compounds can contribute to the benefits of the human body when administrated regularly in the diet, even if their effects are low at the beginning of the consumption. Besides, reducing the risk of chronic diseases is not related to a unique class of bioactive compounds, but is evident and proven when there are addition and interaction of several compounds in a food formulation. In fermented milks, in special, the addition of fruits is very accepted by consumers and may improve the nutritional and functional value. Bioactive compounds are important to enrich the texture and sensory attributes of fermented milks. These compounds are also related to reducing microbial and chemical deterioration, improving the probiotic development, and the quality of these dairy products. Innumerous studies about adding fruit bioactive compounds in fermented milks are performed nowadays providing new perspectives to dairy industries, which are increasingly looking for better quality and acceptance of their products.

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CHAPTER 5

Conventional and alternative concentration processes in milk manufacturing: a comparative study on dairy properties

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Conventional and alternative concentration processes in milk manufacturing: a comparative study on dairy properties

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ABSTRACT

The concentration of dairy products is widely applied in dairy manufacturing due to obtaining products with the high dry matter, added value, reduced volume, and an increase in shelf-life. Traditional thermal concentration processes are the most applied in dairy industries, however, high temperatures can damage the bioactive compounds in milk, in addition to modifying the physicochemical, sensory, and nutritional characteristics of concentrated products. This review summarizes the importance of replacing traditional concentration methods with unconventional non-thermal processes, which can bring an option to dairy industries due to the concentration enabling the preservation of proteins, enzymes, vitamins, color, and flavor of the product. Alternative methods, such as freeze concentration, membrane separation processes, and freeze-drying, compose recent works about new methodologies to concentrate dairy products without changing specific properties and increase the quality, which is one of the main purposes for the dairy industries. Through a comparative study with recent researches, this overview highlights some alternative concentration processes that can improve the yield and increase the quality of concentrated dairy products. With new environmentally sustainable methods and the possibility of reducing the costs of the concentration process, these emerging concentration methods become attractive for dairy industries from a technological and economic perspective.

Practical Application: Improving the quality of concentrated dairy products by non-thermal emerging technologies.

Keywords: non-thermal processing; freeze concentration; membrane separation; freeze-drying; dairy processing; thermolabile compounds.

1 INTRODUCTION

Milk is a highly nutritional valuable food that can be processed, fractionated, and included in dairy products, beverages, or food formulations (AL-HILPHY et al., 2020; MUÑOZ et al., 2018; PRESTES et al., 2021; VARGAS et al., 2021). In addition, dairy products are elucidated as being excellent sources of nutritional compounds, bring health benefits if introduced in a well-balanced diet (FEENEY; LAMICHHANE; SHEEHAN, 2021; VERRUCK et al., 2019a).

Thermal and non-thermal processes implemented in dairy manufacturing have the main purpose to increase the shelf-life and produce a safe, stable, nutritional, and tasty product (AL-HILPHY et al., 2020; MUSINA, 2018; STRATAKOS et al., 2019).

Dairy products contain high water content and, with the purpose to expand the shelf-life, concentration processes are fundamental in dairy industries, since the employed technology can improve the efficiency of milk processing, reducing the volume of production and total costs of shipping and storage (BALDE; AÏDER, 2017; DE LIZ et al., 2020; MUÑOZ et al., 2018). In addition, there is an increase in total dry matter which benefits the added value of a product with high fat and protein content (CARTER et al., 2021; RAO, 2018; VARGAS et al., 2021).

In large-scale production, traditional concentration methods are the most employed in dairy manufacturing, mainly the evaporation and spray drying processes. These unit operations reduce the water content by applying high temperatures during the procedure. However, an intense heat treatment may exceed the heat stability of milk and result in undesired sensory and physiochemical changes, such as separation of milk fat, grittiness, phase separation and sediment formation (DUMPLER; HUPPERTZ; KULOZIK, 2020). Besides, the intense thermal processes may decrease original thermolabile bioactive compounds such as enzymes, vitamins, and proteins (DUMPLER et al., 2018; DUMPLER; HUPPERTZ; KULOZIK, 2020; MOEJES et al., 2020).

Emerging non-thermal technologies are promising alternatives that have been developed and explored in dairy manufacturing. With a purpose to decrease the negative effects of the conventional concentration processes and contribute with dairy products with high quality, these alternative procedures preserve sensory and flavor properties and maintain food pigments, original volatile compounds, vitamins, enzymes, and proteins (DE LIZ et al., 2020; FAION et al., 2019; MACHADO CANELLA et al., 2020; MOEJES et al., 2020; MUÑOZ et al., 2018; STRATAKOS et al., 2019). In recent research about milk concentration processes, technologies such as freeze concentration, membrane separation and freeze-drying are efficient, satisfactory and capable to replace traditional concentration processes and develop concentrated dairy products with high quality (BARROS et al., 2021; CAMELO-SILVA et al., 2021a; DE LIZ et al., 2020; DESHWAL et al., 2020; FAION et al., 2019; MERKEL; VOROPAEVA; ONDRUŠEK, 2021; MUÑOZ et al., 2018; ZHU et al., 2020). Studies can bring essential and attractive information for dairy industries, which can apply different concentration procedures according to the desirable characteristics of their manufacturing processes.

Alternative milk concentration processes have several techniques and different procedures, bringing unique properties to each process. In dairy science and technology, our research group has been operating in the field for over 15 years, with experience in conventional and alternative technologies in milk processing (CAMELO-SILVA et al., 2021a; DANTAS et al., 2021; MAGENIS et al., 2006; MUÑOZ et al., 2018, 2019; PRUDÊNCIO et al., 2014). The purpose of the group, through this review, is to present an essential background of the main emerging technologies of milk concentration and the benefits caused in the organoleptic properties of dairy products concerning the traditional concentration methods.

2 CONVENTIONAL CONCENTRATION PROCESSES IN MILK MANUFACTURING

To produce concentrated or dried dairy products, there are several food processes to remove the liquid fraction, including traditional thermal processes or innovative technologies. The choice will depend on the desired effect of the process on the product's morphology and the extent of concentration required (CHENG; ZHOU; LIU, 2018; MORISON; HARTEL, 2018). Concentration, drying or the combination of these two technologies are the most energy-intensive operations of the dairy industry and, for this reason, the total costs of these concentration processes must also be considered to apply them on a large-scale (MOEJES et al., 2020; RAMIREZ; PATEL; BLOCK, 2006).

2.1 EVAPORATION PROCESS

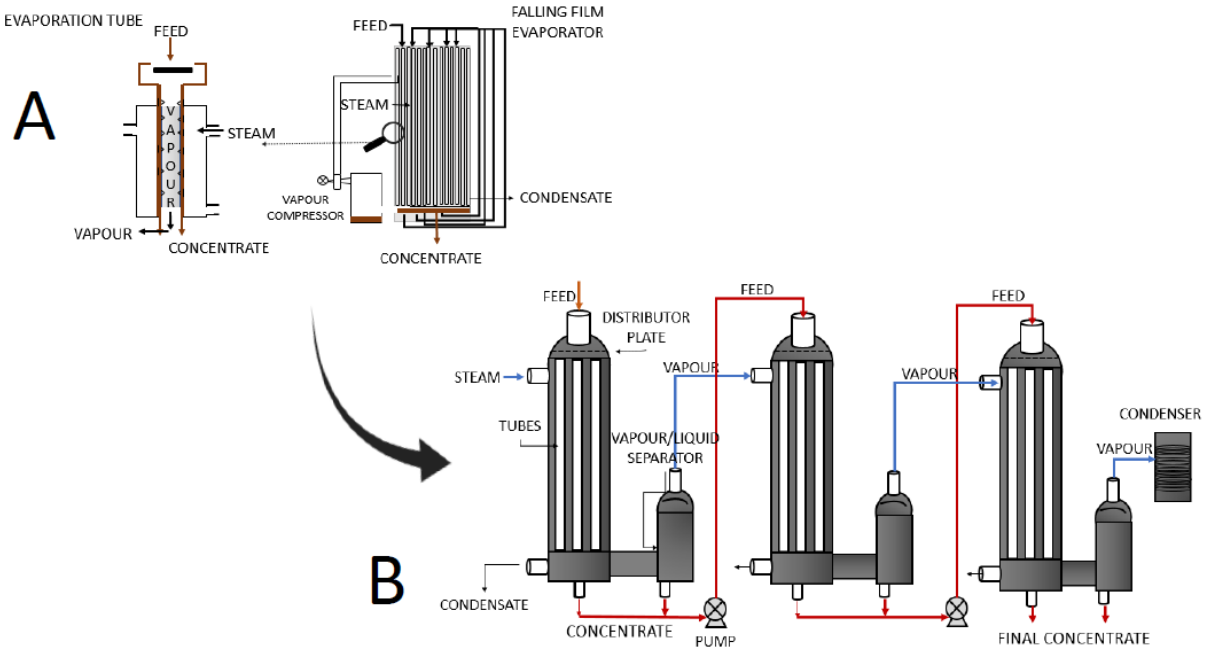
The evaporation of milk has been employed for several years and the main purpose of this unitary operation is to remove the water from the solution to increase the solutes content such as proteins, fat, sugars, and minerals from milk composition. Using high temperatures, the objective is to concentrate on minimum total cost, which is included the energy and cleaning expenditures, capital and operating costs, and product loss (DUMPLER; HUPPERTZ; KULOZIK, 2020; MORISON; HARTEL, 2018). Offering the lowest annual capital cost (approximately € 4M) and high concentration levels, evaporation is considered the most practical concentration approach (Table 8) (ALI et al., 2021; BALDE; AÏDER, 2017; SCHUCK et al., 2015; TANGUY et al., 2015).

Usually, the development of evaporators permits the liquid to be concentrated to flow through a tube in which the heat is applied outside. The liquid is heated up to the boiling point

at ambient pressure (100°C at sea level and 85°C at an altitude approximately 5000m above sea level) and the water is separated from the concentrated fraction. Due to the high latent heat of water evaporation, the energy efficiency is increased with the employment of multiple stages of evaporators or vapor recycling to reuse energy, and decrease this source demand (MORISON; HARTEL, 2018; RAMIREZ; PATEL; BLOCK, 2006).

In dairy industries, the evaporation process is usually done in long-tube vertical falling film evaporators. In this process, the milk (near at its boiling point) is uniformly fed at the top of the inner surface of a tube, which is built side by side with other tubes, fixed, and enclosed by a jacket (Figure 17A). After the milk passes down inside of each tube, forming a thin film, it boils due to the heat applied by the steam. The concentrated liquid is separated at the bottom part of the equipment and the remaining part is removed from the steam in a subsequent separator. In evaporators with multiple effects, the concentrated liquid is pumped to the next stage, while the steam is used to heat the next stage (Figure 17B) (FERNÁNDEZ-SEARA; PARDIÑAS, 2014; GUICHET; JOUHARA, 2020; MORISON; HARTEL, 2018).

Figure 20-A: A long tube vertical falling film evaporator; **B:** Multiple effects of a long tube vertical falling film evaporator.



Source: Figure A: Adapted from Verdurmen and de Jong (2003); Figure B: The authors (2022).

Falling film evaporators offer the advantage of the short residence time of the liquid within the equipment. In addition, to increase energy efficiency, this process is generally operated under vacuum conditions, which is beneficial for milk concentration, since several bioactive compounds can be damaged by extreme heat exposition (DUMPLER; HUPPERTZ; KULOZIK, 2020; MORISON; HARTEL, 2018). In dairy manufacturing, the evaporation process is used as a first step to concentrate products to drying such as whey protein concentrate, lactose, or powdered milk to increase the stability, reduce the volume and production costs, storage, and transportation. Some products such as condensed milk, *dulce de leche*, and evaporated milk are sold as concentrated liquids (DUMPLER; HUPPERTZ; KULOZIK, 2020; MORISON; HARTEL, 2018; VARGAS et al., 2021).

2.1.1 Effects in the dairy matrix composition through the evaporation process

Processing of concentrated dairy products includes several steps that may affect the stability of dairy matrix compounds. Due to the high sensitivity of milk nutritional compounds to intense thermal and mechanical processes, undesirable changes may occur in the physicochemical, sensory, or microbiological characteristics of the dairy matrix and its concentrated products (DUMPLER; HUPPERTZ; KULOZIK, 2020; MASUM et al., 2020; RAFIEE TARI et al., 2021; VERRUCK et al., 2019b; WU et al., 2021).

Evaporators must have time and temperature controlled throughout the procedure. Intense heat exposure of the evaporation process affects the natural pH of milk (approximately 6.6-6.8), resulting in changes in the milk salt equilibrium and denaturation proteins during this procedure and a consequently level of coagulation (DUMPLER; HUPPERTZ; KULOZIK, 2020; LIN et al., 2018; VERRUCK et al., 2019b). A significant decrease in pH during heating is primarily due to acid produced from lactose oxidation at high temperatures, hydrolysis of organic phosphate groups, and precipitation of calcium phosphate (KOUTINA; SKIBSTED, 2015; WU et al., 2021).

Milk proteins directly and indirectly interact with lactose. During any heat treatment, an intense and prolonged heat exposure (above 100°C) results in the formation of early and, in some dairy products, undesired Maillard products with changes in color, texture and flavor aspects (DUMPLER et al., 2018; DUMPLER; HUPPERTZ; KULOZIK, 2020; DUMPLER; KULOZIK, 2015, 2016). The consequent isomerization and thermal degradation of lactose are parallel reactions to the Maillard reaction and, products such as formic and acetic acid, from

lactose oxidation, also lead to a decrease in pH of milk (DUMPLER et al., 2020; FOX; UNIACKE-LOWE; MCSWEENEY, 2015).

The heat stability and the pH sensitivity of milk are often attributed to the presence of casein micelles formed by calcium bridges and linkages by colloidal calcium phosphate through complex hydrogen, hydrophobic bonds, and electrostatic interactions (DUMPLER; HUPPERTZ; KULOZIK, 2020; KOUTINA; SKIBSTED, 2015). During a heat processing of milk below 80°C, whey proteins denature and there are changes in the size and structure of casein micelles, reducing the amount of ionic calcium and phosphate, which will also decrease the pH due to remaining as free ions in the whey fraction for further interactions during acid clot formation (DEETH; BANSAL, 2019; DUMPLER; HUPPERTZ; KULOZIK, 2020; KOUTINA; SKIBSTED, 2015). This consequent coagulum from denaturing whey proteins and casein micelles may result in heat-induced fouling inside of evaporators, increasing the viscosity, decreasing the heat transfer and the energy efficiency of the equipment (DUMPLER; KULOZIK, 2015; WU et al., 2021). In addition, heat-induced protein accumulation on the inner contact surface of the evaporator can induce the proliferation of microorganisms and problematic decreases in product quality, such as changes in color, flavor, and texture. The growth of pathological microorganisms can also be a risk to the consumer's health, with the industries being responsible for all the quality and food safety, from processing to the market.

2.2 SPRAY DRYING PROCESS

Milk powder and powdered dairy products present a globally consolidated market and a particular interest by food industries due to their high acceptability and added value. The milk powder is an interesting solution for those who lack direct access to adequate refrigeration, which characterizes it's practically of consumption (DING et al., 2021; KALYANKAR et al., 2015). Due to the functional and nutritional properties of milk and the high value of its components, powdered milk and powdered dairy products are considered a valuable concentrated ingredient for diverse applications in food formulations, such as bakeries, confectionaries, infant formulas, meat products, and nutritional foods (KALYANKAR et al., 2015; KHAN et al., 2021).

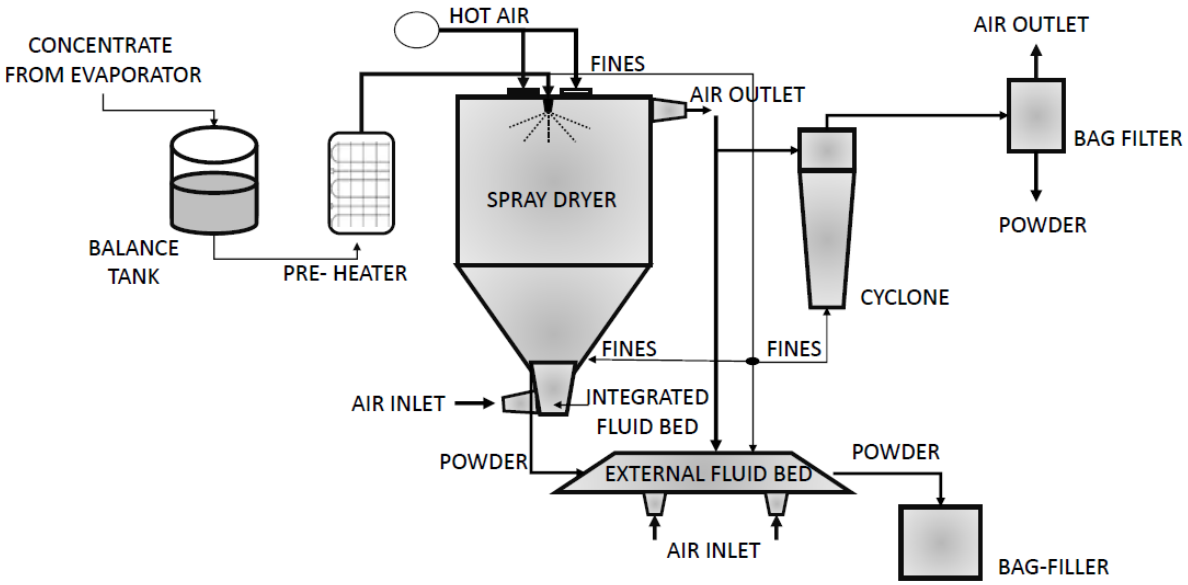
The storage of fresh milk presents obstacles due to the vast volume of dairy processing and the need for refrigerated storage, transport, and marketing. Drying is a conventional way of concentrating food and facilitating logistics, handling milk, increasing the shelf life and

stability of the product. The main purpose of converting fresh milk into milk powder is to transform a liquid perishable matrix into a product that can be stored for years without loss in physicochemical, microbiological, nutritional, and sensory quality (DESHWAL et al., 2020; DING et al., 2021a; KALYANKAR et al., 2015).

The usual process of drying in dairy industries is spray drying and, by definition, is a transformation of feed from a solution, suspension, or paste into a concentrated/dried form by spraying this fluid into a hot drying medium (VERDURMEN; DE JONG, 2003). To improve the thermal efficiency of the drying procedure and avoid overheating of powder particles, the equipment (spray dryer) can consist of one, two, or three stages (Figure 18). Different from evaporators, there is no recovering of latent heat of the vapor in the spray dryer. Drying is responsible for up to 15% of the industrial energy requirement and, if compared to a concentration by evaporation, the energy demand by the spray drying process is 10 - 20 times higher per kilogram of water removed (Table 8). Consequently, it is usual to proceed with a primary concentration by evaporation before drying, however, spray-drying is still the most energy-intensive process in dairy industries and has received much attention (RAMIREZ; PATEL; BLOCK, 2006; SCHUCK et al., 2015; VERDURMEN; DE JONG, 2003).

In the first stage, the preheated feed solution (<100°C) is pumped from a product tank to the atomizing dispositive, which contains the drying chamber. The drying air, composed of filtered atmospheric, is inserted through the hot chamber at 150-250°C by an air disperser. The atomized feed solution meets the hot drying air and occurs the solvent evaporation, which occurs simultaneously with the cooling of the air. The concentrated product is converted into droplets of 10 - 200μm and depending on the dimensions of the spray-dryer, the residence time of the dried particles is around 5 to 30 seconds (SCHUCK et al., 2015; VERDURMEN; DE JONG, 2003). Most powder particles fall to the bottom of the dryer and are submitted to a pneumatic transport and an immediate cooling system, which is essential to preserve better flavor, physicochemical characteristics, and long shelf life. The particles with the smallest diameters remain in the air and, if necessary, the air passes to a cyclone to separate the solid fraction. After drying, the powders are transported to the next drying stage or a packing system (KALYANKAR et al., 2015; MASUM et al., 2020).

Figure 21- Stages of a spray dryer.



Source: Adapted from Verdurmen and de Jong (2003).

2.2.1 Effects in dairy composition through the spray drying process

The dairy concentration by spray drying process is a traditional, important, and economic operation due to the flexibility in handling a variety of products. However, the high temperature needed in this process reduces the heat-sensitive nutritional and sensory components of the original matrix. In addition, the drying air temperature can affect the physicochemical properties of powder milk by changes in the distribution of the majority components, particle morphology, color characteristics, and water activity (DESHWAL et al., 2020; HABTEGEBRIEL et al., 2018; PERUSKO et al., 2021).

One of the components of the solid fraction that is most affected during the spray drying process is the milk fat, which is dispersed in a colloidal system with proteins, water, and soluble components. Aromatic compounds of powdered milk undergo several complex changes during processing and, in spray drying conditions precisely, the thermal procedure can cause damage of droplet shrinkage and release free fat, which is easily oxidized and cause sweet taste, fatty and creamy flavor of powdered milk (FENG et al., 2021). In addition, after drying, the fat is distributed on the surface of whole milk particles and this conformation influences the size of dried flakes and affects the interconnecting among the powder particles, forming an undesirable pasty characteristic (BIRCHAL et al., 2005; HABTEGEBRIEL et al., 2018; LIN et al., 2018). Milk protein is also an important macronutrient that can be used as indicator of milk quality after a technological treatment and is also affected by the intense temperature of

the spray dryer. According to Vincenzetti et al. (2018), who studied the effects of spray drying and freeze-drying processes on the β-lactoglobulin and lysozyme content in donkey milk, the high temperature to which the donkey milk was subjected significantly decreased the lysozyme enzymatic activity (58% of residual activity) and β-lactoglobulin content (6.43 mg/mL in fresh milk vs. 5.51 mg/mL in spray-dried milk). The denaturation of milk proteins can also cause encrustations inside the spray-dryer, reducing the efficiency of heat exchange in the equipment and blocking nozzles, causing a low-quality powder, and requiring product rework (BISTA et al., 2021; CHENG; ZHOU; LIU, 2018; DESHWAL et al., 2020).

The lactose fraction has also proven to be responsible for important roles in the sensory, nutritional, functional, and physicochemical properties of powdered dairy products (FIALHO et al., 2018; PARK; STOUT; DRAKE, 2016; RONGSIRIKUL; HONGSPRABHAS, 2016; ZHOU; LANGRISH, 2021). Depending on the processing conditions, in addition to high temperatures, water evaporation, high concentration of lactose and lysine-rich proteins, Maillard reactions may occur and causes several effects, including the unattractive formation of melanoidins (nitrogen-containing brow pigments), the loss of nutritional value, changes in the sensory properties, presence of off-flavors and formation of potential mutagenic products (PERUSKO et al., 2021; ZHOU; LANGRISH, 2021). Due to the quick changes in the temperature and moisture composition in the hot chamber of the equipment, Maillard reactions in spray dryers may be different from those liquid systems. Furthermore, due to the fast rate of water removal, the lactose structure is converted into its amorphous glassy state, which is unfavorable to the crystallization phenomenon (HABTEGEBRIEL et al., 2018). This lactose form, at high temperatures or moisture content in the storage of powdered dairy products, develops molecular mobility and converts into a rubbery state, which occurs at a temperature range known as glass transition temperature (Tg). If the storage occurs at temperature higher than Tg, the mobility of this lactose conformation increases and the viscosity decreases, initiating the lactose crystallization. According to studies, the extension of Maillard reactions can be reduced by improving the design of spray dryers, as well as the control of dairy concentration processes and all the external variables and internal properties (DESHWAL et al., 2020; HABTEGEBRIEL et al., 2018; MASUM et al., 2020; PERUSKO et al., 2021; ZHOU; LANGRISH, 2021).

Due to these undesirable changes in physicochemical and sensory aspects of concentrated dairy products that may occur in traditional concentration processes, evaporation and spray drying can be replaced recently by new and alternative concentration processes from

the rise of studies of food engineering and food technology, developing alternative and non-thermal technologies which allow the permanence of highly heat-sensitive milk compounds (BALDE; AÏDER, 2017; CAMELO-SILVA et al., 2021a; CASAS-FORERO; ORELLANA-PALMA; PETZOLD, 2020; DE LIZ et al., 2020; GUILLERMO PETZOLD; JORGE J. MORENO, JULIO JUNOD, 2016; MACHADO CANELLA et al., 2020; MOEJES et al., 2020; MUÑOZ et al., 2018). Recently, the dairy sector around the world aims for product quality. Dairy products with high functional and nutritional quality have become the most desired option for consumers, being one of the main focuses of manufacturing. Studies, researches, and development of new food concentration processes allow new choices for industries, improving the production chain.

3. ALTERNATIVE CONCENTRATION PROCESSES IN MILK MANUFACTURING

Non-conventional concentration processes gain increasingly attention from dairy industries as means to decrease the negative effects of conventional processing technologies. Non-thermal technologies maintain the maximum of milk bioactive compounds, obtaining a concentrated dairy product with high quality, nutritional and functional value, accepted sensorially and offsetting the total production costs. The advances of new studies support industries to apply emergent technologies in the dairy manufacturing according to the production flow, method of operation and the type of dairy products, since alternative technologies develop products with specific properties to each type of method, which also becomes a factor of choice for the industrial sector on large-scale application (BARROS et al., 2021; CAMELO-SILVA; BARROS; VERRUCK, et al., 2021; DESHWAL et al., 2020; FRANCE et al., 2021; MACHADO CANELLA et al., 2020; MERKEL et al., 2021; SHABBIR et al., 2021; VERRUCK et al., 2019).

Table 11- Comparation between the traditional and alternative milk concentration processes.

	Process	Advantages	Disadvantages	Studies about energy expenditures
conventional concentration processes	Evaporation	-The most practical milk concentration approach; - Low energy costs using multi-stages evaporators; - Increased shelf-life	- High temperatures may decrease milk bioactive compounds (vitamins, enzymes, proteins); - Denaturation of milk proteins may result in heat-induced fouling inside of evaporators; - Intense heat treatment can affect the pH sensibility and minerals equilibrium; - In specific products, undesirable changes of sensory properties (flavor, color and texture); - High installation and operating costs	3200 kJ. kg^{-1} water removed (single- effect evaporator under partial vacuum and under atmospheric pressure at boiling temperature); 900 $kJ.kg^{-1}$ (pilot-scale roller dryers in a partial vacuum chamber with a mechanical vapor recompression heating system) (RAMIREZ; PATEL; BLOCK, 2006; SCHUCK et al., 2015) 300 $kJ.kg^{-1}$(traditional multi-stages evaporators with mechanical vapor recompression- MVR)(MOEJES et al., 2020; RAMIREZ; PATEL; BLOCK, 2006; TANGUY et al., 2015)
	Spray drying	- Increased shelf-life; - Lower storage and transportation costs; - Reduction of the product volume; -Expansion of logistic distribution; - Requires a noticeably short time	- Decrease in milk thermolabile compounds; - High energy consumption (10 -20 times higher than evaporation process); - To reduce energy costs, there is a need for pre-concentration (90% of the water is removed in the evaporator and only 9–10% in the spray dryer) - Changes in the fat and lactose conformation, resulting in undesirable physicochemical and sensory characteristics	5256 $kJ.kg^{-1}$ for a skim milk spray-dried from 50% to 96% total solids (RAMIREZ; PATEL; BLOCK, 2006; SCHUCK et al., 2015); One single stage :4900 kJ/kg water evaporated; two stages: 4300 kJ/kg; three stages 3400 kJ/kg (RAMIREZ; PATEL; BLOCK, 2006; SCHUCK et al., 2015; VERDURMEN; DE JONG, 2003)
alternative concentration processes	Freeze concentration	- Preservation of highly heat-sensitive milk compounds; - Maintenance of color and flavor; - Low deterioration due to decreased of enzymatic and microbiological activities; - Increased shelf-life; - Non- polluting process; - Without the use of preservatives and additives;	- High investment cost in large-scale production; - High refrigeration and operating costs; - Low production rate; - Loss of soluble solids in the ice fraction	335 $kJ.kg^{-1}$ water removed (which is equivalent to approximately 15% of heat addition in a single-effect evaporator) (SÁNCHEZ et al., 2010)
	Membrane separation	-Low energy consumption; - High flow rates; - Improvement of the yield;	- Membrane pores are often clogged; - Requires high quality water for cleaning; - High maintenance costs	14.0 - 36.0 $kJ.kg^{-1}$ water removed by pressure driven membrane filtration (MOEJES et al., 2020; RAMIREZ; PATEL; BLOCK, 2006) ;

		- Increased shelf-life; -Removal of bacterial pathogens and spores; - Non-polluting process; - Without the use of preservatives and additives		50.08 - 62.54 $kJ.L^{-1}$ of milk retentate, 18.18-21.65 $kJ.L^{-1}$ of permeate and 87.08 – 107 kJ for cleaning at different pressures (BAHNASAWY; SHENANA, 2010); 0.6 - 1.5 $kJs.kg^{-1}$ for ultrafiltration of a Greek-style yogurt (PAREDES VALENCIA et al., 2018)
	Freeze drying	-Preservation of bioactive compounds; -Maintenance of flavor and color; - Increased shelf-life; - Facility of transport and storage; - Non-polluting process, low waste water; - Without the use of preservatives and additives; -Process with absence of oxygen, preventing against oxidative reactions	- High energy consumption; - Inviable to produce in large-scale; - Low production rate; - Requires sterile diluents on reconstitution; - High cost and complexity of the equipment	3820 - 5500 $kJ.kg^{-1}$ water removed. In 24 hours, the energy consumption of all the consumers (cooling chamber, sublimation, and vacuum pump) in a freeze-dryer is 937,177.42 kJ (BANDO et al., 2017; KESELJ et al., 2017)

3.1 FREEZE CONCENTRATION

The freeze concentration process is a non-thermal concentration technology applied in liquid foods based on a solid-liquid separation at low temperatures, which the water fraction is frozen, transformed into pure ice crystals, and removed from the concentrated solution (DING et al., 2021; SÁNCHEZ et al., 2010). This method can be used to concentrate or pre-concentrate heat-sensitive compounds, which is an interesting alternative for dairy manufacturing, due to the maintenance of the nutritional value, volatile compounds, and flavor of dairy products (BARROS et al., 2021; BENEDETTI et al., 2015; CAMELO-SILVA et al., 2021a; DE LIZ et al., 2020; MACHADO CANELLA et al., 2020; MUÑOZ et al., 2018; PRESTES et al., 2022; SÁNCHEZ et al., 2011a). Freeze concentrated dairy products can be used in diverse food formulations or as an intermediate matrix in dairy processing (BALDE; AIDER, 2016; BALDE; AÏDER, 2017; BARROS et al., 2021; CAMELO-SILVA et al., 2021b; MUÑOZ et al., 2018).

The process is based on lowering and controlling the temperature of the solution below its freezing point to avoid freezing all the components simultaneously, at the eutectic point. The purity of ice crystals increases with controlled freezing above the eutectic point of the solution, preserving all the properties of the original liquid matrix. Usually, the upper limit of freeze concentration, concerning the original liquid food, range from 40 to 50% of solid content, depending on the level of soluble solids and the food matrix composition (MORISON; HARTEL, 2018; PRESTES et al., 2022; SÁNCHEZ et al., 2011b).

Since this process does not involve a liquid-vapor interface, there is the maximum preservation of thermolabile compounds, increasing the quality of the concentrated product. In addition, from a thermodynamic point of view, the freeze concentration is highly interesting due the energy consumption is lower when compared to evaporation and spray drying processes. The latent heat of water freezing is almost one-seventh of latent heat of water evaporation (335 $kJ.kg^{-1}$ vs 2260 $kJ.kg^{-1}$), which is a potential for energy saving for de-watering of liquid foods (Table 8) (BALDE; AÏDER, 2017; CASAS-FORERO; ORELLANA-PALMA; PETZOLD, 2020; DING et al., 2021b; PRESTES et al., 2022). In freeze concentration methods, the energy savings can also be related to the possibility of using passive thawing as a recovery step of the concentrated phase, which enhances the energy efficiency and quality of the concentrated products (BALDE; AÏDER, 2017). Since the yield of the concentrate is not high compared to other concentration methods, the separation of frozen water from the concentrate

solution can be carried out once or through several successive freezing steps, in the same liquid food. This procedure, which increases the soluble solids content, will depend on the original composition of the liquid matrix, the desirable objective, and the yield of each freeze concentration stage. In the freeze concentration technology, ice crystals can be formed from liquid solutions in different methods: suspension, progressive, and block freeze concentration, with distinct freeze techniques and ice crystals separation (CASAS-FORERO; ORELLANA-PALMA; PETZOLD, 2020; DING et al., 2021b; SAMSURI; AMRAN; JUSOH, 2018; SÁNCHEZ et al., 2011a, 2011b; ZAMBRANO et al., 2018).

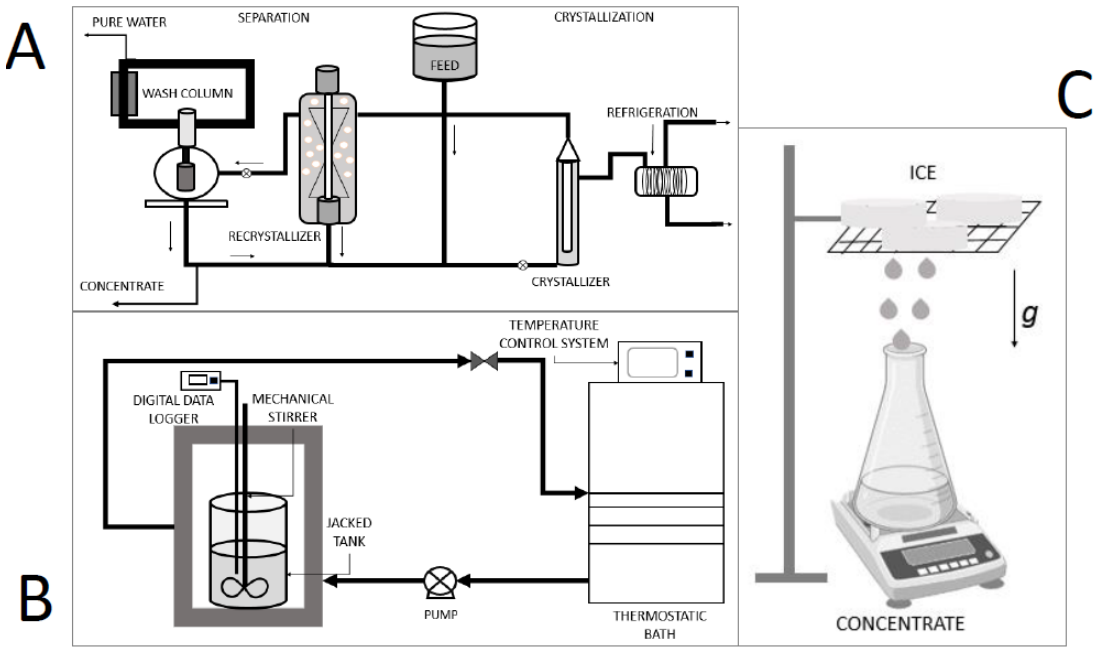
The suspension freeze concentration is the most complex and expansive method of freeze concentration, based on the formation of individual ice crystals when the liquid matrix is submitted at low temperatures. An initial phase of crystallization occurs and the ice crystals position themselves into large ice particles, increasing the volume and, in the second phase, ice nuclei grow inside the solution (MORISON; HARTEL, 2018; SÁNCHEZ et al., 2011a). The size of these ice crystals is limited, and the separation of the concentrate is complex with the use of typical equipment, with specific purposes (Figure 19A). The system is composed of a crystallizer (heat exchanger) to promote the crystals' growth. At a pre-freezing temperature, the liquid matrix is cooled and advances to the crystallizer to form ice crystals, which are removed from the concentrate fraction inside of a separator. The remains of frozen pure water are separated from the concentrated liquid in a wash column with compression to handle the ice crystals with high purity (AIDER; DE HALLEUX, 2009; MORISON; HARTEL, 2018; SÁNCHEZ et al., 2011a).

The progressive freeze concentration is one of the most important methods for concentrating liquid foods and is based on the layer crystallization, in which a large mass of ice is formed on a cold surface, with an easier separation due to the ice crystals adhering to the surface (MOHARRAMZADEH et al., 2021; MORISON; HARTEL, 2018; SAMSURI; AMRAN; JUSOH, 2018). This process is simpler and must be developed according to specific properties of the liquid food, the concentrate fraction, and its yield at the end of the procedure. The costs of operation, equipment, and maintenance are low, which makes this process promising to be applied on a large scale and replace the complexity of a concentration by suspension crystallization (MORISON; HARTEL, 2018; SÁNCHEZ et al., 2011a). This type of freeze concentration has been proven to be efficient in studies about the separation and concentration of dairy products, which systems were developed and improved according to the dairy matrix and specific needs (CHABAROV; AIDER, 2014; DANTAS et al., 2021; MUÑOZ

et al., 2019; SAMSURI; AMRAN; JUSOH, 2018). A progressive freeze concentration system was proposed for the first time in the separation of skimmed milk by Muñoz et al. (2019), proving to be an effective method to concentrate milk and offering interesting energy savings, when compared to the suspension method and nutritional preservation of the concentrated skimmed milk. In this experimental vertical system, the liquid matrix is placed in an agitated tank with a cooling jacket. The low temperature causes the formation of an ice layer on the cooling walls of the tank and a mechanical stirring is applied to decrease the solute accumulation in the ice fraction (Figure 19B) (OJEDA et al., 2017).

In the block freeze concentration technique, the liquid food is frozen and partially thawed by the assisted gravitational defrost method to separate ice and concentrate fractions (ZAMBRANO et al., 2018). At a controlled temperature, the ice block performs as a solid carcase and, by the phenomenon of diffusion, the concentrate is drained (AIDER; DE HALLEUX, 2009) (Figure 19C). About all the freeze concentration methods, this technique is promising due the facility of operation, simpler equipment and the lower total cost, with diverse applications and studies in the dairy sector (AIDER; DE HALLEUX, 2009; BARROS et al., 2021; CAMELO-SILVA et al., 2021a; DE LIZ et al., 2020; MACHADO CANELLA et al., 2020; MORISON; HARTEL, 2018; MUÑOZ et al., 2018; SÁNCHEZ et al., 2011a).

Figure 22- A: Suspension freeze concentration scheme; **B:** Vertical Progressive freeze concentration scheme; **C:** Block freeze concentration system.



Source: A: Adapted from Aider and de Halleux (2009) and Prestes et al. (2022); B: Adapted from Muñoz et al. (2019); C: Block freeze concentration system.

3.1.1 Effect of freeze concentration process in dairy products

Studies and equipment development about freeze concentration of dairy products have resulted in knowledge about the performance of dairy fluids at low temperatures, the impact on the milk physicochemical properties and the influence on the behavior of the main components such as lactose precipitation, protein conformation, and fat dispersion in both the concentrate and the ice fraction (BALDE; AIDER, 2016; BARROS et al., 2021; BARROS et al., 2022; MUÑOZ et al., 2019; SÁNCHEZ et al., 2011a).

During the concentration, there is an increase of soluble solids in the concentrated dairy fraction, with a tendency to increase the viscosity of the concentrate. The high concentration of caseins and their consequent dehydration causes an increase of micelles volume and in the inter-micelles interaction, which strongly contribute to the viscosity of milk. Any chemical or physical effect, as well as the concentration, that may change the aggregation state of casein micelle, certainly will modify the viscosity of milk. As a result, the increase of the viscosity is inversely proportional to the ability of separate the concentrate from the ice fraction, which limits the concentration efficiency (BALDE; AIDER, 2016; BALDE; AÏDER, 2017; BIENVENUE; JIMÉNEZ-FLORES; SINGH, 2003). In addition, high milk soluble solids content (proteins, fat, or lactose) may affect the crystallization and development of pure ice crystals, affecting the efficiency in the separation step and restricting the phenomenon of heat and mass transference.

Alternative studies employing previous treatments to remove fat from whole milk or directing skimmed milk to freeze concentration were carried out to reduce the effect of fat during the concentration and improve the efficiency of ice crystals separation (AIDER; OUNIS, 2012; BALDE; AÏDER, 2017; BARROS et al., 2022; CAMELO-SILVA; BARROS; CANELLA, et al., 2021; MUÑOZ et al., 2019). Compared to skimmed milk, the fat content in the dairy matrix increases the resistance in removing the ice fraction of concentrated whole milk due to the interaction/adsorption of caseins with fat globules. This interaction is responsible for the formation of large particles and the presence of a clumped that interferes in the separation of ice crystals (TRIBST et al., 2020). In addition, the milk fat fraction can also influence the lactose distribution in the concentration process, directing the high lactose content in the ice fraction, which contains most hydrophilic compounds (AIDER; DE HALLEUX, 2009).

The milk color is one of the physical parameters that most influence sensory acceptance, mainly whiteness. In the freeze concentration process, the whiteness and luminosity can be improved according to the conformation of caseins in the concentrate. Comparing the physicochemical parameters of skimmed powdered milk from traditional concentration methods (evaporation) and alternative processes before drying, such as the freeze concentration, Balde and Aïder (2017) obtained products with high luminosity, good flow, and heat stability of milk powders from a previous freeze concentration. The high whiteness and luminosity can be attributed to an aggregation of denatured whey proteins and casein micelles, forming large particles. Compared to the traditional evaporation, significant differences can be attributed to the high temperatures involved in this process, which causes the formation of melanoidins or Maillard Reaction Products (MRP), with a typical caramel-brown color (PERUSKO et al., 2021).

Freeze concentration processes can be easily applied in dairy manufacturing with the purpose to produce stable concentrated products with high dry matter content without the need to enrich the formulation with whey proteins, powdered milk, or additives to improve the color of dairy products (BALDE; AÏDER, 2017). Studies report that freeze concentration is an important technology to promote high lightness, viscosity, and overrun for ice creams with skimmed milk freeze concentrated (CAMELO-SILVA et al., 2021a) and replaced with whey concentrate (BARROS et al., 2021). In addition, recent researches indicate that the high dry matter of the concentrate can offer a large concentration of nutrients and substrates for the development of probiotic cells and be a good carrier in dairy products, ensuring a high quality and functional value (CAMELO-SILVA et al., 2021b; CANELLA et al., 2018; DE LIZ et al., 2020; MUÑOZ et al., 2018).

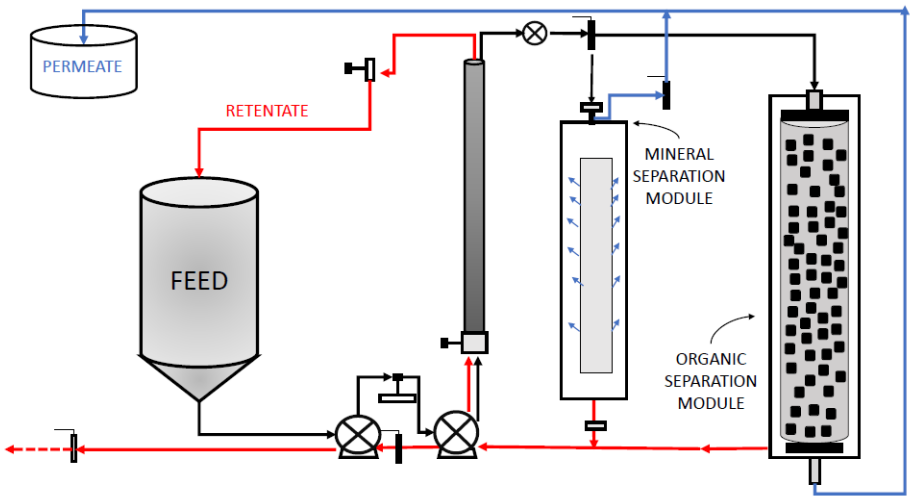
3.2 MEMBRANE SEPARATION PROCESS

Membrane separation is an emerging food processing technology that can provide milk preservation at low temperatures, increasing the shelf-life (CARTER et al., 2021). In dairy manufacturing, this popular separation process can be used to improve the microbial quality of dairy products and maintain the nutritional and functional properties of milk bioactive compounds (HENRIQUES et al., 2020; PRUDENCIO et al., 2014; VERRUCK et al., 2019). Compared to traditional separation processes, the membrane technology is favored due to the operation at moderate temperatures, pressure, and high selectivity during the procedure, which

do not change organoleptic characteristics of dairy products (GALVÃO, 2018; KIM; MIN, 2019; VELPULA, 2017). In addition, unlike conventional methods, membrane separation is a very attractive alternative technology due to the required temperature does not involve solvent phase change, which increases energy savings in this concentration process and represents an energy-efficient alternative to the concentration of dairy products (Table 8) (BAHNASAWY; SHENANA, 2010; FAUCHER et al., 2021; MARX; KULOZIK, 2018; PRUDENCIO et al., 2014).

The principle of this process occurs similarly to a pressure filtration system with membranes (thin film) to separate two solutions acting as a selective barrier. The separation is based on the permeability of membrane pores, separating immiscible solids and soluble solids, and the systems of separation are developed according to the direction of feed flow, which can be tangential or perpendicular. When the liquid passes through the membrane in a single direction, pores clogging may occur. On the other hand, in the tangential system, there are two directions of current passing through the membrane: one that flows parallel to the membrane removing the trapped solids, and the other purified, that passes through it. In this system, the parallel flow assists in the removal of particles that could clog the membrane pores. After passing through the pores, the liquid matrix is separated into two fractions: the permeate or microfiltrated, which is the liquid that passes through the pores, and the retentate (Figure 20). This fraction contains a higher concentration of solids that have a bigger size than the pore diameter of the membrane (BAHNASAWY; SHENANA, 2010; PAREDES VALENCIA et al., 2018; VERRUCK et al., 2019b).

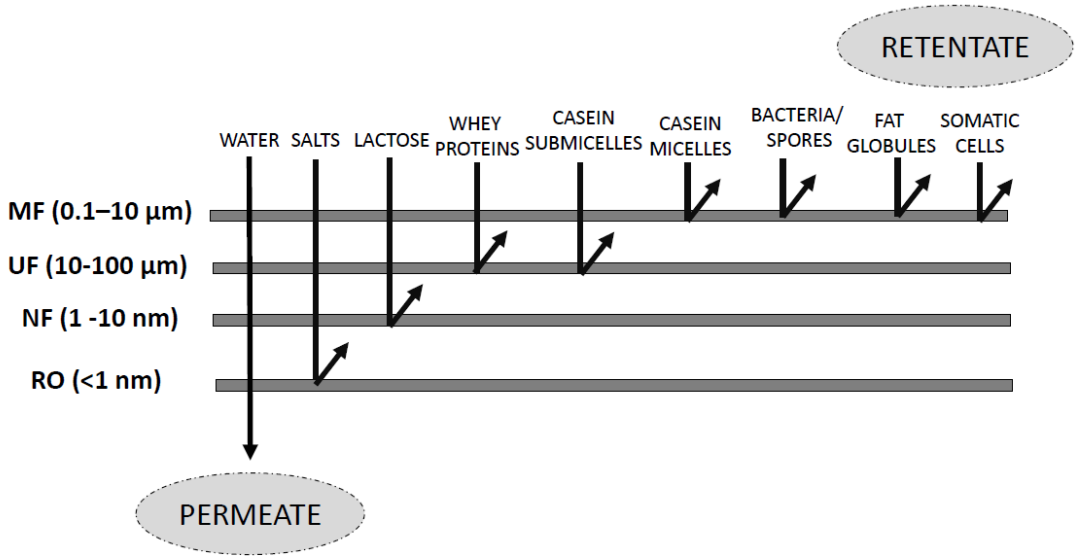
Figure 23- A schematic overview of the membrane separation method.



Source: The authors (2022)

The selectivity in separation depends on the process conditions and the type of membrane, relating the pore size and molecular-weight cutoff. The separation procedure can be classified as Microfiltration (MF), Ultrafiltration (UF), Nanofiltration (NF), and Reverse Osmosis (RO) (Figure 21) (CARTER et al., 2021). In dairy manufacturing, beyond to being used for the separation of solid milk components, MF is widely applied in reducing microbiological counts, with the biggest pore size (0.1–10 µm) and the lowest process pressure (0.01- 0.2 MPa). According to specific pore size, micellar caseins (50-500 nm), whey proteins (3- 6 nm), lactose (1nm), minerals and water can permeate the membrane, while fat globules (10 µm) are separated, and bacteria (10-100 µm), spores (1 µm), and somatic cells are removed (CARTER et al., 2021; SCHÄFER et al., 2018; SMITH, 2013; VERRUCK et al., 2019b). In this case, microfiltration is important in ensuring safe food for consumption, removing spores that are not inactivated in the milk pasteurization/sterilization process, in addition to extending shelf life, since somatic cells induce lipolytic and proteolytic milk reactions , damaging the sensory characteristics, color, flavor, and texture(MA et al., 2000; VERRUCK et al., 2019b).

Figure 24- Passed and rejected dairy components based on membrane pore size.



Source: Adapted from Carter et al. (2021). Note: MF = microfiltration, UF = ultrafiltration, NF = nanofiltration, RO = reverse osmosis.

For UF processes, the pore size ranges from 10 to 100 nm, operating at pressures of 0.1 to 1.0 MPa and, in milk concentration, proteins and fat fraction are retained providing a retentate rich in the dry matter that can be used in the production of high added value concentrated products. The permeate is composed of water, minerals, and lactose, which can be isolated and purified (CARTER et al., 2021; SMITH, 2013; VERRUCK et al., 2019b). The

NF (1- 10 nm of pore size and pressure of 1.5 - 3.0 MPa) is a fractionation technique with a concentration of substances having a molar mass between 100 and 1000 Da (g mol^{-1}) and are applied for whey processing in order to increase protein content (CARTER et al., 2021; MARX; KULOZIK, 2018; MERKEL; VOROPAEVA; ONDRUŠEK, 2021; PRUDÊNCIO et al., 2014; SMITH, 2013). RO is a concentration technique, with the smaller pore size (<1nm) and the higher processing pressure (3.0 – 5.0 MPa), which are related to increasing the stability and the shelf life of whey concentrates (MARX; BERNAUER; KULOZIK, 2018; MARX; KULOZIK, 2018).

3.2.1 Effect of membrane separation processes in dairy products

Membrane separation systems at high-pressure processing have become an interesting alternative to concentrate dairy products, since this technology, in addition to acting on the removal of pathogens and spores, can reduce the loss of nutritional compounds, maintain the maximum of milk properties, enable high flow rates and improve the yield (STRATAKOS et al., 2019; VERRUCK et al., 2019b).

The separation of the milk protein fraction (caseins and whey proteins) is gaining considerable attention from dairy and beverage manufacturers due to its unique protein profile and functionality. Membrane separation technologies, in special the MF and UF, are promising in cheesemaking, which provide a micro/ultrafiltered milk that can be used for protein standardization and improve the texture and yield of cheeses (CARTER et al., 2021; FAION et al., 2019). In a Peccorino cheese production from ultrafiltered sheep's milk by Faion et al. (2019), the ultrafiltered milk provided a nearly fourfold increase in protein (37%) and fat (29%) content, increasing the cheese yield by 17%. In addition, the time and moderate temperature of the ultrafiltration process (22°C for 30 min) can favor the development of lactic acid bacteria for fermented dairy products (Table 9).

In the permeate, minerals and lactose fraction can be affected due to protein interactions and deposition on the membrane surface. According to the specific pore size, these interactions result in a gel layer formation, clogging the pores and exerting resistance to the passage of water, minerals, and lactose, which contains low molecular mass. For minerals, only two-thirds that are bound with micellar proteins (usually calcium phosphate) remain retained in the membrane, while the soluble ones are permeated through it (FAION et al., 2019). Minerals that pass with the whey fraction by interactions or in the form of free ions and salts, may impact

the functional properties of the permeate, in its buffering capacity and influence the cheesemaking (CARTER et al., 2021; MARX; BERNAUER; KULOZIK, 2018; VERRUCK et al., 2019b). In addition, the temperature in membrane separation can influence the distribution of constituents and physicochemical properties of milk. According to France et al. (2021), MF of skimmed milk was performed at 4, 8, and 12°C. During the separation, the mechanical and thermal energy requirements during the MF of skimmed milk were strongly dependent on processing temperature (at 4°C =27.6 KWh, at 8°C= 24.6 KWh and 12°C= 22.9×10^{-3} KWh) with the highest energy requirements for UF at 4°C due to the high viscosity and the generation of heat during pumping, increasing the energy required to maintain the lower temperature. For protein contents, the fouling of the membrane caused by casein micelles decreased the concentration of β-casein, β-lactoglobulin and α-lactalbumin in the permeate throughout MF. However, the dissociation of β-casein into the whey fraction is higher at lower temperatures allowing for increased partitioning thereof into the permeate at 4°C (COPPOLA et al., 2014). The MF at lower temperatures may enable the production of next-generation dairy streams with novel protein fractions (FRANCE et al., 2021).

During the flow of milk through the membranes, the applied pressure minimally affects the protein content and fat globules when compared to a concentration in processes with high temperatures and mechanical impacts (JUKKOLA et al., 2018). The process must be monitored to maintain a suitable functioning of the membranes due to possible cake formation on the surface or pore blocking caused by casein micelle sizes (VERRUCK et al., 2019b). According to studies, whey proteins of pasteurized skimmed milk (> 78°C) can denature and form aggregates with minerals or can adhere to casein micelles, clogging the membrane pores (CARTER et al., 2021; SABOYAINSTA; MAUBOIS, 2000; VERRUCK et al., 2019b). This problem can affect the flux and modify the composition properties of the permeate and the retentate, which will not contain casein micelles but a product similar to milk protein concentrate due to the ratio of casein to whey protein (CARTER et al., 2021). Recently, researches were developed to reduce the membrane fouling caused by casein micelles during the milk ultrafiltration (MIKHAYLIN et al., 2016, 2018). A bipolar membrane electrodialysis was coupled with ultrafiltration, allowing the production of H^+ and OH^- ions from water under the application of an electric current. The milk is acidified in the electrodialysis module and caseins are precipitated and separated from whey proteins, without clogging. In addition, the base generated by the bipolar membrane can be applied in the conversion of insoluble caseins micelles into their soluble form of caseinates, without the use of chemicals and is considered a

more environmentally sustainable process (MIKHAYLIN et al., 2018). Sustainable strategies are also developed for application in ultrafiltration and nanofiltration of whey, with an obtention of nonacidified whey permeate before further application and reducing this major by-product generated by dairy industries (FAUCHER et al., 2021; MERKEL; VOROPAEVA; ONDRUŠEK, 2021). Although membrane fouling is something recurrent, these study alternatives can improve the efficiency of the membrane separation process, which is currently one of the emerging technologies with great potential to replace traditional dairy concentration methods in large-scale.

3.3 FREEZE-DRYING

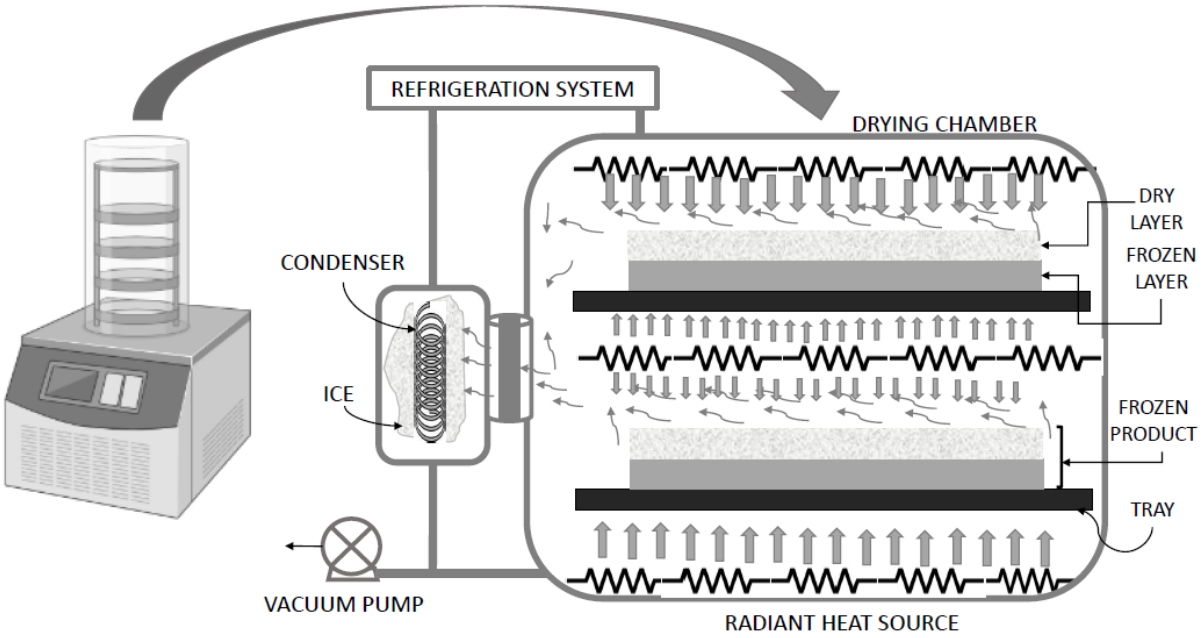
The freeze-drying technology, or lyophilization, is a concentration process based on the phenomenon of water sublimation of the food composition. At low temperatures, the water fraction is separated from the food by crystallization below its triple point (0.01 °C) and then is directly transformed from the solid-state to the vapor phase at high pressures (approximately 611 Pa) (BANDO et al., 2017; WAGHMARE et al., 2021). The concentration at freezing temperatures limits the damage of thermolabile compounds and is an advantageous technology to retain more taste, aroma, and color when compared to other concentration methods, being also an interesting alternative to concentrate dairy products, providing an increase of their quality (DESHWAL et al., 2020; VINCENZETTI et al., 2018; ZHU et al., 2020).

The market of freeze-dried products is increasing; however, it is necessary to reduce the high energy consumption of the process, ranging from 3820 to 5500 $kJ.kg^{-1}$: the highest energy requirement among all the concentration processes (Table 8) (BANDO et al., 2017; KESELJ et al., 2017). Compared to conventional drying, which dehydrates foods in a single stage, the freeze-drying time is longer and consumes large amounts of energy (almost double to remove 1 kg of water) (DUAN et al., 2016). In addition, energy is required to freeze the food, maintain the high pressure, sublimate water crystals and condense water vapor. This concentration process becomes expansive and is usually applied in high value-added products and makes the processing of common and accessible concentrated products unfeasible (GARCIA-AMEZQUITA et al., 2016; WAGHMARE et al., 2021).

The design of a freeze-dryer is composed of four basic components: drying chamber, vacuum pump, heat source, and condenser (Figure 22). For milk processing, it becomes usual to apply a vacuum freeze dryer. In this method, freezing can be done directly in the freeze-dryer

or other equipment that makes this step possible. The vacuum system is a combination of a water circulation pump and oil-sealed pump. During the initial stage of the vacuum process, the air is removed by the high-power oil sealed pump causing a decrease in pressure. After this procedure, the vacuum is maintained by a low-power pump. For the frozen water fraction to change state, passing from the solid-state directly to steam, a heating system is needed to raise the temperature and prevent the change from the solid to the liquid phase. In this system, it can contain plates with steam circulation or hot water at 120°C. Cooling for steam condensation is equipped by a system with liquid refrigerant circulating on plates behind or on the sides of the freeze dryer (DUAN et al., 2016; GARCIA-AMEZQUITA et al., 2016; WAGHMARE et al., 2021).

Figure 25- A typical freeze-dryer system.



Source: Adapted from Garcia-Amezquita et al. (2016)

Table 12- Recent studies on alternative technologies applied to dairy concentration.

Process	Dairy product	Conclusions	Authors
Freeze concentration	Ice cream	The concentrated skim milk provided high viscosity and overrun. The total solids and protein content were high, influencing in the color of ice cream, with high lightness.	(CAMELO-SILVA et al., 2021)
Microfiltration	Skimmed milk	Lower temperatures (4, 8, 12°C) influenced in the MF performance, with the highest membrane fouling at 4°C and the highest energy requirements for separation at 4°C. The permeate, also at 4°C, obtained the highest solid content and whey proteins dissociated.	(FRANCE et al., 2021)
Freeze concentration	Ice cream replaced by whey concentrate	The presence of whey concentrate provided a greenish yellow color in ice cream, increased the viscosity, soluble solids and overrun. The replacement with whey did not affect the microstructure of the ice crystals.	(BARROS et al., 2021)
Ultrafiltration	Milk proteins	Development of bipolar membrane electrodialysis coupled with ultrafiltration enabled the concentration of caseins in their soluble form (caseinates) or precipitated without obstructing the pores of the membranes.	(MIKHAYLIN et al., 2018)
Freeze drying	Petit Suisse cheese	Rheological properties slightly differed between fresh and freeze-dried samples after rehydrating. This concentration method maintained the color properties and influenced in the microstructure of protein conformation, changing the product viscosity.	(SILVA et al., 2021)
Freeze concentration	Fermented lactic beverage	Whey concentrate was used to develop a probiotic and symbiotic (probiotic + inulin) fermented beverage. The use of whey concentrate and inulin contributed to an increase in apparent viscosity and soluble solids. The concentrate provided high macro and micronutrients to probiotic cells development.	(CANELLA et al., 2018)
Ultrafiltration	Greek-style yogurt	A Greek-style yogurt was developed with the retentate of milk ultrafiltration and avoid an acidified whey from permeate and compared to a yogurt ultrafiltered. Both samples obtained similar protein (~10%) and solid content (~17%) and the energy requirement was higher due to the membrane fouling during the procedure.	(PAREDES VALENCIA et al., 2018)
Freeze concentration	Probiotic microcapsules produced with goat's whey concentrate	Powders produced from goat's whey concentrate and inulin (with high total solids content) presented water solubility and cohesiveness and provided high lightness, a greenish color and a good stability of probiotic cells at cold storage.	(DE LIZ et al., 2020)
Freeze drying	Camel milk powder	Freeze-dried camel milk powder had the highest dispersibility (67.15%), solubility (88.77%) and the lowest acidity (0.193% vs 0.211%) when compared to a spray drying process, attributing to the non-thermal concentration process a high stability of camel milk metabolites.	(DESHWAL et al., 2020)
Freeze concentration, reverse osmosis	Skimmed powdered milk	The powdered milk from a previous concentration by freeze concentration and reverse osmosis provided high solubility, whiteness and lightness when compared to an evaporation process. In addition, the powder particle size was highest for the two alternative concentration processes.	(BALDE; AÏDER, 2017)

Freeze drying	Symbiotic yogurt	Survival rates of *L. plantarum* added in microcapsules were higher after freeze -dried when compared to free cells (91.2 *vs* 61.7%). Cryoprotected *L. plantarum* microcapsules tolerate the freeze drying process, classifying the yogurt powder as a probiotic product for 10 weeks at 25 °C.	(JOUKI et al., 2021)
Ultrafiltration	Cheese with concentrated sheep's milk	The sheep milk ultrafiltration (22°C for 30 min at 2bar) increased the Peccorino cheese yield (17%) and provided an approximately fourfold increase in the protein (37%) and fat (29%) content.	(FAION et al., 2019)
Nanofiltration	Sweet and acid whey	Nanofiltration and electrodialysis provided 88% and 91% desalination of sweet and acid whey, which provided a high removal of minerals and organic acids with a decrease by 88% of lactic acid.	(MERKEL; VOROPAEVA; ONDRUŠEK, 2021)

3.3.1 Effects of freeze-drying process in the dairy products composition

Due to the constant consumer demand for foods with higher functional and nutritional quality, the food formulation has been adapted with the replacement of natural components by freeze-dried products. As well as other emerging technologies, freeze-drying in addition to concentrating food can also be considered a method of preserving food products (DESHWAL et al., 2020; VINCENZETTI et al., 2018). Due to the low water activity, microbial development and enzymatic oxidations are delayed, allowing the concentrated product to be stored for a long time at room temperature. Furthermore, especially in dairy manufacturing, the use of low temperatures during the concentration step enables to maintain the color and flavor of the product (DESHWAL et al., 2020; DUAN et al., 2016; SILVA et al., 2021; VINCENZETTI et al., 2018; WAGHMARE et al., 2021).

The first investigation about the effect of freeze-drying on raw milk metabolites was proposed by Zhu et al. (2020). About the contents of some organic acids, amino acids, and dipeptides, slight changes were detected, however, the authors pointed that these alterations may happen as a result of the incomplete re-dissolution process of the freeze-dried milk powder rather than the freeze-drying process. The concentration of orotic acid, a fatty acid naturally occurring in raw milk, was stable after the freeze-drying treatment. To the low storage temperatures (4°C and -20°C), metabolites barely changed when stored in a freezer over a long period, relating the freeze-drying as an effective concentration and preservation method for milk concentration with minimal changes on the metabolites.

Effects in milk composition were also proposed by Deshwal et al. (2020), who compared camel milk powder produced by freeze-drying with the traditional method of spray drying (Table 2). According to this study, freeze-dried camel milk powder had the highest dispersibility (67.15%) and solubility (88.77%). This can be attributed to this process, which makes the products lighter than other drying methods. Low solid feed during freeze-drying results in porous particles as a large amount of water is removed during the stages of drying. The lowest acidity was obtained in freeze-dried camel milk powders when compared to the spray drying method (0.193% vs 0.211%), which can cause an increase of minerals precipitation, lactose degradation, and Maillard reactions with a considerable and irreversible pH decrease. These reactions, due to the application of high temperatures, also decreased the calcium and iron contents of camel milk powders when compared to a freeze-drying process (0.011–0.012 g kg^{-1} vs 13.71 and 15.33 g kg^{-1}, respectively), highlighting the important

maintenance of nutritional and functional compounds in a concentration by freeze-drying. The structure of milk fat globules can also change according to specific concentration methods. In a study about the effects of freeze-drying and spray drying on the microstructure of milk fat globules, Yao et al. (2016) pointed that the surfaces of some fat globules after freeze-drying became thicker than those from raw milk and after spray-drying. This technology can cause the formation of irregular flaky translucent sheets with sharp edges, whereas spray-dried fat globules are spherical particles (ZHU; DAMODARAN, 2011). This phenomenon can be explained due to the amphiphilic phospholipids that tend to accumulate on the surface during freezing and then, the fat globules stick together during the freeze-drying process. In addition, the freeze-drying method caused an increase in fat globules, explained by the authors due to the formation of ice crystals and possible repulsion of foreign material away from the interstitials, causing globule aggregation. In addition, the osmotic pressure of the globules may have caused recombination in larger globules (YAO et al., 2016).

The concentration by freeze-drying also can influence the physicochemical properties of a dairy product. In a development of a freeze-dried Petit Suisse cheese, Silva et al. (2021) pointed that this concentration method can influence the conformation of protein networks of the product composition (Table 9). Microscopy of the freeze-dried samples showed structures with large porosities and low agglomeration, indicating minimal interaction between the particles. This characteristic is attributed to the sublimation process of the larger ice crystals that filled the formed cavities with available water in the protein matrix. The formation of large pores in a protein network may be caused by an increase in the positive electrical charge of casein micelles at pH <4.6, reducing intercellular interactions and resulting in the formation of an opening (porous). The protein dehydration promoted by freezing can change the textural and rheological properties, attributing different viscosities after rehydrating. In addition to changes in the composition and dairy structure, freeze-drying has become an interesting and useful method to extend the shelf life of probiotic bacteria in dairy products. Due to damage to cell membranes by the formation of ice crystals and decreasing cell viability, microencapsulation is an alternative for the incorporation of probiotics into the medium, which was proposed by Jouki et al. (2021) in development of a symbiotic freeze-dried yogurt powder using microencapsulation of *L. plantarum* (Table 9). After freeze-drying, the survival rate of probiotic cells ranged from 67.1 – 91.2%, with minimal effect of this process on microencapsulated cells (9.8–10.6% loss). *L. plantarum* microcapsules enriched tolerated the freeze-drying process, featuring the yoghurt powder as a probiotic product for 10 weeks at 25 °C.

Therefore, the use of freeze-drying technology, as well as all alternative concentration technologies, allows the development of functional dairy products, with their nutritional properties maintained and quality enriched, pointing out these unconventional methods as potential substitutes in the concentration of dairy products in the dairy sector.

4 FUTURE PERSPECTIVES IN THE REPLACEMENT OF TRADITIONAL TECHNOLOGIES BY EMERGING PROCESSES IN THE CONCENTRATION OF DAIRY PRODUCTS

The concentration of dairy products becomes one of the main challenges for the food industry, due to the guarantee of products of high quality and linked to a low-cost process. Recent studies, even if carried out on a small scale, show an industrial potential in replacing traditional concentration technologies by emerging non-thermal alternatives.

New research and equipment refinement must be conducted with adaptations of promising laboratory results for large-volume production, as well as the development of specific equipment at low costs, in addition to increasing speed, yield, and soluble solids content in the concentration/separation steps, which can also be developed into a single operation in the same equipment's section. These improvements would be fundamental mainly for freeze-drying and block freeze concentration technologies that carry out an efficient concentration process, but with low yields and, therefore, are not viable in industrial production today. The application of non-thermal concentration processes, in addition to ensuring a high nutritional and sensory quality to the dairy product, can be linked to processes with an environmental appeal, with the reduction of the use of non-renewable energy resources such as the block freeze concentration, and alternatives for the treatment of by-products from milk manufacturing in recent aspects of membrane separation processes.

With constant research and development in the dairy sector, the industrial replacement of traditional concentration technologies by non-thermal methods becomes a future potential and a differential in the final quality of the product, winning consumer preference and becoming an interesting alternative in the competitive industrial sector from an economic and technological perspective.

5 CONCLUSION

Concentrated dairy products are one of the most important manufactured goods for the dairy industry due to the high added value, reduced volume, and lower transport costs. In addition, concentration processes increase the shelf-life, which can expand distribution logistics. Evaporation and spray drying processes are thermal concentration methods that are still traditional in the dairy industry, however, they are unfeasible processes due to the requirement of high energy expenditure and the decrease of nutritional, functional, and sensory properties of milk and dairy products. Nowadays, studies about the application of emerging non-thermal concentration technologies have been explored as means to decrease the adverse effects of traditional processing and present promising alternatives for dairy industries. Among the emerging dairy concentration methods reported in this review, membrane separation processes are advantageous due to enabling high flow rates, capable of removing bacteria and spores from the matrix, low energy expenditure, and reduced total costs. On the other hand, freeze concentration and freeze-drying, however advantageous in keeping bioactive compounds in milk and enable an increase in the quality of concentrated products, become energy-expensive, have a low yield, and are unfeasible for large-scale production. Recent studies involving the application of unconventional processes in the concentration of dairy products are fundamental for the industrial sector, which makes it possible to implement emerging processes on a large scale and increase the quality of commercialized concentrated products.

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CHAPTER 6

Freeze concentration techniques as alternative methods to thermal processing in dairy manufacturing: a review

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Freeze concentration techniques as alternative methods to thermal processing in dairy manufacturing: A review

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ABSTRACT

Freeze concentration technology is applied to concentrate liquid foods at low temperatures, thus separating pure ice crystals from the final concentrate solution. This method is an interesting alternative to concentrate food with high water levels and significant nutritional value, such as dairy products, since several bioactive compounds are reduced when exposed to elevated temperatures. Considered that, this technique may be a great alternative to concentrating and maintaining both nutritional and sensory characteristics of liquid foods, the present review aims to introduce freeze concentration procedures as an eligible choice on conservating dairy products', also addressing its effects on the dairy matrix.

Keywords: concentration, separation, freezing, dairy products, thermolabile compounds.

1 INTRODUCTION

Dairy manufacturing is a common practice in most countries, also being substantial in several regions of the globe. Since there are many techniques, dairy matrixes, and product aspects/presentations, studies on dairy processing have increased significantly, supporting its production and quality improvement (AL-HILPHY et al., 2020; DING et al., 2021; SÁNCHEZ et al., 2011b).

Currently, dairy industries offer a great variety of products, including butter, different types of cheese, fermented milk, as well as products that require concentrating processes to achieve desirable characteristics, such as condensed milk, powdered milk and evaporated milk, *dulce de leche* (dairy product obtained from heating a sugar-milk mixture under controlled conditions), concentrated lactose, and whey protein. Considering milk and its products contain great amounts of water, submitting them to concentration process can improve milk's processing efficiency, facilitating dry matter obtainment. In addition, such process can extend the product's shelf-life, also granting advantages on packing, transportation, and storage, reducing expenses (BALDE; AIDER, 2016; DE LIZ et al., 2020; FIALHO et al., 2018; MACHADO CANELLA et al., 2020; MUÑOZ et al., 2018; MUSINA, 2018; SÁNCHEZ et al., 2010; VARGAS et al., 2021).

Industrially speaking, evaporation is the mainly operation applied on reducing water content from dairy products, however, it requires thermal energy for the water to boil, enhancing production costs (GUICHET; JOUHARA, 2020; LIN et al., 2018; MORISON; HARTEL, 2018; SÁNCHEZ et al., 2010; SCHUCK et al., 2015). Moreover, evaporation's elevated temperatures may decrease native thermolabile compounds, such as vitamins, enzymes, bioactive compounds, interfering on the product's sensory properties. Other non-thermal processes, such as membrane separation, can be applied, nevertheless, membrane pores can become encrusted and blocked due to the high great number of solids that separate from the liquid matrix (GALVÃO, 2018; SÁNCHEZ et al., 2010, 2011a; VERRUCK et al., 2019).

Over the years, new technologies and processes have been improved to guarantee standardized and high-quality products in the market. Freeze concentration technique is based in liquid foods solid-liquid phases' separation under controlled low temperatures (AIDER; DE HALLEUX, 2009; CASAS-FORERO; ORELLANA-PALMA; PETZOLD, 2020; DING et al., 2021b; SÁNCHEZ et al., 2011a), and might be an interesting non-heated alternative to concentrating products with great protein, enzyme, and vitamin content, since such compounds can be reduced or inactivated when submitted to high temperatures. Also, with these methods, original volatile compounds and food pigments are preserved, maintaining product's flavor and sensory characteristics (AIDER; DE HALLEUX, 2009; BALDE; AIDER, 2019; HENAO-ARDILA; QUINTANILLA-CARVAJAL; MORENO, 2019).

The freezing concentration processes shows several application systems in large-scale, thus acting as an attractive possibility for industries, allowing them to select different methods of said technology accordingly to the characteristics of each of their manufacturing processes. This review aims to present freeze concentration's principles linked to dairy manufacturing, also presenting innovative technology on concentration processes.

2 FREEZE CONCENTRATION

The freeze concentration, or cryoconcentration (from the Greek, *krúos*, meaning "cold"), is a technique applied to concentrate liquid foods over a pre-freezing step followed by the separation of pure ice crystals. Once crystallized, the water fraction is removed, increasing soluble solids content in the solution (MORISON; HARTEL, 2018; OJEDA et al., 2017).

During the process, the liquid containing diluted solutes is cooled below its freezing point under controlled conditions, avoiding eutectic temperature. At this specific moment, the

solvent (i.e., water for liquid foods) and one of the solutes freezes simultaneously (OJEDA et al., 2017; RAVENTÓS et al., 2007). When controlled, liquid food's freezing produces ice crystals above eutectic temperatures, increasing crystallized water's purity, preserving all the original solution's properties. Considering the initial solution, the freeze concentration upper limit goes from 40 to 50% of solid content, which varies accordingly to the food matrix and soluble solids rate (MORISON; HARTEL, 2018). Concentration can be performed repeatedly to reduce water content, although it depends the desirable purpose, yield of each concentration stage and food composition.

The more concentrated a solution is, the lower the temperature needed for it to freeze, since solids tend to reduce the freezing point, making it difficult to crystallize solvents. Freezing time is also related to the concentration solution conteiner's form and material. When in contact with container walls, heat transfers will occur faster in the outermost layers of the solution while, in the inner layers, freezing occurs gradually, until reaching its center. Ice crystals are formed by water particle's incorporation within crystallization nucleus, however, in concentrated solutions, water solidifies next to the soluble solids, forming irregular crystals. In order to prevent irregular ice crystal's formation, freeze concentration's temperature and freezing time must be controlled during all separation steps (HARTEL; CHUNG, 1993; MORISON; HARTEL, 2018).

3 FREEZE CONCENTRATION VERSUS THE TRADITIONAL CONCENTRATION PROCESSES

The main advantage of freeze concentrating liquid foods is the maximum preservation of thermolabile compounds, since it does not involve liquid-vapor procedures, which makes such technique an interesting option for many industries, seeking to improve their product's quality. Evaporation, for example, demands great energy for the water to boil at a higher temperature than the maximum limit for preservation of thermolabile bioactive compounds (Table 10). In addition, when compared to traditional evaporation processes, freeze concentration demands approximately 335 $kJ.kg^{-1}$ of water during freezing, which is lower when compared to the required energy in evaporation processes (~ 2260 $kJ.kg^{-1}$ of water), since the latent heat of vaporization is higher than the latent heat freeze concentration's fusion (JUSOH; YUNUS; HASSAN, 2009; QIN et al., 2021).

Bearing in mind industrial costs, the freeze concentration process, when compared to the evaporation process, demands greater initial investments, anticipated in approximately € 2M for 10 m^3 h^{-1} capacity. Nevertheless, when speaking of an evaporation plant, the annual estimates cost is € 4M (ALI et al., 2021; DADRASNIA et al., 2021). Still, evaporation's operational and productive costs are elevated due to great water outflow (~ 16739 kg h^{-1}), steam (~2764 kg h^{-1}), and cleaning procedures, often exceeding € 156 M, considering a 20 T h^{-1} production of concentrated milk obtained from evaporation (MADOUMIER; AZZARO-PANTEL; GÉSAN-GUIZIOU, 2020).

Another limiting factor of evaporation processes is the common obstruction of heat exchangers when concentrating products with considerable mineral counts. Also, the quality of heat exchanges depends on energy consumption during concentration. In freeze concentration, energy savings is related to the possibility of passive thawing as a recovery step of the concentrated fraction. Therefore, the low energy required in freeze combined with passive thawing enhances process' efficiency and reduces operational costs (BALDE; AÏDER, 2017; DING et al., 2021).

In dairy manufacturing, milk's traditional concentration process must be carried out in multistage evaporators at approximately 75°C in the first section of the equipment. However, when milk is heated at 60°C, an irreversible aggregation of heat-sensitive whey proteins occurs, initiating a denaturation phenomenon, resulting in important losses of water-soluble vitamins. In order to preserve nutritional value, employing non-heated techniques is a great alternative. Membrane technology processes apply preserving temperatures thus conserving sensitive milk components. Nonetheless, membrane pores often get obstructed due to high counts of separated solids, also requiring great amounts of cleaning water (AIDER; DE HALLEUX, 2009; CHABAROV; AIDER, 2014; MUÑOZ et al., 2019).

Before choosing and applying concentration technologies industrially, techniques must be taken under consideration and comparison, aligning all expenses of large-scale production. Speaking of food concentration, especially dairy products, in addition to all processing costs, sensory, physicochemical, and nutritional quality must be also considered. When compared to traditional evaporation processes, freeze concentration is the most suitable and emerging technology due to low temperature application, hence preserving main bioactive compounds found in dairy matrices that are responsible for flavor, sensory aspects, and product's functionality. Still, the ability to provide high sensorial quality, great customer's acceptance and reduced costs are permanent targets of all food industries.

Table 13- Advantages and disadvantages of freeze concentration techniques and the traditional thermal concentration process.

CONCENTRATION TECHNIQUES	ADVANTAGES	DISADVANTAGES
SFC	-High purity of ice crystals; - The scraped-surface heat exchanger (SSHE) is efficient to clean ice scaling from the cooling surface; - Specific ice production rate up to 40 kg h^{-1} m^{-2}; - Preservation of heat-sensitive milk compounds; - Conservation of color, flavor, and nutritional value of dairy products; - Applied industrially as a key technology in liquid food production	- Complexity of the system; - Low crystal growth rates; - Complexity in separating ice crystals; - The most expensive among all the freeze concentration techniques; -High costs of investment and maintenance, which hinders its application on large scale; -Presence of fouling; - Few studies applied in dairy products
PFC	-Simple operation management ; -High crystal growth rates; -Easy ice/concentrate separation by drainage process; -No fouling ; - Equipment with no moving parts (except pumps and valves); -Conservation of color, flavor, and nutritional value of dairy products	-High investment cost on large-scale production; -High energy consumption (35–40 kWh t^{-1}) -Huge cooling surface area is required for practical applications; - The ice layer tends to entrain liquid fractions and causes severe solute loss
BFC	-The cheapest process among all the freeze concentration techniques; - Absence of moving parts -Considered a green technology; -Preservation of color, flavor, and nutritional value of dairy products	- Multistage operations to obtain a high level of concentration; -Ice layer has a poor heat transfer coefficient of less than 0.1 kW m^{-2} K^{-1} - Low yield becomes unfeasible for large-scale production; - Loss of significant soluble solids content in the ice fraction;

EVAPORATION	-The most applied concentration technology in dairy manufacturing; -Capable to recover thermal energy; - Low energy costs using multi-stages evaporators; - Increased shelf-life of concentrated products.	- High installation and operating costs; - Bioactive compounds (proteins, enzymes, vitamins, color and flavor compounds) may decrease at high temperatures ; - Denaturation of milk proteins may result in heat-induced fouling; - Intense heat treatment can affect the minerals equilibrium and pH sensibility; - Undesirable changes of sensory properties (flavor, color, and texture) in specific products;

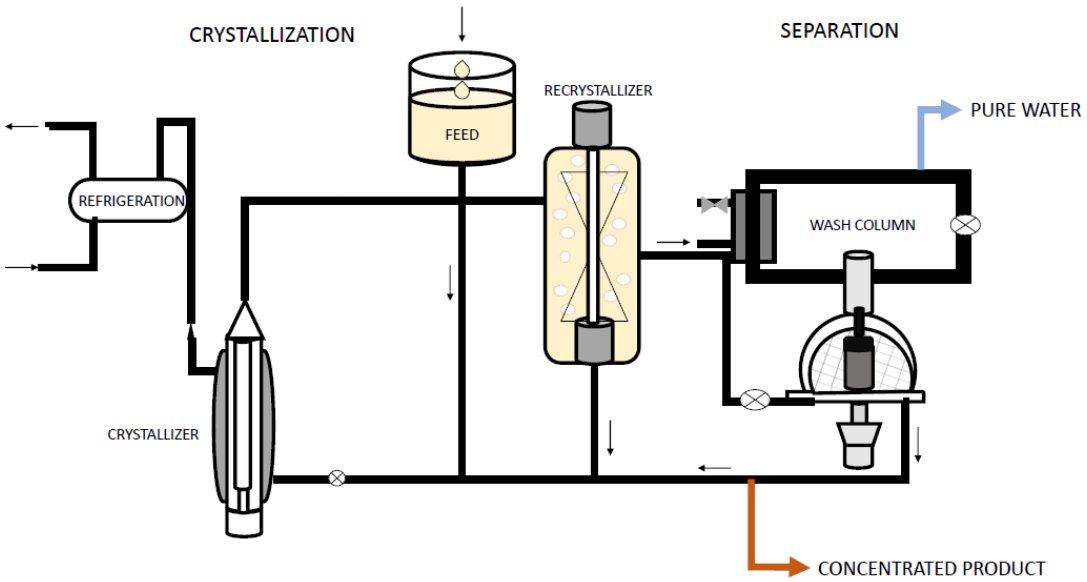
Note: SFC- Suspension Freeze Concentration; PFC- Progressive Freeze Concentration; BFC- Block freeze concentration. Source: Dadrasnia et al. (2021), Dantas et al. (2021), Ding et al. (2021), Madoumier et al. (2020), Peters-Erjawetza et al. (1999), Qin et al. (2003), and Sánchez et al. (2009, 2011).

4 FREEZE CONCENTRATION METHODS

4.1 SUSPENSION FREEZE CONCENTRATION (SFC)

Based in the suspension phenomenon, such technique provides limited sized ice crystals. To efficiently separate the ice from the mother liquor, small ice particles must undergo Otswald ripening, in which small crystals redeposit into larger crystals over time. In such case, there is a need of complex system containing scraped-surface heat exchangers (SSHE) (for feeding ice, cooling the surface, and improving heat transfer coefficient), a re-crystallizer, to increase the size of small crystals, and a washing tower, that allows to separate ice crystals from the final concentrated solution (Figure 23). The suspension process is based on an initial nucleation (crystallization), in which ice crystals reposition themselves into large particles, increasing its volume exponentially. Such phase is followed by ice nuclei in the solution (AIDER; DE HALLEUX, 2009; DING et al., 2021b; SÁNCHEZ et al., 2011a).

Figure 26- Crystallization and separation by suspension freeze concentration.



Source: Adapted from Aider and de Halleux (2009).

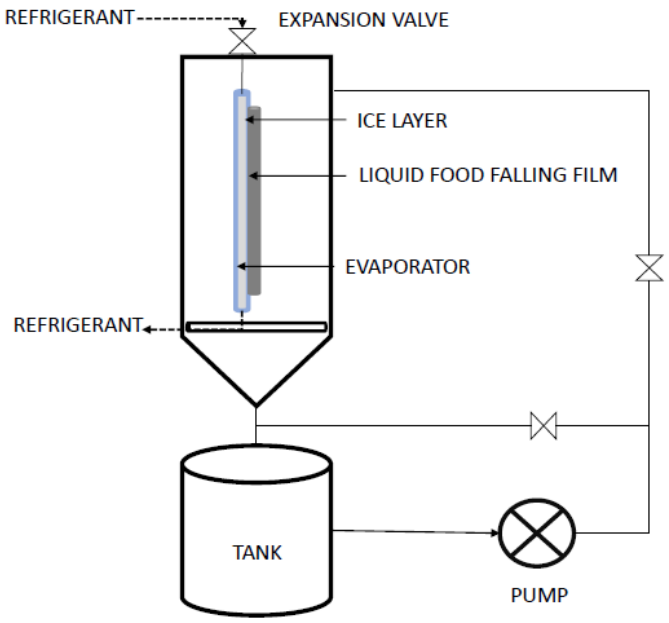
In a complex system such as this, successful separation can form high purity ice crystals (impurity <100 ppm), depending on scraper's speed and solution's concentration. Considering that these parameters are set between 200 and 1000 W m^{-2} K^{-1}, respectively, the production rate of ice crystals is up to 40 kg h^{-1} m^{-2}. Due to its high separation rate, SFC has been applied as key technology when processing liquid foods in industrial environments

(DADRASNIA et al., 2021; DING et al., 2021; QIN et al., 2003), still, it demands many equipment sets and extended operation time, thus being restricted to large scale processes in continuous operation mode. Also, it is the most expensive technique among all freeze concentration procedures, demanding elevated investments, maintenance costs and energy consumption (35–40 kWh t^{-1}) (Table 10) (AIDER; de HALLEUX, 2009; DADRASNIA et al., 2021; DING et al., 2021; MORISON; HARTEL, 2018; QIN et al., 2003; SÁNCHEZ et al., 2011a).

4.2 PROGRESSIVE FREEZE CONCENTRATION (PFC)

Unlike SFC, the progressive freeze concentration is based on layer crystallization, where a large mass layered ice or a single large ice crystals are formed, facilitating separation due to crystal's adhesion to cold surface. Aside from film freeze concentration, this method has become one of the most important ways of concentrating liquid foods, preserving its thermolabile compounds (DANTAS et al., 2021; DE LIZ et al., 2020; MIYAWAKI et al., 2016; MUÑOZ et al., 2019; OJEDA et al., 2017; SAMSURI; AMRAN; JUSOH, 2018; SÁNCHEZ et al., 2010). The process consists in partially freezing the solution under constant agitation, usually employed to decrease solute's on the ice layer. Generally, the ice layer is formed in tank walls (cold surface) and is easily separated from the final concentrated (Figure 24) (DANTAS et al., 2021; OJEDA et al., 2017). This separation can be performed by the same equipment, reducing operational costs, as well as machinery and maintenance expenses (DADRASNIA et al., 2021). However, industrially speaking, investment costs are still high due to the need of great cooling area, essential to achieve significant productivity. Also, the produced ice layers tend to entrain liquid fractions, resulting in severe solute loss (Table 10) (AIDER; DE HALLEUX, 2009; DADRASNIA et al., 2021; MOHARRAMZADEH et al., 2021).

Figure 27- Film freeze concentration system.



Source: Adapted from Raventós et al. (2007).

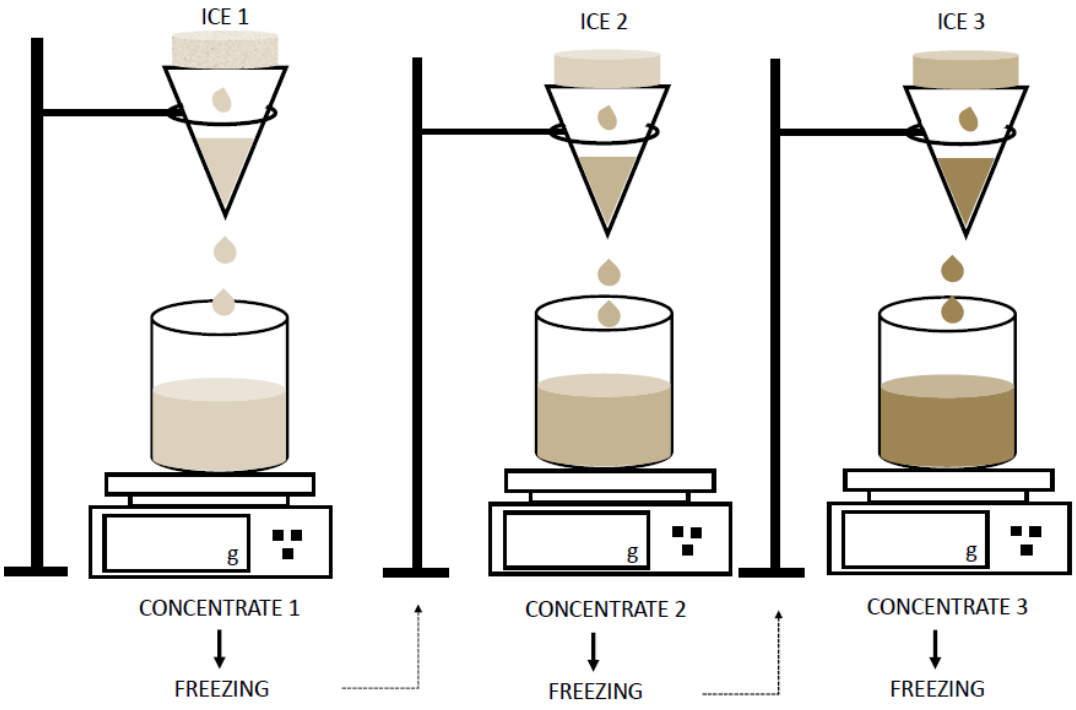
4.3 BLOCK FREEZE CONCENTRATION (BFC)

Block freeze concentration, also known as freezing thawing, consists in completely freezing the solution, that is, the solution's central temperature is below its freezing point. It is followed by partial thawing, performed through an assisted gravitational defrost method (Figure 25) (AIDER; DE HALLEUX, 2009; DADRASNIA et al., 2021; DE LIZ et al., 2020). The ice block acts as a solid matrix, allowing fluids with high soluble solids counts to pass through. Thawing temperature is primordial to increase concentration efficiency, making it possible to overcome a 90% rate, reducing solids content trapped in the ice fraction. Gravity contributes on separate soluble solids through diffusion and, with controlled mass and process time, a gravitational assisted thawing cycle results in an approximated 50% concentration rate (AIDER; DE HALLEUX, 2009). The separation of ice fraction from the final concentrated solution, will associate gravity and supplementary methods, such as vacuum, centrifugal force, annealing, microwave, or ice nucleation protein (INP), improving solute's performance (DADRASNIA et al., 2021; MACHADO CANELLA et al., 2020).

Such technique is reported as the most promising and effective practice to obtain concentrated liquid foods with great nutritional value, also preserving sensory properties, specially in dairy processing (BALDE; AIDER, 2016; BALDE; AÏDER, 2017; BARROS et

al., 2021; CAMELO-SILVA et al., 2021a; CANELLA et al., 2018; DE LIZ et al., 2020; MACHADO CANELLA et al., 2020). One of the main advantages of this process is the absence of moving machinery parts (stirrers or pumps), which reduces production costs. Furthermore, it is an easy to perform technique, making its total cost (energy, operational and equipment expenses) the lowest of all freeze concentration processes (Table 10) (AIDER; DE HALLEUX, 2009; DADRASNIA et al., 2021; MORISON; HARTEL, 2018). Nevertheless, the concentration efficiency of such technique is limited, and the ice layer presents poor heat transfer coefficient (less than 0.1 kW m^{-2} K^{-1}) (DING et al., 2021). However, multiple operations are needed to obtain great concentration levels, also requiring great energy consumption, making it unfeasible to be applied in industrial scale. Many studies have demonstrated efficient results, however, all during laboratory stages of development (BARROS et al., 2021; CAMELO-SILVA et al., 2021a; CANELLA et al., 2019; DADRASNIA et al., 2021; MACHADO CANELLA et al., 2020).

Figure 28- Laboratory scale block freeze concentration system.



Source: Prestes et al. (2022)

5 STUDIES ON FREEZE CONCENTRATION TECHNIQUES APPLIED TO DAIRY MANUFACTURING

Considering freeze concentration techniques' improvement, it is important to develop and adapt processes bearing in mind the specific characteristics of the dairy product, the quality of the final concentrated solution, and the solids yield at the end of the concentration (CHABAROV; AIDER, 2014; MOHARRAMZADEH et al., 2021; OJEDA et al., 2017; SÁNCHEZ et al., 2009)

The efficiency of ice fraction separation and the final concentrated product's quality can be evaluated accordingly to an analytical method in both laboratorial and industrial scales. When developing a concentrated dairy product, it is essential to perform physicochemical, structural, and rheological analyses. Studies that evaluate concentrated dairy matrices and its products' properties prioritize total solids content, soluble solids content, total titratable acidity, and pH analysis, as well as carbohydrate, protein, fat, and ash counts (BARROS et al., 2021; CAMELO-SILVA et al., 2021a, 2021b; CANELLA et al., 2019).

When performing structural analyses on ice fractions and concentrated dairy products, it is important to study the size and behavior of ice crystal's, along with casein micelles and fat globules (BALDE; AIDER, 2016; BALDE; AÏDER, 2017; CAMELO-SILVA et al., 2021a). Moreover, color (determining L*, a*, and b* parameters) and texture analyses performed on specific equipment may grant additional information on the specific product (BALDE; AÏDER, 2017; BARROS et al., 2021). Considering the addition of probiotic strains to dairy products, specific properties must be researched through microbiological tests, gastrointestinal simulations, as well as in vitro evaluation of antioxidant properties (CAMELO-SILVA et al., 2021b; CANELLA et al., 2018; DE LIZ et al., 2020; MUÑOZ et al., 2018).

The analytical techniques mentioned above are recurrent in research involving milk's freeze concentration and dairy products development based on non-thermal processes. Therefore, several studies show improvement adaptations on each freeze concentration method in dairy manufacturing. Such studies should always be encouraged, as they are a key step for the industrial application of these techniques.

5.1 SUSPENSION FREEZE CONCENTRATION IN DAIRY PRODUCTS

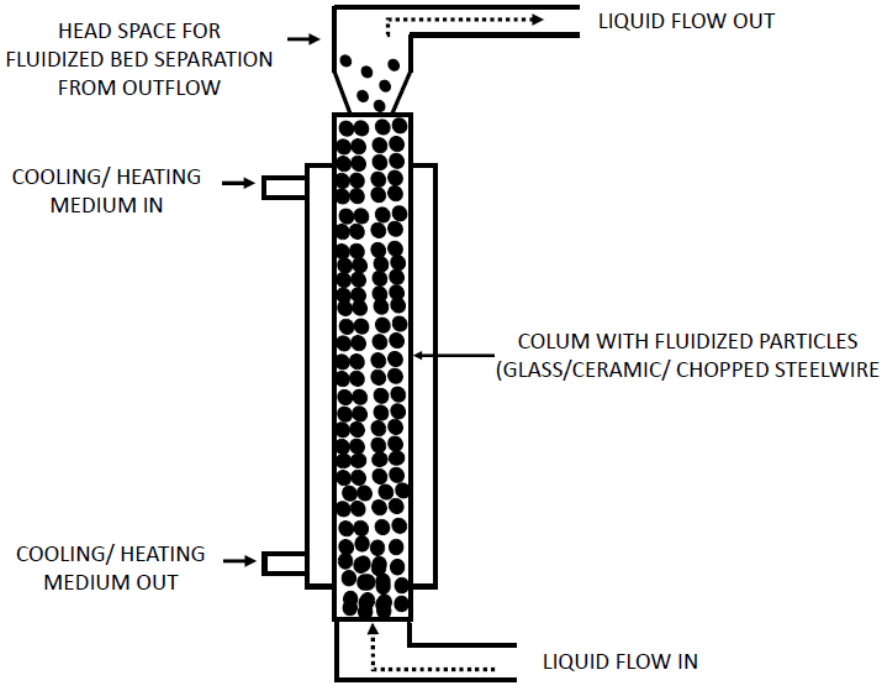
In 1993, Best & Vasavada's pioneer study on suspension freeze concentration's equipment allowed to concentrate whole milk, skimmed milk, whey protein and whey permeate (BEST; VASAVADA, 1993). Due to the variable total soluble solids content of these dairy products, the maximum concentration (w/w) reached was 51% for whey permeate, 46.5% for whey protein, 44% for whole milk, and 40% for skimmed milk. It was verified that whey permeate, containing great amounts of solids, reached the lowest lactose crystallization content (6.70%). An increase of viscosity was observed in products containing casein, such as whole milk and skimmed milk (150 cst and 125 cst for WPC and only 55 cst for permeate), limiting the process. The viscosity's increase during freeze concentration processes is due to the great dehydration of casein micelles during water removal procedures, which increases the volume of its dispersed particles (BALDE; AIDER, 2016). A product's viscosity is inversely proportional to the ability of separating ice fractions from concentrate, acting as a limiter over the maximum reachable concentration. Great casein contents, as well as sugar and fat contents, affects ice crystal's growth and maturity, increasing concentrated dairy products' viscosity, interfering on the crystallization phenomenon, and limiting heat and mass transfer (RAVENTÓS et al., 2007).

A critical subcooling process was executed in order to promote secondary ice nucleation in dairy products (whey extract, whey powder, and skimmed milk) using suspension freeze concentration experimental techniques was carried out with the use of experimental techniques by Hartel and Chung (1993). Among all milk's components, it was noticed that whey protein directly influences critical subcooling temperature. With increased whey protein contents, the critical subcooling temperature also increases linearly, acting as an inhibitor of secondary nucleation, also altering the surface of the ice crystal's microscopic structure. Due to such physicochemical changes and interference on heat and mass transfer phenomena, crystallization and recrystallization adaptions are necessary to reduce undesirable effects when concentrating dairy products through suspension freeze (e.g., process timing, temperature, product's volume, and different crystallization methods).

Several studies have been conducted to enable complex equipment's replacement with simpler machinery, still maintaining high quality production and reducing operational costs. Habib and Farid (2006), for instance, presented a cheaper alternative to SSHE in an experimental study, providing an equipment composed of a fluidized bed heat exchanger

(FBHE) with simpler design, fabrication, and operation settings (Figure 26). Still, this experimental mechanism-maintained scale-free operations inside its heat exchanger during extended periods of time (operated continuously during 22 h), providing considerable heat transferences (850 - 900 W/m^{2}C). With the advent of the FBHE, it was possible to concentrate whole and skimmed milk at different solid content's rates: 13, 14 and 15% for whole milk and 10, 13, 14, 15, and 16% for skimmed milk (HABIB; FARID, 2008). Whole milk's crystals were more resistant to removal when compared to those obtained from skimmed milk. Due to the concentration process, fat globules and casein micelles tend to interact and/or adsorb, increasing large particles' counts and promoting agglutinations, interfering on concentrate and ice fraction separation (TRIBST et al., 2020).

Figure 29- A single tube fluidized bed heat exchanger.



Source: Adapted from Habib and Farid (2006).

Whole milk's fat crystallization occurs when it is submitted to freezing temperatures. The crystallization of fat fractions within aqueous phases can pronouncedly influence on texture, flavor, stability, and appearance of the dairy product. During freeze concentration, milk's temperature decreases rapidly due to intense heat transfers from the product to its freezing surroundings. Generally, faster cooling rates result in unstable and smaller fat crystals that tend to agglutinate, producing voluminous aggregates during crystal's nucleation and growth steps (CEYLAN; OZCAN, 2020; THANASUKARN; PONGSAWATMANIT;

MCCLEMENTS, 2006; WIKING et al., 2009). As a result, the final concentrated milk's viscosity increases, especially under great supersaturation degrees, interfering in mass transfers (by molecular diffusion) and limiting the formation of ice crystals and heat transfers during freeze concentration (WIKING et al., 2009).

Still, milk's fat is responsible for the distribution of milk's matrix components during freeze concentration processes, lending to unique product characteristics. When submitted to low temperatures, fat fractions distribute lactose amounts into two portions (ice and final concentrated milk). Aider et al. (2007) identified that, when freeze concentrating whole whey, lactose and lipids were more present in the ice fraction than in the concentrated fraction (15.37% and 4.22%, respectively). Such phenomenon is possible due to sugar's structural properties, that are capable of increasing mutarotation rates and, consequently, sugar's lipolytic qualities. In addition, milk whey contains relatively high amounts of lactose and phospholipids, which can be solubilized, intensifying lactose-lipid interactions on ice fractions (AIDER; DE HALLEUX; MELNIKOVA, 2009).

It is noted that recent studies on dairy product's suspension freeze concentration are scarce due to its operational complexity, great functioning costs, and the preference for simpler methods. The development of adaptable, easy to operate and accessible equipment should be encouraged to improve suspension freeze concentration technology, allowing its industrial application, since it is a fast process that provides great yield.

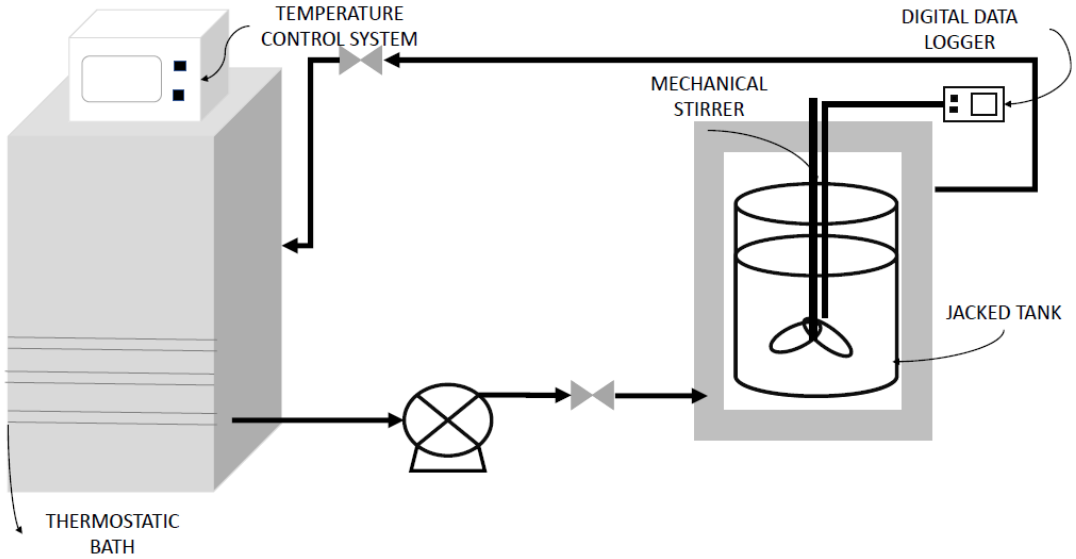
5.2 PROGRESSIVE FREEZE CONCENTRATION IN DAIRY PRODUCTS

The progressive freeze concentration method has been proven as an efficient technique on dairy products' separation. Studies pointed that mass transfer is one of the most interfering phenomena on ice/concentrate ratio, thus supporting the necessity of reducing solute's incorporation into the ice fraction (CHABAROV; AIDER, 2014; DING et al., 2021). Experimental validation and mathematical mass transfer models were proposed by Chabarov & Aider (2014) on skimmed milk's progressive freeze concentration (Table 11). The solute's flow into the ice/concentrate limit layer depends on heat and mass transfers, which increases both ice thickness and ice/concentrate interface's velocity, decreasing process' efficiency (81% efficiency in up to 2 cm ice layers and only 46.5% in 10 cm ice layers). Additionally, ice crystal's growth, combined with high ice/concentrate interface velocity, increases the solute's physical entrapment into the ice fraction, resulting in similar solute concentration in both ice

and concentrate fractions. As well as in the present study, mathematical modeling and experimental validation can be very useful when dealing with freeze concentration simulations, due to parameter predictions and physicochemical phenomena that occurs before operational stages, reducing costs and quickening projects.

A vertical progressive freeze concentration system was proposed by Ojeda et al. (2017) and applied for the first time during skimmed milk concentration performed by Muñoz et al. (2019) (Figure 27) (Table 11). Conditioned in agitated vessels, the highest concentrated yield (82.26%) and efficiency (62.07%) were achieved under 5°C and 1000 $r.min^{-1}$ (approximately 6.5 $g.100g^{-1}$ total solids content), resulting in the purest ice fraction (2.39 $g.100g^{-1}$), thus suggesting that stirring rates influence directly on progressive system's efficacy. Mechanical stirring induces convection and increases solute's mass transfer from the ice fraction to the concentrated fraction. Such vertical system has also proven to be an efficient method on concentrating lactose-free skimmed milk (DANTAS et al., 2021). The process presented 80.81% yield rate and 62.22% efficiency rate at -5°C, 58 min, and 1,035 rpm, as well as 11.68% solids content. Moreover, proteins tended to remain in the ice fraction (final concentrated fraction: 3.80%; ice fraction: 1.05%), while carbohydrates flowed into the concentrated liquid phase (3.39% glucose, 3.22% galactose and 0.12% lactose in the concentrated fraction; 1.21% glucose, 1.60% galactose and 0.02% lactose in the ice fraction). Low weighted carbohydrates showed greater separation and concentration tendencies when compared to proteins, due to their higher molecular weight. Also, solute's mobility can be affected by molecular size and concentration (DANTAS et al., 2021; KAWASAKI; MATSUDA; KADOTA, 2006). Still, such system may be a great technique for future applications involving one or more concentration methods, due to its low equipment and maintenance costs, as well as a relatively fast processing time, since mechanical agitation increases the mass transfer phenomenon and reduces pressure, enhancing the separation process.

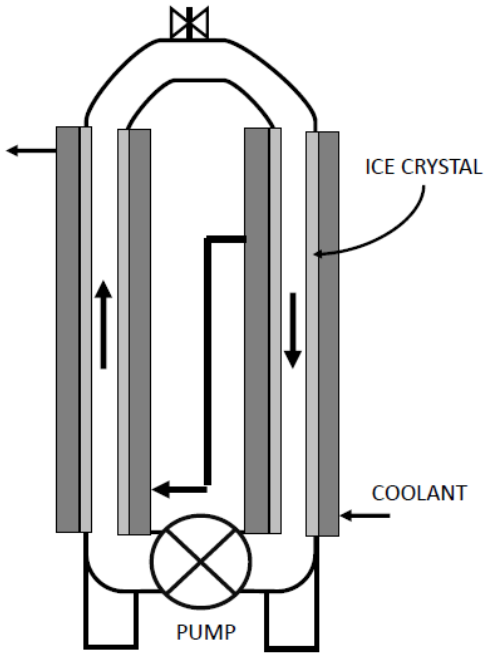
Figure 30- Vertical Progressive freeze concentration.



Source: Adapted from Muñoz et al. (2019).

The vertical progressive freeze concentration is efficient on concentrating milk and dairy products in laboratory scale; however, yield may be lower when compared to suspension freeze concentration (AIDER; DE HALLEUX, 2009; MORISON; HARTEL, 2018). Due to the limiting partition coefficient being dependent on the system's osmotic pressure and flow rate, solute particles are unavoidable retained into the ice fraction when the initial osmotic pressure and solid concentration are elevated. Therefore, a large-scale tubular system was proposed by Miyawaki et al. (2005) where circulating flow enhances concentrate yield and reduces retention of solid fractions in the ice layer (Figure 28). Inside the tubes, ice crystals grow due to a cooling agent. The cooling plate's surface is amplified with serially interconnected tubes, promoting great production (90 to 130 min of operating time, considering a 2.54-3.68 $m.s^{-1}$ flow rate), resulting in approximately 50% of the initial solution as final concentrate. This method can also be an alternative to concentrate dairy products with great yield and reduced costs when compared to the complexity and expensive suspension freeze concentration equipment.

Figure 31- Tubular ice system for progressive freeze concentration.



Source: Adapted from Miyawaki et al. (2005).

5.3 BLOCK FREEZE CONCENTRATION IN DAIRY PRODUCTS

In dairy manufacturing, block freeze concentration is one of the most studied and applied freeze concentration techniques due to its accessible process and interesting results, regarding the maintenance and enhancement of concentrated dairy products' intrinsic characteristics (BALDE; AÏDER, 2017; BARROS et al., 2021; CAMELO-SILVA et al., 2021b; CANELLA et al., 2018; MACHADO CANELLA et al., 2020; MUÑOZ et al., 2018; SÁNCHEZ et al., 2011a).

Studies on dairy products' block freeze concentration are performed on low macronutrient dairy matrices (mainly fat matter), enhancing concentration's efficiency. During milk concentration, the interaction of casein micelles are important to alter the concentrated product's physicochemical, sensory and functional properties of the concentrated product. Balde and Aider (2016) evaluated casein micelle's distribution and its influence on block freeze concentration skimmed milk (Table 11). After three consecutive cycles (total dry matter up to 25.12% and 271.86% efficiency in the third cycle), the block freeze concentration method reduced the size of the casein micelles, increasing concentration stages (160 nm in

unconcentrated skimmed milk to 139 nm after the third concentration) due to the overall contraction, reducing mineral recovery efficiency, especially Ca and Mg (30% and 28%, respectively). Luminosity was also increased after the third freeze concentration cycle (initial skimmed milk L*= 62.68; third cycle L*= 66.97). Milk freeze concentration causes the casein micelles to aggregate, forming larger particles, also related to increased milk luminosity. Whole milk's white color and luminosity are two of the most influent factors to determine purchase intentions by customers. By modifying the conformation of casein, the process can guarantee increased luminosity even in skimmed milk, similarly to whole fresh milk. When developing new products, such visual aspect can enhance global acceptance of reduced fat content milk products.

The block freeze concentration can also be used as a pre-concentration step on powdered dairy product's manufacture, also influencing powder particles and casein micelles' sizes. The conformation of powdered milk particles impacts on its solubility and dispersion in water. Choosing pre-concentration methods is extremely important to produce powdered dairy products with good protein conformation, which is also related to product's final quality. In a study by Balde and Aïder (2017), block freeze concentration was applied as a concentration step to spray drying of skimmed milk during powdered milk production (Table 11). The results were compared to reverse osmosis and vacuum evaporation methods. It was noticed that the size of the powder particles was larger when compared to reverse osmosis and evaporation obtained powders (250 nm, 105 nm, and 62.4 nm, respectively). High evaporation temperatures can cause ruptures and small particle's fragmentation. In addition, block freeze concentrated casein micelles showed higher distribution volumes when compared to reverse osmosis and evaporation processes (66.20, 65.56 and 63.27%, respectively). During freeze concentration, casein micelles are fragmented and can easily associate, forming larger denaturated whey protein and/or casein micelles aggregates.

Recent studies stated block freeze concentration technology's importance to enhance concentrated dairy products' obtainment from non-bovine milk (CANELLA et al., 2019; DE LIZ et al., 2020; MACHADO CANELLA et al., 2020). Currently, research on non-bovine species' milk have escalated, especially due to allergenic properties found in specific conformation protein's present in bovine milk. In addition, differences in quantities and conformation of milk components are related to other mammals' milk increased functionality.

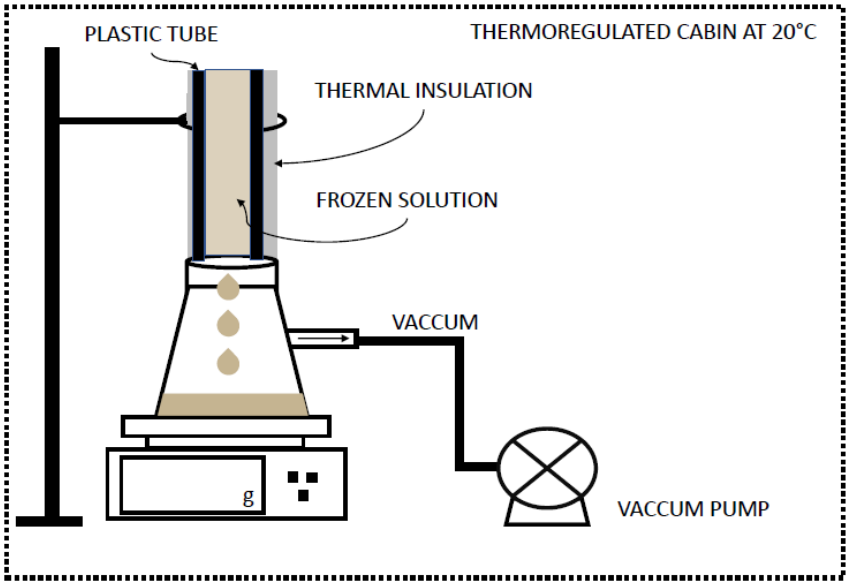
Table 14- Recent studies about freeze concentration in dairy product manufacturing.

Dairy product	Freeze concentration technique	Conclusions	Authors
Skimmed milk	Progressive freeze concentration	Experimental results and a mathematical modeling showed that the process efficiency was freezing rate dependent and the solute flow at the ice/concentrate limit layer was dependent of the heat and mass transfer phenomenon.	(CHABAROV; AIDER, 2014)
Skimmed milk	Block freeze concentration	After three freeze concentration cycles, there was a decrease in the size of casein micelles, color enhancement with high L* values similar to whole milk.	(BALDE; AIDER, 2016)
Skimmed powder milk	Block freeze concentration	Powdered milk particles from the freeze concentration process obtained larger particles and micelles sizes when compared to previous reverse osmosis and evaporation processes. The freeze concentrated skimmed milk was suitable for drying.	(BALDE; AÏDER, 2017)
Probiotic fresh cheese with freeze concentrated milk	Block freeze concentration	The fresh cheese was produced with milk from the second stage of the block freeze concentration. After an *in vitro* gastrointestinal simulation, it was positive the effect of viable cells count of *Bifidobacterium* BB-12 in probiotic and symbiotic cheeses.	(MUÑOZ et al.2018)
Fermented lactic beverage with freeze concentrated cheese whey	Block freeze concentration	Probiotic and symbiotic fermented beverage was developed with concentrated whey from the second cycle of block freeze concentration. The syneresis index, total solids content, color parameters and viscosity were influenced by the addition of inulin and the storage time.	(CANELLA et al. 2018)
Skimmed milk	Progressive freeze concentration	High content of soluble solids and process efficiency at a 5°C and 1000 r/min. The system was applied successfully for the first time in skimmed milk.	(MUÑOZ et al., 2019)
Skimmed goat's milk	Block Freeze concentration	After three cycles of block freeze concentration, the total solids content, total protein, casein, and whey protein increased in both concentrate and ice fractions. The concentrated showed an increase of lightness and color tending to greenish and high viscosity.	(CANELLA et al. 2019)
Goat's whey freeze concentrated as probiotic microcapsules wall materials	Block freeze concentration	Two cycles of block freeze concentration provided powders with high total solids content, stability of *Bifidobacterium BB-12* at cold storage, greenish color, and high lightness. The product presented cohesiveness, water solubility with inulin addition and thermal stability.	(de LIZ et al. 2020)
Semi-skimmed goat's milk	Vacuum-assisted block freeze concentration	Increase in milk solids content. The highest yield in the vacuum equipment was under conditions of 10kPa of pressure, vacuum time of 60 minutes and freezing time of 1 day.	(CANELLA et al. 2020)
Ice cream replaced with whey concentrate	Block freeze concentration	Ice creams were produced with different concentrations of concentrated whey, increasing the total solids content, viscosity, overrun and providing a greenish yellow color. The replacement with whey did not affect the size of the ice crystals and the microstructure of the ice cream.	(BARROS et al. 2021)

Ice cream	Block freeze concentration	The milk from the first cycle of block freeze concentration provided high solids and protein content in the ice cream formulation. Increasing protein content influenced the high lightness, viscosity and overrun.	(CAMELO-SILVA et al. 2021)

An innovative vacuum-assisted block freeze concentration technique performed by Canella et al. (2020) was executed on semi-skimmed goat milk with added NaCl, which influences on freezing and thawing steps, altering ice crystal's conformation (Figure 29) (Table 11). Such concentration system demanded 10 kPa vacuum under 60 min, and 24 hours freezing time. 1.5 to 2% NaCl concentrations provided higher process' efficiency (approximately 90%), also granting the highest protein (10.43 and 10.70 $g.100g^{-1}$) and total solids (35.06 and 36.21 $g.100g^{-1}$) contents. The control sample that didn't receive NaCl demonstrated the highest lactose content (17.42 $g.100g^{-1}$) due to salt's decreasing effect on lactose's solubility, also affecting lactose ice crystal's growth.

Figure 32- Vacuum-assisted block freeze concentration system.



Source: Adapted from Canella et al. (2020).

A freeze concentrated dairy matrix with high total solids' content can also enhance the development of probiotic cells, contributing to a functional appeal (CAMELO-SILVA et al., 2021b; CANELLA et al., 2018; DE LIZ et al., 2020). Muñoz et al. (2018) added a probiotic strain (*Bifidobacterium* BB-12) in concentrated milk after two block freeze concentration cycles (solids content: 19.17 g $100g^{-1}$) in order to develop probiotic cheese (Table 11). The cheese

formulation containing concentrated whey increased dairy products' buffering capacity, also achieving better survival rates of probiotic cells after in vitro gastrointestinal simulations (survival rates: 80.8 - 100.1%). When developing probiotic dairy products with freeze concentrated milk, there is a link between two positive factors: maintaining bioactive compounds through the application of non-thermal concentration processes that benefits the customer's health and the improvement in developing probiotic cells, which is benefited by thermolabile compounds' preservation and the increase of total solids' content, thus making the freeze concentrated dairy matrix a great source of probiotic microorganisms.

Recently, Camelo-Silva et al. (2021) evaluated the usage of freeze concentrated skimmed milk on ice cream production. After the third block freeze concentration cycle (10.27 $g.100g^{-1}$ of total solids and 90.84% efficiency), the concentrated skimmed milk provided an ice cream with great total solids (37.30 $g.100g^{-1}$) and protein (4.92%) contents, as well as luminosity (L*= 84.95), and overrun (63.71%) due to milk's proteins great air retention capacity. Similar behavior was observed by Barros et al. (2021) during ice cream production, where milk was replaced by block freeze concentrated whey (93.35% efficiency and 11.29 $g.100g^{-1}$ of total solids content on the first cycle). The ice cream containing 100% concentrated whey showed lower lightness (L*= 82.45; 50% whey replacement L*= 86.85) due to the absence of casein micelles. The best proportion for whole milk's replacement was 50% whey, with high total solids content (36.98g $100g^{-1}$; control ice cream = 34.75 g 100g-1), which increased ice cream's overrunning (43.85; control ice cream = 27.49). The addition of freeze concentrated milk in ice cream formulation is a marketing differential, allowing flavor and color intensification, as well as preserving bioactive compounds and increasing protein content. Replacing milk fractions with whey provides interesting physicochemical changes, cheaper production and an alternative to whey's disposal, considering it is one of the main polluting residues in the dairy industry.

The block freeze concentration, as well as all the freeze concentration techniques, is a promising technology to be applied in dairy processes and new product's development, providing superior physicochemical and nutritional characteristics, which are attractive to dairy industries.

6 FUTURE PERSPECTIVES ON FREEZE CONCENTRATION IN DAIRY MANUFACTURING

Considering potential large-scale applications of freeze concentration techniques in dairy industries, new research and equipment refinement must be conducted. Developing newer and more efficient crystallizers, especially when it comes to suspension freeze concentration procedures, is extremely important, aiming to reduce equipment and additional costs (DADRASNIA et al., 2021). Furthermore, in view of all freeze concentration methods, there is a need to improve separation's efficiency, as well as to incorporate crystallization and separation steps into a single operation, allowing them to occur in the same equipment's section. Moreover, alternatives on controlling soluble solids' loss from the ice fraction are essential to keep these valuable compounds in the concentrated product (DADRASNIA et al., 2021; HAAS et al., 2022).

In minor production, several studies have shown interesting results obtaining from progressive and block freeze concentration applications on various products, such as lactose-free milk (DANTAS et al., 2021), fermented dairy beverages (CANELLA et al., 2018), different types of fresh cheese (MUÑOZ et al., 2018), ice cream (BARROS et al., 2021; CAMELO-SILVA et al., 2021a), powdered dairy products (BALDE; AÏDER, 2017; DE LIZ et al., 2020) and whey protein (VUIST; BOOM; SCHUTYSER, 2021). Considering such promising results, freeze concentration techniques show great potential to be applied industrially, offering reduction of costs, and providing high-quality products.

CONCLUSION

Freeze concentration is an important technology applied to concentrate liquid foods, maintaining its quality preserving thermolabile compounds, flavor, and color. In dairy industries, this technological approach can significantly contribute to enhancing milk's efficiency, concentrating its total dry matter. Also, such technique provides additional advantages on product's packaging, shipping, and storage. Several studies on progressive, suspension, and block freeze concentrations have been developed to be applied over dairy processing aiming to improve solids content, nutritional and sensory quality through different equipment and separation methods. Nonetheless, a lack of studies on suspension freeze concentration in recent years is noted, which brings the present review an opportunity to expand

information and stimulate new research development on this specific process to achieve constant improvement on dairy products' concentration. Aiming to expand customer's preferences, concentrating dairy products, and maintaining high quality standards can be an attractive proposal to industries from both technological and economic perspectives.

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CHAPTER 7

The use of cold pressing technique associated with emerging non-thermal technologies in the preservation of bioactive compounds in tropical fruit juices: an overview

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THE USE OF COLD PRESSING TECHNIQUE ASSOCIATED WITH EMERGING NON-THERMAL TECHNOLOGIES IN THE PRESERVATION OF BIOACTIVE COMPOUNDS IN TROPICAL FRUIT JUICES: AN OVERVIEW

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ABSTRACT

Thermal processes are necessary to ensure food safety and extend shelf life. However, high temperatures can alter the nutritional and sensory properties of juices, reducing their final quality. The Cold pressing is the most widely used process in the commercial production of juices from fresh fruits, without the addition of heat, preserving all the original nutritional and sensory components, which places these juices in the premium category. Tropical fruits can be a good matrix to produce innovative juices with high amounts of bioactive compounds. These compounds can be reduced or inactivated at high temperatures, making it essential to obtain and preserve these juices through non-thermal processes to ensure maximum maintenance of nutritional quality. Recently, studies showed that a combination of emerging non-thermal technologies before or during pressing could increase the yield of cold-pressed juice, as well as improve its functional properties with a greater release of secondary compounds. The aim of this study was expanding the knowledge of alternatives to produce premium tropical juices, boosting the application of these technologies on an industrial scale.

Keywords: emerging processes, functional compounds, premium fruit juice, tropical fruit, non-thermal processes.

1 INTRODUCTION

Consumption of fresh juices is increasing all over the world. According to Food and Agriculture Organization (FAO), in 2020, world production of tropical fruits was approximately 25 million tons, about 5.2 million tons more when compared to the previous ten years of production (FAOSTAT, 2023). Tropical fruits constitute a comparatively new group in global commodity trade emerged on the international marketplace and comprise a wide spectrum of bioactive compounds such as phenolic compounds, carotenoids, vitamins, and dietary fiber, which are regarded to have functional activities(CÁDIZ-GURREA et al., 2020). Jafari et al. (2023) affirmed that fruits and fruit products, such as fruit juices, are of the best sources of bioactive compounds, which provide a variety of health advantages. However, nowadays people do not consume sufficient amounts of fruits, so the introduction of different formats can help to increase the total consumption of fruit components, as fruit juices (FÁTIMA

BARROSO et al., 2019). Fruit juices are examples of practical and easy-to-eat foods characterized by high pulp juiciness, vitamin C, carotenoids, and polyphenols(GRANONE; HEGEL; PEREDA, 2022a). Therefore, Singh et al.(SINGH et al., 2022) highlighted that fruit juices, due to their excellent nutritional, functional, and therapeutic characteristics, have gained prominence in the everyday diets of people of all ages, classes, and locations. The main successful drivers that are increasing interest of tropical fruits consumption worldwide are appealing sensorial features, exotic character and their undisputable nutritional value related to health promoting activities(CÁDIZ-GURREA et al., 2020).

Several bioactivities have been attributed to phenolic compounds, which could be used for prevention or amelioration diseases, such as antioxidant, anti-allergenic, antiatherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective, vasodilatory, and neuroprotective effects, among others (FÁTIMA BARROSO et al., 2019). For this reason, the growing international demand has driven to an exponential increase in worldwide production of tropical fruits juices in the last years (CÁDIZ-GURREA et al., 2020). For a wide distribution of consumption and preservation, tropical fruit juices can be pasteurized or concentrated by heat treatment. Thermal processes are the most used methods in preserving, concentrating, and increasing shelf life of the tropical fruit juices by the food industry. Heat treatment result in the loss of the nutritional compounds of tropical fruit juices, also generating unpleasant changes in their final taste, flavor, and color degradation. Cádiz-Gurrea et al. (2020) stated that in foods rich in heat-sensitive bioactive compounds there is a decrease in nutritional and functional properties due to intense exposure to heat. Modern consumers expect from food industry products easy and ready to consume, related with health and well-being. Besides, they also expect that processed foodstuffs have the same nutrients than natural foods. Therefore, due to changes in lifestyle, consumers prefer foods with practicality and healthy appeal. The stability of freshness and nutritional compounds increase the industrial interest for the application of non-thermal technologies for extracting fruit juices and, consequently, increasing their quality and yield. Cold pressing is a technique widely used to obtain fresh fruit juices for commercial production, which involves peeling and removing the seeds from the fruit at low temperature, with the differential of fully using the fruit to produce the juice. Compared to traditional processes, Cold pressing is considered an innovative optimized technology to prevent heat and oxidative damages on bioactive compounds(DU et al., 2019; JOHNER; HATAMI; MEIRELES, 2018; KHAKSAR; ASSATARAKUL; SIRIKANTARAMAS, 2019). In this type of processing, the characteristic of the fruit and its maturation stage is extremely important for

the final yield. For tropical fruits, Cold Pressing is common for highly juicy ones, such as citrus (pineapple, acerola, pomegranate, passion fruit, cashew) and non-citrus (mango, melon, watermelon,, coconut, pequi pulp (*Caryocar brasiliense*)) (CORNELIO-SANTIAGO et al., 2022; FERNANDES; SANTOS; RODRIGUES, 2019; JOHNER; HATAMI; MEIRELES, 2018; MARTINS et al., 2020; MURARO et al., 2022; PUTNIK et al., 2019) . However, the Cold Pressing process is not applicable for fruits from the scientific genus *Citrus* (orange, lemon, lime, tangerine, and grapefruit), which constitute a large part of the tropical juice sector, since pressing releases compounds (flavonoids and limonoids) present in the epicarp of these fruits and can generate an undesirable bitter taste for the juice. Thus, the juice is obtained by an extraction method, by squeezing the juicy pulp of the fruits. Table 15 compares these two main methods of obtaining tropical fruit juices.

From an economic point of view, a high tropical juice yield aligned with a high nutritional quality is an extremely important factor for the tropical juice industries and food researchers, who conclude that the quality of juices is highly dependent on the production processes (CARBONELL-CAPELLA et al., 2016; JOHNER; HATAMI; MEIRELES, 2018). The yield of cold juice extraction can be optimized by the fusion of emerging non-thermal technologies that, in addition to increasing yield, allows improving the nutritional, functional and sensory quality of fresh juices. Alternative technologies such as pressure intensified fluid extraction, high pressure processing (HPP), electric pulsed field (PEF), ultrasound, Ultraviolet-C light, and cold plasma are related to “green” concepts and able to provide an increase in juice yield, for being considered efficient methods for the disintegration of plant cells, the reduction of energy requirements compared to thermal treatment of mashes, and an increased release of secondary compounds from fruits (CARBONELL-CAPELLA et al., 2016; JOHNER; HATAMI; MEIRELES, 2018; PUTNIK et al., 2018). It is noteworthy that most of the studies have indicated some effect of UV-C light on phenolic compounds. In some cases, such as in entire tissues, UV-C light may induce an abiotic stress response, activating the phenylalanine ammonia lyase enzyme, which is responsible of the biosynthesis of flavonoids. On the other hand, UV-C light may breakdown the complex phenolic polymers that led to the release of simpler phenolic compounds, which are more easily detectable by the Folin-Ciocalteu reagent (GUERRERO-BELTRÁN; OCHOA-VELASCO, 2021). Despite Folin Ciocalteu being a rapid and typical assay for phenolic determination, however, this method is not as accurate as high-performance liquid chromatography (HPLC) coupled with a photodiode array (DAD) detector. It is worth mentioning that phenolic compounds could be even better determined using

HPLC/DAD, which is considered a powerful method for qualitative and quantitative detection. Typically, the chromatographic conditions of these HPLC methods include the use of a C18 reverse phase column, and a diode matrix detector. The use of HPLC/DAD technique detecting a large number of the simultaneous polyphenolic classes, such as the quantification and the identification, for example, flavonoids, and catechins (DIMCHEVA et al., 2019). Therefore, fruit juices resulting of a non-thermal processing are considered a premium product due to their fresh-like properties and their proven superior quality. In relation to the case of a premium fruit juice, the treatment can be a claim to be positioned in the marked, when compared to the large amount of thermally treated juices present on it. Especially those consumers that are concerned about health and are willing to pay a higher price for premium products(NICOLAU-LAPEÑA et al., 2022).

In order to spread the knowledge about tropical fruits and the obtaining of juices with preservation of their bioactive compounds, and, consequently, its nutritional and functional quality, this review aimed to present recent studies related to the combination of Cold pressing with emerging non-thermal technologies to be a theoretical background for improving the final quality of juices and for industrial technological innovation.

Table 15 - Types of tropical fruit processing.

Process	Tropical fruit	Process conditions	Advantages	Disadvantages
Cold pressing	Citrus and non-citrus fruits: Acerola *(Malpighia emarginata);* Pomegranate; Pineapple; Pequi (*Caryocar brasiliense*); Mango; Passion fruit; Melon; Cashew	- At controlled or room temperature; -Hydraulic press (the most used) : 1- crusher at low rotations per minute; 2- crushed fresh fruits are packed in a thin fabric bag; 3- high power hydraulic press	- More than one pressing method (hydraulic, pneumatic, horizontal basket, continuous plate, belt and screw presses); -Preservation of bioactive compounds, minerals, soluble fibers and enzymes; - Easy operation method; - No temperature oscillations and friction formation; - No presence of pomace and foam; - No water addition; - No solvent or gas addition; - No excessive contact with oxygen; - Positive impact in consumer's choice (healthy appeal)	- It cannot be used for all types of fruits; -It can generate bitterness if applied to fruits of the *Citrus* genus by passing the essential oil from the peel to the juice; - Requires associated methods to increase shelf life
Extraction	Fruits from the scientific genus *Citrus*: Orange; Lemon; Grapefruit; Tangerine; Lime	- At controlled or room temperature: -Two types of methods used by the industry: 1º: the fruit is pressed between two metal cups and the juice is extracted; 2º: the fruit is cut in half and the juice is extracted by moving the two halves against rotating reamers	-Preservation of bioactive compounds (Vitamin C, carotenoids, polyphenols); - No water addition; - No solvent or gas addition; - Obtaining essential oil from fruit peel by analogous processes (a great byproduct) -The pulp (with fiber and insoluble particles) partly disperses in the juice fraction, which causes desirable cloudy and turbid appearance	-It cannot be used for all types of fruits; - Rotating reamers can generate friction and have a small fluctuation in temperature; - Pomace or seed fractions can migrate to the juice fraction, with the need for further separation by finishers, hydrocyclones; - Requires associated methods to increase shelf life

2- COLD PRESSING

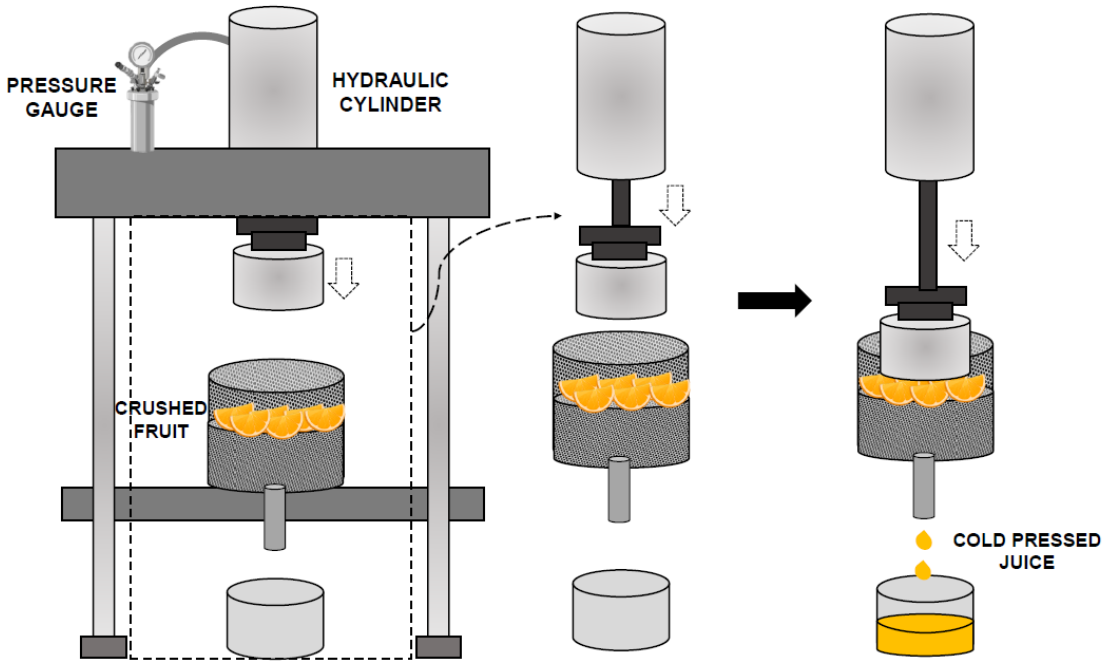
In juice industries, the technique that is currently being used in the extraction of juice from fresh fruits is the Cold Pressing. This technology has become increasingly popular and a large number of industries have started using this method for the production of premium quality juice(MARTINS et al., 2020). The method consists of extracting the juice by crushing the fruits, without adding heat, in order to preserve the enzymes and nutrients as close as possible to their original composition(GHINEA; PRISACARU; LEAHU, 2022). Table 2 shows recent studies (ADEKANYE; ADELAKUN, 2017; DIVYA PRIYA; PYDI SETTY, 2019; LAGUNES-DELGADO et al., 2022; LENEVEU-JENVRIN et al., 2022; MANZI et al., 2022; MONTOYA-ARROYO et al., 2020; OBOH et al., 2015; TIRAVIBULSIN et al., 2021; TÜRKYILMAZ et al., 2013) on obtaining tropical fruit juices (citrus and non-citrus) by cold pressing process, as well as the main benefits in their sensory, physicochemical and nutritional quality.

The most common presses for extracting fresh fruit juices are horizontal basket, membrane, continuous plate with filter, belt, screw, pneumatic and hydraulic presses. Currently, various engineering firms have manufactured these different fresh fruit juice presses, being that each type has a different mode of operation. Horizontal basket presses work by pressing fruits held in a large basket. The press basket has a liquid channeling plate having an arrangement of holes and channels that creates a perimeter seal along a circumference at the bottom of a loaded press basket. The liquid channeling plate traps naturally occurring sludge along the circumference bottom and allows for efficient evacuation of extracted juice from the bottom of the basket as the press mechanism is operated. The basket is usually stainless, though food-grade plastic or wood are options as well. Usually, their advantage in gentleness is a disadvantage in the fruit juice cold pressing. Membrane presses rotate several times before pressing again. This press-rotate-repeat cycle aids in breaking up the pressed-out cake, exposing fruits that might not have been pressed the first time around. The continuous plate with filter press is complete with a feed pump and a hydraulic system, for manually or automatically closing the filter. The filter pack consists of plastic plates with a central manifold for distributing the product to the filter and manifolds at the corners for collecting the filtrate. Each filter plate is complete with a sheet of polypropylene fabric. The juice belt press presents rollers that become smaller and smaller, and the filter belt is S type. They generate diminishing pressure and shear force to achieve one of the best press effects. To improve the pressing rate this equipment has a roller that generates linear and peripheral pressure. Another press used in the

fresh fruit juice obtaining is the screw press. In this press type, the fruit moves forward with the rotation of the screw shaft; along the mud cake outlet direction, the pitch of the screw shaft gradually becomes smaller, the gap between the ring and the ring also gradually becomes smaller, and the volume of the spiral cavity continuously shrinking. Under the action of the back pressure plate at the outlet, the internal pressure gradually increases. Under the continuous driving of the screw-pushing shaft, the juice is squeezed and discharged, and the solid content of the filter cake is continuously increased. However, it is important to emphasize that Kobus et al. (2018) concluded the necessity of further research on the use of screw presses to produce fresh juices with health-promoting properties.

In the pneumatic press, the air is forced into a cylinder, and when pressure is applied the press moves downward. After the press has completed the task at hand, the air is released by valves and springs move the pump back up inside the cylinder. Like hydraulic presses, pneumatic presses leverage compressive forces. Nevertheless, hydraulic, and pneumatic presses are not the same. Like hydraulic presses, pneumatic presses leverage compressive forces. The difference is that hydraulic presses use pressurized liquid, whereas pneumatic presses use pressurized gas or air. On a small scale, in the cold pressing of fruit juices, the hydraulic presses are one of the most used, because provide a good performance and higher yield (Figure 33). However, on an industrial scale, all types of presses mentioned above are used in the production of fresh fruit juices.

Figure 33 - A schematic overview of a hydraulic press for obtaining juices.



The most important parameter of the pressing process is the liquid or 'juice yield', which refers to the percentage of juice pressed out, compared with the amount of raw material that was entered into the system. The juice yield is determined basically by the preparation and pretreatment of the fruit before pressing, and the pressure applied (VATAI, 2010). In general, using the presses mentioned above, fruit juice's yield rate can increase by 20-35% and reach 65-86% based on the total mass, when compared with homemade methods. Some of these presses have been used for different types of fruits, including citrus and non-citrus tropical fruits (ADEKANYE; ADELAKUN, 2017; MURARO et al., 2022). Due to their universality, all presses cited in the present work could be used in the elaboration of all tropical fruit juices; being dragon fruit, pineapple, and mango juice more frequently produced for the belt press; and coconut, pineapple, papaya, and passion fruit for the screw press. It is noteworthy that, among these presses, the belt press has the main advantage of energy saving.

Due to these conditions, this pressing process makes the shelf life of fresh juices very short, requiring storage under refrigeration or by fusion treatments before and/or after pressing, so that the microbiological standard is maintained, as well as the nutritional, functional and sensory quality of freshly pressed juices. Tropical fruit juices are frequently taken owing to their nutritious benefits, fresh appealing flavor, and vibrant color. However, enzymes naturally present in fruit juices, including peroxidase, polyphenol oxidase, pectin methyl esterase, and lipoxygenase, causes quality degradation of fruit juices quality (UMAIR et al., 2022) .Umair et al. (2022) highlighted that the pressure used in cold pressing has a partial effect on covalent-bonds of low-molecular-mass compounds such as pigments, vitamins, and volatile substances as compared to high-molecular-mass molecules including enzymes or proteins. The pressure exerted in this process is not enough to inactivate the enzymes that cause oxidative degradation due to their three-dimensional structure stabilized by covalent/non-covalent interactions. Therefore, series of reactions are needed in the destruction and formation of new linkages, folding, and unfolding thus causes to bring changes in natural structure of enzymes. It has been reported that the degree of enzyme inactivation varies depending on the food type and processing conditions. For same fruit or cultivar, the processing time and temperature can be different depending on the type of technology used for enzyme inactivation. Therefore, in tropical fruit juices, the inactivation of endogenous enzymes is very important to produce high-quality products(GALAVERNA; DALL'ASTA, 2014).

For juice industries, yield is an important factor for commercialization. Before fruit processes, previous parameters that impact the yield must be analyzed, such as the properties

of the raw material and its degree of ripening, degree of pulp milling, mash treatment, and the number of juice drainage channels. During fruit juices processing, various types of enzymes are used, which includes pectinase, cellulase, amylase, tannase, and amyl glucosidase. These enzymes not only assist in breaking down the cell walls of the fruit and vegetables and release the liquids and sugars but also help to improve the storage period of the processed food products. These enzymes find applications in the processing of juice fruit and are extracted from various sources, which include plants, animals, and microorganisms. Enzymes are preferred to chemicals in the fruit and vegetable processing industry due to their high specificity in biochemical reactions(BASHEER; CHELLAPPAN; SABU, 2022).

With the purification and standardization of pectinolytic enzymes, which increase the yield in the extraction of juices, the application of cold pressing on an industrial scale is relevant and can be even more encouraged. In addition, the press can be designed with dimensions for small or large scales, being easily adaptable to produce large volumes of juice by batch. The ease of scaling presses for any production volume that makes the cold pressing process advantageous over other traditional methods of juice extraction. In traditional juice blenders, for example, in addition to the reduced size to produce large volumes of juice, can quickly modify the color of the juice by increasing the area susceptible to oxidative processes, reducing the concentration of bioactive compounds. Hegazi et al. (2021) compared the physicochemical and nutritional aspects of pomegranate juice obtained by cold pressing and with traditional processes such as juice blenders and half-cut hand pressing. In these conventional processes, the juices showed an unpleasant dark color, lower concentration of phenolic compounds, tannins, anthocyanins, and antioxidant activity. In cold-pressed juices, this study revealed that increasing both extraction time and pressure improved fruit juice yield and improved the retention of bioactive compounds (flavonol-anthocyanins adducts) as well as their antioxidant activity. Some beverage manufacturers have introduced cold-pressed juices and have claimed that they are healthier and could be stored for more days than homemade methods, such as the normal centrifugal juices. This process separates the juice extract from fruit flesh by centrifugal force, and when the metal blade spins at a very high speed, it generates heat, which can negatively affect the bioactive compound contents of the juice. On the contrary, the transformation of fruits into juice by cold pressing occurs at a very low speed. This juicing process does not generate heat, usually preserving the health benefits of the juice(KHAKSAR; ASSATARAKUL; SIRIKANTARAMAS, 2019). Health-benefiting properties of fruit juices are described mostly by their bioactive compounds, such as vitamin C, total phenolic content,

carotenoids, tocopherols, anthocyanins content, ellagic acid derivatives, organic acid, saponins, volatile oils, quinones, alkaloids, and tannins, as well as the antioxidant properties (ESMEETA et al., 2022; FERNANDES et al., 2022; KHAKSAR; ASSATARAKUL; SIRIKANTARAMAS, 2019). These natural bioactive compounds are particularly important to produce functional foods, which also have industrial relevance (BANWO et al., 2021). Bioactive compounds are responsible for the wide range of biological properties of fruits, among which the antimicrobial, anticarcinogenic and antiproliferative activity. On the other hand, nowadays, consumers are looking for natural products, such as cold-pressed fruit juices with nutritive and functional value(SCROB et al., 2022) (Table 16).

However, the technology chosen to produce fruit juices depends mainly on the type of fruit and how its tissues and juice are distributed within the pulp, in addition to its maturation stage. The water solubility of pigments and compounds present in fruits should also be considered. In this way, cold pressing is not applicable for all types of citrus or non-citrus fruits (Table 16). Fruits with a high pectin content and with little juicy pulp, such as bananas and avocados, have a low content of liquids in the cellular vacuoles of the plant cell, causing the pressed product to have an appearance and texture of puree instead of juice. In a special case, we can mention the fruits of the scientific genus *Citrus* (orange, lemon, tangerine, grapefruit and lime), which are widely consumed due to its availability, high nutritional values, desirable taste, odor, and color (AGHAJANZADEH; ZIAIIFAR; VERKERK, 2021). In these specific fruits, the epicarp and pericarp have levels of flavonoids, limonoids, and their glycosides (naringin and limonin, for example) that can generate an undesirable bitter taste(BUSTO et al., 2014). In the cold pressing process, the pressed epicarp could release these compounds to the juice fraction, causing an extreme bitter taste and unfeasible for consumption. In this case, the fruit juice from the genus *Citrus* is produced by an extraction process. This process can be performed in two ways: in the first, the fruits are placed between two metal cups equipped with sharpened metal tubes. When the cups press the fruits, the juice is extracted and recovered by the tubes that penetrate the fruits. In the second extraction method, the fruit is cut in half and the juice is extracted by moving the two halves in rotating reamers (GALAVERNA; DALL'ASTA, 2014) In these processes, hydrocyclones and finishers are necessary to remove remnants of seeds and pomace that can migrate to the juice fraction , in addition, analogous processes for recovering the essential oil from the peel are carried out, obtaining a by-product of high added value. Table 15 shows a comparative summary between the two processes for

obtaining juices for the main tropical fruits (cold pressing and extraction), highlighting their main advantages and disadvantages.

Studies show that the fusion of emerging technologies with pressing and extraction techniques can optimize the final characteristics of fresh juices, such as the application of ultraviolet - C light, pulsed electric field, and high-pressure processes. The application of these technologies allows increasing the shelf life of fresh juices (cold-pressed or extracted), which have a short time for consumption. In addition, these processes preserve the color of natural juices and most of their bioactive compounds (vitamins, carotenoids, enzymes and, phenolic compounds), contributing to the preservation of their functional, nutritional and sensorial characteristics(BARBA et al., 2016; DIAS et al., 2020; DZIADEK et al., 2019; EL KANTAR et al., 2018; GALAVERNA; DALL'ASTA, 2014; KOUTCHMA et al., 2016; PUTNIK et al., 2019). In the next topic of this review, the main preservation technologies in the tropical fruit juice will be briefly discussed, whether by thermal or non-thermal processes.

Table 16- Studies on tropical fruit juices obtained by the Cold Pressing technique.

Tropical Fruit	Country	Results
Coconut	Thailand	-The cold pressing (hydraulic pressing machine at 340 bar) combined with ohmic heating inactivated *Clostridium sporogenes* spores more than 5 log cfu/mL at 121.1 °C for 5 min; -The heat treatment preserved desired coconut aromas (alcohols , esters, acids, lactones, aldehydes)
Melon, passion fruit and mango	Brazil	-With cold pressing, the juice yield was between 65.6-73.9%; - Fiber content: 0.84-.091 g/100mL and Antioxidant activity: 129,55- 154,82 µmolTE/100mL); - High sensory acceptance of approximately 86%
Pineapple	France	-In fresh juice, essential oils (0.05%) limited fungal population below 5 log CFU/mL during 14 days; -The low pH of pineapple juice (3.55) probably strengthened the effect of antifungal compounds; - Total acids: 0.83% ± 0.14%, Total soluble solids: 17.0°Brix ±0.1°Brix, L*: 69.2 ± 4.7, a*: 1.3 ± 1.1 and b*: 43.8 ± 8.2. These parameters did not significantly change compared to initial condition after 10 days of refrigerated storage.
Passion fruit	China/ Rwanda	-A mixture of pasteurized cold-pressed passion fruit juice and eucalyptus extracts (40%) inhibited yeasts and mold, total coliform, and total viable count by 100, 58.3 and 100%, respectively, after 3 days of storage (37°C); - After 18 days of storage (37°C), there was no growth of microorganisms for the 20, 30 and 40% juice concentrations.
Mango	Mexico	- After juice pressing from 1kg of fresh unripe mango pulp, It were obtained 37-42g of starch (dry basis); - Isolation procedures resulted in a starch fraction with high purity (87–91%)

3- THERMAL AND NON-THERMAL PROCESSES IN PRESERVING THE QUALITY OF TROPICAL FRUIT JUICES

In the composition of tropical fruit juices there is a high concentration of water and carbohydrates (fructose, glucose, and sucrose), which makes this premium product extremely perishable. Therefore, analogous technologies are needed to increase the shelf life of tropical fruit juices and ensure their food safety. In addition, sensory properties can be negatively altered without the application of preservation technologies after processing juices, such as enzymatic browning and its consequent oxidation of polyphenols, degradation of pigments and original fruit flavor compounds. Table 17 presents a comparative summary of studies involving the application of thermal and non-thermal methods in preserving the quality of tropical fruit juices.

Traditionally, fruit juices are subjected to the thermal pasteurization process to increase shelf life. High - temperature and short-time (HTST) pasteurization (90-95°C; 15-30s) are used in large-scale juice production. Due to tropical fruit juices contain a high concentration of organic acids, they have a low pH (for citrus fruits, for example, pH<4.6). Classified as high-acid products, they can be pasteurized at a temperature below 100 °C, since, in this group of foods, the microorganisms and spores that can contaminate them are thermosensitive (AGHAJANZADEH; ZIAIIFAR; VERKERK, 2021). However, bioactive compounds related to color, antioxidant activity and flavor are reduced or inactivated at high temperatures, which tends to change color, loss of nutritional value, and appearance of "off-flavors" (KOUTCHMA et al., 2016; WANG; XU, 2022).

In the preservation of juices, in addition to traditional pasteurization, other thermal processes are also applied with different methods and reduced temperatures, such as infrared irradiation (IR), microwave, and ohmic heating (OH), which are very efficient methods for reducing microbiological and enzymatic degradation after the production of fresh juices(AGHAJANZADEH; ZIAIIFAR; VERKERK, 2021) (Table 17). Infrared irradiation (IR) is a part of the electromagnetic spectrum in a wavelength range between 0.5 and 100 μm range (AGHAJANZADEH; KASHANINEJAD; ZIAIIFAR, 2016). In food processing, this technology is employed in the inactivation of microorganisms, cooking, blanching, thawing, dehydration, baking, or roasting. In a study with the application of IR in key lime juice and compared to a conventional heat treatment (AGHAJANZADEH; KASHANINEJAD; ZIAIIFAR, 2016), the constant rate of acid ascorbic degradation was lower (0.013 to 0.213

1/min) than the traditional process (0.018 to 0.319 1/min). Concerning color parameters, at higher temperatures, the juice became darker, with a significant effect on Browning Index (approximately 0.20 at 90°C and 0.12 at 60°C) and a 10℃ rise in temperature improved the cloud value of the IR- treated juice (1.44 times higher than the fresh juice). In another relevant study with the application of IR in dried lime juice (ABOUD et al., 2020) (60, 75, and 90°C; 350, 525, and 700 W), there was higher preservation and extraction of ascorbic acid in all temperatures (34.86-36 mg/100mL), as well as for the total phenolic content and antioxidant activity (242.09–244.41 mg/g gallic acid and 33.33%–62.05%, respectively). Results revealed that neither coliform bacteria nor yeast and mold were grown in the IR- treated samples. When compared to traditional heat treatment methods, the IR process becomes advantageous with equipment compactness, faster uniform heating, direct heat penetration, high energy efficiency, faster and uniform heating, and lower degradation of nutritional components.

Microwave heating, also included in the thermal technologies group, is based on the process of energy conversion into heat via friction of dipoles and ionic species that try to follow the oscillating electric field (frequency ranging between 300 MHz to 300 GHz). Due to microwaves generating energy through an entire volume of material in a short time, the processing time becomes reduced when compared to other conventional thermal processes. In addition, there is no need for an intermediate liquid interface for heating, which also prevents the development of unpleasant flavors, and pathogenic microorganisms, and allows the retention of nutritional compounds(KUBO et al., 2019). In "Pera" orange juice treatment by microwave, the retention of vitamin C content was improved (91% for 60s at 70 °C to 85% for 160 s at 90 °C)(AMARO; TADINI, 2021). It also was observed that the residual of PME (Pectin methylesterase) activity decreased with increasing exposure time and temperature (99% inactivation of the PME at 80 °C for at least 20 s). This enzyme is responsible for the degradation and conversion of pectin in low methoxylation degree, which reacts to calcium cation and produces insoluble pectate, causing a colloidal suspension that composes an orange juice cloud. In a study with the application of microwave in tangerine juices, 34s at 700W were optimum parameters to a 5-log reduction in *E. coli* and 5.27, 5.12, and 7.19 log reductions for *S. aureus*, *S. Enteritidis*, and *L. monocytogenes*, respectively(RACOVITA et al., 2022). This process was a potential alternative preservation technique for tangerine juice, resulting in no significant quality depreciation.

Another thermal process also used for preserving fruit juices is ohmic heating (OH). Also known as electrical resistance heating, the basis of this process is the passage of an electric

current, by two electrodes, (electroplasmolysis) through a liquid fraction containing dissolved ionic salts (in this case, fruit juices) and a reduction in electrical conductivity, which enhances the change in the temperature of the juice. In this technology, there is the advantage that the exposure time to electrical conductivity is short and, in addition, electroplasmolysis causes rupture of the cell walls of microorganisms, inactivates enzymes and can also increase the release of secondary compounds from the plant cell (AGHAJANZADEH; ZIAIIFAR; VERKERK, 2021) . In the application of OH in orange juice (ATUONWU et al., 2020) there was no significant difference ($p<0.05$) in the vitamin C content of the juice by OH with the control (approximately 50mg.100mL^{-1}). For the volatile compounds analyzed by GC/MS chromatography, the chromatographs of the control sample and the heat-treated juice were very similar, indicating that there was no change in the compounds related to the aroma of orange juice. In addition, OH showed low energy expenditure (265 kJ/L). In a recent study using ohmic heating as a sterilization process in coconut milk (TIRAVIBULSIN et al., 2021), *Clostridium sporogenes* spores were inactivated more than 5 log CFU/mL at 121.1 °C for 5 min. Color parameters were also improved with the OH treatment when compared to untreated samples (L*= 89.49 and 86.85; a*= -0.43 and -0.44; b*=6.60 and 4.14, respectively).

As efficient as thermal methods are in preserving the quality of tropical fruit juices, the change in food temperature, even if it is minimal, can decrease the levels of the most sensitive bioactive compounds (such as vitamins, carotenoids, and phenolic compounds). Thus, the emerging non-thermal technologies present studies with satisfactory results in the preservation of the nutritional, functional, sensorial, and microbiological quality when applied in fruit juices (Table 17)

Table 17- Thermal and non-thermal processes in the preservation and improvement of tropical fruit juices quality

		Tropical fruit juices	Process conditions	Positive Effects	Negative Effects
Thermal technologies	Pasteurization	Orange	HTST (90-95°C; 15-30s)	- Widely used in large-scale production; -Increase in shelf life; - Reduction of the microorganisms content of the juice	- High energy expenditure; -Thermal treatments negatively affect carotenoids, anthocyanins, and color; - Appearance of "off-flavors"
	Pasteurization	Orange	95°C-60s	- no change in sugar, pH, and acidity levels	-increases of 48.8% in the browning index; - total amino acid reduction (27.65% to 25.42%) - The content of tangeretin decreased significantly after thermal process
	Microwave	"Pera" orange	Heating at 2450 MHz (300W); 70, 75, 80, 85 and 90°C; 0-180s	-Faster heating rate; -Reduced processing time; -No difference ($p<0.05$) in the pH, acidity, and soluble solids content in the juice; - 99% inactivation of the PME in orange juice when exposed to 80 °C for at least 20 s; -Ascorbic acid retention ranged between 91% for 60s at 70 °C to 85% for 160s at 90 °C	-
	Infrared radiation (IR)	Key lime	-Radiant heating chamber with infrared module (1500 W); -60, 70, 80 and 90 °C in 15, 10, 5, 2.5 min using IR	- Compared to a pasteurization process, the IR decreased the processing time (higher energy efficiency); - Equipment compactness ; - Uniform heating ; -During conventional treatment, the constant rate of ascorbic acid degradation (0.018 to 0.319 1/min) was higher than the IR process (0.013 to 0.213 1/min)	-Reduction of acid ascorbic content (by 10°C rising, decreased 2.60 and 2.53 times) ; - The color of the juice became darker at higher temperatures

Table17 (continuation)

		Tropical fruit juices	Process conditions	Positive Effects	Negative Effects
Non-thermal technologies	High pressure processing (HPP)	Orange	550 MPa -5 min	-Compared to pasteurized orange juice, HPP orange juice was presented more freshness ; -Higher preservation of vitamins when compared to thermal processes; -Non-volatile and volatile compounds were higher when compared to pasteurized orange juice	-
	High pressure processing (HPP)	Pineapple	500 MPa ;10 min	- shelf life of 21 days at 4 °C; - No significant difference ($p < 0.05$) for sugar content, pH, titratable acidity, total soluble solids, and sedimentation index; - Greater retention of original color parameters (L*= 80.82; a*= -3.76, b*=20.03) - High retention of antioxidant activity (day 0: DPPH= 252 µMTE/100mL for HPP juice and 250 µMTE/100mL for control juice)	- High juice turbity
	High pressure processing (HPP)	Pomegranate	350, 450, 550 MPa; 1, 3, 5 min	- No difference ($p < 0.05$) of vitamin C content compared to control juice (6.57 mg/L); - No difference of Browning Index (0.39) - No difference of anthocyanin content compared to fresh juice (128.5 mg/L); - No difference in antioxidant activity	-
	Sonication/ Ultrasound (US)	Pomelo	Frequency: 50 kHz, 0–120 min; Temperature:20–70 °C	- decrease of naringin concentration (original content= 1308 µg/mL; US+ enzyme treatment= 430 µg/mL); - high antioxidant activity (hydroxyl scavenging activity = 25.5% for US+ enzyme-treated juice and 20% for fresh juice); - High TPC content for US+ enzyme treatment (approximately 2000 µg GAE/ mL, fresh juice= 1500 µg GAE/ mL)	- TPC was significantly decreased from 1834 µg GAE/ mL to 1542 14.35 µg GAE/ mL; (resin + US treatment)
	UV-C light and HPP	Citrus juice (grapefruit, orange and lemon)	UV-C: 253.7 nm/1000 L/h; 411.4 mJ cm^{-2} HPP: 600 MPa for 3 min	- good retention (< 25% reduction) of ascorbic acid -Higher preservation of original color (L*= 52.25; a*= 1.34; b*= 38.81) , phenolic compounds (30.26mg GAE/100mL for UV-C; 32.92 mgGAE/100mL for HPP), and antioxidant activity (UV-C= 252.42 µMTE/mL; HPP= 278.58 µMTE/mL)	- overdosed UV-C treatments (> 90% reduction of ascorbic; - overdosed UV-C caused undesirable sensory changes to citrus juice

	High pressure processing (HPP)	Orange	550 MPa -5 min	-Compared to pasteurized orange juice, HPP orange juice was presented more freshness ; -Higher preservation of vitamins when compared to thermal processes; -Non-volatile and volatile compounds were higher when compared to pasteurized orange juice	-
	High pressure processing (HPP)	Pineapple	500 MPa ;10 min	- shelf life of 21 days at 4 °C; - No significant difference ($p < 0.05$) for sugar content, pH, titratable acidity, total soluble solids, and sedimentation index; - Greater retention of original color parameters (L*= 80.82; a*= -3.76, b*=20.03) - High retention of antioxidant activity (day 0: DPPH= 252 µMTE/100mL for HPP juice and 250 µMTE/100mL for control juice)	- High juice turbity
	High pressure processing (HPP)	Pomegranate	350, 450, 550 MPa; 1, 3, 5 min	- No difference ($p < 0.05$) of vitamin C content compared to control juice (6.57 mg/L); - No difference of Browning Index (0.39) - No difference of anthocyanin content compared to fresh juice (128.5 mg/L); - No difference in antioxidant activity	-

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High-pressure processing (HPP) has become one of the most emerging methods for inactivating the microorganisms and enzymes of thermolabile foods. High pressures are controlled at room temperature and, in foods, range from 400-600MPa (AGHAJANZADEH; ZIAIIFAR; VERKERK, 2021; ATUONWU et al., 2020; MARTINS et al., 2020; WU et al., 2021) (Table 3). In a recent study with the application of HPP in pineapple juices (WU et al., 2021), good shelf life was obtained (21 days at 4°C), there were no significant differences ($p< 0.05$) for sugars content (fructose = 13.84 g/L; glucose = 12.38 g/L; sucrose= 42. 58 g/L) , pH (4.18), acidity (9.22 g/L), total soluble solids (13.51 °Brix), and no influence in color parameters (L*= 80.82; a*= -3.76, b*=20.03). However, turbidity was higher for juice treated by HPP (day 0= 0.25 for HPP pineapple juice and 0.22 for control; day 28= 0.4 for HPP pineapple juice – approximated results). The HPP treatment could not completely inactivate the activity of pectin methylesterase (PME) and pectin esterase (PE). For antioxidant activity, the HPP retained high activity of free radicals in the pineapple juice (day 0: DPPH= 252 μMTE/100mL for HPP juice and 250 μMTE/100mL for control juice - approximated results). In a study with pomegranate juices, the application of HPP (350, 450, 550 MPa; 1, 3, 5 min), better preserved the anthocyanins (128.5 mg/L), increased their bioaccessibility, and improved the color quality (Browning Index= 0.39) of cloudy juices. Compared to the control juice, there was no difference ($p< 0.05$) in vitamin C content (6.57 mg/L) .

Pulsed Electric Field (PEF) is also a novel non-thermal technology that exerts a high voltage on the juice for a very short time. In foods, typical process conditions are electric field strengths (*E*) between 5–40 kV/cm, and a total pulse time of 20 μs up to 2000 μs. High-intensity PEF can induce electroporation of cell membranes leading to the inactivation of microorganisms, and preserving food safety (TIMMERMANS et al., 2019). In a mathematical study with orange and watermelon juices, with a moderate PEF, the degree of resistance to the electric field of bacteria and yeasts (*E. coli, L. plantarum, S. cerevisiae, S. Senftenberg, L. monocytogenes*) was observed(TIMMERMANS et al., 2019). The temperature parameter is related to the strength of the electric field, indicating that for the inactivation of 50% of the microbiological cells, a higher temperature was necessary. PEF resistance of the species followed the order: *L. monocytogenes* > *E. coli* > *L. plantarum/S. Senftenberg/S. cerevisiae*. The composition and structure of the cell wall may be related to the resistance to the electric field. The thicker peptidoglycan cell walls of Gram-positive bacteria may have resulted in greater resistance to tearing than the thin peptidoglycan layers of the cell wall of Gram-negative bacteria. The application of PEF in a study with cantaloupe melon juice (LI; YANG; ZHAO,

2021) also guaranteed a preservation in the microbiological quality of the juice with the inactivation of , approximately, 6 log of *S. cerevisiae* (30 kV/cm; 400 μs). Concerning the pH of the juice, viable counts were reduced by about 2.8 log and sub-lethally injured cells were generated by less than 1.0 after PEF treatment at pH 4.0.

Supercritical carbon dioxide (SC–CO_2) technology, also called high-pressure carbon dioxide (HPCD) has emerged as a potential non-thermal technology for the inactivation of spoilage and pathogenic microorganisms and endogenous enzymes responsible for the deterioration of fruit and vegetable juices at mild temperatures (lower than 50°C, since the critical temperature and pressure of the CO_2 are 31.2°C and 7.38 MPa, respectively) (PLAZZOTTA; MANZOCCO, 2019; SILVA; MEIRELES; SALDAÑA, 2020).At high pressures, carbonic acid is produced and CO_2 molecules are dissolved in the juice, decreasing the pH, which is temporal until the removal of the pressure. The application of supercritical CO_2 is considerable a sustainable technology because it is a non-flammable, non-toxic, GRAS (generally recognized as safe), and a cheap gas, without causing alteration in the properties of food(AGHAJANZADEH; ZIAIIFAR; VERKERK, 2021). In a comparative study of the physicochemical properties of heat-treated and HPCD-treated banana juices (YU et al., 2013), it was observed a great reduction of Poliphenol oxidase (PPO), one of the main enzymes responsible for undesirable enzymatic browning in fruits and vegetables. Compared to heat-treated banana juice, the residual PPO was 40.7% at 45 °C, which decreased to 11.6% when the temperature increased to 60 °C. For, heat-treatment, only a reduction of 9.1% at 55°C and 20.5% at 60 °C. For color parameters, HPCD-treated juices showed a lower a* value (0.65; for heat-treated juice = 7.89), b* value (9.77; for heat-treated juice=24.51) and higher L* value (91.43 at 60°C; for heat-treated juice= 59.78) at 60°C. In a study with orange juice treated by HPCD at a temperature lower than 40 °C and pressure up to 30 MPa (BRIONGOS et al., 2016), there was an efficient inactivation of pectinmethylesterase (PME) (Residual activity= 0.1 at 21°C; 30MPa). This non-thermal treatment did not change the original physicochemical properties of the juice, such as pH (4.09), total soluble solids (11.35°Brix), and total acidity (0.53g citric acid/100mL).

Sonication/ultrasound (US) is also considered a non-thermal method of treating fruit juices. US may be used alone or in combination with other preservation technologies, at mild heat temperatures, high pressures, and antimicrobials(ZINOVIADOU et al., 2015). It is considered vibrational energy that produces sound energy from mechanical or electrical energy. For fruit juices, it is applicated a low frequency-high power ultrasound (20–100 kHz, 10– 1000

W/cm) is efficient to extract compounds, inactivate microorganisms and enzymes by chemical or physical effects, such as free radical formation or mechanical cavitation (AGHAJANZADEH; ZIAIIFAR; VERKERK, 2021). In pomelo juice, the application of ultrasound was used as a way to reduce the bitterness of the juice in combination with the use of hydrolytic enzymes(KUMAR GUPTA; PRATIM SAHU; MISHRA, 2021). The optimum conditions for sonication with debittering using enzyme were 50 kHz, 2 min, and 45 ℃ while 50 kHz, 60 min, and 60 ℃ were obtained for enzyme hydrolysis. The initial naringin concentration in the juice, one of the main compounds that cause bitterness, was 1308 µg/mL and, after the ultrasound +enzyme treatment, was 430 µg/mL. The US treatment with an enzyme produced a debittered juice with higher content of TPC than fresh juice (approximately 2000 µg GAE/ mL, fresh juice= 1500 µg GAE/ mL), as well as high antioxidant activity (hydroxyl scavenging activity = 25.5% for US+ enzyme-treated juice and 20% for fresh juice). The increase in the bioactivity of the juice could be due to acoustic cavitation produced by US and controlled enzymatic cell wall degradation, enhancing the release of more soluble antioxidant polyphenols into the juice. In a recent work with cashew apple juices treated by US (DELI et al., 2022) (24 kHz, 85W for 15 min), had a residual polyphenoloxidase (PPO) of 16.87%. Ascorbic acid content increased by 22% after sonication and phenolic compounds, such as gallic, syringic, caffeic, chlorogenic acids, and naringenin increased respectively by 125%, 113%, 80%, 31%, and 60% after treatment. This increase can be related to the release of phenolic components throughout intracellular structures (cell wall and membrane) injured by US.

Ultraviolet radiation (UV) is an extremely efficient non-thermal method for food preservation, especially for fruit juices(BAYKUŞ; AKGÜN; UNLUTURK, 2021; DE SOUZA et al., 2020; KOUTCHMA et al., 2016; PREETHA et al., 2021; PUTNIK et al., 2019). The electromagnetic spectrum ranges from 100 to 400 nm (UV-C =200 to 280 nm), however, UV-C 254 nm is suggested to be applied in water and juice treatment. In different microorganisms, UV-C light is capable to rupture the structure of DNA or RNA, which causes cell lysis(AGHAJANZADEH; ZIAIIFAR; VERKERK, 2021). In a recent study with orange and pineapple juices, it was observed the effect of UV-C light on the inactivation kinetics of *Escherichia coli* (PREETHA et al., 2021). At a high fluence rate of 5.6 W/cm^2 there was a *E.coli* reduction in 3.5-3.6 log for the juices. In this work, it was possible to conclude that a photochemical mechanism can cause damage to cellular structures such as the cell walls, cytoplasmic membrane, causing cell death. The inactivation of *E.coli* (5 log) was also provided

by the UV-C pulsed light treatment (312.6–761.4 J cm^{-2}; fluence rate 5.21, 6.59, and 8.46 W cm^{-2}) in a recent study with pomegranate juices (BHAGAT; CHAKRABORTY, 2022). In addition, a total inactivation in polyphenoloxidase and peroxidase activities along with microbial safety was attained for the treatments at 2988 J cm^{-2}.

The fusion of these non-thermal technologies approached with tropical fruit juices obtained by the cold pressing process can optimize the preservation of their nutritional, sensorial and microbiological quality. In the following topic of this review, recent studies with the application of these technologies in cold-pressed tropical juices are briefly discussed.

4- EMERGING NON-THERMAL TECHNOLOGIES COMBINED WITH COLD PRESSING IN TROPICAL FRUIT JUICES PRODUCTION

Emerging non-thermal processes guarantee the production of fruit juices with specific and advantageous in preserving flavor, aroma, and nutritional qualities, with short processing times, reduced energy requirement, eco-friendly techniques, and improvement of food quality and safety. Associated with cold pressing, which has the advantageous action of obtaining fresh juices with maximum maintenance of bioactive compounds at mild temperatures (section 2), the fusion of non-thermal technologies is an interesting addition to this process due to the need to preserve the freshly pressed juices to increase shelf life and ensure freshness and food safety at all stages of the process: production, storage, logistical distribution, and consumption.

In general, emerging technologies can be applied during the first step of cold pressing, when extraction is considerably reduced due to cake compactness and blockage of drainage channels. At this specific point, there is an increase in the permeability of remaining cell membranes, which enhances the extraction of intra-cellular juices. On the other hand, these technologies can also be coupled at the end of pressing for the inactivation of deteriorating or pathogenic microorganisms, as well as enzymatic oxidative processes.

Innovative supercritical and subcritical fluid extractions (SFE) (carbon dioxide ($scCO_2$) or subcritical and supercritical water) are examples of emerging technologies that improve the extraction of fruit juices and their bioactive compounds. Compared to conventional methods, SFE is advantageous in reduced extraction time, less solvent volume, less toxic waste, and better reproducibility. Carbone dioxide CO_2 is a non-polar compound, in its pressurized form, the polarity increases and facilitates the extraction of similar compounds, such as polyphenols.

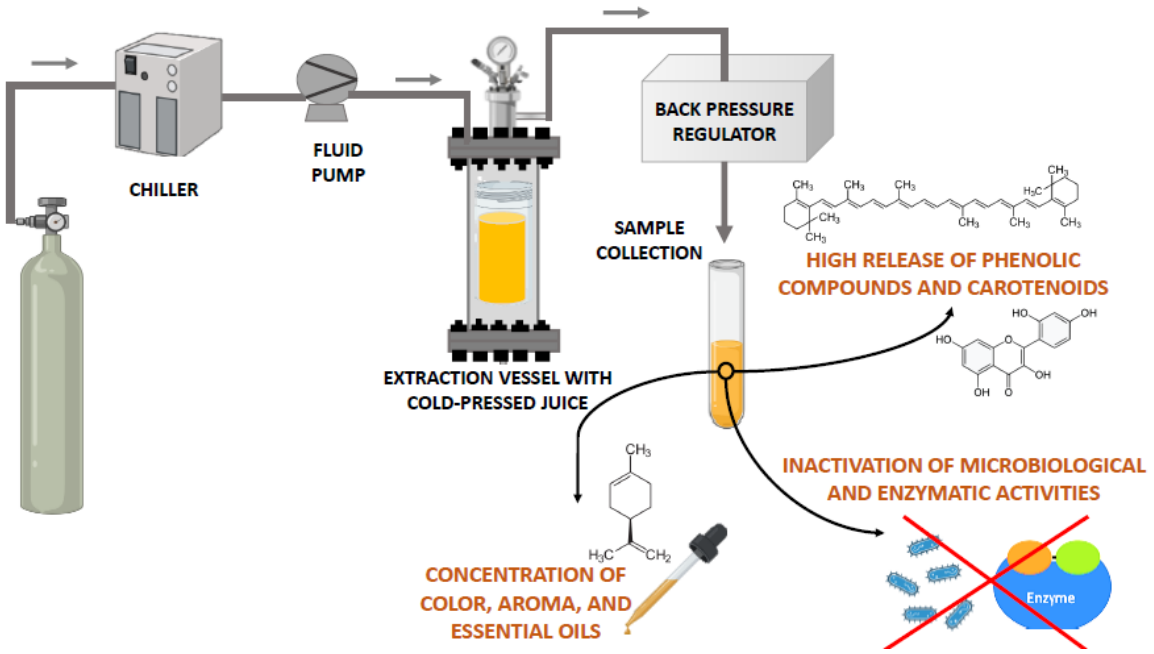
In this emerging process of extraction, usually carried out in an inert atmosphere at low temperatures, there is no light. These factors contribute to prevent thermal degradation, oxidation and/or biochemical changes to phenolic compounds (ARGUN et al., 2022). In pomegranates juices, consolidated with high levels of phenolic compounds and antioxidant activity, the extraction of these bioactive compounds from cold-pressed juices also became improved when associated with processes involving high pressures. Under these conditions, there is a greater efficiency in the release of phenolic compounds from pulps, seeds, peel, and membranes (HEGAZI et al., 2021; YUAN et al., 2022). Associated with a pressing process, Hegazi et al. (HEGAZI et al., 2021) concluded that the release of phenolics, tannins, and anthocyanins in pomegranate juices varied greatly according to the applied pressure and observed that as pressure increases, total phenolics increase (3141 mg/L).

Supercritical fluids also can be applied as an effective pasteurization process in tropical fruit juices. In pomegranate fresh juices treated with $scCO_2$ (45 °C, a treatment time of 40 min and a pressure of 12.7 MPa), Bertolini et al. (BERTOLINI et al., 2020) obtained juices with mesophilic bacteria counts below the detection limit of the plate count method. When carbon dioxide is at supercritical conditions, there is an extreme solubility that allows its penetration into microbial cell and its membrane modification. Thus, the intracellular pH and electrolyte balance are altered, leading to cell inactivation(BERTOLINI et al., 2020).

During cold pressing, supercritical fluid extraction also can increase the extraction of terpene hydrocarbons from citrus fruit peel oil, to prevent the development of undesirable flavors and inactivate oxidative enzymatic activities (GRANONE; HEGEL; PEREDA, 2022b; SILVA; MEIRELES; SALDAÑA, 2020). In a study with the application of $scCO_2$ in fruits, including orange juices (6 to 20 MPa; 25-55°C), the authors observed the inactivation of oxidative enzymes related to a decrease in the quality of fresh juices, such as Polyphenol oxidase (PPO) and pectin methylesterase (PME). Residual enzyme activity was around 26% after 180 minutes of treatment (BENITO-ROMÁN et al., 2020)*. Enzyme inactivation occurs by acidification of the medium by the presence of carbon dioxide in its pressurized form, decreasing the pH, and conformational changes in the secondary and tertiary structure of the enzyme. In addition to inactivating enzymatic reactions, there was also a decrease in dispersed particles, improving the homogeneity of fresh juices (Table 18). Pressure intensified technologies also extract bitter components, such as limonin and naringin, providing a debittering in the fresh juice. In addition, selective green solvents in super/subcritical fluid extraction guarantees high quality and high added value for the by-products of citrus fruit juice

processing, such as concentration of aroma from essential oil from the peel, pectin, carotenoids, and seed oil recovery (Figure 34) (AYDENIZ-GUNESER, 2020; MYRTSI et al., 2022). Supercritical fluid extraction combined with cold pressing also provides a higher yield of fruit pulp extraction, with lower amounts of solvent, which is proved in studies involving the extraction of pequi pulp (*Caryocar brasiliense*), a Brazilian fruit with high lipid contents, with the extract mass eight times higher than the traditional supercritical extraction. A pressurized liquid extraction was used in a study with an environmentally friendly solvent (isopropanol at 60–90 °C; 10.35 MPa, static time: 1-10min; 3 cycles of extraction) to obtain the fat content from the pressed pequi pulp (CORNELIO-SANTIAGO et al., 2022)**. This process was highly efficient, with an approximate recovery of 93% of the lipid content, with a high concentration of total phenolic compounds (64.23–589.46 mg GAE/kg of lipid). The authors also observed that the supercritical extraction of oil from pequi pulp did not change the fatty acid profile with emphasis on the considerable levels of oleic acid (18:1n-9; approximately 51%).

Figure 34- Sub/supercritical fluid extraction in cold-pressed juices.

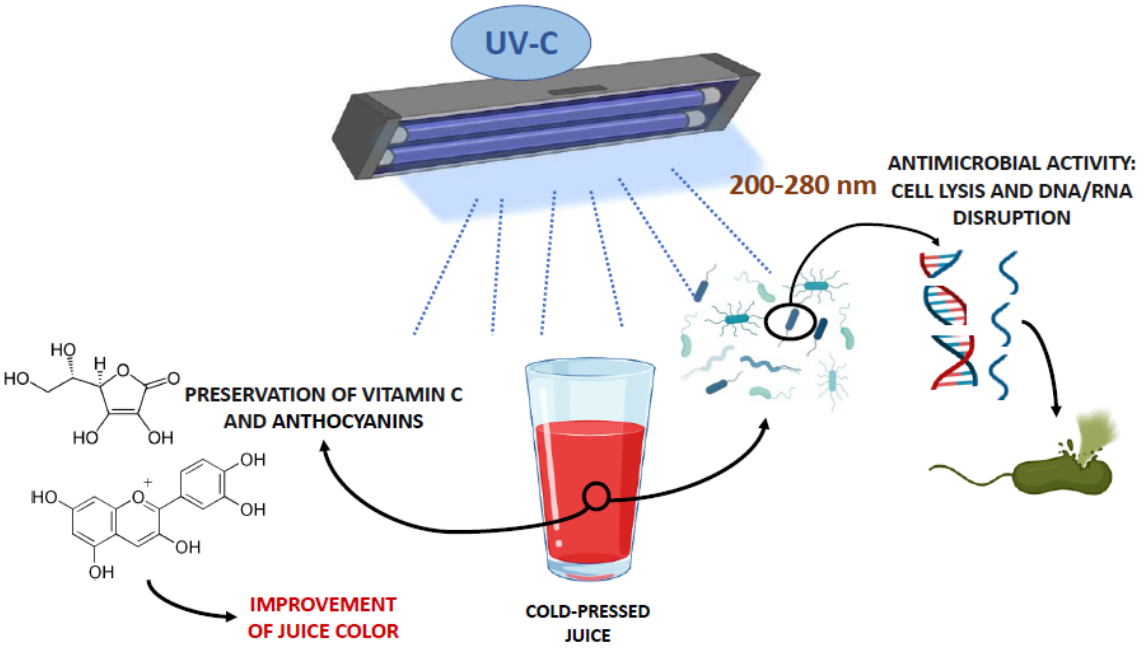


Source: The authors (2023).

Ultraviolet- C (UV-C) light is also an emerging technology that promotes high preservation of phenolic compounds in cold-pressed juices in addition to improving the color of juices, have an effective action in the elimination of bacteria, fungi, spores, and viruses (HEGAZI et al., 2021) (section 3, Figure 35). At a wavelength of 254 nm, the UV-C light is

currently used to disinfect water and foods by causing damage to cellular DNA, promoting the formation of bonds between thymine molecules that hinder DNA replication. Moreover, high doses of UV-C light can destroy the integrity of the plasma membrane, which leads to leakage of intracellular components and eventually to cell death(ANTONIO-GUTIÉRREZ et al., 2017).In a study with orange juice treated with UVC-light (279 nm), the initial population of *S. cerevisiae* was 7.41 log CFU/mL. As exposure time increased under the same average irradiance, the *S. cerevisiae* count were reduced by 0.15, 0.40, 1.19, 2.68, 4.03, and 4.86 log after radiation exposure at doses of 160, 280, 580, 820, 1,180, and 1,420 mJ/cm^2, respectively(NIU et al., 2021). The food safety guaranteed by this technology becomes viable when coupled with the cold pressing process, as it ensures that the fresh juice can be consumed in a longer shelf life, with the same initial properties of a freshly pressed juice. This non-thermal technique, at adequate levels and exposure time, also presents a good effect in the preservation of tropical fruits rich in ascorbic acid. In a study with cold pressed lemon, grapefruit and orange juices (DE SOUZA et al., 2020)* exposed to UV-C light (253.7 nm/1000 L h^{-1}/411.4 mJ cm^{-2}) there was a reduction of less than 25% in ascorbic acid, total phenolic compounds and antioxidant activity, biocomponents highly sensitive to mechanical processes, heat, and oxygen exposure. In this specific study, the authors observed that the effectiveness of the treatment was influenced by juice composition and UV transmission as well as the fluence level (Table 18). The application of UV light on fruits can also improve the release of bioactive compounds contained in plant cells. Through a response to the abiotic stress process, there is activation of the enzyme phenylalanine ammonia lyase enzyme, which synthesizes flavonoids. Complexes of phenolic compounds in polymeric form are also broken down by UV-C light and transformed into simpler compounds with greater antioxidant activity (GUERRERO-BELTRÁN; OCHOA-VELASCO, 2021).

Figure 35- Action of UV-C light in cold-pressed juices.

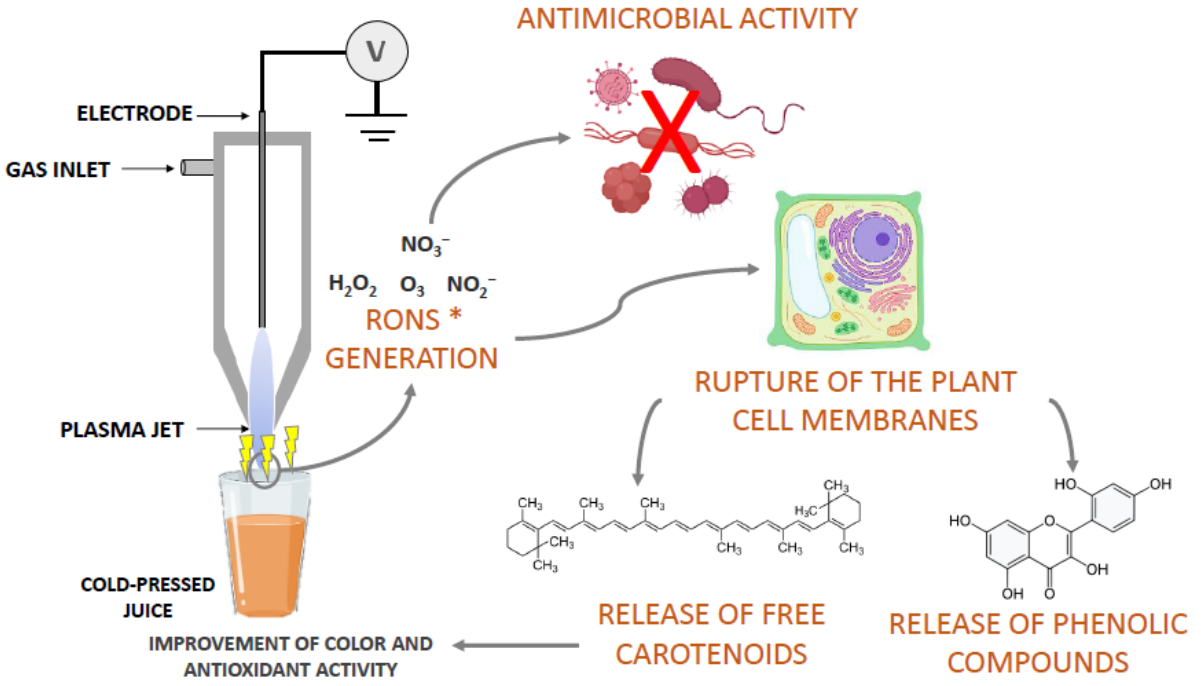


Source: The Authors (2023).

Cold atmospheric pressure plasma (CAPP) is also a prospective novel to improve the quality of fruit juices(POHL et al., 2022). This is an ecofriendly and low-cost technology which, in addition to guaranteeing an increase in the shelf life of products, also contributes to the improvement of the preservation of functional compounds. Considered the fourth state of matter besides the solid, liquid, and gaseous states, plasma is formed when the temperature of a gas is increased to a point where ionization occurs. Thermal plasma can reach 10^7K, cold plasma (below 40°C) inactivates microorganisms and enzymes of fresh products without altering its nutrient and sensory quality (MAYOOKHA et al., 2023). This occurs through the formation of several reactive oxygen and nitrogen species (RONS), in the plasma-liquid interface that can polymerize/depolymerize combined compounds and release them in their free form, potentiating antioxidant, antimicrobial, and anticancer activities (Figure 36). In orange juices, Xu et al.(XU et al., 2017) reported that pectin methylesterase (PME) were reduced in 74% after a CAPP treatment. Enzymatic inactivation occurs by the interaction between amino acids of enzymes and the plasma-induced RONS or free radicals (O_2^-, HO_2, OH, and NO). Furthermore, cold plasma can induce modifications in the secondary structure of enzymes resulted from the interaction between the protein polymer and the reactive species of plasma(MAYOOKHA et al., 2023).

The excitation frequency of cold plasma can also impact the bioactive compounds bioaccessibility. In studies with acerola juice (*Malpighia emarginata*), for example, the application of cold plasma after cold pressing improves the retention of most of the original vitamin C levels, in addition to increasing the carotenoid content, especially for the levels of vitamin A (FERNANDES; SANTOS; RODRIGUES, 2019). Ionized nitrogen species can break bond between pulp cell membranes and carotenoids molecules, which contributes to the release of lipid-soluble components and increases the concentration of free carotenoids in the juice. This increase in carotenoid levels enhances the final orange color of the acerola juice, which enhances its sensory properties. In a study with cashew apple juices, there was also an increase in the bioaccessibility of vitamin C in samples treated with cold plasma. The processed juices presented 820.62 (200 Hz) and 825.7 mg L^{-1} (700 Hz) of vitamin C, compared to control juice (637.97 mg L^{-1}). This increase can be related the conversion of dehydroascorbic acid back to ascorbic acid by the activation of the enzyme dehydroascorbate reductase through the ascorbate–glutathione cycle generated by plasma, which can modify some chemical and physical properties of food matrix (LEITE et al., 2021).

Figure 36- Cold Plasma and Its benefits in cold- pressed Juices.



Source: The Authors (2023). *Note: RONS= Reactive oxygen and nitrogen species.

Table 18- Recent studies on cold pressing and extraction to obtain tropical fruit juices combined with non-thermal technologies.

Emerging technology	Tropical fruit	Process conditions	Conclusion
Supercritical fluid extraction	Pequi (*Caryocar brasiliense*)	Isopropanol at 60–90 °C; 10.35 MPa, static time: 1-10min; 3 cycles of extraction	Pressurized solvent allowed to increase the extraction yield of the lipid content (approximately 93%), about eight times greater than the extraction process without the cold pressing step. (64.23–589.46 mg GAE/kg of lipid).
Pulsed UV- C light	Pomegranate	312.6–761.4 J cm^{-2}, and the fluence rate was 5.21, 6.59, and 8.46 W cm^{-2}. The corresponding levels for voltage were 2.1, 2.4, and 2.7 kV with a treatment time of 60, 90, and 120 s	The UV-C light (2988 J cm^{-2}) retained 97%, 94%, and 83% phenolics, antioxidants, and ascorbic acid, respectively. A complete inactivation in *E. coli* was obtained at 2.7 kV for 90 s (761.4 J cm^{-2})
Pressure intensified technologies (sub/supercritical extraction)	Citrus juice	Temperatures and pressures in a typical range of 308–318 K and 20–50 MPa, respectively	Sub/supercritical fluid extraction can improve the release of secondary bioactive compounds from intracellular cells, as well as increase the quality and add value from citrus juice by-products.
High hydrostatic pressure	Pomegranate	Different pressures (350, 450, 550 MPa) for1, 3, 5 min. Temperature was set at 23 ℃, and the pressure increased at a 500 MPa/min rate	Higher preservation, bioaccessibility of anthocyanins and color quality when compared to thermal processes.
High hydrostatic pressure and UV-C light	Lemon and Citrus juice (grapefruit, orange, and lemon)	UV-C: 253.7 nm/1000 L/h; 411.4 mJ cm^{-2} HPP: 600 MPa for 3 min	- Good retention (< 25% reduction) of ascorbic acid -Higher preservation of original color (L*= 52.25; a*= 1.34; b*= 38.81) , phenolic compounds (30.26mg GAE/100mL for UV-C; 32.92 mgGAE/100mL for HPP), and antioxidant activity (UV-C= 252.42 μMTE/mL; HPP= 278.58 μMTE/mL).
High pressure carbon dioxide (HPCD)	Orange	6 - 18 MPa ; 40 to 55 °C for PME (pectin methylesterase); 6 - 20 MPa; 25 to 45°C for PPO(Polyphenol oxidase); 2-15min	-Inactivation of enzymatic activities (Polyphenol oxidase and pectin methylesterase), resulting in approximately 26% residual activity; - PME is affected by temperature in a greater extent (more resistant than PPO)

As beneficial as the combination of emerging non-thermal technologies with cold pressing of juices may be, there are few recent studies involving the fusion of these methods in tropical fruit processing (including citrus and non-citrus fruits). In addition, other innovative techniques for preserving and increasing food quality (such as pulsed electric field and ultrasound) should also be studied and applied to improve the production of cold-pressed tropical juices. This review aims to encourage researchers and small and large industries to process these fruits by cold pressing combined with recent "green" technologies to obtain new results and increase industrial interest in adopting these technologies. As citrus fruits account for the majority of world production (161.8 million tons in 2021 according to FAO (FAOSTAT, 2023)), and about 20% of the production is processed by juice companies(GRANONE; HEGEL; PEREDA, 2022b), the few recent studies focus on research with citrus juices. Furthermore, citrus processing industries release high volumes of wastewater containing significant amounts of bioactive compounds, in addition to polluting loads for the environment(ARGUN et al., 2022).Thus, the positive results of recent works may cause industrial interest, associating the increase in the final quality of the juices, obtained by these innovative processes, with the great market demand for citrus juices. The industrial interest is also relevant because both the cold pressing process and emerging non-thermal technologies are easily adaptable for small and large scale. Currently, emerging technologies are already present in industrial processes to ensure microbiological safety and maintain the sensory and nutritional quality present in whole fruits, since consumers are increasingly looking for foods with a healthy appeal.

CONCLUSIONS AND FUTURE PERSPECTIVES

The trend of producing fruit juices using the Cold pressing method combined with innovative non-thermal technologies allows the production of premium products with food safety and higher sensory and nutritional quality, mainly for the tropical fruit market, in which they are preferably consumed *in natura*, by most consumers. Pressure intensified technologies, cold plasma, and UV-light can increase the extraction of secondary bioactive compounds from the fruit as well as by-products, increasing their added value and contributing to sustainable production. Recently, there is a lack of studies involving the improvement of the functional and microbiological quality of cold-pressed tropical juices combined with non-thermal alternative

methods. With the encouragement of further research on this topic, the results can be improved for future application on an industrial scale with the possibility of reducing the use of artificial in the preservation of beverages with functional characteristics.

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CHAPTER 8

Influence of guabiroba pulp (*Campomanesia xanthocarpa* O. Berg) added to fermented milk on probiotic survival under in vitro simulated gastrointestinal conditions

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Influence of guabiroba pulp (*Campomanesia xanthocarpa* O. Berg) added to fermented milk on probiotic survival under in vitro simulated gastrointestinal conditions

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ABSTRACT

In fermented milks inoculated with two thermophilic strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*), guabiroba pulp (*Campomanesia xanthocarpa* O. Berg) was added in different concentrations: 5% (I5 sample) and 10% (I10 sample), compared to a control sample, with no pulp addition. In these fermented milks, *Bifidobacterium* BB-12 was added and the samples were submitted to a progressive gastrointestinal simulation in vitro. The cells count was performed, including the survival rates for all the progressive steps of the simulated digestion. Total phenolic content (TPC) and antioxidant activity analysis by FRAP (Ferric Reducing Antioxidant Power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were performed in all the gastrointestinal steps. Before and during the entire gastrointestinal tract, the *Bifidobacterium* BB-12 count was 8 - 9 log CFU $g^{-1,}$ above the recommended for a probiotic product, with a highlight in intestinal colon steps. The I10 sample showed the highest viable cell count, the highest total phenolic content and antioxidant activity throughout the entire gastric steps ($p < 0.05$). The fermented milk proved to be an effective matrix for the probiotic stability and incorporation of guabiroba components. Bioactive compounds present in the guabiroba pulp may have occasioned a prebiotic and protective effect on *Bifidobacterium* BB-12 after gastric conditions. The possible bioconversion of these compounds in more active forms can contribute to the absorption in epithelial cells, enhancing fermented milks with guabiroba pulp as important sources of dietary accessible bioactive compounds.

Keywords: *Campomanesia xanthocarpa* O. Berg, gastrointestinal steps, *Bifidobacterium* BB-12, antioxidant activity, phenolic content, yogurt.

1 INTRODUCTION

Probiotics are recognized by living microorganisms that confer several benefits to the host's health when regularly administrated in adequate amounts (GIBSON et al., 2014). With a property to adhere in gut epithelial cells, probiotics can improve the microbiota and the digestive process, protect against pathogens and generate potential anticarcinogenic properties (RANADHEERA et al., 2018; VERRUCK et al., 2019, 2020). The cells must be viable in the entire gastrointestinal tract including mouth, esophagus, stomach, small and larger intestine to exert benefits to the human body (RANADHEERA et al., 2019; RASIKA et al., 2020). Only

can be considered a probiotic product when the viable cells count is at least 10^6 - 10^7 CFU (Colony-Forming Units) per gram or per milliliter of food at the time of its consumption (GIBSON et al., 2014).

The composition of dairy products contains essential nutrients for the development of probiotic cells, with potential results when added in cheeses, ice creams, frozen yogurts, and fermented milks formulations. (BALTHAZAR et al., 2019; GRANATO et al., 2018; MUÑOZ et al., 2018; VERRUCK et al., 2015, 2020; VERRUCK; DANTAS; PRUDENCIO, 2019). Dairy products also contribute to the probiotic survival in the gastrointestinal tract due to the buffer effect and fat globules, which may protect the viable cells against the extreme acid conditions of the stomach and bile salts of the gut (VERRUCK et al., 2015). *Bifidobacterium* genera is one of the most common probiotic bacteria used in functional dairy products, mainly to the generally recognized-as-safe (GRAS) characteristic, which indicates no or less health risks upon consumption (BALTHAZAR et al., 2017; DE LIZ et al., 2020; RANADHEERA et al., 2019; VERRUCK et al., 2015). In addition, *Bifidobacterium* has been studied in relation its several potential health benefits, including prevention of diarrhea, improvement of lactose digestibility, modulation of immune systems, anticarcinogenic activity and cholesterol reduction (ALESSANDRI et al., 2019; O'CALLAGHAN; VAN SINDEREN, 2016; PRASANNA; GRANDISON; CHARALAMPOPOULOS, 2014).

The dairy market represents a potential to functional foods with the addition of bioactive compounds, enhancing the nutritive value and the development of probiotic cells (ABDOLLAHZADEH et al., 2018; BALTHAZAR et al., 2019; MORAIS et al., 2019). Bioactive compounds are synthesized by vegetables, in fruits, flowers, leaves, seeds or roots composition, in addition, these compounds can be metabolized by some microorganisms and animals (BURSAĆ KOVAČEVIĆ et al., 2020; PATRA et al., 2018; SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018). Including polyphenols, carotenoids, fatty acids, fibers and vitamins, these compounds are capable to act on metabolism and decrease the incidence of degenerative diseases (CUTRIM; CORTEZ, 2018; DE CARVALHO et al., 2019). Sources of bioactive compounds, as fruits extract, pulps and juices, are usually studied as a functional additive in fermented milks, becoming an important source for dairy research and a tendency to industries (BALTHAZAR et al., 2019; CASAROTTI et al., 2018). The addition of fruits bioactive compounds in dairy formulations also enhance the viability and the development of probiotic cells, with a potential prebiotic property (ABDOLLAHZADEH et al.,

2018; BALTHAZAR et al., 2019; CASAROTTI et al., 2018; DE CAMPO et al., 2019; ESPÍRITO SANTO et al., 2010; VICENSSUTO; DE CASTRO, 2020).

Nowadays, native Brazilian fruits are studied in several works related to their composition and consumption benefits (AZEVEDO et al., 2019; DE ARAÚJO PADILHA et al., 2018). Fruits from *Myrtaceae* family are known to their high content of bioactive compounds and antioxidant activity, including the *Campomanesia xanthocarpa* O.Berg, popularly known as "guabiroba" (PEREIRA et al., 2012; SILVEIRA et al., 2019). Considered a functional fruit, this native Brazilian fruit has an acid - sweet flavor, high levels of dietary fiber and antioxidant compounds as polyphenols and Vitamin C (CAPELETTO et al., 2016; PEREIRA et al., 2012; SILVA-RODRIGUES et al., 2020). These properties of guabiroba make the pulp appropriate to consume in natura or in ice creams and beverages compositions, however, today the guabiroba pulp is not an ingredient used commercially (BARBIERI et al., 2018).

In this context, with the purpose of valuing a Brazilian native fruit and enrich a product with bioactive compounds for probiotic development and consumer's health, this study determined the potential of fermented milk added of guabiroba pulp in protecting *Bifidobacterium* BB-12 cells during a simulation of the gastrointestinal process, as well as the levels of total phenolic compounds and antioxidant activity in each step of the gastric conditions.

2 MATERIAL AND METHODS

2.1 MATERIALS

For fermented milk production, it was employed commercial UHT (Ultra High Temperature) whole bovine's milk (30 g/L of fat, Santa Clara®, Carlos Barbosa, RS, Brazil), with a thermophilic started culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, Yoflex®, Chr. Hansen, Hønsholm, Denmark) and guabiroba pulp ceded by Embrapa Florestas (Colombo, PR, Brazil) (with 84.3% of moisture, 0.18% of protein, 7.75% of carbohydrates, 0.88%of fat, 6.26% of dietary fiber). For in vitro simulated gastrointestinal conditions, freeze-dried probiotic culture composed of *Bifidobacterium animalis* subsp. *lactis* BB-12 (Nu-trish® BB-12®, Chr. Hansen, Hønsholm, Denmark), enzymes α-amylase (28.75 U/mg protein), pepsin from porcine gastric mucosa (400 U/mg

protein), pancreatin from porcine pancreas (digestive power – 8 × USP specifications) and the bovine bile salts were purchased from Sigma–Aldrich (St. Louis, USA). MRS Agar (Merck, Darmstadt, Germany), lithium chloride MRS Agar (Merck, Darmstadt, Germany), lithium chloride (Vetec, Rio de Janeiro, Brazil), sodium propionate (Fluka, Neu-Ulm, Germany) and AnaeroGen® (Oxoid, Hampshire, UK) were used for the enumeration of *Bifidobacterium* BB-12. *Streptococcus thermophilus* count was performed in M17 agar (Sigma-Aldrich, St. Louis, MO, USA) and, for *Lactobacillus delbrueckii* subsp. *bulgaricus* count, MRS Agar (Merck, Darmstadt, Germany). Gallic acid (purity ≥ 90%) for total phenolic content analysis and, for antioxidant activities, ferrous sulphate (FeSO4) for FRAP (Ferric Reducing Antioxidant Power) method and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) for DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging.

2.2 INCORPORATION OF GUABIROBA PULP INTO FERMENTED MILK

In the manufacture of the fermented milk, 1000g of UHT bovine's milk was heated at 42 ± 2°C followed by the inoculation of thermophilic culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*), according to the manufacturer's instruction. The incubation for the fermentation step was performed at 42 ± 2°C until reaching pH 4.6 and cooled at 4 ± 2°C for 24 h. The fermented milk was stirred and divided into three samples (control, I5, and I10). The control sample was only fermented milk, prepared with no addition of guabiroba pulp, and I5 and I10 were prepared with an addition of 5% and 10% (w/w) of guabiroba pulp, respectively. The samples were kept under refrigeration (4 ± 2 °C) until the time of the analyses.

2.3 PREPARATION OF *Bifidobacterium* BB-12 SUSPENSION

Before the simulated gastrointestinal analysis, the freeze-dried *Bifidobacterium* BB-12 culture was rehydrated in whole milk and stored in sterile glass vials at - 18 ± 2 °C according to Fritzen-Freire et al. (2012). From this stocked solution, the cell suspension was performed according to Rodrigues et al. (2011) with modifications. The stocked solution was added in MRS broth (with 0.2g 100 g^{-1} of lithium chloride and 0.3 g 100g^{-1} of sodium propionate) and incubated in anaerobic jars with AnaeroGen® at 37 ± 1 °C for 48 h. After this period, the solution was centrifuged (1.000 x *g* - Nova Técnica, São Paulo, Brazil), at 25 ± 1 °C during 10

minutes. The supernatant was discarded, and the solid fraction was washed twice with a saline solution (0.85 g/100g) and suspended in 20 mL of UHT whole milk.

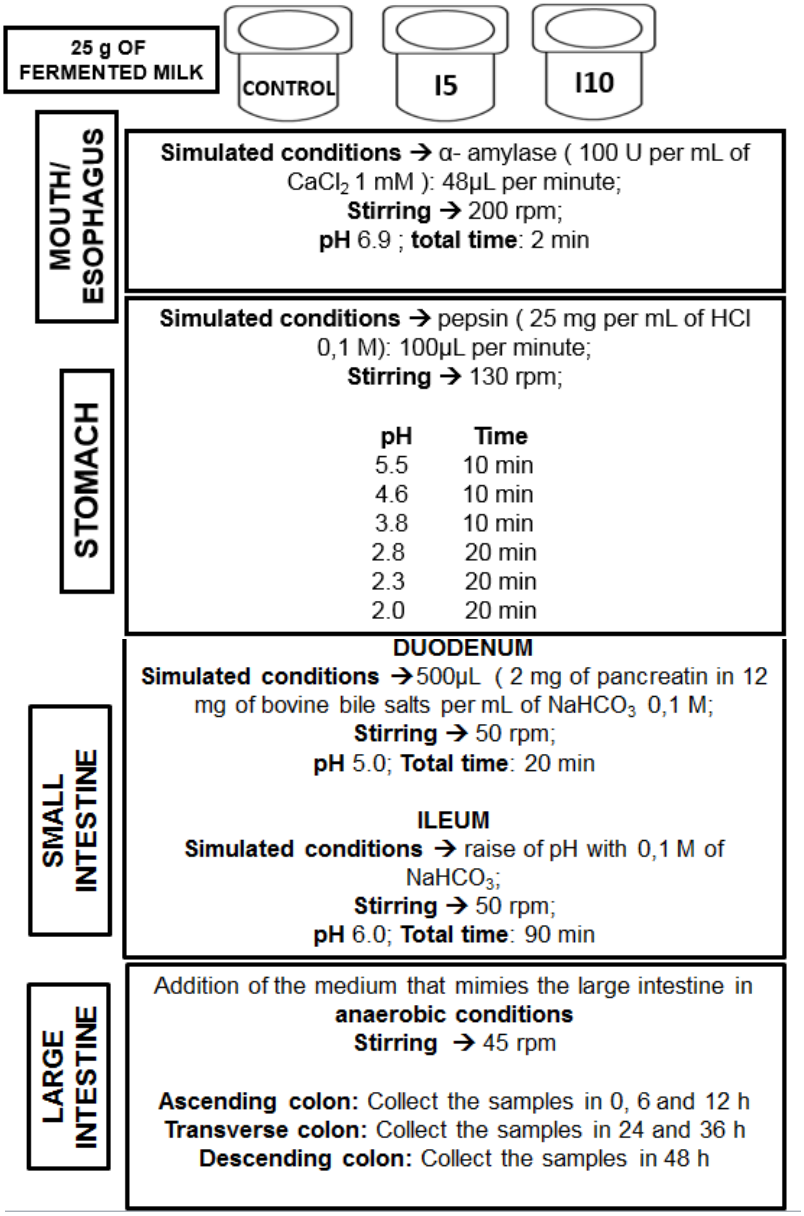
2.4 IN VITRO GASTROINTESTINAL SIMULATION

For in vitro simulated gastrointestinal conditions, the methodology proposed by Verruck et al. (2020) was performed. In the fermented milk (control, I5 and I10) at room temperature, it was added the suspended *Bifidobacterium* BB-12 (0.10 mL L^{-1} of whole bovine milk, which is equivalent to 10 log CFU.g^{-1} and is the initial probiotic concentration in all the fermented milks). Twenty - five grams of each fermented milk with probiotic addition were submitted to similar conditions of the human mouth/esophagus, stomach, duodenum, small and large intestines (Figure 30). The enzyme solutions were filtered-sterilized using a 0.22 μm membrane filter (MF-Millipore, Billerica MA, USA). Before and during all the analysis, the enzyme solutions were kept cooled and gradually added according to the digestion steps. The simulation of a complete digestion was performed once from the same batch of fermented milks. Each step of the gastrointestinal tract was simulated in triplicate.

2.5 MICROBIOLOGICAL ANALYSIS

The microbiological analysis was accomplished by decimal solutions of twenty-five grams of each fermented milk in 225 mL of peptone water (0.1 g 100 mL^{-1}). The *S. thermophilus* count was performed by pour plate technique using M17 agar besides lactose (10 g 100 mL^{-1}), incubated at 37 ± 2 °C for 48 h under aerobic conditions (IDF, 1997). For *L. bulgaricus*, the enumeration of the cells was on MRS agar, and the plates were incubated aerobically at 37 ± 2 °C for 72 h (DAVE; SHAH, 1996). For *Bifidobacterium* BB-12, the diluted samples were plated by pour plate method on the MRS agar with an addition of 0.2 g 100 g^{-1} lithium chloride and 0.3 g 100 g^{-1} sodium propionate (VINDEROLA; REINHEIMER, 1999). In an anaerobic condition, the plates were incubated in jars with AnaeroGen® at 36 ± 1 °C for 72 h. Performed in triplicate, the count of viable cells for the three bacteria was expressed as log colony- forming units per gram (log CFU g^{-1}).

Figure 37- Conditions used in each gastrointestinal steps



Source: adapted from Verruck et al. (2020)

2.6 MICROORGANISMS SURVIVAL RATE

After the simulated gastrointestinal conditions, *Bifidobacterium* BB-12, L. *bulgaricus*, and *S. thermophilus* survival rate were evaluated according to Eq. 1 by Guo et al. (2009):

$$Survival\ rate\ (\%) = \left(\frac{\log CFUN_1}{\log CFUN_0}\right) x\ 100 \qquad (1)$$

Where N_1 represents the total viable count of cells after each step of gastric conditions and N_0 the initial viable count of cells before the gastrointestinal simulation.

2.7. TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY ANALYSIS

The samples (control, I5, and I10) for each step of the gastrointestinal simulation were analyzed for total phenolic content (TPC) according to the Folin-Ciocalteu method (SINGLETON; ROSSI, 1965), with an analysis read at 720 nm in a spectrophotometer. The results were expressed in milligrams of gallic acid equivalent per liter of the sample (mgGAE. L^{-1}) (calibration curve linearity range: $R^2 = 0.99$).

For antioxidant activity, the DPPH method was performed by Brand-Williams et al. (1995). The analysis read was performed in a spectrophotometer, at 515 nm and the results were expressed in micromole of Trolox equivalents per liter of the sample (μmolTE.L^{-1})

For the FRAP method, according to Benzie and Strain (1996), the analysis read was at 595 nm in a spectrophotometer and the results were expressed in micromole of $FeSO_4$ equivalent per liter of the sample (μmol $FeSO_4$.L^{-1}).

All these antioxidant activity analyses were carried up in triplicate.

2.8 STATISTICAL ANALYSIS

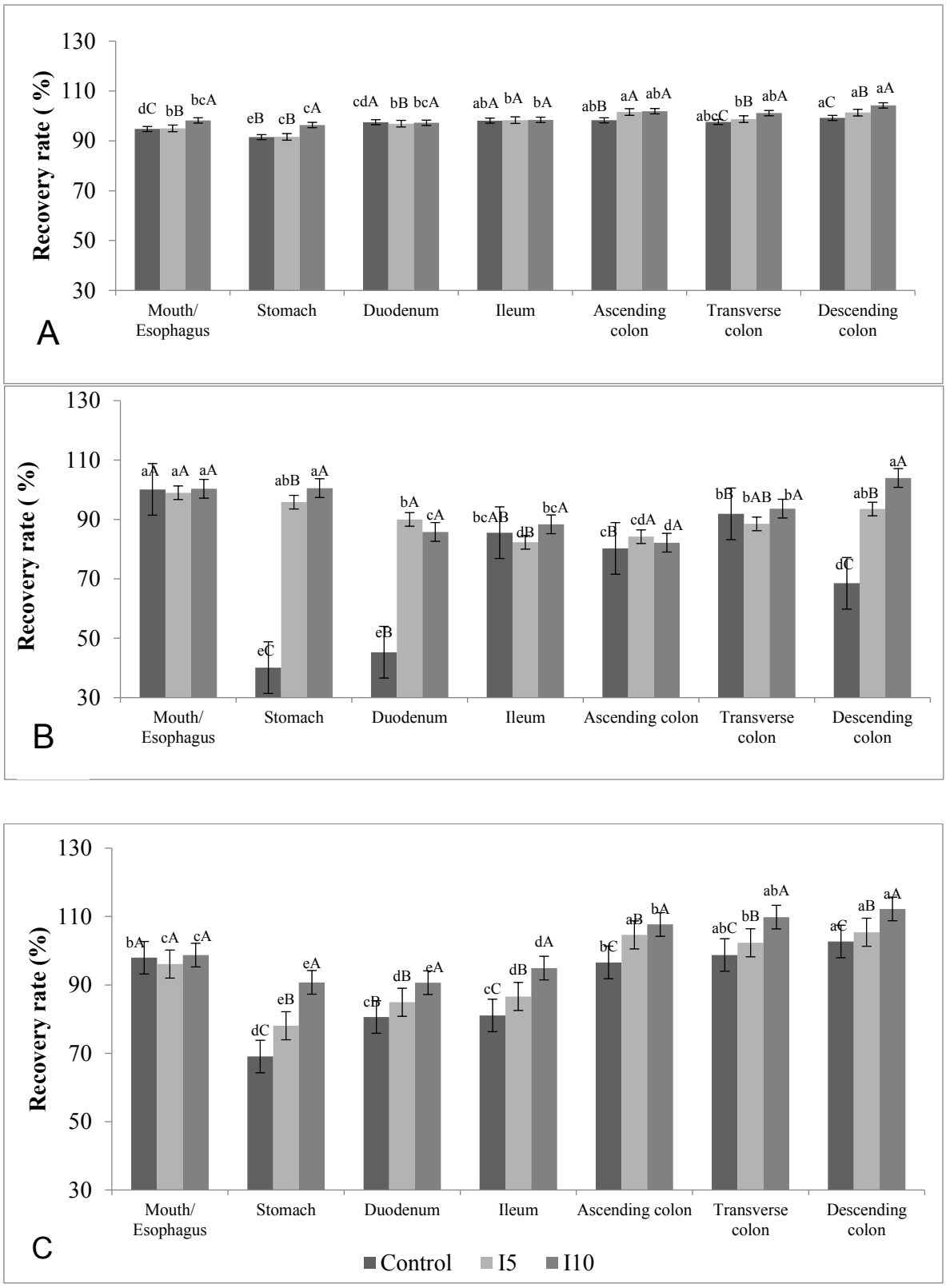
The results were expressed as means ± standard deviation. The data analysis was performed using STATISTICA 7.0 software (StatSoft Inc., Tulsa, USA). To determine the significant differences ($p < 0.05$), analysis of variance (ANOVA) and Fisher LSD was implemented.

3 RESULTS AND DISCUSSION

3.1 MICROBIOLOGICAL VIABILITY THROUGHOUT THE GASTROINTESTINAL TRACT

The viable cells count of *Bifidobacterium* BB-12, *S. thermophilus*, *L. bulgaricus*, and their recovery rates are shown in Table 12,13,14 and Figure 31 A, B, and C, respectively.

Figure 38- Survival rate (%) in gastrointestinal simulation steps.



Source: The authors (2021).

Note: A: Bifidobacterium BB-12; B: Streptococcus thermophilus; C: Lactobacillus bulgaricus.

a - b: Different superscript lowercase letters in the top denote significant differences ($p < 0.05$) among the same sample during all the progressive gastric simulation steps (Mouth/Esophagus, Stomach, Duodenum, Ileum, Ascending colon, Transverse colon and Descending colon).

A – B: Different superscript uppercase letters in the top denote differences ($p< 0.05$) among the same step but in different samples; Significant differences according to ANOVA and Fisher LSD. Triplicate data analysis.

Probiotic products must have at least 6 log CFU. g^{-1} of viable cells to exert benefits in human health (BOYLSTON et al., 2004). During the entire gastrointestinal tract, bifidobacteria maintained a high viability with 8 - 9 log CFU. g^{-1}, classifying the three fermented milks (control, I5, and I10) as probiotic products, mainly in the moment of consumption, stimulated by initial step. Among the three samples, there was no significant difference, in the passage of the fermented milk through the mouth/esophagus, for viable cell count and recovery rate for the three bacteria ($p< 0.05$). The food degradation is gradual by α- amylase enzyme action, present in saliva (HUMPHREY; WILLIAMSON, 2001). For being a low viscous and liquid food, the fermented milk has a little time in contact with the mouth, not interfering in the microbiological count statistics.

Throughout the passage through the stomach, the viable cells count and the survival rate of *L. bulgaricus*, *S. thermophilus* reduced significantly due to hostile conditions of extreme pH simulated by stomach acid. For bifidobacteria, the survival rate decreased only in Control and I5 samples ($p< 0.05$). *L. bulgaricus* and *S. thermophilus* species have an exponential growth under average conditions of pH 5.6 to 6.5 (RAULT; BOUIX; BÉAL, 2009), becoming a challenge for cell development of these lactic - acid bacteria in extreme pH of gastric juice (MARTEAU et al., 1997; Wang et al., 2016). During the development, these thermophilic bacteria produces low concentrations of exopolysaccharides, which is proved to be essential to survival of probiotic bacteria with a protective effect against the extreme acidity or in contact with bile salts of the gut (BOKE; ASLIM; ALP, 2010). Acids and bile salts affect cell membranes, composed by lipids and fatty acids, destroying the membrane permeability and decrease the viable cells, in special to *L. bulgaricus*, with the lowest cells count in the stomach and small intestine. The sensibility to acid surrounding in *L. bulgaricus* also were observed by Chen et al. (2017) and Zeng et al. (2018). According to results of this work, the behavior of the thermophilic culture though the simulated gastric conditions does not potentiate these cells to have a probiotic property.

Table 19- Viable cells of *Bifidobacterium animalis* subsp. *lactis* BB-12 in all the gastrointestinal steps.

***Bifidobacterium BB-12* count (log CFU.g^{-1})**			
Steps	**Control**	**I5**	**I10**
Initial	$9.44 \pm 0.02^{a\ B}$	$9.29 \pm 0.09^{a\ C}$	$9.83 \pm 0.09^{bc\ A}$
Mouth/ Esophagus	$9.40 \pm 0.06^{a\ B}$	$9.12 \pm 0.06^{ab\ C}$	$9.66 \pm 0.15^{cd\ A}$
Stomach	$8.51 \pm 0.08^{d\ C}$	$8.64 \pm 0.14^{c\ B}$	$9.31 \pm 0.01^{e\ A}$
Duodenum	$9.18 \pm 0.01^{c\ B}$	$9.00 \pm 0.15^{b\ C}$	$9.48 \pm 0.01^{de\ A}$
Ileum	$9.11 \pm 0.08^{c\ C}$	$9.30 \pm 0.05^{ab\ B}$	$9.47 \pm 0.10^{de\ A}$
Ascending colon	$9.28 \pm 0.05^{bc\ C}$	$9.43 \pm 0.22^{a\ B}$	$10.24 \pm 0.18^{a\ A}$
Transverse colon	$9.22 \pm 0.12^{bc\ C}$	$9.39 \pm 0.36^{a\ B}$	$9.70 \pm 0.52^{bcd\ A}$
Descending colon	$9.37 \pm 0.26^{ab\ B}$	$9.41 \pm 0.08^{a\ B}$	$10.01 \pm 0.20^{a\ A}$

Source: The authors (2021).

Note: $^{a\ -\ b}$ within a column: means ± standard deviations with different letters denote significant differences ($p < 0.05$) in viable cells count among the samples in progressive steps during all the gastric simulation ;

$^{A\ -\ B}$ within a line: means ± standard deviations with different letters denote significant differences ($p< 0.05$) in viable cells count among the different samples but in the same step of the gastric simulation ; Significant differences according to ANOVA and Fisher LSD; Triplicate data analysis.

Table 20- Viable cells of *Streptococcus thermophilus* in gastrointestinal simulation steps.

***S. thermophilus* count (log CFU.g^{-1})**			
Steps	**Control**	**I5**	**I10**
Initial	$9.07 \pm 0.17^{a\ B}$	$9.15 \pm 0.02^{b\ B}$	$9.24 \pm 0.03^{ab\ A}$
Mouth/ Esophagus	$9.08 \pm 0.01^{a\ A}$	$8.98 \pm 0.01^{bc\ B}$	$9.12 \pm 0.01^{b\ A}$
Stomach	$7.76 \pm 0.17^{bc\ B}$	$7.78 \pm 0.01^{d\ B}$	$8.69 \pm 0.06^{c\ A}$
Duodenum	$8.22 \pm 0.01^{b\ A}$	$8.16 \pm 0.05^{cd\ A}$	$8.01 \pm 0.01^{cd\ B}$
Ileum	$7.28 \pm 0.03^{c\ C}$	$7.46 \pm 0.10^{d\ b}$	$8.40 \pm 0.02^{c\ A}$
Ascending colon	$7.28 \pm 0.20^{c\ C}$	$7.69 \pm 0.18^{d\ B}$	$8.49 \pm 0.04^{c\ A}$
Transverse colon	$8.33 \pm 0.32^{b\ A}$	$6.02 \pm 4.03^{e\ B}$	$8.48 \pm 0.37^{c\ A}$
Descending colon	$6.21 \pm 0.13^{d\ C}$	$9.27 \pm 0.09^{a\ B}$	$9.43 \pm 0.01^{a\ A}$

Source: The authors (2021).

Note: $^{a\ -\ b}$ within a column: means ± standard deviations with different letters denote significant differences ($p < 0.05$) in viable cells count among the samples in progressive steps during all the gastric simulation ;

$^{A\ -\ B}$ within a line: means ± standard deviations with different letters denote significant differences ($p< 0.05$) in viable cells count among the different samples but in the same step of the gastric simulation ; Significant differences according to ANOVA and Fisher LSD; Triplicate data analysis.

Table 21- Viable cells of *Lactobacillus delbrueckii* subsp. *bulgaricus* during all the simulation of gastrointestinal steps.

	***L. bulgaricus* count (log CFU.g^{-1})**		
Steps	**Control**	**I5**	**I10**
Initial	$6.21 \pm 0.13^{c\ B}$	$6.20 \pm 0.18^{cd\ B}$	$6.47 \pm 0.02^{cd\ A}$
Mouth/ Esophagus	$6.09 \pm 0.04^{cd\ A}$	$5.97 \pm 0.08^{cde\ B}$	$6.13 \pm 0.23^{cde\ A}$
Stomach	$4.85 \pm 0.06^{g\ C}$	$5.29 \pm 0.19^{g\ B}$	$5.64 \pm 0.14^{de\ A}$
Duodenum	$5.28 \pm 0.07^{e\ B}$	$5.63 \pm 0.10^{ef\ A}$	$5.01 \pm 0.16^{e\ C}$
Ileum	$5.04 \pm 0.03^{f\ C}$	$5.38 \pm 0.11^{g\ B}$	$5.90 \pm 0.30^{de\ A}$
Ascending colon	$6.00 \pm 0.01^{d\ C}$	$6.50 \pm 0.28^{c\ B}$	$7.56 \pm 0.25^{c\ A}$
Transverse colon	$7.60 \pm 0.07^{b\ B}$	$7.19 \pm 0.21^{b\ C}$	$8.25 \pm 0.38^{b\ A}$
Descending colon	$9.49 \pm 0.01^{a\ C}$	$9.83 \pm 0.14^{a\ B}$	$10.37 \pm 0.09^{a\ A}$

Source: The authors (2021).

Note: $^{a\ -\ b}$ within a column: means ± standard deviations with different letters denote significant differences ($p < 0.05$) in viable cells count among the samples in progressive steps during all the gastric simulation; $^{A\ -\ B}$ within a line: means ± standard deviations with different letters denote significant differences ($p < 0.05$) in viable cells count among the different samples but in the same step of the gastric simulation ; Significant differences according to ANOVA and Fisher LSD; Triplicate data analysis.

For bifidobacteria behavior, even though the count and viable cells recovery have been reduced, the probiotic principle maintained in the digested fermented milk, with counts among 8 to 9 log CFU. g^{-1}. Fat globules in the milk may have a protective effect around the cells and the lipid fraction contributes to fatty acids biosynthesis concentrated around the probiotic plasma membrane (FLORENCE et al., 2016; VERRUCK et al., 2015). The bifidobacteria recovery rate, in adverse conditions, also is related to a genetic transcription attributed to bacteria sigma factor, transcribing genes related to growth, biofilms synthesis and essential metabolites for their survival (PAGET, 2015; ZHANG et al., 2019).

At the colon steps end, it was observed both for *Bifidobacterium* BB-12 and for thermophilic cultures of *L. bulgaricus* e *S. thermophilus*, a significant increase in viable cells and survival rates ($p < 0.05$). For probiotics, it is expected the survival during all the digestion until the large intestine steps (ascendant colon, transverse colon, and descending colon), where it is the site of beneficial action to the host (BOYLSTON et al., 2004). The surrounding with reduced oxygen in colon steps allows the anaerobic bacteria development, represented by bifidobacteria (EL. HADAD et al., 2019). The higher count of viable cells and survival rates (above 100%) may be related to a propitious location with a slightly acid pH (6.0), competitive

inhibition by bacteriocins production, micronutrients, and exopolysaccharides synthesis. These metabolites are concentrated in intestinal epithelial cells and are synthesized for cell survival in adverse gastrointestinal conditions (Table 12, Figure 31A) (RUSSELL et al., 2011; VERRUCK et al., 2020; YAN et al., 2020).

Initially, the *Bifibacterium* BB-12 was added to a high concentration of 10 g. L^{-1} to the fermented milk. In the colon steps, it is probably the viable cells could be in an adaptation phase, in the lag phase. The *Lactobacillus bulgaricus*, possibly would be in exponential phase, with a higher recovery rate at the colon, once these sensible cells are not more exposed to a hostile pH for their development (Figure 31 C, $p<0.05$). Due to the inoculum of three distinct microorganisms in the same product and submitted to the same surrounding conditions and nutrients, the bacteria tend to develop in competition with the other ones and the more sensible tend to reduce the count, as occurs with *S. thermophilus* strain.

3.2 INFLUENCE OF GUABIROBA PULP ON PROBIOTIC SURVIVAL

In all the steps of gastrointestinal simulation, the fermented milk with 10% of guabiroba pulp (I10) provided the highest survival rate and viable cells count ($p< 0.05$) for *Bifidobacterium* BB-12, *L. bulgaricus* e *S. thermophilus* about the product with 5% of the pulp and the control sample (Table 12, 13, 14, Figure 31 A, B, C). For bifidobacteria, moreover, the concentration of 10% of guabiroba pulp was potentially appropriate to provide a protective effect even in adverse stomach conditions, with no significant differences in the survival rate during the passage from the mouth/esophagus to the stomach (Figure 31A).

According to Alves et al. (2013), Pereira et al. (2012) and Rocha et al. (2011) a high phenolic content is in the guabiroba fruit composition, which is higher when compared to conventional and usual fruit which are consumed as apples, grapes or strawberries (ALBERTI et al., 2014; DZHANFEZOVA et al., 2020; MARGRAF et al., 2016). Among the individual phenolic compounds, the composition of fruits from the *Myrtaceae* family contains phenolic acids and flavonoids, which a high molecular mass and glycosylated (FIDELIS et al., 2020). In appropriate concentrations, these bioactive compounds may have had a prebiotic effect on the bifidobacteria and/or protective to the three analyzed microorganisms during all the passage through gastrointestinal simulation, including the extreme stomach acidity. In this digestive step, the addition of 10% of guabiroba pulp resulted in a significant survival rate even to

sensible strains of *S. thermophilus* and *L. bulgaricus*, with rates of 60.4% and 21.7% more than control sample (Figure 31B, C).

An analysis of probiotic fermented milk with phenolic compounds from pomegranate peel, Chan et al. (2018) confirmed that the probiotic development was related to an increase of phenolic compounds content at the end of the fermentation. For total phenolic content (TPC) at Table 4, it is observed that, with the progress of the gastrointestinal simulation in vitro, there is an increase of polyphenols with a higher phenolic concentration in the final colon steps and, about the three fermented milks, the I10 sample showed the highest increase of these compounds ($p < 0.05$) (Table 15). These results emphasized that fermented milks , are an excellent vehicle for phenolic compounds of fruits and plant extracts (DE CARVALHO et al., 2019; GRANATO et al., 2018). The low pH of the matrix and gastric surroundings, enhance the stability of phenolic compounds due to the proteins, peptides, and fat which possibly maintain the integrity of phenolic compounds during digestion (HELAL; TAGLIAZUCCHI, 2018).

Table 22- Total phenolic content in samples of all the gastrointestinal steps.

Total phenolic content (mgGAE.L^{-1})			
Steps	**Control**	**I5**	**I10**
Initial	$89.25 \pm 0.66^{f\,B}$	$82.48 \pm 10.24^{f\,B}$	$161.45 \pm 4.30^{c\,A}$
Mouth/ Esophagus	$96.50 \pm 2.97^{f\,C}$	$103.97 \pm 24.12^{ef\,B}$	$163.79 \pm 1.33^{c\,A}$
Stomach	$154.67 \pm 19.16^{e\,B}$	$162.15 \pm 58.82^{de\,A}$	$162.16 \pm 53.53^{c\,A}$
Duodenum	$171.03 \pm 31.72^{e\,C}$	$173.36 \pm 24.45^{de\,B}$	$229.44 \pm 68.07^{bc\,A}$
Ileum	$185.05 \pm 23.79^{d\,C}$	$225.23 \pm 38.33^{cd\,B}$	$325.23 \pm 80.62^{b\,A}$
Ascending colon	$277.34 \pm 39.98^{c\,B}$	$250.00 \pm 8.59^{bc\,C}$	$297.66 \pm 29.08^{bc\,A}$
Transverse colon	$450.00 \pm 17.84^{a\,C}$	$460.98 \pm 29.41^{a\,B}$	$492.29 \pm 1.99^{a\,A}$
Descending colon	$346.03 \pm 8.26^{b\,B}$	$311.21 \pm 28.42^{b\,C}$	$532.24 \pm 26.43^{a\,A}$

Source: The authors (2021).

Note: $^{a-b}$ within a column: means ± standard deviations with different letters denote significant differences ($p < 0.05$) among the samples in progressive steps during all the gastric simulation ;

$^{A-B}$ within a line: means ± standard deviations with different letters denote significant differences ($p < 0.05$) among the different samples but in the same step of the gastric simulation ; Significant differences according to ANOVA and Fisher LSD; Triplicate data analysis.

During the probiotic cells multiplication along with the simulation and mainly in the colon step, the phenolic compounds, in polymers and oligomers form, might be hydrolyzed in simpler forms for microbial absorption through enzymatic activities (OU; GU, 2014). The high

molecular mass of these bioactive compounds become non-absorbable through the epithelium walls of the gut and, with the enzymatic hydrolysis of probiotics, there is the formation of a metabolite potentially more active and better absorbed by the organism (ESPÍN; GONZÁLEZ-SARRÍAS; TOMÁS-BARBERÁN, 2017). Evaluating the probiotic effect in the gastrointestinal simulation of red pitaya pulp, Morais et al. (2019) associated the intense *Lactobacillus acidophilus* and *Bifidobacterium* BB-12 multiplication with the increase of simple chain phenolic compounds in the intestinal final step. The bioconversion of polyphenols is performed by β- glucosidase enzyme, synthesized by *Lactobacillus* and *Bifidobacterium* genera, able to make hydro soluble tannins free of glucose molecules, forming aglycones which are biologically more active, and it increases the phenolic content through the progressive gastric simulation (DELGADO et al., 2019). This enzyme is capable to decompose chemical compounds of vegetable cells that link polyphenols to cell walls, releasing them to the surrounding and increasing the phenolic activity (HUYNH et al., 2014). This explains why the TPC is five times higher in the descending colon concerning initial step, before being digested, once the colon is the propitious local to bifidobacteria development and it enhances the proliferation and the biosynthesis of compounds which influence in increase of phenolic activity.

3.3 ANTIOXIDANT ACTIVITY IN THE FERMENTED MILK

DPPH free radical scavenging capacity and ferric ion reduction power (FRAP) are shown in Table 16. The three fermented milks digested by gastric steps also have a significate increase of antioxidant activity, mainly for fermented milk with the highest concentration of guabiroba pulp (I10) ($p<0.05$). This sample showed, in the initial step, 275% and 614% more than the control sample for antioxidant activity by FRAP and DPPH assays, respectively. Phenolic compounds can be related directly to the antioxidant activity of the matrix analyzed, emphasizing that with high phenolic content, the antioxidant activity will be also higher (HAMINIUK et al., 2012).

Table 23- Antioxidant activity by FRAP and DPPH assays in gastrointestinal simulation steps.

Antioxidant activity (µmol.L^{-1})						
	FRAP			DPPH		
Steps	**Control**	**I5**	**I10**	**control**	**I5**	**I10**
Initial	314.10 ± 60.28$^{d\,C}$	558.40 ± 20.62$^{e\,B}$	1178.68 ± 60.28$^{cd\,A}$	90.40 ± 43.29$^{e\,C}$	326.00 ± 48.08$^{e\,B}$	646.00 ± 5.65$^{f\,A}$
Mouth/ Esophagus	437.05 ± 44.41$^{d\,C}$	918.51 ± 23.79$^{c\,B}$	1211.20 ± 23.79$^{c\,A}$	94.00 ± 14.17$^{e\,C}$	576.00 ± 77.78$^{c\,B}$	686.00 ± 16.97$^{d\,A}$
Stomach	358.52 ± 10.31$^{d\,C}$	640.10 ± 35.69$^{de\,B}$	1206.44 ± 7.13$^{c\,A}$	82.00 ± 33.94$^{f\,C}$	134.40 ± 41.42$^{f\,B}$	342.00 ± 92.45$^{g\,A}$
Duodenum	324.41 ± 9.51$^{d\,C}$	694.04 ± 48.38$^{d\,B}$	1109.67 ± 19.83$^{d\,A}$	66.40 ± 62.63$^{g\,C}$	130.00 ± 70.71$^{f\,B}$	666.00 ± 25.45$^{de\,A}$
Ileum	352.17 ± 6.34$^{d\,C}$	689.28 ± 19.83$^{d\,B}$	1263.55 ± 23.79$^{c\,A}$	114.00 ± 28.90$^{d\,C}$	413.00 ± 97.92$^{d\,B}$	656.00 ± 31.25$^{ef\,A}$
Ascending colon	2353.21 ± 61.86$^{a\,B}$	2547.76 ± 61.86$^{a\,A}$	2674.63 ± 49.85$^{b\,A}$	226.40 ± 90.51$^{c\,C}$	636.00 ± 35.75$^{b\,B}$	816.00 ± 57.10$^{c\,A}$
Transverse colon	1911.58 ± 38.86$^{b\,C}$	2199.51 ± 81.69$^{b\,B}$	2658.76 ± 33.11$^{b\,A}$	276.00 ± 82.84$^{b\,C}$	416.00 ± 42.40$^{d\,B}$	936.00 ± 49.50$^{b\,A}$
Descending colon	1686.32 ± 61.86$^{c\,C}$	2360.53 ± 63.40$^{a\,B}$	4504.51 ± 63.40$^{a\,A}$	516.00 ± 97.80$^{a\,C}$	1152.00 ± 38.65$^{a\,B}$	1232.00 ± 68.24$^{a\,A}$

Source: The authors (2021).

Note: $^{a\,-\,b}$ within a column: means ± standard deviations with different letters denote significant differences ($p < 0.05$) among the samples in progressive steps during all the gastric simulation ;$^{A\,-\,B}$ within a line: means ± standard deviations with different letters denote significant differences ($p< 0.05$) among the different samples but in the same step of the gastric simulation ; Significant differences according to ANOVA and Fisher LSD; Triplicate data analysis.

During the fermentation in the gastrointestinal tract, different products of phenolic origin are produced by bifidobacteria enzymatic action due to the particular composition of the phenolic matrix, in our case, of the guabiroba pulp. With the bacteria multiplication, mainly in the colon, also occur phenolic transformations, being able to generate compounds with higher antioxidant activity, which explains the progressive increase through the digestion (MORAIS et al., 2019). Besides that, the *Bifidobacterium* genus can form peptides, by enzymatic hydrolysis of milk proteins, with a higher antioxidant and anti-inflammatory activities (CAI et al., 2006). In addition to the antioxidant activity from phenolic compounds of guabiroba pulp, an increase of radical scavenging activity for DPPH assay may be explained due to the formation of the bioactive peptides with antioxidant activity during the proteolytic action in the fermented milk production (RUTELLA; TAGLIAZUCCHI; SOLIERI, 2016).

Balakrishnan and Agrawal (2014) analyzed probiotic fermented milks about their antioxidant activity by the DPPH assay resulting in 79% of radical scavenging activity, highlighting that different peptides of milk protein may be responsible by the increase in antioxidant activity. This explains the significant increase of antioxidant activity in the ascending and transverse colon in the control sample, with no influence of phenolic compounds ($p < 0.05$). The protein hydrolysis is capable to produce amino acids and polyphenols that maintain the energetic balance of the probiotic cell, contributing to an increase of antioxidant activity (CHUGH; KAMAL-ELDIN, 2020). The bifidobacteria metabolism controls activities such as the elimination of free radicals, chelation of metal ions, synthesis of antioxidative enzymes, and inhibition of pro-oxidants to model the circulation of oxidative stress and self-protection against external damage (WANG et al., 2017).

Compounds addition with a potential prebiotic function, as the phenolic compounds of guabiroba, enhances the higher selective fermentation, resulting in a higher concentration of antioxidant metabolites (MADHU; AMRUTHA; PRAPULLA, 2012). Concerning the host's health, more active compounds and with low molecular mass may be absorbed in the blood flow and decrease the incidence of cancer and other non- communicable degenerative diseases.

CONCLUSION

The addition of guabiroba pulp to fermented milks contributed to a higher multiplication and cell survival of *Bifidobacterium* BB-12, *L. bulgaricus*, and *S. thermophilus* in progressive steps of an in vitro gastrointestinal simulation, highlighting the final steps of the

gut, with a surrounding of benefic action of the probiotic strains. The highest concentration of guabiroba pulp to the fermented milk (I10) provided higher cell counts and recovery rates through the simulation among the three samples, highlighting a protective and potentially prebiotic effect of guabiroba bioactive compounds. The addition of guabiroba pulp in fermented milk, until then unpublished, may result in a potential product for the host's health since it benefits the proliferation of the gut microbiota and the absorption of micronutrients by colon epithelium.

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CHAPTER 9

Whey block freeze concentration aiming a functional fermented lactic beverage with the addition of probiotic and guabiroba pulp (*Campomanesia xanthocarpa* O. Berg), a native Brazilian fruit

PRESTES, A. et al. Whey block freeze concentration aiming a functional fermented lactic beverage with the addition of probiotic and guabiroba pulp (*Campomanesia xanthocarpa* O. Berg), a native Brazilian fruit. **Food Science and Technology**, v. 43, p. 2023, 5 out. 2023. **(original writing**

Whey block freeze concentration aiming a functional fermented lactic beverage with addition of probiotic and guabiroba pulp (*Campomanesia xanthocarpa* O. Berg), a native Brazilian fruit

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ABSTRACT

The scientific importance involved in the present study was the use of whey, a co-product of the cheese industry, and its performance during the freeze concentration process. Moreover, the best-concentrated whey from the freeze concentration process, about the total solids, proteins, and mineral contents, was used to prepare two functional fermented lactic beverages. Therefore, whey was subjected to the freeze concentration in blocks with gravitational thawing. Process performance indicated better yields and efficiency for the second stage of freeze concentration. Concentrated whey 2 was used to prepare two fermented lactic beverages added with probiotics; one without adding guabiroba pulp (control) and a beverage incorporated with 10% guabiroba pulp. Containing guabiroba pulp was not enough to modify the total solids, proteins, and mineral contents; however, it decreased pH values, changed the color to an orange hue, and decreased luminosity. The fermented lactic beverage added with probiotic and 10% guabiroba pulp showed 1.61 x more phenolic compounds and an increase of 164% for each evaluated carotenoid content compared to the control beverage.
Keywords: Guabiroba, functional beverage, cheese whey, concentration, bioactive compounds.

1 INTRODUCTION

Whey is an important co-product of the cheese industry, and approximately 197.44 million tons of it are generated worldwide from cheeses made with cow's milk (FAOSTAT, 2023). However, the whey retains about 55% of the solids and 20% of the proteins present in milk, being about 0.6-0.8 g/100g of protein, 0.4-0.5 g/ 100g of fat, 4.5-5g/100g of lactose and 8-10g/100g of mineral salts. Alternatives for using this co-product, aiming at its exploitation, have generated interest both in the small and large industrial sectors and the scientific area. In terms of improving whey's nutritional properties, it can be applied to concentration methods. Among these, Habib and Farid (2008) and Raventós et al. (2007) affirmed that the freeze concentration technology stands out, which employs low temperatures, bringing popularity as an alternative industrial concentration technique of the whey processing, such as vacuum

evaporation and membrane technologies. Therefore, freeze concentration improves quality as it minimizes the effect of heat on sensitive components such as proteins, water-soluble vitamins, and aromatic compounds (MORENO et al., 2015; ROBLES et al., 2016; SÁNCHEZ et al., 2010). Prestes et al. (2022) highlighted that freeze concentration is an important technology applied to focus liquid foods, maintaining their quality and preserving thermolabile compounds, flavor, and color. In dairy industries, this technological approach can significantly enhance milk's and whey's efficiency, concentrating its total dry matter. Also, such a technique provides additional advantages for the product's packaging, shipping, and storage. Barros et al. (2022) evaluated the freeze concentration of whey and observed that proteins mostly represent the higher total solids content of concentrated whey. Therefore, the present study first focuses on using an emerging non-thermal technology in the concentration of a co-product from the dairy industry.

The growing consumer attention for a diet that goes beyond nutritional value, aiming to improve their well-being, has determined a great interest in the food industries in developing products with claims of functional properties. Among these products are probiotics; according to Hill et al. (2014), probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts. These authors further considered two common benefits often associated with probiotics: supporting a healthy digestive tract and immune system. Hill et al. (2014) also acknowledged that evidence supports the beneficial relationship between some foods containing live beneficial microorganisms, especially fermented dairy products, and reduced risk of certain diseases. It accepts the following bacterial species when delivered in food at a level of 1 x 10^9 colony-forming units (CFU) per serving as probiotics for which nonstrain-specific claims might be made: *Bifidobacterium* (*adolescentis, animalis, bifidum, breve*, and *longum*) and *Lactobacillus acidophilus, Lacticaseibacillus casei, Limosilactobacillus fermentum, Lactobacillus gasseri, Lactobacillus johnsonii, Lacticaseibacillus paracasei subsp. paracasei, Lactiplantibacillus plantarum subsp. plantarum, Lacticaseibacillus rhamnosus and Ligilactobacillus salivarius.* This list represents a core group of well-studied species likely to impart some general benefits based on their contribution to healthy gut microbiota (HILL et al., 2014). In addition to using a concentrated product, and therefore with greater nutritional value, as well as a probiotic, this present study envisaged using bioactive compounds from a native Brazilian fruit.

Bioactive compounds are synthesized by plants in the composition of fruits, flowers, leaves, seeds, or roots; these compounds can be metabolized by some microorganisms and

animals (PATRA et al., 2018). Among these compounds are polyphenols and carotenoids, which can act on human metabolism, reducing the incidence of degenerative diseases (CUTRIM; CORTEZ, 2018). Sources of bioactive compounds, such as fruit extracts, pulps, and juices, are often studied as a functional additive in dairy products, becoming an important source for research and a trend for industries (BALTHAZAR et al., 2019; CASAROTTI et al., 2018). Brazilian native fruits have been studied in works related to their composition and consumption benefits (AZEVEDO et al., 2019), such as fruits of the *Myrtaceae* family that are known for their high content of bioactive compounds and antioxidant activity, including *Campomanesia xanthocarpa* O. Berg, popularly known as "guabiroba" (SILVEIRA et al., 2019). Guabiroba is considered a native Brazilian functional fruit that has an acid-sweet taste and antioxidant compounds, such as polyphenols (CAPELETTO et al., 2016). These properties of guabiroba make the pulp suitable for consumption in nature or beverage compositions. However, guabiroba pulp is still not a widely used ingredient in commercial products (BARBIERI et al., 2018). Therefore, this work aimed to use the concentrated whey from freeze concentration in blocks with gravitational thawing technology to elaborate a fermented lactic beverage added with probiotic and guabiroba pulp (*Campomanesia xanthocarpa* O. Berg), aiming to obtain a functional product. At the end of this work, we hope to bring a lactic beverage with probiotics rich in bioactive compounds.

2 MATERIAL AND METHODS

2.1 MATERIAL

The whey was obtained through the manufacture of a fresh Minas Frescal type cheese (whole pasteurized milk; 10.98 g/100g total solids, 2.98 g/100g protein, 4.07 g/100g carbohydrates, and 3.20 g/100g fat, Tirol®, Treze Tílias, Brazil). Clotting enzyme (HA-LA®) with a coagulant power of 1:3000 was purchased from Chr. Hansen (Valinhos, São Paulo, Brazil).The fermented beverages were prepared using a thermophilic culture of *Streptococcus salivarius* subsp. *thermophilus*, *Bifidobacterium* BB-12 and *Lactobacillus acidophilus* LA-5) (BioRich®, Chr. Hansen, Valinhos, São Paulo, Brazil), sucrose (União®, Barra Bonita, São Paulo, Brazil) and glucose (Yoki®, Paranavaí, Paraná, Brazil). The fermented beverage was elaborated with guabiroba pulp addition. The fruits were collected in the “Pinho de Baixo” community, located in the interior of Irati, Paraná state, Brazil (S25o27c56"; W50o37'51").

Guabiroba was pulped and kindly provided for use in this work by EMBRAPA FLORESTAS (Colombo, PR, Brazil), containing the following composition: 15.79 g/100g of total solids, 0.18g/100g of protein, 7.75g/100g of carbohydrates and 0.88 g/100g of fat. Peptone water (Oxoid, Hampshire, UK), AnaeroGen® (Oxoid, Hampshire, UK), MRS Agar (Merck, Darmstadt, Germany), and M17 agar (Sigma-Aldrich, São Paulo, Brazil) were used for the microbiological assays. All reagents were analytical grade. DPPH (1,1-diphenyl-2-picrylhydrazyl), Folin-Ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic) gallic acid and catechin standards were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

2.2 WHEY OBTAINING

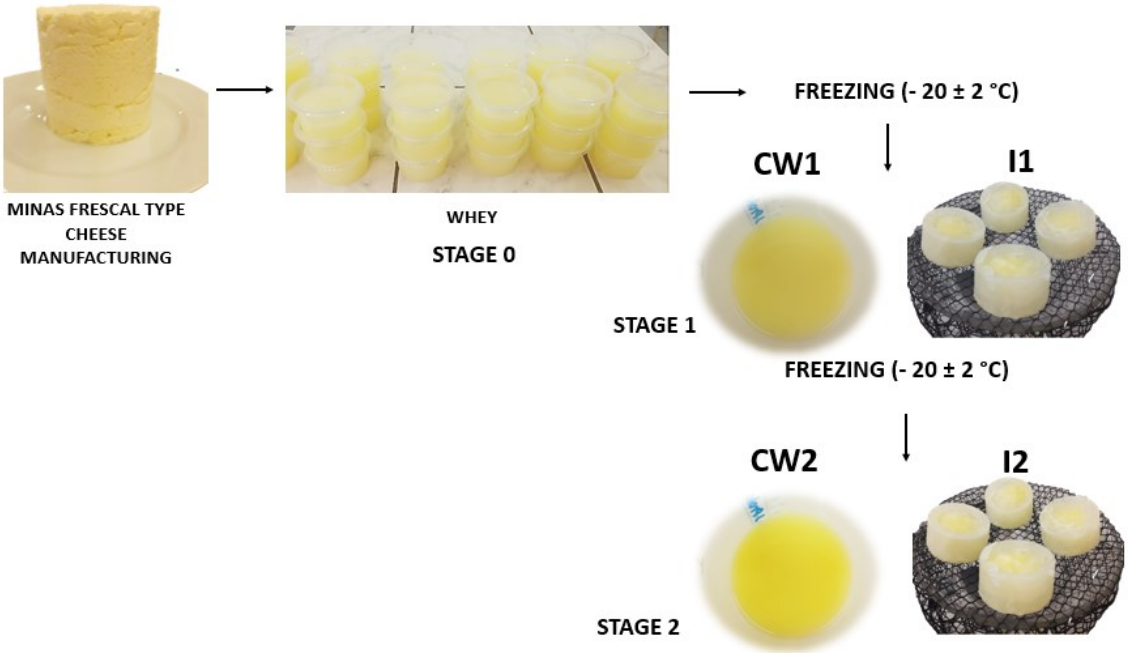
Through a previous production of Minas Frescal type cheese described by Souza and Saad (2009), whey, classified as a residue of the process, was obtained. For cheese production, 48 L of whole pasteurized milk was heated to 37 ± 1 °C for the addition of the coagulating enzyme , followed by an incubation at 37 ± 1 °C for 40 minutes. After this period, the clot was gently cut into cubes, stirred, drained, and placed in perforated cylindrical containers, each with a capacity of 500 g, to separate the whey. A filtration process was carried out with the clot, obtaining the filtered whey which was frozen (- 20 ± 2 °C) until the freeze concentration process and further analysis.

2.3 BLOCK FREEZE CONCENTRATION PROCESS

The block freeze concentration process with gravitational thawing was used in whey concentration, following the methodology described by Canella et al. (2018). At each stage of the freeze concentration process, two fractions were obtained and named concentrated whey (CW) and ice (I) (Figure 39). An initial volume of 7.2 L of whey was separated into pots containing approximately 200 g. Plastic containers containing whey were frozen at - 20 ± 2 °C in a freezer unit (Consul®, Biplex CRD41D, São Bernardo do Campo, Brazil). After complete freezing of whey, 50% of the initial volume was thawed at room temperature (20 ± 2 °C), obtaining two fractions, concentrated whey (CW1) and ice (I1). The concentrated fraction (CW1) was again frozen in plastic pots containing approximately 200 g at - 20 ± 2 °C and used as a feed solution in the second freeze concentration step, resulting in concentrated whey (CW2)

and on ice (I2). After each step, a portion of concentrated (CW1 and CW2) and ice fractions (I1 and I2) were collected and stored at -20 ± 2 °C for further analysis and use in preparing the fermented beverages.

Figure 39- Scheme of the freeze concentration process with gravitational thawing.



Source: The authors (2023).

Concentration fator (CF)

The concentration factor of the freeze concentration process, that is, its yield, was calculated using the following formula, according to Aider and Ounis (2012):

$$CF\% = \frac{\text{TSn}}{\text{TSo}} x100$$

Where TSn is the total solids content (g/100g), mineral salts (g/100g) or proteins (g/100g) in the concentrated whey at each concentration stage, and TSo is the total solids content (g/100g), mineral salts (g/100g) or proteins (g/100g) in the initial whey. The CF value was determined at each concentration stage as a function of the increase in total solids (g/100g), mineral salts (g/100g), and proteins (g/100g) in the cryoconcentrate (TSn), and about whey initial (TSo).

Process efficiency (PE)

The efficiency of the freeze concentration (PE) process was determined based on the increase in total solids in the concentrate (g/100g) concerning total solids, mineral salts (g/100g), or proteins (g/100g) remaining in the ice fraction of each stage of freeze concentration. PE was calculated by the following formula, according to Aider and Ounis (2012):

$$PE\% = \frac{\mathrm{TSn} - \mathrm{TSi}}{\mathrm{TSn}} x100$$

Where TSn is the content of total solids (g/100g), mineral salts (g/100g), or proteins (g/100g) in the concentrated whey fractions, and TSi is the content of total solids (g/100g), mineral salts (g/100g) or proteins (g/100g) on ice.

The concentrated whey used in the preparation of fermented beverages was chosen based on the evaluation of the FC and EP results.

2.4 FERMENTED BEVERAGE ELABORATION

Two fermented beverages were prepared according to the methodology of Almeida et al. (2001), with modifications and previous studies (data not shown) (Table 24).

Table 24- Formulation of the fermented beverage control and the fermented beverage with guabiroba pulp (10%) made with the concentrated whey from the best freeze concentration performance.

Formulation (g)	**Control beverage**	**Guabiroba beverage**
Concentrated whey chosen	879.5	779.5
Sucrose	80.0	80.0
Glucose	40.0	40.0
Thermophilic culture	0.5	0.5
Guabiroba Pulp	0.0	100.0
Total (g)	**1,000.0**	**1,000.0**

The concentrated whey of the stage that presents the best performance of the freeze concentration process was used to prepare the beverages. Two fermented beverages (control

beverage and guabiroba beverage) were prepared by heating concentrated whey to 42 ± 2 ◦C, followed by adding sucrose (8 g/100g), glucose (4 g/100g) to increase the speed of the fermentation process by starter cultures and probiotic cells, and inoculating with 0.05 g/100g of thermophilic culture (*Lactobacillus acidophilus* LA-5, *Bifidobacterium* sp. BB-12 and *Streptococcus thermophilus*), as recommended by the manufacturer. The incubation for the fermentation step was carried out at 42 ± 2 ◦C, measuring the pH, and cooled at 4 ± 2 ◦C for 24 h. The control beverage was prepared only with concentrated whey, without adding guabiroba pulp, and the beverage with guabiroba was ready with 10 g/100g of pulp, as proposed by Prestes et al. (2021). The samples were kept refrigerated (4 ± 1 ◦C) until analysis.

2.5 PHYSICOCHEMICAL ANALYSIS

Proximate Composition

For all samples of concentrated whey, ice fraction, and fermented beverages (control and with guabiroba pulp), the total solids content (g/100 g) was obtained by gravimetry drying in an oven at 105°C about 2g of sample for at least 16h or until until reaching a constant weight(AOAC, 2019). Protein content was performed using the Kjeldahl method. Approximately 0.2 to 0.5 g of sample was added to digester tubes containing the protein catalyst, concentrated sulfuric acid with the process occurring at 100°C (temperature increased every hour until reaching 350°C) until complete digestion. Distillation was carried out with sodium hydroxide and titration with hydrochloric acid. Results were expressed in g/100g (N x 6.38). The hydrogen ion potential of the samples was determined in a digital pH meter (Kasvi®, São Paulo, São Paulo, Brazil) with a previous calibration with buffer solutions (pH= 4.0 and pH= 7.0) at room temperature.

Instrumental analysis

The color parameters of the fermented beverages (control and with guabiroba pulp) were determined using the Minolta Chroma Meter CR-400 colorimeter (Konica Minolta, Osaka, Japan), adjusted to operate with D65 illuminant and observation angle of 10°. The colorimeter was calibrated with a standard white plate, and the CIELab color scale will be used to measure the parameters L*, a*, and b*. The L* parameter varies from 0 to 100 and indicates the brightness (variation from black to white); the b* axis is the change from yellow (+b*) to blue (-b*); and the a* axis shows the transition from red (+a*) to green (-a*). The total color

difference (ΔE*) between the two beverages will be calculated according to Okpala et al. (2010), as described in the following formula:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where ΔL* is the difference in luminosity, Δa* represents the intensity of the red color, and Δb* is the intensity of the yellow color.

Microbiological analysis

For the probiotic count (*Bifidobacterium* BB-12 and *Lactobacillus* LA-5), 25 grams of beverage were diluted in 225 mL of phosphate buffer (pH 7.0, 0.1 mol/L) followed by homogenization using a magnetic stirrer for 10 min. The mixtures were serially diluted in peptone water and inoculated in depth on MRS agar. The inverted plates were incubated at 37°C for 72 h in anaerobic jars using an AnaeroGen® sachet (VINDEROLA; REINHEIMER, 1999). *Streptococcus thermophilus* count was carried out by the pour plate technique using M17 agar with lactose solution (10 g/100 mL) incubated aerobically at 37°C for 48 h (IDF 1997). The total viable count was expressed as colony-forming units per gram of beverages (CFU/g).

Extraction for phenolic analysis and antioxidant activity

Fermented lactic beverages extracts preparation was conducted according to the method used by Shori and Baba (2013). Therefore, 10 g of fermented lactic beverages were mixed with 2.5 mL of distilled water, adjusted to pH 4 using HCl (1 M), and incubated at 45°C for 10 min. In the sequence, the mixture was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was collected, adjusted to pH 7 using 0.1 M NaOH, and centrifuged again at 10,000 rpm for 20 min at 4°C.

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH method was performed according to Brand-Williams et al. (1995). Standard Trolox solution (3000 µmol/L) was used to obtain the calibration curve (linearity range: R^2 = 0.99). 100 microliters of sample were pipetted into tubes with the addition of DPPH solution (0.00336g in 100mL) with a reaction time of 30 min without light and at room temperature. The analysis read was performed in a spectrophotometer (UV-1800, Shimadzu, Brazil) at 515

nm, and the results were expressed in micromoles of Trolox equivalents per liter of the sample (μmol TE/L).

Total phenolic content

The total phenolic content was evaluated using the Folin-Ciocalteu method (SINGLETON; ROSSI, 1965) with a calibration curve obtained with a standard solution of galic acid (1.0-9.0 mg/L). (calibration curve linearity range: $R^2 = 0.99$). In tubes, the sample extract was added (between 0.1 mL and 1.0 mL), with the addition of 1.25 mL of Folin Ciocalteau reagent and 5 mL of 15% sodium carbonate solution. The analysis read was performed in a spectrophotometer at 720 nm in a spectrophotometer. The results were expressed in milligrams of gallic acid equivalent per liter of the sample (mg GAE/ mL).

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation decolorization activity

For the ABTS method, according to Re et al. (1999). Standard Trolox solution (3000 μmol/L) was used to obtain the calibration curve (linearity range: $R^2 = 0.99$). 30 microliters of sample were pippeted into tubes with the addition of 3 mL of analysis solution (ABTS solution 7mmol/L and potassium persulfate 140 mmol/L) with a reaction time of 2 hours without light and at room temperature. The analysis read was at 734 nm in a spectrophotometer, and the results were expressed in micromole of Trolox equivalent per liter of the sample (μmol TE/L).

Carotenoids content

Both fermented beverages (control beverage and guabiroba beverage) were evaluated according to Rodriguez-Amaya (2001), with modifications. To extract carotenoids, 1 g of sample and 20 mL of acetone were weighed in a 50 mL Falcon® tube. After mixing in the vortex (Biomixer®, Jacareí, São Paulo, Brazil), the tube containing the mixture was placed in ultrasound for 30 min. The extract was separated using filter paper and a funnel. In a burette, 4 mL of petroleum ether was added, followed by the extracted liquid and 3 mL of type 2 ultrapure water. The burette was left to rest, waiting for phase separation. When there was no separation, a few drops of NaOH solution were dripped, and separation was awaited. After separation, the lower fraction (colorless) was removed for disposal, keeping only the colored phase in the burette. The colored phase was released to a volumetric flask, passing through a filter paper with sodium sulfate, retaining any aqueous residue. The burette was cleaned with petroleum

ether, avoiding loss of extract. Carotenoid content was obtained in a spectrophotometer, using a wavelength of 450 nm was used for β-carotene; the wavelength of 444 nm for α-carotene; the wavelength of 452 nm for β-cryptoxanthin, and the wavelength of 462 nm for λ-carotene. The carotenoid content was calculated using the following formula:

$$Carotenoids\ content\ [\mu g/100g] = \frac{Abs\ x\ Vol\ mL\ (dilutiion)}{A_{1cm}^{1\%}\ x\ \ weight\ of\ sample}\ x\ 10^6$$

Where, A is Absorbance, V is total extract volume (mL), A1% is molar absorptivity = 2592 (β-carotene), A1% is molar absorptivity = 2800 (α-carotene), A1% is molar absorptivity = 3100 (λ-carotene) and A1% is molar absorptivity = 2386 (β-cryptoxanthin).

Statistical analysis

Results were expressed as mean ± standard deviation. Data were analyzed using the STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, USA). To determine significant differences ($p < 0.05$), an analysis of variance (ANOVA) was carried out, followed by post hoc analysis with the Tukey test. All samples were produced in triplicates, and three parallel measurements were made for each replication.

3 RESULTS AND DISCUSSION

The results obtained for the freeze concentration of whey about the total solids, proteins, and mineral salt contents indicate that these values differed ($p < 0.05$) in the following order: CW2 > CW1 > whey > I2 = I1 (Table 25). It is noteworthy that this study used two stages of freeze concentration because, according to Aider et al. (2007) and Aider and Ounis (2012), from the third and fourth stages of the whey freeze and of the skimmed milk freeze concentration, respectively relatively high amounts of total solids are trapped in the ice fraction. Following these authors, this happens because, with increasing viscosity of the dairy raw material to be concentrated, the ability to obtain pure ice crystals decreases and, therefore, the general efficiency of the freeze concentration process also decreases. These authors also concluded that the increase in the viscosity of the solution resulted in a decrease in the phenomena of mass and heat transfer in the system. On the other hand, Samsuri et al. (2015) also stated that large ice crystals contain fewer impurities and lower solids content than smaller crystals, and such behavior is noted when slow freezing is used, as used in the present study.

The behavior obtained for total solids, proteins, and mineral salt contents of whey in our work was expected compared with these studies cited above. Machado Canella et al. (2020) carried out the freeze concentration process in blocks with vacuum thawing of goat milk, obtaining values for the concentrate yield of ~ 85% for the total solids content in two stages of freeze concentration. The yield of the concentrates obtained in this work were 348.35%, 321.50%, and 300.00%, in the second stage of the freeze concentration, with the total solids, proteins, and mineral salts, respectively. In the study by Canella et al. (2020), it was also observed that the efficiency of the freeze concentration process for the total solids content was around 90%, while our method was verified in the second stage of freeze concentration an efficiency > 95%. For proteins and mineral contents, the efficiency of our freeze concentration process was also approximately 90%. However, in the whey freeze concentration process carried out by Aider et al. (2007), the concentration factor for the total solids content in stage 2 was equal to 351.00% and, therefore, this value was similar to the second stage of the present study. However, in the study realized by these authors, at this same freeze concentration stage, the protein content concentration factor was 213.00% and 24.81% for the somatory of the following salts minerals: potassium, sodium, calcium, and magnesium, being, therefore, lower than values obtained in the present study.

Table 25- Results of the chemical composition and pH (mean ± standard deviation) of whey and samples resulting and the results of the concentration factor and the efficiency for each freeze concentration stage.

		Whey	CW1	I1	CW2	I2	CF1 (%)	PE1 (%)	CF2 (%)	PE2 (%)
Chemical composition (g/100g)	Total solids	$6.06^{c} \pm 0.2$	$11.62^{b} \pm 0.($	$0.93^{d} \pm 0.$	$21.11^{a} \pm 0$	$0.97^{d} \pm 0.0$	190.10	91.93	348.35	95.40
	Proteins	$0.93^{b} \pm 0.($	$1.73^{b} \pm 0.0$	$0.27^{d} \pm 0.$	$2.99^{a} \pm 0.$	$0.31^{d} \pm 0.0$	186.02	84.39	321.50	89.63
	Mineral salts	$0.56^{c} \pm 0,12$	$1.01^{b} \pm 0,03$	$0.14^{d} \pm 0.03$	$1.68^{a} \pm 0.02$	$0.16^{d} \pm 0.01$	180.36	86.14	300.00	90.47
pH		$6.17^{e} \pm 0,01$	$6.25^{c} \pm 0,01$	$6.20^{d} \pm 0.01$	$6.52^{a} \pm 0.05$	$6.41^{b} \pm 0.00$	-	-	-	-

CW1 and CW2 represent concentrated whey from the first and second stages of the freeze concentration process, respectively. I1 and I2 represent ice from the first and second stages of the freeze concentration process. CF1 and CF2 represent the concentration factor from the first and second stages of the freeze concentration process. PE1 and PE2 represent process efficiency from the first and second stages of the freeze concentration process, respectively. $^{a-e}$ Different and superscript lowercase letters, expressed in the same line, indicate significant differences between samples ($p < 0.05$).

Regarding the pH, the data obtained showed a difference ($p < 0.05$) between all values (Table 25). Igartúa et al. (2022) state that the whey pH values are approximately 7.0. According to Ho et al. (2021), pH around 6.0 and 6.5 cannot affect the whey's functional properties, such as solubility, foaming, and emulsifying properties. Through the results obtained in this first stage of the work, that is, due to the higher levels of total solids, proteins, and mineral salts, as well as due to the excellent values obtained for CF and PE, CW2 (concentrated whey 2) was chosen for the elaboration of fermented beverages. The results for the physicochemical composition and color parameters of both beverages elaborated are shown in Table 26.

Table 26- Results of the chemical composition, pH (mean ± standard deviation), and microbiological of the two fermented beverages (control beverage and guabiroba beverage with 10% of pulp), both elaborated from whey concentrate 2 (WC2).

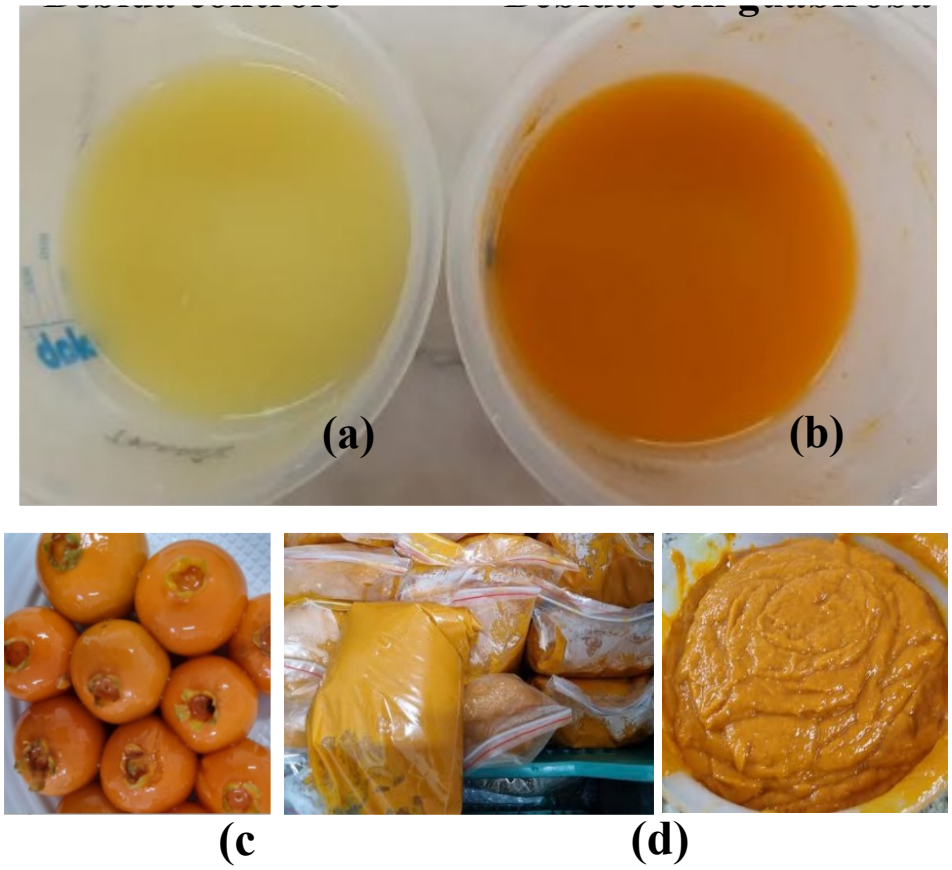
		Control beverage	**Guabiroba beverage**
Composition (g/100g)	Total solids	$28.48^{a} \pm 0.82$	$28.53^{a} \pm 0.28$
	Proteins	$2.99^{a} \pm 0.08$	$2.70^{a} \pm 0.15$
	Mineral salts	$1.66^{a} \pm 0.11$	$1.50^{a} \pm 0.08$
pH	After the fermentation process	$4.79^{a,A} \pm 0.01$	$4.48^{b,D} \pm 0.01$
	Beverages	$4.67^{a,B} \pm 0.01$	$4.56^{b,C} \pm 0.01$
Color parameters	L*	$39.04^{a} \pm 0.03$	$35.13^{b} \pm 2.21$
	a*	$-3.86^{b} \pm 0.02$	$4.52^{a} \pm 0.01$
	b*	$7.12^{b} \pm 0.04$	$21.31^{a} \pm 0.01$
	ΔE*	16.53	
Total count of cultures (CFU/g)	Probiotic culture	5.85×10^{8a}	3.95×10^{8b}
	Streptococcus thermophilus	5.71×10^{8a}	4.01×10^{8b}

The probiotic culture count comprises the count of *Bifidobacterium* BB-12 and *Lactobacillus acidophilus* LA-5, where the total count of cultures was expressed as log colony forming units per gram of beverage ($\log_{10}$ CFU/g). [a,b] Within a row, different superscript lowercase letters denote significant differences ($p < 0.05$) between samples. [A-D] Within a row or column, different superscript uppercase letters indicate significant differences ($p < 0.05$) between samples.

Between the two fermented beverages elaborated with whey concentrate (CW2), no differences were found ($p > 0.05$) between the contents of total solids, proteins, and minerals; that is, the 10% of guabiroba pulp did not show differences about these contents. It is relevant to point out that guabiroba pulp was added after the fermentation process because, according to Ning et al. (2021), some concentrations of organic acids contained in fruit pulps and juices can induce the separation of proteins, in this case, the proteins contained in concentrated whey (CW2). The values for the pH of the fermented beverages after the fermentation process, and therefore, even without the addition of guabiroba pulp, showed differences between them ($p < 0.05$). Likewise, these differences ($p < 0.05$) were observed between the control and fermented beverages incorporated with 10% guabiroba pulp. According to Meena et al. (2022), the difference ($p < 0.05$) in the pH values in the two cases may be due to variations in the different amounts of CW2 used and, subsequently, concerning other factors related to the constitution and enzymatic action of guabiroba, as well as changes in the chemical state of the fruit, transformed into pulp.

Due to the orange color of the guabiroba pulp, the color of the fermented beverage incorporated with the guabiroba pulp presented a color between yellow and red, and the luminosity (L*) decreased ($p < 0.05$). The value for ΔE^* was > 3.00, and according to Dantas et al. (2021), this confirms that the two fermented beverages produced have color differences that can be detected by the human eye, which can also be established in Fig. 40 (a, b).

Figure 40- (a) Control fermented beverage and (b) guabiroba fermented beverage (10% pulp) made from whey concentrate 2 (WC2), (c) "Guabiroba" fruit (Campomanesia xanthocarpa O.Berg), and (d) "Guabiroba" pulp



Regarding the microbiological count, a slight reduction ($p < 0.05$) can be seen for the fermented beverage incorporated with 10% guabiroba pulp. Similar results were obtained by Ning et al. (2021) in yogurts added with passion fruit pulp. These authors credited this behavior to the reduction in pH values and to the higher contents of phenolic compounds presented by the juice, which could negatively affect the viability of the bacteria. However, both fermented beverages could be considered potential probiotic products because their count was $\geq 10^6$ CFU/g. According to Castro et al. (2013), this should be seen as a possible advantage because it demonstrates the possibility of developing products with high whey concentrations and high microorganism counts beneficial to human health, such as probiotic bacteria. These results confirm the ability of the whey beverage to serve as a food matrix or as a food system that could be supplemented with counts of probiotic bacteria capable of delivering human health benefits, mainly toward *Bifidobacterium* BB-12 and *L. acidophilus* LA-5 counts (CASTRO et al., 2013).

This finding corroborated with a highlighted by Farias da Cruz et al. (2022) that dairy products were the most used vehicles for probiotic administration.

Notably, phenolic contents in several dairy products are restricted, and according to Pereira et al. (2012), guabiroba pulp is rich in phenolic compounds. Due to the greater content of these compounds, the fermented beverage with 10% of guabiroba pulp can benefit consumers' health (Table 27). It was possible to verify that the beverage with 10% of guabiroba pulp presented 1.61 times more total phenolic compounds and 2.55 times more DPPH and ABTS results than the control beverage (without the addition of pulp). Thus, the antioxidant potential was also found in the beverage made only with CW2. According to Arranz et al. (2019), whey proteins can exhibit antioxidant activity. Bovine whey proteins are rich in branched chains and sulfur-containing amino acids. Several studies have evaluated whey proteins as antioxidants. They could potentially be used in beverage formulations to deliver much-needed protein and serve to boost the antioxidant intake levels of the elderly consumer (ARRANZ et al., 2019). Bielecka et al. (2022) stated that histidine and other hydrophobic amino acids represent the antioxidant activity of whey. Peptides with high antioxidant capacity are released during whey protein hydrolysis. It is noteworthy that selected bacterial strains and enzymes can be used to stimulate protein hydrolysis and the synthesis of biologically active peptides to design novel products with proven health benefits. Therefore, fermented products are classified as a good source of bioactive peptides (BIELECKA; CICHOSZ; CZECZOT, 2022). Rosa et al. (2023) affirmed that probiotic strains might show different metabolic activities during fermentation, further concentrating on bioactive peptides. These authors observed that probiotic addition, regardless of the probiotic strain, increased the antioxidant activities of whey beverages. More specifically, Rosa et al. (2023) verified that *Lactobacillus* LA-5 and *Bifidobacterium* BB-12 showed higher α-glucosidase inhibition, improvements in the high saturated hypercholesterolemic index, and peptides with angiotensin-converting-enzyme-inhibitory, antimicrobial, immunomodulatory, and antioxidant activities. Their findings suggest that probiotic fermented whey beverages may exert antioxidant properties. Rosa et al. (2023) affirm that the products should be processed with LA-5 or BB-12 for better biological activity. However, the increase in the antioxidant activity was notable for the guabiroba beverage with 10% of pulp. Raphaelli et al. (2021) described that guabiroba has good functional and nutritional properties due to its high antioxidant potential. Prestes et al. (2021), evaluating the influence of guabiroba pulp added to fermented milk, observed that compounds addition with a potential prebiotic function, such as the phenolic compounds of guabiroba, enhances the

higher selective fermentation, resulting in a higher concentration of antioxidant metabolites. Therefore, bioactive substances present in plants have become popular as complementary or alternative therapeutic agents to manage or treat chronic diseases (NING et al., 2021). Paulo Farias et al. (2020) reported that the genus *Campomanesia*, which comprises guabiroba, includes species used against fever, dysentery, and urinary tract diseases, considered a fruit that contains bioactive compounds.

Table 27- Results (mean ± standard deviation) of, and carotenoids contents of two fermented beverages (control beverage and guabiroba beverage with 10% of pulp), both elaborated from whey concentrate 2 (CW2)

		Control beverage	**Guabiroba beverage**
Antioxidant activity	Total phenolic (mg GAE/mL)	$1.68^{b} \pm 0,25$	$2.70^{a} \pm 0,36$
	DPPH (µmol TE/L)	$601.45^{b,B} \pm 70.41$	$1,533.67^{a,A} \pm 117.95$
	ABTS (µmol TE/L)	$681.40^{b,B} \pm 89.10$	$1,741.44^{a,A} \pm 108.23$
Carotenoids (µg/100mL)	β-carotene	$75.90^{b} \pm 0.04$	$200.82^{a} \pm 0.05$
	α-carotene	$70.27^{b} \pm 0.32$	$185.89^{a} \pm 0.06$
	γ-carotene	$63.47^{b} \pm 7.28$	$167.91^{a} \pm 14.01$
	β- cryptoxanthin	$82.46^{b} \pm 9.47$	$218.16^{a} \pm 17.03$

Note: mgGAE/mL = mg of gallic acid per mL. µmol TE/L = micromole of Trolox equivalents per liter. ^{a,b} Within a row, different superscript lowercase letters denote significant differences ($p < 0.05$) between samples. $^{A-B}$ Within a row or column, different superscript uppercase letters indicate significant differences ($p < 0.05$) between samples.

Other bioactive compounds present in guabiroba are carotenoids. Therefore, it can also be observed in Table 4 the results obtained for the carotenoid contents, such as β-carotene, α-carotene, γ-carotene and β-cryptoxanthin of the fermented beverage control and the fermented beverage incorporated with 10% of guabiroba pulp. Carotenoids are natural pigments present in plants that have chemical structures that differ in their functional groups, which allows them to be classified into two groups: xanthophylls, which contain oxygen as an active group, and carotenes that have only the hydrocarbon, without the presence of no functional group. Commonly encountered oxygen substituent groups are hydroxyls, for example, β-

cryptoxanthin. Carotenoids also promote health benefits, even when present in pulps added to products. Carotenoids are phytochemicals among the most important food constituents helping to prevent cancer, in addition to being absorbed and converted by the human body into vitamin A, such as β-carotene. Vitamin A plays an important role in the human body because it directly participates in the chemistry of vision, cell differentiation, the reproduction system, growth, and the formation of organs and bones (DE PAULO FARIAS et al., 2020).

The fermented beverage incorporated with 10% of guabiroba pulp presented an increase of 164% for each evaluated carotenoid compared to those offered by the control beverage. Stinco et al. (2019) assessed the bioaccessibility of twenty-two commercial milk and fruit beverage made in Spain, and all of them had β-carotene (2.50 to 567.70 μg/100mL) in their composition, with twelve containing α-carotene (0.40 to 646.00 μg/100mL) and nine β-cryptoxanthin (2.90 to 475 μg/100mL). These authors concluded that the great variability of carotenoids is related to the food matrix. Even so, when comparing the values obtained in this study with the commercial data and the control sample, verifying that the carotenoid contents were relevant was possible. Finally, this study provides a new and unprecedented approach to a functional product obtained from concentrated whey from the second stage of freeze concentration in preparing a fermented beverage with 10% guabiroba pulp. It is noteworthy that whey beverages are important for dairy industries due to their economic and environmental values because whey is a by-product of the low cost of the cheese industry. In addition, the reduced-cost alternative to conventional yogurt has increased fermented whey beverage consumption. On the other hand, the growing interest in products derived from native plants has gained much attention internationally, which is very important for countries like Brazil, with rich biodiversity.This pioneering work may make it possible in the future to used a by-product of the dairy industry in the preparation of this innovative fermented beverage with potential functional properties. Future studies still must be carried out focusing on the viability of probiotic cells by *in vitro* methods and sensory analysis for the overall evaluation of the product by future consumers.

CONCLUSIONS

The best performance concerning the total solids, proteins, and mineral salt contents was found for the concentrated whey from the second stage of freeze concentration, which was used for elaborating both probiotic fermented beverages (without and with 10% of guabiroba

pulp). Both fermented beverages could be considered potential probiotic products because their count was ≥ 10^8 CFU/g. It was possible to verify that the incorporation of 10% of guabiroba pulp in the probiotic fermented beverage was not enough to modify their contents of total solids, proteins, and minerals. However, a decrease in pH values was observed, besides changing their colors to an orange hue with a reduced luminosity. However, the beverage with 10% of guabiroba pulp addition showed a great advantage, which could classify it as a functional product, because it presented 1.61 times more phenolic compounds and an increase of 164% for each of the evaluated carotenoids (β-carotene, α-carotene, γ-carotene, and β-cryptoxanthin) when compared with the beverage without the addition of guabiroba pulp. Finally, it could be concluded that it was possible to elaborate a functional fermented beverage with 10% guabiroba pulp due to the probiotic count and improving its nutritional properties.

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CHAPTER 10

Production of functional yogurt with cold-pressed guabiroba juice (*Campomanesia xanthocarpa* o. berg) concentrated by freeze concentration process

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PRODUCTION OF FUNCTIONAL YOGURT WITH COLD-PRESSED GUABIROBA JUICE (*Campomanesia xanthocarpa* O. Berg) CONCENTRATED BY FREEZE CONCENTRATION PROCESS

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ABSTRACT

The cold-pressed guabiroba juice was subjected to a block freeze concentration. The best process efficiency was obtained for the first stage of freeze concentration (C1; PE= 75.73%). Yogurt formulations were carried out with C1 (0, 10%, and 15%). For all concentrated juices (C1 and C2), ice fractions (I1 and I2), and yogurts (Control, I10, and I15), were performed physicochemical analyses, antioxidant activity, carotenoid content, total phenolic compounds (TPC), and mineral profile. Total soluble solids contents for the concentrated juices increased by 1.56 to 2 times concerning the cold-pressed guabiroba juice. Furthermore, an increase was observed for TPC (3,434.01 to 9,480.25 $mg.L^{-1}$) and carotenoids (561%). For the I15 sample, TPC increased by 4,556% about the control, as well as increased carotenoids by approximately 226 %, vitamin C to 2,991%, mineral profile (Ca, K, Mg, and Na) of 140 to 225%, also contributing to an increase in the antioxidant activity of 440 to 883%. The addition of concentrated guabiroba juice in yogurt formulations enhances the functional property of this dairy product by maintaining most of the bioactive compounds during cold-pressing associated with the freeze concentration.

Keywords: Fermented milk; *Myrtaceae* family; bioactive compounds; phenolic compounds; antioxidant activity.

1 INTRODUCTION

Yogurt is the main fermented milk consumed around the globe since this dairy product, of different manufacturers, is widely accepted by consumers due to its rich nutritional and functional value, taste, and practicality (ROSA et al., 2021; SFAKIANAKIS; TZIA, 2014). To expand the consumer market, the development of fermented milk with new ingredients is on the rise, with the fusion of new flavors and relevant functional appeal. The addition of juices, pulp, and pieces of fruit are among the ingredients that are most accepted by consumers and, on the other hand, increases the levels of bioactive compounds in the dairy product (AHMAD

et al., 2022; BALTHAZAR et al., 2019; FIDELIS et al., 2020; KARNOPP et al., 2017; NEVES CASAROTTI et al., 2020; PRESTES et al., 2021a).

Bioactive compounds, also called "phytochemicals," are produced by plant defense systems and have a defending action with antimicrobial, insecticidal, and antioxidant properties (DOMÍNGUEZ DÍAZ; FERNÁNDEZ-RUIZ; CÁMARA, 2020; RUIZ RODRÍGUEZ et al., 2021; YAHIA; GARCÍA-SOLÍS; MALDONADOCELIS, 2018). These compounds are classified as 'non-nutrients'. However, when introduced in a healthy dietary routine, it can exert several benefits to the human body, with a reduction in the incidence of chronic non-communicable diseases such as diabetes, obesity, cancer, and cardiovascular and neurological disorders (CUTRIM; CORTEZ, 2018; DOMÍNGUEZ DÍAZ; FERNÁNDEZ-RUIZ; CÁMARA, 2020; YASSIN et al., 2018). Among the bioactive compounds in fruits and vegetables are phenolic compounds, carotenoids, phytosterols, and vitamins (DA SILVA et al., 2022; MELLIDOU et al., 2019; SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018). In fermented milk, bioactive compounds from fruits, in addition to enhancing nutritional, functional, and sensory properties, can improve texture properties and increase the shelf-life of products due to their potential antioxidant and antimicrobial activities (JASTER et al., 2018; LAL et al., 2022; LIMA et al., 2019; PRESTES et al., 2021b). The natural pigment of the fruits, coming from bioactive compounds, can also reduce the addition of synthetic colorants to the formulation, which is extremely important due to recurrent allergenic conditions in people consuming artificial colorings (DE CAMPO et al., 2019; GENGATHARAN; DYKES; CHOO, 2016; KARNOPP et al., 2017; MEDEIROS et al., 2019). Recent studies with unexplored native fruits show their bioactive composition as potential substitutes for artificial food colorants (BACKES et al., 2020; BOHRA et al., 2022; DE PAULO FARIAS et al., 2020).

The guabiroba (*Campomanesia xanthocarpa* O. Berg) is a native Brazilian fruit rich in bioactive compounds, especially phenolic compounds, vitamin C, and carotenoids. The latter characterizes an epicarp with a transparent yellow-orange color due to the high concentrations of β-carotene and cryptoxanthin (DE PAULO FARIAS et al., 2020; SILVA-RODRIGUES et al., 2020). This native fruit has bioactive compounds also related to a potential prebiotic activity in yogurts, according to our previous studies realized by Prestes *et al.* (2021a) and Prestes *et al.* (2022a)

Fruit juices can be extracted from the original matrix and added to fermented milk formulations (BALTHAZAR et al., 2019; JAIMEZ-ORDAZ et al., 2019). With an extraction without high temperatures, the nutritional and sensory compounds of the fruit are preserved.

Cold-pressing processes produce cold-pressed juices, which first crush and press the fruit to extract its juice slowly. This process guarantees an efficient extraction and preserves the nutritional quality of the juice (DE SOUZA et al., 2020; KHAKSAR; ASSATARAKUL; SIRIKANTARAMAS, 2019; PRESTES et al., 2023). The contents of functional compounds, natural pigments and flavorings in fruit juices can be increased when subjected to a concentration process. However, traditional industrial processes, such as evaporation, require high temperatures for water to boil, which reduces the concentration of most native bioactive compounds (CASAS-FORERO; ORELLANA-PALMA; PETZOLD, 2020; PRESTES et al., 2022b). An alternative is the emerging freeze concentration technology in which liquid foods are concentrated over a pre-freezing step followed by the separation of pure ice crystals(MIYAWAKI et al., 2016; ZIELINSKI et al., 2019). The gravitational-assisted block freeze concentration, specifically, is proved to be an efficient and economical technique to concentrate juices from different fruits with high amounts of bioactive compounds (CASAS-FORERO; ORELLANA-PALMA; PETZOLD, 2020; HAAS et al., 2022; JASTER et al., 2018).

To encourage future industrial processes with a native Brazilian fruit, firstly, this study aimed to obtain a cold-pressed guabiroba juice that was concentrated by the gravitational block freeze concentration process. In the sequence, the concentrated of the freeze concentration stage, considered the most efficient, was chosen to be added to the yogurt formulation.

2 MATERIAL AND METHODS

2.1 CHEMICALS

Standards of gallic acid (purity ≥ 90%), ABTS (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were obtained from Sigma-Aldrich (St. Louis, USA). For elemental analysis, standard solutions (1000 $mg.L^{-1}$) of each element (K, Na, Mg, Ca, Fe, Mn, Zn, Se, P, Ni, Cu, Ba, As, and Pb) were obtained from Spex Certiprep Chemical (Metuchen, New Jersey, USA); argon gas was obtained from Linde (Blumenau, Brazil). All analytical grades were obtained from Vertec (Rio de Janeiro, Brazil).

2.2 COLD PRESSING OF GUABIROBA PULP

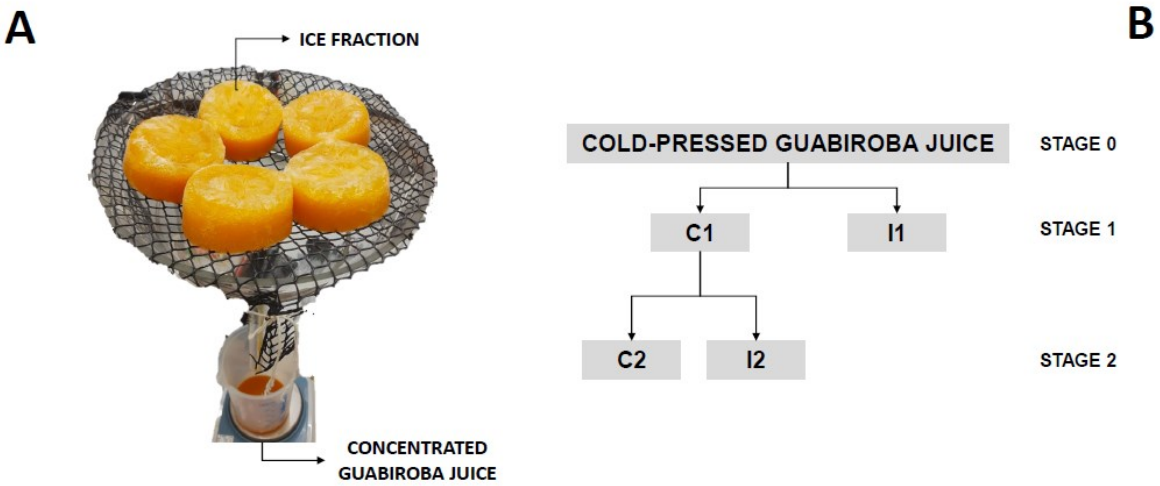
Guabiroba pulp was ceded by Embrapa Florestas (Colombo, PR, Brazil) (with 84.3% moisture, 0.18% protein, 7.75% carbohydrates, 0.88% fat, 6.26% dietary fiber, 0.63% ash). Cold-pressed juice was slowly obtained with a hydraulic press (1 ton) (TE-098, TECNAL, Brazil). The cold-pressed guabiroba juice was cooled at 4 ± 2°C until the next steps.

2.3 BLOCK FREEZE CONCENTRATION PROCESS

According to Canella et al. (2018), the gravitational-assisted block freeze concentration was performed. Firstly, the cold-pressed guabiroba juice was fractionated in plastic containers 200mL and then frozen at - 20 ± 2 °C. After the guabiroba juice was completely frozen, 50 % of the initial volume was defrosted at room temperature (20 ± 2 °C), obtaining two fractions, the concentrate guabiroba juice (C1) and the ice fraction (I1) (Figure 41A).

The defrosted liquid (C1) was used as a feed solution for the second stage, obtaining a second concentrate juice (C2) and ice (I2) (Figure 41B). A portion of each step (C1 and C2) and ice fraction (I1 and I2) was collected and stored at – 20 ± 2 °C until physicochemical, phenolic, and elemental analysis.

Figure 41- A: Block freeze concentration of cold-pressed guabiroba juice; **B:** Diagram of guabiroba juice freeze concentration. C1, C2 refer to concentrates of stages 1 and 2, respectively; I1 and I2 refer to ice fractions of stages 1 and 2, respectively.



Source: The Authors (2023).

2.4 FREEZE CONCENTRATION PARAMETERS

The freeze concentrations parameters were evaluated by the concentration factor (CF) according to the calculus of the methodology proposed by Aider and Ounis (2012) using the following Equation (1):

$$Concentration\ Factor = \frac{TS_n}{TS_0} \quad (1)$$

Where TS_n is the total soluble solids content (g.100mL^{-1}) of the concentrated guabiroba juice, and TS_0 is the total soluble solids content (g.100mL^{-1}) of the initial guabiroba juice.

The process efficiency (PE) was calculated based on the increase of the total soluble solids (TS) in the concentrated juice (g.100mL^{-1}) relative to the TS remaining in the ice (g.100mL^{-1}) from each freeze concentration stage, according to the Equation 2:

$$Process\ Efficiency\ (\%) = \frac{\mathrm{TS}_C - TS_I}{\mathrm{TS}_C}\ x\ 100 \quad (2)$$

Where TSC is the total soluble solids content (g.100mL^{-1}) in the concentrate, and TSI is the total soluble solids content (g.100mL^{-1}) in the ice at the end of each freeze concentration stage.

The experimental mass balance of each stage was calculated and compared to the theoretical value to validate the experimental results (BURDO; KOVALENKO; KHARENKO, 2008; SÁNCHEZ et al., 2011b) using the following Equation 3:

$$Wpred = \frac{C_i - C_c}{C_g - C_c} \quad (3)$$

Where W_{pred} is the predicted ice mass ratio (kg ice. kg concentrated guabiroba juice^{-1}), C_i is the total soluble solids content of the initial guabiroba juice (g100mL^{-1}), C_c is the total soluble solids content of the concentrate fraction (g.100mL^{-1}), and C_g is the total soluble solids content of the ice fraction (g 100mL^{-1}). The root mean square (RMS) deviation was calculated using Equation 4 to determine the variation between experimental and theoretical results.

$$RMS\ (\%) = 100\sqrt{\frac{\Sigma(Wexp-Wpred/Wexp)2}{N}} \quad (4)$$

Where W_{exp} and W_{pred} are the ratios of experimental and predicted ice mass, respectively, and N is the number of test repetitions.

2.5 YOGURT PRODUCTION

For yogurt production, it was employed commercial UHT (Ultra High Temperature) skimmed milk (4.5% carbohydrate, 3.75% protein, 0% lipid, and 8.05% total solids content) in Florianópolis, Santa Catarina state, Brazil, with an incorporation of thermophilic started culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, Yoflex®, Chr. Hansen, Hønsholm, Denmark) according to the manufacturer's instruction. The incubation for the fermentation step was performed at 42 ± 2°C until reaching pH 4.6 and cooled at 4 ± 2°C for 24 h. The yogurt was stirred and divided into three samples (control, I10, and I15). The control sample was prepared with no addition of concentrated guabiroba juice, and I10 and I115 were ready with 10% and 15% concentrate ($m.m^{-1}$), respectively, of the most efficient stage of freeze concentration. The samples were stored at - 20 ± 2 °C until further analysis.

2.6 PHYSICOCHEMICAL ANALYSIS

For all the samples (C1, C2, I1, I2, and the yogurt formulations: control, I10, and I15), the total solids content ($g.100g^{-1}$) was obtained by the oven drying method until constant weight at 105 ± 2 ºC (315 SE- Fanem, Brazil) (AOAC, 2019). Crude protein was determined by the Kjeldahl method (AOAC, 2019), fat content by the Soxhlet method (AOAC, 2019), and fixed mineral reside (ash) by subjecting the samples to 550°C (J 200 – Jung, Brazil)(AOAC, 2019). According to the Association of Official Analytical Chemist-AOAC (2019), the titratable acidity was also determined.

The pH analysis was performed with a digital pH meter DM 20 (Digimed, São Paulo, Brazil), and total soluble solids (°Brix) were measured at 20 °C (±1 °C) on a Tropen model I refractometer. Color intensity was measured with a spectrophotometer at 420 nm on a U-1800 UV–Vis (Hitachi, Kyoto, Japan), and the total color difference (ΔE*) between the samples were performed according to the Equation 5 (OKPALA; PIGGOTT; SCHASCHKE, 2010) :

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (5)$$

Where ΔL* is the luminosity difference, Δa* represents the intensity of the red color, and Δb* is the intensity of the yellow color.

A glass pycnometer was used for density analysis (g/cm^3) and calculated according to Equation 6:

$$\rho s = \frac{(m_3 - m_1)}{(m_2 - m_1) x \rho H_2 0} \quad (6)$$

Where ρs is the density of solutions, m_1 is the mass of empty pycnometer (g), m_2 is the mass of pycnometer with water (g), m_3 is the mass of pycnometer with solutions (g), and pH_2O is the density of water.

The concentration of vitamin C was determined by Tillman's method (AOAC, 2019), which consists of reducing 2,6-dichlorophenolindophenol by ascorbic acid, and the results were expressed as mg ascorbic acid.100 mL^{-1}.

2.7 PHENOLIC AND ANTIOXIDANT ANALYSIS

The samples (C1, C2, I1, I2, and control, I15 and I10 yogurts formulations) were analyzed for total phenolic content (TPC) according to the Folin-Ciocalteu method (SINGLETON; ROSSI, 1965), at 720 nm in a spectrophotometer (UV-1800, Shimadzu, Brazil). The results were expressed in milligrams of gallic acid equivalent per liter of the sample (mgGAE.mL^{-1}) (calibration curve linearity range: $R^2 = 0.99$).

For antioxidant activity, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed by Brand-Williams et al. (1995). The analysis read was performed in a spectrophotometer at 515 nm, and the results were expressed in micromole of Trolox equivalent per liter of the sample (μmolTE.L^{-1}). For the ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay, according to Re et al. (1999), the analysis read was at 734 nm in a spectrophotometer, and the results were expressed in micromole of Trolox equivalent per liter of the sample (μmolTE.L^{-1}). All these analyses were performed in triplicate.

2.8 CAROTENOIDS CONTENT

The carotenoid content was performed according to the methodology proposed by Rodriguez-Amaya (2001) with modifications. In a spectrophotometer, total alpha-carotene, beta-carotene, gamma-carotene, and cryptoxanthin contents were obtained at the following wavelengths: 450, 444, 452, and 462 nm, respectively. The results were expressed in micrograms of carotenoids per 100 grams of sample ($\mu g.100mL^{-1}$).

2.9 ELEMENTAL PROFILE BY OPTICAL EMISSION SPECTROMETER (ICP OES)

The multi-mineral composition of concentrate guabiroba juice, ice fractions, and yogurt formulations was determined using an inductively coupled plasma optical emission spectrometer (ICP OES). The spectrometer (iCAP 6300 DUO) had a concentric nebulizer, a cyclone spray chamber, and an automatic sampler (CETAC ASX-520-Thermo Scientific, Waltham, Massachusetts, USA). The samples were digested with nitric acid and hydrogen peroxide in a microwave oven (DGT-100 Plus Provecto microwave oven -Provecto Analítica, São Paulo, Brazil). Argon was used as the main, auxiliary, and nebulizer gas. The monitored elements were K, Na, Mg, Ca, Fe, Mn, Zn, Se, P, Ni, Cu, Ba, As, and Pb. The operating parameters were 1300 W radio frequency power, the auxiliary gas flow of 1.0 $L.min^{-1}$, and the nebulizer gas flow rate of 0.38 $L.min^{-1}$. All determinations were performed in triplicate, and three-step recovery tests were performed to check the method's accuracy.

2.10 STATISTICAL ANALYSIS

The results were expressed as means ± standard deviation. The data analysis was performed using STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, USA). One-way analysis of variance (ANOVA) was implemented to determine the significant differences ($p< 0.05$). All samples were produced in triplicates, and three parallel measurements were made for each replication.

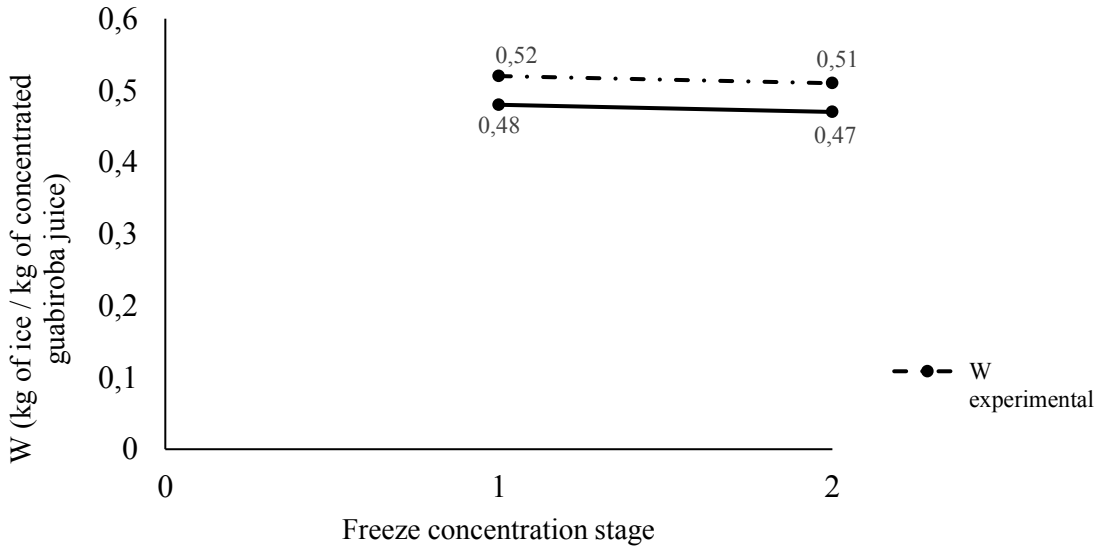
3 RESULTS AND DISCUSSION

3.1 MASS BALANCE AND THE PERFORMANCE OF THE BLOCK FREEZE CONCENTRATION

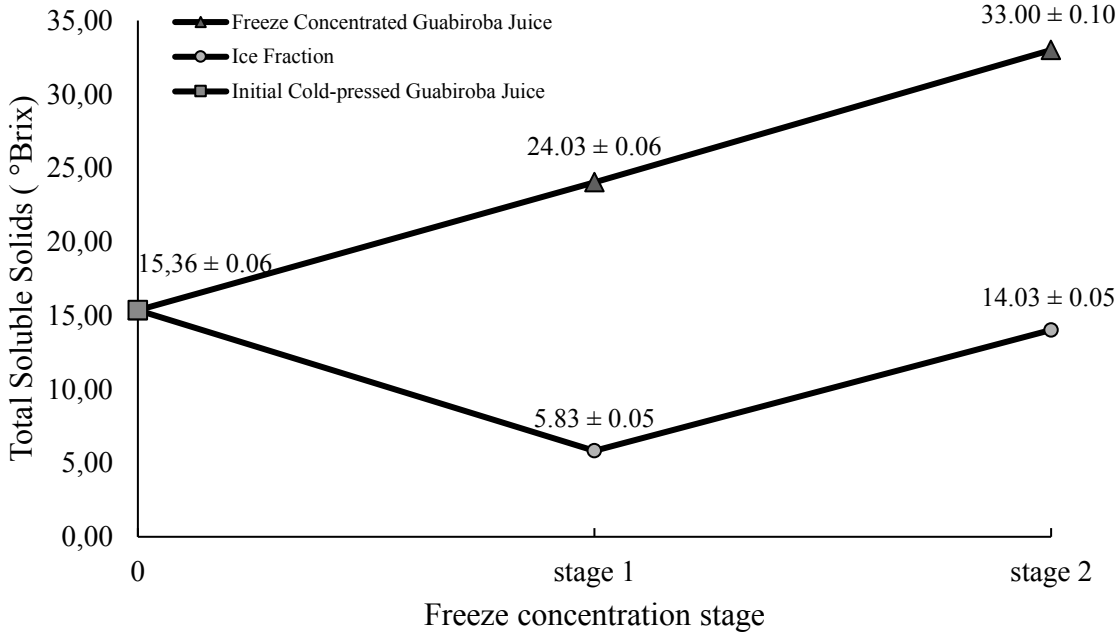
The mass balance of the freeze concentration process is shown in Figure 42A. the Mass balance of theoretical data (W predicted) in each freeze concentration stage was compared with experimental data (W experimental). It is possible to observe that a good agreement was reached. With the RMS, it is possible to determine the deviation between theoretical and experimental results with a good adjustment observed in the first stage (8.10%) and the second (7.92%). These concentration stages obtained deviations below 25%, which becomes an acceptable adjustment according to Lewicki (2000). The RSM results are also in agreement and close to the values obtained in studies by Canella *et al.* (2019) (6.9%), Hernández *et al.* (2010) (7.3%) and Petzold *et al.* (2016) (9.5%).

Figure 42- A- Experimental and predicted ice mass ratios as a function of freeze concentration stages of guabiroba juice. **B-** Changes in soluble solids of concentrated guabiroba juice and ice fractions obtained by two-stage block freeze concentration.

A



B



Source: The Authors (2023).

Table 28 shows the performance of the freeze concentration process with values of concentration factor and process efficiency.

Table 28- Process efficiency and concentration factor of block freeze concentration of cold-pressed guabiroba juice.

Stages	Samples	Total soluble solids ($g.100mL^{-1}$)	CF	PE (%)	RSM (%)
Stage 0	Cold-pressed guabiroba juice	15.36 ± 0.06^{cA}	-	-	-
Stage 1	C1	24.03 ± 0.06^{b}	1.56 ± 0.01	75.73 ± 0.18	8.10
	I1	5.83 ± 0.05^{C}	-	-	
Stage 2	C2	33.00 ± 0.10^{a}	2.14 ± 0.01	57.47 ± 0.01	7.92
	I2	14.03 ± 0.05^{B}	-	-	

Note: Results expressed as a mean ± standard deviation, among two batches performed in triplicate for each freeze concentration stage, with three repetitions for total soluble solids, CF and PE; ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p<0.05$) between the guabiroba juice and the C1 and C2; $^{A, B, C}$ Within a column, different superscript uppercase letters denote significant differences ($p<0.05$) between the guabiroba juice and the I1 and I2 fractions.

These calculated parameters were based on total soluble solids content, which ranged from 15.36 to 33.0 g.100mL^{-1} for the guabiroba juice and the concentrates, statistically differing in each stage of concentration (Figure 42B) ($p<0.05$). With the increase in freeze concentration stages, there was a progressive increase in concentration factor (1.56 to 2.14), directly related to the total soluble solids in the concentrated fraction (CANELLA et al., 2019). Similar results were obtained in studies with the freeze concentration of orange juice by Haas *et al.* (2022), with concentration factors ranging from 1.61 to 3.53.

With the decrease in the availability of free water with the advancement of the freeze concentration stages, there is a greater retention of solids content in the concentrated juices concerning the original juice, increasing the concentration factor (PETZOLD et al., 2016). On the other hand, process efficiency decreases throughout the freeze concentration stages due to the increase of total solids not only in the concentrated juice, but also in its ice fraction as the stages of the process pass (5.83 g.100mL^{-1} for I1 and 14.03 g.100mL^{-1} for I2) (HAAS et al., 2022). According to Raventós *et al.* (2007) and Sánchez *et al.* (2011b), the viscosity of the concentrate solution increases as the total solids content increases (Table 29), and these particles tend to be retained at the ice-liquid interface, decreasing the speed of the diffusion process and also the process efficiency.

In the freeze concentration process of fruit juices, the total soluble solids content (°Brix) is a good indicator of the process efficiency and how much solids are retained both in the concentrated and the ice fractions, being an important parameter of the number of stages of this process to be carried out. In our preliminary studies with whey and milk, process efficiency is high up to the second or third stage of block freeze concentration (BARROS et al., 2021; CAMELO-SILVA et al., 2021; CANELLA et al., 2018; DE LIZ et al., 2020). In this study, the highest process efficiency of the freeze concentration of cold-pressed guabiroba juice was obtained in stage 1 (75.73%), similar to the same stage obtained for freeze-concentrated orange juice (81.80%) Haas *et al.* (2022). In addition, Table 29 shows a significant difference ($p<0.05$) between the total solids retained in the ice fractions (4.28 g.100mL^{-1} for I1 and 9.72 g.100mL^{-1} for I2), which emphasizes the decrease of the process efficiency for the stage 2. Therefore, C1 was chosen to be added to the formulation of yogurts at different concentrations (0, 10, and 15%).

3.2 PHYSICOCHEMICAL PROPERTIES AND MINERAL PROFILE OF CONCENTRATED GUABIROBA JUICE

Table 29 shows the physicochemical parameters for cold-pressed guabiroba juice, the freeze concentrates, and the ice fractions.

Table 29- Physicochemical parameters for cold-pressed guabiroba juice and its freeze concentrated.

Analysis	Cold-pressed juice (J)	C1	I1	C2	I2
Moisture ($g.100mL^{-1}$)	84.39 ± 7.26^{Ca}	81.93 ± 0.87^{b}	95.62 ± 0.04^{A}	75.54 ± 0.36^{c}	90.28 ± 0.04^{B}
Total solids ($g.100mL^{-1}$)	15.61 ± 7.26^{Ab}	18.07 ± 0.87^{c}	4.38 ± 0.12^{C}	24.46 ± 0.37^{a}	9.72 ± 0.82^{B}
Protein ($g.100mL^{-1}$)	0.40 ± 0.01^{Ac}	0.43 ± 0.01^{b}	0.20 ± 0.01^{C}	0.61 ± 0.02^{a}	0.36 ± 0.02^{B}
Lipid ($g.100mL^{-1}$)	<0.01	<0.01	<0.01	<0.01	<0.01
Ash ($g.100mL^{-1}$)	1.50 ± 0.35^{Ac}	3.41 ± 2.55^{b}	0.01 ± 0.01^{C}	4.94 ± 0.95^{a}	0.29 ± 0.01^{B}
Titratable acidity ($g.100mL^{-1}$)	0.96 ± 0.04^{Ac}	1.28 ± 0.28^{b}	0.76 ± 0.63^{C}	2.10 ± 0.05^{a}	0.88 ± 0.01^{B}
pH	3.87 ± 0.03^{Ba}	3.75 ± 0.01^{b}	3.87 ± 0.02^{B}	3.72 ± 0.03^{c}	3.90 ± 0.02^{A}
Density ($g.cm^{3}$)	1.07 ± 0.01^{Ab}	1.09 ± 0.05^{b}	1.01 ± 0.01^{B}	1.13 ± 0.10^{a}	1.02 ± 0.01^{B}
L*	33.33 ± 0.18^{a}	33.06 ± 0.13^{a}	33.00 ± 0.16	32.69 ± 0.17^{b}	33.41 ± 0.33
a*	3.82 ± 0.07^{Ab}	3.90 ± 0.02^{b}	3.44 ± 0.03^{B}	4.62 ± 0.03^{a}	3.46 ± 0.10^{B}
b*	16.69 ± 0.18^{A}	16.62 ± 0.15	16.13 ± 0.03^{B}	16.90 ± 0.03	15.64 ± 0.15^{C}
ΔE (JxC1)			0.29		
ΔE (JxC2)			1.04		
ΔE (C1xC2)			0.86		
ΔE (JxI1)			0.73		
ΔE (JxI2)			1.11		
ΔE (C1xI1)			0.73		
ΔE (C2xI2)			1.85		
ΔE (JxC1)			0.29		

Note: Results expressed as a mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p<0.05$) between the cold-pressed guabiroba juice (J) and Concentrated 1 (C1) and Concentrated 2 (C2). $^{A, B, C}$ Within a column, different superscript uppercase letters denote significant differences ($p<0.05$) between the cold-pressed guabiroba juice and ice fractions 1 and 2 (I1 and I2). ΔE is the total color difference between two different samples.

For moisture results (Table 29), the freeze-concentrated juices had significantly lower levels (75.54 to 81.9 g.100mL^{-1}) compared to the original juice (84.39 g.100mL^{-1}) ($p<0.05$). These data were expected, since at each stage of the block freeze concentration, about 40 to 50% of the free water content is directed to the ice fraction (MORISON; HARTEL, 2018; PRESTES et al., 2022b). From this, there is a significant increase ($p<0.05$) in the total solids content (18.07 g.100mL^{-1} for C1 and 24.46 g.100mL^{-1} for C2) and ashes (3.41 g.100mL^{-1} for C1 and 4.49 g.100mL^{-1} for C2) as the freeze concentration stages advance.

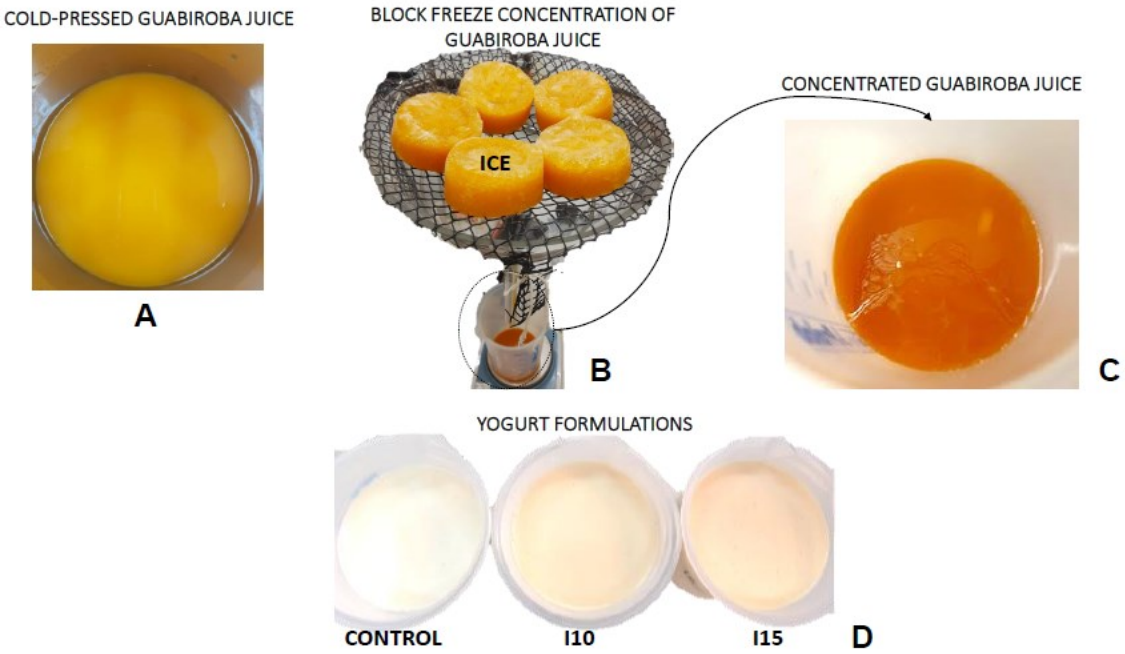
Within the measured total solids, the protein content also increased with the evolution of the block freeze concentration stages (0.43g.100mL^{-1} for C1 and 0.61g.100mL^{-1} for C2). The protein content of the whole guabiroba fruit is around 0.4 to 5.5 g.100g^{-1} (DE OLIVEIRA RAPHAELLI et al., 2021; PRESTES et al., 2022a) and, considering that the cold-pressed juice does not contain other parts of the fruit such as pulp, peel and seeds, the protein content is lower. Based on this principle, in the cold pressing process, the juice is extracted from the guabiroba pulp without the presence of peel and seeds, which contain the lipid content of the fruit (1.5 to 1.9 g.100g^{-1})(PRESTES et al., 2022a; VALLILO et al., 2008). Due to the absence of extracting the lipid fraction of the peels and seeds for the juice, the total lipid content was below the quantification limit of the method (<0.01 g.100mL^{-1}; Table 29).

Freeze concentration caused significant effects ($p<0.05$) both for pH and total titratable acidity in concentrated juices and ice fractions (Table 29). The increase in acidity is related to the rise in the concentration of organic acids as the stages of this process advance(CASAS-FORERO; ORELLANA-PALMA; PETZOLD, 2020). Due to the attention to solids content, the density of C2 increased ($p<0.05$) in the cold-pressed juice. This result emphasizes the efficiency of solids retention by the process described in Table 28.

For color parameters, C2 differed significantly from C1 and cold-pressed juice. For the a* coordinate, the progress of the process intensifies the orange color of the concentrated juices (C2= 4.62; C1= 3.90; juice= 3.82) (Figure 3C; Table 29), but decreases the luminosity, characterized by L* (C2= 32.69; C1= 33.06; juice= 33.33; Table 29). The b* coordinate did not differ statistically ($p<0.05$) to C1 and cold-pressed juice, indicating a yellow hue for all samples. The yellow/orange color of both the cold-pressed guabiroba juice and the concentrated juice is related to the high concentration of carotenoids in the fruit (DE PAULO FARIAS et al., 2020; PRESTES et al., 2022a) (Table 30). Since part of the solid content is also retained in the ice fraction, a yellow-orange color is also observed for I1 and I2 (I1: a*= 3.44; b*= 16.13; I2: a*= 3.46; b*= 15.64; Figure 43B). The intensity of the color is so noticeable that for the total color

difference, expressed by ΔE*, in all comparison pairs between samples, the values were less than 3.0. Martínez-Cervera *et al.* (2011) reported that when ΔE* is lower than 3, it cannot be visually perceived by the human eye, which is notable in Figures 43A, B, and C.

Figure 43- Cold-pressed guabiroba juice (**A**), Ice fraction from freeze concentration process (**B**), concentrated guabiroba juice (**C**), and yogurt formulations (**D**).



Source: The authors (2023).

The levels of total phenolic content (TPC) ranged from 3,434.01 to 9,480.25 mg.L^{-1} (Table 30), increasing as the block freeze concentration stages progressed, with an approximate increase of 276% in the last stage of freeze concentration (C2). Similar behaviors were observed in orange juice (HAAS et al., 2022), apple juice (ZIELINSKI et al., 2019), and blueberry juice (CASAS-FORERO; ORELLANA-PALMA; PETZOLD, 2020).

Guabiroba is one of the fruits of the *Myrtaceae* family with one of the highest levels of phenolic compounds in its composition (9,033.20 mg.100g^{-1}) (PEREIRA et al., 2012; PRESTES et al., 2022a). The cold-pressed juice, even if it does not contain fractions of the peel, seeds, and pomace, where the phenolic compounds are also concentrated, contains high levels of phenolic compounds (3,434.01 mg.L^{-1}) when compared to orange juice (715.70 mg.L^{-1}), and apple juice (740 to 873 mg.L^{-1}) (HAAS et al., 2022; ZIELINSKI et al., 2019). In the same way, the content of individual carotenoids increased with the progress of the concentration stages,

with an average increase of 561% for each carotenoid content for C2 (α, β, γ- carotene, and cryptoxanthin; Table 30).

Table 30- Total phenolic content, carotenoids content, antioxidant activity and vitamin C of cold-pressed guabiroba juice, freeze concentrated juices (1 and 2), and ice fractions (1 and 2).

Analysis	Cold-pressed juice	C1	I1	C2	I2
TPC ($mg.L^{-1}$)	3,434.01 ± 0.26^{Ac}	5,944.98 ± 0.23^{b}	2,392.16 ± 0.11^{C}	9,480.25 ± 2.37^{a}	2,954.05 ± 0.79^{B}
α- carotene ($\mu g.100mL^{-1}$)	357.10 ± 0.80^{Ac}	651.96 ± 0.18^{b}	155.59 ± 3.21^{C}	2,003.88 ± 2.91^{a}	212.61 ± 1.17^{B}
β-carotene ($\mu g.100mL^{-1}$)	385.76 ± 2.49^{Ac}	704.28 ± 7.94^{b}	168.80 ± 4.75^{C}	2,164.68 ± 3.22^{a}	229.68 ± 6.34^{B}
γ-carotene ($\mu g.100mL^{-1}$)	322.54 ± 4.01^{Ac}	588.87 ± 2.01^{b}	140.53 ± 5.10^{B}	1,809.96 ± 9.31^{a}	192.04 ± 2.08^{C}
Cryptoxanthin ($\mu g.100mL^{-1}$)	419.06 ± 7.19^{Ac}	765.08 ± 5.41^{b}	249.51 ± 2.23^{B}	2,351.58 ± 6.34^{a}	182.59 ± 8.13^{C}
DPPH ($\mu molTE.L^{-1}$)	2,304.00 ± 138.59^{Ac}	5,240.00 ± 93.40^{b}	93.40 ± 40.24^{C}	16,640.00 ± 131.37^{a}	1,015.07 ±40.46^{B}
ABTS ($\mu molTE.L^{-1}$)	41,477.78 ± 688.61^{Ac}	58,700.00 ± 648.31^{b}	1,067.00 ± 470.14^{C}	101,477.78 ± 554.09^{a}	2,177.00 ± 330.00^{B}
Vitamin C ($mgAA.100mL^{-1}$)	466.66 ± 46.19^{Ac}	533.33 ± 23.09^{b}	293.33 ± 23.10^{C}	666.66 ±20.30^{a}	373.33 ± 24.00^{B}

Note: Results expressed as a mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p<0.05$) between the guabiroba juice and the C1 and C2. $^{A, B, C}$ Within a column, different superscript uppercase letters denote significant differences ($p<0.05$) between the guabiroba juice and the I1 and I2 fractions. TPC= Total phenolic content. TE= Trolox equivalent. AA= Ascorbic Acid.

According to Santos (2012) and Schmidt *et al.* (2019), *Campomanesia xanthocarpa* O.Berg is a potential source of carotenoids, with the β-carotene contents of the whole fruit (12.30 to 3,400.00 $mg100\ g^{-1}$) being related to a great potential of vitamin A when compared to other fruits (papaya= 0.04 $mg.100g^{-1}$; orange 0.09 $mg.100g^{-1}$). The intensity of the orange color, on the other hand, is related to the significant levels of cryptoxanthin (419.06 $\mu g.100mL^{-1}$ for cold-pressed juice; 765.08 $\mu g.100mL^{-1}$ for C1, and 2,351.58 $\mu g.100mL^{-1}$ for C2; Table 3),

also in prominence when compared to other fruits (papaya= 0.50 mg.100 g^{-1} and apricot= 0.60 mg.100 g^{-1}) (PRESTES et al., 2022a).

Fruits from the *Myrtaceae* family are elucidated to contain high levels of vitamin C. The whole guabiroba fruit has average levels of 17.80 to 233 mg.100g^{-1} (PRESTES et al., 2022a). For the cold-pressed juice and concentrates, the contents ranged from 466.66- 666.66 mg.100mL^{-1} (Table 30). In orange juice's block freeze concentration process, Haas *et al.* (2022) obtained levels of the second stage of 40 mg.100 mL^{-1}. On the other hand, the concentrated juice of the second stage of this work (C2) reached 666.66 mg.100mL^{-1}, corresponding to more than 16 times the content of orange juice. This content offers potential benefits to human health due to its antioxidant capacity, which acts on the mechanism of scavenging free radicals related to aging processes and degenerative diseases(PRESTES et al., 2022a; SCHMIDT et al., 2019). Thus, high levels of vitamin C, total phenolics, and carotenoids in both juices and concentrates are related to the great antioxidant activity among all samples. For the DPPH assay, the C2 content reached 16,640.00 µmolTE.$L^{-1,}$ and for the ABTS method, 101,477.78 µmolTE.L^{-1} (Table 30), indicating that the antioxidant activity also increased with the progress of the freeze concentration. Similar behavior was also obtained in apple juices, according to Zielinski *et al.* (2019), and in orange juices by Haas *et al.* (2022). Since it is a non-thermal process, the freeze concentration preserves bioactive compounds and increases antioxidant activity levels. In addition, the cold-pressing process prevents heat and oxidative damage to bioactive compounds due to pressing the whole fruit at low/room temperatures (PRESTES et al., 2022a).

The mineral profile of cold-pressed juice, concentrates, and ice fractions is shown in Table 31.

The levels of four macroelements (Ca, K, Mg, Na) and seven microelements (Fe, Se, Zn, Cu, Mn, P, Ba) varied significantly ($p<0.05$) between the juice and concentrates. With the advancement of the freeze concentration stages and with the high concentration of solids and removal of water, there was a greater concentration of the main macroelements for C2 (K: 2,609 µg.mL^{-1}; Mg: 290 µg.mL^{-1}; Na: 79 µg.mL^{-1}; Ca= 52 µg.mL^{-1}, with increases of 185%, 146%, 493%, and 148%, respectively). These results are in agreement with the literature since the guabiroba fruit is a good source of Potassium (208.40 mg.100g^{-1}) which is naturally present in fruit in the form of potassium tartrate, and Calcium (28.40 mg.100g^{-1}) (DE PAULO FARIAS et al., 2020; VALLILO et al., 2008). For micronutrients, which are also essential to maintain proper functioning of the body's biological functions, guabiroba is rich in iron (0.60 mg.100g^{-1}), phosphorus (14.90 mg.100g^{-1}), manganese(13.50 mg.100g^{-1}) and zinc (0.40 mg.100g^{-1}

[1])(PRESTES et al., 2022a; VALLILO et al., 2008). In the present study, interesting values of these micronutrients were also obtained for stage 2 (P=223 μg.mL^{-1}; Fe=1.9 μg.mL^{-1}; Mn=3.3 μg.mL^{-1}; Zn= 5.3 μg.mL^{-1}, with approximate increases of 155%). Arsenic and Pb were below the detection and quantification limits.

Table 31- Elemental profile of cold-pressed guabiroba juice, freeze concentrated juices 1 and 2 (C1 and C2), and ice fractions 1 and 2 (I1 and I2) obtained from block freeze concentration process.

Elements (μg.mL^{-1})	**Cold-pressed guabiroba juice**	**C1**	**I1**	**C2**	**I2**
Ca	35.00 ± 2.00^{Ab}	18.00 ± 1.00^{c}	21.00 ± 1.00^{B}	52.00 ± 4.00^{a}	26.00 ± 2.00^{B}
K	1409.00 ± 26.00^{Ac}	1483.00 ± 21.00^{b}	573.00 ± 14.00^{C}	2609.00 ± 45.00^{a}	1028.00 ± 30.00^{B}
Mg	198.00 ± 3.00^{bA}	173.00 ± 3.00^{c}	58.00 ± 1.00^{C}	290.00 ± 3.00^{a}	118.00 ± 4.00^{B}
Na	16.00 ± 1.00^{Ac}	30.00 ± 1.00^{b}	5.00 ± 1.00^{C}	79.00 ± 2.00^{a}	14.00 ± 1.00^{B}
Fe	0.22 ± 0.02^{c}	0.80 ± 0.10^{b}	< LOQ	1.90 ± 0.10^{a}	<LOQ
Mn	2.20 ± 0.10^{Ab}	1.42 ± 0.10^{c}	0.74 ± 0.10^{C}	3.30 ± 0.10^{a}	1.96 ± 0.10^{B}
Se	0.28 ± 0.04^{b}	0.26 ± 0.08^{b}	< LOD	1.19 ± 0.03^{a}	<LOD
Zn	3.41 ± 0.10^{Ab}	2.21 ± 0.10^{c}	1.26 ± 0.10^{C}	5.30 ± 0.10^{a}	3.14 ± 0.10^{B}
P	143.00 ± 1.00^{Ab}	126.00 ± 3.00^{c}	40.00 ± 2.00^{C}	223.00 ± 5.00^{a}	86.00 ± 2.00^{B}
Ni	0.14 ± 0.06	< LOD	< LOD	0.11 ± 0.01	< LOD
Cu	0.84 ± 0.03^{Ab}	0.53 ± 0.02^{c}	0.34 ± 0.01^{C}	1.43 ± 0.05^{a}	0.73 ± 0.01^{B}
Ba	0.69 ± 0.02^{Ab}	0.55 ± 0.01^{c}	0.33 ± 0.01^{B}	0.97 ± 0.03^{a}	0.39 ± 0.01^{B}
As	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pb	<LOQ	<LOQ	< LOD	< LOD	<LOQ

Note: Results expressed as a mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p<0.05$) between the guabiroba juice and the C1 and C2. $^{A, B, C}$ Within a column, different

superscript uppercase letters denote significant differences ($p<0.05$) between the guabiroba juice and the I1 and I2 fractions. LOD = limit of detection. LOQ = limit of quantification.

These results are important because metallic elements are potentially toxic at a low level of exposure to the human body, affecting the respiratory and vascular systems, kidneys, heart, and liver (JAISHANKAR et al., 2014).

These results show that, as in any process, the block freeze concentration can generate total solid losses that tend to be retained in the ice fraction. Considerable contents of phenolic compounds, carotenoids, and some minerals such as Na, Mg, Fe, and P, make this byproduct interesting to be applied in other food formulations, as a result of the retention of functional and nutritional compounds, and also by the great orange color, related to the concentration of carotenoids (Table 30, Table 31, Figure 43B). Therefore, ice fractions can be reused and destined for processing beverages, jams, or candies, adding a new functional and sensory value.

3.3 PHYSICOCHEMICAL, BIOACTIVE PROPERTIES AND MINERAL PROFILE FOR YOGURT FORMULATIONS

The best process efficiency obtained in stage 1 was the determining factor for adding C1 in the yogurt formulation, containing 0%, 10%, and 15% (control, I10, and I15) of the concentrated juice, with the physicochemical results shown in Table 32.

With a gradual increase in the concentration of C1 between the samples, there was an increase in the content of total solids (10.99 to 11.98 $g.100mL^{-1}$), total soluble solids (6.60 to 10.50 $g.100mL^{-1}$), ash (0.74 to 0.79 $g.100mL^{-1}$), and density (0.97 to 1.01 $g.cm^3$), with the highest levels found for yogurt with 15% of concentrated guabiroba juice ($p<0.05$).

Dairy products are sources of protein with average levels of $3.4g.100mL^{-1}$ for milk and $5g.100mL^{-1}$ for yogurt (MARINI et al., 2022). However, when concentrated guabiroba juice was added to the formulation, there was a dilution of protein contents in samples I10 ($3.51g.100mL^{-1}$) and I15 ($3.43g.100mL^{-1}$), when compared with the control sample (3.75 $g.100mL^{-1}$), statistically differing ($p<0.05$, Table 32). Similar results were also obtained by Bianchini *et al.* (2020) in yogurts with uvaia pulp (3.12 to 3.35 $g.100mL^{-1}$ of protein), from the *Myrtaceae* fruit family, with a control sample (4.02 $g.100mL^{-1}$ of protein). For the total lipid content, skimmed milk was used to manufacture yogurts, and, in the concentrated guabiroba juice, there were not detected by the method; thus, the results of the lipid content were not

significant for all samples. The organic acids in the concentrated form in C1 increased the acidity between samples (0.9 to 1.04g.100mL^{-1}), with a slight decrease in pH value (4.31 to 4.19).

Table 32- Physicochemical properties for yogurts formulations.

Analysis	Control (C)	I10	I15
Moisture (g.100mL$^{-1)}$	88.41 ± 0.32^{a}	88.09 ± 0.09^{b}	88.01 ± 0.03^{c}
Total solids (g.100mL^{-1})	10.99 ± 0.32^{c}	11.59 ± 0.08^{b}	11.98 ± 0.18^{a}
Total soluble solids (g.100mL^{-1})	6.60 ± 0.10^{c}	8.83 ± 0.04^{b}	10.50 ± 0.01^{a}
Protein (g.100mL^{-1})	3.75 ± 0.39^{a}	3.51 ± 0.01^{b}	3.43 ± 0.16^{c}
Lipid (g.100mL^{-1})	<0.01	<0.01	<0.01
Ash (g.100mL^{-1})	0.74 ± 0.08^{c}	0.77 ± 0.03^{b}	0.79 ± 0.03 ^{a}
Titratable acidity (g.100mL^{-1})	0.90 ± 0.04^{c}	0.99 ± 0.02^{b}	1.04 ± 0.02^{a}
pH	4.31 ± 0.01^{a}	4.23 ± 0.02^{b}	4.19 ± 0.01^{c}
Density (g.cm^{3})	0.97 ± 0.01^{b}	1.01 ± 0.03^{a}	1.01 ± 0.10^{a}
L*	78.58 ± 2.91^{a}	74.13 ± 0.01^{b}	73.78 ± 0.04^{b}
a*	-3.23 ± 0.12^{ac}	-0.59 ± 0.24^{b}	-0.09 ± 0.02^{a}
b*	8.69 ± 0.19^{c}	14.16 ± 1.75^{b}	16.40 ± 0.03^{a}
ΔE (CxI10)		7,52	
ΔE (CxI15)		9,65	
ΔE(I15xI10)		2,33	

Note: Results expressed as a mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p<0.05$) between the samples. ΔE = total color difference between two different samples. *AA= Ascorbic Acid. C is the control yogurt sample, without cold-pressed guabiroba juice concentrated from the first stage of freeze concentration process. I10 and I15 are yogurts with 10% of cold-pressed guabiroba juice concentrated from the first stage of freeze concentration process, and with 15% of cold-pressed guabiroba juice concentrated from the first stage of freeze concentration process, respectively.

Color parameters are important factors in improving the quality of yogurts that are added with pulp or fruit juices due to influences on the sensory acceptance of the product. In addition, the predominant color of fruits can decrease or end with the addition of artificial pigments that are potential allergens in a wide range of people(ZIELINSKI et al., 2019). The addition of C1 decreased the lightness (L*; 78.58 to 73.78) of the yogurts ($p<0.05$) and increased the tendency to red (a*; -3.23 to -0.09) and yellow (b*; 8.69 to 16.40) (Table 32).

These parameters were expected due to the intense orange pigmentation of guabiroba juice (Figure 43). For the total color difference (ΔE), the comparison between the control sample with the yogurts added with C1 showed a value higher than 3 (Table 32) and, according to Dantas *et al.* (2021b), this confirms that the three yogurts produced have color differences that the human eye can detect. However, for I10 and I15 samples, the value of ΔE was 2.33. Therefore, both samples have no visual perception (Figure 43D).

Regarding the bioactive compounds shown in Table 33, there was an increase with the addition of guabiroba juice concentrate, with the highest values obtained for the I15 sample ($p<0.05$). TPC ranged from 208.96 $mg.L^{-1}$ to 9,506.58 $mg.L^{-1}$, with a representative increase of 45.5 times (approximately 4,556%) for I15, compared with the control sample. The presence of phenolic compounds in fermented milk is related to several benefits to the human body, including anticarcinogenic, antidiabetic, antimicrobial, prebiotic, and antioxidant properties (BANWO et al., 2021; NING et al., 2021; PRESTES et al., 2021a).

These results also potentiate the functional activity of the guabiroba composition since studies with fermented milk added from fruits of the same scientific family did not obtain the same high concentration of total polyphenols. Bianchini *et al.* (2020) found TPC content equal to 118.0 $mg.100g^{-1}$, while Fidelis *et al.* (2020) obtained TPC content similar to 43.6 $mg.100g^{-1}$, for yogurts with uvaia and camu-camu extracts, respectively. According to our previous studies, from the concentration of 10% of guabiroba pulp in probiotic yogurts, there was an increase in the concentration of total phenolic compounds and antioxidant activity after gastric simulation, which characterized the fermented milk as a potential symbiotic product, attributing prebiotic characteristics to the bioactive compounds present in the guabiroba composition (PRESTES et al., 2021a). Individual carotenoids in yogurts increased significantly ($p<0.05$) with the addition of C1, mainly for the sample I15, increasing ~ 226% for α, β, γ-carotene, and cryptoxanthin, when compared to the control sample (Table 33). In addition to the visible change in the color of yogurts, due to the high concentration of carotenoids in concentrated guabiroba juice (Figure 43D), the supplementation of these bioactives is extremely beneficial to health. The ingestion of β-carotene, the precursor activity of vitamin A, and other specific carotenoids promote antioxidant effects. It reduces the incidence of non-communicable degenerative diseases, such as cancer, cardiovascular and ocular diseases (STINCO et al., 2019).

Table 33- Total phenolic content, carotenoids content, antioxidant activity and vitamin C of yogurt formulations.

Analysis	Control	I10	I15
TPC ($mg.L^{-1}$)	208.96 ± 0.07^{c}	879.13 ± 0.18^{b}	9506.58 ± 1.30^{a}
α- carotene ($\mu g.100mL^{-1}$)	120.46 ± 27.94^{c}	270.57 ± 31.56^{b}	273.07 ± 18.41^{a}
β-carotene ($\mu g.100mL^{-1}$)	130.13 ± 16.21^{c}	292.28 ± 14.50^{b}	294.13 ± 28.14^{a}
γ-carotene ($\mu g.100mL^{-1}$)	108.80 ± 14.21^{c}	244.39 ± 10.98^{b}	246.64 ± 12.76^{a}
Cryptoxanthin ($\mu g.100mL^{-1}$)	141.36 ± 9.31^{c}	317.52 ± 15.77^{b}	320.45 ± 10.49^{a}
DPPH ($\mu molTE.L^{-1}$)	200.91 ± 40.69^{c}	685.40 ± 49.50	884.00 ± 60.93
ABTS ($\mu molTE.L^{-1}$)	885.33 ± 30.36^{c}	$1{,}750.33 \pm 69.60^{b}$	$7{,}558.89 \pm 90.29^{a}$
Vitamin C ($mgAA.100mL^{-1}$)	0.83 ± 0.58^{c}	17.16 ± 2.02^{b}	24.83 ± 0.58^{a}

Note: Results expressed as a mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences between the samples ($p<0.05$).TPC= Total phenolic content. DPPH is (2,2-diphenyl-1-picrylhydrazyl) assay. ABTS is [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay. TE= Trolox equivalent. AA= Ascorbic Acid. C is the control yogurt sample, without cold-pressed guabiroba juice concentrated from the first stage of freeze concentration process. I10 and I15 are yogurts with 10% of cold-pressed guabiroba juice concentrated from the first stage of freeze concentration process, and with 15% of cold-pressed guabiroba juice concentrated from the first stage of freeze concentration process, respectively.

The concentration of vitamin C with the addition of C1 was very positive for the composition of yogurts (with an increase of 2,991% for I15 to the control; Table 33); since dairy products are not sources of vitamin C, the incorporation of natural additives rich in this bioactive enriches the nutritional composition and functional properties of the product. According to the Brazilian pediatric society (DEPARTAMENTO DE NUTROLOGIA DA SOCIEDADE BRASILEIRA DE PEDIATRIA, 2018), the recommended daily intake of vitamin C is 65 mg for ages 14 to 18 years; therefore, the consumption of 100g of yogurt with 15% concentrated juice provides approximately 38.2% of the daily intake for this vitamin. Antioxidant activities differed ($p<0.05$) with the addition of concentrated guabiroba juice, with an increase of 440% for DPPH and 854% for ABTS for I5 when compared to the control sample (Table 33). The high contents of phenolic compounds, carotenoids, and vitamin C of the guabiroba composition are related to high antioxidant properties. Furthermore, during fermentation in fermented milk, *L. bulgaricus* and *St. thermophilus* can release bioactive peptides through endogenous enzymatic pathways. These bioactive peptides have specific

structures according to their composition, size, amino acid sequence and also have antioxidant properties (AGUILAR-TOALÁ et al., 2017; BANIHASHEMI et al., 2020), which explains the presence of antioxidant activities for both DPPH (200.91 μmolTE.L^{-1}) and ABTS (885.33 μmolTE.L^{-1}) assays in the control yogurt. Regarding the multielement profile of yogurts, four macroelements (Ca, K, Mg, and Na) and six microelements (Mn, Se, Zn, P, Cu, and Ba) differed ($p<0.05$) among the samples. Fe, Ni, As, and Pb values were below the detection limit and the method's quantification (Table 34).

Table 34- Elemental profile of yogurts with or withou concentrated guabiroba juice from stage 1 of block freeze concentration process.

Elements (μg.mL^{-1})	Control	I10	I15
Ca	776.00 ± 11.00^{c}	1,089.00 ± 63.00^{b}	1,094.00 ± 34.00^{a}
K	780.00 ± 12.00^{c}	1,521.00 ± 95.00^{b}	1,758.00 ± 63.00^{a}
Mg	74.00 ± 4.00^{c}	141.00 ± 9.00^{b}	160.00 ± 4.00^{a}
Na	260.00 ± 3.00^{c}	407.00 ± 12.00^{b}	430.00 ± 24.00^{a}
Fe	0.05 ± 0.03	<LOQ	< LOQ
Mn	0.03 ± 0.01^{c}	0.43 ± 0.10^{b}	0.62 ± 0.10^{a}
Se	0.30 ± 0.02^{c}	0.78 ± 0.08^{b}	0.93 ± 0.04^{a}
Zn	2.85 ± 0.20^{c}	4.93 ± 0.30^{b}	5.23 ± 0.10^{a}
P	520.00 ± 11.00^{c}	800.00 ± 50.00^{b}	814.00 ± 26.00^{a}
Ni	< LOD	<LOQ	< LOD
Cu	0.04 ± 0.01^{c}	0.21 ± 0.01^{b}	0.28 ± 0.01^{a}
Ba	0.07 ± 0.01^{b}	0.26 ± 0.01^{a}	0.26 ± 0.01^{a}
As	<LOQ	<LOQ	<LOQ
Pb	<LOQ	<LOQ	<LOQ

Note: Results expressed as a mean ± standard deviation; ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p<0.05$) between samples; LOD = limit of detection; LOQ = limit of quantification. C is the control yogurt sample, without cold-pressed guabiroba juice concentrated from the first stage of freeze concentration process. I10 and I15 are yogurts with 10% of cold-pressed guabiroba juice concentrated from the first stage of freeze concentration process, and with 15% of cold-pressed guabiroba juice concentrated from the first stage of freeze concentration process, respectively.

Due to its higher solids content, yogurt with 15% concentrated guabiroba juice had the highest concentration of these elements. The supplementation of 15% guabiroba juice promotes the main minerals of its composition: Sodium (430 $\mu g.mL^{-1}$), Calcium (1,094 $\mu g.mL^{-1}$), Potassium (1,758 $\mu g.mL^{-1}$), Manganese (160 $\mu g.mL^{-1}$) and Phosphorus (814 $\mu g.mL^{-1}$), and can be delivered to the customers by the addition in the yogurt. Dairy products are a source of Calcium, which explains the high contents among the samples (776 to 1,094 $\mu g.mL^{-1}$). For children and adolescents between 14 and 18 years, the ideal dose of calcium consumption is 1,300 mg per day (DEPARTAMENTO DE NUTROLOGIA DA SOCIEDADE BRASILEIRA DE PEDIATRIA, 2018). A portion of 100g of yogurt I15 can provide about 8.41% of the required daily Calcium intake. The ingestion of this mineral is important due to composes the structure of bones and teeth, blood plasma, regulates blood pressure, and helps the development of the muscles (MATERA et al., 2018). Potassium also is in high concentration in guabiroba composition; therefore, the I15 can provide about 5% of the recommended daily intake in a portion of 100g. This mineral is the main intracellular cation that helps the normal functioning of the cells (BEZERRIL et al., 2021; DEPARTAMENTO DE NUTROLOGIA DA SOCIEDADE BRASILEIRA DE PEDIATRIA, 2018). The same portion of I15 yogurt can provide approximately 4% and 6.5% of the daily intake for manganese and phosphorus, respectively. The ingestion of these micronutrients is also essential for the human body. Manganese is present in the formation of bones and reactions involving amino acids, cholesterol, and carbohydrates. Phosphorus composes all cell membranes and is part of the structure of bones and teeth (BEZERRIL et al., 2021).

The results of the present study show that concentrated guabiroba juice improved the components naturally present in yogurt, obtaining a dairy product with higher functional and nutritional properties.

4 CONCLUSIONS

The freeze concentration of the cold-pressed guabiroba juice provided satisfactory process efficiency for the first stage and can be used to formulate yogurts. The bioactive compounds and minerals of the freeze-concentrated juice provided yogurts with a high concentration of phenolic compounds, carotenoids, vitamin C, and antioxidant activity, emphasizing the yogurt produced with 15% of concentrated juice from the first stage. The non-

thermal processes of cold-pressing and its consequent freeze concentration allowed for maintaining most of the bioactive compounds sensitive to high temperatures, with a high concentration in the yogurt composition. The innumerable benefits of the bioactives present in guabiroba can encourage future applications of this pioneering study on an industrial scale since the consumer market craves products with healthy and functional appeal. Furthermore, the ice fractions, considered by-products of the freeze concentration process, can be reused in the formulation of several food products since there were significant retentions of bioactive compounds, minerals, and the orange color of the guabiroba juice, which makes reuse interesting from an economic point of view.

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CHAPTER 11

The use of ice fraction from the freeze concentration process of cold-pressed guabiroba juice aiming to prepare functional carbonated beverage: a smart management of this waste

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THE USE OF ICE FRACTION FROM THE FREEZE CONCENTRATION PROCESS OF COLD-PRESSED GUABIROBA JUICE AIMING TO PREPARE FUNCTIONAL CARBONATED BEVERAGE: A SMART MANAGEMENT OF THIS WASTE

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ABSTRACT

The freeze concentration of liquid foods generates a by-product with few academic studies and no industrial application: the ice fraction of each concentration stage. From the freeze concentration of cold-pressed guabiroba juice, sugar-free carbonated beverages were formulated containing 20% of the residual ice fraction from the first stage of the process (I120), from the second stage (I220), and the original guabiroba juice (J20). Physicochemical properties, contents of phenolic compounds, carotenoids, vitamin C, and antioxidant activity were determined for all samples. There was no significant difference ($p<0.05$) between J20 and I220 for the total solids content (TS = 11.45 g.100mL^{-1} and 10.98 g.100mL^{-1}, respectively) and for total soluble solids (TSS = 12.82 g.100mL^{-1} and 11.91 g.100mL^{-1}, respectively). For total phenolic compounds (TPC), the I220 content was 151.3% higher than the original juice J20 and, for antioxidant activity, 295.8% higher for ABTS and 130.2% higher for DPPH. The I220 beverage presented 159% more vitamin C content than the beverage containing juice (J20). The same behavior was observed for each carotenoid content, with 168% more for the 1220 sample. The total color difference (average $\Delta E=2.75$) revealed no difference in colors visible to the naked eye for the three formulated beverages. The promising results of the bioactive compounds from guabiroba juice retained in the ice fraction can add value to this waste process in the formulation of new products due to the remaining functional appeal of the original fruit matrix.

Keywords: *Myrtaceae* family. Non-alcoholic beverage. Process waste. Carotenoids. Phenolic compounds. Antioxidant activity.

1 INTRODUCTION

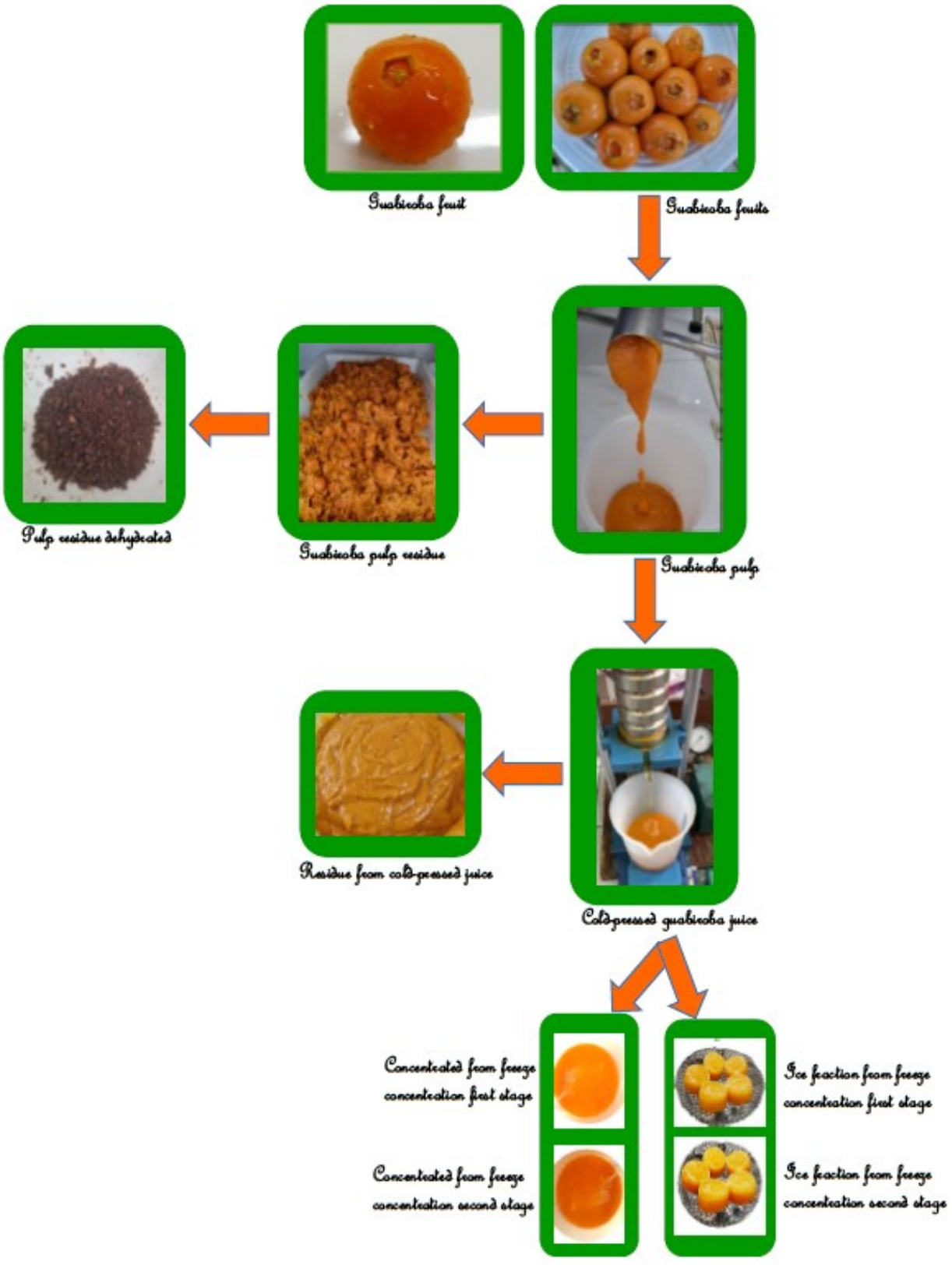
Campomanesia xanthocarpa O. Berg, popularly known as "guabiroba" or "gabiroba" is a native Brazilian fruit from the scientific family *Myrtaceae*, elucidated for containing fruits of high nutritional and functional value and high concentrations of bioactive compounds (de PAULO FARIAS et al., 2020; DONADO-PESTANA et al., 2018; FIDELIS et al., 2018; PRESTES et al., 2022). However, due to the small and regional production aimed at rural producers, there is no commercialization of guabiroba on an industrial scale since knowledge

about the characteristics of the fruit is not widespread. Due to its rich nutritional and functional composition, recent studies have aimed to explore the guabiroba fruits and apply them in various products to spread knowledge about this native fruit (CRISTOFEL ET AL., 2021; LEONARSKI ET AL., 2020, 2021; MALHERBI ET AL., 2019; PRESTES ET AL., 2021; RAMOS MESSIAS et al., 2021). Table 35 shows several products in studies carried out with guabiroba, using different parts of the fruit, including the whole fruit, pulp, pomace, dehydrated residue, and juice (Figure 44).

Table 35- Products developed with guabiroba fruit and its fractions

Guabiroba fractions	**Product**
Guabiroba fruit	*In natural* fruit
Guabiroba pulp	Juice Cold-pressed juice Licor Jam Ice cream *Dulce de leche* Cheese Fermented milk Cake Frozen pulp
Guabiroba pulp residue	Pulp dehydrated residue Cake Cookies
Pulp residue dehydrated	Guabiroba flour Cookies Meat seasonings and sauces Cereal bar Cake Bread
Cold-pressed juice	Refrigerated cold-pressed juice Fermented lactic beverage Ice cream Concentrated from 2 stages of the freeze concentration process Ice fraction from 2 stages of the freeze concentration process
Concentrated 1 and 2	Fermented milk
Ice fractions 1 and 2	Not yet applied

Figure 44- Flowchart describing guabiroba fractions.



Source: The authors (2023).

Emerging non-thermal technologies must be applied to maintain the fruit juice's nutritional aspects because it usually maintains the high contents of bioactive compounds in guabiroba products in industrial processes. The cold-pressing process allows extracting the juice from the whole fruit through slow crushing and simultaneous pressure at room temperature (PRESTES et al., 2023). For the concentration of liquid products rich in bioactive compounds, technologies that operate at low temperatures are also fundamental for preserving the nutritional value of the fruit. The gravitational block freeze concentration process is an efficient and economical technology that can concentrate fruit juices through a previous freezing step followed by separating pure ice crystals (CASAS-FORERO; ORELLANA-PALMA; PETZOLD, 2020; HAAS et al., 2022). This technique, which can be carried out in several stages, concentrates approximately 50% of the solids fraction contained in the juice. Depending on the process efficiency, there may be significant losses of the solids content in the ice fraction, which is considered a process waste and does not have, to date, technological applications for its reuse (Figure 44; Table 35). The applicability of the residual ice fraction in the development of new products can contribute to the circular economy, in which economic development is associated with better use of natural resources, optimization of manufacturing processes with less dependence on raw materials, prioritizing recyclable inputs and renewable sources, and becoming a key to the development of sustainability (FATIMAH et al., 2023). Through previous studies with the freeze concentration of cold-pressed guabiroba juice (data not shown), there was a significant retention of bioactive compounds in the ice fraction, which can be reused in the formulation of other food products, such as beverages in general.

Carbonated beverages are among the non-alcoholic beverages most consumers, including children and adults, because they are sensorially accepted. Typically, this drink is formulated to offer the consumer a pleasant fruity flavor, and reconstituted fruit juices from 2 to 20% of the volume of the product can be added. Carbohydrates are the traditional source of sweetness, mainly high fructose corn syrup (HFCS) or sucrose(ABU-REIDAH, 2020; ASHURST, 2016). However, diet and healthier versions are formulated without added sugars, replacing them with synthetic sweeteners (saccharin, aspartame, sucralose, among others). In addition to the replacement of sugars by their substitutes of natural origin (stevia and polyols), there is a tendency to add fruits or functional plants as raw material in carbonated beverages, with emphasis on sources rich in bioactive compounds and antioxidants, being a fastest-growing segment of the functional food market (BOCHNAK-NIEDŹWIECKA; ŚWIECA, 2020; DI CAGNO et al., 2019; TANGULER; SENER, 2022).

The main purpose of this work is to develop an innovative functional carbonated beverage with an employment of the ice fraction of the freeze concentration from a cold-pressed guabiroba juice, increasing its added value and becoming a novel product that can be produced on a large scale with a technological application to process waste.

2 MATERIAL AND METHODS

2.1 CHEMICALS

Standards of gallic acid (purity ≥ 90%), ABTS (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) from Sigma-Aldrich (St. Louis, USA). Xylitol (Growth supplements, Brazil), citric acid (ACS cientifica, Brazil), sodium benzoate (Vogler, Brazil), potassium sorbate (ACS cientifica, Brazil), and EDTA (ethylenediaminetetraacetic acid) (ACS cientifica, Brazil).

2.2 COLD-PRESSING AND FREEZE CONCENTRATION PROCESSES OF GUABIROBA JUICE

In partnership with Embrapa Florestas (Colombo, PR, Brazil), guabiroba pulp was obtained (84.3% moisture, 0.18% protein, 7.75% carbohydrates, 0.88% fat, 6.26% dietary fiber, 0.63% ash) and, with a hydraulic press (1 ton) (TE-098, TECNAL, Brazil), the cold-pressed juice was obtained and frozen at -20 ± 2°C until the next steps.

According to Canella et al. (2018), the gravitational-assisted block freeze concentration was performed. Figure 45 contains a schematic view of the process: the cold-pressed guabiroba juice (J) was fractionated in plastic containers 200 mL and then frozen at -20 ± 2 °C. After completely freezing the juice, 50 % of the initial mass was defrosted at room temperature (20 ± 2 °C), obtaining two fractions at the first stage: the concentrated guabiroba juice (C1) and the ice fraction (I1). The defrosted liquid (C1) was used as a feed solution for the second stage, obtaining a second concentrated juice (C2) and ice (I2). For stage 1 of the freeze concentration process, the concentration factor (CF) and process efficiency (PE) were 1.6 and 76%, respectively. For stage 2, CF= 2.1 and PE= 58%, according to Aider and Ounis (2012).

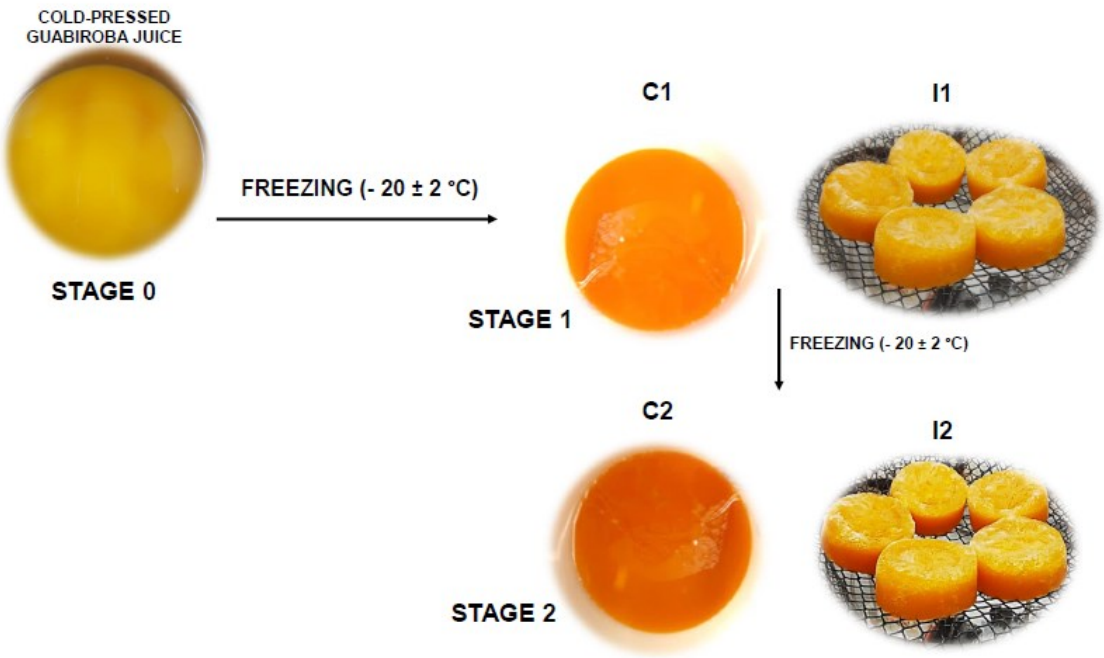
The concentrated guabiroba juice (C1 and C2) was used for other food applications (composition shown in Table 36). In contrast, the ice fraction, which is the residue of the process and is not yet applicable, was used in this work and stored for future analysis (Table 35).

Table 36- Mean nutritional composition of guabiroba fruit and guabiroba fractions

Guabiroba fractions	**Total solids (g 100g^{-1})**	**Protein (g 100g^{-1})**	**Lipid (g 100g^{-1})**	**Carbohydrate (g 100g^{-1})**	**Ash (g 100g^{-1})**	**Fiber (g 100g^{-1})**
Guabiroba fruit	19.6	1.3	1.3	8.3	0.6	8.1
Guabiroba pulp	15.7	0.2	0.9	7.8	0.6	6.2
Guabiroba pulp residue	3.9	1.1	0.4	0.5	<0.1	1.9
Pulp residue dehydrated	27.8	2.2	5.8	4.5	0.7	14.6
Cold-pressed juice	15.6	0.4	10	0.8	2.5	1.9
*Concentrated 1	18	0.4	<0.1	14.1	3.4	<0.1
*Concentrated 2	24.5	0.6	<0.1	35.5	4.9	<0.1
*Ice fraction 1	4.4	0.2	<0.1	4	<0.1	<0.1
*Ice fraction 2	9.7	0.4	<0.1	8.9	0.3	<0.1

Note: *Concentrated 1 and 2 come from the first and second stages of the cold-pressed guabiroba juice freeze concentration process, respectively. Ice fractions 1 and 2 come from the first and second stages of the cold-pressed guabiroba juice freeze concentration process, respectively.

Figure 45- Visual scheme of block freeze concentration carried out with the cold-pressed guabiroba juice in two stages. Note: C1 and C2 refer to concentrates of stages 1 and 2, respectively; I1 and I2 refer to ice fractions of stages 1 and 2, respectively.



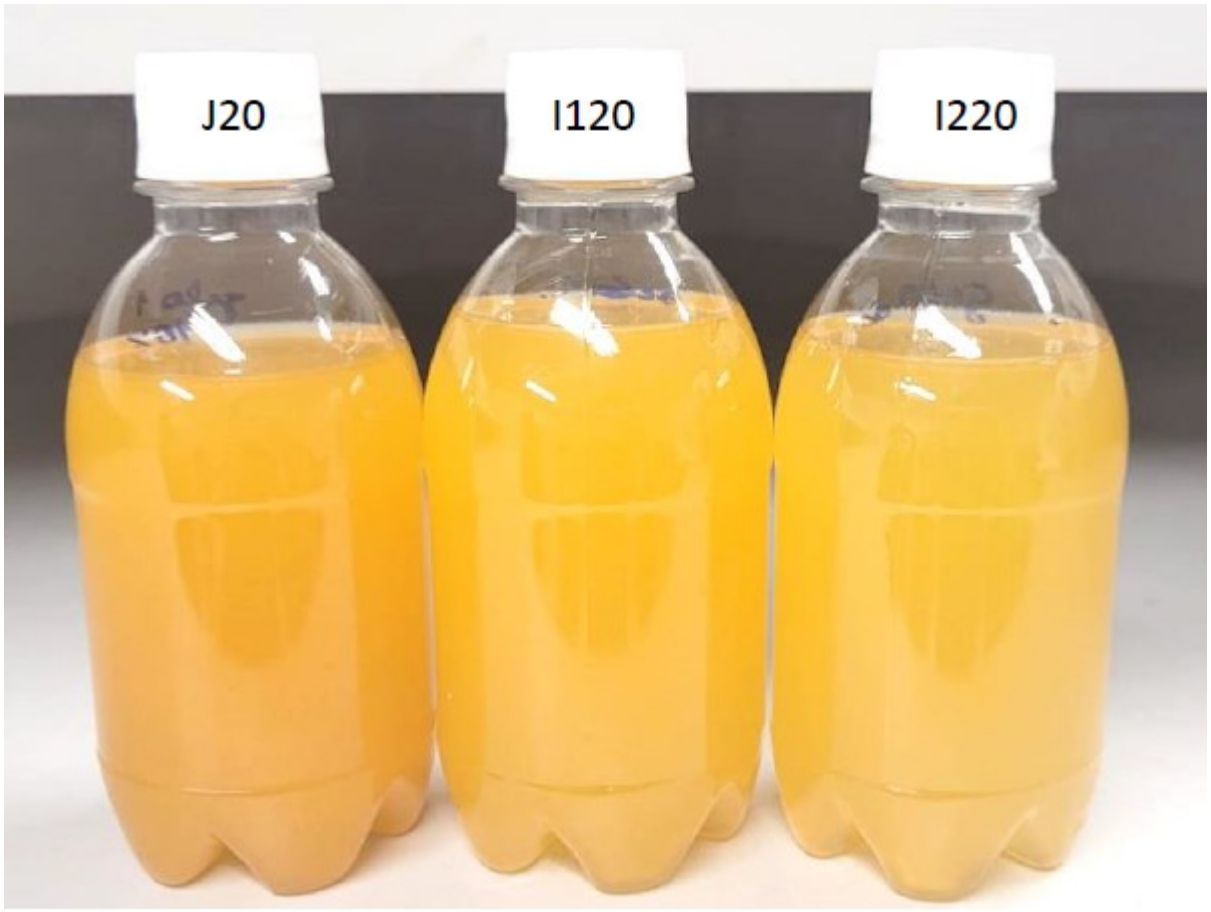
2.3 CARBONATED BEVERAGE MANUFACTURING

Firstly, concentrated syrup formulations were obtained separately, containing 20% of the original cold-pressed guabiroba juice (J), ice fraction from the first stage (I1), and the second stage (I2) from the freeze concentration process (Table 37). There was an addition of 7.5 g $CO_2.L^{-1}$ at 2.1 to 4.5°C in the beverages with a carbonator (Omve, CF 121, Netherlands). 100L of three different beverages (containing 40 kg of concentrated syrup) were produced, obtaining a carbonated beverage with the original cold-pressed guabiroba juice (J20) and two others containing the ice fraction from the first stage (I120) and from the second stage of the freeze concentration process (I220) (Figure 46).

Table 37- Formulation of 40 kg of concentrated syrup with cold-pressed guabiroba juice (J) (20%) and ice fractions (I1 and I2 - 20%) from the block freeze concentration process.

Formulation (kg)	
Water	11.61
Xylitol	8.25
Citric acid	0.10
Sodium benzoate	0.02
Potassium sorbate	0.01
EDTA	0.002
J, I1 or I2	20.00
Total (kg)	**40.00**

Figure 46- Carbonated beverages with 20% of cold-pressed guabiroba juice (J20), ice fraction from the first stage of freeze concentration (I120), and ice fraction from the second stage of freeze concentration (I220).



Source: The authors (2023).

2.4 PHYSICOCHEMICAL ANALYSIS

For all the beverages (I120 and I220), the total solids content ($g.100g^{-1}$) was obtained by the oven drying method until constant weight at 105 ± 2 ºC (315 SE- Fanem, Brazil)(AOAC, 2019). Crude protein was determined by the Kjeldahl method (AOAC, 2019), fat content by the Soxhlet method (AOAC, 2019), and fixed mineral reside (ash) by subjecting the samples to 550°C (J 200 – Jung, Brazil) (AOAC, 2019). The titratable acidity was also determined, according to AOAC (2019).

Color analysis was determined with a spectrophotometer at 420 nm on a U-1800 UV–Vis (Hitachi, Kyoto, Japan), and the total color difference (ΔE*) between the samples was performed according to the Equation 1 (OKPALA; PIGGOTT; SCHASCHKE, 2010) :

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Where ΔL* is the luminosity difference, Δa* represents the intensity of the red color, and Δb* is the intensity of the yellow color.

For each sample, the concentration of Vitamin C was determined according to the Association of Official Analytical Chemists (AOAC, 2019) through the reduction of 2,6-dichlorophenolindophenol by ascorbic acid. The results were expressed as mg ascorbic acid.100 mL^{-1}.

2.5 TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY

Total phenolic content (TPC) was measured for all the carbonated beverages (I120 and I220) according to the Folin-Ciocalteu method (SINGLETON; ROSSI, 1965) at 720 nm in a spectrophotometer (UV-1800, Shimadzu, Brazil). The results were expressed in milligrams of gallic acid equivalent per liter of the sample (mgGAE.mL^{-1}) (calibration curve linearity range: $R^2 = 0.99$).

For antioxidant activity analysis, a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed according to Brand-Williams, Cuvelier and Berset (1995).In a spectrophotometer, the analysis read was performed at 515 nm, and the results were expressed in micromole of Trolox equivalent per liter of the sample (μmolTE.L^{-1}). For the ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay (RE et al., 1999), the analysis read was at 734 nm, and the results were expressed in micromole of Trolox equivalent per liter of sample (μmolTE.L^{-1}). All these analyses were performed in triplicate.

2.6 CAROTENOIDS CONTENT

The carotenoid content was measured according to Rodriguez-Amaya (2001) with modifications. Total α-carotene, β-carotene, γ-carotene, and cryptoxanthin contents were obtained at the following wavelengths in a spectrophotometer: 450, 444, 452, and 462 nm,

respectively. The results were expressed in micrograms of carotenoids per 100 milliliters of sample ($\mu g.100\ mL^{-1}$).

2.8 STATISTICAL ANALYSIS

All the results were expressed as means ± standard deviation. One-way analysis of variance (ANOVA) was used to determine the significant differences ($p<0.05$), followed by *post hoc* analysis with Tukey's test. All samples were produced in triplicates, and three parallel measurements were made for each replication. The data analysis was performed using STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, USA).

3 RESULTS AND DISCUSSION

3.1 EFFECTS OF FREEZE CONCENTRATION ON SOLIDS RETENTION OF CARBONATED BEVERAGES

During the freeze concentration process, as the stages advance, there is a progressive increase in the concentration factor (1.60 for C1 and 2.10 for C2), directly related to the increase in the concentration of total solids in the concentrated fraction (Figure 45). This behavior for two stages was also similar in studies with gravitational block freeze concentration for orange juice (1.61 and 3.53 for C1 and C2, respectively) (HAAS et al., 2022), for blueberry juice (1.70 and 2.6, in unidirectional conditions) (ORELLANA-PALMA et al., 2017), and for *Morinda citrifolia* L. tea (1.51 and 2.95)(ALMEIDA et al., 2023). Petzold et al. (2016) described that free water availability in the concentrated fraction decreases with the advancement of freeze concentration stages, leading to greater solid retention of the original juice and an increase in the concentration factor. However, the process efficiency tends to decrease as the freeze concentration steps advance (PE= 76% for stage 1 and 58% for stage 2). Sánchez et al. (2011) describe this phenomenon as the increase in viscosity in the concentrated fraction as the solids content also increases, which declines the particle diffusion speed, retaining them at the ice-liquid interface. For the freeze concentration of orange juice, Haas et al. (2022) obtained similar behavior for process efficiency (81.80% for stage 1 and 76.47% for stage 2) as well as in studies

with pineapple juice (59.0% and 54.9%) and blueberry juice (66.08% and 58.01%)(PETZOLD et al., 2015).

In works that use the concentrated fraction, applying the concentrate from the first stage of freeze concentration is interesting due to its significant efficiency and solids retention (CAMELO-SILVA et al., 2021). However, as this study focuses on reusing the residual ice fraction in developing new products, the highest solids retained in this waste are the most suitable for its applicability. Thus, the technological aptitude of this process allows the use of all its fractions.

According to the physicochemical results of the formulated beverages (Table 38), the soluble solids levels ranged from 10.58-12.82°Brix. In agreement with the standards established by Brazilian legislation (MAPA, 2013), non-alcoholic carbonated beverages must have a minimum content of 10.5 °Brix. Similarly, total acidity must have a minimum content of 0.1 g of citric acid. 100 mL^{-1} and a pH between 2.7- 3.5. According to Table 4, the acidity between the samples ranged from 0.35- 0.51g.100mL^{-1} and pH 3.88-4.03, which follows established legislation. Casas-Forero et al. (2020) described that the freeze concentration is a method that can cause significant effects both in total acidity and pH due to the increase of organic acids as the process stages advance.

There was no difference ($p < 0.05$) between the samples for total protein content. During the cold pressing process to obtain guabiroba juice, there is no migration of pulp, peel, or seeds into the juice, which could cause a decrease in its protein content. Based on the same fact, cold-pressed juice does not contain seed fractions, which are the portions of the guabiroba fruit that contain low-fat content (1.5-1.9 g.100g^{-1}) (Prestes et al., 2022), which led to obtaining beverages with lipid levels below the quantification limit of the applied method (<0.01 g.100mL^{-1}; Table 38).

Table 38- Physicochemical properties for carbonated beverages.

Analysis	J20	I120	I220
Moisture (g.100mL^{-1})	89.81 ± 0.10^{b}	91.06 ± 0.10^{a}	88.55 ± 0.22^{c}
Total solids (g.100mL^{-1})	11.45 ± 0.10^{a}	8.94 ± 0.10^{b}	10.98 ± 0.22^{a}
Total soluble solids (g.100mL^{-1})	12.82 ± 0.30^{a}	10.18 ± 0.15^{b}	11.91 ± 0.29^{a}
Protein (g.100mL^{-1})	0.27 ± 0.10	0.24 ± 0.10	0.27 ± 0.10
Lipid (g.100mL^{-1})	<0.01	<0.01	<0.01
Ash (g.100mL^{-1})	0.30 ± 0.10^{a}	0.18 ± 0.10^{b}	0.25 ± 0.10^{a}
Titratable acidity (g.100mL^{-1})	0.43 ± 0.10^{b}	0.35 ± 0.10^{c}	0.51 ± 0.10^{a}
pH	3.88 ± 0.10^{b}	4.03 ± 0.10^{a}	3.95 ± 0.10^{b}
L*	39.40 ± 0.72^{b}	41.82 ± 0.48^{a}	36.80 ± 0.25^{c}
a*	2.14 ± 0.10^{b}	1.19 ± 0.10^{c}	2.57 ± 0.10^{a}
b*	20.83 ± 0.14	20.12 ± 0.77	20.35 ± 0.86
ΔE (J20xI120)		2.91	
ΔE (J20xI220)		2.65	
ΔE(I120xI220)		2.69	

Notes: Results are expressed as a mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($p<0.05$) between the samples. ΔE = total color difference between two different samples. J20 is a carbonated beverage with 20% of the original cold-pressed juice. I120 corresponds to the carbonated beverage with a 20% ice fraction from the first stage of block freeze concentration; I220 corresponds to the carbonated beverage with 20% from the second block freeze concentration.

3.2 CARBONATED BEVERAGE FUNCTIONALITY

The formulation of carbonated beverages was carried out per Brazilian legislation that covers the standards for producing soft drinks and non-alcoholic carbonated beverages (Table 3) (MAPA, 2013, 2021). Since guabiroba is a native Brazilian fruit, all products obtained from this fruit must be within the standards established by local legislation, which regulates non-alcoholic carbonated beverages with the addition of a minimum amount of 5% fruit juice or pulp, carbonation with pure industrial carbon dioxide equal to or greater than 2.5v (MAPA, 2013), the desired amount of xylitol and citric acid, a maximum of 500 mg.L^{-1} and 800 mg.L^{-1} of sodium benzoate and potassium sorbate, respectively, and a maximum of 35 mg.L^{-1} of EDTA (ethylenediaminetetraacetic acid), for preservative and stabilizing properties (ANVISA, 2023). The addition of a natural sweetener (xylitol) was chosen to completely replace sucrose, fructose,

or glucose to guarantee a healthy appeal to the product. According to Zhang et al.(2020), consuming sugar-containing carbonated beverages is extremely common worldwide and contains large amounts of fructose. This consumption is positively associated with hyperuricemia, the precursor of gout, and is related to cardiovascular disease, hypertension, and renal disease. In addition, Chun et al. (2016) reported that high consumption of sugary carbonated beverages is associated with a predisposition to heart disease, even in healthy individuals without a genetic predisposition or history of coronary heart disease, cancer, or diabetes. Thus, in relation only to the added natural sweetener, the formulation of the carbonated beverages in this study can be targeted for consumption by adults and children without restrictions. Furthermore, beverages containing bioactive compounds such as carotenoids, phenolic compounds, and vitamins can provide numerous health benefits (TANGULER; SENER, 2022). Nowadays, this implementation becomes important due to the daily intake of fruit by the world population (at least 400 g of fruit and vegetables per day) being lower than that recommended by WHO (World Health Organization) and FAO (Food and Agriculture Organization), which leads to functional appeal strategies for industrial formulations containing fruit and being well accepted by the consumer market (DIAS et al., 2018).

Usually, the ice fraction from the freeze concentration process is not reused. However, data in Tables 38 and 39 prove that using ice remaining from the guabiroba juice concentration can be an intelligent strategy on an industrial scale due to the significant levels of solids and bioactive compounds that were retained. For total phenolic compounds (Table 39), the beverage containing 20% of the ice fraction from the second stage (I220) stood out when compared to the other formulations ($p<0.05$). Concerning the carbonated beverage containing guabiroba juice (J20) (543.11 $mgGA.L^{-1}$), TPC for I220 was higher by 151.3%. Originally, the guabiroba fruit stood out for containing high levels of phenolic compounds (9,033.20 $mg.100g^{-1}$), classified as one of the fruits from the *Myrtaceae* family with the highest levels of these compounds which, consequently, are transferred to the fruit fractions (PEREIRA et al., 2012; PRESTES et al., 2022). Furthermore, obtaining the guabiroba juice by cold pressing in this work maintains most of the nutritional compounds sensitive to high temperatures, being also a beneficial technology for improving the product's functionality. The higher concentration of bioactive compounds for sample I220 is due to the successive concentration of solids, mostly retained at the solid-liquid interface since the process efficiency in concentrating solid particles in the concentrated fraction is lower for stage 2. It should be noted that the first stage concentrate was used in the second stage of the freeze concentration process (Figure 46). In this way, feed

with a higher solids content was used. As the freeze concentration stages consist of reducing the mass of the feed by 50%, when using a liquid with a higher solids content, it is possible that a higher content of bioactive compounds will be retained in the ice fraction (stage 2, I220) (Table 39), as well as for the total solids content of I220 (Table 38).

Table 39- Total phenolic, carotenoid, Vitamin C, and antioxidant activity of carbonated beverages.

Analysis	J20	I120	I220
TPC (mgGA.L^{-1})	543.11 ± 96.82^{c}	548.28 ± 33.97^{b}	821.59 ± 181.46^{a}
α- carotene (µg.100mL^{-1})	31.18 ± 3.21^{c}	42.52 ± 1.17^{b}	71.42 ± 0.80^{a}
β-carotene (µg.100mL^{-1})	33.76 ± 4.75^{c}	45.93 ± 6.34^{b}	77.15 ± 2.49^{a}
γ-carotene (µg.100mL^{-1})	28.11 ± 5.10^{c}	38.41 ± 2.08^{b}	64.51 ± 4.01^{a}
Cryptoxanthin (µg.100mL^{-1})	36.52 ± 2.23^{c}	49.91 ± 8.13^{b}	83.81 ± 7.19^{a}
DPPH (µmolTE.L^{-1})	159.15 ± 0.33^{c}	205.27 ± 0.96^{b}	207.16 ± 1.57^{a}
ABTS (µmolTE.L^{-1})	83,998.33 ± 5,831.24^{c}	13,6350 ± 8,267.21^{b}	248,460.00 ± 7,839.71^{a}
Vitamin C (mgAA.100mL^{-1})	58.66 ± 2.74^{c}	74.66 ± 3.25^{b}	93.33 ± 4.85^{a}

Results are expressed as a mean ± standard deviation. ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences between the samples ($p<0.05$).TPC Total phenolic content. DPPH is 2,2-diphenyl-1-picrylhydrazyl assay. ABTS is 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay. GA= Galic Acid; TE= Trolox equivalent; AA= Ascorbic Acid. J20 is a carbonated beverage with 20% of the original cold-pressed juice. I120 corresponds to the carbonated beverage with a 20% ice fraction from the first stage of block freeze concentration; I220 corresponds to the carbonated beverage with 20% from the second block freeze concentration.

This behavior is related to the decrease in process efficiency observed in stage 2 of the freeze concentration process. Furthermore, TPC in the ice fractions of the second stage of guabiroba juice freeze concentration is so prominent (1220= 821.59 mgGA.L^{-1}) that, when compared to orange juice freeze concentration by Haas et al. (2022), only 20% of the addition of the ice fraction were superior to whole orange juice with 715.7 mgGA.L^{-1}. The I220 beverage also obtained higher TPC levels when compared to studies with Red Delicious apple juice, also subjected to a freeze concentration process (740 mgCA.L^{-1}) (ZIELINSKI et al., 2019). The same behavior applies to the antioxidant activity, directly related to TPC and other bioactive components such as carotenoid content and Vitamin C (HAMINIUK et al., 2012). I220 sample presented the highest antioxidant activity ($p<0.05$) among the three samples for both the DPPH

(207.16 μmolTE.L^{-1}) and ABTS (248,460.00 μmolTE.L^{-1}) assays and when compared to the J20 beverage, the activities were higher by 130.2% and 295.8% for DPPH and ABTS, respectively (Table 39). Compared to studies with whole apple juice (1,255.0 μmolTE.L^{-1}) and concentrated juice by two stages of freeze concentration (8,254.0 μmolTE.L^{-1}) (ZIELINSKI et al., 2019), only 20% of the ice fraction of guabiroba juice in the beverages promoted greater antioxidant activity for ABTS essay, highlighting the intense functional property of the original fruit fractions.

Among the formulated beverages, there was a considerable vitamin C content (58.66 - 93.33 mgAA.100mL^{-1}), also related to the significant antioxidant activity due to its ability to capture and neutralize free radicals. One of the most important nutritional properties of *Campomanesia xanthocarpa* O.Berg is the high content of vitamin C in its composition, ranging from 17.80 to 233 mg.100g^{-1} according to our previous studies (PRESTES et al., 2022). Orange juice is a common product in consumers' daily lives and is a routine source of vitamin C. According to Haas et al. (2022), the vitamin C content of orange juice is 33.3 mgAA.100mL^{-1} and, compared to carbonated beverages, the I120 sample presented 2.2x more and, for the I220 sample, 3x the vitamin C content than a pure orange juice.

The functional prominence of the 1220 sample can also be discussed about the carotenoid levels (α-, β-, γ- carotene and cryptoxanthin). With the J20 beverage, the average content of all carotenoids was 168% higher. Santos (2012) and Schmidt et al. (2019) described the guabiroba fruit as a potential source of carotenoids, mainly for β-carotene concentration, a precursor of Vitamin A (retinol), (12.30 to 3,400.00 mg100 g^{-1}) which stands out when compared to the levels of other conventional fruits, such as papaya with 0.04 mg.100g^{-1} (Prestes et al., 2022). The beverage containing only 20% of the ice fraction (I220 = 0.07 mg.100mL^{-1}) surpassed the β-carotene content found in whole papaya fruit. In addition to contributing to the increase in antioxidant activity, the intensity of the orange color of both guabiroba fruit and its fractions (Figure 45) (which can also be noted in the carbonated beverages in this work in Figure 46) is also related to the high levels of carotenoids, mainly for cryptoxanthin content, with 36.52 - 83.81 μg.100mL^{-1} among the samples. According to Table 4, the a* parameter, related to the intensity of the red color, was greater for the I220 sample (I220= 2.57; J20 = 2.14; I120 =1.19), correlating the higher carotenoid contents with the intense orange color of both the guabiroba juice and its respective ice fractions. For the b* parameter, which relates the intensity of the yellow color, there was no significant difference for all three samples, emphasizing the color intensity of the guabiroba pulp remaining in the fruit fractions. Therefore, for the total color

difference, expressed by ΔE* (Table 38), there was no noticeable difference to the naked eye due to the pairwise comparison values between samples were less than 3.0. Martínez-Cervera et al. (2011) described that when the total color difference is lower than 3.0, the change in the color of the samples or their tone is not visually noticeable, as shown in Figure 46. This characteristic makes the beverage formulation even more functional due to the lack of need to use dyes from synthetic sources, which can be allergenic. In addition to the health benefits brought by the carotenoid content, these carbonated beverages can be aimed at all groups of people, as food allergies among consumers are increasingly evident.

4 CONCLUSION

The ice fraction from a freeze concentration process has no academic or industrial applicability, being considered a process waste. This unprecedented work combined the development of a new product with functional properties with an intelligent reuse of this residual fraction. The ice resulting from the block freeze concentration of cold-pressed guabiroba juice had a significant retention of polyphenols, Vitamin C, and carotenoids, with the latter compounds responsible for the residue's striking orange color. The formulation of a carbonated beverage without added sugar with 20% of the ice fraction from the second freeze concentration stage (I220) showed higher levels of bioactive compounds due to lower process efficiency of solids migration to the concentrated fraction, resulting in greater contents in the ice fraction. Thus, in addition to developing a carbonated beverage containing guabiroba juice, the reuse of ice fractions from a block freeze concentration becomes an outstanding strategy due to the high retention of bioactive compounds from the original fruit. This innovative product has an interesting economic appeal due to the reuse of process waste and its healthy properties due to the absence of sugars and synthetic dyes. Therefore, in the future, both the incorporation of the freeze concentration process and the development of products with guabiroba fractions have the potential to be applied on a large scale, disseminating knowledge of this native fruit with a rich functional composition and contributing to waste management and circular economy.

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FINAL CONSIDERATIONS

The nutritional and functional capacity of the bioactive compounds in the guabiroba fruit was evidenced and highlighted in this work. The high concentration of these fruit compounds, when inserted in the formulation of probiotic fermented milks, proved the potential prebiotic activity of the matrix, previously unknown in academia world. In the published chapters of this thesis, it was discussed that only 10% of the addition of guabiroba fruit fractions is possible to obtain a prebiotic property, contributing to the development of symbiotic dairy products. Furthermore, these compounds are related to high antioxidant activity in all fractions of the fruit, highlighting the health benefits when included in a dietary routine.

The study with fractions of the guabiroba fruit, through the fusion of emerging non-thermal technologies, improved the concept of technological innovation. Cold pressing of fruit juices, an innovative topic published in this thesis through a literature review, guarantees the maintenance of compounds sensitive to high temperatures. Obtaining guabiroba juice through this process combined with the concentration of compounds through the emerging technology of freeze concentration allows us to obtain products with unprecedented methods for application in products, contributing to innovation in food engineering and food science. The economic appeal was also explained in this work, being an interesting factor for a possible industrial application. The reuse of process residues, such as the ice fraction from freeze concentration technology, in addition to by-products from the dairy sector, such as whey, makes it possible to apply them in new products with an increase in the added value of these co-products.

The work involved in these four years of development of this Ph.D. thesis resulted in publications of literature reviews on innovative concepts in food engineering and food science, in addition to the development of unreleased products: probiotic fermented milks added with guabiroba pulp, a functional dairy beverage with concentrated whey added with guabiroba pulp, fermented milks added with freeze concentreated cold-pressed guabiroba juice, and a carbonated non-alcoholic beverage added with the residual ice fraction from the freeze concentration of guabiroba juice. As an extension project, in partnership with EMBRAPA Florestas, a workshop on the development of innovative products added with guabiroba pulp was developed for small rural producers. In the future, new projects can be held aimed at family farming with the development of other products with guabiroba, providing a free means of dissemination for fruit producers in different regions.

For future work, the possibilities for applying the guabiroba fruit and its fractions are broad in the development of innovative products, such as dairy beverages, ice creams, cheeses, in addition to the extraction of its evident functional compounds, such as carotenoids that highlight the natural orange color of the fruit.

The choice of a native Brazilian fruit as the subject for a Ph.D. thesis becomes important for the dissemination of the functional, nutritional, and technological benefits of a matrix not known in academia and industry, which becomes a free gateway to international knowledge through our work.

Annex A- Publications in scientific journals

First page off the article "Potential Properties of Guabiroba (*Campomanesia xanthocarpa* O. Berg) Processing: A Native Brazilian Fruit" in the journal Advances in Food Technology and Nutritional Sciences

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Review

Potential Properties of Guabiroba (*Campomanesia xanthocarpa* O. Berg) Processing: A Native Brazilian Fruit

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ABSTRACT

Guabiroba (*Campomanesia xanthocarpa* O. Berg) is a native Brazilian fruit with an important nutritional value and a great economic potential for processing. This fruit is a source of fibers, carbohydrates, potassium, and bioactive compounds, such as polyphenols, carotenoids, and Vitamin C. The phytochemicals of guabiroba are elucidated regarding their high antioxidant activity, which is related to human health benefits when introduced into a dietary routine. In addition, the antioxidant property of this native fruit can act as a natural preservative against oxidative and enzymatic reactions, and microbiological spoilage, extending the shelf-life of food. Thus, the addition of guabiroba in the development of new products, in addition to improving the functionality of the food, can reduce the use of chemical additives. Studies related to encouraging the use of guabiroba in food formulation, as well as the use of emerging technologies in the processing of this native fruit, become the basis of this review that aims to expand the knowledge of this Brazilian fruit and enhance its application in the food industry.

Keywords

Myrtaceae family; Gavirova; New products; Emerging technologies; Technological approach.

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How to improve the functionality, nutritional value and health properties of fermented milks added of fruits bioactive compounds: a review

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Abstract

Fermented milks, with diverse manufacturing, fermentations and specific strains, have been consumed around the world, with a millennial knowledge of their production. These dairy products have a potential nutritional value, taking food industries to invest, nowadays, in dairy products with a functional and healthy appeal due to the changes in the habits and diet of the population. The addition of natural ingredients from vegetables and fruits into fermented milks is a tendency nowadays. The inclusion of natural additives may change the texture, composition, sensory attributes and increase of the shelf life since some compounds are related to have a high antioxidant activity, which decreases the development of deteriorating microorganisms. These called bioactive compounds are synthesized by plants and also may contribute to the fermented milk formulation, in special from fruits, which increase the sensory acceptance. Several classes of fruits bioactive compounds are associated to several health benefits and are a base of many studies about functional fermented milks, reported in this review. This theory background becomes essential for future studies and dairy products development.

Keywords: dairy products; functional food; natural additives; antioxidant activity; prebiotics.

Practical Application: Potential functional properties of fermented milks added of fruit bioactive compounds.

1 Introduction

The dairy products manufacturing is known since antiquity, with the fermentation process as a traditional approach to food preservation. Nowadays, dairy products are substantial in most dairying countries where produce, in large demand, fermented products including butter, cheeses and fermented milks (Surono & Hosono, 2011). Due their nutritional value and taste, fermented milks are considered a product with high potential for the development of new dairy products, being explored by dairy industries (El Hatmi et al., 2018).

Fermented milks, with an old knowledge and appreciation around the world, have an acidic property due to the specific microorganism's development. The low pH of the product prolongs the shelf-life of the milk and the fermentative process generates physicochemical and organoleptic changes due to synthesized metabolites by inoculated strains, contributing to sensory characteristics pleasant to the taste. Beyond sensory attributes, fermented milks, mainly yogurts, are very appreciated by consumers due to practicality and better digestibility compared to the milk. This dairy matrix contains all the necessary components to cell growth, becoming an ideal vehicle for the development of a probiotic product with a functional appeal (Granato et al., 2018; Verruck et al., 2019).

Foods with a functional appeal are those that, besides to promote basic nutrients, when consumed in a routine, produce benefic effects on the organism (da Costa, 2017). The search for healthy food, with nutritive functions and health benefits, is quite common nowadays, once a bad diet induces an increase of cardiac diseases, diabetes, and obesity. Changes in the diet are increasingly present in everyday life and food industries invest in the development of products with functional appeal. In these products, there is a tendency to add natural ingredients in the formulation, including those that are in the fruits and vegetables composition; the bioactive compounds (Domínguez Díaz et al., 2020; Fazílah et al., 2018; Yassin et al., 2018).

Bioactive compounds are synthesized by vegetables, including leaves, fruits, seeds, or roots which promote health benefits when consumed regularly. The consumption of these natural compounds is related to a decrease in the incidence of noncommunicable diseases, diabetes, cardiovascular diseases, and the reduction of carcinogenic cells (Cutrim & Cortez, 2018; Yassin et al., 2018). Among the bioactive metabolized by plants, there are unsaturated and polyunsaturated fatty acids, several classes of polyphenols, carotenoids, vitamins, phytosterols, and dietary fibers (Septembre-Malaterre et al., 2018). Emerging studies about adding bioactive compounds from fruits, juices or extracts in dairy matrices have interesting and promising results, once that milk is a good vehicle

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Conventional and alternative concentration processes in milk manufacturing: a comparative study on dairy properties

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Abstract

The concentration of dairy products is widely applied in dairy manufacturing due to obtaining products with the high dry matter, added value, reduced volume, and an increase in shelf-life. Traditional thermal concentration processes are the most applied in dairy industries, however, high temperatures can damage the bioactive compounds in milk, in addition to modifying the physicochemical, sensory, and nutritional characteristics of concentrated products. This review summarizes the importance of replacing traditional concentration methods with unconventional non-thermal processes, which can bring an option to dairy industries due to the concentration enabling the preservation of proteins, enzymes, vitamins, color, and flavor of the product. Alternative methods, such as freeze concentration, membrane separation processes, and freeze-drying, compose recent works about new methodologies to concentrate dairy products without changing specific properties and increase the quality, which is one of the main purposes for the dairy industries. Through a comparative study with recent researches, this overview highlights some alternative concentration processes that can improve the yield and increase the quality of concentrated dairy products. With new environmentally sustainable methods and the possibility of reducing the costs of the concentration process, these emerging concentration methods become attractive for dairy industries from a technological and economic perspective.

Keywords: non-thermal processing; freeze concentration; membrane separation; freeze-drying; dairy processing; thermolabile compounds.

Practical Application: Improving the quality of concentrated dairy products by non-thermal emerging technologies.

1 Introduction

Milk is a highly nutritional valuable food that can be processed, fractionated, and included in dairy products, beverages, or food formulations (Al-Hilphy et al., 2020; Muñoz et al., 2018; Prestes et al., 2021; Vargas et al., 2021). In addition, dairy products are elucidated as being excellent sources of nutritional compounds, bring health benefits if introduced in a well-balanced diet (Feeney et al., 2021; Verruck et al., 2019a).

Thermal and non-thermal processes implemented in dairy manufacturing have the main purpose to increase the shelf-life and produce a safe, stable, nutritional, and product (Al-Hilphy et al., 2020; Musina et al., 2018; Stratakos et al., 2019).

Dairy products contain high water content and, with the purpose to expand the shelf-life, concentration processes are fundamental in dairy industries, since the employed technology can improve the efficiency of milk processing, reducing the volume of production and total costs of shipping and storage (Balde & Aider, 2017; Liz et al., 2020; Muñoz et al., 2018). In addition, there is an increase in total dry matter which benefits the added value of a product with high fat and protein content (Carter et al., 2021; Rao, 2018; Vargas et al., 2021).

In large-scale production, traditional concentration methods are the most employed in dairy manufacturing, mainly the evaporation and spray drying processes These unit operations reduce the water content by applying high temperatures during the procedure. However, an intense heat treatment may exceed the heat stability of milk and result in undesired sensory and physiochemical changes, such as separation of milk fat, grittiness, phase separation and sediment formation (Dumpler et al., 2020). Besides, the intense thermal processes may decrease original thermolabile bioactive compounds such as enzymes, vitamins, and proteins (Dumpler et al., 2018, 2020; Moejes et al., 2020).

Emerging non-thermal technologies are promising alternatives that have been developed and explored in dairy manufacturing. With a purpose to decrease the negative effects of the conventional concentration processes and contribute with dairy products with high quality, these alternative procedures preserve sensory and flavor properties and maintain food pigments, original volatile compounds, vitamins, enzymes, and proteins (Liz et al., 2020; Faion et al., 2019; Canella et al., 2020; Moejes et al., 2020; Muñoz et al., 2018; Stratakos et al., 2019). In recent research

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CONCISE REVIEWS & HYPOTHESES IN FOOD SCIENCE

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Freeze concentration techniques as alternative methods to thermal processing in dairy manufacturing: A review

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Abstract: Freeze concentration technology is applied to concentrate liquid foods at low temperatures, thus separating pure ice crystals from the final concentrate solution. This method is an interesting alternative to concentrate food with high water levels and significant nutritional value such as dairy products, since several bioactive compounds are reduced when exposed to elevated temperatures. Considered that, this technique may be a great alternative to concentrating and maintaining both nutritional and sensory characteristics of liquid foods. The present review aims to introduce freeze concentration procedures as an eligible choice for conserving dairy products', also addressing its effects on the dairy matrix.

KEYWORDS
concentration, dairy products, freezing, separation, thermolabile compounds

Practical Application: This study reports the main techniques of freeze concentration applications in dairy products, to be used both on an industrial and laboratory scale, aiming to improve the nutritional quality of the products obtained.

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Influence of guabiroba pulp (*campomanesia xanthocarpa* o. berg) added to fermented milk on probiotic survival under *in vitro* simulated gastrointestinal conditions

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ABSTRACT

In fermented milks inoculated with two thermophilic strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*), guabiroba pulp (*Campomanesia xanthocarpa* O. Berg) was added in different concentrations: 5% (I5 sample) and 10% (I10 sample), compared to a control sample, with no pulp addition. In these fermented milks, *Bifidobacterium* BB-12 was added and the samples were submitted to a progressive gastrointestinal simulation *in vitro*. The cells count was performed, including the survival rates for all the progressive steps of the simulated digestion. Total phenolic content (TPC) and antioxidant activity analysis by FRAP (Ferric Reducing Antioxidant Power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were performed in all the gastrointestinal steps. Before and during the entire gastrointestinal tract, the *Bifidobacterium* BB-12 count was 8–9 log CFU g^{-1}, above the recommended for a probiotic product, with a highlight in intestinal colon steps. The I10 sample showed the highest viable cell count, the highest total phenolic content and antioxidant activity throughout the entire gastric steps ($p < 0.05$). The fermented milk proved to be an effective matrix for the probiotic stability and incorporation of guabiroba components. Bioactive compounds present in the guabiroba pulp may have occasioned a prebiotic and protective effect on *Bifidobacterium* BB-12 after gastric conditions. The possible bioconversion of these compounds in more active forms can contribute to the absorption in epithelial cells, enhancing fermented milks with guabiroba pulp as important sources of dietary accessible bioactive compounds.

1. Introduction

Probiotics are recognized by living microorganisms that confer several benefits to the host's health when regularly administrated in adequate amounts (Hill et al., 2014). With a property to adhere in gut epithelial cells, probiotics can improve the microbiota and the digestive process, protect against pathogens and generate potential anticarcinogenic properties (Ranadheera et al., 2018; Verruck et al., 2019, 2020). The cells must be viable in the entire gastrointestinal tract including mouth, esophagus, stomach, small and larger intestine to exert benefits to the human body (Ranadheera et al., 2019; Rasika et al., 2020). Only can be considered a probiotic product when the viable cells count is at least 10^6–10^7 CFU (Colony-Forming Units) per gram or per milliliter of food at the time of its consumption (Hill et al., 2014).

The composition of dairy products contains essential nutrients for the development of probiotic cells, with potential results when added in cheeses, ice creams, frozen yogurts and fermented milks formulations. (Balthazar et al., 2019; Granato et al., 2018; Muñoz et al., 2018; Verruck

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The use of cold pressing technique associated with emerging nonthermal technologies in the preservation of bioactive compounds in tropical fruit juices: an overview

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The cold pressing is the most widely used process in the commercial production of juices from fresh fruits, without the addition of heat, preserving all the original nutritional and sensory components, which places these juices in the premium category. Tropical fruits can be a good matrix to produce innovative juices with high amounts of bioactive compounds. These compounds can be reduced or inactivated at high temperatures, making it essential to obtain and preserve these juices through nonthermal processes to ensure maximum maintenance of nutritional quality. Recently, studies showed that a combination of emerging nonthermal technologies before or during pressing could increase the yield of cold-pressed juice, as well as improve its functional properties with a greater release of secondary compounds. The aim of this study was expanding the knowledge of alternatives to produce premium tropical juices, boosting the application of these technologies on an industrial scale.

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Introduction

Consumption of fresh juices is increasing all over the world. According to Food and Agriculture Organization (FAO), in 2020, world production of tropical fruits was approximately 25 million tons, about 5.2 million tons more when compared with the previous ten years of production [1]. Tropical fruits constitute a comparatively new group in global commodity trade emerged on the international marketplace and comprise a wide spectrum of bioactive compounds such as phenolic compounds, carotenoids, vitamins, and dietary fiber, which are regarded to have functional activities [2]. Jafari et al. [3] affirmed that fruits and fruit products, such as fruit juices, are of the best sources of bioactive compounds, which provide a variety of health advantages. However, nowadays, people do not consume sufficient amounts of fruits, so the introduction of different formats can help to increase the total consumption of fruit components, as fruit juices [4]. Fruit juices are examples of practical and easy-to-eat foods characterized by high-pulp juiciness, vitamin C, carotenoids, and polyphenols [5], with excellent nutritional, functional, and therapeutic characteristics, and have gained prominence in the everyday diets of people of all ages, classes, and locations. The main successful drivers that are increasing interest of tropical fruit consumption worldwide are appealing sensorial features, exotic character, and its undisputable nutritional value related to health- promoting activities [2].

Several bioactivities have been attributed to phenolic compounds, which could be used for prevention or amelioration diseases, such as antioxidant, antiallergenic, antiatherogenic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective, vasodilatory, and neuroprotective effects, among others [4]. For this reason, the growing international demand has driven to an exponential increase in worldwide production of tropical fruit juices in the last years [2]. For a wide distribution of consumption and preservation, tropical fruit juices can be pasteurized or concentrated by heat treatment. Thermal processes are the most used methods in preserving, concentrating, and increasing shelf life of the tropical fruit juices by the food industry. Heat treatment

First page of the article "Whey block freeze concentration aiming a functional fermented lactic beverage with addition of probiotic and guabiroba pulp (*Campomanesia xanthocarpa* O. Berg), a native Brazilian fruit" in Food Science and Technology

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Whey block freeze concentration aiming a functional fermented lactic beverage with the addition of probiotic and guabiroba pulp (*Campomanesia xanthocarpa* O. Berg), a native Brazilian fruit

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Abstract

The scientific importance involved in this study was the use of whey, a co-product of the cheese industry, and its performance during the freeze concentration process. Moreover, the best-concentrated whey from the freeze concentration process, about the total solids, proteins, and mineral contents, was used to prepare two functional fermented lactic beverages. Therefore, whey was subjected to the freeze concentration in blocks with gravitational thawing. Process performance indicated better yields and efficiency for the second stage of freeze concentration. Concentrated whey 2 was used to prepare two fermented lactic beverages added with probiotics: one without adding guabiroba pulp (control) and a beverage incorporated with 10% guabiroba pulp. Containing guabiroba pulp was not enough to modify the total solids, proteins, and mineral contents. However, it decreased pH values, changed the color to an orange hue, and decreased luminosity. The fermented lactic beverage added with probiotic and 10% guabiroba pulp showed 1.61× more phenolic compounds and an increase of 164% for each evaluated carotenoid content compared with the control beverage.

Keywords: Guabiroba; functional beverage; cheese whey; concentration; bioactive compounds.

Practical Application: Production of a fermented dairy beverage with the reuse of dairy waste, aiming to increase its functionality by adding native fruit pulp, with a rich composition of bioactive compounds.

1 INTRODUCTION

Whey is an important co-product of the cheese industry, and approximately 197.44 million tons of it are generated worldwide from cheeses made with cow's milk (FAOSTAT, 2023). However, the whey retains about 55% of the solids and 20% of the proteins present in milk, being about 0.6–0.8 g/100 g of protein, 0.4–0.5 g/100 g of fat, 4.5–5 g/100 g of lactose, and 8–10 g/100 g of mineral salts. Alternatives for using this co-product, aiming at its exploitation, have generated interest in both the small and large industrial sectors and the scientific area. In terms of improving whey's nutritional properties, it can be applied to concentration methods. Among these, Habib and Farid (2008) and Raventós et al. (2007) affirmed that the freeze concentration technology stands out, which employs low temperatures, bringing popularity as an alternative industrial concentration technique of the whey processing, such as vacuum evaporation and membrane technologies. Therefore, freeze concentration improves quality as it minimizes the effect of heat on sensitive components such as proteins, water-soluble vitamins, and aromatic compounds (Moreno et al., 2015; Robles et al., 2016; Sánchez et al., 2010). Prestes et al. (2022) highlighted that freeze concentration is an important technology applied to focus liquid foods, maintaining their quality and preserving thermolabile compounds, flavor, and color. In dairy industries, this technological approach can significantly enhance milk's and whey's efficiency, concentrating its total dry matter. Also, such a technique provides additional advantages for the product's packaging, shipping, and storage. Barros et al. (2022) evaluated the freeze concentration of whey and observed that proteins mostly represent the higher total solids content of concentrated whey. Therefore, this study first focuses on using an emerging non-thermal technology in the concentration of a co-product from the dairy industry.

The growing consumer attention for a diet that goes beyond nutritional value, aiming to improve their well-being, has determined a great interest in the food industries in developing products with claims of functional properties. Among these products

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Article submission letter to the journal Industrial Crops & Products

“ The use of ice fraction from the freeze concentration process of cold-pressed guabiroba juice aiming to prepare functional carbonated beverage: a smart management of this waste”

Please verify your contribution to THE USE OF ICE FRACTION FROM THE FREI CONCENTRATION PROCESS OF COLD-PRESSED GUABIROBA JUICE AIMING PREPARE FUNCTIONAL CARBONATED BEVERAGE: A SMART MANAGEMENT WASTE - [EMID:9c2ab8cdb7456db7] Caixa de entrada ×

Industrial Crops and Products <em@editorialmanager.com> 28 de set. de 2023, 1
para mim

This is an automated message.

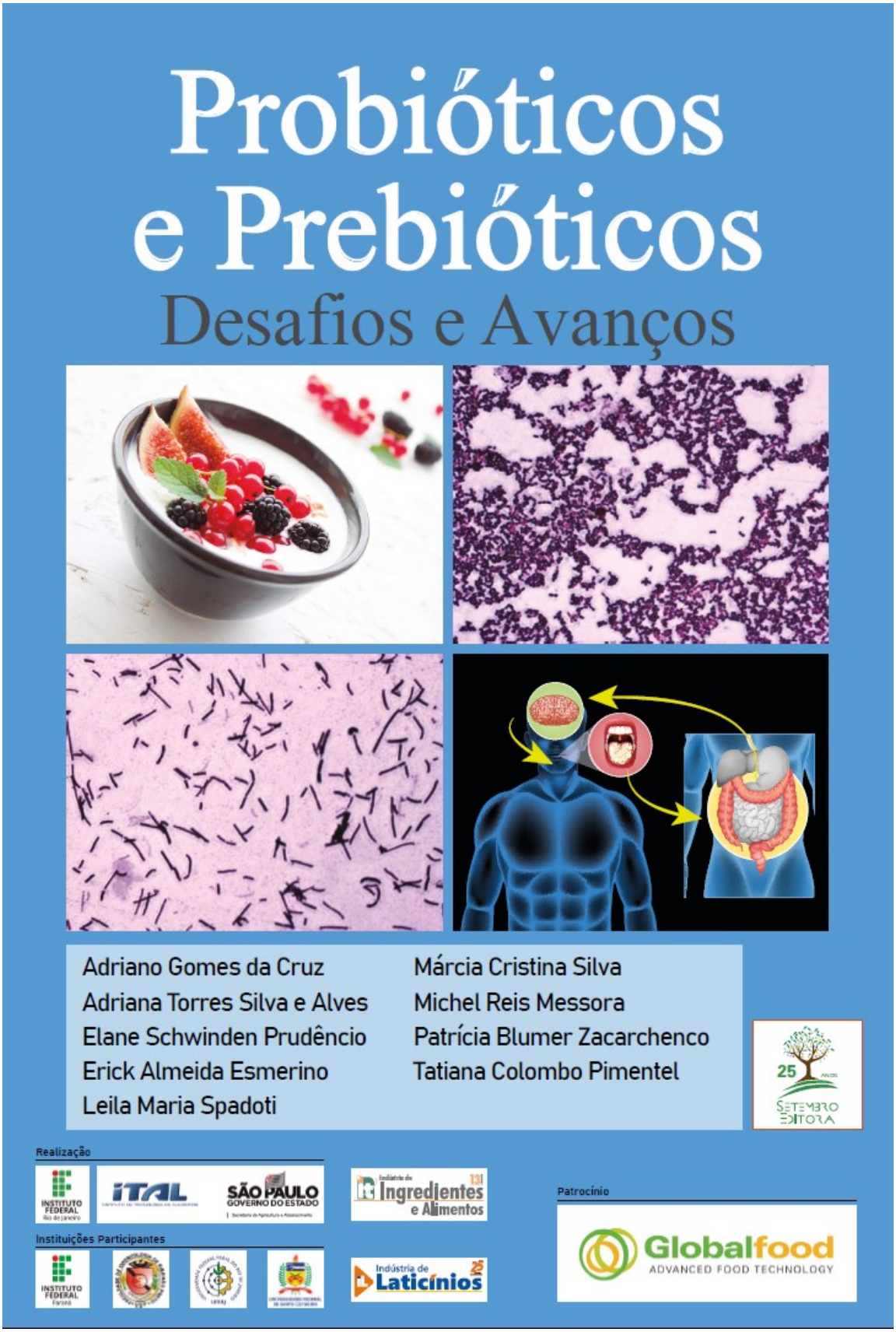
Journal: Industrial Crops & Products
Title: THE USE OF ICE FRACTION FROM THE FREEZE CONCENTRATION PROCESS OF COLD-PRESSED GUABIROBA JUI FUNCTIONAL CARBONATED BEVERAGE: A SMART MANAGEMENT OF THIS WASTE
Corresponding Author: Dr Elane Schwinden Prudencio
Co-Authors: Amanda Alves Prestes; Karine Marafon; Dayanne Andrade; Cristiane Vieira Helm; Bruna Wanderley; Renata Amboni
Manuscript Number: INDCRO-D-23-06284

Dear Dr. Amanda Alves Prestes,

The corresponding author Dr Elane Schwinden Prudencio has listed you as a contributing author of the following submission via E for Industrial Crops & Products.

Submission Title: THE USE OF ICE FRACTION FROM THE FREEZE CONCENTRATION PROCESS OF COLD-PRESSED GUAI PREPARE FUNCTIONAL CARBONATED BEVERAGE: A SMART MANAGEMENT OF THIS WASTE

Book chapters



Leites e Derivados Probióticos e Prebióticos de Espécies Não Bovinas

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Amanda Alves Prestes
Silvani Verruck
Maria Helena Machado Canella
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Celso Fasura Balthazar
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Resumo

Este capítulo engloba a utilização de leites de búfalas, cabras, ovelhas e de outras espécies como matrizes benéficas para o desenvolvimento de produtos probióticos. Na descrição das características dos leites, são enfatizadas suas composições no favorecimento da multiplicação de células probióticas como também seus aspectos contribuintes na finalidade prebiótica. Ao final, foram abordados estudos em destaque sobre o desenvolvimento de produtos lácteos, de cada espécie em particular, e seus resultados no beneficiamento seletivo de microrganismos probióticos.

1. Introdução

O leite de vaca é o leite mais consumido em todo o mundo, dominando a produção mundial de leite com 675 milhões de toneladas em 2017. Assim, como pode ser visualizado na Figura 1, em 2017 81,61% da produção mundial de leite é bovino, seguido por leites de outras espécies, como o de búfala (14,54%), cabra (2,25%), ovelha (1,26%) e de camela (0,34%) (FAOSTAT, 2020). Esses dados foram apresentados pela Organização das Nações Unidas para a Alimentação e a Agricultura (FAO) em 2019. No entanto, as fazendas produtoras de leite de espécies não bovinas representam uma parte significativa da economia agrária em muitos países onde a produção de vacas leiteiras não consegue ser explorada (CLARK; GARCÍA, 2017). O

Kefir e Kombucha: Alimentos Probióticos Emergentes

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Maria Helena Machado Canella
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Erick Almeida Esmerino
Patrícia Blumer Zacarchenco
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Resumo

Neste capítulo, serão tratados aspectos tecnológicos voltados ao processamento de *kefir* e *kombucha*. São apresentados os principais efeitos benéficos relacionados ao consumo desses produtos, bem como os principais microrganismos envolvidos na sua produção. É realizada também uma revisão sobre a metagenômica e as interações dos microrganismos com a matriz *kefir* e *kombucha*. Por fim, este capítulo aborda os principais conceitos envolvidos na produção de *kefir* e *kombucha* e sua perspectiva de utilização como produtos funcionais em escala industrial.

1. Introdução

Alimentos fermentados tradicionais, como o *kefir* e a *kombucha*, têm ganhado espaço na mesa dos consumidores. Os microrganismos probióticos vêm sendo selecionados naturalmente nesses produtos ao longo dos anos. Os efeitos dos probióticos nesses produtos estão sendo avaliados em um crescente número de estudos.

Para exemplificar como há crescimento do interesse do consumidor e, portanto, na produção desse segmento, o mercado de *kefir* foi estimado ao redor de 130 milhões de dólares americanos em 2014, enquanto, nos anos de 1990, ele era desprezível. Algumas indústrias importantes, mundialmente, no segmento de *kefir* apontadas pela Future Market Insights são: Lifeways Foods, Danone, Nourish Kefir, Babushka Kefir, Kenmare Living Foods, Happy Kombucha, Valio Eesti AS, Lifehouse Foods, Wallaby Yogurt Company, Best of Farms LLC, entre outros, (FUTURE MARKET INSIGHTS, 2019). No Brasil, as empresas Keiff e A Leiteria produzem *kefir*.

Bebidas Vegetais Probióticas e Prebióticas

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Adriano Gomes da Cruz

Resumo

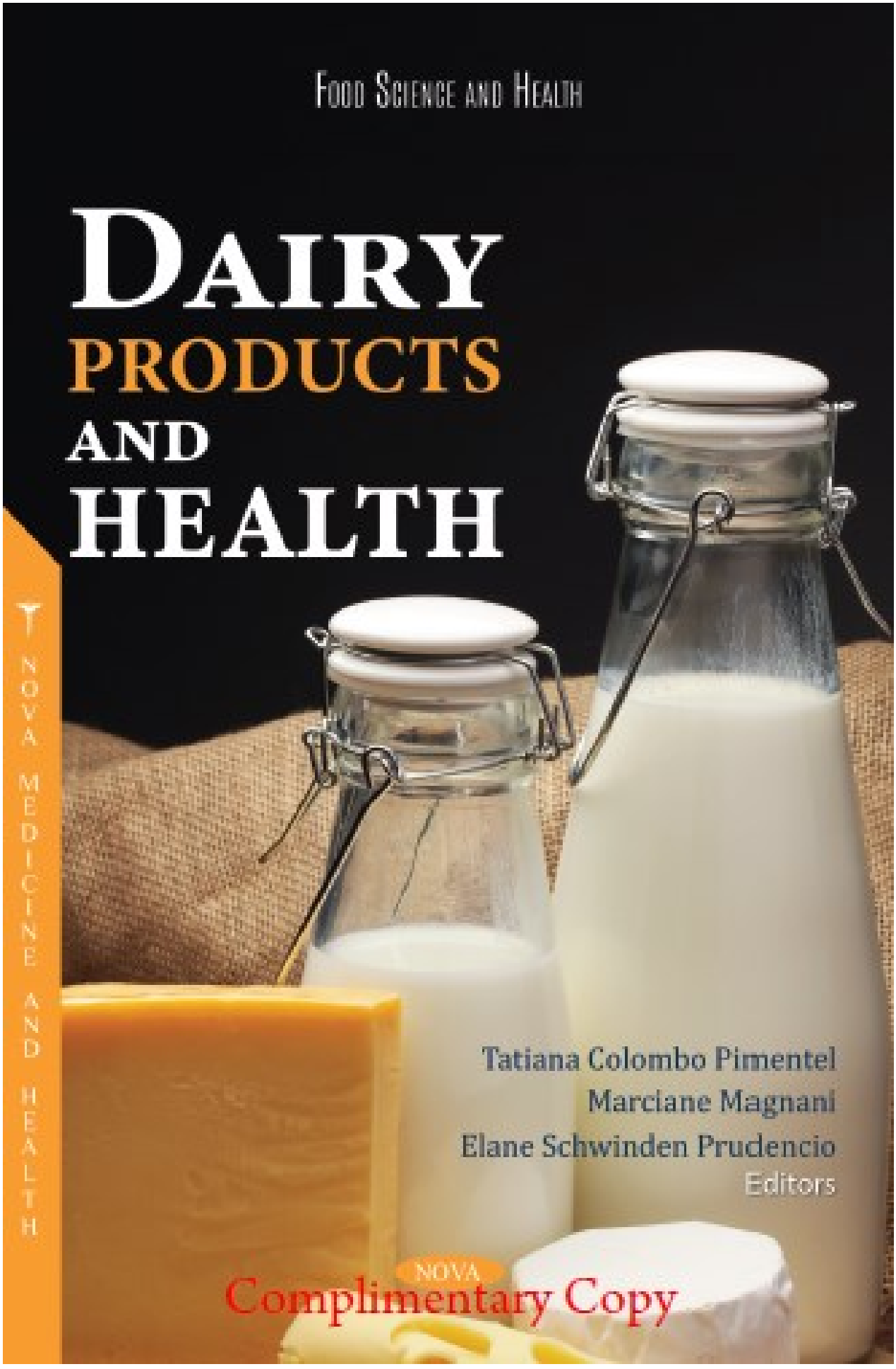
As bebidas, muitas vezes, não são consumidas pelo seu valor alimentar, mas sim como fonte de energia. Entretanto, as bebidas tornaram-se uma tendência alimentar, porque representam para todas as gerações de consumidores uma forma de dieta simplificada a fim de obter maior nutrição. Até o momento, a literatura relata vários exemplos de bebidas com a adição de diferentes nutrientes. Inúmeros objetivos e aspectos foram levados em consideração para garantir fortificações bem sucedidas, inclusive, aceitando novos desafios tecnológicos. Este capítulo fornecerá uma abordagem sobre o valor de prebióticos e/ou probióticos em bebidas de origem vegetal, englobando uma ampla gama de produtos, como bebidas à base de soja, cereais, vegetais, frutas, soja e frutas, e frutas e vegetais. Também serão abordados os principais tipos de prebióticos e probióticos presentes em bebidas vegetais, bem como as principais tecnologias envolvidas no desenvolvimento de bebidas vegetais adicionadas de prebióticos e/ou probióticos.

1. Introdução

O interesse do consumidor em nutrição e saúde contribuiu para o aumento no desenvolvimento de produtos classificados como probióticos, prebióticos e simbióticos. De acordo com Hill *et al.* (2014), probióticos são microrganismos vivos que, quando administrados em quantidades adequadas, conferem um benefício à saúde do hospedeiro. A grande maioria dos trabalhos científicos classificaram como culturas probióticas espécies de *Lactobacillus* e *Bifidobacterium*. Com a recente reclassificação de espécies de microrganismos que pertenciam ao gênero *Lactobacillus*, contudo, há espécies probióticas realocadas em outros gêneros. Por exemplo, o *Lactobacillus casei* e o *Lactobacillus reuteri* estão, atualmente, reclassificados, respectivamente, como *Lacticaseibacillus casei* e *Limosilactobacillus reuteri*, pertencendo, portanto, a outros gêneros. Este capítulo manteve a nomenclatura de espécies que pertenciam ao gênero *Lactobacillus*, que passou por reclassificação publicada nos primeiros meses do ano de 2020. Estão também colocados os nomes das espécies após a reclassificação.

Food Science and Health

Dairy Products and Health



Tatiana Colombo Pimentel
Marciane Magnani
Elane Schwinden Prudencio
Editors

Nova Medicine and Health

Chapter 3

Prebiotics, Probiotics, Synbiotics, and Postbiotics in Dairy Products

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Abstract

In the last few years, the interest of consumers for healthy products has increased, and the high capacity of probiotics and prebiotics to provide beneficial health effects led to the growing scientific and commercial interests facing the probiotics and prebiotics administration as a health promoting strategy. Since the first definition of what is probiotics, the research on functional foods advanced significantly, at the point of arising new emerging concepts, as postbiotics to refer to the non-viable

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Chapter 4

The Improvement of the Functional Potential of Dairy Products Using Fruits and Plant Extracts

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Chapter 5

An Approach to the Innovative Better-for-You Ice Cream Category

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Abstract

Foods with claims of functional and health properties must consistently demonstrate the association between the food or its constituent and the metabolic or physiological effect beneficial to the health of the human organism. Labels that obtain authorization to use claims of functional and health properties must present the text of the claim exactly as approved in the evaluation process, including warnings and other required information. Among these foods, a new category of ice cream adopted by the industries stands out, classified as better for you. The ice creams in this category have presented remarkable commercialization growth, with many ice cream companies launching as alternatives to traditional

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Chapter 6

Brown Cheese (Brunost): Health and Technological Aspects

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Abstract

The chapter focuses on brown cheeses' health and technological aspects. This chapter involves the definition, origin, and nutritional composition of brown cheeses. The highlight of this chapter was the possibility to elaborate the brown cheeses from milk, cream and/or whey, either from cow or goat. Another relevant point of this chapter is the use of whey by industry, due to the large volume generated in the manufacture of cheeses and, therefore, its low cost. In addition, whey has the availability of nutrients from proteins, being a source of essential amino acids, bioactive peptides, antioxidants and immunostimulants, and on the other hand be considered an environmental pollutant. Finally, it is possible to conclude that a dairy industry has equipment such as pasteurizer, falling film

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Sensory Profiling of Dairy Products

Edited by John J. Tuohy

WILEY Blackwell

SDT Society of Dairy Technology
Passion for Dairy

SOCIETY OF DAIRY TECHNOLOGY

6 Sensory Attributes of Liquid Milk Products

Elson R. Filho, Amanda A. Prestes, Maria H. Canella, Elane S. Prudencio, Mônica Q. Freitas, Tatiana C. Pimentel, Erick A. Esmerino and Adriano G. da Cruz

6.1 Introduction

High-quality milk is a balanced, delicate and pleasantly sweet food. There is also a tendency toward neutrality and the absence of aftertaste. Such characteristics are reflections of a balanced nutritional composition, rich in proteins, fats, minerals and sugar (lactose) (Clark et al. 2009). However, the inherent blandness means that milk does not have highly pronounced attributes, which has led to the preoccupation of sensory investigations with possible deviations from this neutrality.

At the beginning of the twentieth century, the sensory analysis of milk and other dairy products focused on quality measurement using standardised methods, such as the Dairy Product Judging and the American Dairy Science Association (ADSA) scorecard system (Schiano et al. 2017). Such methods focused their efforts on identifying the most likely defects and off-flavours in the milk by expert judges, who analysed the samples in search of signs of contamination or deterioration.

The perishability of milk is caused by its high water activity and nutritional richness. Therefore, sensory deviations resulting from contamination are quickly manifested in the 'flavour' of the product, deviating it from the expected neutrality. Although in common sense, flavour and taste are interchangeable words, they have different meanings in sensory analysis. In this chapter, we present a basic overview of the definitions of taste and flavour due to the growing trend in the use of sensory methods based on consumer perception and the need to increasingly reduce the training hours of specialists (trained tasters/panellists). In both cases, confusion about these definitions can impair the performance of sensory methods.

The concept of flavour can be better understood when broken down into five related terms (taste, flavour, somatosensation, odour and aroma) and two human senses: taste and smell. Taste can be described as the phenomenon resulting from the stimulation of taste buds distributed in the mouth, especially those on the tongue. Although not restricted to these, in sensory analysis, taste is primarily related to the manifestation of its five primary forms: sweet, sour, salty, bitter and umami. Other somatosensations such as metallic, greasy, spicy, refreshing and astringent are also perceived in the oral cavity when stimulated by some foods. Somatosensations can be understood as

Sensory Profiling of Dairy Products, First Edition. Edited by John J. Tuohy.

7 Sensory Profile of Yoghurt and Related Products

Amanda A. Prestes, Elane S. Prudencio, Maria H. Canella, Mônica Q. Freitas, Elson R. Filho, Tatiana C. Pimentel, Erick A. Esmerino and Adriano G. da Cruz

7.1 Introduction

Fermented milks have been manufactured and enjoyed from antiquity. Fermentation has been used for milk preservation since it was discovered that fermented milk was a stable and nutritious food that could be safely stored for a period of time. Fermented milks production expanded and improved over the centuries with an evolution from domestic production to industrial manufacturing with defined strain starter cultures, production protocols and large-scale hygienically designed equipment.

Fermented milk products are produced by the fermentation of lactose (milk sugar) by lactic acid bacteria (LAB), via various metabolic pathways without the involvement of oxygen or its agents (Tamime 2006). LAB strains are able to hydrolyse the lactose molecules, into its constituent glucose and galactose monosaccharides, from which lactic acid is produced. Lactic acid development reduces the pH of the milk until the isoelectric point of casein around pH 4.6 is reached and gelation occurs, due to casein precipitation and binding with calcium and phosphorus. Gelation is the basis of texture development while flavour and aroma are primarily attributable LAB. Importantly, acidification by LAB is responsible for inhibiting the growth of spoilage and pathogenic bacteria and contributing to the shelf life of fermented milk products.

Ferment milk products can be classified into two broad categories, based on fermentation temperature: cultured milks, such as cultured buttermilk, kefir, koumiss and a range of indigenous Scandinavian cultured milk products, are produced using starter cultures comprising mesophilic LAB and typical incubation temperatures in the range 20–25 °C and yoghurts, produced using LAB capable of fast growth at typical incubation temperatures in the range 37–45 °C. This chapter deals exclusively with the sensory properties of yoghurt and yoghurt products and explores the application of sensory analysis methodology in relation to product acceptability, quality improvement and innovative new product development.

Sensory Profiling of Dairy Products, First Edition. Edited by John J. Tuohy.

Book chapters submitted to Blucher publisher:

CAPÍTULO 1
QUEIJOS: ORIGEM E MERCADO CONSUMIDOR

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CAPÍTULO 5
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CAPÍTULO 7
QUEIJOS DE MASSA FILADA

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CAPÍTULO 10
QUEIJOS FUNCIONAIS

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Annex B- Work presentations

Work presentation certificate "Efeito protetivo de iogurte adicionado de polpa de guabiroba (*Campomanesia* sp.) na sobrevivência de *Bifidobacterium animalis* subsp. *lactics BB-12* submetida às condições gástricas" at the XV Southern Regional Meeting of Food Science and Technology (XV ERSCTA)- 2019

XV Encontro Regional Sul de Ciência e Tecnologia de Alimentos
"Caminhos da produção de alimentos: Biodiversidade e Inovação"

CERTIFICADO

Certificamos que

AMANDA ALVES PRESTES, SILVANI VERRUCK, EULÁLIA LOPES DA SILVA BARROS, LUAN VALDEMIRO ALVES DE OLIVEIRA, CRISTIANE VIEIRA HELM, ELANE SCHWINDEN PRUDÊNCIO

Participou(aram) da XV ERSCTA - Encontro Regional Sul de Ciência e Tecnologia de Alimentos, realizado no período de 28 e 29 de novembro de 2019, em Curitiba - PR.

Na qualidade de autor(es) do Trabalho Científico: EFEITO PROTETIVO DE IOGURTE ADICIONADO DE POLPA DE GUABIROBA (CAMPOMANESIA SP.) NA SOBREVIVÊNCIA DE BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS BB-12 SUBMETIDA ÀS CONDIÇÕES GÁSTRICAS

Curitiba, 29 de Novembro de 2019.

Leomara Floriano Ribeiro
Coordenadora da Comissão Científica do XV ERSCTA

Agnes de Paula Scheer
Presidente do XV ERSCTA

Promoção

Organização

Speech certificate “Produtos lácteos probióticos e prebióticos de espécies não bovinas” at the online symposium “Probiotics and prebiotics: challenges and advances”– 2020

CERTIFICADO

Certificamos que
AMANDA ALVES PRESTES
atuou como instrutora no Simpósio On-line :
PROBIÓTICOS E PREBIOTICOS: AVANÇOS E DESAFIOS
promovido pelo Centro de Tecnologia de Laticínios
e realizado no dia 01 de novembro de 2020, em Campinas/SP,
com duração de 30 minutos.

Campinas, 01 de novembro de 2020

Adriana Torres
Coordenação Técnica

Diretora Geral do Ital

REGISTRO Nº TL-101220-B006

ITAL
INSTITUTO DE TECNOLOGIA DE ALIMENTOS

TecnoLat

Workshop preparation “Use of Guabiroba fruit in development of products with a focus on *dulce de leche*”

Certificado de Participação

Certifico que a Doutoranda

Amanda Alves Prestes

Ministrou a oficina: "Aproveitamento do fruto de Guabiroba no desenvolvimento de produtos com enfoque em doce de leite", como parte das atividades do Projeto Guabiroba em parceria com a Heide Extratos Vegetais, Embrapa Florestas e Fundação Cargill. Realizada no dia 28/04/2023 das 09h às 16h na localidade do Pinho de Baixo no município de Irati/PR.

Rodrigo Heemann
Coordenador do Projeto