Giustino Tribuzi

DESENVOLVIMENTO DE ALTERNATIVAS TECNOLÓGICAS PARA O PROCESSAMENTO E CONSERVAÇÃO DA CARNE DE MEXILHÃO

Tese submetida ao Programa de Pós-Graduação em Engenharia de Alimentos da Universidade Federal de Santa Catarina para a obtenção do Grau de Doutor em Engenharia de Alimentos.
Orientador: Prof. Dr. João Borges Laurindo

Florianópolis
2013

Ficha de identificação da obra elaborada pelo autor,
através do Programa de Geração Automática da Biblioteca Universitária da UFSC.

Tribuzi, Giustino
Desenvolvimento de alternativas tecnológicas para o processamento e conservação da carne de mexilhão / Giustino Tribuzi ; orientador, João Borges Laurindo - Florianópolis, SC, 2013.
174 p.

Tese (doutorado) - Universidade Federal de Santa Catarina, Centro Tecnológico. Programa de Pós-Graduação em Engenharia de Alimentos.

Inclui referências

1. Engenharia de Alimentos. 2. Carne cozida de mexilhão. 3. Alternativas tecnológicas. I. Borges Laurindo, João. II. Universidade Federal de Santa Catarina. Programa de Pós-Graduação em Engenharia de Alimentos. III. Título.

FOLHA DE APROVAÇÃO – PGEAL

Ai miei cari nonni.

AGRADECIMENTOS

Ao Professor Dr. João Borges Laurindo, pela orientação, pelo suporte, pelo esforço e pela paciência que dedicou em me ajudar a realizar este trabalho e em me tornar melhor pesquisador.
Aos professores membros das bancas de qualificação e de defesa pelo tempo e atenção despendidos nas correções e sugestões dadas ao trabalho: Professor Marcelo Cristianini, Professora Glaucia Maria Falcão de Aragão, Professor Marco Di Luccio, Professora Franciny Campos Schmidt, Professora Alcilene Rodrigues Monteiro Fritz, Professor Bruno Augusto Mattar Carciofi.
A todos os professores do Departamento de Engenharia Química e Engenharia de Alimentos.
A todos os colegas do laboratório PROFI e dos outros laboratórios do EQA e da UFSC que colaboraram de alguma forma na realização deste trabalho.
À Elizabeth por ter estado pacientemente no meu lado nos últimos anos.
A todas as pessoas que de alguma forma me ajudaram neste caminho.
Ao Programa de Pós-Graduação em Engenharia de Alimentos da Universidade Federal de Santa Catarina.
A todos os responsáveis do projeto Erasmus Mundus - EBWII por permitir a viabilização desta experiência.
À CAPES, à FINEP e ao projeto Erasmus Mundus-EBW II pelo apoio financeiro.

Dum loquimur, fugerit ínvida aetas:
carpe diem, quam minimum credula postero.
(Horácio)

RESUMO

A comercialização dos mexilhões no Brasil é realizada principalmente como produto fresco, ou cozido (resfriado ou congelado), limitando a difusão e o beneficiamento deste molusco às regiões próximas à área de produção, uma vez que o mexilhão é muito perecível, necessitando de transporte rápido e refrigerado. Desta forma, pesquisas são necessárias para o desenvolvimento de processos alternativos que estendam a vida útil desse tipo de produtos, agregando valor e melhorando as condições de comercialização. Com este objetivo, no presente trabalho foram estudadas técnicas de conservação, como a salga e a marinação, o tratamento térmico em embalagens flexíveis termoesterilizáveis e a desidratação, aplicados à carne cozida de mexilhão. Na primeira etapa, realizou-se o tratamento da carne de mexilhão em salmouras e em salmouras com adição de ácido. Foram obtidas informações sobre o comportamento da carne de mexilhão quando submetida a diferentes concentrações de sal (5, 10, 15, 20 e 26 % m/m) e ácido acético (0,5, 2 e 6 % m/m) durante 24 horas de processo. Foi observado que os maiores ganhos de água foram obtidas com as salmouras com concentrações de 5 e 10 % de sal e as maiores perdas de água foram verificadas em salmouras com elevada concentração de sal ou com a presença de ácido acético na salmoura que apresentou um forte efeito desidratante. Foi possível ajustar modelos matemáticos empíricos aos dados de ganho de sal e ganho de água. Na segunda etapa do trabalho foi avaliada a possibilidade da utilização de embalagens flexíveis termoesterilizáveis para o desenvolvimento de uma conserva de carne de mexilhão. Nesse estudo foi avaliado o efeito da temperatura da autoclave no rendimento do produto e no valor de cozimento, sendo que a melhor condição encontrada foi na temperatura da autoclave de 118 °C e com um tempo total de processo de 57 min. Baseando-se nos resultados obtidos no primeiro estudo deste trabalho, a respeito de salga e marinação, foram adicionados sal e ácido acético aos produtos, avaliando-se o efeito destas substâncias durante um ano de armazenamento a 25 °C. O lote aditivado com sal mostrou melhor rendimento, embora não tenham sido observadas diferenças nos demais parâmetros físico-químicos avaliados. Na última etapa do trabalho, foi desenvolvido um aparato experimental que permite registrar a massa e a temperatura das amostras durante a liofilização, para diferentes condições de fornecimento de calor. As curvas de desidratação foram obtidas com a placa aquecida (suporte das amostras) nas temperaturas de 15, 30 e 40 °C e com o sistema de aquecimento desligado (temperatura

ambiente). O sistema desenvolvido ofereceu uma boa reprodutibilidade nas condições estudadas. O ligeiro aquecimento do suporte (15 °C) apresentou-se como a melhor condição na liofilização de carne de mexilhão, sendo que causou um significativo incremento na taxa de secagem sem modificar as características visuais das amostras. Também foram avaliados os métodos de secagem a vácuo e convectiva e o efeito dos três diferentes métodos na capacidade de reidratação do produto seco. O produto liofilizado apresentou os melhores resultados, recuperando, durante a reidratação, mais de 90 % da água perdida durante a secagem.

Palavras-chaves: *Perna perna*. Carne cozida de mexilhão. Salga. Marinação. Cinética. Tratamento térmico. Embalagens flexíveis termoesterilizáveis. Secagem.

ABSTRACT

In Brazil, mussels are mainly commercialized fresh or cooked (refrigerated or frozen). Since this product is a highly perishable food and needs refrigerated transport, the diffusion and processing of this mollusk are limited to coastal area. Investigations are necessary to develop alternative processes to extend the shelf life of this product, adding value and improving the commercialization conditions. With this aim, in this work, different conservation techniques of conservation of cooked mussel meat were studied, as the salting and the marination, the heat treatment in retort pouches and the dehydration. In a first step of this work, the pre-cooked mussel meat was treated in brine and marinades. Thus, data were obtained about the mussel meat behavior when submitted to different salt (5, 10, 15, 20, and 26 % w/w) and acetic acid (0, 5, 2, and 6 % w/w) concentrations during 24 hours of process. The highest water gains were obtained in brines at the concentrations of 5 and 10 % of salt and the highest water losses were obtained with high concentrations of salt or when the acetic acid was added to the brine. Moreover, it was possible to fit empiric mathematical models to the data of the salt and water gain. In the second part of this work, the possibility of using retort pouch to develop a conserve of mussel meat was evaluated. The retort temperature effect in the product yield and in the cook value was evaluated. The temperature of the retort of 118 °C resulted the best condition, with a total process time of 57 minutes. Based on the results obtained in a first study of this work about the salting and the marination, salt and acetic acid were added to the products, evaluating the effect of these substances during the one year on storage at 25 °C. The salted batch showed better yield during storage; although differences in the other physicochemical parameters evaluated have not been observed. In the last part of this work, an experimental equipment was built. This equipment allows the online monitoring of the weight and the temperature of the samples during the freeze drying process at different temperatures. The dehydration curves were obtained with the heated plate (sample holder plate) at 15, 30 e 40 °C and with the heating system switched off (room temperature). The developed system offered a good reproducibility at the studied conditions. The heating of the sample holder plate (15 °C) was the best condition in the freeze drying of the mussel meat, causing a significant increase in the drying rate without modifying the external aspect of the dried product. Other drying methods as the vacuum and the air drying were also

studied. The rehydration capacity of the dried product was evaluated for all the drying methods and the best results were obtained with the freeze dried mussels that recovered during rehydration about 90 % of the water lost during drying.

Keywords: *Perna perna*. Pre-cooked mussel meat. Salting. Marination. Kinetic. Heat treatment. Retort pouch. Drying.

LISTA DE TABELAS

Table 3.1 - Physicochemical properties of pre-cooked mussel meat..... 59

Table 3.2 - Equations and determination coefficient values observed for the fit of WG as function of salt concentration (Figure 3.3) for different immersion times. 64

Table 3.3 - Equations and determination coefficient values observed for the fit of SG as function of salt concentration (Figure 3.5) for different immersion times. 69

Table 3.4 - Equations and determination coefficient values observed for the fit of S % and M % of mussels treated in brines and marinades as function of salt concentration for different immersion times. 70

Table 3.5 - Changes in mussels WHC during the first 4 h of treatment*. 78

Table 3.6 - Parameters of Peleg's model, Weibull type model and Zugarrumurdi and Lupin model fitted to experimental data of SG. 84

Table 3.7 - Parameters of Peleg's model, Weibull type model and Zugarrumurdi and Lupin model fitted to experimental data of WG. 85

Table 4.1 - Preliminary test conditions..... 113

Table 4.2 - Chopped mussel meat characterization (proximate composition, physicochemical properties and total viable count). 118

Table 4.3 - Heat seal strength test. 119

Table 4.4 - Results from process calculations at the retort temperature of 110, 118, and 121 °C. 121

Table 4.5 - Yield of chopped mussel meat processed at the retort temperature of 110, 118, and 121 °C. 123

LISTA DE FIGURAS

Figure 2.1 - Lateral view of a dissected *Perna perna* mussel from the left side. (1) valve internal side; (2) muscular scars; (3) gill ; (4) mantle; (5) visceral mass; (6) byssus; (7) foot. 37

Figure 2.2 - *Perna perna* mussels. Raw (a); raw opened (b); cooked male (c); cooked female (d)..... 40

Figure 3.1 - Salting and marination experimental device sketch..... 53

Figure 3.2 - Kinetics of WG of mussel treated in brines with different C_s (a) and in marinades with different C_{ms} and C_{ma} (b)..... 60

Figure 3.3 - The WG of pre-cooked mussel meat treated in brines with salt concentration from 5 % to 20 % (a) and in marinades with salt concentration from 5 % to 20 % and acetic acid concentration of 2 % (b) as function of the salt concentration. 63

Figure 3.4 - Kinetics of SG of mussel treated in brines with different C_s (a) and in marinades with different C_{ms} and C_{ma} (b)..... 66

Figure 3.5 - The SG of pre-cooked mussel meat treated in brines with salt concentration from 5 % to 20 % (a) and in marinades with salt concentration from 5 % to 20 % and acetic acid concentration of 2 % (b) as function of the salt concentration. 68

Figure 3.6 - Operational diagram of pre-cooked mussel meat immersed in brines (a) and marinades (b). The gray lines are related to salt, while the dark lines represent the moisture content. 71

Figure 3.7 - Evolution of the acid concentration in mussel treated in marinades with different concentrations of salt and acetic acid. 73

Figure 3.8 - Kinetics of the pH in mussel treated in marinades with different concentrations of salt and acetic acid. 74

Figure 3.9 - Changes of water activity (aw) during salting (a) and marination..... 76

Figure 3.10 - Kinetics of MG of mussel treated in brines with different Cs (a) and in marinades with different Cm (b). 79

Figure 3.11 - Scanning electron microscopy of the mantle of female mussels (magnification: x50). 81

Figure 3.12 - Scanning electron microscopy of the mantle of female mussels: (a) untreated; (b) treated 4 hours with brine with C_s=5 % (c) treated 4 hours with brine with C_s=20 %; (d) treated 4 hours with marinade with C_{ms}=5 % and C_{ma}= 2 % (magnification, x250)..... 82

Figure 4.1 - Example of a heat penetration curve measured in the SP and of the lethality curve. 100

Figure 4.2 - Flow chart representing the whole mussel treatment, from the industry to heat-treated product..... 106

Figure 4.3 - Experimental device. 1- retort; 2-tray with pouches; 3-retort controller; 4- pressure transducer; 5- pressurized air pipe line; 6-cooling water line..... 108

Figure 4.4 - Lateral (a) and superior (b) view of the tray with the RPs. The numbers 1 to 5 indicate the position of the thermocouples during the temperature distribution tests. 109

Figure 4.5 - Measurement locations for seal strength tests. 111

Figure 4.6 - Device used to determine residual air in the pouches..... 112

Figure 4.7 - Lateral (a) and frontal (b) view of the RP with the thermocouple..... 114

Figure 4.8 - Simulated heat penetration curve (*Revisited General Method* -Case 1)..... 115

Figure 4.9 - Temperature distribution test for the tested conditions. Thermocouple 1 (—), *2* (—), *3* (—), *4*(—), and 5(—)..... 120

Figure 4.10 - Heat penetration characteristics for retort pouches in the retorting conditions a, b, c, and d. Slowest heating point temperature (—), retort temperature (—), L_0 (—), F_0 (—) and C_T ($C100\ °C33.1\ °C$) (—)..... 122

Figure 4.11 - Changes in total yield (% mass) of chopped mussel meat (B0, Bs, and Bm) on storage at 25 °C. 124

Figure 4.12 - Changes in WHC of chopped mussel meat (B0, Bs, and Bm) during storage at 25 °C. 125

Figure 4.13 - Changes TVB-N and TMA-N of chopped mussel meat (B0, Bs, and Bm) on storage at 25 °C. 127

Figure 4.14 - Changes in pH of chopped mussel meat (B0, Bs, and Bm) on storage at 25 °C..... 128

Figure 5.1 - Drying rate curve. 138

Figure 5.2 - Stability map for reactions as a function of water activity. 141

Figure 5.3 - Phase diagram of water. 145

Figure 5.4 - Diagram of a material on a tray during freeze drying. N_w is the vapor flux trough the dry layer; q_r is the radiation heat flux; and q_c is the conductive heat flux. 147

Figure 5.5 - Freeze dryer 150

Figure 5.6 - Vacuum chamber – detail. 151

Figure 5.7 - Freeze-drying curves of pre-cooked mussel meat at the SHP temperature of 15, 30, 40 °C and Non-Heated condition (five replicates). 156

Figure 5.8 - Freeze drying curves (a), freeze drying rate as function of time (b), and freeze drying rate as function of moisture content (c) of pre-cooked mussel meat freeze-dried at the plate temperature of 15 °C (—), 30°C (—), 40 °C (—) and Non-Heated (—). 157

Figure 5.9 - Air drying curves (AD) and vacuum drying curves (VD) of pre-cooked mussel (three replicates). 159

Figure 5.10 - Drying curves (a), drying rate as function of time (b), and drying rate as function moisture content (c) of pre-cooked mussel meat air dried (—) and vacuum dried (—). 160

Figure 5.11 - Temperature of the samples during drying of pre-cooked mussel meat. Freeze drying with SHP temperature of 15 °C (—), 30°C (—), 40 °C (—), Non-Heated (—), and Vacuum drying (—) 161

Figure 5.12 - Effect of the process temperature on the external aspect of the freeze-dried samples. 162

Figure 5.13 - External aspect of air and vacuum dried samples. 162

Figure 5.14 - Rehydration capacity kinetics of dried mussels obtained for the different drying methods (FD, VD, and AD) and rehydrated in water at 20 °C (●) and 80 °C (□). 163

LISTA DE ABREVIATURAS

EPAGRI	Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina
FDA	Food and Drug Administration
FAO	Food and Agriculture Organization
C_s	NaCl concentration of the brine
C_m	Marinade concentration (salt and acetic acid)
C_{ma}	Acid concentration of the marinade
C_{ms}	NaCl concentration of the marinade
WHC	Water holding capacity
SG	Salt gain
WG	Water gain
MG	Mass gain
a_w	Water activity
M %	Mussel moisture content during salting and marination (%)
S %	Mussel salt content during salting and marination (%)
RP	Retort pouch
F_r	Accumulated lethality (Required)
F_0	Accumulated lethality for *C. botulinum* type A (Calculated)
L_0	Lethality for *C. botulinum* type A (Calculated)
C_T	Cooking value for thiamine (Calculated)
SP	Slowest heating point of the packaging
TVB-N	Total volatile basic nitrogen
TMA-N	Trimethylamine nitrogen
SHP	Sample holder plate
FD	Freeze drying
AD	Air drying
VD	Vacuum drying
RC	Rehydration capacity

SUMÁRIO

1. INTRODUÇÃO..... 31
1.2 OBJETIVO 33
2. MUSSEL GENERALITIES 35
2.1 MORPHOLOGY 35
2.2 NUTRITIONAL COMPOSITION 37
2.3 PRODUCTION AND COMMERCIALIZATION 39
2.3.1 Production technology 40
2.4 REFERENCES 42
3. MASS TRANSFER DURING SALTING AND MARINATION PROCESSES OF PRE-COOKED MUSSEL MEAT..... 45
3.1 INTRODUCTION 45
3.2 LITERATURE REVIEW..... 46
3.2.1 Salting..... 46
3.2.2 Marination 47
3.2.3 Equilibrium relations during the osmotic treatment of food.....48
3.2.4 Study of the of mass transfer rates 49
3.2.4.1 Modeling of the mass transfer 50
3.3 MATERIAL AND METHODS 52
3.3.1 Sample preparation..... 52
3.3.2 Experimental setup 52
3.3.3 Experimental methods 53
3.3.4 Analytical determinations 54
3.3.4.1 Moisture content..... 54
3.3.4.2 Water activity 54
3.3.4.3 pH 54

3.3.4.4 Sodium chloride analysis 54
3.3.4.5 Acid content – titratable acidity 55
3.3.4.6 Water-holding capacity (WHC) 55
3.3.4.7 Microstructure Examination 56
3.3.5 Process parameters 56
3.3.5.1 Mathematical modeling of salt and water gain 57
3.3.5.2 Statistical analysis 58
3.4 RESULTS AND DISCUSSION 59
3.4.1 Raw material 59
3.4.2 Kinetics of the water gain 59
3.4.3 Kinetics of the salt gain 64
3.4.4 Operational diagram 69
3.4.5 Effect of the marinade acetic acid and salt concentration on the acid concentration and ph of pre-cooked mussel meat 72
3.4.6 Effect of the salt and acid concentration on the a_w of pre-cooked mussel meat 75
3.4.7 Effect of salt and acid concentration on the whc of pre-cooked mussel meat 77
3.4.8 Mass gain rates 78
3.4.9 Microstructure analysis 80
3.4.10 Mathematical modeling 82
3.5 FINAL CONSIDERATIONS 86
3.6 REFERENCES 87
4. THERMAL PROCESSING OF MUSSEL MEAT IN RETORT POUCHES 95
4.1 INTRODUCTION 95
4.2 LITERATURE REVIEW 96
4.2.1 Thermal processing 96

4.2.1.1 Thermal processing parameters 97
4.2.1.1.1 Thermal process evaluation 99
4.2.1.2 Retort Pouch 101
4.3 MATERIALS AND METHODS 106
4.3.1 Raw material 106
4.3.2 Experimental method 106
4.3.2.1 Sample preparation 107
4.3.2.2 Experimental device 107
4.3.2.3 Retort pouches 109
4.3.2.3.1 Filling and sealing 109
4.3.2.3.2 Retort pouch quality control 110
4.3.2.3.2.1 Mechanical seal test 110
4.3.2.3.2.2 Residual Air Test 111
4.3.2.4 Thermal processing 112
4.3.2.5 Thermal treatment parameters 113
4.3.3 Physicochemical analysis of the chopped mussel 115
4.3.3.1 Yield 116
4.3.3.2 Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) 116
4.3.4 Microbiological analysis 117
4.3.5 Sterility test 117
4.3.6 Statistical analysis 118
4.4 RESULTS AND DISCUSSION 118
4.4.1 Pre-cooked mussel meat characterization 118
4.4.2 Retort pouch properties 119
4.4.3 Thermal processing 119
4.4.4 Storage study 123

4.4.4.1 Yield and WHC monitoring during storage 123
4.4.4.2 Changes of the physicochemical parameters during storage 126
4.4.5 Sterility test 128
4.5 FINAL CONSIDERATIONS 130
4.6 REFERENCES 130
5. EVALUATION OF DIFFERENT DEHYDRATION METHODS OF COOKED MUSSELS MEAT 135
5.1 INTRODUCTION 135
5.2 LITERATURE REVIEW 136
5.2.1 Drying 136
5.2.1.1 Principles of drying 136
5.2.1.2 Dried product properties 140
5.2.1.2.1 Pre-treatments 142
5.2.1.2.2 Rehydration 143
5.2.1.3 Air drying (Convective drying) 143
5.2.1.4 Vacuum drying 144
5.3 MATERIALS AND METHODS 149
5.3.1 Sample preparation 149
5.3.2 Freeze drying 149
5.3.2.1 Experimental device 149
5.3.2.2 Freeze drying 152
5.3.3 Air drying 153
5.3.4 Vacuum drying 153
5.3.5 Drying kinetics 153
5.3.6 Rehydration 153
5.3.7 Analytical determinations 154
5.3.7.1 Moisture content 154
5.3.7.2 Water activity 154

5.3.7.3 Statistical analysis..... 154
5.4 RESULTS AND DISCUSSION 154
5.4.1 Drying kinetics 154
5.4.2 Rehydration 163
5.5 FINAL CONSIDERATIONS 165
5.6 REFERENCES 166
6. CONCLUSÕES.....173

ESTRUTURA DO TRABALHO

O presente trabalho está estruturado da seguinte forma:
O Capítulo 1 é uma breve introdução da matéria estudada no trabalho e dos objetivos gerais.
O Capítulo 2 aborda brevemente as características, a produção e o beneficiamento da matéria prima utilizada.
Os Capítulos 3, 4 e 5 apresentam uma introdução, uma revisão da literatura relacionada com o estudo, material e métodos, resultados e discussão, considerações finais e referências bibliográficas.
O Capítulo 3 apresenta um estudo da transferência de massa durante a salga e a marinação de carne de mexilhão cozida.
O Capítulo 4 apresenta um estudo do processamento térmico de carne de mexilhão embalada em embalagens flexíveis termoesterilizáveis.
O Capitulo 5 apresenta um estudo sobre a secagem de carne de mexilhão cozida.
O Capitulo 6 apresenta as conclusões gerais do trabalho e sugestões para trabalhos futuros.

1. INTRODUÇÃO

O estado de Santa Catarina é o maior produtor de mariscos do Brasil (mais de 90 % da produção nacional). Em particular, a produção de mexilhões no ano de 2012 foi de 21027 toneladas, representando uma importante realidade para a economia da região (EPAGRI, 2013). A importância do mexilhão não é somente econômica, uma vez que este alimento representa também uma importante fonte de aminoácidos essenciais, ácidos graxos ômega-3, minerais e vitaminas (SCHAAFSMA, 2008).

Os mexilhões, como os bivalves em geral, são organismos que se alimentam filtrando a água do mar. Portanto, podem apresentar uma elevada carga microbiana que, em conjunto com uma natural biodisponibilidade dos nutrientes na carne, provocam uma rápida deterioração deste produto se não adequadamente tratado ou armazenado.

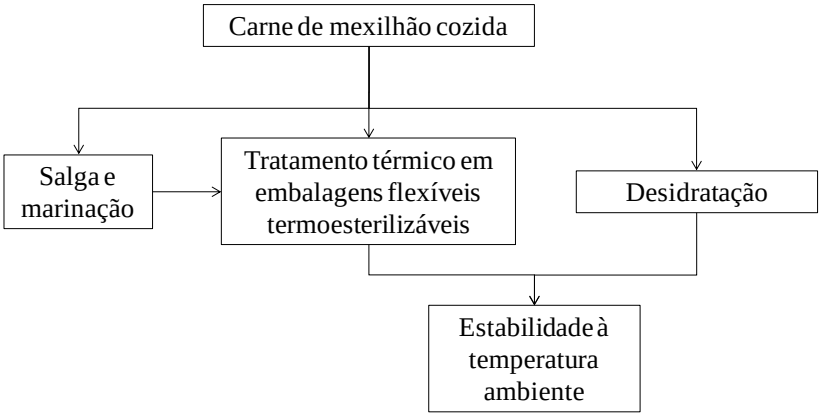
No Brasil, os mexilhões são comercializados principalmente frescos, cozidos e desconchados, mantidos sob refrigeração. Isso dificulta uma ampla difusão deste produto no mercado nacional, em virtude da necessidade de transporte rápido e refrigerado. Dessa forma, a comercialização do produto é concentrada nas áreas próximas às áreas de produção, tornando o produto muito pouco consumido no interior do país. Nesse contexto, o presente trabalho visa contribuir cientificamente e tecnologicamente para o desenvolvimento de alternativas para um melhor beneficiamento dos mexilhões através de processos que permitam a extensão da vida útil deste produto, como a salga e a marinação, o tratamento térmico em embalagens flexíveis termoprocessáveis e a desidratação.

A salga é um dos métodos mais antigos de preservação de alimentos. Aumentando o conteúdo de sal e diminuindo a atividade de água, o produto permanece estável por longos períodos. Para obter esta estabilidade, são necessárias altas concentrações de sal que impedem que o produto seja consumido diretamente, mas apenas após uma etapa de dessalga. Para diminuir o conteúdo de sal, deixando inalterada a estabilidade do produto, é possível combinar outros processos à salga, como por exemplo a diminuição do pH (CHIRALT et al., 2001). O tratamento de produtos com soluções de sal e ácidos orgânicos (marinação) permite a obtenção de produtos prontos para o consumo e com uma certa estabilidade a temperaturas de refrigeração.

Para a obtenção da estabilidade à temperatura ambiente, é necessário, após a salga ou a marinaçao dos mexilhões, submetê-los a

um tratamento térmico de esterilização comercial. A qualidade dos produtos tratados termicamente é fortemente afetada pelo binômio tempo de processo - temperatura. As embalagens flexíveis termoesterilizáveis oferecem boas caraterísticas de penetração de calor, permitindo a diminuição do tempo de proceso e uma menor degradação do produto. Embora alimentos processados nestes tipos de embalagens possam ter alta qualidade nutricional, a forma e a estutura dos produtos pode ser afetada, pois as "pouches" não oferecem suporte estrutural, sendo particularmente indicadas para produtos onde não seja preciso manter a forma original (produtos ralados ou triturados). A estabilidade dos mexilhões à temperatura ambiente também pode ser obtida por desidratação. A desidratação, porém, provoca mudanças importantes no produto final, dependendo do processo utilizado. Um dos processos que menos afeta a qualidade do produto final é a liofilização (RATTI, 2011). Este processo permite ainda a obtenção de produtos com reidratação instantânea, utilizáveis em várias preparações culinárias.

A Figura 1.1 - apresenta um diagrama com as alternativas de processo propostas no presente estudo.



1.2 OBJETIVO

O objetivo principal deste trabalho foi o desenvolvimento de alternativas tecnológicas para o processamento e conservação da carne de mexilhão.

Os objetivos específicos foram:

- Estudar a transferência de massa durante a salga e a marinação de carne de mexilhão cozida;
- Estudar o processamento térmico e a estabilidade físico-química durante o armazenamento da carne de mexilhão triturada acondicionada em embalagens flexíveis termoprocessáveis ("*retort pouches*");
- Estudar diferentes métodos de desidratação aplicados à carne de mexilhão.

2. MUSSEL GENERALITIES

Mussels are edible bivalves belonging to the *Mytilidae* family, in particular to the *Mytilus* genus (true mussels) and the *Perna* genus (warm water mussels). Adult specimens belonging to these genus are taxonomically differentiated by the presence (*Mytilus*) or absence (*Perna*) of the anterior adductor muscle and by the variation on the number of retractor muscle scars. In the genus *Perna* there are two retractor muscle scars when in *Mytilus* only one (SIDDAL, 1980; NARCHI and GALVÃO-BUENO, 1997).

Two of the most cultivated species, the Blue mussel, *M. edulis*, and the Mediterranean mussel, *M. galloprovincialis*, belong to the *Mytilus* genus (DAILIANIS, 2011). Other species from this genus that also have a strong commercial interest include the *M. californianus*, the *M. chilensis* and the *M. trossulus*. In relation to the *Perna* genus the species of highest commercial value are the *P. perna*, the *P. vidris* and the *P. canaliculus* (SIDDAL, 1980).

For the human consumption, mussels are intensively caught and bred worldwide. On the Atlantic coast of South America, the most cultivated mussel is the Brown mussel (*Perna perna*). The areas of highest cultivation are in Venezuela, in Brazil (from Espírito Santo to Rio Grande do Sul) and also in Uruguay. Moreover, the Brazilian state of Santa Catarina is where the production is greatest (SIDDAL, 1980).

2.1 MORPHOLOGY

The mussel is basically constituted by two parts. An external part, the shells, and an internal part, which constitutes the edible part. The shell is composed of two calcareous asymmetrical valves with broad, rounded ends. In the *Perna perna*, these are black-brown with concentric growing lines. Their internal side is often characterized by a lustrous nacreous or rather pearly lining. Externally, on all mussel species, the byssus, a set of 40-100 threads (each one of 0.1 mm in diameter and 2-4 cm in length), is clearly visible. This anatomical part has adhesive, locomotive and protective functions for the animal. The protein substances from which the byssus is composed are secreted by a set of glands present inside of the foot. These substances act as glue and became insoluble after the contact with sea water (WAITE, 1992a).

Removing shells and byssus, the remaining part, is edible and it is composed of:

- The mantle skirts: The inner lining surface of the two valves, consisting of epithelial tissues that form the chamber called "mantle cavity". While alive, the mantle cavity stays filled with seawater. The two mantle skirts are connected to each other dorsally and are also attached to the valves along the pallial line (MARQUES, 1998). The mantle is thin in young animals and thick in adults, especially when they are in the reproductive season, due to the increasing of the gametes. Its thickness can vary from less of 1 mm to more than 5 mm in cooked mussels. The mantle also has a function in energy storage.

- The gonads: Oppositely to many other bivalves, the gonadal system of mussels is not a single organ. It is composed of a set of genital ducts spread on the entire surface of the mantle, around the abductor muscles and on the visceral mass (MAGALHÃES and FERREIRA, 1997). The color of the gonadal tissue allows the identification of the animal gender. The gonads of a male specimen are creamy beige, while the ones of a female are reddish. (RUPPERT, FOX, and BARNES, 2004). This difference is due to the fact that female *P. perna* stores carotenoids in order to protect developing ova from oxidative damage (LOUDA et al., 2008).

- The muscular system: Mussels need to maintain their muscle tension at a low metabolic cost, to be able to actively hold together the two shells for long time when exposed to tides or dangers. This capacity is given by a special form of muscles composed of large paramyosin filaments. Slow to contract, inelastic and very isotonic, they are capable of maintaining tension for very different lengths of time. Mussels muscular tissue is also composed of smooth invertebrate helical muscles and occasionally, fast-contracting striated muscle. (NARCHI and GALVÃO-BUENO, 1997; MARQUES, 1998).

- The gills (ctenidia): This organ has respiratory and feeding functions. There are two gills or halobranchs, one for each valve that extends throughout the length of the mantle cavity, covering both sides of the visceral mass. The demibranchs hang freely into the mantle cavity and are attached to the body wall on the central axis. Each halobranch consists of two demibranchs, or half gills, one medial and one lateral. Each of the two surfaces of a demibranch is a lamella and it is covered by gills filaments or cilia which move water inside the shells, thus increasing enormously the surface in contact with water, allowing a good gas exchange and water filtration for the selection of nutrients (RUPPERT, FOX, and BARNES, 2004).

- The foot: A motion organ located at the middle of the ventral margin of the visceral mass, it has a role in the formation and the

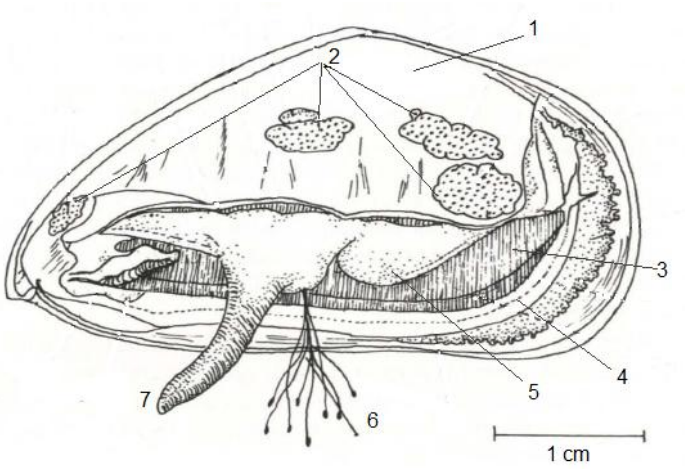
manipulation of the byssal threads. It is constituted of a muscular outer layer of circular muscles shaping around an inner core of longitudinal muscles and also some oblique fibers (SIDDAL, 1980).

- The visceral mass or mesosoma: It is located in the center of the mantle cavity, between the two gills. The tissue of the mesosoma shows the same coloration of the mantle tissue. This anatomical part is important for energy storage and reproduction.

- The digestive apparatus or gut: It is where the digestion of the feed occurs. It is embedded in the dorsal region of the foot and visceral mass.

The Figure 2.1 shows the image of the lateral view of a dissected raw mussel.

Figure 2.1 - Lateral view of a dissected *Perna perna* mussel from the left side. (1) valve internal side; (2) muscular scars; (3) gill ; (4) mantle; (5) visceral mass; (6) byssus; (7) foot.



Source: Modified from Narchi and Galvão-Bueno (1997).

2.2 NUTRITIONAL COMPOSITION

Shelled molluscs constitute an excellent source of many nutrients such as essential amino acids, long chain omega-3 (n-3) fatty acids, minerals (in particular iodine and selenium) and vitamins (A, C, D and B group vitamins). Furthermore, shellfish is a food with a low calorie

intake in comparison with its high nutritional value, and thus indicated in hypocaloric diets (SCHAAFSMA, 2008).

The composition of mussels meat was analyzed by many authors (ORBAN et al., 2002; TAYLOR and SAVAGE, 2006; VERNOCCHI et al., 2007; PARISENTI et al., 2008; FUENTES et al., 2009; GOPALAKRISHNAN and VIJAYAVEL, 2009; FURLAN et al., 2011) presenting a considerable variability in the content of proteins, lipids, carbohydrates and minerals. This variability is also clear in the amino acidic and fatty acids profile. It is justified considering different factors as the season, the geographical area of farming (water temperature, salinity, sea currents, pollution, distance from the coast and rivers), the development stage of the animal, the sex and the specie.

The composition and the caloric value of cooked *Perna perna* mussels, bred in the Brazilian coast was studied by Cordeiro (2005), Parisenti et al. (2008) and Lima (2010) presenting a moisture content variable in between 76 % and the 83 %, a protein content of 7.2 - 19 %, a fat content lower than the 5 % and a high glycogen content, characteristic of this animal, that vary among 1 % and 7 % (Nifext fraction).

Even though the fatty acid composition is extremely variable, particularly among seasons, the predominance of some fatty acids was clearly demonstrated (VERNOCCHI et al., 2007; NARVÁEZ et al., 2008; FUENTES et al., 2009). Among the saturated fraction, the Palmitic acid (16:0) was predominant constituting with the Stearic acid (18:0) more than half of the saturated fatty acids fraction. The most abundant mono-unsaturated fatty acid was the Palmitoleic acid (16:1n-7). The polyunsaturated fraction constitute the 36 - 48 % of the total fatty acid content and the omega-3 fatty acid eicosapentaenoic (EPA; 20:5 n-3) and docosahexaenoic (DHA; 22:6 n-3) are predominant.

Omega-3 polyunsaturated fatty acids are characterized by the first double bond at the third position counted from the methyl end of the molecule. These fatty acids are considered essentials because the human body cannot synthesize them and their ingestion play a crucial role in maintaining health. Essential fatty acids can be divided in two groups: the short chain n-3 fatty acids and the very long-chain n-3 fatty acids. To the first group belongs the alpha-linolenic acid (C18:3 n-3) that is commonly found in vegetable oil. To the second group belong the eicosapentaenoic acid and the docosahexaenoic acid that are commonly found in seafood (BROUWER, 2008).

Many authors have studied the effects of n-3 fish fatty acid on health. In general, n-3 fatty acids have a role in the decreasing of the

incidence of cardiovascular diseases and tumors (BROUWER, 2008). The mussel fatty acids have shown an anti-inflammatory effect on diseases as rheumatoid and osteoarthritis (GIBSON and GIBSON, 1998; TRESCHOW et al., 2007; SINGH et al., 2008; WAKIMOTO et al., 2011) and a positive effect in the treatment of asthma (EMELYANOV et al., 2002).

2.3 PRODUCTION AND COMMERCIALIZATION

The most recent data about the Brazilian aquiculture has shown that in 2009 it represented only the 1.7 % of the world's annual production. Considering only the mussels production (all edible species), Brazil represents only the 0.6 %, when China, Thailand, Spain and Chile are the main world producers (FAO, 2011). Although the Brazilian mussel production does not constitute a high percentage in the global context, it is the second greater producer in Latin America, after Chile (SOUZA et al., 2009).

In Brazil the mussels aquiculture is mainly located in the Southern Region and the state of Santa Catarina is the principal national producer (≈ 95 %). In the last twenty years the production has had an extraordinary development passing from the 190 t produced in 1990 to the 21027 t produced in 2012. Considering the mean price paid to the producers, the association EPAGRI has estimated that in 2012 the economic value of mussel production was about R$ 31,330,230.00 (EPAGRI, 2013).

The production of mussels is particularly advantageous wherever the geographic and climatologic conditions are favorable to an economic and a rapid start up and a good growth rate of the animals. The mussels are organisms that feed by filtration of seawater, not requiring further nutrients and extended surfaces for their production. Furthermore, since the production is carried out in an open environment, subjected to a continuous and natural action of marine currents, the diseases are not much common (MAGALHÃES and FERREIRA, 1997).

Another advantage of mussels farming is the easiness of the reproduction. Even if recommended, the production of so called mussel seeds in laboratory is not strictly necessary. Actually, it is possible to collect the young mussels directly in the sea, just with some particular but simple devices. Moreover, it is important to remark that the mussels belonging to the *Perna perna* genus are particularly well adapted to the environmental condition of the Brazilian coast (Santa Catarina bay,

especially), showing a good growing rate if compared with mussels farmed in other countries (MAGALHÃES and FERREIRA, 1997).

2.3.1 Production technology

After the harvest, from the sea structures (farming holding support), the mussels are submitted to some operations, prior to the commercialization. The mussels' production process used in small and medium companies is detailed as following (HUBER, 2004).

The mussels are mechanically separated from the farming holding support and are washed for the first time. Then, they are moved to the plant, where another and more careful cleaning is done. Then, raw mussels are cooked with steam, for different times depending upon the equipment used and the amount and size of the product. This operation has the objective of coagulating the protein constituent the mussel meat and opening the valves to facilitate the separation of the meat from the shells. This operation also allows a strong decrease of the natural and contaminant microbiota. Mussels can be also commercialized raw without any cooking operation. The Figure 2.2 shows the image of both raw and cooked mussel.

Figure 2.2 - *Perna perna* mussels. Raw (a); raw opened (b); cooked male (c); cooked female (d).

Source: Author.

After cooking, the mussels are cooled by immersion in cold water and then, the meat is manually or mechanically removed from the shells. Thus, the mussels meat can be refrigerated, frozen, packaged in modified atmosphere or submitted to others processes.

In Brazil, the most diffused mussel products are the frozen and the refrigerated and packaged in modified atmosphere mussel meat. Contrary to others countries, the mussels commercialized alive are not strongly present in the Brazilian market, principally because of

problems in the distribution chain combined with the extremely rapid perishableness of this seafood. Furthermore, on the market is possible to find the mussel meat as smoked, canned with sauces or oil and semi-preserved (pasteurized, marinated, etc.) products. Few of these products are currently present on the Brazilian market, mainly because of the lack of habit of the Brazilian people to consume canned and semi-preserved high price products and to the technology required to process this kind of products.

2.4 REFERENCES

BROUWER, I. A. Fish, omega-3 fatty acids and heart disease. In: T. Borresen, **Improving seafood products for the** consumer (pp. 165-166). Cambridge, UK: Woodhead Publishing Limited, 2008.

CORDEIRO, D. **Qualidade do mexilhão Perna perna submetido ao processo combinado de cocção, congelamento e armazenamento.** Piracicaba, Brazil: Escola Superior de Agricultura Luiz de Queiroz – USP, 2005.

DAILIANIS, S. Environmental impact of Anthropogenic Activities: the use of musselas a relable tool for monitoring marine pollution. In: **Mussel: anatomy, habitat and environmental impact.** (pp. 43-72). New York: Lauren e McGevin, 2011.

EMELYANOV, A., FEDOSEEV, G., KRASNOSCHEKOVA, O., ABULIMITY, A., TRENDELEVA, T., and BARNES, P. Treatment of asthma with lipid extract of New Zealand green-lipped mussel: a randomized clinical trial. **European Respiratory Journal**, 20, 596-600, 2002.

EPAGRI – Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina - Centro de Desenvolvimento em Aqüicultura e Pesca. SANTOS, A. A., NOVAES, A. L. T., SILVA, F. M., SOUZA, R.V., COSTA, S.W., GUZENSKI, J. **Síntese Informativa da Maricultura**. Retrieved 30/08/2013 from www.epagri.sc.gov.br, 2012.

FDA **Food and Drug Administration**. Retrieved 12/08/2013 from www.fao.org, 2011.

FUENTES, A., FERNÁNDEZ-SEGOVIA, I., ESCRICHE, I., and SERRA, J. Comparison of physico-chemical parameters and composition of mussels (Mytilus galloprovincialis Lmk.) from different Spanish origins. **Food Chemistry**, 112(2), 295-302, 2009.

FURLAN, E., GALVÃO, J., SALÁN, E., and OETTERER, M. Composição centesimal e valor calórico de mexilhões Perna perna cultivados no litoral norte de São Paulo, **Brasil. Bol. Inst. Pesca**, 37(1), 85-93, 2011.

GIBSON, S., and GIBSON, R. The treatment of arthritis with a lipid extract of Perna canaliculus: a randomized trial. **Complementary Therapies in Medicine**, 6, 122-126, 1998.

GOPALAKRISHNAN, S., and VIJAYAVEL, K. Nutritional composition of three estuarine bivalve mussels, Perna viridis, Donax cuneatus and Meretrix meretrix. **International Journal of Food Sciences and Nutrition**, 60(6), 458-463, 2009.

HUBER, E. **Resfriamento a vácuo de cortes de carnes após o cozimento**. Dissertation, Universidade Federal de Santa Catarina, Florianópolis, 2004.

LIMA, M. **Avaliação das condições de processamento de mexilhões Perna perna pré-cozidos e resfriados**. Florianopolis: Universidade Federal de Santa Catarina, 2010.

LOUDA, J., NETO, R., MAGALHAES, A., and SCHNEIDER, V. Pigment alterations in the brown mussel Perna perna. **Comparative Biochemistry and Physiology**, Part B , 150, 385-394, 2008.

MAGALHÃES, A. R., and FERREIRA, J. **Mexilhões: biologia e cultivo.** Universidade Federal de Santa Catarina, Florianópolis, 1997.

MARQUES, H. L. **Criação comercial de mexilhões.** São Paulo, Brazil, 1998.

NARCHI, W., and GALVÃO-BUENO, M. S. Anatomia funcional de *Perna perna (Linné)* (Bivalvia, Mytioidae). **Revta bras. Zol.**, 14(1), 135-168, 1997.

NARVÁEZ, M., FREITES, L., GUEVARA, M., MENDOZA, J., and GUDERLEY, H. Food availability and reproduction affects lipid and fatty acid composition of the brown mussel, Perna perna, raised in suspension culture. **Comparative Biochemistry and Physiology**, Part B (149), 293-302, 2008.

ORBAN, E., DI LENA, G., NEVIGATO, T., CASINI, I., MARZETTI, A., and CAPRONI, R. Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites. **Food Chemistry**, 1(77), 57-65, 2002.

PARISENTI, J., TRAMONTE, V., and FACCIN, G. Composição centesimal de mexilhõesPerna perna, crus e cozidos, coletados emdiferentes estações do ano na cidade de Florianópolis/SC. **Revista Higiene Alimentar**, 22(159), 84-88, 2008.

RUPPERT, E., FOX, R., and BARNES, R. **Invertebrate Zoology, A functional evolutionary approach**, 7th ed. Belmont CA: Brooks Cole Thomson, 2004.

SCHAAFSMA, G. Introduction to Part II: health benefits of seafood. In: T. Borresen, **Improving seafood products for the consumer** (pp. 113-115). Cambridge, UK: Woodhead Publishing Limited, 2008.

SIDDAL, S. E. A clarification of the genus perna (*mytilidae*). **Bulletin of marine science**, 30(4), 858-870, 1980.

SINGH, M., HODGES, L., WRIGHT, P., CHEAH, D., WYNNE, P., KALAFATIS, N., et al. The CO(2)-SFE crude lipid extract and the free fatty acid extract from *Perna canaliculus* have anti-inflammatory effects on adjuvant-induced arthritis in rats. **Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology**, 2, 251-258, 2008.

SOUZA, R. V., NOVAES, A. L., SANTOS, A. A., ROPP, G. S., and SILVA, F. M. Controle Higiênico-sanitário de moluscos bivalves. **Panorama da Aqüicultura**, 54-59, 2009.

TAYLOR, A., and SAVAGE, C. Fatty acid composition of New Zealand green-lipped mussels, *Perna canaliculus:* Implications for harvesting for n-3 extracts. **Aquaculture**, 261(1), 430-439, 2006.

TRESCHOW, A., HODGES, L., WRIGHT, P., WYNNE, P., KALAFATIS, N., and MACRIDES, T. Novel anti-inflammatory omega-3 PUFAs from the New Zealand green-lipped mussel, *Perna canaliculus*. **Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology**, 147(4), 645-656, 2007.

VERNOCCHI, P., MAFFEI, M., LANCIOTTI, R., SUZZI, F., and GIARDINI, F. Characterization of Mediterranean mussels (Mytilus galloprovincialis) harvested in Adriatic Sea (Italy). **Food Control**, 18(12), 1575-1583, 2007.

WAITE, J. H. The formation of mussel byssus: anatomy of a natural manufacturing process. **Results Probl. Cell Differ.**, 19, 27-54, 1992a.

WAKIMOTO, T., KONDO, H., NII, H., KIMURA, K., EGAMI, Y., OKA, Y., et al. Furan fatty acid as an anti-inflammatory component from the green-lipped mussel Perna canaliculus. **Proceedings of the National Academy of Sciences of the U.S.A.**, 108(42), 17533-17537, 2011.

3. MASS TRANSFER DURING SALTING AND MARINATION PROCESSES OF PRE-COOKED MUSSEL MEAT

3.1 INTRODUCTION

Salting is one of the oldest fish preservation methods. It consists of treating foods with a salted solution or dry salt in order to increase the salt concentration of the final product and reduce its water activity, leading to an increased shelf life. In fact, the low water activity and the high salt concentration have an inhibitory action on the microorganism growth. The shelf life of the salted product can be increased combining the salting operation with other treatments as marination, smoking and drying (CHIRALT et al., 2001). The marination process consists in immersing foods in solutions of wine, vinegar, organic acids, fruit juices or pulps or plant extracts, called marinades. Industrially, the marinades are commonly a mixture of water, weak acids (lactic, acetic and citric) and salt (YUSOP, KERRY, and KERRY, 2010). Marinated products are stable for medium/long periods in refrigerated conditions.

The treatment of foods in brine and marinades involves the mass transfer of water, salt, and acid between the product and the solution. The direction of the mass transfer is affected by different factors. The most important is the difference between the concentration of the solutes in the liquid phase of the product and the concentration of the soaking solution. The direction of the mass transfer is also conditioned by other factors as proximate composition, microstructure, size and shape of the product (BARBOSA-CANOVAS and VEGA-MERCADO, 1996). The knowledge of the transport phenomena represents a very important link between the food process and the final quality and safety of the processed foods and is essential to a good process design.

In this context, the objective of the study presented in this chapter was the evaluation of the mass transfer phenomena during salting and marination of pre-cooked mussel meat. The specific objectives were to:

- Study the effect of brines with various concentrations of salt and acetic acid on the parameters of salt gain, water gain, mass gain, and changes of water activity and pH of pre-cooked mussel meat during processing;
- Evaluate the water holding capacity of mussels treated in brines and marinades with various concentrations of salt and acetic acid;
- Evaluate the microstructure of the mantle of treated mussels;
- Investigate the applicability of different empirical models (Peleg's model, the Weibull type model and the Zugarrumurdi and Lupin

model) on the prediction of water gain/loss and salt gain of cooked mussels immersed in solutions with different salt and acetic acid concentrations.

3.2 LITERATURE REVIEW

3.2.1 Salting

Salting of foods is one of the oldest preservation methods and together with drying has been used for thousands years to obtain preserved products, thus allowing the access to safe and nutritious foods in seasons or areas where its availability was limited (MARTINEZ-ALVAREZ and GOMEZ-GUILLEN, 2005; LEROI et al., 2008). Nowadays, the demand for salted products has changed. In fact, they are commercialized rather for the improved sensorial quality (taste and aroma) than for safety reasons (MARTINEZ-ALVAREZ and GOMEZ-GUILLEN, 2005). The preservative effect of salting is basically provided by the reduction of water activity that improves the microbiological and physicochemical stability of the final product. This preservative effect can be increased following the salting operation with further processes as fermentation, smoking, acidification and drying (CHIRALT et al., 2001).

There are three salting methods used for meat and fish products, which are classified basing on the procedure by which the salt is added to the product: the dry salting, for which the food is covered by an excess of dry salt and the exudate is constantly removed; the brine salting, where the product is immersed in a salted solution (brine); and a combination of both dry and brine salting, in which the food is covered by salt and put in containers where the exudate remains in contact with the product (BELLAGHA et al., 2007; FUENTES, 2007).

Collignan et al. (2001) define as osmotic treatment the traditional processes where the product is placed in contact with a concentrated solution containing osmotic agents as salts, sugars, phosphates, and acids, among others. Normally, when the product is immersed in dilute solutions, water and solutes are incorporated in the processed food, causing an osmotic hydration (LEMOS, NUNES, and VIANA, 1999). On the other hand, when the product is immersed in a concentrate solution the water present in the food is partially removed and part of the solutes is incorporated in the food, leading to a phenomenon called osmotic dehydration (SCHMIDT, CARCIOFI, and LAURINDO, 2008b; COLLIGNAN et al, 2001; FITO, 1994). The direction of the mass

transfer of water and solutes from a product immersed in an osmotic solution is controlled principally by the concentration of the solution (SCHMIDT, CARCIOFI, and LAURINDO, 2008b), but also by composition, microstucture, size and shape of the product, and mass ratio, temperature and stirring intensity of the solution (FITO, 1994; BOUDHRIOUA et al., 2009; BELLAGHA and KECHAOU, 2009; CZERNER and YEANNES, 2012)

The salting process of seafood has been widely studied, particularly for fishes that are traditionally commercialized salted as cod (BARAT et al., 2002; LAURITZSEN et al., 2004; BARAT et al., 2006; NGUYEN et al., 2010; among others), salmon (SIGURGISLADOTTIR et al., 2000; GALLART-JORNET et al., 2007a; GALLART-JORNET et al., 2007b), sardine (CORZO and BRACHO, 2006; BOUDHRIOUA et al.; 2009), anchovy (CZERNER and YEANNES, 2012) and seabass (FUENTES et al., 2008).

On the other hand, no many studies regarding salting of shellfish were found in literature. Turan et al. (2007) discussed the effects of brine and dry salting on mussel meat chemical and sensorial quality, stored at 4 ± 1 °C for 4 months. The concentration of the brine used in that study was 26.4 g NaCl/100 mL. In dry salting, the ratio 4:1 (sample weight/NaCl weight) was used. The salt content of the samples raised from 0,87 % before the treatment to 21,01 % after the brine salting and 25.26 % after the dry salting. The authors concluded that the salting method influenced the sensorial evaluation and the total volatile basic nitrogen content of the final product. The salted mussel could be consumed for a period of 4 months of refrigerated storage.

Cavalheiro (2010) studied the mass transfer kinetics of vacuum cooled pre-cooked mussel meat immersed in brines with NaCl concentration between 5 and 20 % (g NaCl/100 g brine). The author was observed that the salt concentrations of 5 and 10 % caused the highest water gains, recovering some of the water lost during cooking-cooling process.

3.2.2 Marination

The marination process consists in immersing foods in solutions of salt, sugar, spices, oil and organic acids also called marinades. Marinated meat and fish products are widely consumed worldwide, because of their convenience, taste and extended shelf life (BJORKROTH, 2005). The preserving capacity of marination involves the simultaneous action of salt and organic acids that, increasing the

ionic strength and decreasing the pH of the treated product, limits the growth of pathogenic bacteria and spoilage bacteria (KILINC and CAKLI, 2004; BJORKROTH, 2005). The aim of marination is not only the microbiological stability but also the enhancement of the product quality (LEMOS, NUNES, and VIANA, 1999; GÖKOĞLU, CENGIZ, and YERLIKAYA, 2004). Indeed, this process improves the tenderness, juiciness, flavour and aroma of meat, poultry, seafood and also vegetables (CADUN, CAKLI, and KISLA, 2005; GOLI et al., 2011)

Small sized foods as meat cubes, small fish filets and shellfish usually are processed using the still-marination method in which the product is immersed in a marinade until the required pH and solute concentration are reached (GOLI et al., 2011). On the other hand, for products with larger size other techniques, such as injection and tumbling are commonly used (LEMOS, NUNES, and VIANA, 1999).

The positive effect on the shelf life extension of salted and marinated seafood has been clearly demonstrated with shrimp (CADUN, CAKLI, and KISLA, 2005), warty venus (KILINC et al., 2008) anchovies (POLIGNE and COLLIGNAN, 2000), sardine (GÖKOĞLU, CENGIZ, and YERLIKAYA, 2004), and saury (SALLAM et al., 2007).

Aveiro et al. (2008) studied the effect of the marination treatment on the microbiological, physicochemical and sensorial quality of pre-cooked mussel meat (*Perna perna*). The experimental data analysis demonstrated that marinated mussel meat was stable on storage at 4 °C for 50 days. The sensorial tests showed how the appearance, taste, texture and odour remained stables during storage. The authors affirm that the marination process is able to retard microbial growth and physicochemical changes, extending the shelf-life of the mussel meat.

3.2.3 Equilibrium relations during the osmotic treatment of food

During the osmotic treatment, the difference of chemical potential of a solute (μ) between the product and the soaking solution represents the mass transfer driving force. This driving force is directly related to the difference between the solute concentration in product liquid phase and in the external solution. It is also related to the microscopic pressure gradients, to the capillary forces and to the molecular interactions (BARBOSA-CANOVAS and VEGA-MERCADO, 1996).

The thermodynamic equilibrium will be achieved when the chemical potential of the solute in the product is equal to the chemical potential in the solution (BARBOSA-CANOVAS and VEGA-

MERCADO, 1996). The equilibrium of the water flux will be achieved when the chemical potential is the same in the water in the osmotic solution and in the liquid phase of the product (LP) (Equation 3.1)

$$\mu_w|_{LP} = \mu_w|_{solution} \quad (3.1)$$

The water chemical potential (μ_w) in the liquid phase of a porous medium at a certain temperature (Equation 3.2) is the sum of the contributions of the potential related to the solute (osmotic potential, $RTlna_w$), of the pressure potential ($\bar{V}p$) and of the medium matrix potential related to water (interaction among the water and the matrix, through the capillary forces, sorption and electrical forces, $\bar{V}\Psi$) (FITO and CHIRALT, 1997; REICHARDT, 1985):

$$\mu_w = RTlna_w + \bar{V}p + \bar{V}\Psi \quad (3.2)$$

in which R is the ideal gas constant, T is the system absolute temperature, a_w is the water activity, $\bar{V}$ is the partial molar volume of the water, p is the system pressure and Ψ is the matrix potential.

When the osmotic treatment is carried out at atmospheric pressure there is not a pressure gradient and the matrix potential of the product is function of the attraction forces and capillarity (SCHMIDT, 2006).

3.2.4 Study of the of mass transfer rates

The knowledge of the mass transfer rates during salting and marination is of great importance. In fact, estimating the exact amount of solutes and water in the final product is important to optimize the process to obtain a product with desired quality and safety properties (SCHMIDT, CARCIOFI, and LAURINDO, 2008b).

Goli et al. (2011) studied the mass transfer phenomena in turkey breast meat cubes soaked in marinades with various concentrations of acetic acid (0 - 5.6 % (w/w)) and NaCl (0 - 8 % (w/w)). The mass, water, salt, and acid gain were determined as well as the velocities of water, salt and acid diffusion. The presence of acid in the marinade of NaCl had a negative influence on the mass gain, since the acetic acid showed a positive effect on the mass gain of turkey meat cubes. The salt gain was widely influenced by the marinade salt concentration and by

the process time. The acid gain was negatively influenced by salt proportionally to its concentration in the marinade.

Nguyen et al. (2010) evaluated the effect of brines with different salt concentrations (6, 15, 18 and 24 % (w/w)) on the mass transfer phenomena during brine salting of cod loin. The increase of brine concentration increased the water loss and the salt gain. On the other hand, the mass gain increased with the reduction of the salt concentration.

The diffusion of NaCl and acetic acid in anchovy fillets was investigated by Casales, Capaccioni and Yeannes (2009). The equilibrium times and diffusion coefficients of acid and NaCl were studied to optimize the marination process of anchovy fillets. The changes on the marinade solute concentration were avoided establishing a ratio of 10:1 (marinade weight/product weight). The marination solution was composed of 3 % of acetic acid and 10 % w/v of NaCl with an initial pH of 2.5. The effect of the stirring of the external solution was evaluated. The acetic acid and the NaCl uptake followed the Flik's law only when the solution was stirred. Agitation during marination did not reduce the equilibrium times or changed the final water and salt concentrations.

3.2.4.1 Modeling of the mass transfer

The classical mathematical models used to describe the mass transfer during the osmotic treatment are based on the diffusion equation and on the use of apparent diffusion coefficients. These equations have analytical solution only for classical geometries. On the other hand, simple empirical models that have no geometric restrictions for their application have been reported for describing mass transfer in osmotically treated foods (CHIRALT et al., 2001; MUJAFFAR and SANKAT, 2003; CORZO and BRACHO, 2006; VOLPATO et al., 2007; SCHMIDT, CARCIOFI, and LAURINDO, 2008a; among others).

Peleg (1988) used a two parameters empirical model to represent the water adsorption of milk powder and whole rice grains. This equation was used to describe the kinetics of sorption that approaches equilibrium asymptotically. This model has been widely used to describe sorption and desorption curves of osmotically treated vegetables (SOPADE, AJISEGIRIB, and BADAU, 1992; ABU-GHANNAM, and MCKENNA, 1997; TURHAN, SAYAR, and GUNASEKARAN, 2002; MASKAN, 2002; GARCÍA-PASCUAL et

al., 2006; JIDEANI, and MPOTOKWANA, 2009), fruits (SACCHETTI, GIANOTTI, and DALLA ROSA, 2001), meat (SCHMIDT, CARCIOFI, and LAURINDO, 2009; CORZO, BRACHO, and RODRÍGUEZ, 2012) and fishes (SOBUKOLA and OLATUNDE, 2001; CORZO and BRACHO, 2006; CORZO et al., 2007; CZERNER and YEANNES, 2012). Although in the literature there is a lack about the informations referent to the application of the Peleg model to describe kinetics of mass transfer of osmotic treated meat and fishes, it is demonstred that this model is usefull to represent the kinetics of water and salt gain as well as to estimate a "equilibrium" moisture content (CORZO et al., 2007; SCHMIDT, CARCIOFI, and LAURINDO, 2009).

In 1980, Zugarramurdi and Lupın proposed a two parameter exponential model, which allows estimating equilibrium values of water and salt contents. This model was specifically developed to describe the mass transfer rates during the salting process of fish. The authors checked the model prediction capacity with experimental data of various fish species as anchovy, haddock and sardine and with published data of other fish species. This model has been also used in other papers to describe kinetics of salt and water gain in other fishes as cod (ESCRICHE et al., 1998) and sardine sheets (CORZO and BRACHO, 2005).

The three parameter exponential model based on the Weibull type equation has been recently used to represent sorption and desorption curves of foods, demonstring that this model can represent well the moisture and salt content of foods inmersed in osmotic solutions (DENG and ZHAO, 2008; CORZO and BRACHO, 2008; SCHMIDT, CARCIOFI, and LAURINDO, 2009).

3.3 MATERIAL AND METHODS

3.3.1 Sample preparation

Live mussels (*Perna perna)* were purchased from aquaculture farms on the coast of Santa Catarina (Brazil). Fresh samples were rapidly transported to the laboratory in polystyrene boxes with ice. At the laboratory, the live mussels were stored in a refrigerator at temperature of 4 ± 1 °C before sample preparation.

Batches of 2 kg of live mussels were cleaned from superficial incrustations and cooked for 5 minutes in steam at 100 °C at atmosphere pressure. After cooking, the mussels were cooled by immersion in a water and ice mixture for 5 minutes, enough to allow the cooling of the mussels meat to 8 ± 2 °C. Then, the mussel meat was carefully separated from the shells with a knife, preserving its integrity. The byssus was also removed, cutting at its base.

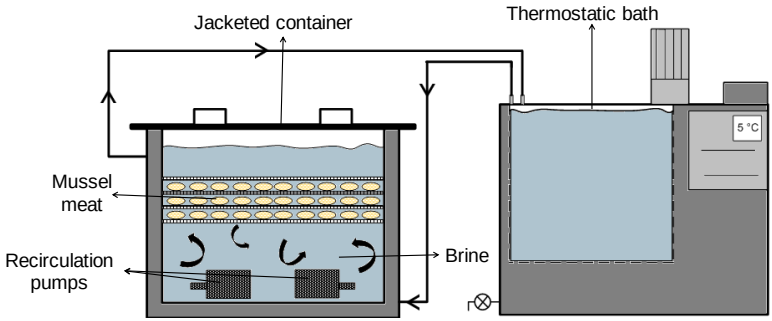
Cooked-cooled mussel meat sample, with weight of 10 ± 3 g, were transferred to 42 pouches, each one with 32 mussels. All pouches were quickly deep frozen (-80 °C) and stored at -18 °C, aiming to obtain samples with uniform characteristics. Twenty-four hours before the experiment, a batch of samples (one pouch) was slowly defrosted in a refrigerator.

The solutions (brines and marinades) were prepared from commercial sodium chloride, acetic acid ≥ 99 % (Sigma-Aldrich Co., USA), and distilled water. The concentration of NaCl and acetic acid (CH_3COOH) was expressed in percentage (%) [(solute weight / solution weight)·100]. A mass ratio of 1:20 (w/w) of pre-cooked mussel meat and solution was used in all experiments in order to avoid significant changes of salt and acid concentration during the processing.

3.3.2 Experimental setup

The experiments of salting and marination of cooked mussels were performed with the device showed in Figure 3.1. This device consists in a jacketed container (with internal volume of 14.4 L) with the temperature controlled by water at constant temperature, circulated through a thermostatic bath (Quimis, model Q214M2, Brazil). The solution stirring was performed by two immersed pumps (Atman, model AT-10 with flow rate of 1200 $L.h^{-1}$) that circulated internally the brine during experiments.

Figure 3.1 - Salting and marination experimental device sketch.



Source: Author.

3.3.3 Experimental methods

The effect of different brine concentrations (C_s = 0 (only water), 5, 10, 15, 20, and 26 % of NaCl) on the behaviour of pre-cooked mussel meat submitted to the salting process was evaluated. In the marination process, two effects on the behaviour of pre-cooked mussel meat were evaluated. The first one was the influence of the marinade acid concentration, which was studied using three concentration of acetic acid (C_{ma} = 0.5, 2, and 6 %) and fixing the marinade salt concentration (C_{ms} = 15 %). The second effect evaluated was the influence of the presence of acetic acid (C_{ma} = 2 %) at different salt concentrations (C_{ms} = 5, 10, 15, and 20 %).

For all evaluated conditions, the pre-cooked mussel samples were weighed individually, identified and put in nylon net bags (each one with four samples). Seven net bags were immersed in the stirred osmotic solution (brine or marinade) at 5 ± 0.5 °C inside the jacketed container. For predefined immersion times (0.5, 1, 2, 3, 4, 6, and 24 hours), one nylon net bag was withdrawn from the osmotic solution. Then, the mussels were placed in a metal sifter for five minutes (to remove the excess of solution on their surface), weighed individually, and prepared for the analyses described in the sections 3.3.4. All the experiments of salting and marination of cooked mussels meat were performed in triplicate.

3.3.4 Analytical determinations

3.3.4.1 Moisture content

The moisture content of treated samples was determined in triplicate, according to the gravimetric method (AOAC, 2000). The sample weighting was done with an electronic balance (Shimadzu, model AY220, Japan).

3.3.4.2 Water activity

Water activity of treated mussels was determined in triplicate with a hygrometer (Aqualab model Series 3, Decagon Devices Inc., Pullman, USA).

3.3.4.3 pH

The pH was measured using a pH meter (Quimis, model Q400A, Brazil). Treated mussels were mixed with distillated water in proportion 1:1 (w/w) and minced. The pH was measured directly on the formed solution (AOAC, 1995). The pH electrode was calibrated with standard buffers at pH 4.0 and pH 7.0 prior to its use. All measurements were made on triplicate.

3.3.4.4 Sodium chloride analysis

The sodium chloride content was determined with a chloride analyzer (Cole Parmer, model MKII Chloride Analyzer, USA). Two grams of sample were homogenized in distillated water, using an Ultra-Turrax (Ika Laboratory and Analytical Equipment, Ika T25, Germany) at 12000 rpm for 3 min. The solution was made up at 100 mL with distilled water and centrifuged at 9055 g for 10 minutes at 20 °C (SIGMA Laborzentrifugen GmbH, model SIGMA 4-16K, Germany). An aliquot of 500 µL of supernatant liquid was analyzed in the chloride analyzer. The system gives the value of mg of Cl^-/L of solution. Starting with this value it was possible to calculate the NaCl concentration (Equation 3.3)

$$\%NaCl = \frac{IM \cdot V \cdot 55.453}{m \cdot 35.453 \cdot 1000} \quad (3.3)$$

in which *IM* is the measure provided by the equipment, *V* is the volume of the solution of water and mussel (0.1 L) and *m* is the sample mass used to make the solution.

3.3.4.5 Acid content – titratable acidity

In measuring titratable acidity, the amount of acid that is capable of reacting with a known amount of base is determined. The acid content was determined by NaOH (0.05 M) titration (the NaOH solution was standardized with potassium hydrogen phthalate). Five grams of mussel were homogenized in 50 mL of distillated water, using an Ultra-Turrax (Ika Laboratory and Analytical Equipment, Ika T25, Germany). This solution was titrated using a pH meter up to the endpoint at pH 8.2. All determination was performed in triplicate. The result was corrected considering the acidity of the blank solution (50 mL of distilled water).The results were expressed in g of acetic acid/100 g of sample and calculated as follows (Equation 3.4).

$$\%Acid = \frac{M \cdot f \cdot V \cdot eqW}{sW} \cdot 100 \quad (3.4)$$

in which the value $M \cdot f$ is the real molarity of the NaOH solution, *V* the volume of the NaOH solution used in the titration, *eqW* is the equivalent weigh of the acetic acid (60.05) and *sW* is the sample weigh.

3.3.4.6 Water-holding capacity (WHC)

The WHC was determined in pre-cooked mussel meat after 1, 2, 3, and 4 h of immersion in various brine concentrations. Mussels were withdrawn from the brine and coarsely chopped (to avoid individual differences among mussels). Then, five grams of sample were wrapped in filter paper and transferred to 50 mL falcon tubes, which bottoms were filled with cotton wool, and centrifuged (SIGMA Laborzentrifugen GmbH, model SIGMA 4-16K, Germany) at 465 g for 10 min at 4 °C. The WHC was estimated as liquid retention (LL) and expressed as percentage of weight of liquid retained (Equation 3.5) (OFSTAD et al., 1993).

$$WHC\ (g\ H_2O/g\ dry\ matter) = \frac{(w_b * x_w) - (w_b - w_a)}{w_b(1 - x_w)} \quad (3.5)$$

in which w_b and w_a are the weight of samples before and after centrifugation, respectively. x_w is the sample moisture content (g water / g sample) before centrifugation. Mean values were calculated from 6 replicates for each experimental point.

3.3.4.7 Microstructure Examination

Untreated samples and samples obtained after 4 hour of treatment in brines with C_s of 5 and 20 % and 4 hours of treatment marinades with C_{ms} of 5 % and C_{ma} 2 % were analyzed. The specimens were obtained cutting small sections of the mussel mantle using a chirurgical knife. Specimens were freeze dried for 24 hours to remove all the residual moisture. Then, the mussel mantle sections were mounted on aluminum planchets and coated with gold in an anion-sputtering apparatus (LEICA, model EM SCD500, Germany) and finally examined with a JEOL JSM 6390LV (Japan) scanning electron microscope, operating at 10 kV. The microstructure analyses were performed in the Electronic Microscopy Central Laboratory (LCME), UFSC.

3.3.5 Process parameters

The mass transfer that took place between mussels and brines was studied determining the values of salt gain (SG), mass gain (MG), and water gain (WG) that were calculated as follows (Equations 3.6, 3.7, 3.8).

$$SG = \frac{W_{si} - W_{s0}}{m_0} \cdot 100 \quad (3.6)$$

$$MG = \frac{m_i - m_0}{m_0} \cdot 100 \quad (3.7)$$

$$WG = \frac{W_{wi} - W_{w0}}{m_0} \cdot 100 \quad (3.8)$$

in which W_{si} is the sample NaCl content at the time t, W_{si} is the initial NaCl content, m_i is the sample mass at the time t, m_0 initial mussel mass, W_{wi} is the water content at the time t and W_{w0} is the initial water content. In marinated samples the W_w was deduced (Equation 3.9) considering that the acetic acid is volatilized during the drying operation of the moisture determination method (GOLI et al., 2011).

$$W_w = 1 - W_{dm} - W_a \quad (3.9)$$

in which W_a is the mass of acetic acid and W_{dm} is the mass dry matter.

3.3.5.1 Mathematical modeling of salt and water gain

The fitting of the three models to the experimental data of salt gain and water gain/loss of pre-cooked mussel meat during salting and marination was performed using the software MATLAB® R2007a (Math Works Inc., Natick, MA, USA). The adaptation of the Peleg (1988) mathematical model used in the present work is given by the following equation (Equation 3.10).

$$XG_i = \pm \frac{t}{k_1 + k_2 t} \quad (3.10)$$

in which XG_i is the salt or the water gain at the time *t*.

When the model was used to describe the salt gain the sign of the Equation 3.10 becomes positive, considering that the salt gain is always positive. On the other hand, when the model was used to describe the water gain the sing ‘‘±” became ‘‘+” if the process was the hydration and ‘‘-” if the process was the dehydration.

According to this model, the reciprocal value of k_1 is the initial rate (t = 0) of the *XG* (Equation 3.11).

$$\left.\frac{d(XG)}{dt}\right|_{t=0} = \pm\frac{1}{k_1} \quad (3.11)$$

The reciprocal value of k_2 (Equation 3.12) allows the determination of the salt concentration at equilibrium (XG^{∞})($t \rightarrow \infty$).

$$XG_i^{\infty} = \pm\frac{1}{k_2} \quad (3.12)$$

An exponential model with three parameters has been used for describing water and salt gain during treatments. This model is a Weibull-type model and is given by Equation 3.13 (CUNHA et al., 2001).

$$\frac{XG_i}{XG_i^{\infty}} = 1 - exp\left[-\left(\frac{t}{\beta}\right)^{\alpha}\right] \quad (3.13)$$

in which β is a shape parameter that is a behavioural index, α is a scale parameter that is associated with the process rate (the time required for XG_i/XG^{∞} to reach a value of $1\text{-}e^{-1}$).

The mathematical model proposed by Zugarramurdi and Lupín (1980), is described hereafter by the Equation 3.14.

$$\frac{dXG_i}{dt} = -k_i(XG_i^{\infty} - XG_i) \tag{3.14}$$

in which k_i [$(g/g_{db}).h^{-2}$] is the specific rate constant for the variation of XG_i. The integration of the Equation 3.14 with the initial condition $XG_i(0) = XG_i$, results in the Equation 3.15:

$$XG_i = XG_i^0(e^{-k_i t}) + XG^{\infty}(1 - e^{-k_i t}) \tag{3.15}$$

3.3.5.2 Statistical analysis

For each studied case, the goodness of fit was determined the determination coefficient (R^2) and the root mean square error (RMSE, Equation 3.16).

$$RMSE = \frac{1}{n}\left[\sum_n \left(SG_{predicted} - SG_{exp}\right)^2\right]^{0.5} \tag{3.16}$$

The variance analysis (one-way ANOVA) with probability of 90 % was performed using the software Statistica® (Statistica 8.0, StatSoft, USA). In case of significant effects ($p < 0.1$) the means were compared using the Tukey test.

3.4 RESULTS AND DISCUSSION

3.4.1 Raw material

The values of pH, water activity, moisture content, salt content, titratable acidity and water holding capacity of the pre-cooked mussel meat used in the present study are shown in Table 3.1.

Table 3.1 - Physicochemical properties of pre-cooked mussel meat.

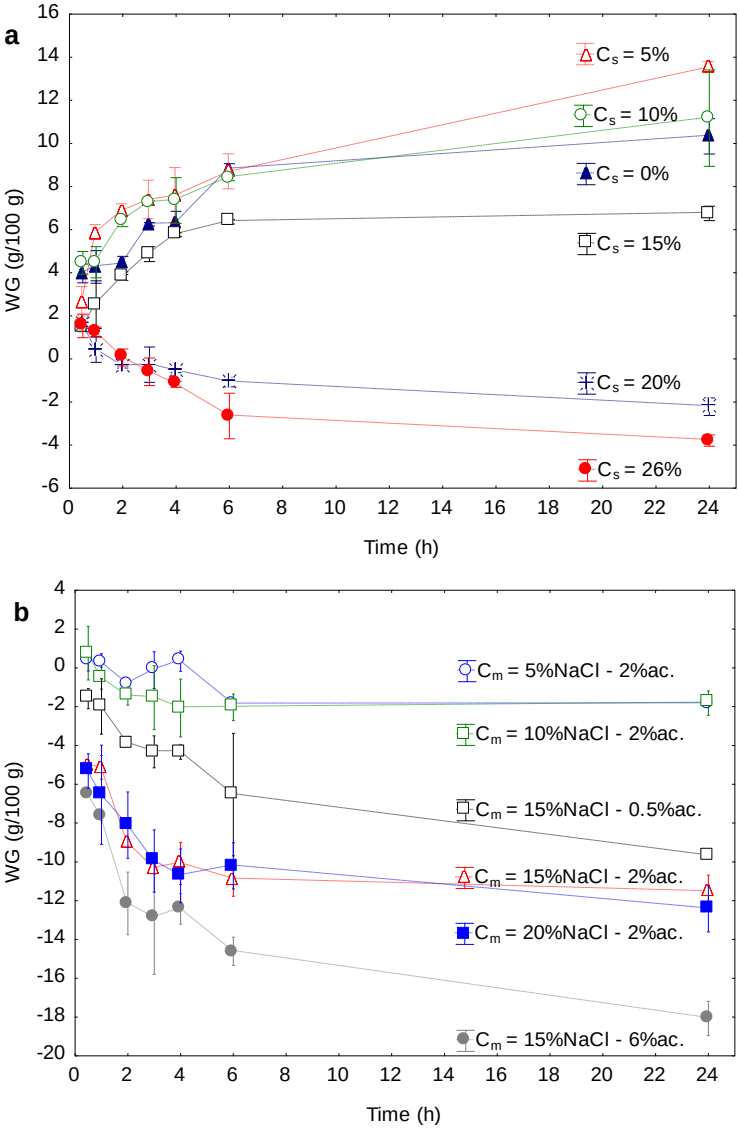
	Values* Mean ± standard deviation
pH	6.63 ± 0.10
Water activity (a_w)	0.994 ± 0.003
Moisture content [g/100 g]	76.9 ± 1.02
Salt content [g/100 g]	1.08 ± 0.09
Titratable acidity [g/100 g]	0.2 ± 0.05
WHC (g H_2O/ g dry matter)	2.21 ± 0.15

The a_w of the solutions used was of 0.975, 0.943, 0.899, 0.842, and 0.771 respectively for the brines with C_s of 5, 10, 15, 20, and 26 % of NaCl and the addition of acetic acid did not affect significantly the a_w of the solutions.

3.4.2 Kinetics of the water gain

The effect of different salt concentrations (C_s) of the brine on the kinetic of WG of mussel meat is given in Figure 3.2a. The WG was greatly influenced by the C_s and by the duration of the salting process. Samples treated in solutions with 5 % NaCl showed the highest WG, reaching 13.50 ± 0.22 g of water/100 g of sample after 24 h of treatment. Samples treated in brines with C_s of 10 % presented, in the first 6 hours of treatment, values of WG slightly lower than the values presented by mussels treated in brine with C_s of 5 %. This difference increased during the process and after 24 hour it was about 17 %.

Figure 3.2 - Kinetics of WG of mussel treated in brines with different C_s (a) and in marinades with different C_{ms} and C_{ma} (b).



Samples immersed in brines with C_s of 20 % and 26 % presented water loss from the second hour of treatment, due to the high osmotic

forces between the solution and the liquid phase of the sample. Such forces generated larger water flows to the exterior of the samples, overcoming the intake flows generated by the action of the capillary forces and sorption forces between the water and the mussel proteins. The degree of dehydration for mussels immersed in brines with C_s of 20 % was -2.22 ± 0.35 g of water/100 g of sample and for the C_s of 26 % was -3.79 ± 0.22 g of water/100 g of sample, after 24 h of immersion. The low values of water loss observed in mussel meat, when treated in brines with high salt concentration, could be explained considering their physical morphology, particularly the extension of the exposed surface. In fact, gills and visceral cavity together with the spongy structure of some anatomical part offer an ideal surface to a strong water adsorption.

Offer and Trinick (1983) suggested that the water gain in salted meat is positively affected by the Cl^-, when the NaCl concentration is low. This behavior was justified with the capacity of Cl^- to establish bonds with actin and myosin filaments, increasing negative charges, which amplify the repelling forces among the filaments, leading to muscle swelling. This behavior was also reported by Schmidt, Carciofi and Laurindo (2008b) for salted chiken breast cuts. Although in mussels the amount of muscular fibers is limited, the WG was particularly high at low C_s suggesting that the negative charge of Cl^- may have also an influence in the non-muscular tissues.

The kinetics of WG of mussel meat immersed in marinades with different concentrations of salt (C_{ms}) and acetic acid (C_{ma}) are shown in Figure 3.2b. The WG was affected by the C_{ms}, the C_{ma} and by the duration of the treatment. A negative WG was observed for all studied concentrations of the marinade. Even for C_{ms} of 5 % and 10 % and C_{ma} of 2 %, the WG was around -1.8 g of water/100 g of sample after 24 hours of treatment. For both C_{ms}, hydration was observed only for the first hour of treatment. The C_{ma} showed a strong dehydrating action. The difference between WG of mussels treated in solutions with C_{ma} of 6 % and 0.5 % at the same C_{ms} was of 8.39 g of water/g of sample.

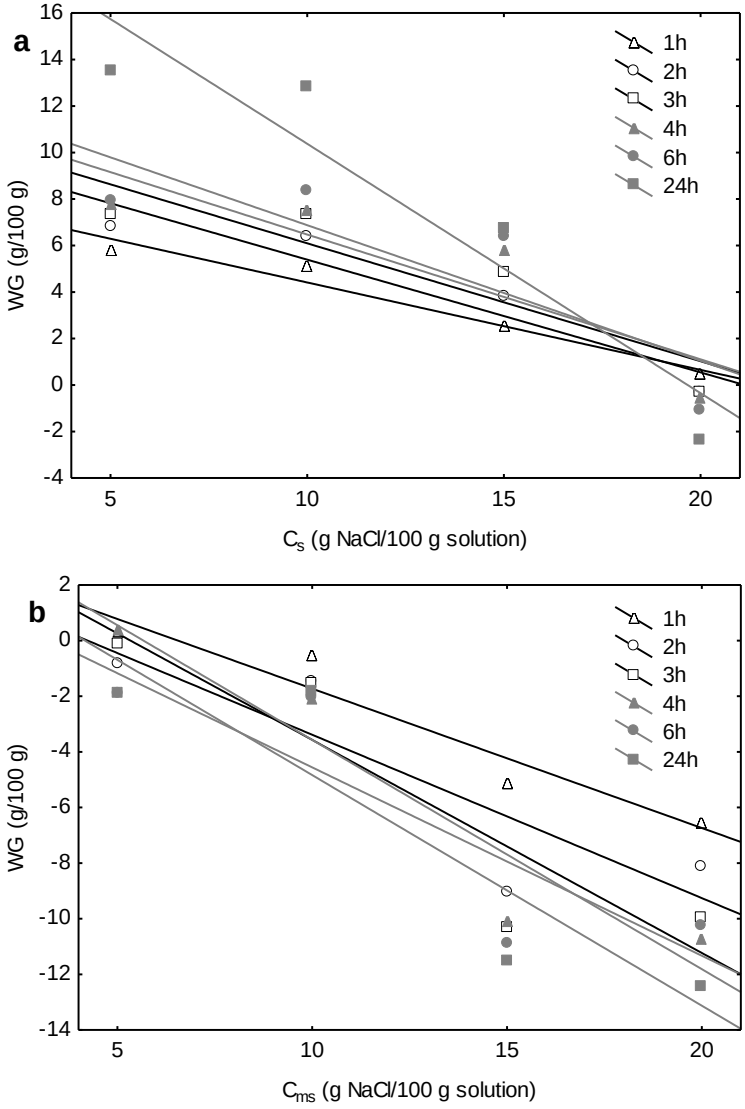
During the salting process the WG was controlled principally by the salt concentration of the solution and by the process time, again during marination a synchronous action of the salt and the acetic acid on the WG was shown. Indeed, during marination the salt apparently conditioned the WG only between the concentrations of C_{ms} 10 and 15 %. On the other hand, with the salt concentrations of 5 and 10 % and 15 and 20 % the WG did not show significant differences ($p< 0.1$).

The effect of acetic acid on the water gain of marinated turkey meat cubes was investigated by Goli et al. (2011). In contrast to what

occurs with mussels, the authors found that the presence of acetic acid caused protein swelling and the increasing the water gain. This phenomenon is due to the reduction of attraction forces between myofibrillar proteins when the pH decreases below its isoelectric point (KE et al., 2009). This reduction of attraction forces opens the meat protein matrix, increasing their water holding capacity. The mussel structure is complex considering the presence of reproductive cells (mantle and visceral mass), muscles, organs and gills (Chapter 2). For this reason, the mechanisms that control the WG of mussels treated in marinades are not exactly defined. Probably, there are two principal reasons that justify this phenomenon: firstly the acetic acid degrades the connective tissue and the cellular membranes, decreasing the water holding capacity, and secondly, the acid could denature proteins and affect its solubility (MACHADO et al., 2007), changing chemical equilibrium protein - water, resulting in additional water release and in less water adsorption.

The WG of pre-cooked mussel meat treated in brines and marinades with salt concentration from 5 % to 20 % as function of the solution salt concentration is shown in Figure 3.3.

Figure 3.3 - The WG of pre-cooked mussel meat treated in brines with salt concentration from 5 % to 20 % (a) and in marinades with salt concentration from 5 % to 20 % and acetic acid concentration of 2 % (b) as function of the salt concentration.



The equations of the linear fit of the WG of salted and marinated mussel meat found in the present study are presented in the Table 3.2.

Table 3.2 - Equations and determination coefficient values observed for the fit of WG as function of salt concentration (Figure 3.3) for different immersion times.

Time (h)	Salting		Marination	
	Equation	R^2	Equation	R^2
1	$WG = 8.17 – 0.37C_s$	0.960	$WG = 3.28 - 0.50C_{ms}$	0.926
2	$WG = 10.24 – 0.48C_s$	0.895	$WG = 2.49 - 0.58C_{ms}$	0.872
3	$WG = 11.16 – 0.50C_s$	0.829	$WG =4.09 - 0.77C_{ms}$	0.934
4	$WG = 11.83 – 0.53C_s$	0.790	$WG = 4.67 - 0.82C_{ms}$	0.902
6	$WG = 12.70 – 0.58C_s$	0.823	$WG = 2.21 - 0.68C_{ms}$	0.843
24	$WG = 21.10 – 1.07C_s$	0.887	$WG = 3.45 - 0.83C_{ms}$	0.830

Schmidt, Carciofi, and Laurindo (2008b) observed a linear behavior between the water gain of salted chicken breast cuts and the brine concentration, with determination coefficients between 0.957 and 0.997. Cavalheiro (2010) studied the effect of the salt concentration on the water gain of pre-cooked mussel meat cooled by vacuum application. For processing time of 4 hour, the author reported determination coefficients values between 0.991 and 0.956. The low values of R^2 found in this study revealed that this fit cannot be used to predict accurately the WG during salting and marination of pre-cooked mussel meat. It was not possible to preview with exactness the transition zone between hydration ($WG > 0$) and dehydration ($WG < 0$) regimes, although it is situated between the C_s of 15 % and 20 % for the salting process and around C_{ms} of 5 % for marination process.

3.4.3 Kinetics of the salt gain

Two main mechanisms are involved in the mass transfer of salt between brine and product. The salt transfer by diffusion due to the gradient of concentration between brine and sample liquid phase (intercellular solution) and liquid solution transfer caused by capillary forces (hydrodynamic mechanism) lead to the simultaneous distribution of salt and water in the food porous space (SCHMIDT, 2006).

The Figure 3.4a shows the average values of SG of pre-cooked mussel meat immersed in brines with C_s of 5, 10, 15, 20, and 26 %. The SG was significantly affected by the C_s and by the processing time. It was intense in the first stage of the process, when the gradient between

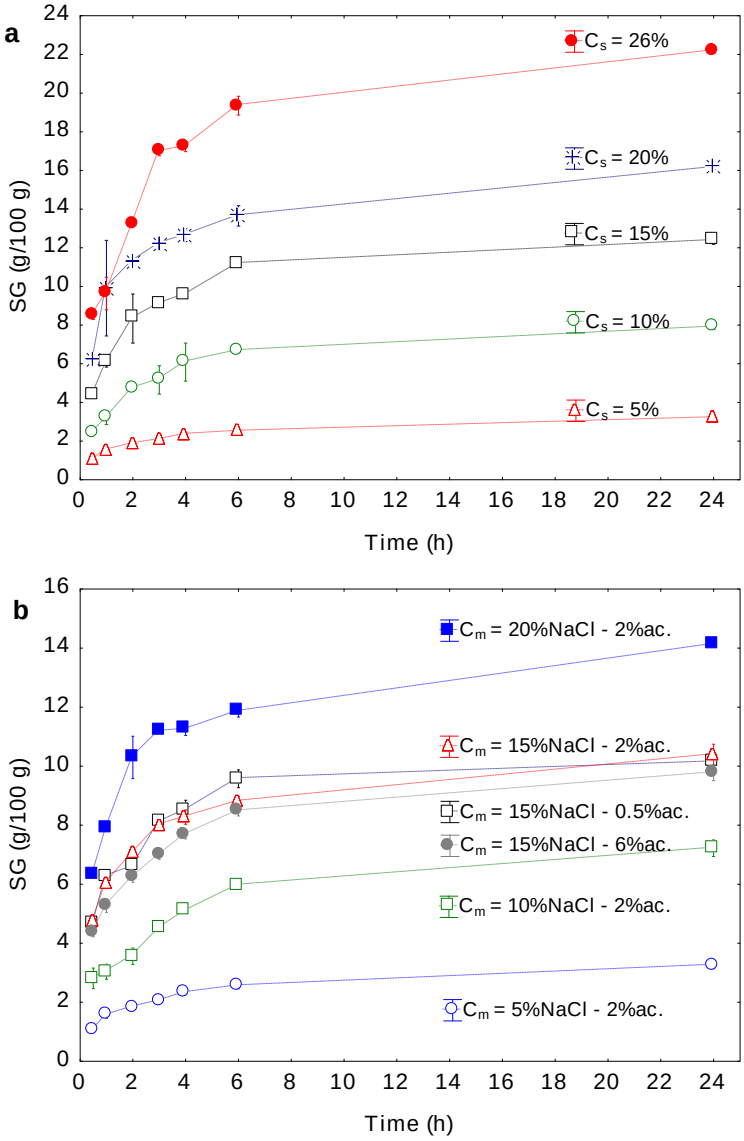
the C_s and the salt concentration in mussels liquid phase was particularly high. Afterwards, the gradient between salt concentration of intercellular solution and the brine decreased, reaching the equilibrium during the treatment. For C_s lower than 15 % the equilibrium was reached after around six hours of treatment.

The Figure 3.4b shows the average values of SG of pre-cooked mussel meat treated in marinades with C_{ms} of 5, 10, 15, and 20 % and C_{ma} of 2 % and with C_{ma} of 0.5 and 6 % and C_{ms} of 15 %. The SG was influenced by salt concentration and by processing time and only a tendency of a negative action of the acetic acid concentration was observed.

By the comparison of the SG of mussels immersed in salt solutions with and without acetic acid, it appears that the SG values were slightly higher for mussels immersed in brines than for mussels immersed in marinades. Goli et al. (2011) investigated the effect of acetic acid on the salt impregnation of turkey meat cubes and reported that this acid did not have a significant effect on salt impregnation.

Salt concentration in seafood is an important factor for the reduction of microbial activity. In any case, this inhibitory action can be found only with high salt concentrations. For example to inhibit the growth of *C. botulinum*, when no other hurdles are present, at least 20 % of salt concentrations is required (FDA, 2011). On the other hand, for fish that will be consumed directly without any desalting process, the maximum salt concentration cannot exceed 3.5 % and the optimum is considered about 2 %, considering the negative effect on the human health of a diet with high sodium intake (PIGOTT and TUCKER, 1990). For salted mussel meat, only samples treated in brines with C_s of 26 % reached the final NaCl concentration of about 20 %, enough to avoid the grow of *C. botulinum*. Probably, if the product processed will be stored at room temperature and without any other treatments, other salting methods (dry salting) or a longer brine salting should be used.

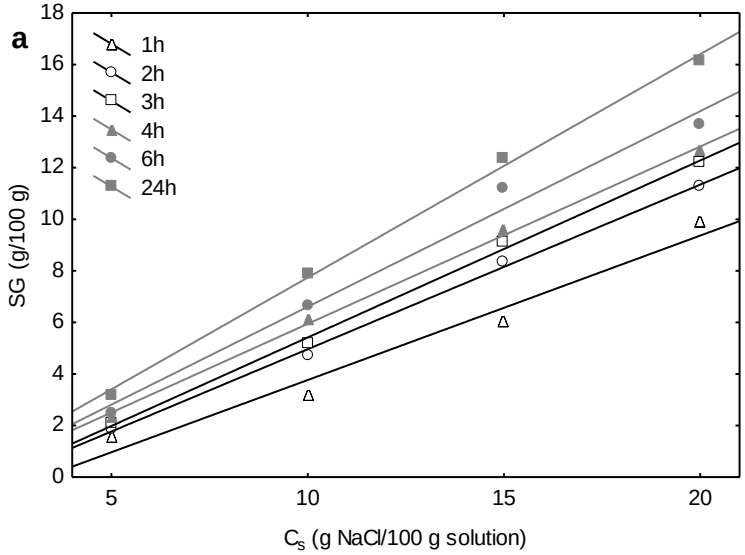
Figure 3.4 - Kinetics of SG of mussel treated in brines with different C_s (a) and in marinades with different C_{ms} and C_{ma} (b).

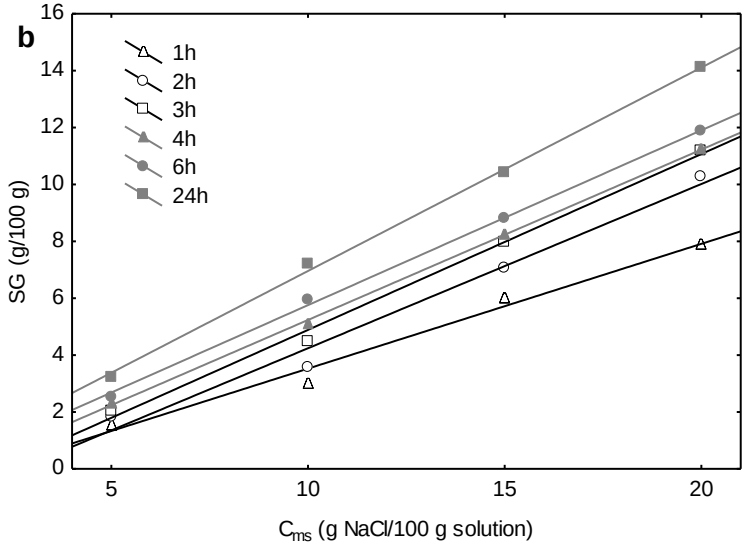


The Figure 3.5a shows the SG of cooked mussels meat treated in brines with C_s from 5 to 20 % as function of the solution salt concentration. The difference of SG between the end of the process and

the first hour was of 1.67, 4.71, 6.33 and 6.24 g/100 g for the C_s of 5, 10, 15, and 20 %, respectively. The Figure 3.5b shows the SG of pre-cooked mussel meat treated in marinades with C_{ms} from 5 to 20 % and C_{ma} of 2 % as function of $C_{ms.}$ In this case, the difference of SG among the end of the process and the first hour was 1.70, 4.19, 4.36, and 6.25 for the C_{ms} of 5, 10, 15 and 20 %, respectively.

Figure 3.5 - The SG of pre-cooked mussel meat treated in brines with salt concentration from 5 % to 20 % (a) and in marinades with salt concentration from 5 % to 20 % and acetic acid concentration of 2 % (b) as function of the salt concentration.





The equations of the linear fitting of the SG of salted and marinated mussel meat and the determination coefficients (R^2) are presented in Table 3.3.

Table 3.3 - Equations and determination coefficient values observed for the fit of SG as function of salt concentration (Figure 3.5) for different immersion times.

Time (h)	Salting		Marination	
	Equation	R^2	Equation	R^2
1	$SG = -1.83 + 0.56C_s$	0.969	$SG = -0.86 + 0.43C_{ms}$	0.984
2	$SG = -1.42 + 0.63C_s$	0.998	$SG = -1.53 + 0.58C_{ms}$	0.982
3	$SG = -1.44 + 0.68C_s$	0.998	$SG = -1.29 + 0.62C_{ms}$	0.995
4	$SG = -0.93 + 0.68C_s$	0.998	$SG = -0.75 + 0.59C_{ms}$	0.999
6	$SG = -0.98 + 0.75C_s$	0.986	$SG = -0.39 + 0.61C_{ms}$	0.998
24	$SG = -0.92 + 0.86C_s$	0.998	$SG = -0.20 + 0.71C_{ms}$	0.998

The behavior of SG as a function of the solution concentration was approximately linear. The determination coefficient between the equation of the linear fitting data and the experimental was between 0.969-0.999. Considering the high values of the R^2, these equations are a very useful tool to preview the salt gain of salted and marinated mussels in the range of salt concentration between 5 % and 20 %.

3.4.4 Operational diagram

The experimental data of water and salt gain of salted and marinated pre-cooked mussel meat presented a non linear behavior, for this reason it was not possible to build the operational diagram from these data. On the other hand, the values of moisture content "M %" and salt content "S %" can be calculated from the data of water and salt gain, substituting the experimental values on the equations 3.16 and 3.17, neglecting the soluble solid loss (SCHMIDT, CARCIOFI, and LAURINDO, 2008).

$$M\ \% = \frac{WG + m_0}{SG + WG + 100} \tag{3.16}$$

$$S\ \% = \frac{SG}{SG + WG + 100} \tag{3.17}$$

The behavior of M (%) and of S (%) *versus* the salt concentration of the soaking solution was approximately linear for both treatments (salting and marination). The equations of the linear fitting and the determination coefficient (R^2) are presented in Table 3.4.

Table 3.4 - Equations and determination coefficient values observed for the fit of S % and M % of mussels treated in brines and marinades as function of salt concentration for different immersion times.

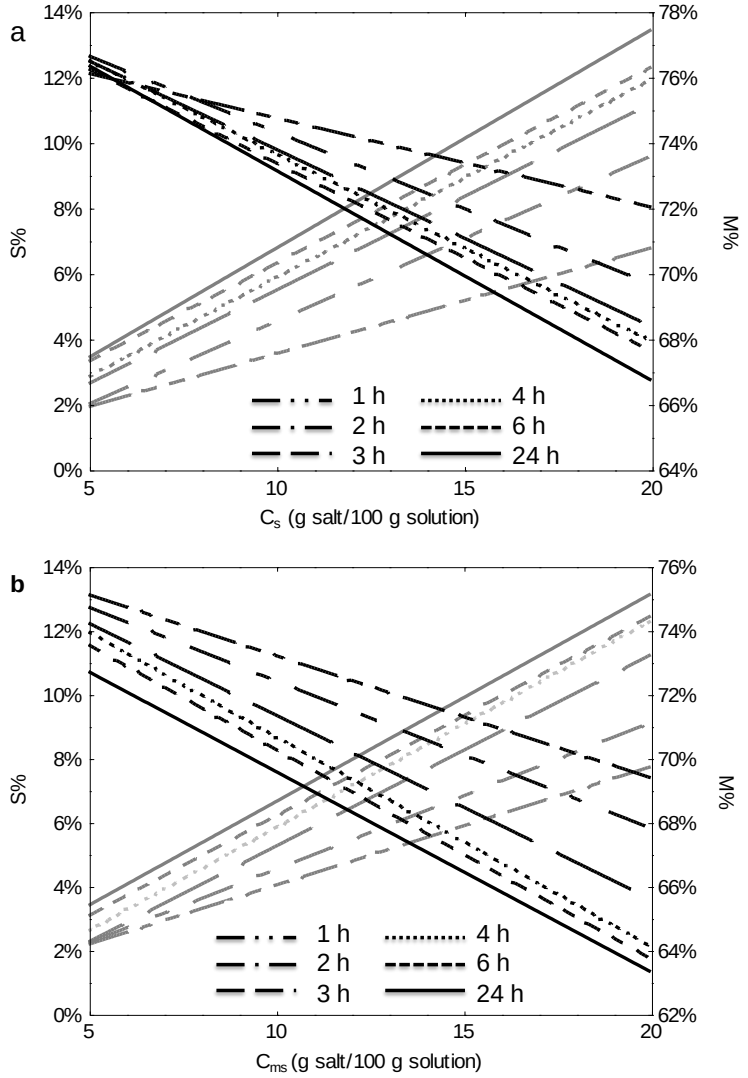
	Time (h)	S %		M %	
		Equation	R^2	Equation	R^2
Salting	1	$S = 0.26 + 0.32C_s$	0.992	$M = 77.46 - 0.27C_s$	0.959
	2	$S = -0.53 + 0.51C_s$	0.980	$M = 78.97 - 0.47C_s$	0.977
	3	$S = -0.24 + 0.57C_s$	0.997	$M = 79.19 - 0.57C_s$	0.993
	4	$S = -0.20 + 0.60C_s$	0.998	$M = 79.37 - 0.57C_s$	0.990
	6	$S = 0.30 + 0.60C_s$	0.999	$M = 79.11 - 0.58C_s$	0.992
	24	$S = 0.07 + 0.67C_s$	0.996	$M = 79.55 - 0.64C_s$	0.997
Marination	1	$S = 0.32 + 0.37C_{ms}$	0.986	$M = 77.02 - 0.38C_{ms}$	0.963
	2	$S = -0.70 + 0.46C_{ms}$	0.980	$M = 77.02 - 0.46C_{ms}$	0.980
	3	$S = -0.75 + 0.60C_{ms}$	0.981	$M = 77.10 - 0.58C_{ms}$	0.947
	4	$S = -0.64 + 0.65C_{ms}$	0.992	$M = 77.22 - 0.66C_{ms}$	0.959
	6	$S = -0.70 + 0.63C_{ms}$	0.998	$M = 76.81 - 0.65C_{ms}$	0.975
	24	$S = 0.14 + 0.65C_{ms}$	0.999	$M = 75.84 - 0.63C_{ms}$	0.962

The accuracy of the linear fitting is proved considering that the determination coefficient between the equation of the linear fitting and the experimental data was between 0.980 and 0.999 for the S %, and between 0.959 and 0.997 for the M % during salting. The determination coefficient demonstrated the accuracy of the fitting also during marination. Indeed for S % the R^2 was between 0.980 and 0.999 and between 0.947 and 0.980 for M %. Considering the high values of the R^2 these equation represent a very useful tool to predict the salt content and the moisture content of salted and marinated mussels in the range of salt concentration in between 5 % and 20 % for different immersion times.

Figure 3.6 shows the salting and marination operational diagrams built using the equations presented in Table 3.4. In these diagrams, the black lines are related with the M% and the grey lines with the S%.

Same kinds of lines (solid, dashed, etc.) indicate similar immersion times (1, 2, 3, 4, 6, and 24 h).

Figure 3.6 - Operational diagram of pre-cooked mussel meat immersed in brines (a) and marinades (b). The gray lines are related to salt, while the dark lines represent the moisture content.



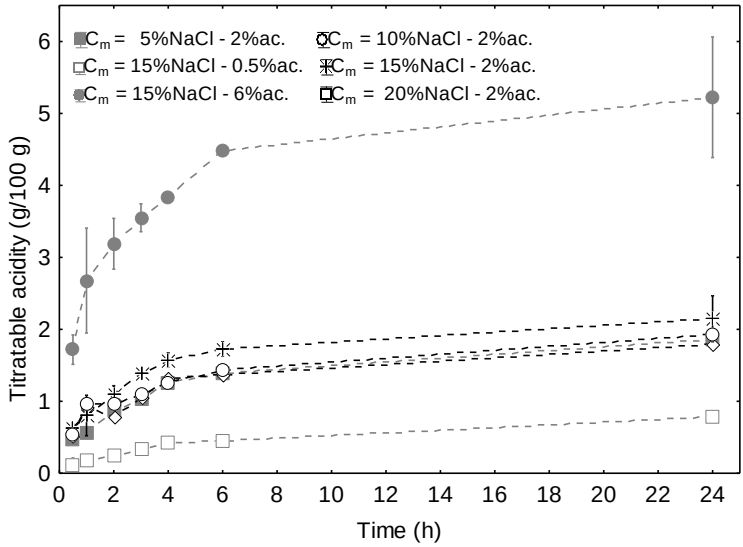
Such operational diagrams represent a very useful graphical tool that can be used to optimize a process of salting or marination of cooked mussel meat, establishing previously the required salt or water concentration in the final product. For example, by using the operational diagrams, it is possible to determine the salt concentration of the soaking solution that minimize the process time required to obtain mussels with salt content of 3.5 % and moisture content close to 75 %. In the case of a salting process (Figure 3.6a), a brine with bs close to 10 % NaCl and immersion time of 1 h would be enough to achieve the desired salt content with a final moisture content of 74.8 %. On the other hand, in the marination process (Figure 3.6b), aiming to obtain the same salt content with the same process time, a solution with 8.5 % NaCl would be required (this value is lower than the one obtained for the salting process because of the dehydration caused by the acid addition) and the moisture content of the final product would be of 73.8 %. This kind of operational diagrams are specific for the process on which they are built. In fact, any variation on the product geometry, process temperature, stirring level and on other possible solutes added to the solution could modify the mass transfer (LAWRIE, 2005; VOLPATO et al., 2007; SCHMIDT, CARCIOFI, and LAURINDO, 2008b).

3.4.5 Effect of the marinade acetic acid and salt concentration on the acid concentration and ph of pre-cooked mussel meat

The Figure 3.7 shows the effect of the marinade concentration (C_m) on the acid content of pre-cooked mussel meat treated for 24 hours. The acid concentration calculated as titratable acidity was influenced exclusively by the acid concentration and by the processing time. The salt concentration had not significant influence ($p > 0.1$) on the acidity of traded mussels.

The pH of fresh fish is between 6.6 and 6.8 and in the *post-mortem* period it tends to increase due to the microbial and enzymatic activities that bring to the decomposition of nitrogenous compounds (SHENDERYUK and BYKOWSKI, 1990). Mussels store glycogen as a source of energy, and in the *post-mortem* period it is hydrolyzed by the initial microbial and enzymatic flora, leading, contrarily to what happen in fish muscle, to a pH decreasing.

Figure 3.7 - Evolution of the acid concentration in mussel treated in marinades with different concentrations of salt and acetic acid.

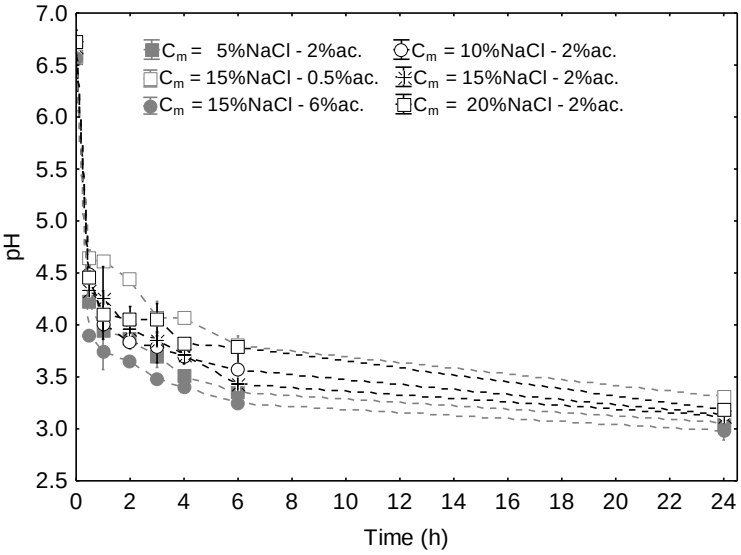


The changes of the pH in mussel meat during marination are presented in Figure 3.8. The acid content and the processing time had a negative effect on the pH of mussel meat. The difference among the pH of mussels immersed in marinades with various C_{ma} decrease with time. For example, the difference on the pH of mussels treated in marinades with C_{ma} of 0.5 % and 6 % was of 1 pH unit after 0.5 h of processing and of 0.4 pH unit after 24 h.

At the end of the process (24 h) the difference between mussels and marinade pH was around 0.4 pH unit, suggesting that the equilibrium of H^+ concentration was not reached, while the equilibrium of acid content was reached, as clearly showed in Figure 3.7. This result agrees with the study of Goli et al. (2011), in which is reported that, when the equilibrium of the acid concentration, between marinated turkey breasts cuts and marinade, is reached at the end of the process there is still a difference between meat and solution on the pH. The authors suggested that the continue denaturation of the meat proteins exposes buffering chemical groups that limits the pH decrease. Moreover the buffering effect tends to be lost during the process. After 6 h of processing the acid content is very near to the equilibrium, while

the pH was about 0.9 pH units far from the marinade pH (this difference fall to about 0.4 after 24 h).

Figure 3.8 - Kinetics of the pH in mussel treated in marinades with different concentrations of salt and acetic acid.



Mussel meat immersed in marinades with high C_{ms} presented higher values of pH than those immersed in marinades with low C_{ms}. These differences are significant ($p < 0.1$) until the sixth hour of processing, losing this significance at the twenty-fourth hour of processing. This discrepancy was probably due to the buffering action of some chemical groups that were exposed by the denaturation caused by the NaCl.

The microbiological stability of marinated products is largely affected by pH and acidity values. When the pH of a product is reduced below the minimum limit for microbial grow (e.g. *C. botulinum*, $pH < 4.5$), cells stop growing and also reduce viability. The undissociated molecules of weak acids are lipophilic, and can enter into the microbial cell and then dissociate to generate H^+ in the cytoplasm, causing a reduction in its internal pH, which ultimately destroys the proton gradient between the inside and the outside of the cells. It dissipates proton-motive force as well as the ability of the cells to generate energy (ADAMS and MOSS, 2008). In marinated pre-cooked

mussel meat, pH values lower than 4.5 (able to inhibit the *C. botulinum* growth) were reached after 0.5 h of processing for C_{ma} of 2 and 6 %, and for C_{ma} of 0.5 % the value of pH of 4.5 was reached after 2 h of processing.

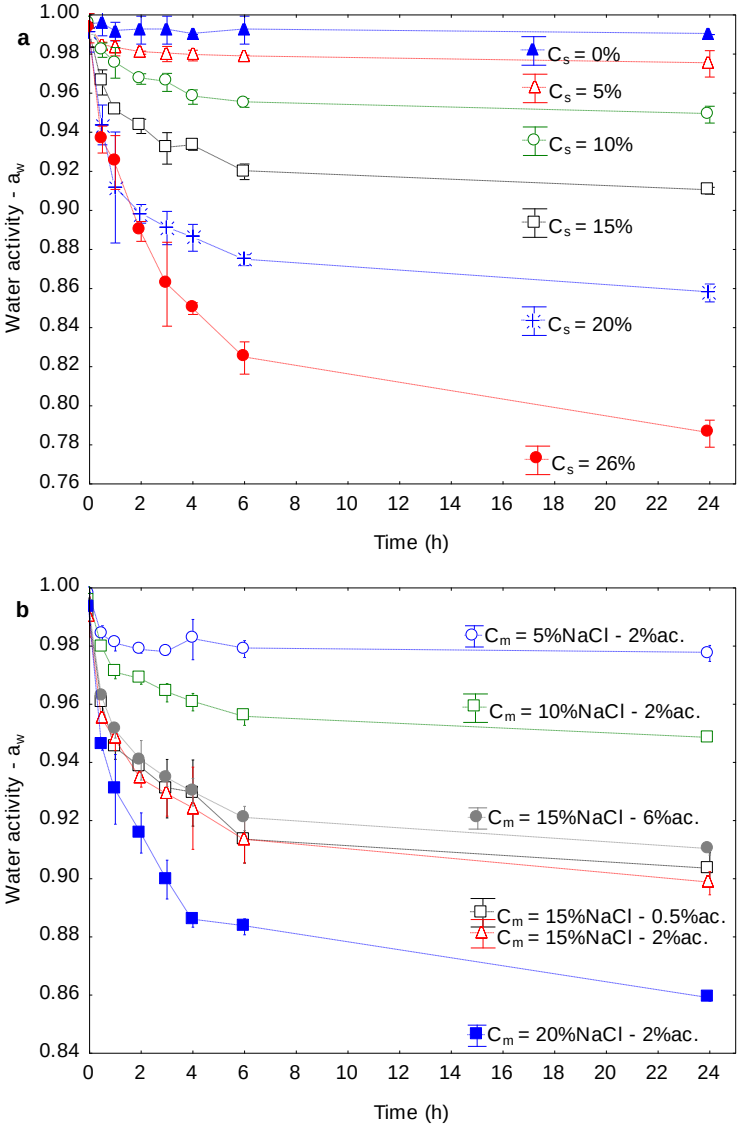
3.4.6 Effect of the salt and acid concentration on the a_w of pre-cooked mussel meat

The effect of the investigated treatments on the a_w of pre-cooked mussel meat is shown in Figure 3.9. As expected, the salt depressed the a_w proportionally to its concentration in the soaking solution. Even with C_s of 5 % and 10 %, while the product gain much water, the decrease of the a_w was evident and is explained by increase of the salt concentration on the liquid phase of the product. As shown in Figure 3.9b, the presence and the concentration of the acetic acid in the marinade had not any significant effect on the a_w, confirming that its reduction was due only to the meat salt uptake.

The initial a_w of pre-cooked mussel meat was of 0.994 ± 0.003. After 24 hours of processing the a_w was of 0.975 ± 0.003 for C_s of 5 %, 0.949 ± 0.002 for C_s of 10 %, 0.910 ± 0.001 for C_s of 15 % and 0.857 ± 0.002 for C_s of 20 %. The minimum value of 0.785 ± 0.003 of a_w was reached in pre-cooked mussel meat soaked for 24 h in brine with C_s of 26 %.

Values of a_w lower than 0.90 inhibit the pathogenic bacteria growth. Only with brine with C_s of 20 % and 26 % this a_w value was reached for pre-cooked mussel meat after 2 h of processing. However, the critical limits of a_w for microbiological growth may also be shifted to higher levels by the low pH values of the final product (FELLOW, 2000).

Figure 3.9 - Changes of water activity (aw) during salting (a) and marination.



3.4.7 Effect of salt and acid concentration on the whc of pre-cooked mussel meat

The water holding capacity (WHC) is one of the most important characteristics of seafood and is related to the texture and the juiciness of the product, having a strong influence in the acceptation by the consumers. The WHC is directly affected by the pore and capillary size, pH, charges in the protein matrix, modification in the protein structure, among others factors (OLSSON, OFSTAD, and OLSEN, 2003; SKIPNESET al., 2007).

The results of WHC variation in the mussel samples subjected to salting and marination processes for 4 hours in solutions with different salt and acetic acid concentrations are presented in Table 3.5. Except for the samples subjected to the salting process with C_s of 5 %, all samples subjected to the remainder studied conditions presented values of WHC significantly smaller ($p < 0.1$) than those observed for the pre-cooked samples (approximately 2.21 gH_2O/g dry matter, Table 3.1). Moreover, the reduction of WHC was larger for the samples subjected to the salting with higher values of C_s. Gallart-Jornet et al. (2007) studied the salting process of Atlantic salmon and also verified a reduction in the WHC with the increase of brine concentration. This reduction was associated to protein denaturation that occurs at high salt concentrations.

The values of WHC of mussels subjected to marination were lower than those of the mussels subjected to salting with the same salt concentration. This can be explained by the solubilization of the collagen in dilute acids solutions (AIDOS, LIE, and ESPE, 1999) and denaturation of the protein, increasing the water release. On the other hand, the effect of the marinade acid concentration on the WHC of the mussels was not significant.The water holding capacity (WHC) is one of the most important characteristic of seafood. It is strictly related to the juiciness and the texture that have a strong influence in the acceptance of the product by the consumers.

Table 3.5 - Changes in mussels WHC during the first 4 h of treatment*.

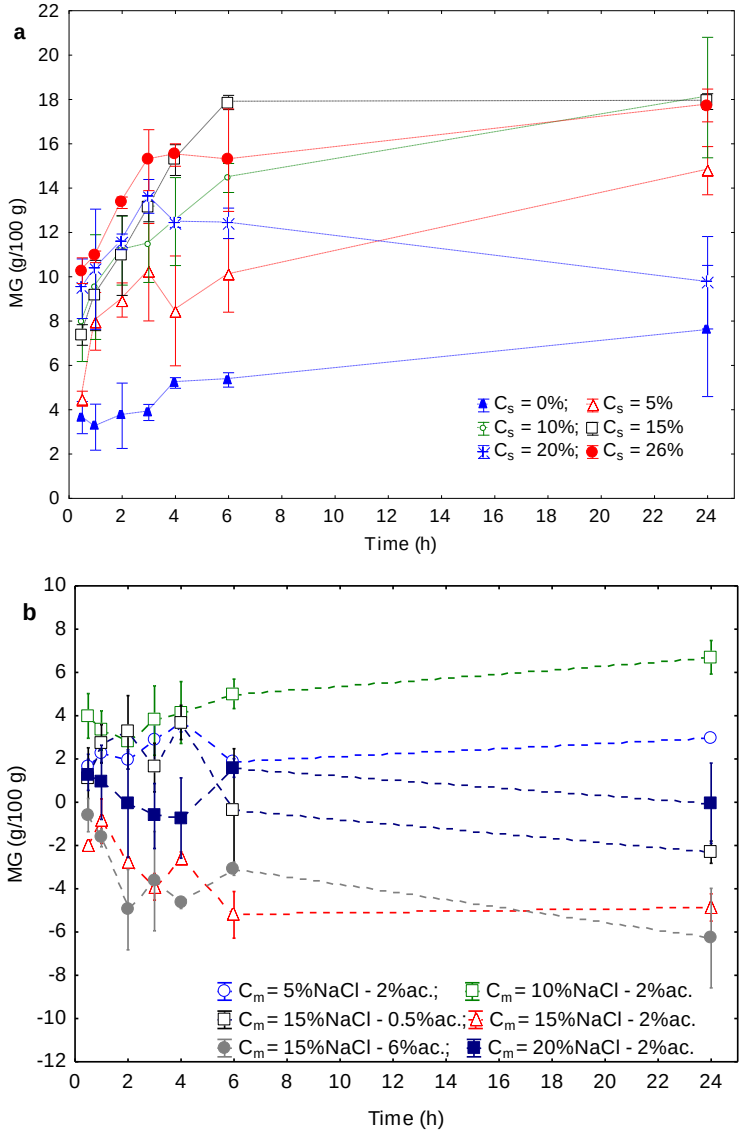
Concentration	Time			
	1 h	2 h	3 h	4 h
5 %NaCl	$2.14\pm0.07^{a,A}$	$2.14\pm0.06^{a,A}$	$2.19\pm0.06^{a,A}$	$2.25\pm0.05^{a,A}$
10 %NaCl	$1.86\pm0.17^{a,BCD}$	$1.79\pm0.05^{ab,B}$	$1.70\pm0.04^{ab,BC}$	$1.57\pm0.08^{b,BC}$
15 %NaCl	$1.73\pm0.11^{a,CDE}$	$1.52\pm0.10^{b,CD}$	$1.38\pm0.05^{b,D}$	$1.49\pm0.06^{b,CD}$
20 %NaCl	$1.92\pm0.08^{a,ABC}$	$1.22\pm0.07^{c,F}$	$0.96\pm0.08^{b,E}$	$1.11\pm0.07^{c,EF}$
5 %NaCl – 2 %ac.	$2.02\pm0.11^{a,AB}$	$2.00\pm0.09^{a,A}$	$1.87\pm0.18^{ab,B}$	$1.69\pm0.12^{b,B}$
10 %NaCl – 2 %ac.	$1.80\pm0.05^{a,BCD}$	$1.64\pm0.07^{ab,BCD}$	$1.63\pm0.12^{b,C}$	$1.52\pm0.05^{b,BCD}$
15 %NaCl – 0.5 %ac.	$1.81\pm0.08^{a,BCD}$	$1.59\pm0.07^{b,BCD}$	$1.41\pm0.04^{c,D}$	$1.35\pm0.08^{c,DE}$
15 %NaCl – 2 %ac.	$1.52\pm0.09^{a,EF}$	$1.45\pm0.09^{a,DE}$	$1.38\pm0.06^{a,D}$	$1.20\pm0.08^{b,E}$
15 %NaCl – 6 %ac.	$1.66\pm0.14^{a,DE}$	$1.66\pm0.10^{a,BC}$	$1.58\pm0.10^{ab,CD}$	$1.35\pm0.06^{b,DE}$
20 %NaCl – 2 %ac.	$1.27\pm0.10^{a,F}$	$1.25\pm0.11^{ab,EF}$	$1.09\pm0.03^{bc,E}$	$1.00\pm0.07^{c,F}$

$^{a–c}$ Means in the same row with the same letter do not differ significantly ($p > 0.1$).
$^{A–F}$ Means in the same column with the same letter do not differ significantly ($p > 0.1$).
* Results are presented as means ± standard deviations.

3.4.8 Mass gain rates

The rates of mass gain during salting and marination of pre-cooked mussel meat are shown in Figure 3.10. Mussels immersed in brines with different C_s (Figure 3.10a) gained mass, for all studied concentrations. After 24 h of processing, the mass gain varied from a minimum of 6.73 ± 0.37 (g/100 g) for C_s=0 % to a maximum of 18.09 ± 1.70 (g/100 g) for C_s=10 %.

Figure 3.10 - Kinetics of MG of mussel treated in brines with different Cs (a) and in marinades with different Cm (b).



When mussels were treated in marinades the mass gain decreased, following the same tendency of the water loss. After 24 h of

processing, mussels treated in marinades with C_{ms}=5 % and 10 % showed a mass gain of 2.95 ± 0.10 and 6.70 ± 0.77 (g/100g). Mussels treated in marinades with C_{ms}=15 % and different C_{ma} presented negative mass gain. The marinade with C_{ms} of 20 % resulted in a null mass gain of treated mussels, after 24 h of processing.

The high standard deviation values of the MG of salted and marinated pre-cooked mussel meat can be explained considering the natural variability of the product as well as the fragility of its structure. Indeed, small amounts of mantle or gut may be lost during processing, resulting in discrepancies of the MG.

3.4.9 Microstructure analysis

The microstructure of mussels depends on the season, gender and development stage, and also on the tissue part (MANCEBO et al., 1992). Trying to avoid the influence of this variability, only transversal sections of the mantle of female specimens, apparently in the same sexual development stage, were analysed. The Figure 3.11 shows the scanning electron microscopy (magnification of 50x) of a section of a female mantle (cooked as described earlier). The microstructure of the mantle was very porous since vesicular and adipoglonular cells (1) were occupying most of the mantle tissue. Mancebo et al. (1992) described the same structure in fresh mantle sections of mussels from the species *M. galloprovincialis.*

Figure 3.11 - Scanning electron microscopy of the mantle of female mussels (magnification: x50).

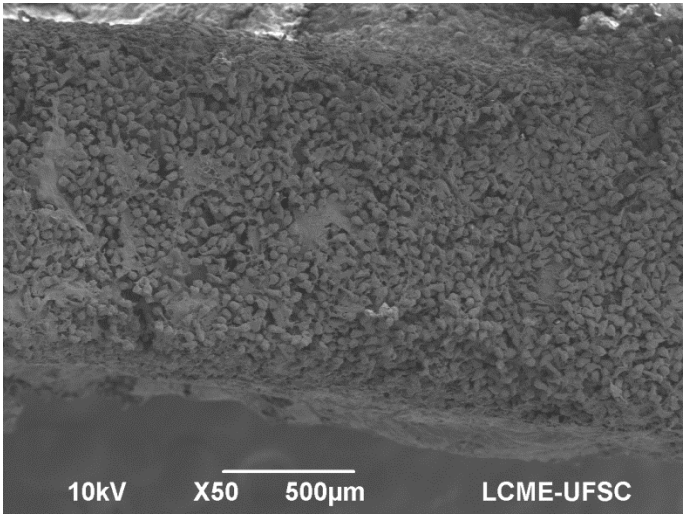
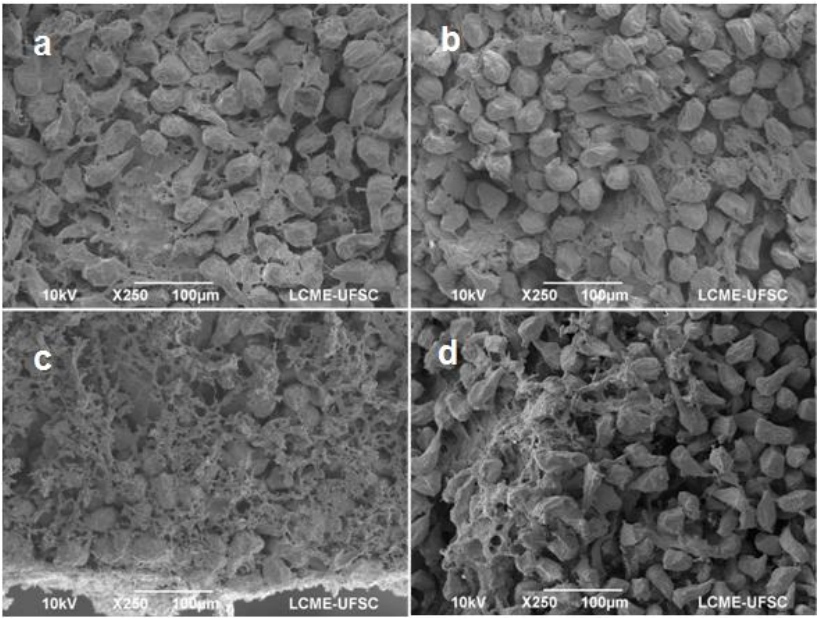


Figure 3.12 shows the scanning electron microscopy at the magnification x250 of transversal sections of the mantle of female pre-cooked mussel that was performed with untreated samples (a) and after 4 hours of treatment in brines with C_s of 5 (b) and 20 % (c) and marinades with C_{ms} of 5 % and C_{ma} of 2 % (d).

Although the microstructure of the mussel treated in brine with C_s of 5 % appears similar to the microstructure of the untreated mussel, the cells and the connective tissue appears swollen in the treated mussel. This fact could justify the high WG showed by the mussels when treated with brines with C_s of 5 %. The mussel treated in brine with C_s of 20 % apparently presented signs of denaturation of the connective tissue and salt incrustations on the surface of the cut. The microstructure of the sample treated in marinade presented cells shrinkage and consequent increase of the intercellular space. This aspect could be explained by the denaturation and probably by the melting of the connective tissue promoted by the acetic acid, which could also justify the low values of WG and WHC.

Figure 3.12 - Scanning electron microscopy of the mantle of female mussels: (a) untreated; (b) treated 4 hours with brine with C_s=5 % (c) treated 4 hours with brine with C_s=20 %; (d) treated 4 hours with marinade with C_{ms}=5 % and C_{ma}= 2 % (magnification, x250).



3.4.10 Mathematical modeling

The parameters of the Peleg's model, the Weibull type model and the Zugarrumurdi and Lupin model fitted to experimental data of SG of salted and marinated mussels are presented in Table 3.6.

The parameter k^s_1 of the Peleg's model is related to the initial salt transfer rate. Small values of k^s_1 represent higher salt gain in the first stage of the process. High initial rates of SG for higher C_s and C_{ms} values can be explained by the higher gradients of salt concentration between the osmotic solution and product liquid phase.

The statistical values R^2 and MRSE quantify the goodness of fit related to the models used to estimate the values of SG during salting and marination processes. The exponential model had the better performance that could be attributed to its three parameters. The Peleg's model adequately predicted the salt gain for salt concentration lower than 20 %, even if it tended to overestimate the salt gain at the

equilibrium (SG_P^{∞}). On the other hand, the Zugarrumurdi and Lupin model had a worse performance compared with the other two models. In particular, this model described accurately the first six hours of treatment, but largely underestimates the salt gain at the equilibrium (SG_Z^{∞}). The SG_Z^{∞} predicted by this model showed values of about 15 % lower than the SG predicted by the exponential model (SG_W^{∞}).

The parameters of Peleg's model, exponential model and Zugarrumurdi and Lupin model fitted to experimental data of WG are presented in Table 3.7. The three models were not capable to adequately represent the experimental data of WG at the C_s of 20 % and 26 % and the C_m of 5 % and 10 % of NaCl and 2 % acetic acid, probably due to the changes in the water flow direction in the sample during the 24 h of immersion.

The parameter k^w_1 did not show any clear correlation with the WG in the first stage of the treatments. The high standard deviations observed especially in the first stages of the treatments could cause this lack of relation between k^w_1 and WG. The statistical parameters that qualify the goodness of fit (R^2 and RMSE) indicated that the values of WG_W^{∞} estimated by the exponential model are more reliable than the values of WG_P^{∞}. The Zugarrumurdi and Lupin model tends to largely underestimate the WG_Z^{∞}.

Table 3.6 - Parameters of Peleg's model, Weibull type model and Zugarrumurdi and Lupin model fitted to experimental data of SG.

SALT GAIN

SOLUTION CONC.	PELEG'S MODEL					WEIBULL MODEL					ZUGARRAMURDI AND LUPIN MODEL			
	ESTIMATED PARAMETERS			STATISTICAL PARAMETERS		ESTIMATED PARAMETERS			STATISTICAL PARAMETERS		ESTIMATED PARAMETERS		STATISTICAL PARAMETERS	
	K^S_1	K^S_2	SG_P^∞	R^2	RMSE	A	B	SG_W^∞	R^2	RMSE	K^S_1	SG_Z^∞	R^2	RMSE
5 %NACL	0.400	0.316	3.17	0.955	0.161	0.492	3.252	3.43	0.996	0.057	0.604	2.78	0.822	0.320
10 %NACL	0.183	0.120	8.31	0.986	0.248	0.662	2.526	8.00	0.994	0.186	0.497	7.35	0.931	0.553
15 %NACL	0.085	0.078	12.78	0.987	0.351	0.623	1.896	12.50	0.993	0.287	0.695	11.27	0.909	0.917
20 %NACL	0.047	0.063	15.76	0.961	0.676	0.496	1.581	16.32	0.967	0.688	1.077	13.84	0.841	1.361
26 % NACL	0.052	0.044	22.99	0.962	1.071	0.614	2.115	22.56	0.979	0.896	0.618	20.35	0.889	1.839
5 %NACL - 2 %AC.	0.417	0.310	3.22	0.945	0.182	0.486	3.715	3.55	0.993	0.071	0.557	2.84	0.809	0.341
10 %NACL - 2 %AC.	0.211	0.136	7.35	0.894	0.578	0.477	4.507	8.19	0.965	0.373	0.444	6.66	0.779	0.833
15 %NACL - 2 %AC.	0.070	0.098	10.22	0.936	0.542	0.518	1.405	10.40	0.965	0.449	1.020	9.11	0.790	0.983
15 %NACL - 0.5 %AC.	0.068	0.100	10.03	0.951	0.453	0.433	1.687	10.82	0.997	0.125	1.132	8.87	0.779	0.955
15 %NACL - 6 %AC.	0.087	0.105	9.55	0.933	0.532	0.449	2.132	10.36	0.993	0.185	0.910	8.43	0.752	1.019
20 %NACL - 2 %AC.	0.050	0.073	13.76	0.964	0.537	0.477	1.461	14.31	0.983	0.414	1.111	12.20	0.836	1.146

Table 3.7 - Parameters of Peleg's model, Weibull type model and Zugarrumurdi and Lupin model fitted to experimental data of WG.

WATER GAIN

SOLUTION CONC.	PELEG'S MODEL					WEIBULL MODEL					ZUGARRAMURDI AND LUPIN MODEL			
	ESTIMATED PARAMETERS			STATISTICAL PARAMETERS		ESTIMATED PARAMETERS			STATISTICAL PARAMETERS		ESTIMATED PARAMETERS		STATISTICAL PARAMETERS	
	K^{W}_{1}	K^{W}_{2}	WG_{P}^{∞}	R^2	RMSE	A	B	WG_{W}^{∞}	R^2	RMSE	K^{W}_{1}	WG_{Z}^{∞}	R^2	RMSE
5 %NACL	0.166	0.074	13.53	0.889	1.193	0.426	22.470	20.85	0.955	0.852	0.325	12.17	0.775	1.701
10 %NACL	0.121	0.093	10.79	0.878	0.893	0.376	10.290	14.97	0.981	0.395	0.574	9.50	0.705	1.388
15 %NACL	0.236	0.129	7.73	0.968	0.391	0.987	2.301	6.82	0.996	0.151	0.436	6.80	0.996	0.136
15 %NACL - 2 %AC.	0.426	0.087	-11.55	0.971	0.517	0.706	7.986	-10.96	0.980	0.486	0.194	-9.65	0.957	0.629
15 %NACL - 0.5 %AC.	0.072	0.081	-12.40	0.922	0.826	0.772	1.331	-11.52	0.957	0.826	0.814	-11.07	0.918	0.843
15 %NACL - 6 %AC.	0.065	0.056	-17.92	0.940	1.058	0.521	2.608	-18.75	0.963	0.935	0.691	-15.57	0.827	1.798
20 %NACL - 2 %AC.	0.067	0.081	-12.42	0.951	0.607	0.545	1.576	-12.49	0.965	0.574	0.895	-11.01	0.850	1.060

The Peleg model was used by Corzo and Bracho (2006) to describe experimental data of salt gain and water loss during osmotic dehydration of sardine sheets. The authors found values of R^2=0.997-0.999 that confirmed the high performance of this model in the studied case.

The Weibull exponential model was applied by Corzo and Bracho (2008) for predicting water and salt gain of sardine sheets during the osmotic dehydration with application of vacuum pulse. A high regression coefficient (R^2>0.99) was found, indicating the very good performance of this three parameters model for representing the experimental data.

Sobukola and Olatunde (2001) compared the performances of the Peleg model and of the Zugarramurdi and Lupin model in representing values of salt gain of African catfish treated osmotic solutions. Both models adequately predict the values of salt gain. However, the performance of the Peleg model (R^2=0.930-0.999) was better than the Zugarramurdi and Lupin model (R^2=0.847-0.996).

3.5 FINAL CONSIDERATIONS

The mass transfter of pre-cooked mussel meat treated in brines and marinades is strongly affected by salt and acetic acid concentrations on the solution. The salt gain is mainly affected by solution salt concentration, while the water gain is affected by both the salt and the acid concentrations.

The mussel meat treated in marinades showed negative water gain in all cases. On the other hand, only samples treated in brines with high salt concentration (Cs=20 % and 26 %) showed dehydration during processing.

The addition of acetic acid to the brines resulted in a greater modification of the properties and microstructure of the mussel meat. However, both treatments reduced the WHC and modify consistently the microstructure of mussel meat.

Three models were fitted to the experimental data of salt and water gain. The Weibull exponential model was able to describe acccurately the experimental data as well as the Peleg model, except for the range of salt concentration that characterizes the inversion hydration–dehydration. Zugarramurdi and Lupın model tends, in all cases, to underestimate the mass gain at the equilibrium and presented lower regression coefficients, and was not able to predict the salt and water gain of salted and marinated mussels.

From the operational diagrams it is possible to predict, the final concentration of salt and the final moisture of samples subjected to processes of salting and marination under certain given conditions (temperature, agitation level, etc.). These results show that the variables salt concentration in the solution and immersion time can be manipulated to obtain the desired concentration of NaCl and moisture on the final product. Thus, this work presents results that can be useful for comprehending the mass transfer process during the osmotic treatment of pre-cooked mussel meat, which can be used to improve industrial processes.

3.6 REFERENCES

ABU-GHANNAM, N., and MCKENNA, B. The Application of Peleg's Equation to Model Water Absorption During the Soaking of Red Kidney Beans (*Phaseolus vulgaris L.*). **Journul of' Food Enginrrring**, 32, 391-401, 1997.

ADAMS, M. R., and MOSS, M. O. **Food Microbiology** (Third Edition ed.). Cambridge, Uk: RSC Publishing, 2008.

AIDOS, I., LIE, Ø., and ESPE, M. Collagen Content in Farmed Atlantic Salmon (*Salmo salar L.*). **Journal of Agricultural and Food Chemistry**, 47(4), 1440-1444, 1999.

AVEIRO, M., PELLIZZARO, Q. C., AMBONI, R. D., BATISTA, C. R., BEIRÃO, L. H., and BARRETO, P. L. Chemical, microbiological and sensory changes of marinade mussel (*Perna perna*, linné 1758) storage at 4 ºC. **Alimentos e Nutrição**, 18(2), 121-126, 2008.

BARAT, J., GALLART-JORNET, L., ANDRES, A., AKSE, L., CARLEHOG, M., and SKJERDAL, O. Influence of cod freshness on the salting, drying and desalting stages. **Journal of Food Engineering**, 73, 9-19, 2006.

BARAT, J., RODRÍGUEZ-BARONA, S., ANDRÉS, A., and FITO, P. Influence of increasing brine concentration in the cod salting process. **Journal of food science**, 67(5), 1922-1925, 2002.

BARBOSA-CANOVAS, G., and VEGA-MERCADO, H. **Dehydration of Foods.** Chapman and Hall, 1996.

BELLAGHA, S., SAHLI, A., FARHAT, A., KECHAOU, N., and GLENZA, A. Studies on salting and drying of sardine (*Sardinella aurita*): Experimental kinetics and modeling. **Journal of Food Engineering**, 78, 947–952, 2007.

BJORKROTH, J. Microbiological ecology of marinated meat products. **Meat Science**, 70(3), 477-480, 2005.

BOUDHRIOUA, N., DJENDOUBI, N., BELLAGHA, S., and KECHAOU, N. Study of moisture and salt transfers during salting of sardine fillets. **Journal of Food Engineering**, 94, 83-89, 2009.

CADUN, A., CAKLI, S., and KISLA, D. A study of marination of deepwater pink shrimp (*Parapenaeus longirostris*, Lucas, 1846) and its shelf life. **Food Chemistry**, 90, 53-59, 2005.

CASALES, M., CAPACCIONI, M. E., and YEANNES, M. Obtenção dos tempos de equilíbrio e coeficientes de difusão de ácido e de sal para desenhar o processo de marinado de filés de *Engraulis anchoita*. **Ciência e Tecnologia de Alimentos**, 29(4), 933-937, 2009.

CAVALHEIRO, D. **Estudo de alternativas para o processamento de mexilhoes** (*Perna perna*).Florianopolis: Universidade Federal de Santa Catarina, 2010.

COLLIGNAN, A., BOHUON, P., DEUMIER, F., and POLIGNÉ, I. Osmotic treatment of fish and meat products. **Journal of Food Engineering**, 49, 153-162, 2001.

CORZO, O., and BRACHO, N. Osmotic dehydration kinetics of sardine sheets using Zugarramurdi and Lupín model. **Journal of Food Engineering**, 66, 51-56, 2005.

CORZO, O., and BRACHO, N. Application of Peleg model to study mass transfer during osmotic dehydration of sardine sheets. **Journal of Food Engineering**, 75, 535-541, 2006.

CORZO, O., and BRACHO, N. Application of Weibull distribution model to describe the vacuum pulse osmotic dehydration of sardine sheets. **LWT - Food Science and Technology**, 41, 1108-1115, 2008.

CORZO, O., BRACHO, N., and RODRÍGUEZ, J. Comparison of Peleg and Azuara et al. models in the modeling mass transfer during pile salting of goat sheets. **LWT - Food Science and Technology**, 46, 448-452, 2012.

CORZO, O., BRACHO, N., RODRIGUEZ, J., and GONZALEZ, M. Predicting the moisture and salt contents of sardine sheets during vacuum pulse osmotic dehydration. **Journal of Food Engineering**, 80, 781-790, 2007.

CUNHA, L., OLIVEIRA, F., ABOIM, A., FRIAS, J., and PINHEIRO-TORRES, A. Stochastic approach to the modelling of water losses during osmotic dehydration and improved parameter estimation. **International Journal of Food Science and Technology**, 36, 253-262, 2001.

CZERNER, M., and YEANNES, M. Modelling the effect of temperature and lipid content on anchovy (Engraulis anchoita) salting kinetics. **Journal of Food Engineering**, In Press, Accepted Manuscript, 2012.

CHIRALT, A., FITO, P., BARAT, J., ANDRÉS, A., GONZÁLEZ-MARTÍNEZ, C., ESCRICHE, I., et al. Use of vacuum impregnation in food salting process. **Journal of Food Engineering**, 49, 141-151, 2001.

DENG, Y., and ZHAO, Y. Effects of pulsed-vacuum and ultrasound on the osmodehydration kinetics and microstructure of apples (Fuji). **Journal of Food Engineering**, 85(1), 84-93, 2008.

ESCRICHE, I., SERRA, J. A., FITO, P., and RIVERO, E. Estudio de la influencia de la deshidratacion osmotica a vacıo en el salado del bacalao. **Actas del II Congreso Iberoamericano de Ingenierıa de Alimentos**. (CD-Rom).Bahıa Blanca, Argentina, 1998.

FDA. Clostridium botulinum toxin formation. Ch. 13. In **Fish and Fishery Products Hazards and Controls Guidance.** Washington, DC: Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, 2011.

FITO, P. Modelling of vaccum osmotic dehydration of food. **Journal of Food Engineering**, 22, 313-328, 1994.

FITO, P., and CHIRALT, A. Osmotic Dehydration An Approach to the Modeling of Solid Food-Liquid Operations. **Journal of Food Engineering**, 231-257, 1997.

FUENTES, A. **Desarrollo de productos ahumados a partir de lubina (*Dicentrarchus labrax L.*).** Valencia, Spain: Universidad Politecnica de Valencia, 2007.

FUENTES, A., BARAT, J., FERNÁNDEZ-SEGOVIA, I., and SERRA, J. Study of sea bass (*Dicentrarchus labrax L.*) salting process: Kinetic and thermodynamic control. **Food Control**, 19(8), 757-763, 2008.

GALLART-JORNET, L., BARAT, J., RUSTAD, T., U., E., ESCRICHE, I., and FITO, P. Influence of brine concentration on Atlantic salmon fillet salting. **Journal of Food Engineering** , 80, 267–275, 2007b.

GALLART-JORNET, L., BARAT, J., RUSTAD, T., U., E., ESCRICHE, I., and FITO, P. A comparative study of brine salting of Atlantic cod (Gadus morhua) and Atlantic salmon (*Salmo salar*). **Journal of Food Engineering**, 79, 261–270, 2007a.

GARCÍA-PASCUAL, P., SANJUAN, N., MELIS, R., and MULET, A. *Morchella esculenta* (morel) rehydration process modelling. **Journal of Food Engineering**, 72, 346-353, 2006.

GÖKOĞLU, N., CENGIZ, E., and YERLIKAYA, P. Determination of the shelf life of marinated sardine (*Sardina pilchardus*) stored at 4 °C. **Food Control**, 15(1), 1-4, 2004.

GOLI, T., BOHUON, P., RICCI, J., TRYSTRAM, G., and COLLIGNAN, A. Mass transfer dynamics during the acidic marination of turkey meat. **Journal of Food Engineering**, 104, 161-168, 2011.

JIDEANI, V., and MPOTOKWANA, S. Modeling of water absorption of Botswana bambara varieties using Peleg's equation. **Journal of Food Engineering**, 92, 182-188, 2009.

KE, S., HUANG, Y., DECKER, E., and HULTIN, H. Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle. **Meat Science**, 82(1), 113-118, 2009.

KHECHINASHIVILI, N., JANIN, J., and RODIER, F. Thermodynamics of the temperature induced unfolding of globular proteins. **Protein Science**, 4, 1315-1324, 1995.

KILINC, B., and CAKLI, S. Chemical, microbiological and sensory changes in thawed frozen fillets of sardine (*Sardina pilchardus*) during marination. **Food Chemistry**, 88, 275–280, 2004.

KILINC, B., CAKLI, S., CADUN, A., DINCER, T., and TOLASA, S. Chemical, microbiological, sensory and color changes in warty wenus (*Venus verrucosa)* flesh during marination. **Journal of Muscle Foods**, 19(4), 385-398, 2008.

LAURITZSEN, K., AKSE, K., JOHANSEN, A., JOENSEN, S., SØRENSEN, N., and OLSEN, R. Physical and quality attributes of salted cod (*Gadus morhua L.*) as affected by the state of rigor and freezing prior to salting. **Food Research International**, 37, 677-688, 2004.

LAWRIE, R. **Ciencia da carne** (sixth ed.). Porto Alegre: Artmed, 2005.

LEMOS, A., NUNES, D., and VIANA, A. Optimization of the still-marination process of chicken parts. **Meat Science**, 52, 227-234, 1999.

LEROI, F., JOFFTAUD, J., ARBOLEYA, J., AMARITA, F., CRUZ, F., IZURIETA, E., ET AL. Hurdle technology to ensure the safety of seafood products. In: T. Bùrresen, **Improving seafood products for the consumer** (pp. 400-405). Cambridge, England, 2008.

MACHADO, M. F., OLIVEIRA, F. A., and CUNHA, L. M. Effect of milk fat and total solids concentration on the kinetics of moisture uptake by ready-to-eat breakfast cereal. **International Journal of Food Science and Technology**, 34 (1), 47-57, 1999.

MANCEBO, M., TREVIÑO, M., FERRAN, E., and ESPINOSA, J. Pattern of the mantle adenylate cyclase activity during the reproductive cycle of the female *Mytilus galloprovincial*. **General and comparative endocrinology**, 86(2), 184-188, 1992.

MARTINEZ-ALVAREZ, O., and GOMEZ-GUILLEN, M. The effect of brine composition and pH on the yield and nature of water-soluble proteins extractable from brined muscle of cod (*Gadus morhua*). **Food Chemistry**, 92, 71-77, 2005.

MASKAN, M. Effect of processing on hydration kinetics of three wheat products of the same variety. **Journal of Food Engineering**, 52, 337-341, 2002.

MUJAFFAR, S., and SANKAT, C. The mathematical modeling of the osmotic dehydration of shark fillets at different brine temperatures. **International Journal of Food Science and Technology**, 40, 1-12, 2003.

NGUYEN, M. V., ARASON, S., THORARINSDOTTIR, K. A., THORKELSSON, G., and GUDMUNDSDOTTIR, A. Influence of salt concentration on the salting kinetics of cod loin (*Gadus morhua*) during brine salting. **Journal of Food Engineering**, 100, 225-231, 2010.

OFFER, G., and TRINICK, J. On the mechanism of water holding in meat – the swelling and shrinking of myofibrils. **Meat Science**, 8, 245–281, 1983.

OLSSON, G., OFSTAD, R., B, L. J., and OLSEN, J. Changes in water-holding capacity of halibut muscle during cold storage. **LWT - Food Science and Technology**, 36(8), 771-778, 2003.

PELEG, M. An empirical model for the description of moisture sorption curves. **Journal of Food Science**, 53, 1216–1219, 1988.

PIGOTT, G. M., and TUCKER, B. **Seafood: effects of technology on nutrition.** (362, Trad.) New York: Marcel Dekker, 1990.

POLIGNE, I., and COLLIGNAN, A. Quick Marination of Anchovies (*Engraulis enchrasicolus*) Using Acetic and Gluconic Acids. Quality and Stability of the End Product. **LWT - Food Science and Technology**, 33(3), 202-209, 2000.

REICHARDT, K. **Processos de Transferência no Sistema Solo-Planta-Atmosfera**. 4ª ed. rev. Campinas, Fundação Cargill, 466p. 1985.

SACCHETTI, G., GIANOTTI, A., and DALLA ROSA, M. Sucrose–salt combined effects on mass transfer kinetics and product acceptability. Study on apple osmotic treatments. **Journal of Food Engineering**, 49(2-3), 163-173, 2001.

SALLAM, K., AHMED, A., ELGAZZAR, M., and ELDALY, E. Chemical quality and sensory attributes of marinated Pacific saury (*Cololabis saira*) during vacuum-packaged storage at 4 °C. **Food Chemistry**, 102, 1061–1070, 2007.

SCHMIDT, F. **Estudo das trocas de masa durante o tratamento de cortes de peito de frango com soluçoes salinas**. Florianopolis: Universidade Federal de Santa Catarina, 2006.

SCHMIDT, F. C., CARCIOFI, B. A., and LAURINDO, J. Application of Diffusive and Empirical Models to Hydration, Dehydration and Salt Gain During Osmotic Treatments of Chicken Breast Cuts. **Journal of Food Engineering**, 91, 553-559, 2009.

SCHMIDT, F., CARCIOFI, B., and LAURINDO, J. Efeito da impregnação a vácuo na transferência de massa durante o processo de salga de cortes de peito de frango. **Ciência e Tecnologia de Alimentos**, 28, 366–372, 2008a.

SCHMIDT, F., CARCIOFI, B., and LAURINDO, J. Salting operational diagrams for chicken breast cuts: hydration–dehydration. **Journal of Food Engineering**, 88, 36-44, 2008b.

SHENDERYUK, V., and BYKOWSKI, P. Salting and marination of fish. In: Z. Sikorski, **Seafood: resources, nutritional composition and preservation** (pp. 147-162). Boca Raton, FL: CRC Press. INC, 1990.

SIGURGISLADOTTIR, S., SIGURDARDOTTIR, M., TORRISSEN, O., VALLET, J., and HAFSTEINSSON, H. Effects of different salting and smoking processes on the microstructure, the texture and yield of Atlantic salmon (*Salmo salar*) fillets. **Food Research International**, 33(10), 847-855, 2000.

SKIPNES, D., LUND ØSTBY, M., and HENDRICKX, M. A method for characterising cook loss and water holding capacity in heat treated cod (*Gadus morhua*) muscle. **Journal of Food Engineering**, 80(4), 1078-1085, 2007.

SOBUKOLA, O., and OLATUNDE, S. Effect of salting techniques on salt uptake and drying kinetics of African catfish (*Clarias gariepinus*). **Food and bioproducts processing**, 89, 170-177, 2001.

SOPADE, P., AJISEGIRIB, E., and BADAU, M. The Use of Peleg's Equation to Model Water Absorption in Some Cereal Grains During Soaking. **Journal of Food Engineering**, 15, 269-283, 1992.

TURAN, H., SÖNMEZ, G., ÇELIK, M. Y., YALÇIN, M., and KAYA, Y. Effects of different salting process on the storage quality of Mediterranean mussel (*Mytilus galloprovincialis* L. 1819). **Journal of Muscle Foods**, 18, 380-390, 2007.

TURHAN, M., SAYAR, S., and GUNASEKARAN, S. Application of Peleg model to study water absorption in chickpea during soaking. **Journal of Food Engineering**, 53, 153-159, 2002.

VOLPATO, G., MICHIELIN, E., FERREIRA, S., and PETRUS, J. Kinetics of the diffusion of sodium chloride in chicken breast (*Pectoralis major*) during curing. **Journal of Food Engineering**, 79, 779–785, 2007.

YUSOP, S., KERRY, J., and KERRY, J. Effect of marination time and low pH on marinade performance and sensory acceptability of poultry meat. **Meat Science**, 85, 657-663, 2010.

ZUGARRAMURDI, A., and LUPIN, H. M. A model to explain observed behavior on fish salting. **Journal of Food Science**, 45, 1305–1311, 1980.

4. THERMAL PROCESSING OF MUSSEL MEAT IN RETORT POUCHES

4.1 INTRODUCTION

The use of retort pouches (RP_s) for the heat treatment of foods represents an interesting alternative to the traditional packaging materials as can and glass. The RPs are flexible or semi-rigid pouches formed by assembling different material films which are laminated together giving good mechanical and thermal resistance, optimal gas barrier and efficient sealing properties (HOLDSWORTH and SIMPSON, 2007).

The RPs offer many advantages when compared to the traditional materials, such as the rapid heat penetration during the thermal treatment, which can reduce the processing time and result in better nutritional and sensorial characteristics of the processed food, the easiness to store, transport and open. On the other hand, this kind of packaging requires particular attention on the thermal process parameters control. Moreover RPs cannot be used with some products, particularly when the shape of the food must be conserved because of the lack of structural support.

Although the application of RP for retorted seafood is widely diffused in many countries, in Brazil this packaging is found in the market only for tuna-based products. However, scientific literature have reported results on the use of retort pouch packaging for different fish and shellfish species, as tuna (CRISTIANINI, 1998), fried mussel meat (BINDU et al., 2004), clam (BINDU et al., 2007), salmon (BYUN et al., 2010), among others. Cavalheiro (2010) studied the thermal treatment for the cooking and pasteurization of raw mussels in retort pouches evaluating different combinations of temperature and time, obtaining a product with microbiological stability of 21 days under refrigeration.

The pre-cooked mussel meat remains industrially unexplored in Brazil. In fact, most of the production is consumed near to the farming area and presents few commercialization alternatives. In this context, the investigation presented in this chapter had the main objective of studying the thermal processing and the physicochemical stability on storage at room temperature of mussel meat in retort pouches. The specific objectives were to:

- Study the thermal process parameters of mussel meat in RPs;
- Study the mechanical properties of the RPs;

- Study the effect of the retort temperature on both the yield and the cook value of the processed mussels;
- Study the effect of salt and acetic acid on the yield and on the physicochemical properties of the product during storage at 25 °C.

4.2 LITERATURE REVIEW

4.2.1 Thermal processing

The modern era of thermal food preservation began in 1810 in France, when Nicholas Appert invented the art of canning, in order to gain a prize offered by the French Directoire. Appert heat-treated perishable food in glass jars, thus making one of the most important advances in the history of food processing. The British responded directly to this development when Donkin and Hall began to can foods using tin plate instead of glass. In 1813 they set up the first commercial canning factory in Bermondsey, London. At this time, the reasons of the stability of heat treated foods were not completely understood. Actually, Appert in his book *"Art of Conserving all kinds of Animal and Vegetable Matter for several Years*" justified the conservation from the elimination of air from the product. This fact caused many problems in the next 50 years, until another French scientist, Louis Pasteur, established the relation between microbiological activity and food deterioration (ADAMS and MOSS, 2008). In this way, the two words employed on thermal processes aiming food stability took names related to these two great Figures: pasteurization and appertization (commercial sterilization).

The objective of the pasteurization process is the inhibition of all the vegetative pathogens cells and a large amount of the spoiling microorganisms and enzymes. The lowest combination between time and temperature ($T \leq 100$ °C) is used to achieve the correct microbiological inactivation with the minimal thermal damage of treated foods. This process is ineffective against thermophilic microorganisms and spores. However, it is possible to extend the shelf life of a pasteurized product applying other safety factors, as refrigeration, modified atmosphere packaging, addition of preservative compounds, reduced a_w, low pH and high NaCl concentration (RAY and BHUNIA, 2008).

The food appertization, or more correctly, commercial sterilization, is the process for which a large amount of thermophilic microorganisms and spores are inactivated and that allows a high

stability of the product at ambient temperature. In the Codex Alimentarius Commission (1993), the commercial sterility is defined as *"the absence of microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during manufacture, distribution and storage."*

After definition of the thermal treatment to which a canned food may be submitted, the choice of the packaging material assumes a great importance. It must be adequately chosen to perform the following basic functions (PAGE, EDWARDS, and MAY, 2003):

- Preserve and protect the product;
- Resist to chemical actions of the product;
- Resist to the handling and processing conditions;
- Resist to the external environment conditions;
- Have the required shelf display properties at the sales point;
- Allow easy opening and simple/safe product removal;
- Be constructed from recyclables materials.

The most widely used packaging material is the metal can. It can be used both with foods (lacquered tin-free steel or tin-plated steel) and beverages (aluminum or thin steel). This material offers advantages as the lightness, the low production costs and the mechanical resistance and disadvantages as the possibility of internal and external corrosion and interactions with the food. Another material traditionally used, mainly in high quality foods and beverages, is the glass. Glass jars offer consistent advantages, especially considering the appearance of the product and the very low interaction between packaging material and food. On the other hand, this material has also some disadvantages due to its heaviness and to its extreme fragility. Actually it requires a particular and more careful management in the processing and transport operations. Rigid and flexible plastic materials are also used as packaging. In the last two decades, retort pouch (RP) packaging has been gaining a renewed impulse in heat treated foods. Retort pouches are taking popularity over the traditional packaging, due to the better handling, the capacity to withstand thermal processing as pasteurization or sterilization and the possibility of reheating before consumption (HOLDSWORTH and SIMPSON, 2007).

4.2.1.1 Thermal processing parameters

The definition of the target microorganism characteristics is the first step to establish the condition of the heat treatment. The thermal

characteristics of a microorganism are the *D-value* (decimal reduction time), defined as the time at the reference temperature in which the microbial surviving population is reduced of one logarithmic cycle and the *z*-value that refers to the temperature variation required for a one log change of *D-value*.

Through the values mentioned above, it is possible to define the exact process time required to allow the specified lethality desired for the target microorganism, the required *F- value* (F_r).

Normally, the target of the sterilization process is the most heat-tolerant pathogen, *C. botulinum*. In fact, if present the thermo-resistant spore of this microorganism could germinate in low-acid canned product and produce a lethal exotoxin (lethal doses $\approx 10^{-8}$ g) (ADAMS and MOSS, 2008).

The *C. botulinum* strains can be divided into two groups, the proteolytic group and the nonproteolytic group. To the first group belong the most heat resistant strains of *C. botulinum*, the type A and B ($D_{121.1\,°C}$ = 0.21 min). They are generally found in land and occasionally in water. To the second group belongs the *C. botulinum* type E that is relatively heat sensitive ($D_{80\,°C}$ = 3.3 min) but could represent a risk in low acid pasteurized or semi preserved foods because it can grow at low temperature without any modification of the product. This strain is the most commonly found in freshwater and marine environments (ADAMS and MOSS, 2008; FDA, 2011). Although the *C. botulinum* type E is very common in seafood, the types A and B may also be found in fish and shellfish viscera. So the FDA (2011) recommends that *C. botulinum* type A should be assumed as present in any raw fishery product.

The minimum thermal process required to provide safety from the survival of *C. botulinum* type A in low acid food (pH > 4.6) is equivalent to 12*D*. Thus, an adequate sterilization degree is reached when the temperature of 121.1 °C is maintained for at least 2.52 min (FAO, 1988). However, in common industrial processes the temperature of the product change from the room temperature to the retort temperature during the heating step and the reverse in the cooling step. Therefore, it is mandatory to define the real lethality of each sterilization process studied, calculating the *F-value* of the process (F_0 for the *C. botulinum* and F^{z}_{Tref} for other microorganisms) (WILHELM, SUTER, and BRUSEWITZ, 2005; FDA, 2011).

4.2.1.1.1 Thermal process evaluation

Different methods to calculate the process *F-value* (F^{z}_{Tref}) have been developed during the last century. The method, that is still today the most used, was proposed in 1920 by Bigelow et al. and is known as "*General method*". The experimental basis of this method is the heat penetration curve measured in the slowest heating point of the packaging (SP). This method is extremely simple and useful and is based on the graphical calculation of the integral presented in the Equation 4.1.

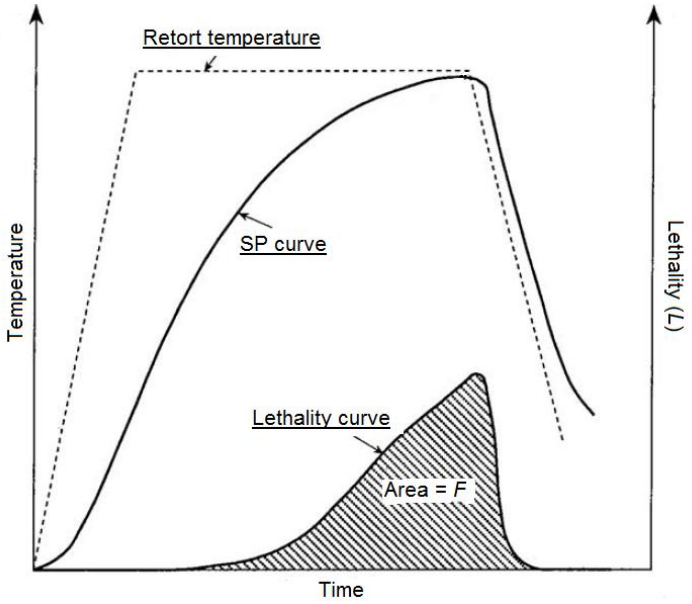
$$F^{z}_{Tref} = \int_{t_0}^{t_t} L^{z}_{Tref}\, dt \tag{4.1}$$

in which *L* is the quantitative measure of the inactivation rate at a given temperature, and is defined as the lethality (Equation 4.2).

$$L^{z}_{Tref} = 10^{(T-T_{ref})/z} \tag{4.2}$$

in which *T* is the temperature in the SP of the packaging and T_{ref} is the reference temperature of the target microorganism. The Figure 4.1 shows an example of the heat penetration curve and the relative lethality curve.

Figure 4.1 - Example of a heat penetration curve measured in the SP and of the lethality curve.



Source: modified from Holdsworth and Simpson (2007).

The choice of the optimum target F_r for a product is mandatory to combine the food safety with good sensory attributes. Actually, it was demonstrated (BRODY, 2003) that shorter heat treatments allow lower product degradation, providing an improvement of the food general quality.

The thermal treatment has a considerable effect on the microorganism population as well as on the general quality of the treated food (nutrients, texture, flavor, *etc*.). Mansfield (1962) introduced the *C*-value (cook value) (Equation 4.3) that is the parameter currently used to quantify the quality degradation of a thermal processed food.

$$C_{Tref}^{z} = \int_{t_0}^{t_t} 10^{(T-T_{ref})/z_c} dt \qquad (4.3)$$

in which z_c is the thermal destruction rate, analogue to the microbiological inactivation *z*-value.

Many substances as proteins, enzymes, vitamins as well as sensory factors can be used as reference of *C*-value (HOLDSWORTH and SIMPSON, 2007). For convenience, the most used reference substance to calculate the *C*-value is the Vitamin B1-thiamine (z_c = 33.1 °C and T_{ref} = 100 °C).

The effect of the heat treatment on the nutritional quality of proteins and amino acids was studied by García-Arias et al. (2004) in canned tuna during three year of storage. The authors found no differences between the different heat treatments studied. On the other hand, the sterilization time-temperature combination showed a considerable influence in the free fatty acid profile in canned albacore and salmon (PEREZ-MARTIN et al., 1988; RODRÍGUEZ et al., 2009).

4.2.1.2 Retort Pouch

Food companies are gradually replacing metallic cans and others rigid packaging by retort pouches and a wide variety of ready-to-eat foods are being manufactured by several multinational companies using these pouches. Particularly, Japan, USA and some countries of EU have traditionally used retort pouches in food industry (HOLDSWORTH and SIMPSON, 2007). In the Brazilian market, the use of RPs for sterilized products is relatively new and not much developed. Actually, just few products are found, as meat, beans, tuna fish, sauces and some other ready-to-eat products.

The first application of the retort pouch concept dates back to the 1940s, when shelf-stable packaging was examined for military rations, although it was given up basically because of mechanical and technological problems presented by this kind of packaging. In the last two decades, the appeal for the RP grow up, for many reasons, including changes in consumer lifestyles, progress in packaging technology and shortage in metal cans, brought it to a predominant position (BRODY, 2003).

Kebede et al. (1996) studied the heat penetration during the thermal treatment of three different RPs and a can using a model system formed by a betonite suspension with ascorbic acid. In this investigation, the effect of the heat treatment on the ascorbic acid, including in the storage period were also evaluated. The process time was of 32 min for the pouches and 52 min for the cans, without significative differences between the diferent pouches. The ascorbic acid retention was 83 % in pouches and 75 % in cans. During storage, the

ascorbic acid content decreased both in can and in RP but the difference between samples did not changed significantly.

The quality of commercial sterilized seafood in can and in RP was compared by Adams and Otwell (1982). In this study, it was demonstrated that heat treated flake tuna, shrimp, crab meat and fish filets packaged in RPs presented higher flavor retention than the canned samples. Only shrimp and fish filets presented texture degradation in RP.

Kirwan and Strawbridge (2003) defined in a complete manner, advantages and disadvantages of retorting in RPs, as follow:

- Advantages:
  - Less energy is required to manufacture pouches compared with cans;
  - Transport of empty containers is cheaper (85 % less space required than cans);
  - Filling lines are easily changed to a different size;
  - Rapid heat penetration and faster process results in better nutrition/flavor;
  - Packed pouch is more compact, requiring about 10 % less shelf space;
  - Less brine or syrup used, pouches are lower in mass and cheaper to transport;
  - Fast reheating of contents by immersion of the package in hot water;
  - Opens easily by tearing or cutting;
  - Ideal for single portion packaging and serving size control;
  - Retort pouch materials are non-corrosive;
  - Convenient for outdoor leisure and military rations use.
- Disadvantages:
  - A major investment is required to achieve equivalent cannery production efficiency;
  - New equipment for filling and processing is required;
  - Production speed on single filler/sealer is usually less than half that of common can sealers;
  - New handling techniques have to be adopted and may be difficult to introduce;
  - Heat processing is more critical and more complex (overpressure control is necessary to limit the pouch expansion);

- For rapid heat penetration there are limitations on pouch dimensions;
- Some form of individual outer wrapping is usually required, adding costs;
- Being non-rigid packaging some product can lost their shape;
- Being a new concept, education of the consumer, as correct storage and use is required during marketing.

One of the most important parameter that affect the complexity of the retort processing is the over pressure control. Indeed, during the retort processing, if the system overpressure is not correctly controlled, a volumetric expansion of the pouch content (blow up) may occur. This phenomenon may strongly affect the temperature distribution inside the pouch and finally the effectiveness of the thermal process. Moreover, it could cause lack of integrity of the physical structure of the pouch. In order to avoid any of the mentioned problems, the pressure difference between the inner side of the pouch and the retort should be less than 0.1 atm. The adequate value of the overpressure may be established adding 0.2-0.3 atm to the water vapor pressure at the product temperature. This statement can be accepted only if the residual air content in the pouch is less of the 2 % of the total pouch content (BINDU, RAVISHANKAR, and SRINIVASA GOPAL, 2007; DAL BELLO, 2007).

RPs are produced by a lamination process in which different films are stick together. These films can be made of: PET (Polyethylene terephthalate), PA (Polyamide or Nylon), Al (Aluminum), PET alox (PET with aluminum oxide), PET siox (PET with siliceous oxide), PP (Polypropylene - with sealing properties) and other polyolefins as LDPE (low-density polyethylene), LLDPE (linear low-density polyethylene), HDPE (high-density polyethylene), CPP (cast polypropylene) OPP and BOPP (orientated and biaxially oriented polypropylene) (GOMES DE CASTRO and SÉRGIO POUZADA, 2003; DAL BELLO, 2007). All these materials must be inert, heat sealable, dimensionally stable, physically strong and heat resistant to at least 130 °C for typical process times. They must present a low permeability to light, oxygen and water vapor and have good ageing properties. Others important properties of RP constituents are a balance between toughness and stiffness, aesthetic properties and printability, processability and machineability (balanced surfaced friction), and recyclability (TUCKER and FEATHERSTONE, 2011).

In relation to the product quality, one of the most important characteristics of the RP film is the oxygen permeability. Byun et al.

(2010) tested the effect of various RPs composition on thermal processed vacuum packed salmon filets, considering the sensory quality, lipid oxidation and shelf life. The authors found that oxygen permeability of PET/silicon oxide-coated nylon/CPP increased significantly during the thermal processing and storage, so the salmon packaged in this material had higher lipid oxidation and less acceptability than salmon packaged in PET/aluminum foil/CPP and aluminum oxide-coated PET/nylon/CPP. On the other hand, the mechanical properties of the aluminum foil are worse than to the other two films.

There are basically two format of RPs: pillow pouch and stand-up pouch. The first is the standard pouch used since the beginning of this technology, and is made by heat sealing on three sides, leaving the forth opened to allow the filling operation. Pillow pouches are commonly used for ambient self-stable food with a low amount of liquid fraction, as tuna fish, meat, poultry, beans, among others. The stand-up pouches are designed with a bottom gusset that allows the vertical position of the pouch without external support. It is used principally for dried products, sauces, liquid foods and chilled products. Its manufacture is quite complex and could present problems and stress due to gravity and uneven weight distribution of the content (TUCKER and FEATHERSTONE, 2011).

Basically, the materials and format of the RPs should be chosen following these criteria (GOMES DE CASTRO and SÉRGIO POUZADA, 2003):

- Protection degree needed for the packed food (for example: oxygen and water barrier, resistance retort temperature);
- Careful evaluation of historical data and experiences about the interactions between product, material and machine;
- Expected use of the packaged product, with special attention for the mechanical resistance, size and shape of the packaging;
- Importance of the material for the external graphical project.

The RPs use was widely studied with commercial sterilized traditional Indian culinary preparations of clam (BINDU, RAVISHANKAR, and SRINIVASA GOPAL, 2007), anchovy (BINDU et al., 2010), mackerel (SRINIVASA GOPAL et al., 2001) and tilapia (DHANAPAL et al., 2010). The comparison among the quality of prawn ‘kuruma’ (Indian white shrimp) sterilized in RPs and in cans was also studied. The difference in the process time (RPs resulted in 35.67 % and 56.56 % reduction in process time compared with cans, depending on

the size) showed the lower quality of the canned product, particularly on color, firmness, hardness, chewiness and overall acceptability (MOHAN et al., 2008).

Bindu et al. (2004) developed a processing method to prepare ready-to-eat fried mussel meat packed in retort pouch. The mussel meat was seasoned, fried and vacuum packed in retort pouch and thermal treated with an F_0 of 9.6 min and $C_{100\,^{\circ}C}^{33.1\,^{\circ}C}$ of 90 min. Physicochemical and sensorial properties were analyzed during 12 months of storage at room temperature. The results showed that the product maintained a good quality during the analyzed period and it could represent an interesting alternative to the common mussel trade in India.

4.3 MATERIALS AND METHODS

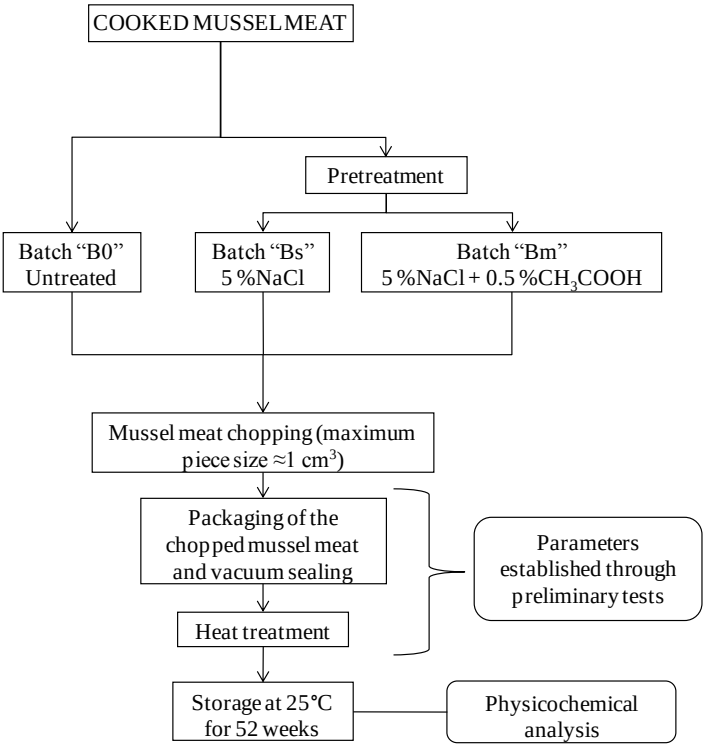
4.3.1 Raw material

The pre-cooked mussel meat used in this study was purchased in a local industry (Cavalo Marinho S.A., Palhoça (SC), Brazil). In the industrial plant, the raw mussels were cooked for 5 min in steam at 100 °C, chilled until 8 °C and then manually separated from the shells. The separated mussel meat was transported to the laboratory in 5 kg bags covered with ice and processed within 24 h.

4.3.2 Experimental method

The overall procedures used in this study are sketched in Figure 4.2.

Figure 4.2 - Flow chart representing the whole mussel treatment, from the industry to heat-treated product.



4.3.2.1 Sample preparation

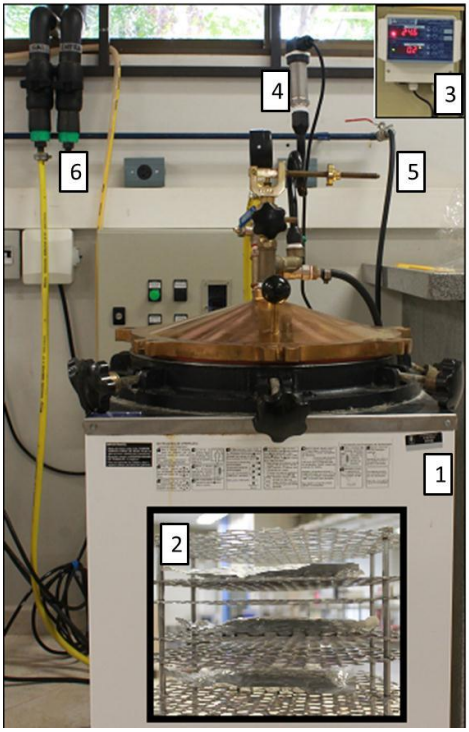
Pre-cooked mussel meat was divided in three batches (12 kg each). One batch was immersed for 30 min in brine with 5 % NaCl (batch "Bs"). Other batch was immersed for 30 min in a marinade with 5 % NaCl and 0.5 % acetic acid (batch "Bm").The batch "B0" was processed without any pretratement.

The final concentration of additives (salt and acetic acid) was, in the batch "Bs" of approximately 2 % NaCl and in the batch "Bm" of approximately 2 % NaCl and 0.2 % acetic acid. These additive concentrations are characteristic of canned shellfish (ADAMS and OTWELL, 1982). The results presented in Chapter 3 were used to determine the pretreatment parameters, in order to obtain the desired concentration of salt and acetic acid in the chopped mussel meat. After pretreatements, the mussels were manually chopped with a knife and then carefully weighed, vacuum packed in RPs and thus heat treated. Samples used in all preliminary tests were prepared as the batch "B0".

4.3.2.2 Experimental device

A vertical discontinuous retort (AV-50, Phoenix, Brazil) was adapted for the RP processing (Figure 4.3). Forty litres of water were used as heating medium. The original power of the retort electrical resistance (3000 W) was increased up to 4500 W, substituting one of the two original resistances with one of double electrical power. The temperature was controlled with a control system (Climflex PLUS, Expectron, Brazil) connected to a thermocouple positioned on the bottom of the retort and to both resistances. To manage the pressure during processing, a compressed air line were connected to the cover of the retort. A cool water line was also connected to the cover or the retort to allow the cooling of the system at the end of the sterilization process.

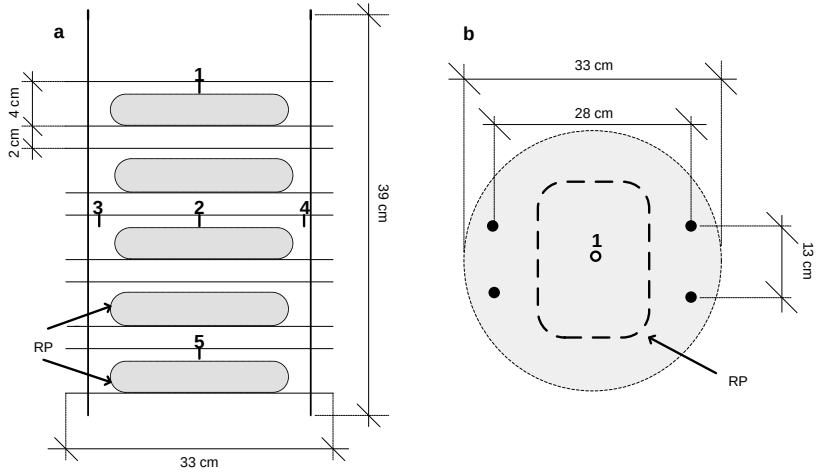
Figure 4.3 - Experimental device. 1- retort; 2-tray with pouches; 3-retort controller; 4- pressure transducer; 5- pressurized air pipe line; 6-cooling water line.



Source: Author.

Stainless steel perforated trays were built (CAVALHERO, 2010) to hold the pouches (Figure 4.4), maintaining the good circulation of hot water during the process. The retort was pre-tested in fully loaded operational conditions (including tray and filled RPs) to verify the uniformity of the temperature distribution and the presence of cold points. Thus, five calibrated thermocouples, T-type (IOPE, TX-TF-TF-R-32AWG, IOPE, Brazil), connected with a data logger (34970A, Agillent, Malaysia) were distributed in strategic positions, indicated by the numbers 1 to 5 in Figure 4.4. Three complete cycles were carried out. All thermocouples were calibrated against an ASTM mercury-in-glass thermometer (Incoterm, Brazil)

Figure 4.4 - Lateral (a) and superior (b) view of the tray with the RPs. The numbers 1 to 5 indicate the position of the thermocouples during the temperature distribution tests.



Source: Author.

4.3.2.3 Retort pouches

The retort pouches used in this study were acquired from the company ICB packaging (ICB, São Paulo, Brazil) and produced by the Korean KSP (KSP, Seoul, Korea). The characteristics of the RPs were:

- Composition: PET 12 µm, Al 9 µm, BOPP 15 µm, CPP 80 µm;
- Size: 190 mm x 240 mm;
- Seal length: 170 mm.

4.3.2.3.1 Filling and sealing

Preliminary tests were carried out in order to determine the optimum filling quantity and vacuum level. The optimal amount of mussels was defined basing on the producers recommendation and verifying that the RP content did not interfer with the sealing operation and did not modify the shape of the pouch. Thus, approximately 400 g of chopped mussel meat was considered as optimum filling quantity. The optimum vacuum level was established testing different vacuum times, according to the characteristics of the vacuum sealer (Selovac 200b adapted with a bi-active sealing system, São Paulo, Brazil). The

suitable heat sealing condition (sealing time) was reached testing the sealing process empirically. The seal quality was visually evaluated following the procedure proposed by Oliveira and Alves (1992) apud Cristianini (1998), which characterizes five different seal quality level.
Degree 1 (D1): no appreciable defects:
Degree 2 (D2): little creasing covering until 50 % of the sealed surface;
Degree 3 (D3): little creasing or irregularities covering between 50 % and 100 % of the sealed surface;
Degree 4 (D4): medium creasing or irregularities on 100 % of the sealing surface and twinkle loss;
Degree 5 (D5): strong creasing or irregularities in on 100 % of the sealing surface and total twinkle loss.

4.3.2.3.2 Retort pouch quality control

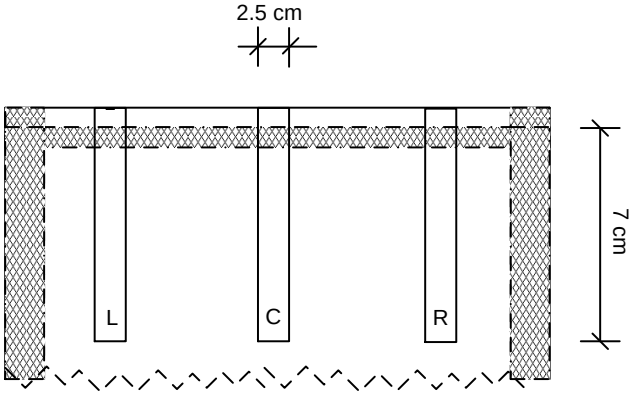
A careful visual evaluation of filled and sealed RPs was performed before and after the heat treatment (CFIA, 2002). The evaluation considered:

- The inspection of each seal for any visible evidence of product liquor or defects;
- The inspection of the RPs and the seals for signs of creep or delamination;
- The inspection of evidence of volumetric expansion of the pouch content (blow up), due to lack of overpressure after the thermal treatment.

4.3.2.3.2.1 Mechanical seal test

Tensile strength of the RP sealed area was measured using a texturometer (Stable Micro System, mod. TA.XT *Plus*, UK), following the method described by ASTM (1988). From each analyzed RP, three sections of the sealed area (2.5 cm in width and 7 cm in length) were taken (Figure 4.5) Samples were conditioned for 24 h at 25 °C and 50 ± 5 % relative humidity before the tests. The rate of loading was 25 mm/min. The force required to pull the seal apart was recorded in N/m of width.

Figure 4.5 - Measurement locations for seal strength tests.

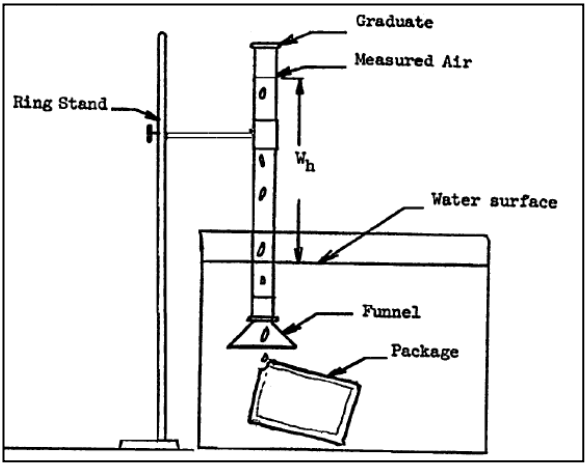


Source: Adapted from CFIA (2002).

4.3.2.3.2.2 Residual Air Test

The residual air content of the vacuum sealed RP was evaluated to verify the effectiveness of the vacuum packaging. The filled and sealed RPs were submitted to low pressure (15 kPa) for 15 s, in the vertical position, with the aim to enforce the residual air to go to the upper section of the pouch. Then, following the method reported in CFIA (2002), the RP was held under water below a funnel attached to a graduate cylinder. The pouch was cut in one of its corners and the air was squeezed out into the funnel (Figure 4.6).

Figure 4.6 - Device used to determine residual air in the pouches.



Source: CFIA (2002).

The amount of residual air was corrected through the Boyle's law as follows (Equation 4.4)

$$V_1 = \frac{(P_a - W_h) * V_m}{P_a} \quad (4.4)$$

in which V_1 is the volume of air at atmospheric pressure (mL), P_a the atmospheric pressure (mmHg), W_h the pressure of water level in graduated cylinder (mmHg) and V_m the volume of measured air (mL).

4.3.2.4 Thermal processing

During the process, the overpressure was maintained at approximately 0.3 atm above of the vapor pressure of water at the retort temperature. In the cooling stage, the overpressure was maintained at approximately 0.3 atm above the temperature of the slowest heating point of the RP to avoid problems with the pouches such as the volumetric expansion of the pouch content (blow up). Before treatment, the filled RPs were conditioned for 30 min in water at room temperature to set the product temperature at ≈20 °C, to uniform the initial temperature of the product.

- Definition of the heat treatment parameters

Although, in the canning industry a F_0 lower than 10 min is rarely used, in tuna fish (CRISTIANINI, 1998) and shrimp (MALLICK et al., 2010) packaged in RPs, a F_0 of ≈7 min was reported as adequate, allowing an optimal commercial sterility, maintaining an acceptable quality of the product. In mussels, a F_r for *C. botulinum* of 7 min appears as adequate considering that the product is very sensible to long heat treatments and that the initial contamination is relatively low (due to the pre-cooking operation).

Tests were carried out to define the total process time that was determined as the heating time (including come-up time) and cooling time required to achieve an F_0 of about 7 min, calculated using the *General method*. The optimal retort temperature was determined by testing the conditions reported in Table 4.1 and evaluating the process $C_{100\,°C}^{33.1\,°C}$ (C_T) and the product yield.

Table 4.1 - Preliminary test conditions.

Test	Initial retort temperature (°C)	Final retort temperature (°C)
a	20	110
b	100	110
c	100	118
d	100	121

- Storage study

The RPs filled with chopped mussel meat from the three batches (B0, Bs, Bm) were heat processed for 57 min (come up time of 19 min, cook time of 23.5 min and cooling time of 14.5 min) at the retort temperature of 118 °C (test C). After the thermal process, the samples were stored at 25 °C.

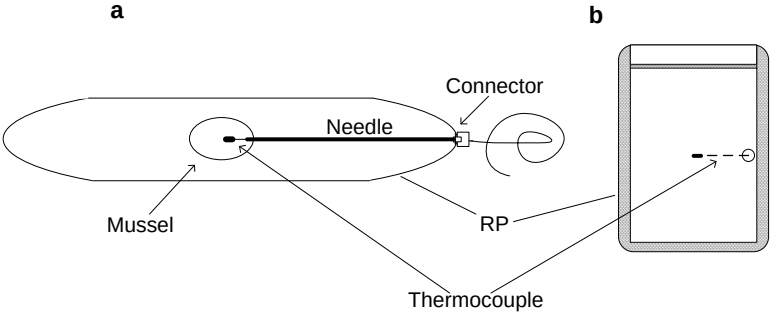
4.3.2.5 Thermal treatment parameters

The calculation of the process lethality was based on results of heat penetration curves, considering the *C. botulinum* type A as the target microorganism.

For the measurement of the retort temperature during processing, two calibrated T-type thermocouples were positioned on the tray (position 1 and 3 Figure 4.4). For the measurement of the temperature at the slowest heating point of the RP, a calibrated T-type thermocouple

was inserted into a needle (80 mm) and fixed on the RP with a Teflon connector developed by Cavalheiro (2010) and sealed with heat resistant silicone. The thermocouple tip was inserted into an entire mussel positioned in the geometric center of the pouch (Figure 4.7). Thermocouple outputs were recorded every 10 seconds by a data logger (34970A, Agillent, Malaysia) connected to a CPU.

Figure 4.7 - Lateral (a) and frontal (b) view of the RP with the thermocouple.



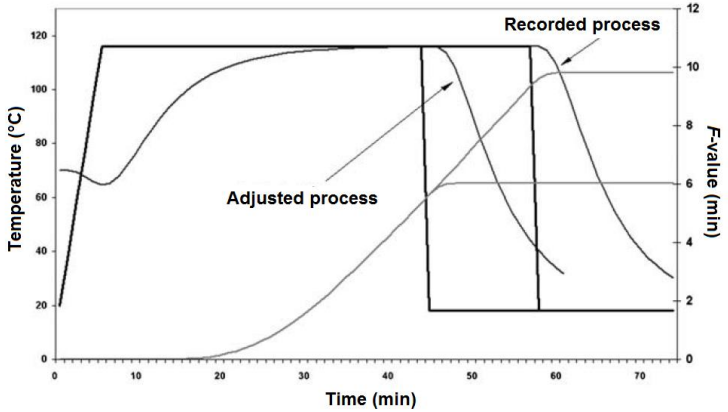
Source: Author.

The heat penetration curve was repeated minimum 4 times for every condition tested (one time to estimate the total processing time and three times to verify the repeatability of the processing conditions).

The *General method* was used to calculate the F_0. In this method the area under the lethality curve is calculated using the trapezoidal rule. The process F_0 was set at about 7 min using the method reported by Simpson, Almonacid, and Teixeira (2003), called *Revisited General Method*. The heat penetration tests for which the final lethality is bigger than the required lethality were included in the "Case 1" of this method (Figure 4.8). In this case, the processing time has to be shortened to find a new processing time.

The cook value "C_T" was also calculated with the *General method* but using thiamine as the reference substance (z_c = 33.1°C and T_{ref} = 100 °C).

Figure 4.8 - Simulated heat penetration curve (*Revisited General Method* - Case 1).



Source: Simpson, Almonacid, and Teixeira (2003).

4.3.3 Physicochemical analysis of the chopped mussel

Before the heat treatment the proximate composition of pre-cooked mussel meat was determined. The protein content was calculated by converting the nitrogen content determined by Kjeldahl's method (6.25×N). The fat content was determined by the method described by the AOAC (1997) using the Soxhlet extractor system. The ash content was determined by ashing the sample in a furnace at 525 °C for 24 h (AOAC, 1997). The sodium chloride content was determined according to the method described in section 3.3.4.4.

Physicochemical analyses of heat treated chopped mussel meat were carried out in duplicate for each batch, after 1 week from the heat treatment and each 4 weeks during 52 weeks on storage.

Summarizing, the following determinations were performed in the storage study:

- Yield;
- Moisture content (Item 3.3.4.1.);
- Water activity (Item 3.3.4.2.);
- pH (Item 3.3.4.3.);
- Water-holding capacity (WHC) (Item 3.3.4.6.);
- Total volatile basic nitrogen (TVB-N);
- Trimethylamine nitrogen (TMA-N);

4.3.3.1 Yield

The chopped mussel meat content of each RP was weighed before the filling operation. When the pouch was selected to the analysis, the exudate was carefully separated from the mussel meat and the yield was calculated by Equation 4.5

$$Yield\ \% = \left(\frac{DW}{IW}\right) \cdot 100 \quad (4.5)$$

in which *IW* is the initial filling weight and *DW* is the weight of the drained product after processing.

4.3.3.2 Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N)

Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) content are usually used to determine the spoilage level of seafood during storage. The determination of TVB-N and TMA-N was carried out following the Conway micro-diffusion method (Conway, 1968 *apud* Özogul and Özogul, 2000). Twenty-five grams of sample were homogenized with 25 mL of trichloroacetic acid 10 % (v/v) in an ultra-turrax® (Ika, T25, Germany). Then, the homogenized was centrifuged (SIGMA Laborzentrifugen GmbH, SIGMA 4-16K, Germany) for 5 minutes at 11630 g. Two milliliters of the obtained supernatant were placed in the outer ring of the micro diffusion Conaway plate. A 1 % boric acid solution containing the Conway indicator was then pipetted into the inner ring. Solid Vaseline was spread of the Conaway plate edge. Then, to initiate the reaction, two milliliters of K_2CO_3 saturated solution were transferred in the Conaway plate outer compartment and the plate was immediately closed with the specific cap. The plate was placed in a convective oven at 36 °C for 2 h.

The TVB-N values were estimated by titration of the content of the inner compartment with 0.01 M HCl solution using a micro-burette. The TMA-N values were estimated using the same method proposed for the TVB-N with the difference that, before the sealing of the plate, 20 drops of formaldehyde solution 35 % (v/v) were distributed in the outer compartment. The values of TVB-N and TMA-N were calculated by the Equation 4.6 and 4.7, respectively.

$$TVB\text{-}N(mg/100g) = \frac{V*M*14*100*(T+X)}{Va*W} \quad (4.6)$$

$$TMA\text{-}N(mg/100g) = \frac{V' * M * 14 * 100 * (T+X)}{Va * W} \quad (4.7)$$

in which *V* is the volume of HCl used in the titration of the TVB-N; *V'* is the volume of HCl used in the titration of the TMA-N; *M* the HCl solution molarity; *14* is the nitrogen equivalent weight; *T* is the volume of trichloroacetic acid used; *X* is the sample moisture content; *Va* is the extract volume analyzed; and *W* is the sample weight.

4.3.4 Microbiological analysis

Total viable count of the pre-cooked mussel meat before thermal processing was determined according to method reported by Vanderzant and Splittstoesser (1992), in order to determine the microbiological status of the product.

4.3.5 Sterility test

The effectiveness of the commercial sterilization was verified carrying out a sterility test after one week and at the end of the storage study (52 weeks). The Brazilian legislation referred to the sterility test in the resolution RDC number 12, 02/01/2001 of ANVISA (2001) (*Agência Nacional de Vigilância Sanitária* - National Health Surveillance Agency). Moreover, the normative instruction number 62, 26/08/2003 of the MAPA (2003) (*Ministério da Agricultura, Pecuária e Abastecimento* - Ministry of Agriculture, Livestock and Supply) defines procedures to the verification of the effectiveness of the sterilization process applied to low acidic food.

Twelve RPs were randomly selected, accurately cleaned and identified. The samples were divided in two groups and incubated at 36 ± 1 ºC for 10 days and at 55 ± 1 ºC for 5 days. The samples were positioned between two filter paper sheets in order to detect any eventual leakage or product loss. At the end of the incubation period the RPs were visually analyzed to detect eventual swelling and product loss. Then, the samples were conditioned at room temperature and opened to verify the presence of off-odors and alteration on the aspect of the product. Following the guideline of the legislation, the pH of the product was measured and the test was considered negative only if the samples did not present any sign of spoilage and if the pH did not present variation higher than 0.2 (ANVISA, 2001; MAPA, 2003).

4.3.6 Statistical analysis

The variance analysis (one-way ANOVA) with probability of the 95 % was performed using the software Statistica® (Statistica 8.0, StatSoft, USA). In case of significant differences ($p < 0.05$) the means were compared using the Tukey test.

4.4 RESULTS AND DISCUSSION

4.4.1 Pre-cooked mussel meat characterization

The proximate composition, water holding capacity, pH and total viable count of the samples used in this study are shown in Table 4.2.

Table 4.2 - Chopped mussel meat characterization (proximate composition, physicochemical properties and total viable count).

Proximate composition (g/100 g)	
Moisture	77.69±0.98
Proteins	14.67±0.805
Lipids	1.94±0.09
Ash	1.99±0.12
Physicochemical parameters	
WHC (g H_2O/ g dry matter)	2.37±0.06
pH	6.63±0.05
Microbiological analysis	
Total viable count (Log CFU/g)	3.97±0.12

Each value represents the mean ± standard deviation of at least triplicate.

The results of the proximate composition (moisture content, protein, lipids and ash) of mussels used in this study were similar to those reported by the literature to the same species (PARISENTI, TRAMONTE, and FACCIN, 2008; LIMA, 2010).

The pH is an important quality parameter of shellfish. In contrast to what is common in the *post mortem* degradation processes of fish, for which the pH tends to increase due to microbiological and enzymatic activity, in mussels the pH decrease due to the high concentration of glycogen. The values found for chopped cooked mussels were high and

consistent with the findings of other authors for the same product (SALAN, 2005; CAVALHEIRO, 2010; FURLAN et al., 2011).

The microbiological quality evaluation of the product used in this study was estimated through the total viable count. The Brazilian legislation does not establish limits of TVC for shellfish, but for the European legislation (CEE, 2005a) the legal limit of TVC for seafood commercialization is of 10^6 CFU/g. Thus, it was possible to consider that the mussels used in this study had a good microbiological quality.

4.4.2 Retort pouch properties

The optimum performance of the sealing condition was found with 30 seconds of vacuum and 5 seconds of heat sealing time (D1 on the seal quality level scale (Item. 4.3.2.3.1)). Higher vacuum levels caused leakage of liquid from RP contaminating the sealing area and causing sealing defects. For the established conditions, the residual air content was of 1.92 ± 0.35 mL, resulting in 0.5 % - mL air/100 g of sample. Bindu et al. (2007) reported that an amount of residual air inferior to 2 % indicates the effectiveness of vacuum, which avoids the blow up.

The results of the heat seal strength test recorded in three sections of the sealed area are shown in Table 4.3.

Table 4.3 - Heat seal strength test.

	Values (N/m)*
Central section (C)	3930 ± 350
Right section (R)	4148 ± 178
Left section (L)	3969 ± 208

*Mean ± standard deviation of 4 readings.

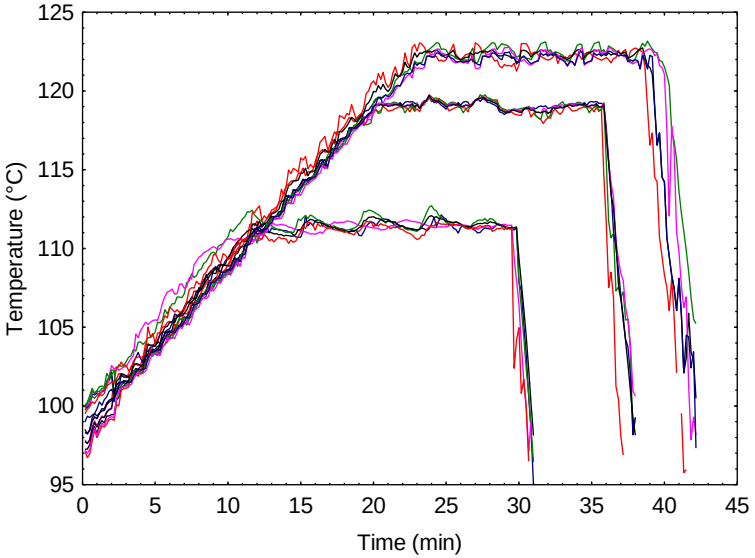
Lampi (1980) established that the value of 3000 N/m of width is the minimum requirement of heat seal strength. The retort pouches used in this study presented higher heat seal strength, which was satisfactory for resisting the heat treatment in the overpressure retort.

4.4.3 Thermal processing

Preliminary tests were carried out to evaluate the performance of the retort. Figure 4.9 shows the temperature data recorded by the five thermocouples used to perform the temperature distribution test. The

temperature distribution was tested at all the reference temperature used in this study considering only temperature above 100 °C.

Figure 4.9 - Temperature distribution test for the tested conditions. Thermocouple 1 (—), *2* (—), *3* (—), *4*(—), and 5(—).



When the retort reaches the set point temperature (cook period), the maximum difference between the thermocouples was 0.9 °C. The FDA (1997) reported that for water immersion retorts, during the cook period, it is normally expected to find all thermocouples at or above the set point temperature and the difference between the minimum and maximum recorded temperature should be no greater than 1.1 °C.

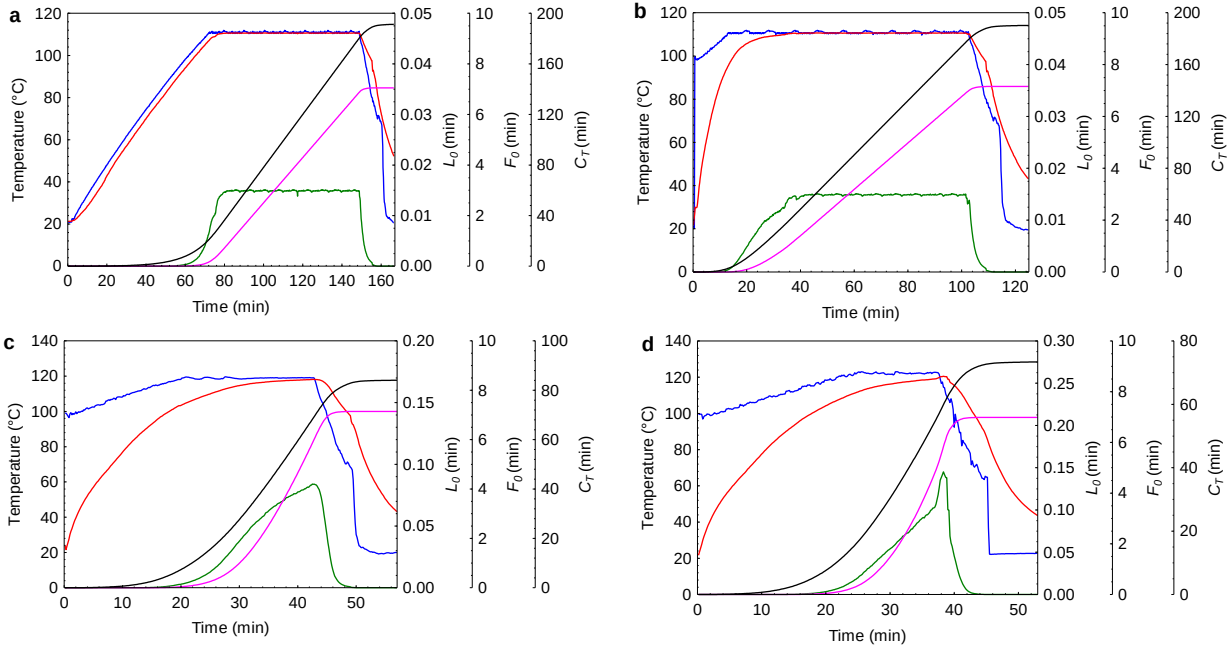
Although the temperature distribution test did not show any cold point in the retort, it was preferred to place the RP with the thermocouple in the highest position of the tray, considering that the cooling phase could be faster in that position and that the overpressure air line inlet is placed in the cover of the retort.

The results of the heat penetration into RPs filled with 400 g of chopped mussel, at the retort temperatures of 110 °C, 118 °C and 121 °C are presented in Table 4.4 and in Figure 4.10, for initial retort temperatures of 20 °C and 100 °C.

Table 4.4 - Results from process calculations at the retort temperature of 110, 118, and 121 °C.

Test	Initial retort temperature (°C)	Final retort temperature (°C)	Come-up time (min)	Cook-Period (min)	Total process time (min)	F_0 (min)	C_T (min)
a	20	110	71	82	165	7.07± 0.05	197.9± 0.3
b	100	110	13	92	117	7.07± 0.09	194.5± 3.1
c	100	118	19	23.5	57	7.10± 0.04	84.3± 1.9
d	100	121	22	15	53	7.04± 0.11	72.9± 2.1

Figure 4.10 - Heat penetration characteristics for retort pouches in the retorting conditions a, b, c, and d. Slowest heating point temperature (—), retort temperature (—), L_0 (—), F_0 (—) and C_T ($C_{100\,°C}^{33.1\,°C}$) (—).



The cook value is a measurement of the heat treatment with respect to nutrient degradation and textural changes that occur during processing. A C_T of 100-200 min is considered a range beyond of which the food quality is impaired (AWUAH, RAMASWAMY, and ECONOMIDES, 2007). It can be seen that in the processes "a" and "b", for which the retort temperature was set at 110 °C, the C_T was high. The difference between the cooking values was not affected by the initial retort temperature. On the other hand, the processes "c" and "d", with retort temperatures of 118 °C and 121 °C, presented adequate cook values (less than 100 min).

The yields of the product processed at different retort temperatures are presented in Table 4.5.

Table 4.5 - Yield of chopped mussel meat processed at the retort temperature of 110, 118, and 121 °C.

Test code	Yield (%)
a	73.5±2.2
b	73.4±1.5
c	78.1±1.3
d	78.2±0.9

The yield was significantly affected by the retort temperature. The yield of the processes "a" and "b" was on average 6.5 % lower than the yield of the processes "c" and "d".

The process "c" was chosen for the storage study, since the value of yield and C_T were better than in the processes "a" and "b". The process "d" was discarded because of difficulties in the overpressure control and cooling water injection caused by the high pressure reached inside the retort during processing.

4.4.4 Storage study

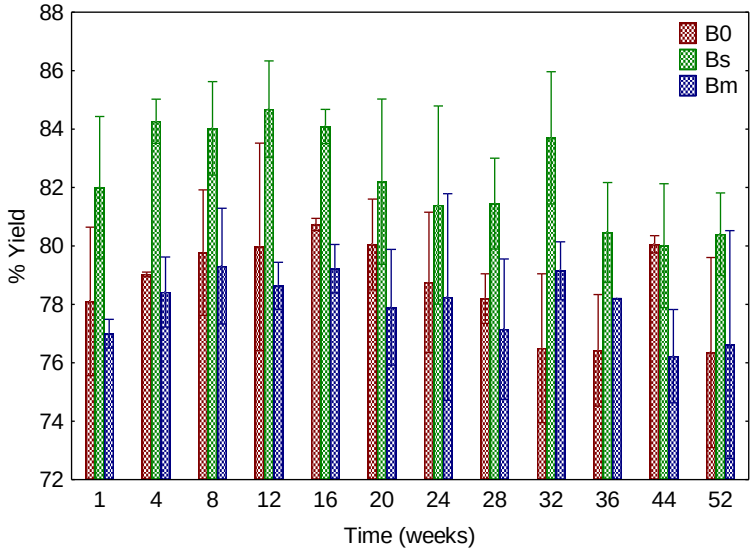
4.4.4.1 Yield and WHC monitoring during storage

During retorting, chopped mussel meat lose water and other water-soluble substances, because of the heat-induced protein denaturation. The amount of this liquid loss is affected by pre-treatments, retorting conditions and salt concentration (ALMONACID et al., 2012).

The changes in yield of chopped mussel meat for the batches B0, Bs and Bm stored at 25 °C are shown in Figure 4.11. The product lost

about 20 % of its weight in liquids by exudation after processing. Moreover, the yield for chopped mussels of the batch "Bs" was significantly ($p < 0.05$) higher than the yield of the batches "B0" (≈5.5 %) and "Bm" (≈6.5 %) for the 52 weeks of storage.

Figure 4.11 - Changes in total yield (% mass) of chopped mussel meat (B0, Bs, and Bm) on storage at 25 °C.



Kong et al. (2008) reported that salt addition reduced cook loss in thermally processed salmon fillets. This study reported that the addition of salt at a concentration of 1.5 % resulted in a consistent reduction in cook loss when compared to control samples having no salt added. The authors supposed that salt solubilize proteins, resulting in increased protein–protein and protein–water interactions. These interactions could bring to a gelation process after the retort processing

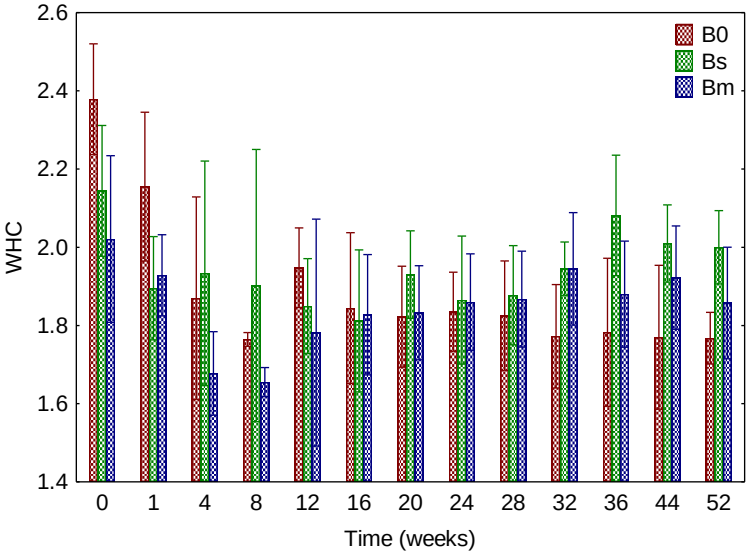
The positive effect of salt on the yield was limited when the acetic acid was added to the product (Bm) during the pre-treatment. Aidos, Lie and Espe (1999) proved that salmon collagen is particularly soluble in dilute acids, which could explain the low yield of product pre-treated in brine with 0.5 % of acetic acid.

The Figure 4.12 shows the WHC of the chopped mussel meat before and after the retort processing on storage at 25 °C. The values of WHC before processing were influenced by the pre-treatment,

confirming the results reported in item 3.4.7. The heat treatment caused the reduction (≈9 %) of the WHC values in all batches.

During the storage period, the WHC of the chopped mussel meat remained practically constant and no significant differences were found among the three different batches. Only in the weeks 36, 44 and 52 the WHC of the batch Bs showed a tendency of higher values in respect to the other two batches.

Figure 4.12 - Changes in WHC of chopped mussel meat (B0, Bs, and Bm) during storage at 25 °C.



Almonacid et al. (2012) studied the yield and the water holding capacity (WHC) of canned mussel meat. An experimental design was used to test various precooking times (1, 4, 7 min), various retort temperatures (110, 116, 122 °C) and various filling brine NaCl concentrations (1, 2, 3 % NaCl). Total process yield was obtained as a measurement of total loss of water from the mussel meats occurring during the precooking and retort sterilization processes, because of heat-induced protein denaturation. The best results were obtained with a retort temperature of 116 °C, with precooking time of 4 min and a 2 % NaCl in the salt solution.

4.4.4.2 Changes of the physicochemical parameters during storage

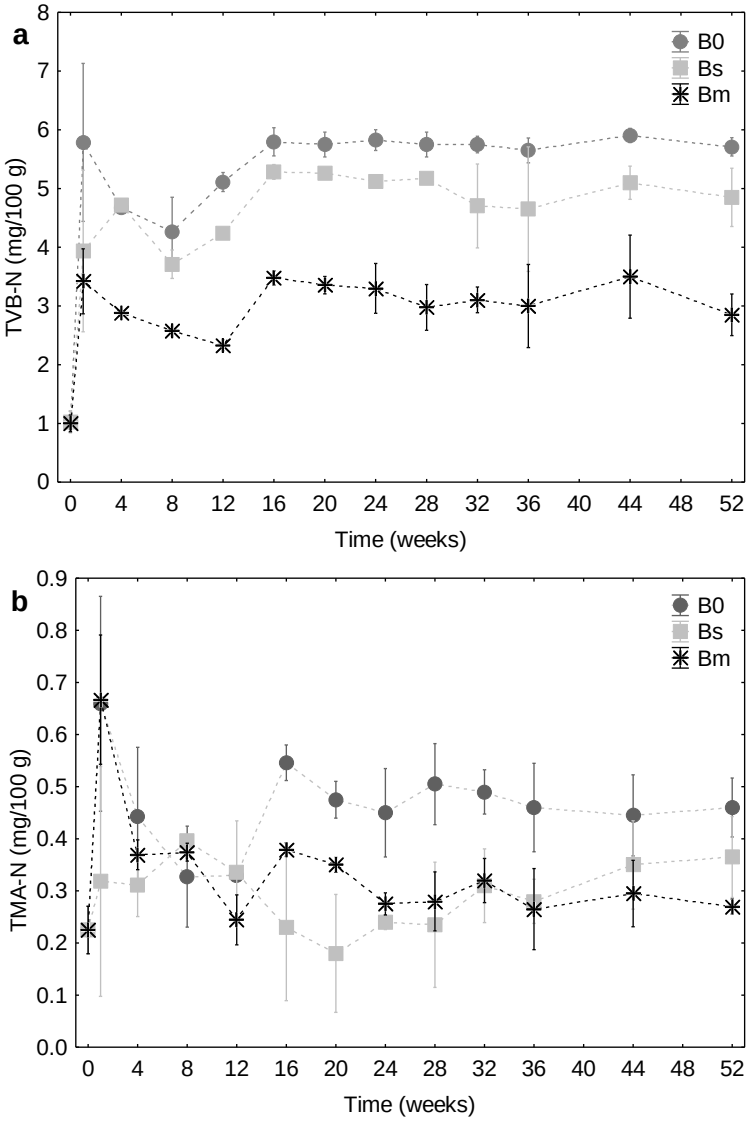
The changes of TVB-N of chopped mussel meat stored at 25°C are shown in Figure 4.13a. The TVB-N of the chopped mussel meat at the day 0 (untreated mussels) was of 1.01 mg N/100 g. After the thermal treatment the values increased significantly. On the other hand, during storage, the values of TVB-N remained rather stables.

TVB-N is used to determine the spoilage level of seafood during storage. Its value is affected by the species, catching season, region, age and sex of mussels (TURAN et al., 2007). Although acceptability limit set by the brazilian legislation is 30 mg N/100 g. Dalgaard (2000) asserts that these limits can change greatly depending on the fish species. In fact, the TVB-N content of 75 mg N/100 g was found in herring with good sensorial quality, while the content of 10-20 mg N /100 g of canned shrimp were found inappropriate to the consumption. In any case the results found in the present study were largely lower than the cited limits.

The changes of TMA-N of chopped mussel meat stored at 25 °C are shown in Figure 4.13b. Before the treatment the TMA-N value was of 0.22 mg N/100 g of sample. The TMA-N content increased slightly with the thermal treatment and remained at low levels during the storage period. TMA-N results from the reduction of TMA-N oxide by bacterial activity and partly by intrinsic enzymes and is often used as an index of quality of marine fish (PÉREZ-VILLARREAL and POZO, 1990). In fact, trimethylamine (TMA), trimethylamine oxide and N,N-dimethyl-formamide are known to contribute to ammonia odour.

TMA can be produced from TMAO by thermal breakdown during the cooking and sterilisation steps (RODRÍGUEZ et al., 2009). The sterilization process did not show a great influence on the TBA-N, probably because of the initial pretreatment. In any case, the values found in this study are lower than the value of 5–10 mg N/100 g sample that is considered as an acceptable limit for fish consumption (SHENDERYUK and BYKOWSKI, 1990).

Figure 4.13 - Changes TVB-N and TMA-N of chopped mussel meat (B0, Bs, and Bm) on storage at 25 °C.

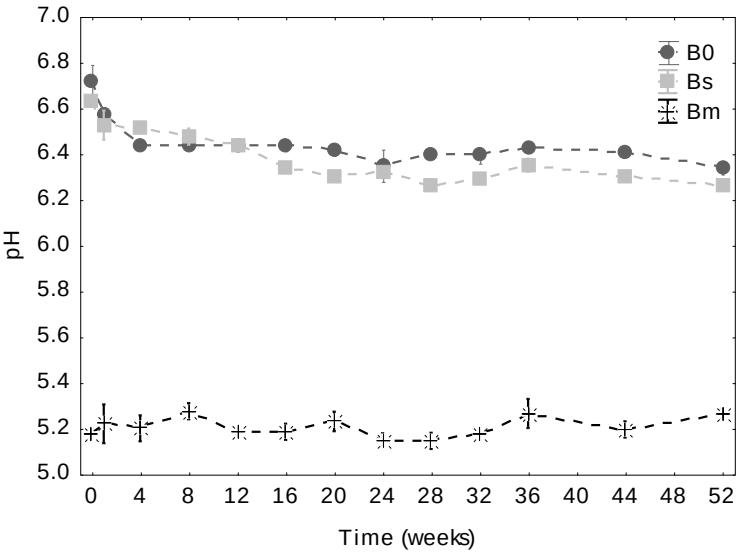


The changes of the pH during storage at 25 °C of chopped mussel meat are presented in Figure 4.14. Before processing, the pH of the batches B0 and Bs were respectively of 6.72 and 6.63. The pH of the

batch Bm was 5.18, due to the acidification occurred on the pretreatment. After the heat treatment, the pH decreased slightly in the batches B0 and Bs and remained constant in the chopped mussels of the batch Bm. After the first week of storage, the pH values remained practically constant for the whole storage period with values of 6.39 ± 0.05 for the batch B0, 6.37 ± 0.09 for the batch Bs and 5.22 ± 0.09 for the batch Bm.

The pH of the marinated batch is 5.22, largely higher than the limit for *C. botulinum* (pH < 4.6) indicating that the heat treatment realized was necessary to allow a safe storage at room temperature.

Figure 4.14 - Changes in pH of chopped mussel meat (B0, Bs, and Bm) on storage at 25 °C.



4.4.5 Sterility test

The sterility test is based on the verification of the germination and growth of thermophilic spores at the temperature of 36 ± 1 ºC and 55 ± 1 ºC (ANVISA, 2001). This test represents an essential tool to verify the commercial sterility of the retorted product. Indeed, the spoilage of low acid foods is basically caused by thermophilic sporeformes microorganisms. These microorganisms can cause three

types of deterioration of the canned product: the flat sour spoilage, the thermophilic anaerobic spoilage, and the sulfide spoilage. The flat sour spoilage is caused by the growth of the *B. stearothermophilus*. It does not affect the aspect of the packaging but the product present a pH markedly lowered and may present off odors and sometimes a cloudy liquor. The thermophilic anaerobe spoilage is caused by the growth of the *C. thermosaccharolyticum*. It produces gas and causes the relative swelling of the pouch. The product commonly presents fermented, sour, cheesy or butyric odor. The sulfide spoilage is caused by *Desulfotomaculum nigrificans*. This microorganism does not cause any swelling of the packaging but the product present a darkened aspect with rotten egg odor due to the production of high amount of H_2S (JAY, 2000; FDA, 2001).

A sterility test was carried out with the samples (RPs) elaborated in this study. From the visual analysis of retort pouches after the incubation period (36 ± 1 ºC for 10 days and 55 ± 1 ºC for 5 days) any swollen or product loss was found both for the samples stored for 1 and 52 weeks. After the conditioning at room temperature, the RPs were opened and the content was visually analyzed looking for off-odors or changes of the normal aspect of the retorted chopped mussel meat, then the pH was measured. The analyzed samples did not present any visual or olfactory sign of spoilage. The maximum variation of the pH between the incubated samples and the non incubated samples (showed in Figure 4.14, week 1 and week 52) was of 0.07, 0.02, and 0.09 for the batches B0, Bs and Bm, respectively, for samples incubated at 36 °C and of 0.05, 0.06, and 0.08 for the batches B0, Bs, and Bm, respectively, for samples incubated at 55 °C, for the test realized at the first week of storage. In the test realized at the week 52 differences of 0.03, 0.08, and 0.05 were found for the batches B0, Bs and Bm, respectively, for samples incubated at 36 °C and of 0.06, 0.07, and 0.06 for the batches B0, Bs, and Bm, respectively, for samples incubated at 55 °C.

According to the Brazilian legislation (ANVISA, 2001), the sample can be considered commercially sterile since the pH did not presented changes higher than 0.2, from the pH of the samples before the incubation and the product did not presents any other sign of spoilage.

4.5 FINAL CONSIDERATIONS

The retort pouches used in this study showed good sealing properties and an optimal performance during the retort processing.

The modified retort was appropriate to process the retort pouches at all the tested temperatures. Only at the temperature of 121 °C, the overpressure control was uneasy because of the equipment limitations.

The product processed at the retort temperatures of 118 °C and 121 °C presented better yields and cook values than the product processed at the temperature of 110 °C. The retort temperature of 118 °C was selected as optimal.

The yield of the product was affected by the pre-treatments. In particular the salting of the mussel meat increased the value of yield respect to the marination pre-treatment and to the control batch.

The retorted products were physicochemically stables for one year on storage at 25°C, and the results of the sterility test confirm that the applied thermal process was sufficient to avoid any microbial growth in the product.

4.6 REFERENCES

ADAMS, J. P., and OTWELL, W. S. **Suitability of seafood to retort pouch processing**. University of Florida, Department of Food Science and Human Nutrition, Gainesville, Florida, 1982.

ADAMS, M. R., and MOSS, M. O. **Food Microbiology** (Third Edition ed.). Cambridge, Uk: RSC Publishing, 2008.

AIDOS, I., LIE, Ø., and ESPE, M. Collagen Content in Farmed Atlantic Salmon (Salmo salar L.). **Journal of Agricultural and Food Chemistry**, 47(4), 1440-1444, 1999.

ALMONACID, S. B., SIMPSON, R., URTUBIA, A., PINTO, M., and TEIXEIRA, A. Commercially Sterilized Mussel Meats (Mytilus chilensis): A Study on Process Yield. **Journal of Food Science**, 77(6), 127-135, 2012.

AOAC. **Official Methods of analysis** 16th Ed. Arlington, Virginia.: Association of Official Analytical chemists, 1997.

AWUAH, G., RAMASWAMY, H., and ECONOMIDES, A. Thermal processing and quality: principles and overview. **Chemical Engineering and Processing**, 46, 584–602, 2007.

BIGELOW, W. D., BOHART, G. S., RICHARDSON, A. C., and BALL, C. O. **Heat penetration in processing canned foods**. Bulletin No. 16L, 1920.

BINDU, J., RAVISHANKAR, C. N., SRINIVASA GOPAL, T. K., and MALLICK, A. K. Investigation of shelf life and heat penetration attributes of ready-to-eat "fish peera" from anchovy (Stolephorous commersoni) in retort pouches. **Journal of Food Processing and Preservation**, 34, 207-222, 2010.

BINDU, J., RAVISHANKAR, C., and SRINIVASA GOPAL, T. Shelf life evaluation of a ready-to-eat black clam (*Villorita cyprinoides*) product in indigenous retort pouches. **Journal of Food Engineering**, 78, 995–1000, 2007.

BINDU, J., SRINIVASA GOPAL, T., and NAIR, T. Ready-to-eat Mussel Meat Processed in Retort Pouches for the Retail and Export Market. **Packaging Technology and Science**, 17, 113-117, 2004.

BRODY, L. A. The return of the retort pouch. **Food technology feature**, 57(6), 76-79, 2003.

BYUN, Y., BAE, H., COOKSEY, K., and WHITESIDE, S. Comparison of the quality and storage stability of salmon packaged in various retort pouches. **LWT - Food Science and Technology**, 43, 551-555, 2010.

CAVALHHEIRO, D. **Estudo de alternativas para o processamento de mexilhoes** (*Perna perna*).Florianopolis: Universidade Federal de Santa Catarina, 2010.

CEE. Reglamento (CE) nº 2073/2005 de la Comisión de 15 de noviembre de 2005 relativo a los criterios microbiológicos aplicables a los productos alimenticios. **Diario Oficial de la Unión Europea**, L338, 1, 2005a.

CFIA, C. F. **Flexible Retort Pouch Defects - Identification and Classification Manual**.Canada, 2002.

CODEX ALIMENTARIUS COMMISSION, C. **Code of hygienic practice for aseptically processed and packaged low-acid foods**. Rome: FAO/WHO, 1993.

CRISTIANINI, M. Estudo da distribuição de temperaturas durante o processo de esterilização de atum (*Katsuwonus pelanis*) em bolsa flexivel. Universidade Estadual de Campinas, **Ciencia dos alimentos**, Campinas (SP), Brazil, 1998.

DAL BELLO, M. **Viabilidade técnico econômica da substituição da lata por "retort pouch".** Escola de Engenharia Mauá do Centro Universitário do Instituto Mauá de Tecnologia, São Caetano do Sul, Brazil, 2007.

DALGAARD, P. **Freshness, quality and safety in seafoods.** Flair-Flow Europe Technical Manual. F-FE 380A/00, 2000.

DHANAPAL, K., BASU, G. R., SHASHIDHAR, S., VENKATESHWARLU, K., and CHOUKSEY, M. Quality of Ready to Serve Tilapia Fish Curry with PUFA in Retortable Pouches**. Journal of Food Science**, 75(7), 348-354, 2010.

FAO. Principles of canning. In D. Warne, **Manual ou fish canning.** Rome: Food and Agriculture Organization of the United Nations, 1988.

FROTT, R., and LEWIS, A**. Canning of meat and fish products.** London, UK.: Chapman Hall, 1994.

GARCÍA-ARIAS, M., NAVARRO, M., and GARCÍA-LINARES, M. Effects of different thermal treatments and storage on the proximate composition and protein quality in canned tuna. **Archivos Latinoamericanos de Nutrición**, 54(1), 112-117, 2004.

GOMES DE CASTRO, A., and SÉRGIO POUZADA, A. **Embalagens para a industria alimentar.** Lisboa: Istituto Piaget, 2003.

HOLDSWORTH, D., and SIMPSON, R. **Thermal Processing of Packager Foods** (Second Edition ed.). Springer, 2007.

KEBEDE, E., MANNHEIM, C. H., and MILTZ, J. Heat Penetration and Quality Preservation during Thermal Treatment in Plastic and Metal Cans. **Journal of food engineering**, 30, 109-115, 1996.

KIRWAN, M., and STRAWBRIDGE, J. Plastics in food packaging. In: R. Coles, D. McDowell, and M. Kirwan, **Food packaging technology** (pp. 224-232). CRC press, 2003.

KONG, K., J., T., LIN, M., and RASCO, B. Thermal effects on chicken and salmon muscles: Tenderness, cook loss, area shrinkage, collagen solubility and microstructure. **LWT - Food Science and Technology**, 41, 1210–1222, 2008.

LIMA, M. **Avaliação das condições de processamento de mexilhões *Perna perna* pré-cozidos e resfriados.** Florianopolis: Universidade Federal de Santa Catarina, 2010.

LAMPI, R. Retort pouch: The development of a basic packaging concept in today's high technology era. **Journal of Food Process Engineering**, 1-18, 1980.

MALLICK, A., SRINIVASA GOPAL, T., RAVISHANKAR, C., and GEETHALAKSHMI, V. Changes in instrumental and sensory properties of indian white shrimp in curry medium during retort pouch processing at different F_0 values. **Journal of Texture Studies**, 41, 611-632, 2010.

MANSFIELD, T. **High-temperature-short-time sterilisation**. 4, p. 311. London: Gordon and Breach, 1962.

MOHAN, C. O., RAVISHANKAR, C. N., SRINIVASA GOPAL, T. K., and BINDU, J. Thermal processing of prawn 'kuruma' in retortable pouches and alluminium cans. International **Journal of Food Science and Technology**, 43, 200-207, 2008.

ÖZOGUL, F., and ÖZOGUL, Y. Comparision of Methods Used for Determination of Total Volatile Basic Nitrogen (TVB-N) in Rainbow Trout (*Oncorhynchus mykiss*). **Turk J Zool**, 24, 113-120, 2000.

PAGE, B., EDWARDS, M., and MAY, N. Metal cans. In: R. Coles, D. McDowell, and M. Kirwan, **Food Packaging Technology** (pp. 120-151). CRC press, 2003.

PEREZ-MARTIN, R., FRANCO, J., AUBOURG, S., and GALLARDO, J. Changes in free amino acids content in albacore. **Zeitschrift für Lebensmitteluntersuchung und -Forschung A**, 185(5), 432-435, 1988.

RAY, B., and BHUNIA, A. **Fundamental of food microbiology**. Fourth edition. New York: CRC press, 2008.

RODRÍGUEZ, A., CARRILES, N., GALLARDO, J. M., and AUBOURG, S. P. Chemical changes during farmed coho salmon (*Oncorhynchus kisutch*) canning: Effect of a preliminary chilled storage. **Food Chemistry.** 112, 362-368, 2009.

SHENDERYUK, V., and BYKOWSKI, P. Salting and marination of fish. In: Z. Sikorski, **Seafood: resources, nutritional composition and preservation** (pp. 147-162). Boca Raton, FL: CRC Press. INC, 1990.

SIMPSON, R., ALMONACID, S., and TEIXEIRA, A. Bigelow's General Method Revisited: Development of a New Calculation Technique. **Journal of Food Science**, 68(4), 1324-1333, 2003.

SMITH, P. **Introduction to Food Process Engineering** (Second Edition ed.). New York: Springer, 2011.

SRINIVASA GOPAL, T., VIJAYAN, P., BLACHANDRAN, K., MADHAVAN, P., and IYER, T. Traditional Kerala style fish curry in indigenous retort pouch. **Food Control**, 12, 523-527, 2001.

TUCKER, G., and FEATHERSTONE, S. **Essential of thermal processing**. Oxford, UK: Wiley-Blackwell, 2011.

TURAN, H., SÖNMEZ, G., ÇELIK, M. Y., YALÇIN, M., and KAYA, Y. Effects of different salting process on the storage quality of Mediterranean mussel (*Mytilus galloprovincialis* L. 1819). **Journal of Muscle Foods**, 18, 380-390, 2007.

WILHELM, L., SUTER, D., and BRUSEWITZ, G. **Food and Process Engineering Technology**. Amer Society of Agricultural, 2005.

5. EVALUATION OF DIFFERENT DEHYDRATION METHODS OF COOKED MUSSELS MEAT

5.1 INTRODUCTION

Food dehydration is an important achievement in the human history. One of the oldest drying methods is the air drying, in which a product is exposed to an air flow at a higher temperature. Although dehydrated products present increased shelf life in respect to the original product, they present reduced nutritional and sensorial quality in comparison to the raw material (RATTI 2001; FIGIEL, 2009).

In the last century, the application of vacuum drying processes were introduced to improve the quality of dried products and to reduce drying time. Indeed, the reduction of the water vapour pressure reduces the process temperature, facilitates the moisture evaporation and, in this way, the time required to reach a desired moisture content. In the vacuum drying, the heat energy required to vaporize water of the product is normally provided by conduction and radiation. Recently, other heating systems have been studied to improve the efficiency of the vacuum drying process, keeping the high quality of the products. The application of microwave heating during vacuum drying, for example, has shown good results with fruit, vegetables (DROUZAS and SCHUBERT, 1996; SUNJKA, 2003; FIGIEL, 2009; BAI-NGEW, THERDTHAI, and DHAMVITHEE, 2011; among others) and fish (ZHANG et al., 2007).

When the vacuum drying is carried out at very low pressure and with the food in the frozen state (freeze drying) the product obtained normally has a highly porous structure and recovers the original shape, taste and aroma when rehydrated (IBARZ and BARBOSA-CÁNOVAS, 2003). In literature, only few studies on the freeze drying of seafood were found (DONSÌ, FERRARI, and DI MATTEO, 2001; SARKARDEI and HOWELL, 2007; CRAPO et al., 2010; DUAN et al., 2010). Moreover, studies of freeze drying rates, important to the optimization of the process, are commonly carried out discontinuosly with a limited number of experimental data. The use of an online weighting system integrated to the freeze dryer could represent an useful tool for the investigation of the drying curve, as reported by Barresi et al. (2009) and Menlik, Özdemir, and Kirmaci (2010).

As the pre-cooked mussel meat is extremely perishable, the dehydration seems to be an interesting alternative to increase its shelf life. In this context, it is of great importance to study the drying of pre-

cooked mussel meat. For this reason different drying processes were investigated in this study, aiming to obtain high quality pre-cooked mussel meat. The specific objectives of this section were to:

- Modify a commercial freeze drying equipment to record continuously the sample weight and to control temperatures of the sample-holder plate;
- Study the drying rates of the freeze drying, vacuum drying and convective drying at different process conditions;
- Study the rehydration rates of the products dried with the three methods listed above and the resulting water holding capacity.

5.2 LITERATURE REVIEW

5.2.1 Drying

The removal of moisture from foods is one of the most ancient food preservation techniques. It has been used by the humanity since prehistoric times. When the water content and water activity are reduced to low levels, the microbiological growth and other deteriorative reactions are reduced during the storage period, thus promoting longer shelf life (IBARZ and BARBOSA-CÁNOVAS, 2003).

Many drying technologies are available at the industry. Basically, the drying processes may be divided into two large groups: in-air (atmospheric) and in-vacuum drying. The most diffused and traditional process is the air-drying. In this process the moisture content is reduced raising the temperature, reducing the relative humidity of the gas and increasing the gas flow. The vacuum drying started to be used in the first half of the twentieth-century. In this method the moisture removal is facilitate by the pressure gradient and is preferable when the presence of air and high temperatures should be avoided, due to undesirable degradation of nutritive and functional properties of the processed product (e.g. freeze drying, conductive vacuum drying, microwave vacuum drying, among others) (CHEN and MUJUMDAR, 2008).

5.2.1.1 Principles of drying

Commonly, the studies of the drying processes are carried out through the evaluation of the variation of the product weight during processing. To understand the behavior of a material during drying, the mass variation must be represented as moisture content. The moisture content of a dried product is defined as the ratio between the water

content of the food and the weight of the dry matter (dry basis moisture content) (Equation 5.1) (BARBOSA-CANOVAS and VEGA-MERCADO, 1996):

$$X_t = \frac{W_t - W_{dm}}{W_{dm}} \quad (5.1)$$

in which X_t is the moisture content expressed as g water/g dry sample, W_t is the total weight of the material at a determined time, W_{dm} is the weight of the dry matter.

The free moisture content (X) is an important parameter that should be known to study a drying process (Equation 5.2):

$$X = X_t - X_\infty \quad (5.2)$$

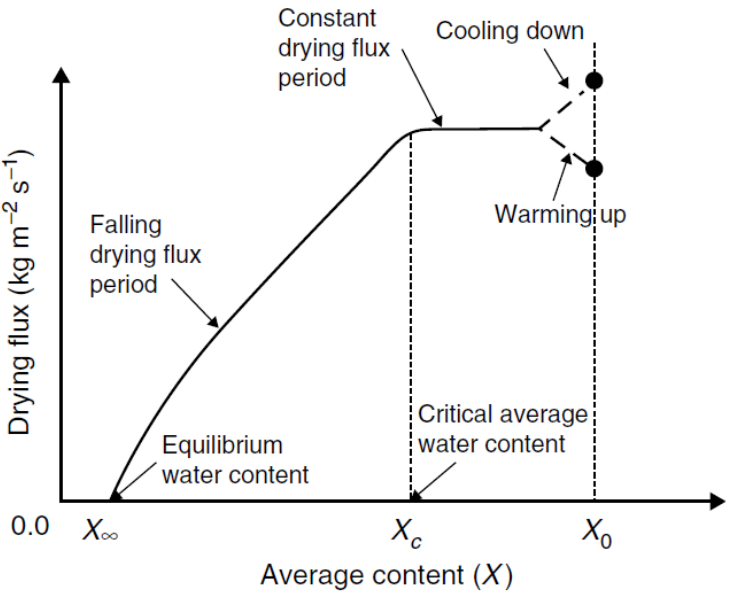
in which X_∞ is the equilibrium moisture content at constant drying conditions, which depends on the air humidity and temperature.

By convention, the drying flux (R) is defined by the Equation 5.3.

$$R = -\frac{W_t}{A}\frac{dX}{dt} \quad (5.3)$$

in which t is the drying time, A is the evaporation area and R is the drying rate [$kg \cdot m^{-2} \cdot h^{-1}$]. If the area is not known, then the drying rate may be expressed in kg water evaporated per hour [$kg \cdot h^{-1}$]. The plot of *R versus X* (or X_t) is the so-called drying rate curve (Figure 5.1).

Figure 5.1 - Drying rate curve.



Source: Chen and Mujumdar, (2008).

This curve represents a drying process of a typical material and can be divided in three characteristics stages. In the early stage of drying, the product temperature rises until reach approximately the wet bulb temperature of the gas. Then, a significant reduction of the moisture content will occur at constant rate. The product temperature remains constant and the unbound water is removed. In this phase the drying rate is principally controlled by the heat transfer to the food surface. When the water in the product surface is not sufficient to maintain the equilibrium between mass and heat transfer, a "critical moisture content" (this value depends on the drying conditions) is reached and the last drying period begins. In this period, the drying rate decreases with time (falling-rate drying period) since the water migration from the interior of the product to the surface is not sufficient to maintain the constant evaporation rate (the internal mass transfer control the drying rate). The drying is completed when the product reaches its equilibrium moisture content that depends on the humidity and temperature of the air (IBARZ and BARBOSA-CÁNOVAS, 2003). Intricate phenomena are involved in this drying period. Basically, water

must move from the interior of the food to the surface, where it evaporates and is removed by the drying air. The movements of water from the interior to the surface are due to capillary forces and concentration gradients. The removal of water from the surface of the food involves the evaporation of water into the air (CHEN and MUJUMDAR, 2008; VAN ARSDEL and COPLEY, 1963 apud IBARZ and BARBOSA-CÁNOVAS, 2003).

The understanding of the heat transfer mechanisms is essential to a better understanding of the drying process. In this section, the basic principles of heat conduction, heat convection and thermal radiation will be briefly presented.

-Heat Conduction:

When a solid material (a plate for example) is submitted to a temperature difference across its faces, this gradient is the driving force promoting the heat transfer from one face to the other. The heat transfer equation is known as Fourier's law (Equation 5.4)

$$q_c = -k\frac{dT}{dx} \tag{5.4}$$

in which q_c (W/m^2) is the heat transfer flux; *dT/dx* (K/m) is the temperature gradient in the *x* direction and *k* is the thermal conductivity (W·k^{-1}·m^{-1}) (WELTY et al., 2001).

- Heat Convection:

The convection heat transfer occurs between a fluid in motion (e.g. air) and a bounding surface when both are at different temperatures (INCROPERA et al., 2006). Considering the nature of the fluid flow, a distinction should be made between forced convection and free convection. In the first a fluid is made to flow past a solid surface by an external agent such as a fan. On the other hand, the free convection occurs when a warmer fluid next to the solid boundary causes circulation due to the density difference resulting from the temperature variation throughout a region of the fluid (WELTY et al., 2001).

The heat flux equation for convective heat transfer is known as Newton´s Law of cooling and is expressed by the Equation 5.5.

$$q_h = h\left(T_s - T_f\right) \tag{5.5}$$

in which q_h (W/m^2) is the convection heat flux; $(T_s\text{-}T_f)$ is the temperature difference between the surface, T_s, and the fluid, T_f, while h is the convective heat transfer coefficient (W/m^2·K)

-Radiation heat transfer:

The radiation is the only heat mechanism that does not need a medium to occur. It is usually predominant at high temperatures and/or under vacuum. The Stefan-Boltzman law (Equation 5.6) describes the radiation heat flow, q_r, between non-black bodies (BIRD, STEWART, and LIGHTFOOT, 1960).

$$q_r = \sigma \frac{(T_{s_1}^4 - T_{s_2}^4)}{1/\epsilon_1 + 1/\epsilon_2 - 1} \tag{5.6}$$

in which σ is the Stefan-Boltzmann constant ($5.676 \cdot 10^8$ W·m^{-2}·K^{-4}), T_s (K) are the temperatures of the surfaces of the two non-black bodies (1 and 2), and ε the material emissivity.

5.2.1.2 Dried product properties

The quality of dried foods is directly related to the amount and activity of water in the product, which affects enzymatic activity, microbial deterioration, texture, viscosity, aroma and taste. For this reason, moisture content, water activity, glass transition and sorption isotherm are important indirect indicators of dried food quality (CHEN and MUJUMDAR, 2008; IBARZ and BARBOSA-CÁNOVAS, 2003).

The knowledge of the moisture content alone is not sufficient to predict the stability of foods. Another very important parameter for determining microbial, enzymatic or chemical activity is the availability of water, measured as water activity (FELLOWS, 2000). This parameter is defined as the ratio of the water vapor pressure in the food and the pure water vapor pressure, at the same temperature (Equation 5.7).

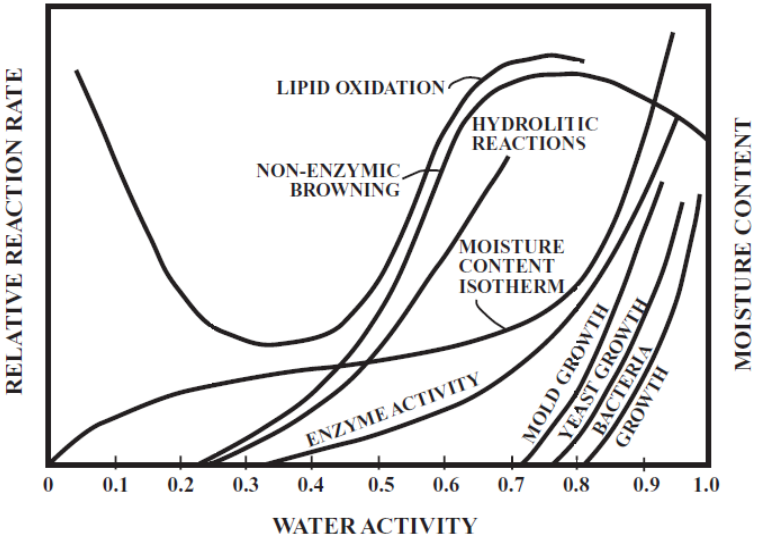
$$a_w = \left[\frac{P}{P_0}\right]_{P,T} \tag{5.7}$$

in which a_w is the water activity, P is equilibrium partial vapor pressure of water in the food and P_0 is the vapor pressure of liquid water at the same temperature.

The relation between a_w, deteriorative reactions and microbial growth has been widely studied. Labuza et al. (1970) developed the food

stability map (Figure 5.2) that indicates qualitatively some reactions as function of a_w. This diagram is a very useful tool to understand the effectiveness of drying upon physicochemical reaction and microbiological growth. Values of water activity lower than 0.90 normally prevent the pathogenic bacteria growth. For other bacteria, molds and yeast the limit of a_w is around 0.60-0.70. Other reactions are also limited by lower levels of a_w as the lipid oxidation, the non-enzymatic browning (Maillard reactions) and enzymatic reactions. However, the critical limits of water activity may also be shifted to higher or lower levels by other factors, such as pH, salt, antimicrobial agents, heat treatment and temperature.

Figure 5.2 - Stability map for reactions as a function of water activity.



Source: Labuza et al. (1970)

Another parameter related with the deterioration mechanisms during processing and the food safety of dried food is the glass transition temperature (T_g). It can be defined as the temperature at which an amorphous system changes from the glassy to the rubbery state (PELEG and CHINACHOTI, 1996). The T_g can be used to establish parameters as processability, quality, stability and safety of the dried food. During drying, the product temperature should be maintained

below the T_g for a specific moisture content, in order to avoid shrinkage. Only freeze-drying process takes place at low temperature but contrarily to the expected, the product temperature is below T_g only at the end of the drying. If only the average moisture content of the product is considered, the absence of shrinkage is not easily explicable. Actually, the portion of the solid that is in the highest temperatures is the dry layer, which has low moisture content and a T_g corresponding closely to that of the dry solids (RATTI, 2001).

The sorption isotherm is the graphical representation of the absolute water content of a food as function of the water activity (equilibrium relative humidity) at a given temperature and when the system has reached the equilibrium (CHEN and MUJUMDAR, 2008). The sorption isotherm can be obtained by the rehydration of a dried sample (adsorption isotherms) or by the dehydration of a wet sample (desorption isotherms). The adsorption isotherm of a completely dried food is obtained subjecting the sample to head-spaces of progressively higher relative humidity (hermetical containers with saturated solutions of various salts) and when the equilibrium is reached the corresponding moisture contents is determined. On the other hand, the desorption isotherm is obtained subjecting the wet sample to progressively lower relative humidity, allowing the moisture loss (BARBOSA-CANOVAS and VEGA-MERCADO, 1996; CHEN and MUJUMDAR, 2008).

5.2.1.2.1 Pre-treatments

Highly perishable foods are usually pre-treated before drying to avoid microbiological and physicochemical degradation during the early stages of the drying process. Cooking, smoking, salting and addition of others preservatives are, among others, the most used pre-treating methods. In particular, cooking before drying could be considered as one of the most important pre-treatments for the dehydration of shellfish. In cooked shellfish the microbiological contamination is considerably lower than in the raw material. Moreover, the microbiological advantage, the formation of a superficial layer (case-hardening) could be avoided by precooking. In fact, in the cooked tissue, the cells normally become more permeable to water diffusion reducing this phenomenon (RAHMAN, 2006).

5.2.1.2.2 Rehydration

The rehydration capacity is one of the most important properties of a dried product. It is a complex process that occurs when the dried foods are immersed in water and it is aimed at the restoration of raw material properties usually before its cooking or consumption (LEWICKI, 1998; RAHMAN and PERERA, 2007).

A very important parameter in the rehydration process is the rehydration capacity or rehydration ratio. This is a value related to the amount of water that is absorbed by the dried product during soaking (LEWICKI, 1998; TAIWO, ANGERSBACH, and KNORR, 2002). Many factors affect the rate of the rehydration of the foods such as the porosity, the presence of trapped air, the soaking water temperature and pH (RAHMAN and PERERA, 2007). Moreover, the water removed from the product during drying cannot be replaced in the same way when the food is rehydrated. In fact, the properties of the rehydrated material can be influenced by the loss of cellular osmotic pressure, changes in cell membrane permeability, solute migration, crystallization of polysaccharides and coagulation of cellular proteins can cause volatile loss and irreversible changes in texture and water holding capacity (FELLOWS, 2000).

The rehydration of dried seafood is not widely discussed in literature, only few papers reported data of rehydration of cod (BJØRKEVOLL, OLSEN, and OLSEN, 2004; BARAT et al., 2004; NGUYEN et al., 2012a; NGUYEN et al., 2012b), jumbo squid (VEGA-GÁLVEZ et al., 2011), tilapia (DUAN et al., 2011) and sea cucumber (DUAN et al., 2010).

5.2.1.3 Air drying (Convective drying)

The air-drying is one of the most ancient food conservation technologies and still today it is the most used. When a wet material is placed in a stream of forced heated air, the heat is transferred to its surface mainly by convection and the water vapor is carried away from the material surface by the air flow (Brennan, 2006).

This technology is very inexpensive but presents many disadvantages depending on the process parameters and on the food nature (FELLOWS, 2000):

- Damage to sensory characteristics and nutritional properties caused by long drying times and overheating at the surface;

- Low rates of heat transfer due to the low thermal conductivity of dry food, that results in long drying times;
- Oxidation of pigments and vitamins due to prolonged exposure to high temperatures and oxygen;
- The formation of the case-hardening (hard and impermeable skin) that reduces the drying rate.

In the convective drying process the vaporization heat is provided by a flow of air so a vapor pressure gradient is established between the wet sample and the dryer air. The drying rate in convective air drying depends mainly upon nature, size, shape and arrangement of the food pieces and upon the wet bulb depression or relative humidity of the dryer air as well as its temperature and velocity (WILHELM, SUTER, and BRUSEWITZ, 2005).

Vega-Gálvez et al. (2011) investigated the influence of air temperature on the drying kinetics, color, rehydration capacity, total volatile basic nitrogen, antioxidant capacity and texture of salted jumbo squid fillets during convective dehydration at temperatures from 50 to 90 °C. The effect of the drying temperature was noticeable on the drying rate and on the indexes of color, rehydration, and texture of the dried squid. High drying temperatures showed a negative effect on the color and the rehydration index. On the other hand, the texture index was positively affected by increasing of the air-drying temperature probably because of changes in food protein matrix.

Niamnuy, Devahastin, and Soponronnarit (2007) studied the drying process of shrimps in a jet-spouted bed dryer. The effect of various parameters (concentration of salt, cooking time, drying air temperature and size) was investigated upon the kinetics of drying and various quality attributes of shrimps during drying. The drying temperature showed a significant effect on the drying kinetics increasing the rate of dehydration. In terms of quality, it was found that higher concentration of salt solution, longer boiling time, and larger size of shrimp led to more shrinkage and toughness of dried shrimp but to less rehydration ability.

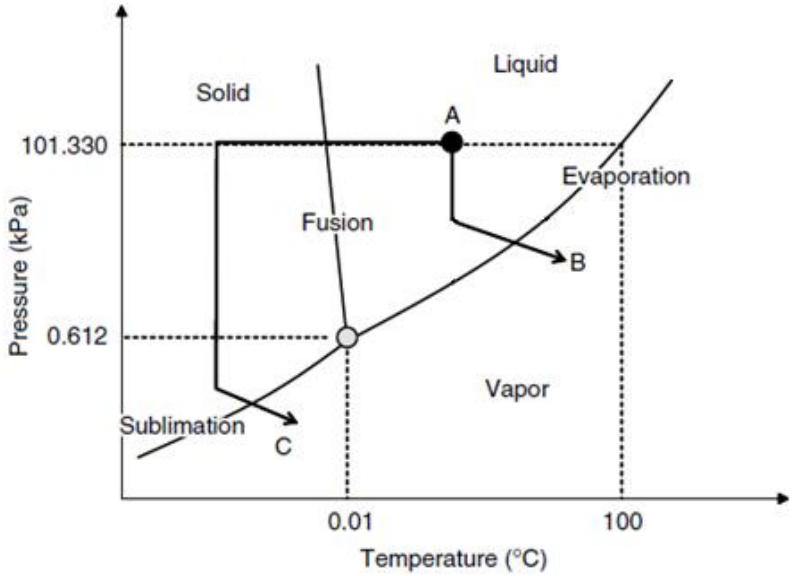
5.2.1.4 Vacuum drying

The use of vacuum to dry foods and other sensible substances started to be studied and to have some industrial applications in the 1930s. Although this technique presents problems, related principally to the vacuum condition and its high cost, the low operational temperature and the reduction of oxygen during the process allow the achievement of

great quality foods that justify the use of this process. However, the choice of this technique should be accurately evaluated considering the characteristic and the market of the vacuum dried products (FLOSDORF and TEASE, 1958; RATTI, 2008).

A typical phase diagram of water is illustrated in Figure 5.3. In these diagrams, it is possible to define the area where the vacuum drying takes place, namely when the pressure is maintained under the 101.330 kPa. Following the line from A to B or, in other words, decreasing the pressure and maintaining the liquid water state, the water evaporation occurs (ordinary vacuum drying). Otherwise, if from the point A is followed the line up to the point C (the pressure is drastically decreased after the freezing of the food material) sublimation of the water from the solid state occurs (freeze drying).

Figure 5.3 - Phase diagram of water.



Source: modified from Ratti (2008).

If the temperature of the product during vacuum drying is lower than the water boiling point at the process pressure, then the drying mechanisms are similar to those encountered during atmospheric conventional drying. On the other hand, if during drying the material

temperature is at the boiling point of water, then internal evaporation is obtained with the consequent increase of the drying rate.

There are various methods of vacuum drying which differs basically on the vacuum level, the temperature, the state of water in the product and the heating system. Following, the method used in the present study are briefly described.

- Freeze drying

Some foods are very sensitive to heating, which can cause serious damage to the physicalchemical characteristics of final products. During freeze drying, the frozen state of the water in the product maintains a structural rigidity avoiding the collapse and developing a highly porous structure. In fact, the freeze dried food recovers the original shape, taste and aroma when rehydrated, resulting in high quality products (IBARZ and BARBOSA-CÁNOVAS, 2003). These advantages are balanced by the energy-intensive aspects of the product freezing and vacuum requirements (SINGH and HELDMAN, 2009).

The freeze dried products present high retention of the original nutritional and sensorial properties. The rehydration process is normally extremely quick due to the porous structure. The texture of the rehydrated product is very similar to the original product. Furthermore, comparing the dried product with the original one, only small changes in the vitamin content are found (RATTI, 2008). However, the porous structure makes the freeze dried food fragile to mechanical stress (RATTI, 2008) and the oxygen can penetrate easily causing oxidation in the lipid compounds, that in dried condition are more sensible to oxidation (KAREL, LABUZA, and MALONEY, 1967).

The freeze drying process consists essentially of two stages: the freezing (-30 to -60 °C), and the drying under high vacuum removing the water by sublimation.

The freezing stage has a primary role on the pores formation, size and distribution in the dried layer of the food. Indeed, if the freezing stage is slow (>1 °C/min), large ice crystals are formed in the extracellular location modifying greatly the microstructure and the properties of the product. On the other hand, if the freezing stage is fast, the crystallization is uniform, the ice crystals are small, discontinuous and the microstructure of the product is just slightly altered (AGUILERA and STANLEY, 1990; LIAPIS and BRUTTINI, 2006).

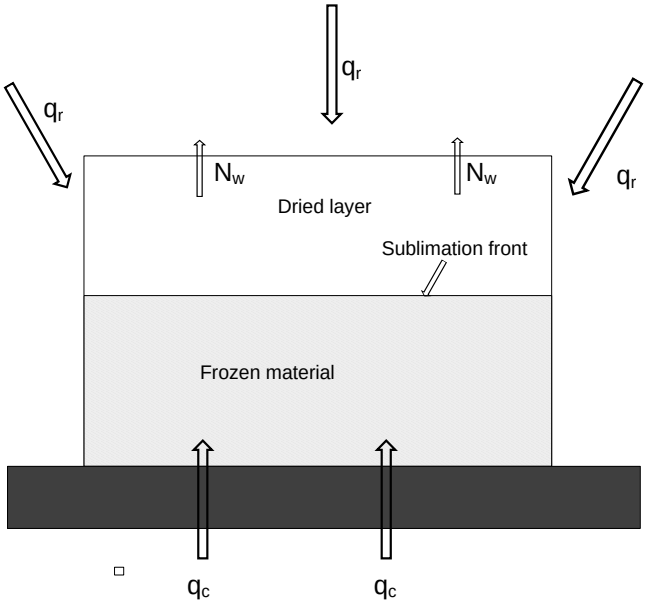
The drying stage is performed at very low pressures (0.01 to 1 mbar) in order to sublime the ice. During the freeze drying operation, a dried layer formed around the frozen product, acting as an insulator,

affecting the heat transfer thus decreasing the drying rate (GEANKOPLIS, 1993; IBARZ and BARBOSA-CÁNOVAS, 2003).

The drying stage is divided in two phases. The first phase is governed by two transport mechanisms: the heat transfer to transform ice into water vapor (between –21 and –30 °C approximately 2805 kJ/kg) and the mass transfer of the water vapor from the sublimation surface through the already dried product into the drying chamber to the condenser (OETJEN and HASELEY, 2004). The second drying phase involves the removal of bounded water that did not freeze (about 10-35 % of the initial moisture content). This phase is not totally independent from the first one. Actually, a small amount of bounded water is removed by desorption from the dried layer during the primary drying stage (LIAPIS and BRUTTINI, 2006).

The energy required to sublimate ice is supplied by radiation and/or conduction through the dry and the frozen layer as it is shown in Figure 5.4 (BARBOSA-CANOVAS and VEGA-MERCADO, 1996).

Figure 5.4 - Diagram of a material on a tray during freeze drying. N_w is the vapor flux trough the dry layer; q_r is the radiation heat flux; and q_c is the conductive heat flux.



Source - modified from Liapis and Bruttini (2006).

The amount of the heat flux cannot be increased drastically. Indeed, if the temperature is increased upper certain limits, the product could present loss of bioactivity, colour change (non-enzymatic browning), chemical and biochemical derivative reactions and structural deformation of the dried layer (LIAPIS and BRUTTINI, 2006).

Even though seafood as well as other high protein foods are very sensible to non-enzymatic browning and to structural collapse during conventional drying, in literature there are few studies on the use of freeze-drying for protein based foods.

Babic et al. (2009) studied the effect of freeze drying process parameters on the quality of chicken breast meat. The influence of meat thickness, freezing speed, time of drying phases and pressure were evaluated in relation to physical and sensory properties of the dried and rehydrated meat. The results showed that sample thickness was critical for the determination of process conditions and that the product quality was influenced by the process temperature and by the small differences on the sublimation pressure.

Crapo et al. (2010) developed a method for producing freeze-dried fish cubes using 3 different salmon species. The process was divided in two stages studying the effect of temperature variation on the drying kinetics and on physical characteristics of the final product including bulk density, shrinkage, hardness, colour, and rehydration kinetics. The processing time required to reach moisture content lower than 10 % and a water activity lower than 0.4 was between 8.5 to 11 hours. The developed product presented instantaneous rehydration properties.

5.3 MATERIALS AND METHODS

5.3.1 Sample preparation

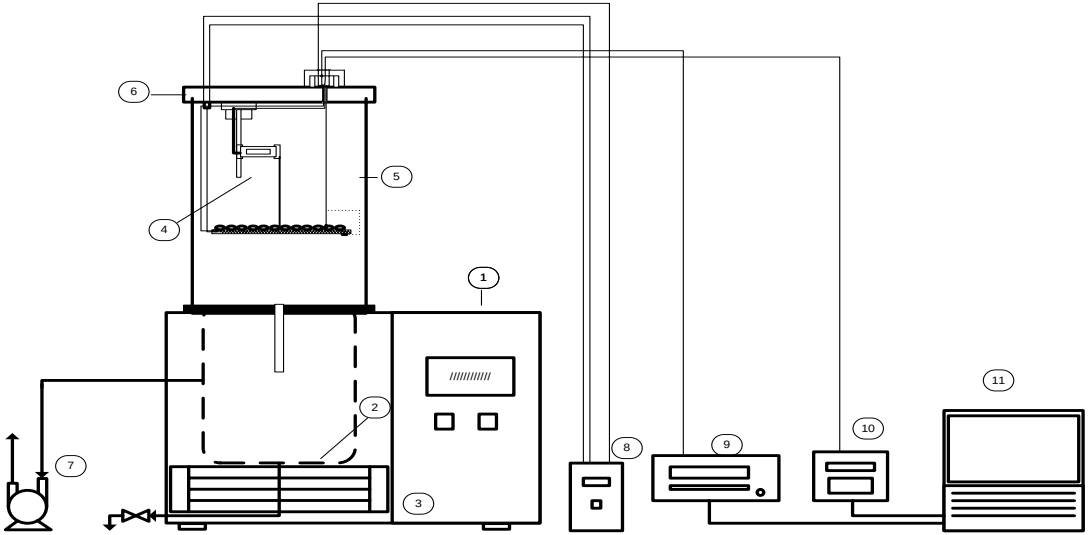
Mussels are prepared as described in section 3.3.1. Samples with weight of 8 ± 2 g and anatomical integrity were selected and stored in a refrigerator at 4 ± 1°C until processing.

5.3.2 Freeze drying

5.3.2.1 Experimental device

The freeze drying curves were determined continuously from the sample weight inside the chamber during the process. The experimental device used for that purpose is illustrated in Figure 5.5 and 5.6.

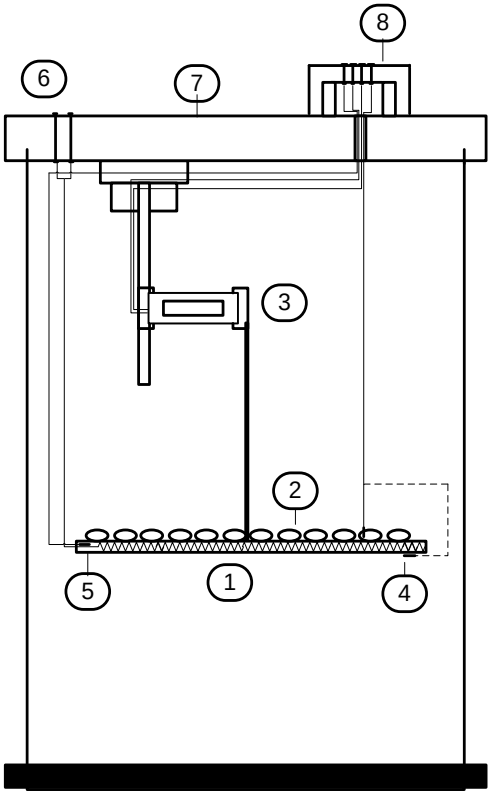
Figure 5.5 - Freeze dryer



1	Compressor;	5	Freeze drying chamber;	9	Data logger (thermocouple);
2	Condensation surface;	6	Modified cover – PMMA;	10	Data logger (load cell);
3	Condenser;	7	Vacuum pump;	11	Personal computer;
4	Weight measurement system;	8	Plate temperature controller;		

Source: Author.

Figure 5.6 - Vacuum chamber – detail.



1 Heat plate (SHP);
2 Sample;
3 Load cell;
4 Thermocouple (sample and plate);
5 Thermocouple (plate temperature controller);
6 Electrical connections;
7 Modified cover – PMMA;
8 Load cell and thermocouples Connections.

Source: Author.

This device was built by modifying commercial freeze dryer equipment (Liotop, L101, Brazil). This freeze dryer consist in a vacuum chamber connected to a condensation system that maintain the temperature in the condenser surface at -60 ± 1°C. The vacuum chamber was divided in two parts: the superior, where the drying process occurs, in PMMA (Polymethyl-methacrylate) with diameter of 250 mm and volume of 15.8 dm^3, and the inferior (bottom) in stainless steel that is the condensation surface for the vapor released by the samples. The diameter of this chamber is 220 mm and the volume is approximately of 8 dm^3. The two chambers are separated by a perforate plate in PMMA that has a structural function. The vacuum in the chamber is produced by a vacuum pump (RC.8D, D.V.P. Vacuum technology, Italy),

reaching a final and constant pressure of 0.2 ± 0.05 mbar in 10 minutes since the operation start.

The modified part of this equipment is the cover of the upper chamber and the support for the samples. The cover is built in PMMA, with diameter of 270 mm and thickness of 22 mm. The vacuum condition is maintained by an O-ring. The cover is the physical support for the weight measurement system and for the heating plate used for the samples freeze-drying.

The measurement system was constituted by a single-point load cell (Alfa Instrumentos, Load Cell, Mod. GL, Brazil), with nominal capacity of 2 kg and readability of 0.1 g, connected to a signal conditioning system (Alfa Instrumentos, Indicador de pesagem 3101C, Brazil), which is in turn connected to a computer.

The sample holder plate (SHP) is in aluminum with diameter of 210 mm and thickness of 10 mm and connected to the load cell by a stem. The plate is heated by an electric resistance and its temperature is measured by a calibrated thermocouple (mod. PT100) and controlled by a PID control system (Dist, Produtos para Laboratório, Brazil). Another thermocouple, connected to a data logger (Agilent 34970A.Software Agilent Benchlink Data Logger 3; 4.00.00.), was used to measure the sample and plate temperature. Both thermocouples were calibrated against an ASTM mercury-in-glass thermometer (Incoterm, Brazil) in the whole temperature range used in the experiments.

The system was tested in freeze drying condition without samples to assess the operational reaction of the load cell under vacuum.

5.3.2.2 Freeze drying

Before freeze-drying, 120 ± 2g of cooked samples were frozen. Mussels were placed in direct contact with the condensation surface for 4 h, reaching in the core, where the thermocouple tip was placed, the temperature of -50 ± 5°C.

Frozen samples were equally and rapidly distributed in the aluminum plate connected to the load cell and the vacuum pump was started. After 10 minutes, the chamber pressure reached operative pressure of about 0.2 mbar. After 20 h of freeze drying, the atmospheric pressure in the chamber was restored, the sample was removed and the final moisture content and a_w were determined.

The temperature of the heating plate during the freeze drying process was set in four levels: Non-heated, 15, 30 and 40 °C.

The experiments were repeated five times for all studied condition. The average value (triplicate) of the moisture content (initial and final) and the sample weight curves for each process were used to calculate the dry base moisture content during the process and plot the drying curves.

5.3.3 Air drying

The online weighting system, showed in Figure 5.6 item 4, was adapted to an air circulation and renovation oven (TECNAL-TE 394/2, Brazil). Approximately 120 of cooked samples were spread on the sample holder plate and dried at 40 °C. The air velocity was of ≈1 m/s, measured with a compact thermal anemometer (Testo, 425, Germany) and the RH was of 55 % ± 5 measured with a thermohygrometer (Texto, 610, Germany).

5.3.4 Vacuum drying

The vacuum drying was performed in a vacuum oven (TECNAL-TE 395, Brazil). Approximately 120 ± 2 g of cooked mussels were vacuum dried at the pressure of ≈15 mbar and at the temperature of the oven set at 40° C. The weighing of the samples during the experiment was carried out adapting the online weighting system showed in Figure 5.5 item 4.

5.3.5 Drying kinetics

The experimental drying curves obtained from the three drying methods were treated with a smoothing filter, in order to smooth the experimental data and perform the derivatives. For that, the Matlab function *"sgolayfilt"* was used. The smoothed curves were derived and presented as the drying rate as a function of the time and as function of moisture content.

5.3.6 Rehydration

Dried samples were rehydrated and the rehydration capacity was calculated. Samples obtained using different drying process, with average moisture of 10 %, were packaged in nylon nets and totally immersed in distillated water (1:50 - dried mussel weight:water weight). The water temperature effect was also investigated at 20 °C and 80 °C.

Sample weight was measured after 1, 2, 3, 4, 6, 8, 10, 15, 20, 25 and 30 minutes of immersion for freeze dried samples and after 3, 6, 10, 15, 20, 25, 30, 40, 50, 60, 90, and 120 minutes for vacuum and air dried samples to calculate the rehydration capacity (*RC*) (Equation 5.13)

$$RC = \frac{W_a}{W_l} * 100 \quad (5.13)$$

in which W_a is the mass of water absorbed during rehydration and W_l is the water lost during drying (LEWICKI, 1998).

5.3.7 Analytical determinations

5.3.7.1 Moisture content

The moisture content was determined with the method described in Section 3.3.4.1.

5.3.7.2 Water activity

The a_w was determined with the method described in Section 3.3.4.2.

5.3.7.3 Statistical analysis

The variance analysis (one-way ANOVA) with probability of 90 % was performed using the software Statistica® (Statistica 8.0 , StatSoft, USA). In case of significant effects ($p < 0.1$) the means were compared using the Tukey test.

5.4 RESULTS AND DISCUSSION

5.4.1 Drying kinetics

Figure 5.7 shows the freeze-drying curves of pre-cooked mussel meat recorded at different SHP temperature (non-heated, 15, 30, and 40 °C).

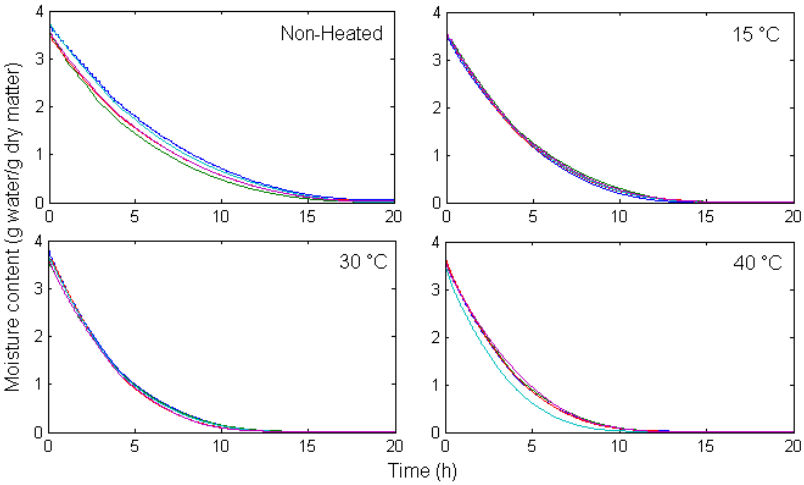
The SHP temperature significantly affected the drying rate. The equilibrium moisture content was reached after 19.8, 16.5, 15.9, and 15 hours with the SHP set in the conditions of non-heated, 15, 30, and 40 °C, respectively. The moisture content at the end of the process was of

0.017, 0.014 and 0.015 (g water/g dry matter) with the SHP at 15, 30 and 40 °C, respectively. The final moisture content of mussels dried with the non-heated SHP was of 0.043 (g water/g dry matter).

Despite the fact that the shape and the weight of mussels were different, the final moisture content of each mussel did not differ significantly from each other. This fact could be explained considering that, on average, the thickness of the mantel and of other anatomical parts of mussels does not differ much, particularly when the weight differences are not significant. Moreover, the experimental data recorded by the online system developed for this study presented good reproducibility.

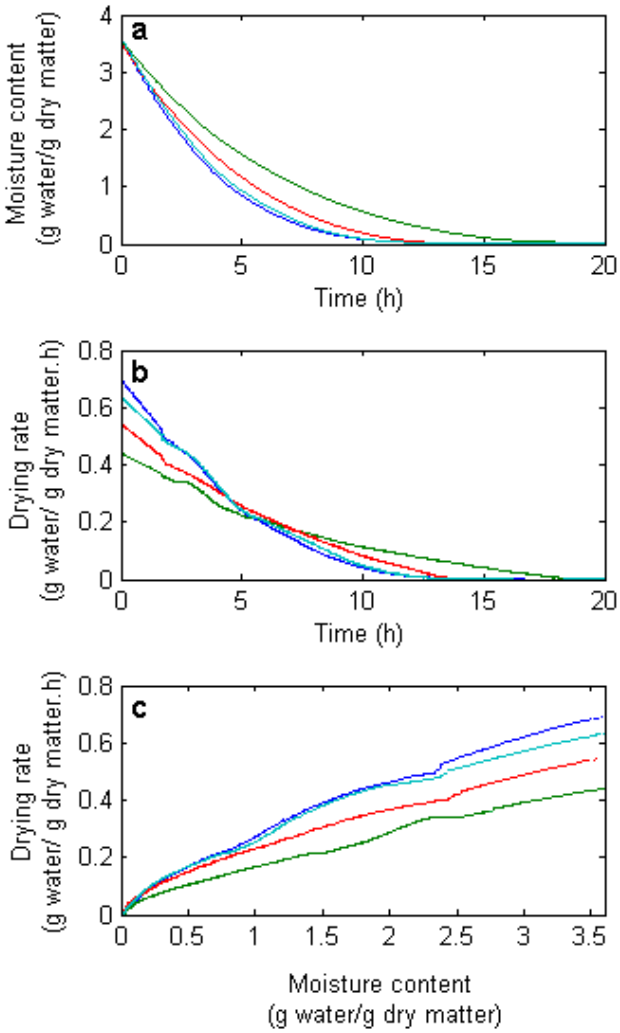
The moisture content of 0.11 ± 0.01 (g water/g dry matter) is considered acceptable for dried seafood (CRAPO et al., 2010). In freeze dried mussel meat this value was reached after 11.3, 10.4 and 9.3 hours of drying at the SHP temperature of 15, 30, and 40 °C, respectively. When the heating system was switched off, the drying time required to reach the objective moisture content was of 15.1 hours. The a_w of the products at this moisture content was not influenced by the SHP temperature resulting on average of 0.270 ± 0.053. This a_w can be considered optimal, basing on the study of Labuza (1970).

Figure 5.7 - Freeze-drying curves of pre-cooked mussel meat at the SHP temperature of 15, 30, 40 °C and Non-Heated condition (five replicates).



The experimental data of moisture content as function of drying time for the different SHP temperature (non-heated, 15, 30, and 40 °C) were filtered with the Matlab's smoothing function *sgolayfilt* (Figure 5.8a). This function was derived and presented as drying rate as function of time (Figure 5.8b) and as function of the moisture content (Figure 5.8c). For better visualization, only one representative experimental curve for each condition is presented.

Figure 5.8 - Freeze drying curves (a), freeze drying rate as function of time (b), and freeze drying rate as function of moisture content (c) of pre-cooked mussel meat freeze-dried at the plate temperature of 15 °C (—), 30°C (—), 40 °C (—) and Non-Heated (—).



The drying rates presented an initial period of adaptation (≈15 min). In this stage, the drying rate was particularly high, due to the superficial ice and to the condensation on the sample surface. After this

initial stage, a decreasing drying rate was observed for all the studied conditions. The primary and secondary periods of drying were not clearly identifiable in Figure 5.8c. This fact confirms that in the freeze drying of biological materials, these drying stages occur simultaneously (LIAPIS and BRUTTINI, 2006).

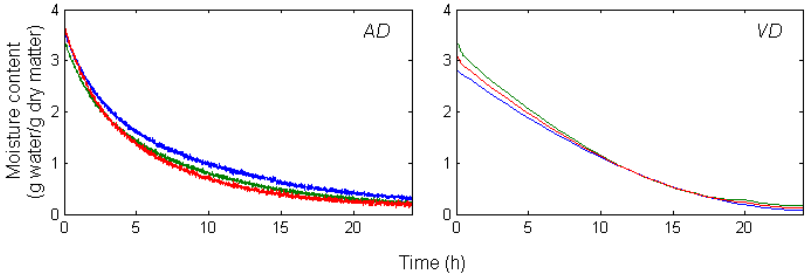
The temperature of the SHP affected significantly the drying rate, particularly in the early stages of drying. After ≈ 5 h of drying the drying rate of the samples dried at the SHP temperature of 15, 30, and 40 °C becomes similar.

The latent heat required to the ice sublimation was provided by conduction (SHP) and radiation (SHP and environment). As the drying process proceeds a dry layer is formed on the surface of the sample. This dry layer behaves as an insulating material, reducing the contribution of the radiation on the heat transfer to the sublimation front.

Figure 5.9 shows the experimental data of moisture content during air and vacuum drying mussel meat at 40 °C. In both cases the experimental data presented good reproducibility. The data recorded during drying in the convective oven presented some interference due to the vibration of the system and to the air flux that interfered on the stability of the load cell. The moisture content of the dried mussel after 24 h of drying was of 0.18 ± 0.05 and 0.11 ± 0.03 (g water/g dry matter) respectively for air and vacuum dried samples.

The a_w of AD and VD mussels after 24 h of drying was of 0.401 ± 0.015 and 0.250 ± 0.012 respectively. The values of a_w of vacuum dried mussels did not differed significantly from those of obtained in freeze dried samples. On the other hand, the values of a_w of air dried mussel was significantly ($p > 0.1$) higher of that of the mussels obtained in FD and VD processes. However, considering that the a_w value of 0.6 is the limit for microbial growth (LABUZA et al. 1970) the air dried mussels can be considered microbiologically stables at room temperature, however other degradative reactions (lipid oxidation and enzymatic activity) could take place on storage.

Figure 5.9 - Air drying curves (AD) and vacuum drying curves (VD) of pre-cooked mussel (three replicates).



The experimental data of moisture content as function of time were filtered with the Matlab's smoothing function *sgolayfilt* (Figure 5.10a). This function was derived and presented as drying rate as function of time (Figure 5.10b) and as function of the moisture content (Figure 5.10c).

The drying method (AD and VD) influenced significantly the drying rate of pre-cooked mussel meat. The drying rates (Figure 5.10 b and c) show clearly two falling rate periods for both AD and VD methods. The drying rate during AD was higher than that of VD in the first five hours of drying. Then, the AD rate decreased and stabilized to lower levels with respect to the VD rate. This rate change started approximately after 4 hours of process. The absence of a constant drying rate period could be justified by the formation of the case hardening on the samples. This layer formed on the sample surface make difficult the diffusion of water to the surface decreasing the drying rate. This phenomenon is more intense in convective drying (RATTI, 2001) than in vacuum drying, as confirmed by the higher drying rate presented in the second part of the VD.

Figure 5.10 - Drying curves (a), drying rate as function of time (b), and drying rate as function moisture content (c) of pre-cooked mussel meat air dried (—) and vacuum dried (—).

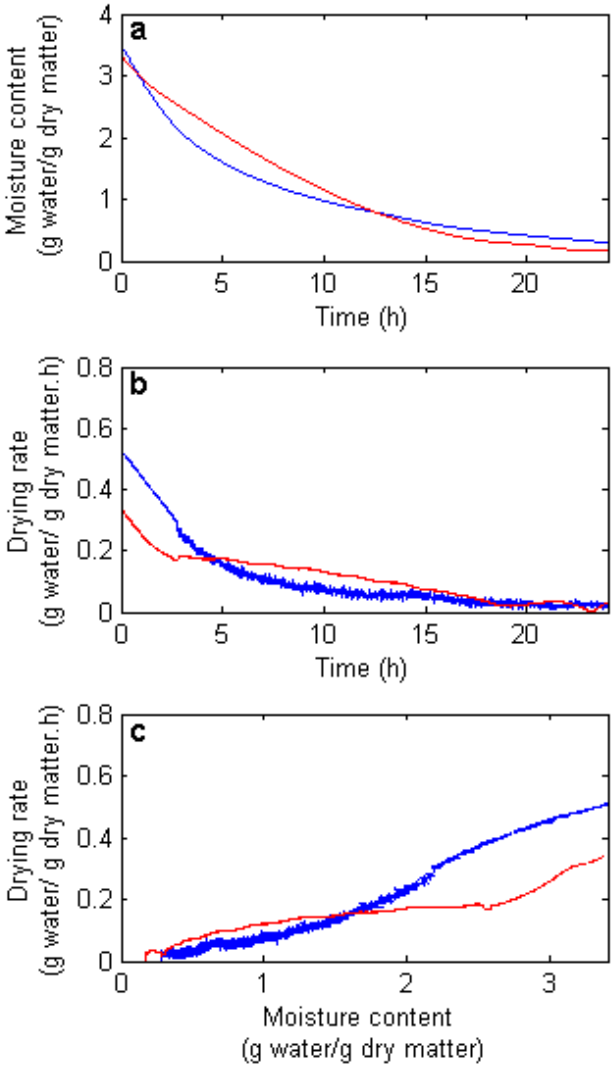


Figure 5.11 shows the temperature profiles of the samples during freeze drying and vacuum drying at the studied conditions. The temperature of the sample was recorded in its centre. During freeze

drying, the temperature should be considered as an average temperature. Indeed, all the free water continues in the frozen state due to the low pressure until the end of the first period of drying. In the vacuum drying (black line), two periods are clearly identifiable. In the first one the temperature is in equilibrium with the pressure of the oven, due to the water evaporation on the wet surface of the product. Then, in correspondence to the change on the drying rate showed in Figure 5.10, the surface started to dry and the sample temperature increased.

Figure 5.11 - Temperature of the samples during drying of pre-cooked mussel meat. Freeze drying with SHP temperature of 15 °C (—), 30°C (—), 40 °C (—), Non-Heated (—), and Vacuum drying (—)

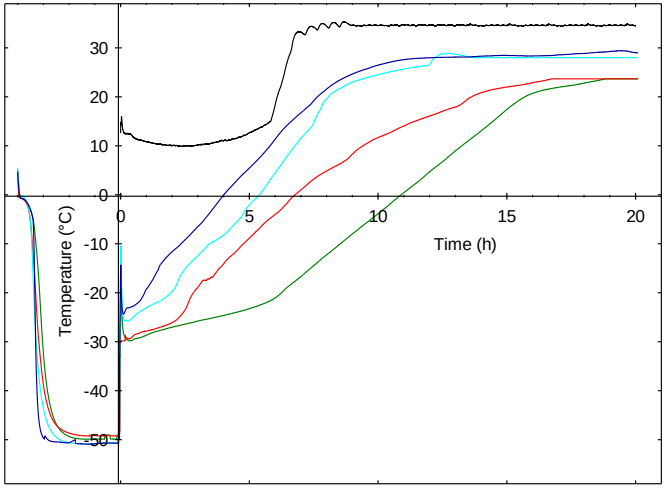
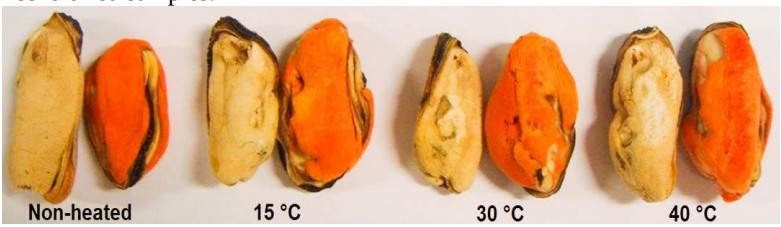


Figure 5.12 shows images of freeze dried mussels at different SHP temperatures. A color change and a "burned" aspect were identifiable in the surface of the mussels in contact with the SHP when the temperatures were maintained at 30 and 40 °C.

Heating during freeze drying contributed significantly to the drying rates. On the other hand, the processing temperature should be maintained below the maximum temperature that the frozen and the dried layers can tolerate. The melting at the sublimation interface, or any melting that could occur in the frozen layer, can cause gross material faults such as puffing, shrinking, and other physical defects (LIAPIS and BRUTTINI, 2006).

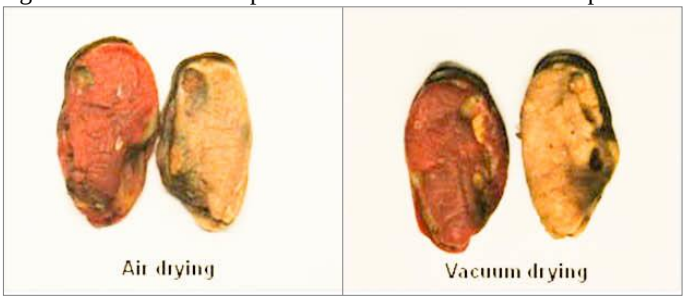
Figure 5.12 - Effect of the process temperature on the external aspect of the freeze-dried samples.



Source: Author.

Figure 5.13 shows images of the pre-cooked mussel meat after 24h of AD and VD. The drying method had an influence on the external aspect of dried mussel meat. The air dried mussel presents a surface rougher than the vacuum dried mussel. In both cases the shrinkage of the product is higher than in freeze dried samples and the color is more intense and dark.

Figure 5.13 - External aspect of air and vacuum dried samples.

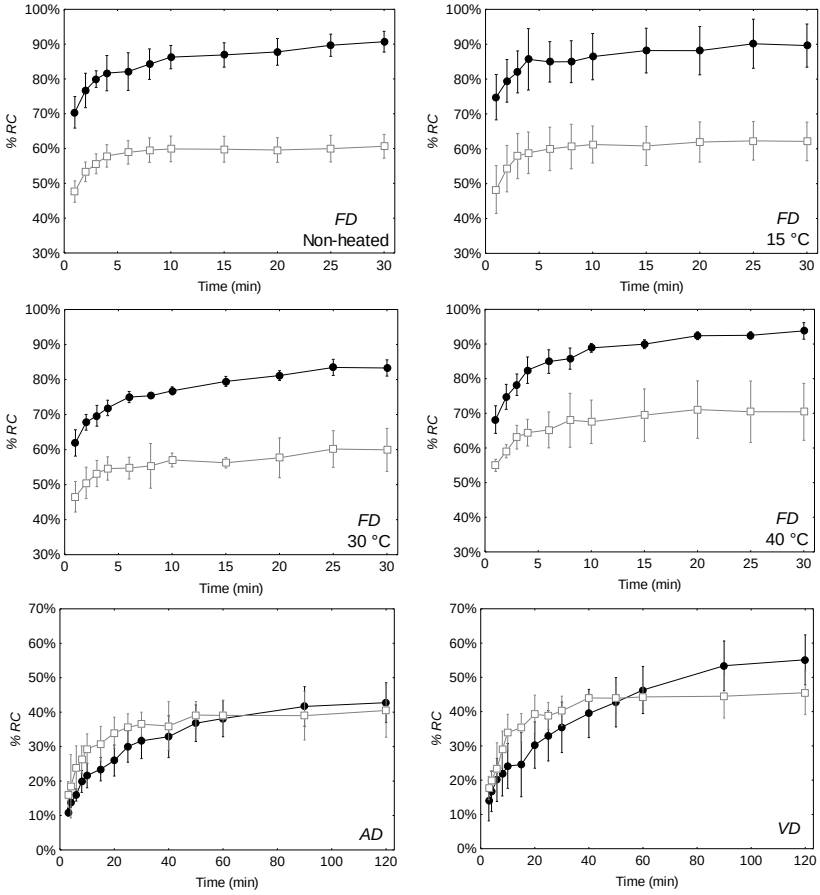


Source: Author.

5.4.2 Rehydration

The mussels dried with the different methods were rehydrated in distilled water at 20 and 80 °C. The results were expressed in rehydration capacity (RC) and are presented in Figure 5.14. The rehydration capacity represents the percentage of water absorbed during rehydration with respect to the water lost during drying.

Figure 5.14 - Rehydration capacity kinetics of dried mussels obtained for the different drying methods (FD, VD, and AD) and rehydrated in water at 20 °C (●) and 80 °C (□).



The water temperature affected significantly ($p < 0.1$) the RC of the freeze dried mussel meat. The freeze dried mussels showed higher RC (≈20 %) at lower water temperature when compared to the samples rehydrated at higher temperature. On the other hand, the SHP temperature during FD did not show significant ($p > 0.1$) effect on the RC. For mussels dried with the other two methods the RC was higher with warm water in the first hour of rehydration, than the situation revert and the RC presented higher values for mussels rehydrated in cold water. The VD presented a tendency of a higher rehydration capacity than the air dried mussels.

The RC of the AD and VD mussels was on average 30 % lower than the RC of FD mussels at the water temperature of 20°C and of about 10 % lower at the water temperature of 80 °C at the end of the respective processes.

These results agrees with those reported by Hernando et al. (2008) that studied the rehydration of freeze dried and convective dried *Boletus edulis* mushrooms at two different water temperatures (20 and 70 °C). In that study, the authors found that the cold water allowed better rehydration in both cases although in the freeze dried mushrooms this difference was greater. The authors analyzed the microstructure of the product, finding that in freeze dried samples rehydrated in cold water the rehydration occurs at intra and inter cellular level. On the other hand, in hot water the rehydration was in extracellular level and incomplete. Moreover, it is possible to suppose that at the higher water temperature the protein structure of the mussel suffer an intense denaturation that could cause the smaller water uptake.

The lower values of the RC found in VD and AD mussels with respect to the values of RC found form FD mussels are easily explainable considering the great modification of the microstructure that occurs during VD and AD. Deng et al. (2013) studied the water distribution during rehydration of freeze dried and air dried squid filets, using nuclear magnetic resonance analysis. The water migration from the periphery to the internal region of the product was affected by the microstructure. At the same rehydration time, in freeze dried samples the water was uniformly distributed in the product, when in air dried samples the water penetration was barely visible.

5.5 FINAL CONSIDERATIONS

The experimental device, developed to determine continuously the sample weight and temperatures during freeze drying, provided a very good reproducibility of results in the studied conditions.

The temperature of the sample holder plate affected significantly the drying rate.

A mild heating of the sample-holder plate (15°C) produced a considerable increase in the drying rate without modifying the aspect of the sample.

The samples air and vacuum dried presented a greater modification of the external aspect and reached acceptable moisture content only after 24 h of processing.

The rehydration capacity of the dried sample is influenced by both the drying method and the temperature of the water. With water at higher temperature, the RC was considerably lower than with cold water. The maximum rehydration capacity (about 90 % of the initial weigh) was obtained with freeze dried mussel in water at 20 °C.

5.6 REFERENCES

AGUILERA, J., and STANLEY, D. **Microstructural Principles of Food Processing and Engineering**. USA: Elsevier Science Publishing Co. Inc, 1990.

ALIBAS OZKAN, I., AKBUDAK, B., and AKBUDAK, N. Microwave drying characteristics of spinach. **Journal of Food Engineering**, 78, 577–583, 2007.

BABIC, J., CANTALEJO, M., and ARROQUI, C. The effects of freeze-drying process parameters on Broiler chicken breast meat. **LWT - Food Science and Technology**, 42, 1325–1334, 2009.

BAI-NGEW, S., THERDTHAI, N., and DHAMVITHEE, P. Characterization of microwave vacuum-dried durian chips. **Journal of Food Engineering**, 104, 114-122, 2011.

BARAT, J., RODRÍGUEZ-BARONA, S., ANDRÉS, A., and VISQUERT, M. Mass transfer analysis during the cod desalting process. **Food Research International**, 37(3), 203-208, 2004.

BARBOSA-CANOVAS, G., and VEGA-MERCADO, H. **Dehydration of Foods**. Chapman and Hall ,1996.

BARRESI, A., PISANO, R., FISSORE, D., RASETTO, V., VELARDI, S., VALLAN, A., et al. Monitoring of the primary drying of a lyophilization process in vials. **Chemical Engineering and Processing: Process Intensification**, 48(1), 408-423, 2009.

BJØRKEVOLL, I., OLSEN, J., and OLSEN, R. Rehydration of salt-cured cod using injection and tumbling technologies. **Food Research International**, 37(10), 925-931, 2004.

BRENNAN, J. Evaporation and Dehydration. In: J. Brennan, **Food Processing Handbook** (pp. 85-86). WILEY-VCH Verlag GmbH and Co. KGaA, 2006.

CHEN, X. D., and MUJUMDAR, A. S. **Drying Technologies in Food Processing**. Wiley-Blackwell, 2008.

CHOU, S., and CHUA, K. New hybrid drying technologies for heat sensitive foodstuff. **Trends in Food Science and Technology**, 12, 359–369, 2001.

COHEN, J., and YANG, T. C. Progress in food dehydration. **Trends in Food Science and Technology**, 61, 20-25, 1995.

CRAPO, C., OLIVEIRA, A., NGUYEN, D., BECHTEL, P., and FONG, Q. Development of a Method to Produce Freeze-Dried Cubes from 3 Pacific Salmon Species. **Journal of Food Science**, 74(5), 267-275, 2010.

CUI, Z., XU, S., and SUN, D. Microwave–vacuum drying kinetics of carrot slices. **Journal of Food Engineering**, 65, 157–164, 2004.

DARVISHI, H., AZADBAKHT, M., REZAEIASL, A., and FARHANG, A. Drying characteristics of sardine fish dried with microwave heating. **Journal of the Saudi Society of Agricultural Sciences**, In Press ,2012.

DENG, Y., LUO, Y., WANG, Y., YUE, J., LIU, Z., ZHONG, Y., ZHAO, Y., and YANG, H. Drying induced protein and microstructure damages of squid fillets affected moisture distribution and rehydration ability during rehydration, **Journal of Food Engineering**. Accepted manuscript, 2013.

DONSÌ, G., FERRARI, G., and DI MATTEO, P. Utilization of combined processes in freeze-drying of shrimps. **Trans IChemE**, 79, 2001.

DOYMAZ, I. Convective air drying characteristics of thin layer carrots. **Journal of Food Engineering**, 61, 359-364, 2004.

DROUZAS, A. E., and SCHUBERT, H. Microwave Application in Vacuum Drying of Fruits. **Joumul of Food Engineering,** 20, 203-209, 1996.

DUAN, X., ZHANG, M., MUJUMDAR, M., and WANG, S. Microwave freeze drying of sea cucumber (*Stichopus japonicus*). **Journal of Food Engineering**, 96, 491–497, 2010.

DUAN, Z., JIANG, L., WANG, J., YU, X., and WANG, T. Drying and quality characteristics of tilapia fish fillets dried with hot air-microwave heating. **Food and Bioproducts Processing**, 89(4), 472-476, 2011.

FELLOWS, P. **Food processing technology - Principles and Practice**. Cambridge, England: Woodhead Publishing limited, 2000.

FIGIEL, A. Drying kinetics and quality of vacuum-microwave dehydrated garlic cloves and slices. **Journal of Food Engineering**, 94, 98–104, 2009.

FIGIEL, A. Drying kinetics and quality of beetroots dehydrated by combination of convective and vacuum-microwave methods. **Journal of Food Engineering**, 98, 461–470, 2010.

FLOSDORF, E., and TEASE, S. Vacuum drying. **Vacuum**, 6, 89-112, 1958.

GEANKOPLIS, C. **Transport Processes And Unit Operations** (Third edition ed.). Prentice-Hall International, 1993.

HERNANDO, I., SANJUAN, N., PEREZ-MUNUERA, I., AND MULET, A. Rehydration of Freeze-Dried and Convective Dried Boletus edulis Mushrooms: Effect on Some Quality Parameters. **Journal of food science**—Vol. 73, Nr. 8, 2008.

IBARZ, A., and BARBOSA-CÁNOVAS, B. **Unit operations in food engineering.** CRC Press LLC, 2003.

INCROPERA, F., DEWITT, D., BERGMAN, T., and LAVINE, A. **Fundamentals of Heat and Mass Transfer** (Sixth Edition ed.). Wiley, 2006.

KAREL, M., LABUZA, T. P., and MALONEY, J. F. Chemical changes in freeze dried foods and model systems. **Cryobiology**, 3 (4), 288-296, 1967.

KNUTSON, K., MARTH, E., and WAGNER, M. Microwave heating of food. **Lebensmittel-Wisseinscaft und-Technologie**, 20, 101-110, 1987.

KUDRA, T., and MUJUMDAR, A. **Advanced drying technologies**. New York: Marcel Dekker Inc, 2002.

LABUZA, T., TANNENBAUM, S., and KAREL, M. Water content and stability of low moisture and intermediate. **Food Technology**, 24, 543–550, 1970.

LEWICKI, P. Some Remarks on Rehydration of Dried Foods. **Journal of Food Enginerring**, 36, 81-87, 1998.

LIAPIS, A., and BRUTTINI, R. Freeze Drying. In: A. Mujumdar, **Handbook of Industrial Drying** (Third Edition ed., pp. 257-271). CRC Press, 2006.

LOMBRAÑA, J., RODRÍGUEZ, R., and RUIZ, U. Microwave-drying of sliced mushroom. Analysis of temperature control and pressure. Innovative **Food Science and Emerging Technologies**, 11(4), 652–660, 2010.

MASKAN, M. Drying, shrinkage and rehydration characteristics of kiwifruits during hot air and microwave drying. **Journal of Food Engineering**, 48(2), 177-182, 2001.

MENLIK, T., ÖZDEMIR, M., and KIRMACI, V. Determination of freeze-drying behaviors of apples by artificial neural network. **Expert Systems with Applications**, 37, 7669–7677, 2010.

NGUYEN, M., JONSSON, J., THORKELSSON, G., ARASON, S., GUDMUNDSDOTTIR, A., and THORARINSDOTTIR, K. Quantitative and qualitative changes in added phosphates in cod (Gadus morhua) during salting, storage and rehydration. **LWT - Food Science and Technology**, 47, 126-132, 2012a.

NGUYEN, M., THORARINSDOTTIR, K., THORKELSSON, G., GUDMUNDSDOTTIR, A., and ARASON, S. Influences of potassium ferrocyanide on lipid oxidation of salted cod (*Gadus morhua*) during processing, storage and rehydration. **Food Chemistry**, 131, 1322–1331, 2012b.

NIAMNUY, C., DEVAHASTIN, S., and SOPONRONNARIT, S. Effects of Process Parameters on Quality Changes of Shrimp During Drying in a Jet-Spouted Bed Dryer. **Journal of Food Science**, 72(9), 553-563, 2007.

OETJEN, G., and HASELEY, P. **Freeze-Drying**. WILEY-VCH Verlag GmbH and Co. KGaA, 2004.

PELEG, M. An empirical model for the description of moisture sorption curves. **Journal of Food Science**, 53, 1216–1219, 1988.

PELEG, M., and CHINACHOTI, P. On modeling changes in food and biosolids at and around their glass transition temperature range. **Critical Reviews in Food Science and Nutrition**, 36(1-2), 49-67, 1996.

RAHMAN, M. Drying of Fish and Seafood. In: A. Mujumdar, **Handbook of Industrial Drying** (Third edition ed., pp. 552-553). Taylor and Francis Group, 2006.

RAHMAN, M., and PERERA, C. O. Drying and food preservation. In: M. S. RAHMAN, **Handbook of Food Preservation** (Secon edition ed., pp. 403-430). New York.: CRC Press, 2007.

RATTI, C. Hot air and freeze-drying of high-value foods: a review. **Journal of Food Engineering**, 49, 311-319, 2001.

RATTI, C. Freeze and vacuum drying of foods. In: X. Chen, and A. Mujumdar, **Drying Technologies in Food Processing** (pp. 225-243). Blackwell Publishing, 2008.

SARKARDEI, S., and HOWELL, N. The effects of freeze-drying and storage on the FT-Raman spectra of Atlantic mackerel (Scomber scombrus) and horse mackerel (*Trachurus trachurus*). Food Chemistry, 103 (1), 62–70, 2007.

SINGH, R., and HELDMAN, D. **Introduction to Food Engineering** (Fourth Edition ed.). London: Academic Press Inc, 2009.

SUNJKA, P. **Microwave/vacuum and osmotic drying of cranberries.** McGill University, 2003.

TAIWO, K. A., ANGERSBACH, A., and KNORR, D. Rehydration studies on pretreated and osmotically dehydrated apple slices. **Journal of Food Science**, 67(2), 842-847, 2002.

THERDTHAI, N., and ZHOU, W. Characterization of microwave vacuum drying and hot air drying of mint leaves (*Mentha cordifolia Opiz ex Fresen*). **Journal of Food Engineering**, 91(3), 482-489, 2009.

VEGA-GÁLVEZ, A., MIRANDA, M., CLAVERÍA, R., QUISPE, I., VERGARA, J., URIBE, E., et al. Effect of air temperature on drying kinetics and quality characteristics of osmo-treated jumbo squid (*Dosidicus gigas*). **LWT - Food Science and Technology**, 44(1), 16-23, 2011.

VENKATESH, M., and RAGHAVAN, G. An Overview of Microwave Processing and Dielectric Properties of Agri-food Materials. **Biosystems Engineering**, 88(1), 1-18, 2004.

WANG, J., and SHENG, K. Far-infrared and microwave drying of peach. **LWT - Food Science and Technology**, 39(3), 247-255, 2006.

WELTY, J., WICKS, C., WILSON, R., and RORRER, G. Fundamentals of Momentum, **Heat, and Mass Transfer** (Fourth Edition ed.). Jhon Wiley and Sons, Inc, 2001.

WILHELM, L., SUTER, D., and BRUSEWITZ, G. **Food and Process Engineering Technology.** Amer Society of Agricultural, 2005.

WU, T., and MAO, L. Influences of hot air drying and microwave drying on nutritional and odorous properties of grass carp (*Ctenopharyngodon idellus*) fillets. **Food Chemistry**, 110, 647–653, 2008.

ZHANG, J., ZHANG, M., SHAN, L., and Z., F. Microwave-vacuum heating parameters for processing savory crisp bighead carp (*Hypophthalmichthys nobilis*) slices. **Journal of Food Engineering**, 79(3), 885-891, 2007.

6. CONCLUSÕES

Os estudos realizados neste trabalho contribuem cientificamente e tecnologicamente para o desenvolvimento de alternativas para um melhor beneficiamento dos mexilhões por meio de processos que permitem a extensão da vida útil deste produto.

O estudo da salga e da marinação da carne de mexilhão cozida realizado na primeira parte desse trabalho permite otimizar o processo de produção de mexilhões salgados ou marinados, e os modelos matemáticos permitiram estimar o ganho de sal e de água desejado no produto final e os diagramas operacionais permitem otimizar o processo de produção de mexilhões salgados ou marinados

A conserva de carne de mexilhão triturada processada em embalagens flexíveis termoesterilizáveis, desenvolvida na segunda etapa deste trabalho, apresenta-se como uma interessante alternativa para facilitar a difusão desse produto no país, especialmente em regiões onde não há infraestrutura adequada para o transporte rápido e refrigerado, necessário para a distribuição do produto fresco.

A carne de mexilhão desidratada, estudada na última etapa do trabalho, é outro processo que agrega valor ao produto e pode diversificar as formas de comercialização. Em especial a liofilização da carne de mexilhão cozida destaca-se quanto as características do produto final, apresentando melhor aspecto visual e maior capacidade de reidratação em relação aos outros processos de secagem estudados.

Sugestões para trabalhos futuros

As alternativas para a produção e comercialização de carne de mexilhão estudadas no presente trabalho despertaram interesse e sugestões para trabalhos futuros, entre as quais podemos elencar:

- Avaliar o efeito de diferentes ácidos e realizar um estudo de análise sensorial para identificar as quantidades ótimas para a obtenção de um produto pronto para o consumo;
- Realizar um estudo sobre a qualidade nutricional do produto embalado em embalagens flexíveis termoprocessáveis e tratado termicamente (esterilização comercial) para verificar a hipótese de que a carne de mexilhão triturada em *retort pouch* seja um produto de alto valor nutricional (ácidos graxos polinsaturados e vitaminas);
- Avaliar diferentes condições e outros métodos de desidratação da carne de mexilhão;
- Realizar um estudo sobre a estabilidade da carne de mexilhão desidratada por diferentes métodos no armazenamento e avaliar a influência que o processo tem na qualidade nutricional e microbiológica do produto.