UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ENGENHARIA DE ALIMENTOS

NATÁLIA BRUNNA MORESCO FERREIRA

MODELAMENTO MATEMÁTICO DO EFEITO DA ATIVIDADE DE ÁGUA E DO PROCESSAMENTO À ALTA PRESSÃO HIDROSTÁTICA NA RESISTÊNCIA DE *L. MONOCYTOGENES* E DE BACTÉRIA ÁCIDO LÁTICA EM EMULSÃO CÁRNEA

CAMPINAS

2022

NATÁLIA BRUNNA MORESCO FERREIRA

MODELAMENTO MATEMÁTICO DO EFEITO DA ATIVIDADE DE ÁGUA E DO PROCESSAMENTO À ALTA PRESSÃO HIDROSTÁTICA NA RESISTÊNCIA DE *L. MONOCYTOGENES* E DE BACTÉRIA ÁCIDO LÁTICA EM EMULSÃO CÁRNEA

Dissertação apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em Tecnologia de Alimentos

Orientador: Prof. Dr. Marcelo Cristianini

ESSE TRABALHO CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO DEFENDIDA PELA ALUNA NATÁLIA BRUNNA MORESCO FERREIRA E ORIENTADA PELO PROF. DR. MARCELO CRISTIANINI

CAMPINAS

2022

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

Ferreira, Natália Brunna Moresco, 1990-
F413m Modelamento matemático do efeito da atividade de água e da alta pressão hidrostática na resistência de *L. monocytogenes* e de bactéria ácido lática em emulsão cárnea / Natália Brunna Moresco Ferreira. – Campinas, SP : [s.n.], 2022.

Orientador: Marcelo Cristianini.
Dissertação (mestrado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Emulsões. 2. Alta pressão hidrostática. 3. Atividade de água. 4. *Listeria monocytogenes*. 5. Bactérias produtoras de ácido láctico. I. Cristianini, Marcelo. II. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Mathematical modeling of the effect of water activity and high hidrostatic pressure processing on the resistance of *L. monocytogenes* and lactic acid bacteria in meat emulsion
Palavras-chave em inglês:
Emulsions
High hydrostatic pressure
Water activity
Listeria monocytogenes
Lactic acid bacteria
Área de concentração: Tecnologia e Inovação
Titulação: Mestra em Tecnologia de Alimentos
Banca examinadora:
Marcelo Cristianini [Orientador]
Dirce Yorika Kabuki
Ana Lúcia da Silva Corrêa Lemos
Data de defesa: 28-07-2022
Programa de Pós-Graduação: Tecnologia de Alimentos

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: 0000-0003-1490-3625
- Currículo Lattes do autor: http://lattes.cnpq.br/402831451213557

BANCA EXAMINADORA

Prof. Dr. Marcelo Cristianini
FEA/UNICAMP
(Presidente)

Prof. Dra. Dirce Yorika Kabuki
FEA/UNICAMP
(Membro Titular)

Prof. Dra. Ana Lucia da Silva Corrêa Lemos
ITAL
(Membro Titular)

A ata da defesa com as respectivas assinaturas dos membros encontram-se no processo de vida acadêmica do aluno.

Dedico esse trabalho aos meus pais por tudo que deixaram de ter para que eu tivesse, sobretudo, educação.

AGRADECIMENTOS

Agradeço à Deus pela vida e por sempre ter colocado pessoas boas no meu caminho.

Agradeço em especial meu amigo e orientador, Marcelo pela confiança, paciência, disposição e incentivo infindáveis. Sou muito grata por ter me proporcionado o conhecimento não apenas racional, mas a manifestação do caráter e afetividade da educação no processo de formação profissional e pessoal. Obrigada por me guiar e me ajudar a evoluir a cada dia.

Aos meus amores, Di, Joca e Amora por serem meu porto seguro, compartilharem meus sonhos, celebrarem minhas conquistas, terem me acolhido nas decepções e aguentarem diariamente meu "jeitinho".

Aos meus pais, meus maiores mentores, formadores do meu caráter, que se doaram inteiros para sempre me proporcionar um estudo de qualidade e por me encorajarem na constante busca pelo saber.

À BRF em nome do Fabio Bagnara e da Rodicler Bortoluzzi pelo apoio, incentivo e suporte em toda a pesquisa realizada. Em especial, à toda equipe da planta piloto (Itamar, Gabi, Luiza, Everton, Karine, Giovani) e laboratório (Niéli, Jacson, Analice, Sabrina, Paulo, Isabelly) e a Carolina pela ajuda com o Minitab, o meu eterno obrigada.

Ao Departamento de Tecnologia de Alimentos da UNICAMP pelo suporte à minha formação no curso de Mestrado. Em especial ao Fabiano por toda ajuda com os processamentos no HPP.

À professora Maria Isabel pelo carinho e paciência na divisão do conhecimento sobre o DOE.

Agradeço aos meus amigos, que em todos os momentos necessários me socorreram, acolheram e também celebraram comigo. Em especial, ao Vini, pelo apoio desde o início. À Raquel, por me lembrar quem eu sou. Ao PZ pelas conversas lindas sobre emulsão e sobre a vida. Ao Lucas pelo apoio, por dividir seu conhecimento e por toda ajuda sempre que precisei. À Bruna por todas as vezes que me disse "calma" nos momentos difíceis e "que orgulho" em cada pequena conquista. À Deisy pela companhia nas idas à Campinas para as aulas e na correria dos dias divididos entre trabalhar e estudar. Quem tem amigos tem tudo.

Agradeço aos meus familiares que de uma maneira ou outra, contribuíram nessa trajetória, seja com carinho ou palavras de incentivo. Aos primos Nayara e Robson por entenderem cada “não posso” nos encontros de final de semana. E em especial, à Letícia, que trouxe beleza ao trabalho.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

A todos que me ajudaram direta ou indiretamente meu muito obrigada!

RESUMO

A indústria de carnes é constantemente desafiada a buscar novas tecnologias que atendam a demanda dos consumidores por produtos seguros e de qualidade. Controlar micro-organismos é um desafio para a indústria e pode ser enfrentado com o uso do processamento à alta pressão (HPP- High Pressure Processing). Todavia, o efeito baroprotetor causado pela atividade de água (a_w) têm sido estudado em diferentes produtos, micro-organismos e parâmetros de processo, porém, informações são limitadas e em algumas vezes contraditórias. Assim, o objetivo dessa pesquisa foi avaliar o efeito da a_w em uma emulsão cárnea modelo na inativação microbiana sob alta pressão através de uma metodologia de superfície de resposta. O trabalho foi conduzido separadamente para o estudo de um patógeno e um deteriorante representados por *Listeria monocytogenes* e uma bactéria ácido lática (BAL) (*Latilactobacillus sakei*) isolada de um produto cárneo deteriorado, respectivamente. Os parâmetros utilizados para avaliação de *L. monocytogenes* foram 400-600MPa de pressão, tempo de 180-420s e a_w entre 0,94-0,96. As três variáveis influenciaram significativamente (p<0,05) a inativação de *L. monocytogenes* pela alta pressão na emulsão cárnea modelo, e a redução logarítmica variou entre 0,74 a 5,18 log UFC/g. Contudo, a variável de maior influência foi a a_w (R^2 94,54%) confirmando a baroproteção causada pela baixa a_w. Por outro lado, no estudo conduzido com a BAL (pressão: 400-600MPa; tempo: 180-480s; a_w: 0,94-0,98), demonstrou que a a_w não tem influência significativa (p>0,05) na inativação do micro-organismo e a redução logarítmica variou entre 0,99 e 4,12 log UFC/g. Com o presente trabalho foi possível concluir que os parâmetros físico-químicos, de processamento e o micro-organismo interferem no efeito da alta pressão na inativação microbiana. Os modelos matemáticos criados e validados experimentalmente nesse trabalho favorecem a indústria de carnes na otimização do processo, garantia da segurança alimentar, extensão da vida de prateleira, redução do desperdício de alimentos e atendimento de legislações.

Palavras-chave: Emulsão cárnea. Alta Pressão Hidrostática. Atividade de Água. *Listeria monocytogenes*. Bactéria Ácido Lática.

ABSTRACT

The meat industry is constantly challenged to seek new technologies that meet consumer demand for safe and quality products. Controlling pathogenic and spoilage microorganisms is a challenge for the industry and can be faced with the use of High Pressure Processing (HPP). However, the baroprotective effect caused by water activity (a_w) has been studied in different products, microorganisms, and process parameters, however, information is limited and sometimes contradictory. Thus, the objective of this research was to evaluate the effect of a_w in a model meat emulsion on microbial inactivation under high pressure through a response surface methodology. The work was carried out separately for the study of a pathogen and a spoilage represented by *Listeria monocytogenes* and a lactic acid bacteria (LAB) (*Latilactobacillus sakei*) isolated from a spoiled meat product, respectively. The parameters used for the evaluation of *L. monocytogenes* were 400-600MPa of pressure, time of 180-420s and a_w between 0.94-0.96. The three variables significantly ($p<0.05$) influenced the inactivation of *L. monocytogenes* by high pressure in a model meat emulsion, and the log reduction ranged from 0.74 to 5.18 log CFU/g. However, the most influential variable was a_w (R^2 94.54%) confirming the baroprotection caused by the low a_w. On the other hand, in the study conducted with LAB (pressure: 400-600MPa; time: 180-480s; a_w: 0.94-0.98), it was shown that a_w has no significant influence ($p>0.05$) on the inactivation of the microorganism and the logarithmic reduction varied between 0.99 and 4.12 log CFU/g. With the present work, it was possible to conclude that the physical-chemical parameters, processing parameters and the microorganism interfere in the effect of high pressure on microbial inactivation. The mathematical models created and experimentally validated in this work favor the meat industry in optimizing the process, ensuring food safety, extending shelf life, reducing food waste and complying with legislation.

Keywords: Meat emulsion. High Hydrostatic Pressure. Water activity. *Listeria monocytogenes*. Lactic Acid Bacteria.

LISTA DE TABELAS

CAPÍTULO 2

Table 1 - Selected variables (factors) and the corresponding five levels used in the Central Composite Rotational Design. 46

Table 2 - Combinations of selected variables for laboratory validation of the mathematical model created. 49

Table 3 - Results of inactivation of *L. monocytogenes* ATCC 19111 after HPP according to combinations of factors proposed by CCRD. 53

Table 4 - Results of predicted and obtained in three conditions chosen to experimental validation. 57

CAPÍTULO 3

Table 1 - Selected variables (factors) and the corresponding five levels used in the Central Composite Rotational Design. 68

Table 2 - Results of inactivation of *L. sakei* after HPP according to combinations of factors proposed by CCRD. 75

LISTA DE FIGURAS

CAPÍTULO 2

Figure 1 - Predicted versus observed (experimental) values of inactivation of *L. monocytogenes* in a meat emulsion model with different values of a_w submitted to HPP according to the model developed. 55

Figure 2 - Response surface and contour plots of the inactivation of *L. monocytogenes* in a model meat emulsion with different values of a_w and submitted to HPP according to the model developed. (a) Effects of pressure and a_w; (b) Effects of pressure and time; (c) Effects of time and a_w. The factor not included in each plot was maintained at the central value of the CCRD (300s in the plot (a); a_w of 0.96 in the plot (b); and 500MPa in the plot (c)).....57

CAPÍTULO 3

Figure 1 - Response surface graphs (a) and contour plots (b) of the effect of pressure and time on the inactivation of *L. sakei* in a model meat emulsion according to the model developed. The factor not included in the graph was maintained at the central value of the CCRD, a_w of 0.96.....77

SUMÁRIO

INTRODUÇÃO 14

OBJETIVOS 18

OBJETIVO GERAL 18

OBJETIVOS ESPECÍFICOS 18

CAPÍTULO 1 – *Revisão Bibliográfica* 19

1. REVISÃO BIBLIOGRÁFICA 20

1.1 *LISTERIA MONOCYTOGENES* 20

1.2 BACTÉRIAS ÁCIDO LÁTICAS 21

1.3 EMULSÃO CÁRNEA 23

1.4 ATIVIDADE DE ÁGUA 24

1.5 PROCESSAMENTO À ALTA PRESSÃO HIDROSTÁTICA 25

1.5.1 Efeito da alta pressão nos micro-organismos 27

1.5.2 O efeito da atividade de água em matrizes submetidas à alta pressão 30

REFERÊNCIAS BIBLIOGRÁFICAS 32

CAPÍTULO 2 - *Modeling the effect of water activity of a meat emulsion matrix model on Listeria monocytogenes inactivation by high pressure processing* 42

ABSTRACT 43

1. INTRODUCTION 44

2. MATERIAL AND METHODS 45

2.1 EXPERIMENTAL DESIGN 45

2.2 BACTERIAL STRAIN AND CULTURE PREPARATION 46

2.3 MEAT EMULSION MODEL AND SAMPLE PREPARATION 46

2.4 HIGH PRESSURE PROCESSING 48

2.5 MICROBIOLOGICAL DETERMINATIONS 48

2.6 STATISTICAL ANALYSIS AND MATHEMATICAL MODELING 49

2.7 MODEL VALIDATION 49

3 RESULTS AND DISCUSSION 50

3.1 CHARACTERIZATION OF THE MEAT EMULSION MODEL 50

3.2 INACTIVATION OF *L. MONOCYTOGENES* 50

3.4 MODEL VALIDATION 57

3.5 MODEL APPLICATION 57
4. CONCLUSION 59
ACKNOWLEDGMENTS 59
REFERENCES 59

CAPÍTULO 3 - *Effect of high pressure processing and water activity on pressure resistant spoilage lactic acid bacteria in a ready-to-eat meat emulsion model* 64
ABSTRACT 65
1. INTRODUCTION 65
2. MATERIAL AND METHODS 67
2.1 BACTERIAL ISOLATION, IDENTIFICATION, AND CULTURE PREPARATION ... 67
2.2 EXPERIMENTAL DESIGN 68
2.3 MEAT EMULSION MODEL 68
2.4 SAMPLE PREPARATION AND HIGH PRESSURE PROCESSING 70
2.5 MICROBIOLOGICAL DETERMINATIONS 70
2.6 STATISTICAL ANALYSIS AND MATHEMATICAL MODELING 71
3. RESULTS AND DISCUSSION 71
3.1 MEAT EMULSION MODEL CHARACTERISTICS 71
3.2 LACTIC ACID BACTERIA INACTIVATION 72
3.3 REGRESSION MODELING OF HP-INACTIVATION DATA AND SURFACE PLOTS 75
4. CONCLUSION 77
ACKNOWLEDGMENTS 78
REFERENCES 78

DISCUSSÃO GERAL 82
CONCLUSÕES GERAIS 83
REFERÊNCIAS BIBLIOGRÁFICAS 84

INTRODUÇÃO

A necessidade de desenvolver e melhorar produtos para que atendam cada vez mais a exigência dos consumidores por produtos de qualidade em um mercado cada vez mais competitivo é clara para a agroindústria da carne. Um dos principais objetivos dos fabricantes de alimentos é desenvolver e empregar tecnologias de processamento que garantam a segurança dos alimentos, retenham ou criem as qualidades nutricionais, reduzam as mudanças indesejáveis devido ao processamento e prolonguem a vida de prateleira dos produtos.

Devido à pública relevância de *Listeria monocytogenes* em produtos prontos para consumo RTE (*Ready-to-eat*), onde não são necessários processos térmicos antes do consumo, esse patógeno é a prioridade se tratando de critérios microbiológicos da indústria de alimentos para produção de produtos seguros representado o principal patógeno-alvo de controle (BAHRAMI et al., 2020a; HEREU et al., 2014). O patógeno é resistente a uma série de condições ambientais, como baixas temperaturas, alto teor de sal ou alta acidez em alimentos, bem como baixa umidade e baixo oxigênio em ambientes alimentares (FSIS, 2014). Além de resistir a diversas barreiras, o micro-organismo também é considerado um grande desafio para a indústria por sua alta capacidade de se adaptar, persistir e multiplicar em diversos ambientes (RUGNA et al., 2021)

O patógeno oportunista *L. monocytogenes* é causador da doença listeriose, que causa sintomas graves com alta hospitalização e altos índices de mortalidade em idosos, recém nascidos e imunossuprimidos (BAHRAMI et al., 2020a; GANDHI; CHIKINDAS, 2007). Recentemente, em outubro de 2020, os Estados Unidos reportaram um total de 10 pessoas infectadas por *L. monocytogenes* acarretando em uma morte devido ao consumo de produtos cárneos como mortadela e salame (CDC, 2020). Em 2019, um surto registrado pelos EUA, resultou em uma morte e 10 hospitalizações e foi associado ao consumo de produtos cárneos fatiados contaminados por *L. monocytogenes* (CDC, 2019). No total, em 2018, os estados membros da União Europeia registraram 2.549 casos confirmados de listeriose humana causando 229 mortes, confirmando assim, a tendência de aumento de casos que vem sido notificada desde 2009 (EFSA, 2019a). Nos estados Unidos da América, calcula-se que *L. monocytogenes* cause cerca de 1.600 doenças a cada ano, com mais de 1.500 hospitalizações e 260 mortes relacionadas (CDC, 2016). Em 2008, 57 casos de listeriose que resultaram em 24 mortes no Canadá, foram associados aos produtos cárneos prontos para o consumo originários de uma única planta produtora. A estimativa econômica feita sobre o caso apontou um prejuízo

total de 242 milhões de dólares canadenses (THOMAS et al., 2015). Dados dessa magnitude colocam em evidência a necessidade de estudos sobre o comportamento e barreiras que contenham este micro-organismo que farda as autoridades, indústrias e responsáveis.

A deterioração de um alimento é associada à rejeição e perda dos produtos. De acordo com a FAO (2011), um terço dos alimentos produzidos para o consumo humano no mundo são perdidos ou desperdiçados. As bactérias ácido láticas (BAL) são a microbiota deteriorante mais encontrada em produtos cárneos prontos para o consumo, causando problemas como sabor azedo, odor estranho, limosidade, cor esverdeada e formação de gás. Para estender a vida de prateleira de produtos cárneos é preciso um severo controle dos micro-organismos deteriorantes durante o armazenamento do produto (HYGREEVA; PANDEY, 2016). O aumento da vida de prateleira do produto traz benefícios aos fabricantes, pois leva à possibilidade de comercialização em locais distantes de sua produção, garantindo acesso a mais consumidores.

Na atualidade, o crescimento do mercado de produtos cárneos prontos para consumo é significativo; no entanto, considerando as características intrínsecas dessa categoria de produtos que apresenta alta a_w, pH favorável e rica composição nutricional somados ao formato de consumo, fatiado embalado, onde, o produto durante o processo de fatiamento, torna-se vulnerável à contaminação por micro-organismos presentes no ambiente e superfícies. Por tratar-se de um produto que não necessita de aquecimento prévio ao consumo, o emprego de barreiras tecnológicas que atendam às necessidades de segurança e qualidade são necessárias (HYGREEVA; PANDEY, 2016; TSALOUMI et al., 2021).

A a_w é um dos fatores intrínsecos dos alimentos e é uma medida quantitativa que possibilita avaliar a disponibilidade de água livre que são necessárias as diversas reações físicas, químicas e biológicas. A água livre correlaciona-se às taxas de crescimento microbiano e às taxas de reações de degradação, assim, a a_w é indicativo de estabilidade e segurança microbiana do produto (DAMODARAN; PARKIN; FENNEMA, 2008). O principal efeito da redução da a_w é o aumento da fase lag e a redução das taxas de multiplicação microbiológica, devido ao efeito conhecido como estresse osmótico, pois quando o micro-organismo se encontra em um ambiente de baixa a_w, a água intracelular migra para fora da célula, onde a concentração de soluto é maior. Essa perda de água resulta na perda da estrutura rígida celular paralisando o crescimento microbiano (LEISTNER, 1994).

Diferentes tecnologias têm sido desenvolvidas para estender a vidad de prateleirae eliminar patógenos em produtos cárneos prontos para consumo (BAHRAMI et al., 2020a; BOVER-CID et al., 2015; HYGREEVA; PANDEY, 2016). A utilização do processamento à alta pressão (HPP- *High Pressure Processing*) é uma tendência inovadora para a conservação de alimentos por se tratar de uma tecnologia de pasteurização a frio (não-térmica), reconhecida como a tecnologia comercialmente mais importante para alimentos, sendo utilizada satisfatoriamente nas indústrias de processamento de produtos cárneos no pós-processo para a extensão de vida útil, atendimento de legislações, garantia da segurança alimentar e eliminação de conservantes sem alterar as características sensoriais e nutricionais do produto. Os produtos cárneos são os que representam o maior crescimento de aplicação dessa tecnologia nos últimos anos (HYGREEVA; PANDEY, 2016).

Resumidamente, o tratamento se dá em submeter o produto embalado à altas pressões (entre 100 - 600MPa) através de um vaso pressurizado utilizando um meio que transfira a pressão de uma forma uniforme e quase instantânea ao produto por determinado tempo a uma certa temperatura. A eficácia do tratamento depende dos parâmetros de processo (pressão, tempo e temperatura), características do produto (como a_w, pH e composição) e condições do micro-organismo (como o tipo de cepa e fase de crescimento) (BOVER-CID et al., 2011; GEORGET et al., 2015). Nos EUA, os órgãos competentes recomendam a tecnologia no pós-processamento de produtos cárneos prontos para o consumo, principalmente para controle de *L monocytogenes* (USDA/FSIS, 2003). Assim, o processamento de alta pressão é o mais viável para estender prazo de validade, manter o frescor, mantendo a qualidade sensorial e nutricional e melhorar a segurança alimentar, em produtos sensíveis ao calor sem a necessidade de aditivos ou conservantes artificiais suportando inovações e reinvenção de produtos cárneos.

A a_w é um parâmetro importante do produto para o processo de inativação microbiana por alta pressão. Produtos cárneos com a_w reduzida podem não responder à inativação microbiana do HPP bem como aqueles com maior a_w. A dificuldade de se prever o efeito da a_w na inativação microbiana pelo tratamento com alta pressão é grande, pois, sua redução (a_w) parece criar um efeito protetor nos micro-organismos contra a letalidade por processo de alta pressão na maioria dos casos (BALAMURUGAN et al., 2020; BOVER-CID et al., 2015; RUBIO et al., 2018), tornando-se mais perceptível quando a a_w cai para valores abaixo de 0,95 (YORDANOV; ANGELOVA, 2010). Por outro lado, a recuperação das células injuriadas após o processamento pode ser inibida por um baixo valor de a_w e influenciada pela

temperatura de armazenamento. Portanto, a mesma propriedade físico-química que cria o efeito protetor nos micro-organismos em relação à pressão é barreira para a recuperação de células injuriadas após o processamento. O efeito baroprotetor associado à a_w está sendo estudado em diferentes micro-organismos e matrizes (BALAMURUGAN et al., 2020; BOVER-CID et al., 2015; PÉREZ-BALTAR et al., 2020b; RUBIO et al., 2018), porém, informações sobre o efeito da a_w são limitadas e em algumas vezes contraditórias (BUERMAN; WOROBO; PADILLA-ZAKOUR, 2020; BULLARD et al., 2018). Até hoje, não existem estudos considerando a inativação microbiana por alta pressão de um emulsionado cárneo cozido com diferentes valores de a_w.

Nesse contexto, o presente estudo tem por objetivo avaliar o efeito da a_w na inativação de *Listeria monocytogenes* e uma BAL em uma emulsão cárnea modelo submetida à alta pressão através de uma metodologia de superfície de resposta. A emulsão cárnea foi desenvolvida para que nenhum outro ingrediente interferisse na resposta do micro-organismo. A BAL foi isolada de um produto cárneo que foi submetido ao HPP. Os resultados dessa pesquisa interessam tanto aos cientistas como aos fabricantes de alimentos e órgãos de saúde pública.

O presente trabalho foi dividido em três etapas, descritas a seguir:

1) Revisão bibliográfica considerando todas as variáveis do trabalho e o estado da arte do efeito da atividade de água e o processamento à alta pressão (Capítulo 1);

2) Avaliação do efeito da a_w e do processamento à alta pressão em *L. monocytogenes* (Capítulo 2);

3) Avaliação do efeito da a_w e do processamento à alta pressão em uma bactéria ácido lática resistente à pressão isolada de um produto cárneo deteriorado (*L. sakei*) (Capítulo 3).

OBJETIVOS

OBJETIVO GERAL

Avaliar o efeito da atividade de água e dos parâmetros de processamento a alta pressão hidrostática, pressão e tempo, na inativação de um micro-organismo patógeno e um deteriorante em um produto emulsionado cárneo cozido.

OBJETIVOS ESPECÍFICOS

1) Avaliar o efeito da atividade de água, pressão e tempo de processamento na inativação de *L. monocytogenes* e de *Latilactobacillus sakei* isolada de um produto cárneo emulsionado cozido através de uma metodologia de superfície de resposta;

2) Desenvolver e validar um modelo matemático considerando as variáveis que tem interferência significativa na inativação dos micro-organismos propostos.

CAPÍTULO 1

REVISÃO BIBLIOGRÁFICA

REVISÃO BIBLIOGRÁFICA SOBRE AS VARIÁVEIS INDICADAS CONSIDERADAS NO ESTUDO E O ESTADO DA ARTE DA PESQUISA PROPOSTA

1. REVISÃO BIBLIOGRÁFICA

1.1 *LISTERIA MONOCYTOGENES*

L. monocytogenes é um micro-organismo anaeróbio facultativo, não formador de esporos, Gram-positivo, conhecido como um dos principais patógenos da indústria de carnes por sua habilidade de crescer em vários tipos de ambientes e em baixas temperaturas (-4 a 50°C) (ALÍA et al., 2019). *L. monocytogenes* resiste à altas concentrações de cloreto de sódio (10 a 30%), tem faixa de pH de multiplicação de 4,3 a 9,6 e sobrevive a ambientes com a_w menor que 0,93 (JAY, JAMES M.; LOESSNER, MARTIN J.; GOLDEN, 2005).

A listeriose, doença causada por *L. monocytogenes*, pode apresentar diversas manifestações clínicas, que pode resultar em aborto, endocardite, conjuntivite, meningite, entre outras doenças (BUCHANAN et al., 2017; GANDHI; CHIKINDAS, 2007). A listeriose é considerada como uma doença oportunista, os indivíduos com saúde normal podem não desenvolver sintomas ou apresentar uma forma entérica muito leve da doença. No entanto, é altamente fatal para fetos, recém-nascidos, idosos, mulheres grávidas e pessoas imunocomprometidas, como aqueles com câncer, doenças renais, doenças cardíacas e AIDS (FARBER et al., 2021; GANDHI; CHIKINDAS, 2007; ZHANG et al., 2021). Sintomas gastrointestinais como náusea, vômitos e diarreia, podem preceder ou acompanhar as manifestações mais graves da doença (GANDHI; CHIKINDAS, 2007).

O relatório da Autoridade Europeia para Segurança dos Alimentos (EFSA) e do Centro Europeu de Prevenção de Controle de doenças, apresentou os resultados das atividades de monitoramento de zoonoses realizadas em 2018 em 36 países europeus. Este relatório mostra que o número de casos de listeriose confirmados relatados aumentou ainda mais em 2018, no total foram relatados 5146 surtos com 229 mortes, transmitidos por alimentos e água (EFSA, 2019a). Ainda em 2018, foram registrados 2.549 casos de listeriose invasiva em humanos, com uma taxa de notificação da União Europeia de 0,47 casos por 100.000 habitantes. As infecções por *Listeria* foram mais comumente relatadas na faixa etária acima de 64 anos e, particularmente, na faixa etária acima de 84 anos (EFSA, 2019). São poucas as investigações de surtos de listeriose no Brasil, sendo a doença normalmente subdiagnosticada e subnotificada, o que evidencia uma deficiência na caracterização dos agentes responsáveis no Brasil (DESTRO, 2006; SILVA et al., 2016). Portanto, os números mostram que frequentemente surtos acontecem em países desenvolvidos preocupando sobre a situação de países em

desenvolvimento os quais o micro-organismo e a doença não são notificados e diagnosticados (ZHANG et al., 2021).

Considerando os eminentes riscos, a fim de garantir a segurança alimentar evitando contaminações e surtos, regulamentos foram aplicados em produtos prontos para consumos na maioria dos países. Para os membros da União Europeia, o regulamento (CE) 2073/2005 estabelece um máximo de 100 UFC/g de *L. monocytogenes* durante a vida útil do produto (EUROPEAN COMISSION, 2005). O mesmo é aplicado pela regulamentação canadense (HEALTH CANADA, 2011), Nova Zelândia (AUSTRALIAN GOVERNMENT, 2017) e Brasil (MINISTERIO DA SAÚDE, 2019). Diferentemente, nos Estados Unidos da América, foi imposta a "tolerância zero", o que significa que os produtos não devem ser liberados se tiverem contaminados com o patógeno em qualquer quantidade (FSIS, 2015). Tal medida faz com que qualquer positividade seja motivo de recall, expondo a marca, desperdiçando produtos e trazendo prejuízos econômicos para a empresa.

L. monocytogenes pode ser eliminada por processos térmicos, porém, produtos prontos para o consumo (RTE) ficam expostos ao ambiente e podem assim, facilmente serem contaminados em qualquer etapa do processamento pós cozimento (BUCHANAN et al., 2017; RUGNA et al., 2021; VORST; TODD; RYSER, 2006). *Listeria* tem grande capacidade de sobreviver e se adaptar ao ambiente devido à sua característica biológica de formar biofilmes dificultando sua eliminação (ZHANG et al., 2021). Todavia, uso de sanitizantes, desinfetantes e antibióticos nem sempre apresentam bons resultados contra a bactéria ou então causa problemas em equipamentos como a corrosão (ZHANG et al., 2021).

1.2 BACTÉRIAS ÁCIDO LÁTICAS

A deterioração por micro-organismos na indústria de alimentos é um problema crítico devido às inúmeras perdas de produto (TAKAHASHI et al., 2021). A vida de prateleira de um produto é o período em que suas características de qualidade permanecem inalteradas e assim, aptas para consumo. A perda de qualidade de um produto pode ser causada por alterações físicas, reações químicas ou crescimento microbiológico (IULIETTO et al., 2015).

A deterioração proveniente do aumento da concentração de micro-organismos é o resultado de atividades do metabolismo microbiano que formam moléculas (metabólitos) e estas causam alterações indesejáveis na cor, sabor, odor, textura e aparência do produto levando ao desperdício de alimentos (REMENANT et al., 2015; TAKAHASHI et al., 2021). É

importante reconhecer alterações que deterioram o produto e desenvolver barreiras para minimizá-las pois, a deterioração é um julgamento do consumidor que pode ser influenciado pela cultura e economia, bem como nas percepções sensoriais (NYCHAS et al., 2008). Portanto, controlar os micro-organismos deteriorantes é comercialmente importante para garantir a qualidade do produto e extensão de sua vida de prateleira.

Os principais fatores que impactam no desenvolvimento de bactérias em produtos cárneos são: a composição gasosa dentro da embalagem (vácuo ou gases), composição dos produtos (aw, pH, quantidade de sal, nitritos) e a temperatura de armazenamento, considerada a variável mais importante (NYCHAS et al., 2008; REMENANT et al., 2015; SAMELIS; KAKOURI; REMENTZIS, 2000). As contaminações bacterianas de produtos cárneos são originárias inicialmente do animal e abatedouro e então do ambiente de processamento e dos colaboradores do processo (KORKEALA; BJÖRKROTH, 1997; POTHAKOS et al., 2015) podendo se adaptar ao meio e causar inúmeros casos de contaminação nos produtos (TAKAHASHI et al., 2021).

As bactérias ácido láticas (BAL) são bactérias Gram-positivas, catalase negativa, não-esporuladas, no formato de bacilus ou coccus e que produzem ácido lático (TAKAHASHI et al., 2021). São identificadas como o maior grupo de deteriorantes em produtos cárneos embalados à vácuo (NYCHAS et al., 2008; REMENANT et al., 2015). Porém, controversamente, algumas espécies são utilizadas de forma benéfica para produção como o de processos de fermentação para contribuir com sabores, texturas e até na vida de prateleira do produto (BARCENILLA et al., 2022; IULIETTO et al., 2015; REMENANT et al., 2015). Também, em outra aplicação, por produzirem ácido, baixam o pH do produto e inibem o crescimento de outros micro-organismos que deterioram o produto com maior velocidade (ZHANG et al., 2018) ou até patógenos garantindo a segurança do alimento (BARCENILLA et al., 2022).

Dentre as BAL deteriorantes, as espécies encontradas em carnes são classificadas em heterofermentativas, como os *Lactobacilli* (principalmente *L. curvatus* e *L. sakei*) e *Leuconostoc,* ou homofermentativas, como os *Lactobacillus* e *Pediococcus* (IULIETTO et al., 2015; REMENANT et al., 2015). As BAL homofermentativas produzem praticamente apenas ácido lático (característica que originou o nome do grupo) enquanto as heterofermentativas produzem dióxido de carbono, etanol, ácido acético entre outros metabólitos responsáveis pela deterioração (IULIETTO et al., 2015). Em presunto cozido fatiado embalado em atmosfera

modificada, *Lactobacillus sakei* e *Leuconostoc carnosum* foram os micro-organismos deterioradores encontrados dominantemente (RAIMONDI et al., 2019).

BAL são micro-organismos de difícil controle, pois suportam temperaturas de refrigeração, ambientes com pouco oxigênio e resistem à presença de nitrito, sal e defumação (fumaça) (CAYRÉ; VIGNOLO; GARRO, 2003; NYCHAS et al., 2008). As atividades metabólicas das BAL resultam na deterioração dos alimentos, aparecendo como sabores ácidos, ausência de sabor e odor, exsudação, limosidade, formação de gás e esverdeamento (IULIETTO et al., 2015). A taxa de deterioração é regida pela combinação de fatores intrínsecos e extrínsecos, como a composição do produto, forma de embalagem e temperatura de armazenamento e o aparecimento das características de deterioração é proporcional ao aumento da concentração dos micro-organismos (KAMENÍK et al., 2015). A concentração de 10^7 UFC/g de BAL é considerada determinante para o limite de deterioração do produto e consequentemente para sua vida de prateleira (MENEZES et al., 2018). A maioria das modificações organolépticas nos produtos ocorrem quando LAB atinge a fase estacionária de crescimento (IULIETTO et al., 2015).

Devido à importância do controle das BALs em produtos cárneos, para evitar perdas de produto e consequentemente econômicas para a indústria de produtos cárneos, ingredientes como nisina (ARAÚJO et al., 2018; LARANJA; MALHEIROS; TONDO, 2019) e óleos essenciais (KHORSANDI et al., 2018; MENEZES et al., 2018), embalagens ativas (PATEIRO et al., 2019) e processos como a alta pressão (BALAMURUGAN et al., 2018) ou ultrassom (AGUILAR et al., 2021) são estudados para evitar a proliferação das BAL.

1.3 EMULSÃO CÁRNEA

A emulsão cárnea é uma matriz complexa por ser influenciada por vários fatores, como a composição e o processo, que alteram sua estabilidade e/ou característica do produto. Os três principais objetivos de uma emulsão cárnea são: aumentar a estabilidade do produto; criar um perfil sensorial típico (aparência, cor, textura, sabor); e criar valor agregado à carne pela utilização de cortes de baixo valor (TOLDRÁ, 2010).

Emulsão é o resultado da mistura de dois líquidos imiscíveis, um na forma dispersa e outro na forma contínua (FORREST et al., 1975). A emulsão cárnea é considerada uma emulsão óleo em água, onde pequenas partículas de gordura são ligadas com a água e envolvidas por uma matriz proteica, porém, por não apresentarem características claras de uma

emulsão, como o tamanho da fase dispersa, são consideradas suspensões coloidais complexas (SANTHI; KALAIKANNAN; SURESHKUMAR, 2017).

A formulação básica de uma emulsão é composta por proteína, gordura, água e sal, porém, outros ingredientes podem ser utilizados (TOLDRÁ, 2010). O sal (cloreto de sódio) está associado a várias funções nas emulsões como a capacidade de retenção de água, sabor, odor, textura e com a segurança microbiológica do alimento. O sal causa muitas alterações na miofibrila da carne, pois a força iônica altera as cargas das proteínas resultando em maior solubilização. A funcionalidade da miosina pode ser limitada pela baixa força iônica (TOLDRÁ, 2010).

A estabilidade de uma emulsão depende de vários fatores como o tipo e a quantidade de proteína, de água e de gordura adicionada além de outros ingredientes cárneos e não cárneos e do processo (SANTHI; KALAIKANNAN; SURESHKUMAR, 2017). A capacidade de emulsionar a proteína solúvel é influenciada pela quantidade de proteína solubilizada; pH da carne, sendo 6,5 o valor ótimo; velocidade de mistura; temperatura final da emulsão; e a quantidade de gordura adicionada inicialmente (SANTHI; KALAIKANNAN; SURESHKUMAR, 2017; TOLDRÁ, 2010).

O processo mecânico utilizado para a elaboração de emulsões influência nas características do produto devido às condições durante o processamento como a temperatura final e o tamanho da partícula (TOLDRÁ, 2010). O processo de cozimento (tempo e temperatura) também interfere nas características da emulsão onde quanto mais lento o processo melhor por permitir maior tempo em condições favoráveis para a interação proteica (SANTHI; KALAIKANNAN; SURESHKUMAR, 2017).

1.4 ATIVIDADE DE ÁGUA

Um alimento é microbiologicamente estável e seguro em razão da presença de barreiras particulares conforme suas características em termos de natureza e intensidade. Essas barreiras ou o conjunto delas mantém os micro-organismos patogênicos e deteriorantes sob controle porque estes não ultrapassam esses obstáculos presentes no alimento (LEISTNER; GORRIS, 1995).

A água é vital para todos os tipos de vida e é o maior componente da maioria dos sistemas de alimentos (GEORGET et al., 2015). A água presente nos alimentos pode se

presentar na forma de molécula livre ou associada à um substrato. O termo “atividade de água (a_w) ou aquosa (Aa)” foi desenvolvido para indicar a intensidade com a qual a água associa-se a constituintes não aquosos, referindo-se à água não ligada (DAMODARAN; PARKIN; FENNEMA, 2008).

A a_w é a razão entre a pressão de vapor a certa temperatura (graus Celsius) sobre a pressão de vapor da água pura na mesma temperatura (FEINER, 2006). É avaliada numa escala de 0 a 1, onde zero corresponde a ausência de água livre e 1 representa a água pura. Atividade de água é o maior fator de preservação em alimentos (LEISTNER, 1994). Quanto maior o valor de a_w, maior o risco de deterioração do alimento. A a_w em temperaturas menores é menor, pois a pressão de água é menor. Assim, a a_w combinada com baixas temperaturas, reduz ainda mais a multiplicação de micro-organismos.

A água livre é disponível para o crescimento de micro-organismos e para a realização de diferentes reações químicas e bioquímicas (MATHLOUTHI, 2001). Quando as bactérias são expostas em condições com baixa a_w, a pressão de turgescência pode ser perdida e a célula é afetada negativamente se tornando flácida ou plasmolisada (GEORGET et al., 2015). Algumas bactérias, chamadas de osmotolerantes, tem um mecanismo para se adaptar e reconstruir a pressão de turgescência interna, se adaptando, assim, à baixa a_w (GEORGET et al., 2015).

A a_w pode ser reduzida nos alimentos através da adição de solutos (sal, açúcar), presença de óleos e gorduras ou remoção física da umidade pela desidratação (BUCUR et al., 2018; GEORGET et al., 2015).

1.5 PROCESSAMENTO À ALTA PRESSÃO HIDROSTÁTICA

A busca dos consumidores por produtos seguros, frescos, nutritivos e saborosos impulsiona os estudos com novas tecnologias. Novas tecnologias surgiram para contribuir com produtores de alimentos para atender os desejos do consumidor pois nem todos os produtos são possíveis de usar tecnologias onde o princípio de funcionamento é o calor (SOLADOYE; PIETRASIK, 2018). A emergente tecnologia de processamento à alta pressão (HPP – *High Pressure Processing*) é um processo não-térmico, descoberto por Hiter em 1899 que vem crescendo na tecnologia de alimentos como a mais promissora tecnologia principalmente por contribuir para a inativação de micro-organismos com mínimas alterações na qualidade do

produto (BALASUBRAMANIAM; MARTÍNEZ-MONTEAGUDO; GUPTA, 2015; GONZÁLEZ-ANGULO et al., 2021; YANG et al., 2021).

Por seus bons resultados e viabilidade industrial, tal tecnologia não-térmica tem sido muito bem aceita e é a mais implementada por indústrias de diversos setores, entre eles sucos e bebidas, peixes e frutos do mar e por sua alta perecibilidade, a alta pressão é aplicada em produtos cárneos (GONZÁLEZ-ANGULO et al., 2021; TONELLO-SAMSON; QUEIRÓS; GONZÁLEZ-ANGULO, 2020). Os níveis de pressão aplicados para pasteurização de carnes e produtos cárneos vão de 400-600MPa com curtos períodos de tempo de 3–7 minutos a temperatura ambiente (BAJOVIC; BOLUMAR; HEINZ, 2012). Ainda, a tecnologia é altamente sustentável por ser limpa, natural e de baixo consumo de energia (HUANG et al., 2017; PINTON et al., 2021).

O tratamento consiste em submeter o produto já embalado em câmaras, através de vasos apropriados, a elevadas de pressões, por algum período de tempo a determinada temperatura utilizando como fluido pressurizador, fluídos de baixa compressibilidade (geralmente água). A pressão é transmitida de forma instantânea e o produto é comprimido uniformemente em toda sua área. O tempo total de pressurizar, segurar (processo em si) e despressurizar refere-se a um ciclo (BAJOVIC; BOLUMAR; HEINZ, 2012; CAMPOS; DOSUALDO; CRISTIANINI, 2003; CHEFTEL; CULIOLIB, 1997; DA CRUZ et al., 2010; SUN, 2014; TAO et al., 2007). Independentemente do tamanho ou formato, temperaturas aumentam em torno de 3°C a cada 100MPa dependendo da composição do alimento (FRANCO-VEGA et al., 2020).

As alterações que o processo causa no produto são governadas com base no princípio de Le Chatelier, onde as reações associadas a uma diminuição no volume molecular são favorecidas, enquanto as reações acompanhadas pelo aumento de volume são inibidas (BAHRAMI et al., 2020a; CHEFTEL; CULIOLIB, 1997). Assim sendo, o processamento pode alterar ligações não covalentes, modificar a conformação de macromoléculas, ativar ou inativar enzimas, alterar as propriedades dos carboidratos, gorduras, polímeros e modificar a taxa de reações químicas sem afetar pequenas moléculas físico-químicas e nutricionais como as de sabor e vitaminas, respectivamente (SOLADOYE; PIETRASIK, 2018). Portanto, a tecnologia promove a destruição de micro-organismos na forma vegetativa e inativação de enzimas sem afetar significativamente as moléculas do alimento que contribuem para seus aspectos qualitativos e nutricionais, e com isso, pode aumentar a durabilidade do produto mantendo frescor e qualidade.

O processo também segue o princípio isostático, que indica que a pressão é transmitida de forma uniforme e quase instantânea e, portanto, independente do seu volume e formato (CAMPOS; DOSUALDO; CRISTIANINI, 2003). Devido à aplicação uniforme da alta pressão acontecer em todas as partes do alimento, esta tecnologia tem vantagem significativa sobre outros métodos de processamento pois permitem aos sólidos reter o seu formato original e é aplicada após o processo de embalagem, evitando riscos de recontaminação (BAJOVIC; BOLUMAR; HEINZ, 2012; CAMPOS; DOSUALDO; CRISTIANINI, 2003; CHEFTEL; CULIOLIB, 1997).

Países desenvolvidos como os Estados Unidos da América (FDA, 2001; FSIS, 2012), Canadá (HEALTH CANADA, 2020) e países da União Europeia (EUROPEAN COMISSION, 2015) reconhecem e regulamentam o uso da alta pressão. A regulamentação aumenta a confiabilidade de consumidores e favorece a disseminação da aplicação da tecnologia nas indústrias.

1.5.1 Efeito da alta pressão nos micro-organismos

O principal uso da alta pressão em alimentos é para a inativação de patógenos e deteriorantes para garantir a segurança do alimento e proporcionar extensão da vida de prateleira sem impactar na qualidade do produto (BAJOVIC; BOLUMAR; HEINZ, 2012; GONZÁLEZ-ANGULO et al., 2021; RITZ et al., 2002; SUN, 2014).

A eficácia da tecnologia para inativação microbiana depende de vários fatores como a pressão, temperatura, tempo de processamento, tipo de micro-organismo e fase de crescimento, bem como de características do produto como a_w, pH, quantidade de sal e presença de antimicrobianos (BAHRAMI et al., 2020a; PALOU et al., 1997; RUBIO et al., 2018).

Diferentes cepas de mesma espécie e em diferentes substratos podem ter comportamentos diferentes sob pressão, concluindo que a resistência dos micro-organismos é extremamente variável frente a alta pressão pois depende de uma série de fatores (BAJOVIC; BOLUMAR; HEINZ, 2012; PATTERSON et al., 1995). De modo geral, bolores e leveduras são mais sensíveis à alta pressão do que células procarióticas (BOLUMAR et al., 2021; CHEFTEL, 1995; GEORGET et al., 2015); as células vegetativas na fase de crescimento são mais sensíveis do que as células nas fases estacionárias ou dormentes (CHEFTEL, 1995; SMELT, 1998); assim como, as bactérias gram-negativas são mais sensíveis do que as gram-positivas (BOLUMAR et al., 2021; CHEFTEL, 1995; GEORGET et al., 2015). Pressões mais

baixas retardam o crescimento microbiano, enquanto pressões maiores promovem a inativação e redução da divisão celular (BAJOVIC; BOLUMAR; HEINZ, 2012; BOLUMAR et al., 2021) devido ao desdobramento ou desnaturação de importantes enzimas e proteínas celulares (GEORGET et al., 2015).

A inativação pela alta pressão nos micro-organismos é um fenômeno complexo que envolve mais do que uma reação fisiológica (MOLINA-GUTIERREZ et al., 2002). A pressão causa danos aos micro-organismos que podem levá-los a morte. Alterações nas funções básicas da célula como sua morfologia, estruturas subcelulares e na bioquímica, físico-química e genética são os principais fatores da inativação microbiana pela alta pressão (BOLUMAR et al., 2021; CHEFTEL, 1995). O princípio básico da inativação pela alta pressão hidrostática sob os micro-organismos está relacionado à desnaturação proteica que, por sua vez, causa impactos na membrana celular, em aspectos bioquímicos e em mecanismos genéticos desestabilizando a célula (BAJOVIC; BOLUMAR; HEINZ, 2012; FARKAS; HOOVER, 2000; SOLADOYE; PIETRASIK, 2018). Os impactos na membrana celular são um dos principais motivos da morte celular (CHEFTEL, 1995). A permeabilidade da membrana é afetada pois a alta pressão causa a fase de transição e alteração na fluidez (BAJOVIC; BOLUMAR; HEINZ, 2012). A redução o pH intracelular está atrelada à inativação de enzimas e a danos à membrana celular (GEORGET et al., 2015; SMELT, 1998). Portanto, a inativação microbiana pelo HPP é, provavelmente, resultado de uma combinação de fatores.

A inativação de micro-organismos patógenos pela alta pressão é exaustivamente estudada em produtos cárneos para que produtores possam garantir a segurança dos alimentos e cumprir com legislações de seus países. Para carnes processadas prontas para consumo como a linguiça fermentada fatiada (a_w: 0,75; 0,91; 0,93), 600MPa/18°C por 10-12min se provou efetivo para *L. monocytogenes* (POSSAS et al., 2019). Células inoculadas de *L. monocytogenes* em presunto cozido fatiado foram inativadas por HPP com 600MPa e 20°C por 1 minuto onde as reduções logarítmicas variaram entre 1,2 e 5 log UFC/g dependendo da espécie (HAYMAN et al., 2004) e com 400MPa e 17°C por 10 minutos (AYMERICH et al., 2005).

De acordo com Pereira et al., (2018), a recuperação de *L. monocytogenes* após tratamento à alta pressão tem sido relatada nos últimos anos. O processo causa injúria à célula e sugere a impossibilidade de seu crescimento, porém, ainda pouco estudado, a célula pode se recuperar e crescer novamente quando em condições adequadas de ambiente estão disponíveis. Cepas de *L. monocytogenes* inoculadas em presunto cozido mostraram a recuperação de suas células após 4 semanas do tratamento com HPP (500 ou 600MPa e 17-32°C, por menos de 8

minutos) (TEIXEIRA et al., 2016). O estudo do efeito do processamento com alta pressão (500MPa por 10min) em *L. monocytogenes* inoculada (contagem inicial de 5log10 UFC/g) em presunto cozido fatiado armazenado a 10°C foi estudado mostrou que após o processamento com pressão, as contagens de *L. monocytogenes* chegaram ao nível mínimo de detecção do método (menor ou igual a 10 células). Contudo, durante o período de armazenamento (70 dias) as contagens aumentaram gradualmente chegando a valores maiores do que o inoculado incialmente (7-8 log10 UFC/g) (KOSEKI; MIZUNO; YAMAMOTO, 2007).

Embora o maior foco dos estudos do processo de alta pressão seja micro-organismos patogênicos, o processo também é empregado sobre a microbiota deteriorante de produtos cárneos com a finalidade de aumentar a de vida de prateleira. De modo geral, a alta pressão pode aumentar a vida de prateleira de produtos cárneos de 3 a 4 vezes quando comparada com o produto sem processamento na mesma condição de estocagem (GONZÁLEZ-ANGULO et al., 2021). Avaliando o efeito do HPP (200/300/400MPa por 5, 10, 15min) sobre BAL (10^7 UFC/g) em presunto fatiado embalado à vácuo e estocadas a 8°C foi observado que a pressão e o tempo influenciaram no comportamento dos micro-organismos. Tratamentos a 400MPa por 15min a 8°C aumentaram a vida de prateleira de 19 (controle não pressurizado) para 85 dias na amostra processada por HPP (SLONGO et al., 2009).

O efeito do processo de alta pressão (600MPa por 5min a temperatura ambiente) em salsichas cozidas embutidas em dois diferentes tipos de tripa (natural 30-32mm e colágeno 19mm), embaladas à vácuo ou sob atmosfera modificada e armazenadas a 4°C por 35 dias foi estudado e concluiu-se que, independentemente da embalagem e da tripa, as contagens de BAL foram significativamente (p<0,001) menores nas amostras processadas com HPP, provando a eficácia do processo. Em relação aos atributos qualitativos (cor, sensorial e oxidação lipídica), nenhum resultado foi significativo (p>0,001) em relação à amostra controle (KAMENÍK et al., 2015).

Para potencializar a inativação microbiana, outras barreiras podem ser utilizadas em sinergia com a alta pressão como moderada ou elevada temperatura, baixo pH e antimicrobianos naturais como enzimas, óleos essenciais, peptídeos e bacteriocinas (AYMERICH et al., 2005; BAJOVIC; BOLUMAR; HEINZ, 2012; LI et al., 2020; YANG et al., 2021). Também, pode ser utilizada para atender as demandas dos consumidores que apelam por produtos com o rótulo mais “limpo”, na substituição de aditivos e conservantes da composição dos produtos (PINTON et al., 2021), por exemplo, nitrito de sódio e sal em presunto e peito de peru (MYERS et al., 2013), redução de sal em peito de peru (DE OLIVEIRA

et al., 2015; PIETRASIK; GAUDETTE; JOHNSTON, 2017) e redução de fosfatos em emulsões cárneas suínas (FERNÁNDEZ-MARTÍN et al., 2002).

1.5.2 O efeito da atividade de água em matrizes submetidas à alta pressão

A alta pressão pode ser tratada como uma barreira para micro-organismos e pode ser combinada com outras barreiras (como pH, a_w e conservantes), porém, para garantir a eficiência do tratamento, é necessário explorar a interação dessas variaveis e a cinética de inativação de cada micro-organismo (FRANCO-VEGA et al., 2020).

Conhecer como os micro-organismos se comportam em determinados valores de a_w das diversas matrizes é necessário para desenhar uma adequado processamento para controlá-los. Como citado anteriormente, a a_w pode ser alterada pela adição de solutos, por alterações na composição de gordura e pelo processo de desidratação. Todavia, reduzidos valores de a_w em matrizes são reconhecidos por proteger os micro-organismos contra a pressão na maioria casos (FRANCO-VEGA et al., 2020; RUBIO et al., 2018; SMELT, 1998).

Matrizes de baixo valor de a_w, como os produtos que tem processo de desidratação, tem se mostrado complexas para descontaminação microbiológica pois a ausência da água como um meio de transmitir a pressão aumenta o desafio do HPP (FRANCO-VEGA et al., 2020; GEORGET et al., 2015). Pérez-Baltar et al., (2020), reportaram o efeito de diferentes valores de a_w (0,92, 0,88 e 0,84) ajustadas com solução saturada de cloretos em presunto cru (matriz de baixa a_w) tratados com 450 MPa/10 min e 600 MPa/5 min de duas cepas de *L. monocytogenes* (S4-2-sorotipo 1/2b e S12-1-sorotipo 1/2c) monitoradas por 30 dias a 4°C. Os efeitos antimicrobianos do HPP (600MPa/5min) tiveram melhores resultados com valores de a_w de 0,92. Concluindo, portanto, que a inativação do micro-organismo é favorecida na maior a_w e maior pressão. Em outro estudo, Balamurugan et al., (2020), avaliaram o processo à alta pressão (600 MPa por 3 minutos) combinado com o processo de fermentação e secagem de linguiças poderia inativar *E. coli* O157:H7 durante o processamento e também a recuperação das células durante o armazenamento a 4°C e concluíram que, a eficácia do processo a alta pressão na inativação do patógeno foi significativamente (p<0,05) reduzida com a baixa da a_w (<0,9).

Matrizes que associam altas quantidades de gordura com baixa a_w (reduzida pelo NaCl), são complexas e o efeito da alta pressão nos micro-organismos deve ser explorado (FRANCO-VEGA et al., 2020). Para avaliar o efeito do processo de alta pressão em Queijo

tipo hispânico fresco sem sal e desidratado (feito pelos pesquisadores) e Queijo Mahón maduro (comprado em mercado) sob a barotolerância de *L. monocytogenes* Scott A, Morales et al. (2006) alteraram a a_w dos produtos através da adição de sal (NaCl) (de 0 até 5%) no queijo tipo hispânico fresco e de água destilada (de 0 a 40%) no queijo Mahón maduro e, após inocularem o micro-organismo, embalaram os produtos à vácuo e mantiveram armazenados a 8°C. Os parâmetros utilizados no processo de alta pressão foram de 400MPa a 12°C por 10min. Os autores concluíram que, em ambos os queijos, quanto menor o valor de a_w, maior o efeito de baroproteção no produto na faixa de a_w de 0,925 até 0,965. Porém, na faixa de a_w de 0,945 até 0,965 a barotolerância em equivalentes valores de a_w dos queijos foi significativamente ($p<0,05$) maior no queijo fresco (2,2 logs para a_w de 0,956) onde a hipótese levantada para justificar a maior barotolerância de *L. monocytogenes* Scott A em queijo fresco do que no queijo maduro poderia ser causada pelos diferentes acúmulos de solutos devido ao tratamento com NaCl para reduzir a a_w.

Efeitos contraditórios à hipótese de baroproteção do micro-organismo causada pela redução de a_w foram encontrados em literatura. Ao analisar o efeito da combinação do processo de alta pressão (586MPa, 180s a 5°C) com dois valores de a_w obtidos através do processo de secagem (0,91 e 0,85) em *L. monocytogenes* inoculada (3 e 6 log UFC/g) em barras de carne de peru armazenadas à temperatura ambiente, Bullard et al., (2018), concluíram que a a_w impactou na inativação do patógeno. O valor de a_w de 0,85 mostrou potencial para controlar crescimento de *L. monocytogenes* com contagem inicial de 3 log UFC/g. O mesmo efeito foi encontrado ao avaliar o comportamento de diferentes espécies de fungos e leveduras em diferentes faixas de a_w (0,94; 0,96; 0,98 e 1,0) e dois valores de pH (4,6 ou 7) em suco de maçã tratado à 450MPa por 1,5min e para as espécies mais resistentes à pressão, a 600MPa por 1,5 ou 3min. Interessantemente, os autores verificaram uma maior resistência à pressão nas amostras com menores valores de a_w e maior pH com exceção ao *A. niger* pois seu comportamento foi igual no menor (0,94) e maior (1,0) valor de a_w (P = 0,66 e pH 4,6) (BUERMAN; WOROBO; PADILLA-ZAKOUR, 2020).

Devido à importância da relação entre as características dos produtos (diferentes matrizes) e parâmetros do HPP, modelos matemáticos são ótimas ferramentas para descrever e predizer a inativação microbiana devido à influência de determinadas condições. A modelagem pode ser uma ferramenta útil para realizar avaliações de risco, simular, otimizar e validar o processo contribuindo com o melhor desenho das condições para cada produto. O modelo proposto e validado em função da pressão, tempo e temperatura na inativação de *L.*

monocytogenes em presunto cru permitiu uma avaliação quantitativa da influência dos três fatores na performance do processo demonstrando a alta influência da pressão e do tempo em tais produtos com baixa a_w (BOVER-CID et al., 2011).

Ao identificar o modelo de inativação de *L. monocytogenes* CTC1034 em presunto cru com diferentes parâmetros de a_w e gordura com o processo de HPP, Bover-Cid et al., (2015), utilizaram como parâmetros para o equipamento de alta pressão 347–852 MPa, 5 min/15 °C, variações de a_w de 0,86–0,96 alteradas com água e parâmetros de gordura, alterados adicionando gordura moída para alcançar valores de 10–50%. Os autores concluíram que a a_w e a gordura tem uma significativa ($p<0,05$) influência na inativação do micro-organismo pelo HPP onde entre o menor e o maior valor de a_w tiveram diferença de aproximadamente 4log. Portanto, conforme a a_w foi reduzida e a gordura aumentada, houve uma tendência no micro-organismo de ser mais resistente ao processo de HPP. Os efeitos de baroproteção pela a_w também foram encontrados em estudo similar, porém alterando o micro-organismo para *Salmonella enterica*. Todavia, nesse caso, a variação da gordura não teve impacto significativo no micro-organismo (BOVER-CID et al., 2017).

Através de um Delineamento Composto Central Rotacional (DCCR) Rubio et al., (2018) avaliaram a relação entre valores de a_w atingidos durante o processo de secagem (0,79-0,92), e o processo à alta pressão variando pressão (349-600MPa a 18°C) e tempo (0-12,53min) em chouriço espanhol contaminado com 10^6 UFC/g de *L. monocytogenes*. Os autores concluíram que todas as variáveis influenciaram significativamente ($p<0,05$) na inativação do micro-organismo. Ainda, observaram que baixos valores de a_w parecem formar um efeito protetor no patógeno. Um modelo matemático foi criado para auxiliar processadores de alimentos a melhorar a eficácia do equipamento a alta pressão e cumprimento de legislação.

REFERÊNCIAS BIBLIOGRÁFICAS

AGUILAR, C. et al. Influence of high power ultrasound on natural microflora, pathogen and lactic acid bacteria in a raw meat emulsion. **Ultrasonics Sonochemistry**, v. 72, p. 105415, abr. 2021.

ALÍA, A. et al. Combined effect of temperature, water activity and salt content on the growth and gene expression of Listeria monocytogenes in a dry-cured ham model system. **Meat Science**, v. 155, n. March 2018, p. 16–19, 2019.

ARAÚJO, M. K. et al. Combination of garlic essential oil, allyl isothiocyanate, and nisin Z as bio-preservatives in fresh sausage. **Meat Science**, v. 143, n. 2017, p. 177–183, set. 2018.

AUSTRALIAN GOVERNMENT. **Australia New Zealand food standards code – Standard 1.6.1– Microbiological limits in food, schedule 27.**, 2017. Disponível em: <https://www.legislation.gov.au/Details/F2017C00323>

AYMERICH, T. et al. Inhibition of Listeria monocytogenes and Salmonella by Natural Antimicrobials and High Hydrostatic Pressure in Sliced Cooked Ham. **Journal of Food Protection**, v. 68, n. 1, p. 173–177, 1 jan. 2005.

BAHRAMI, A. et al. Efficiency of novel processing technologies for the control of Listeria monocytogenes in food products. **Trends in Food Science and Technology**, v. 96, p. 61–78, 2020.

BAJOVIC, B.; BOLUMAR, T.; HEINZ, V. Quality considerations with high pressure processing of fresh and value added meat products. **Meat Science**, v. 92, n. 3, p. 280–289, nov. 2012.

BALAMURUGAN, S. et al. Effects of High Pressure Processing and Hot Water Pasteurization of Cooked Sausages on Inactivation of Inoculated Listeria monocytogenes, Natural Populations of Lactic Acid Bacteria, Pseudomonas spp., and Coliforms and Their Recovery during Storage at 4 a. **Journal of Food Protection**, v. 81, n. 8, p. 1245–1251, 1 ago. 2018.

BALAMURUGAN, S. et al. High pressure processing during drying of fermented sausages can enhance safety and reduce time required to produce a dry fermented product. **Food Control**, v. 113, n. March, p. 107224, 2020.

BALASUBRAMANIAM, V. M. (BALA); MARTÍNEZ-MONTEAGUDO, S. I.; GUPTA, R. Principles and Application of High Pressure–Based Technologies in the Food Industry. **Annual Review of Food Science and Technology**, v. 6, n. 1, p. 435–462, 10 abr. 2015.

BARCENILLA, C. et al. Application of lactic acid bacteria for the biopreservation of meat products: A systematic review. **Meat Science**, v. 183, p. 108661, jan. 2022.

BOLUMAR, T. et al. High-pressure processing of meat: Molecular impacts and industrial applications. **Comprehensive Reviews in Food Science and Food Safety**, v. 20, n. 1, p. 332–368, 2021.

BOVER-CID, S. et al. Model for Listeria monocytogenes inactivation on dry-cured ham by

high hydrostatic pressure processing. **Food Microbiology**, v. 28, n. 4, p. 804–809, jun. 2011.

BOVER-CID, S. et al. Modeling the protective effect of a w and fat content on the high pressure resistance of Listeria monocytogenes in dry-cured ham. **Food Research International**, v. 75, p. 194–199, set. 2015.

BOVER-CID, S. et al. Modelling the impact of water activity and fat content of dry-cured ham on the reduction of Salmonella enterica by high pressure processing. **Meat Science**, v. 123, p. 120–125, 2017.

BUCHANAN, R. L. et al. A review of Listeria monocytogenes: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. **Food Control**, v. 75, p. 1–13, 2017.

BUCUR, F. I. et al. Resistance of Listeria monocytogenes to Stress Conditions Encountered in Food and Food Processing Environments. **Frontiers in Microbiology**, v. 9, p. 1–18, 13 nov. 2018.

BUERMAN, E. C.; WOROBO, R. W.; PADILLA-ZAKOUR, O. I. High pressure processing of spoilage fungi as affected by water activity in a diluted apple juice concentrate. **Food Control**, v. 107, n. July 2019, p. 106779, jan. 2020.

BULLARD, B. R. et al. Effect of High Pressure Processing and Water Activity on the Survival of Listeria Monocytogenes on Ready-to-Eat Shelf-Stable Turkey-Based Meat Bars. **Meat and Muscle Biology**, v. 2, n. 2, p. 134–134, 1 jan. 2018.

CAMPOS, F. P.; DOSUALDO, G. L.; CRISTIANINI, M. Utilização da Tecnologia de Alta Pressão no Processamento de Alimentos. **Brazilian Journal of Food Technology**, v. 6, p. 351–357, 2003.

CAYRÉ, M. E.; VIGNOLO, G.; GARRO, O. Modeling lactic acid bacteria growth in vacuum-packaged cooked meat emulsions stored at three temperatures. **Food Microbiology**, v. 20, n. 5, p. 561–566, out. 2003.

CHEFTEL, J. C. Review : High-pressure, microbial inactivation and food preservation. **Food Science and Technology International**, v. 1, n. 2–3, p. 75–90, 1995.

CHEFTEL, J. C.; CULIOLIB, J. Effects of high pressure on meat A review. **Meat Science**, v. 46, n. 3, p. 211–236, 1997.

DA CRUZ, A. G. et al. High pressure processing and pulsed electric fields: potential use in

probiotic dairy foods processing. **Trends in Food Science & Technology**, v. 21, n. 10, p. 483–493, out. 2010.

DAMODARAN, S. .; PARKIN, K. L.; FENNEMA, O. R. Química de Alimentos de Fennema. In: ARTMED EDITORA S.A (Ed.). . **Química de Alimentos de Fennema**. 5th. ed. São Paulo: [s.n.]. p. 1104.

DE OLIVEIRA, T. L. C. et al. Phenolic carvacrol as a natural additive to improve the preservative effects of high pressure processing of low-sodium sliced vacuum-packed turkey breast ham. **LWT - Food Science and Technology**, v. 64, n. 2, p. 1297–1308, 2015.

DESTRO, M. T. **Listeria monocytogenes consumidor na cadeia produtiva final de alimentos : da produção primária ao**. [s.l.] Universidade de São Paulo, 2006.

EFSA. **The European Union One Health 2018 Zoonoses Report: European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC)**. Disponível em: <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5926>. Acesso em: 16 ago. 2020.

EUROPEAN COMISSION. **Commission Regulation (EC) n° 2073/2005 of 15 November 2005 on microbiological criteria for food stuffs.** Disponível em: <https://eur-lex.europa.eu/eli/reg/2005/2073/oj>.

EUROPEAN COMISSION. Regulation (UE) 2015/2283, of 25 november 2015. **Official Journal of the European Communities**, 2015.

FARBER, J. M. et al. Alternative approaches to the risk management of Listeria monocytogenes in low risk foods. **Food Control**, v. 123, p. 107601, maio 2021.

FARKAS, D. F.; HOOVER, D. G. High Pressure Processing. **Journal of Food Science**, v. 65, p. 47–64, 2000.

FDA. **CRF part 120.24**. Disponível em: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50&showFR=1&subpartNode=21:1.0.1.1.20.2>. Acesso em: 2 fev. 2022.

FEINER, G. **Meat products handbook: pratical science and technology**. Cambrigde: Woodhead Publishing Limited, 2006.

FERNÁNDEZ-MARTÍN, F. et al. Salt and phosphate effects on the gelling process of

pressure/heat treated pork batters. **Meat Science**, v. 61, n. 1, p. 15–23, maio 2002.

FORREST, J. C. . et al. **Principles of meat science.** United States: W. H. Freeman and Company, 1975.

FRANCO-VEGA, A. et al. Baroprotective Effect from Reduced a w. In: **Water Activity in Foods**. [s.l.] Wiley, 2020. p. 357–383.

FSIS. HIGH PRESSURE PROCESSING (HPP) AND INSPECTION PROGRAM PERSONNEL (IPP) VERIFICATION RESPONSIBILITIES. p. 1–5, 2012.

FSIS. **9 CFR part 430: Control of Listeria monocytogenes in ready-to-eat meat and poultry products.**Federal Register, 80 (118), 35178–35188., , 2015.

GANDHI, M.; CHIKINDAS, M. L. Listeria: A foodborne pathogen that knows how to survive. **International Journal of Food Microbiology**, v. 113, n. 1, p. 1–15, 2007.

GEORGET, E. et al. Inactivation of microorganisms by high isostatic pressure processing in complex matrices: A review. **Innovative Food Science and Emerging Technologies**, v. 27, p. 1–14, 2015.

GONZÁLEZ-ANGULO, M. et al. Food and Beverage Commercial Applications of High Pressure Processing. In: **Innovative Food Processing Technologies**. [s.l.] Elsevier, 2021. v. 1p. 39–73.

HAYMAN, M. M. et al. Effects of High-Pressure Processing on the Safety, Quality, and Shelf Life of Ready-to-Eat Meats. **Journal of Food Protection**, v. 67, n. 8, p. 1709–1718, 1 ago. 2004.

HEALTH CANADA. **Policy on Listeria monocytogenes in ready-to-eat foods (DF-FSNP 0071).**F. D. Bureau of Microbial Hazards, Health Products and Food Branch 74, , 2011.

HEALTH CANADA. **Foods treated with high pressure processing (HPP)**. Disponível em: <https://inspection.canada.ca/preventive-controls/high-pressure-processing/eng/1498504011314/1498504256677>. Acesso em: 2 fev. 2022.

HUANG, H.-W. et al. Current status and future trends of high-pressure processing in food industry. **Food Control**, v. 72, n. 12, p. 1–8, fev. 2017.

IULIETTO, M. F. et al. Meat Spoilage: A Critical Review of a Neglected Alteration Due to Ropy Slime Producing Bacteria. **Italian Journal of Animal Science**, v. 14, n. 3, p. 4011, 17

jan. 2015.

JAY, JAMES M.; LOESSNER, MARTIN J.; GOLDEN, D. A. **Modern Food Microbiology**. 7th. ed. [s.l: s.n.].

KAMENÍK, J. et al. The effect of high pressure on the microbiological quality and other characteristics of cooked sausages packed in a modified atmosphere orvacuum. **Food Control**, v. 57, n. 1333, p. 232–237, 2015.

KHORSANDI, A. et al. Antibacterial effect of essential oils against spoilage bacteria from vacuum-Packed cooked cured sausages. **Journal of Food Protection**, v. 81, n. 8, p. 1386–1393, 2018.

KORKEALA, H. J.; BJÖRKROTH, K. J. Microbiological Spoilage and Contamination of Vacuum-Packaged Cooked Sausages. **Journal of Food Protection**, v. 60, n. 6, p. 724–731, 1 jun. 1997.

KOSEKI, S.; MIZUNO, Y.; YAMAMOTO, K. Predictive modelling of the recovery of Listeria monocytogenes on sliced cooked ham after high pressure processing. **International Journal of Food Microbiology**, v. 119, n. 3, p. 300–307, nov. 2007.

LARANJA, D. C.; MALHEIROS, P. DA S.; TONDO, E. C. Effective use of nisin to control lactic acid bacterial spoilage in sliced cooked ham. **Journal of Food Processing and Preservation**, v. 43, n. 10, p. 1–5, 30 out. 2019.

LEISTNER, L. Further Developments in the Utilization of Hurdle Technology for Food Preservation. In: **Water in Foods**. [s.l.] Elsevier, 1994. v. 22p. 421–432.

LEISTNER, L.; GORRIS, L. G. M. Food preservation by hurdle technology. **Trends in Food Science & Technology**, v. 6, n. 2, p. 41–46, fev. 1995.

LI, H. et al. Control of pathogenic and spoilage bacteria in meat and meat products by high pressure: Challenges and future perspectives. **Comprehensive Reviews in Food Science and Food Safety**, v. 19, n. 6, p. 3476–3500, 20 nov. 2020.

MATHLOUTHI, M. Water content, water activity, water structure and the stability of foodstuffs. **Food Control**, v. 12, n. 7, p. 409–417, 2001.

MENEZES, N. M. C. et al. Modeling the effect of oregano essential oil on shelf-life extension of vacuum-packed cooked sliced ham. **Meat Science**, v. 139, p. 113–119, maio 2018.

MINISTERIO DA SAÚDE. **Regulamento Técnico Sobre Os Padrões Microbiológicos para Alimentos.**BRASILDiário Oficial. Brasília, DF. 26 de dezembro de 2019, , 2019. Disponível em: <https://www.in.gov.br/en/web/dou/-/resolucao-rdc-n-331-de-23-de-dezembro-de-2019-235332272>

MOLINA-GUTIERREZ, A. et al. Effect of sucrose and sodium chloride on the survival and metabolic activity of Lactococcus lactis under high-pressure conditions. In: **Progress in Biotechnology**. [s.l: s.n.]. v. 19p. 295–302.

MORALES, P. et al. Effect of Cheese Water Activity and Carbohydrate Content on the Barotolerance of Listeria monocytogenes Scott A. **Journal of Food Protection**, v. 69, n. 6, p. 1328–1333, 1 jun. 2006.

MYERS, K. et al. The effect of high hydrostatic pressure, sodium nitrite and salt concentration on the growth of Listeria monocytogenes on RTE ham and turkey. **Meat Science**, v. 93, n. 2, p. 263–268, 2013.

NYCHAS, G.-J. E. et al. Meat spoilage during distribution. **Meat Science**, v. 78, n. 1–2, p. 77–89, jan. 2008.

PALOU, E. et al. Kinetic Analysis ofZygosaccharomyces bailiiInactivation by High Hydrostatic Pressure1. **Lebensmittel-Wissenschaft und-Technologie**, v. 30, n. 7, p. 703–708, nov. 1997.

PATEIRO, M. et al. Antioxidant active packaging systems to extend the shelf life of sliced cooked ham. **Current Research in Food Science**, v. 1, p. 24–30, nov. 2019.

PATTERSON, M. F. et al. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. **Journal of Food Protection**, v. 58, n. 5, p. 524–529, 1995.

PEREIRA, S. A. et al. The Impact of Environmental Stresses in the Virulence Traits of Listeria monocytogenes Relevant to Food Safety. In: **Listeria Monocytogenes**. [s.l.] InTech, 2018.

PÉREZ-BALTAR, A. et al. Impact of Water Activity on the Inactivation and Gene Expression of Listeria monocytogenes during Refrigerated Storage of Pressurized Dry-Cured Ham. **Foods**, v. 9, n. 8, p. 1092, 10 ago. 2020.

PIETRASIK, Z.; GAUDETTE, N. J.; JOHNSTON, S. P. The impact of high hydrostatic pressure on the functionality and consumer acceptability of reduced sodium naturally cured

wieners. **Meat Science**, v. 129, p. 127–134, 2017.

PINTON, M. B. et al. Green technologies as a strategy to reduce NaCl and phosphate in meat products: an overview. **Current Opinion in Food Science**, v. 40, p. 1–5, 2021.

POSSAS, A. et al. High hydrostatic pressure processing of sliced fermented sausages: A quantitative exposure assessment for Listeria monocytogenes. **Innovative Food Science & Emerging Technologies**, v. 52, n. January, p. 406–419, mar. 2019.

POTHAKOS, V. et al. Lactic acid bacteria and their controversial role in fresh meat spoilage. **Meat Science**, v. 109, p. 66–74, nov. 2015.

RAIMONDI, S. et al. Microbiota of sliced cooked ham packaged in modified atmosphere throughout the shelf life. **International Journal of Food Microbiology**, v. 289, n. September 2018, p. 200–208, jan. 2019.

REMENANT, B. et al. Bacterial spoilers of food: Behavior, fitness and functional properties. **Food Microbiology**, v. 45, n. PA, p. 45–53, fev. 2015.

RITZ, M. et al. Physiological damages of Listeria monocytogenes treated by high hydrostatic pressure. **International Journal of Food Microbiology**, v. 79, n. 1–2, p. 47–53, 2002.

RUBIO, B. et al. Model for Listeria monocytogenes inactivation by high hydrostatic pressure processing in Spanish chorizo sausage. **Food Microbiology**, v. 69, p. 18–24, fev. 2018.

RUGNA, G. et al. Distribution, virulence, genotypic characteristics and antibiotic resistance of Listeria monocytogenes isolated over one-year monitoring from two pig slaughterhouses and processing plants and their fresh hams. **International Journal of Food Microbiology**, v. 336, n. October 2020, p. 108912, 2021.

SAMELIS, J.; KAKOURI, A.; REMENTZIS, J. Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4°C. **Food Microbiology**, v. 17, n. 3, p. 329–340, jun. 2000.

SANTHI, D.; KALAIKANNAN, A.; SURESHKUMAR, S. Factors influencing meat emulsion properties and product texture: A review. **Critical Reviews in Food Science and Nutrition**, v. 57, n. 10, p. 2021–2027, 3 jul. 2017.

SILVA, H. R. et al. Listeriose: uma doença de origem alimentar pouco conhecida no Brasil. **Higiene Alimentar**, v. 30, n. 262–263, p. 17–20, 2016.

SLONGO, A. P. et al. Modeling the growth of lactic acid bacteria in sliced ham processed by high hydrostatic pressure. **LWT - Food Science and Technology**, v. 42, n. 1, p. 303–306, jan. 2009.

SMELT, J. P. P. . Recent advances in the microbiology of high pressure processing. **Trends in Food Science & Technology**, v. 9, n. 4, p. 152–158, abr. 1998.

SOLADOYE, O. P.; PIETRASIK, Z. Utilizing High Pressure Processing for Extended Shelf Life Meat Products. **Reference Module in Food Science**, p. 1–8, 2018.

SUN, D. W. **Emerging Technologies for Food Processing, Second Edition**. Second Edi ed. Dublin, Ireland: Academic Press, 2014.

TAKAHASHI, H. et al. Evaluation of the antibacterial activity of allyl isothiocyanate, clove oil, eugenol and carvacrol against spoilage lactic acid bacteria. **LWT**, v. 145, n. March, p. 111263, jun. 2021.

TAO, Y. et al. **High-Pressure Processing of Foods**. Second Edi ed. [s.l.] Elsevier Ltd, 2007.

TEIXEIRA, J. S. et al. The effect of growth temperature, process temperature, and sodium chloride on the high pressure inactivation of Listeria monocytogenes on ham. **European Food Research and Technology**, v. 242, n. 12, p. 2021–2029, 2016.

TOLDRÁ, F. **Handbook of meat processing**. 1th. ed. Iowa USA: John Wiley & Sons, 2010.

TONELLO-SAMSON, C.; QUEIRÓS, R. P.; GONZÁLEZ-ANGULO, M. Advances in high-pressure processing in-pack and in-bulk commercial equipment. In: **Present and Future of High Pressure Processing**. [s.l.] Elsevier, 2020. p. 297–316.

VORST, K. L.; TODD, E. C. D.; RYSER, E. T. Transfer of Listeria monocytogenes during slicing of turkey breast, bologna, and salami with simulated kitchen knives. **Journal of Food Protection**, v. 69, n. 12, p. 2939–2946, 2006.

YANG, P. et al. High pressure processing combined with selected hurdles: Enhancement in the inactivation of vegetative microorganisms. **Comprehensive Reviews in Food Science and Food Safety**, v. 20, n. 2, p. 1800–1828, 16 mar. 2021.

ZHANG, X. et al. Review controlling Listeria monocytogenes in ready-to-eat meat and poultry products: An overview of outbreaks, current legislations, challenges, and future prospects. **Trends in Food Science & Technology**, v. 116, n. June, p. 24–35, out. 2021.

ZHANG, Y. et al. Bio-protective potential of lactic acid bacteria: Effect of Lactobacillus sakei and Lactobacillus curvatus on changes of the microbial community in vacuum-packaged chilled beef. **Asian-Australasian Journal of Animal Sciences**, v. 31, n. 4, p. 585–594, 1 abr. 2018.

CAPÍTULO 2

MODELING THE EFFECT OF WATER ACTIVITY OF A MEAT EMULSION MATRIX MODEL ON *LISTERIA MONOCYTOGENES* INACTIVATION BY HIGH PRESSURE PROCESSING

Submetido à revista Innovative Food Science and Emerging Technologies em 01.06.22

Modeling the effect of water activity of a meat emulsion matrix model on *Listeria monocytogenes* inactivation by high pressure processing

ABSTRACT

High-pressure processing (HPP) is an efficient preservation technology for inactivating *L. monocytogenes*, one of the main pathogens that concern public health and the food industry. However, studies mention that the efficiency of the technology depends on the processing parameters and characteristics of the food matrix. The objective of this study was to build and validate a mathematical model that relates and characterizes the behavior of *L. monocytogenes* ATCC19111 as a function of HPP parameters and water activity (a_w) in a meat emulsion model through response surface methodology. Processing parameters used were: pressure (400-600MPa), a_w (0.94-0.98), and holding time (180-420s), according to the proposed Central Composite Rotational Design (CCRD). The relationship between the listed variables was described through a polynomial equation. According to the obtained equation that best fitted the terms (R^2 94.54%), the three variables influenced the inactivation of the microorganism, where a_w showed the highest influence (p <0.05), considering the range of this study. Processing pressure and holding time showed a positive influence on the inactivation of the microorganism. On the other hand, the pathogen inactivation decreased with the reduction of the a_w in the emulsified meat product, showing a clear baroprotection of the microorganism by the lower a_w. The results highlight the negative influence of lower a_w on the lethality of the microorganism when the product is subjected to high pressure. Therefore, to guarantee the effectiveness of the inactivation process of *L. monocytogenes*, the HPP implementation must be designed and validated considering the intrinsic characteristics of the product and processing parameters. The mathematical model created in this research may contribute to the industry on ensuring food safety, compliance with legislation, and process optimization.

Keywords: High Hydrostatic Pressure. *Listeria monocytogenes.* Food Safety. Meat Products. Response Surface Methodology

1. INTRODUCTION

L. monocytogenes is one of the main pathogens that threaten public health (EFSA, 2019b; USDA/FSIS, 2022). *L. monocytogenes* has a great ability to survive and grow under different stress conditions such as low temperatures, high concentrations of salt, and low a_w, in addition to adapting to the environment due to its biological characteristic of forming biofilms, making their elimination difficult and increasing the risks to consumer health (BUCHANAN et al., 2017). Ready-to-eat meat products (RTE) are one of the main food associated with outbreaks that the microorganism carries (ZHANG et al., 2021) and are considered high risk by government organizations (EFSA BIOHAZ PANEL, 2018). *L. monocytogenes* contamination in RTE meat products is favored by the processing steps after cooking, such as slicing and packaging, where the product is exposed to the environment, providing cross-contamination (POSSAS et al., 2019; VORST; TODD; RYSER, 2006). Thus, *L. monocytogenes* is considered the main target pathogen of control when it comes to microbiological criteria of the RTE meat industry for safe food production (BAHRAMI et al., 2020).

To delay growth and/or eliminate *L. monocytogenes* from products, food processors are forced to adapt and search for new technologies that help ensure food safety. HPP is an attractive non-thermal technology for food preservation with increasing industrial implementation in RTE meat (BOLUMAR et al., 2021; GONZÁLEZ-ANGULO et al., 2021; PÉREZ-BALTAR et al., 2020a). HPP can be applied as a final conservation measure after packaging, such as cold pasteurization. The benefits of the process go beyond the inactivation of pathogens and spoilages that guarantee food safety and shelf-life extension and include the maintenance of the organoleptic and nutritional characteristics of the product (BALASUBRAMANIAM; MARTÍNEZ-MONTEAGUDO; GUPTA, 2015; HYGREEVA; PANDEY, 2016). The technology is recognized and recommended as a potential conservation process by regulatory authorities in countries such as the USA, Canada, and Europe (EUROPEAN COMISSION, 2015; FSIS, 2021; HEALTH CANADA, 2020), favoring its implementation and consumer acceptance of products processed with HPP.

There is clear evidence that HPP is an effective conservation alternative for meat products. However, the implementation and validation of this technology at industrial level requires evaluation, once studies show that the efficiency of HPP does not depend only on the processing parameters and the microorganism, but also on the characteristics of the food matrix (BOVER-CID et al., 2015; GEORGET et al., 2015; HEREU et al., 2014). While the reduction

in pH favors the effect of high-pressure (BLACK et al., 2007), products with a reduced water activity (a_w) present a particular challenge due to the surprising antagonistic baroprotective effect observed in the available studies (BOVER-CID et al., 2015; GEORGET et al., 2015; RUBIO et al., 2018; SMELT, 1998).

Complex matrices, such as meat emulsions, which have high amounts of fat and salt, instigate interest in the effect of high pressure on microbial inactivation, mainly because emulsions can create regions with low a_w even if containing large amounts of free water (GEORGET et al., 2015). To date, no study has demonstrated the a_w impact on the lethality of microorganisms in emulsified meat products subjected to high-pressure, creating a scientific lacuna to be researched.

To improve food safety management decisions, mathematical approaches are built to describe the behavior of microorganisms in function of certain relevant processing and product design variables (ALLENDE; BOVER-CID; FERNÁNDEZ, 2022). In this context, this research aims to build and validate a mathematical model of the inactivation of *L. monocytogenes* ATCC 19111 in a meat emulsion model as a function of the technological parameters of high pressure processing and physicochemical product: pressure, holding time, and a_w. The availability of a mathematical model on *L. monocytogenes* inactivation in complex matrices will greatly contribute to the food industry in the application of the optimized process condition in an efficient way, ensuring food safety and reducing processing costs.

2. MATERIAL AND METHODS

2.1 EXPERIMENTAL DESIGN

For the process optimization a Central Composite Rotational Design (CCRD) was proposed to evaluate the effect of the independent variables: pressure level, holding time, and a_w on the proposed inactivation of *L. monocytogenes*. A 2^3 factorial design, including 6 axial points and 4 repetitions at the central point, totaling 18 trials was considered (RODRIGUES; IEMMA, 2015). The experimental design with levels and factors is shown in Table 1.

Table 1 - Selected variables (factors) and the corresponding five levels used in the Central Composite Rotational Design.

Factors	Levels[a]				
	-1.68	-1	0	+1	+1.68
Pressure (MPa)	400	440	500	560	600
Time (s)	180	229	300	371	420
a_w	0.940	0.948	0.960	0.972	0.980

[a] Considering the central composite rotational design for three factors, the scaled value of α for the coded ±1 values is 1.68 ($2^{3/4}$).

2.2 BACTERIAL STRAIN AND CULTURE PREPARATION

The *L. monocytogenes* ATCC 19111 strain was used for sample inoculation. To prepare the inoculum, the stored culture (stored in BHI + 30% glycerol at -20°C) was reactivated by transferring it to an appropriate broth for growth (BHI – Brain Heart Infusion) and subsequently incubated at 37°C until the turbidity of the broth. After growth, 8 plates were washed and transferred to a tube containing 5mL of BHI broth. The inoculum volume was standardized by the *McFarland Standard Scale* to 10^{10} CFU/mL. The cell suspension was plated with an ALOA® culture medium to confirm the exact quantification of the population.

2.3 MEAT EMULSION MODEL AND SAMPLE PREPARATION

To represent the RTE meat product, a meat emulsion was designed to simulate the product without the interference of other ingredients in the sensitivity of the microorganism. The meat emulsion model with different values of a_w was prepared through the elaboration of a "base batter" composed of lean pork meat – 83%; pork fat – 16%; salt (sodium chloride) – 0.5%; sodium tripolyphosphate (E451i) - 0.35%; and sodium acid pyrophosphate (E450i) – 0.15%. The raw materials and ingredients were chopped in a bowl cutter (model KRAMER GREBE, capacity 30Kg, Germany) until they reached an approximated temperature of 5°C. Subsequently, a sample was taken (approximately 100g) and moisture analysis was performed in duplicate by rapid method (microwave) according to the methodology of AOAC 985.14 in the CEM equipment (Smart Turbo 5 model). From the "base batter" different treatments were elaborated with the values of a_w proposed by the CCRD. Equation 1 proposed by Krispien;

Rodel; Leistner, (1979) was used to calculate the amount of salt (sodium chloride) to be added in each sample considering the average moisture value. The initial amount of salt added was discounted.

$$a_w = -8E\text{-}08x^6 + 4E\text{-}06x^5 - 9E\text{-}05x^4 + 0.0009x^3 - 0.0042x^2 + 0.0019x + 0.9917 \quad (1)$$

Where:

a_w = water activity

x = brine concentration of the product (%NaCl*100)/(%NaCl + % Moisture).

The "base batter" was divided into 5 portions and each one was individually processed in the bowl cutter with the remaining salt for each treatment. In the bowl cutter, each portion was emulsified until reached 10 to 12°C. After, the meat emulsion was stuffed (INCOMAF, capacity 15Kg) into plastic polyamide casings (Viskase ®, Brazil) with 61-63mm diameter. Samples were cooked in a chamber with appropriate staggered temperature until reached 72°C in the center of the product (AGUILAR et al., 2021). Subsequently, samples were cooled in water and ice until 25±0.3°C and stored in temperature-controlled chambers at 4±0.3°C for further treatment. To confirm the a_w result of the proposed method, samples were analyzed in quintuplicate in AquaLab Model 3TE equipment (Decagon Devices, USA) at 25±0.3ºC (error ±0.005). Also, the physicochemical characterization of the product was performed in duplicate, where the pH was measured after initial dilution (10g of sample in 100g of distilled water) and homogenization followed by the introduction of meter electrodes in homogenized slurries for pH readings. Moisture (ISO 1442, 1997), protein (AOAC 928.08, 2019) and fat (AOAC 991.36, 2019) were also determined.

For sample preparation the pieces were sliced, packed and sealed in plastic packaging compounded by nylon/polyethylene with permeability <5g/m^2.24h at 38°C (200x150mm Selovac, Brazil) simulating a commercial packaging composition of sliced products. The microbial analytical units were composed of one slice of 25±1g (approximately ±8mm thickness). The slices were pasteurized (75°C x 15min) in a moist steam oven to eliminate the existing microbiota (decontamination was validated through the microbiological analysis of mesophiles – Petrifilm 3M 48h (AOAC, 2007)). Then, in a laminar flow chamber, 250µl of the suspension (to final inoculum of ~10^8 CFU/g on slice) was spread on the product slice and let for 15min (in the laminar flow chamber) before vacuum packaging. Samples were stored at 4±0.3°C for 24h before HPP (DE OLIVEIRA et al., 2015). Triplicate samples were

performed for each combination of CCRD factors (N – number of survivals), as well as for the contaminated and unprocessed samples (positive control) (N_0 – initial count) and uncontaminated samples (negative control) of each of the 5 proposals from a_w. All samples were analyzed in duplicate, totaling 168 analyses.

2.4 HIGH PRESSURE PROCESSING

After 24 hours of packaging the samples of each proposed treatment by the CCRD were submitted to HPP (QFP 2L-700, Avure Technologies®, USA), with a maximum operating pressure of 690 MPa, temperature control from 0 to 90°C, and pressurization chamber of 2 liters volume (inner vessel of 100x254mm). The unit is equipped with an intensifier (Intensifier, Flow International, Kent, WA). The system was operated using deionized water and a single operating cycle. Two thermocouples located at the top and middle of the treatment chamber monitored the temperature of the pressure transmitting fluid, and another thermocouple monitored the temperature of the water jacket surrounding the pressure vessel. The mean pressurization rate was 5±0.30 $MPa \cdot s^{-1}$. In all tests, the initial water temperature was approximately 18±1°C, the high pressure treatment was achieved in approximately 2 min and the decompression was instantaneous. After processing, all samples were immediately refrigerated for further analysis.

2.5 MICROBIOLOGICAL DETERMINATIONS

Samples were analyzed After 24 hours post-HPP. For microbiological determinations, each sample was opened aseptically with sterile scissors, and 225ml of 0.1% peptone water (1:10) was added to the same plastic bag used for the process and homogenized in a Stomacher homogenizer device (Metroterm®, Brazil) with 490 strokes/ 2min at room temperature. After, a serial decimal dilution was performed (up to 10^{-6} in 0.1% peptone water) and plated in a Petri plate (90x15mm) containing selective and differential medium for Listeria spp., ALOA® (*Agar Listeria according to Ottaviani & Agosti*) and incubated at 37°C±1°C for 48h±2h (ISO 11290-2, 2017).

2.6 STATISTICAL ANALYSIS AND MATHEMATICAL MODELING

The inactivation of *L. monocytogenes* was analyzed in terms of logarithmic reductions as Log (N_0 /N). To quantify the effect of the studied variables on the reduction of *L. monocytogenes*, the Response Surface Methodology (RSM) was applied. The equation that best fitted the inactivation results was obtained through a linear regression where only statistically significant terms (p<0.05) were maintained analyzed by the Protimiza Experiment Design Software (http://experimental-design.protimiza.com.br). Analysis of variance (ANOVA) of the models was performed to assess the significance and goodness of fit through the coefficient of determination (R^2), F test, and Lack of Fit test. The significance of the differences was determined based on an analysis of variance using the Tukey test ($p < 0.05$) using Minitab (version 20) at a confidence interval of 95%.

2.7 MODEL VALIDATION

New experiments were conducted for the experimental validation of the mathematical model created. Three different conditions of the treatments used for the CCRD were selected from combinations of the significant variables according to Table 2. The meat model emulsion elaborated were used for the validation were the same used for the CCRD assays. Triplicate samples were performed for each of trial factors (N – number of survivals), as well as for the contaminated combination and unprocessed samples (positive control) (N_0 – initial count) and uncontaminated samples (negative control). Samples were prepared according to section 2.3, processed according to section 2.4 and analyzed according to section 2.5.

Table 2 - Combinations of selected variables for laboratory validation of the mathematical model created.

Pressure (MPa)	Time (s)	a_w
540	350	0.972
420	400	0.94
580	200	0.96

3. RESULTS AND DISCUSSION

3.1 CHARACTERIZATION OF THE MEAT EMULSION MODEL

The meat emulsion model was designed to simulate a ready-to-eat cooked emulsified meat product in which other variables (such as spices and additives) did not interfere with the results of the proposed study. According to the equation used, the amount of salt (NaCl) to obtain the a_w of 0,94; 0,948; 0,96; 0,972 and 0,98 were 5,65%; 4,86%; 3,62%; 2,45%; and 1,72% respectively. Therefore, as expected, the 5 samples prepared for treatments with different values of a_w (according to CCRD) had similar physical-chemical results. The average values of moisture were 61.64±1.04%, 16.5±0.97% of lipids, 17.06±0.55% of protein, and a pH of 6.1±0.07. The physicochemical results of the model meat emulsion are comparable to RTE emulsified meat products typically found in the local market (HEREU et al., 2014; SALDAÑA et al., 2015; SANTHI; KALAIKANNAN; SURESHKUMAR, 2017).

3.2 INACTIVATION OF *L. MONOCYTOGENES*

Table 3 presents the inactivation of *L. monocytogenes* ATCC 19111 in the meat emulsion model expressed in log (N_0/N) for all combinations of pressure, holding time, and a_w, according to the proposed in the CCRD. Depending on the treatment, the reductions ranged from 0.74 to 5.18 log CFU/g, with the highest inactivation achieved in the a_w combination of 0.972, 560MPa of pressure and holding time of 371s (trial 8, Table 3). Under the conditions applied, total inactivation of the pathogen was not achieved in any of the experiments, even with the highest pressures and times applied. Ritz et al., (2006) observed total inactivation of *L. monocytogenes* inoculated at concentrations of 10^8 –10^9 CFU/mL in laboratory media or buffered solutions under conditions of 400-600MPa for 10 min. Complete inactivation of the pathogen was also found by Bover-Cid et al., (2011) in dry-cured ham, however, it was necessary to use higher pressures that are not available in industrial equipment (852MPa for 9.03min at 16°C). This fact ensures the impact of the matrix on the inactivation of the pathogen.

As expected, the reduction of a_w impacted the inactivation of the pathogen. The highest inactivation of *L. monocytogenes* ATCC 19111 occurred in samples where a_w was higher than 0.96 (αof +1 and +1.68). While comparing trials 2 and 6, which have the same pressure (560MPa) and the same time (229s), but different a_w (0.948 and 0.972, respectively), is possible to observe that the lowest a_w showed the lower inactivation (1.95 log), and the

highest a_w, the higher inactivation (4.76 log CFU/g). The same can be observed comparing trials 13 and 14. The results found in this study are in agreement with previous observations carried out in other meat matrices that the reduction of a_w creates a protective effect on *L. monocytogenes* (BOVER-CID et al., 2015; PÉREZ-BALTAR et al., 2020a; RUBIO et al., 2018). The reasons for the baroprotection created by the low a_w are not completely clear and the main hypothesis relates the low a_w to the stabilization of proteins (enzymes), preventing denaturation, and reducing the effectiveness of treatment with HPP (GEORGET et al., 2015; HAYMAN et al., 2008). On the other hand, it should be pointed out that the recovery of sublethally injured cells can be inhibited by a low a_w, which means that the same physicochemical parameter that creates protection in the microorganism against high-pressure serves as a barrier against microbial recovery post-processing with HPP.

The lethal effect of HPP on *L. monocytogenes* is highly dependent on the characteristics of the matrix and the physiological state of the microorganism (HEREU et al., 2014). The inactivation of *L. monocytogenes* CTC1034 found in the study conducted by Hereu et al., (2014) in mortadella (an emulsified meat product) of a_w: 0.976 ±0.002; pH: 6.3 ±0.1; and lipids: 24.9% ±4.6 under 400MPa pressure conditions for 5 minutes was 4.21 logs CFU/g for the cold-adapted strain. In the present study, a close logarithmic reduction (4.45 log CFU/g) was found at a value of close a_w (0.975) but higher pressure (500MPa) in the same period of pressurization time (trial 14, Table 3). In the study conducted by Bover-Cid et al., (2015) using dry-cured ham contaminated with *L. monocytogenes* CTC1034, a similar reduction (4.18 log CFU/g) was reported in parameters of a_w of 0.922 (achieved by the addition of water starting from an initial a_w product of 0.85); 30.18% fat; and 600MPa of pressure for 5min at 15°C. Differently, in another meat matrix, Spanish chorizo sausage (pH between 4.8 and 5.3), the parameters used were a_w ranging from 0.79 to 0.92 (achieved through drying process); pressure intensity between 349 to 600MPa at 18°C; and a retention period ranging from 0 to 12.53min, a logarithmic reduction similar to these studies was not found. The highest inactivation found in the study was 3.71log CFU/g (a_w: 0.90; the pressure of 550MPa and 10min holding time) (RUBIO et al., 2018). All the works cited above concluded that the lower a_w creates the baroprotective effect in *L. monocytogenes*. However, they observed that similar logarithmic reductions were found, or not, in different processing parameters and product compositions, reinforcing that the physicochemical characteristics of the matrix, the differences in species and condition of the microorganism (adapted or not adapted to cold), and also the means of meeting

the desired a_w (addition of solutes, addition of water or drying) may have interfered with microbial inactivation (GEORGET et al., 2015).

In general, the lethality of *L. monocytogenes* ATCC19111 increased with increasing time and pressure at higher values of a_w. The relationship can be evidenced while comparing trials 9 and 10 with the same a_w and time. The logarithmic reduction of trial 9 (lowest pressure/400MPa) was 0.74 log CFU/g, lower than the 4.04 log CFU/g found in trial 10 (higher pressure/600MPa). In the present study, the inactivation under pressure conditions equal to or lower than 450MPa, such as the inactivation of trial 5 (440MPa/229s/0.972) and trial 7 (440MPa/371s/0.972), were representative (2.34 and 3.86, respectively). However, when in a_w lower than 0.96, the inactivation was also smaller or close to 1 log, similar to the studies cited. In contrast, Rubio et al., (2018) and Bover-Cid et al., (2011), found low levels of inactivation at low pressures, being lower or close to 1 log even when long retention periods were applied (≤15.75min), thus, it can be concluded that for a_w lower than 0.96 and pressure conditions lower than or equal to 450MPa, the level of inactivation of *L. monocytogenes* will reduce. Interestingly, in the study by Bover-Cid et al., (2015), in the condition of 450MPa/5min and a_w of 0.939, inactivation was high (3.92 log CFU/g) in dry-cured ham with a high amount of fat (42.18%). Therefore, further studies should be carried out to better understand the relationship between pressure and fat.

Finally, regarding the pressurization time, it is possible to observe that the increase in holding time where the pressure and a_w were maintained, it increased the logarithmic reduction. For example, increasing the pressurization time from 229 to 371 seconds at a pressure of 440 MPa and a_w of 0.948 (trials 1 and 3) resulted in higher inactivation (0.85 and 1.04 log CFU/g, respectively). The same effect can be observed comparing trials 2 and 4; 5 and 7; 6 and 8; and 11 and 12. Higher pressure and longer processing time were also found in raw ham (BOVER-CID et al., 2011), and turkey breast ham (DE OLIVEIRA et al., 2015).

In the present study, the logarithmic reductions of trials 15, 16, 17, and 18, which correspond to the central points of the CCRD (pressure 500MPa, time of 300 seconds, and a_w of 0.96), were very similar and there is no statistical difference (p <0.05) among them, where the mean inactivation of *L. monocytogenes* ATCC 19111 was 2.64 log CFU/g. The proximity of the results to the central points of the experimental design means high reliability in the experiment.

Table 3 - Results of inactivation of *L. monocytogenes* ATCC 19111 after HPP according to combinations of factors proposed by CCRD.

Trial	Pressure (MPa)	Time (s)	a_w[a]	Inactivation log (N_0/N)[b]
1	440	229	0.948 (0.946)	0.85 (0.88/0.84/0.82)^{j}
2	560	229	0.948 (0.946)	1.95 (1.82/2.3/1.72)^{f,g,h,i,j}
3	440	371	0.948 (0.946)	1.04 (1.16/0.98/0.98)^{i,j}
4	560	371	0.948 (0.946)	2.66 (2.61/2.75/2.61)^{d,e,f,g}
5	440	229	0.972 (0.969)	2.34 (2.43/2.37/2.21)^{e,f,g,h,i}
6	560	229	0.972 (0.969)	4.76 (4.65/4.44/5.20)^{a,b}
7	440	371	0.972 (0.969)	3.86 (3.83/3.93/3.82)^{a,b,c,d}
8	560	371	0.972 (0.969)	5.18 (5.45/5.64/4.46)^{a}
9	400	300	0.96 (0.959)	0.74 (0.72/0.72/0.77)^{j}
10	600	300	0.96 (0.959)	4.04 (3.25/4.58/4.29)^{a,b,c}
11	500	180	0.96 (0.959)	1.54 (1.63/1.35/1.63)^{g,h,i,j}
12	500	420	0.96 (0.959)	3.69 (2.93/3.25/4.89)^{b,c,d,e}
13	500	300	0.94 (0.940)	1.07 (1.38/0.87/0.96)^{h,i,j}
14	500	300	0.98 (0.975)	4.45 (4.05/4.02/5.26)^{a,b}
15	500	300	0.96 (0.959)	2.73 (2.62/2.43/3.14)^{c,d,e,f,g}
16	500	300	0.96 (0.959)	2.38 (2.46/2.48/2.19)^{e,f,g,h,i}
17	500	300	0.96 (0.959)	2.42 (2.60/2.35/2.31)^{e,f,g,h}
18	500	300	0.96 (0.959)	3.02 (2.93/2.41/3.73)^{c,d,e,f}

[a] The column of a_w reports target theorical values according to CCRD; in parenthesis, is reported the actual measured value.

[b] The mean of the inactivation values of three replicates (inactivation value 1/ inactivation value 2/ inactivation value 3). Values with different superscript letters are statistically different according to Tukey's test (p <0.05).

3.3 REGRESSION MODELING OF HP-INACTIVATION DATA AND SURFACE PLOTS

Due to the importance of the relationship between product characteristics (different matrices) and HPP parameters, mathematical models are great tools to describe and predict

microbial inactivation due to the influence of certain conditions. Modeling can be a useful tool for carrying out risk assessments, simulating, optimizing, and validating the process, contributing to the best design of conditions for each product. The present study developed a new mathematical model to predict the interaction between a_w, pressure, and time in the inactivation of *L. monocytogenes* in an emulsified meat product. The results referring to the survivor's ratio log were applied in a multiple regression analysis to obtain the equation to quantify the impacts of each of the statistically significant variables (p<0.05). The approach used resulted in a linear equation according to Equation 4.

$$\text{Log } (N_0/N) = 2.71 + 0.88.P + 0.47.t + 1.12a_w \quad (4)$$

where, Log (N_0 /N) represents the logarithmic reduction of *L. monocytogenes* ATCC 19111; P, the pressure level (MPa); t, the HPP holding time (seconds) (at 18°C); and a_w, the water activity values of the elaborated meat emulsion model.

The mathematical approach allows knowing the intensity with which each of the proposed variables interferes with the study's response. The results showed that the estimated logarithmic reduction of *L. monocytogenes* ATCC 19111 is significantly (p <0.05) affected by the three variables listed in this work: a_w, pressure level, and holding time and are linearly present in the model created. However, it was also possible to observe the influence of a_w is higher than that of pressure, and the influence of pressure is higher than the impact of holding time on the inactivation of *L. monocytogenes* for the range studied. Within the pre-established study range, the highest logarithmic reduction of *L. monocytogenes* was found at one of the highest a_w, and one of the highest pressures and holding times of this study indicated by the CCRD (trial 8, Table 3).

According to ANOVA, the regression was highly significant where the R^2 value obtained was 94.54% for the dependent variable; the calculated F = 80.5 (p <0.001) and the value obtained from the lack of fit of 1.5 (p<0.5) were higher than the respective tabulated F, indicating that the lack of fit is not significant in relation to the pure error. Thus, it is possible to conclude that the model fits well with the experimental data and that the correlation between the predicted and experimental values is satisfactory (Figure 1).

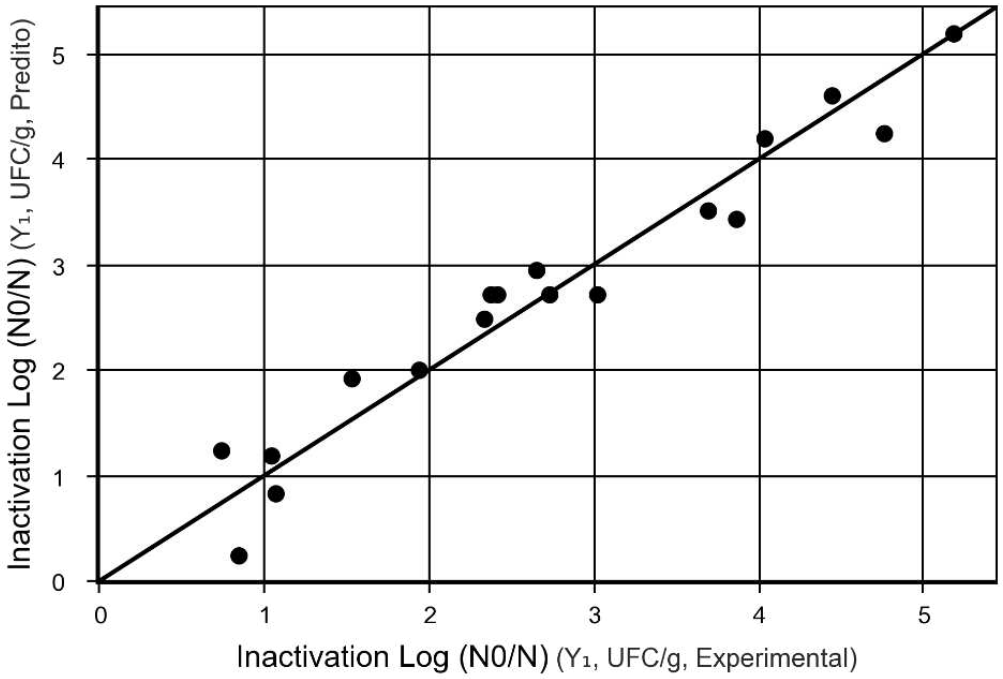
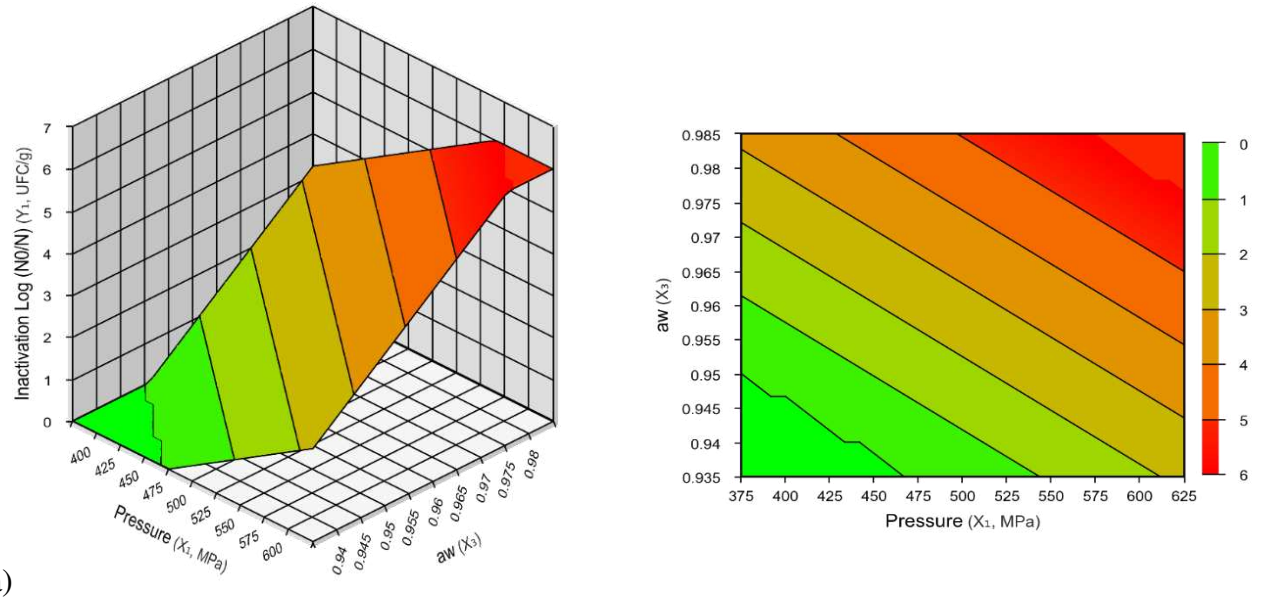
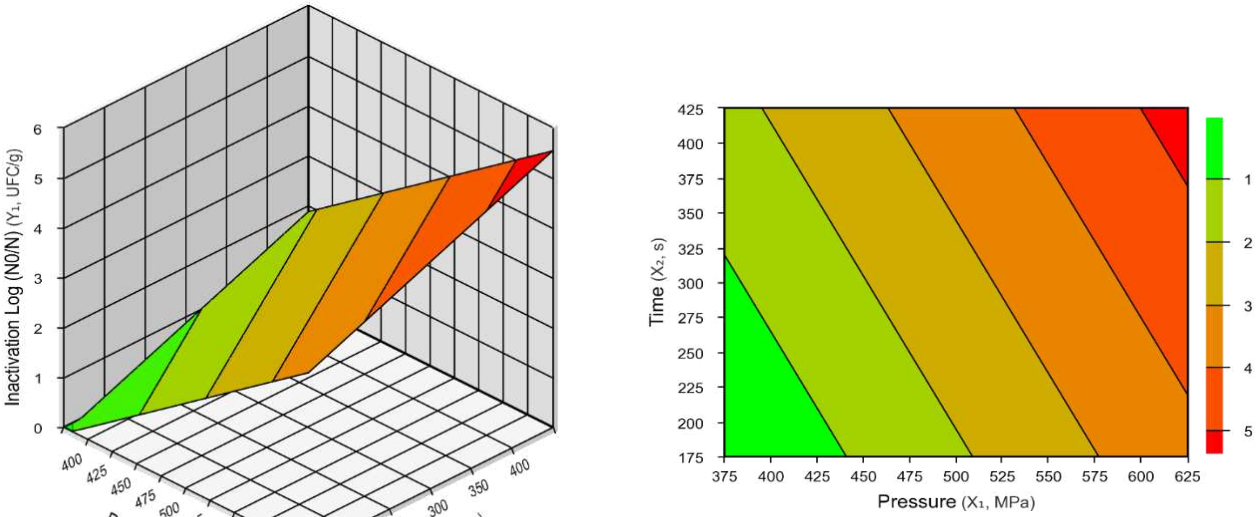


Figure 1 - Predicted versus observed (experimental) values of inactivation of *L. monocytogenes* in a meat emulsion model with different values of a_w submitted to HPP according to the model developed.

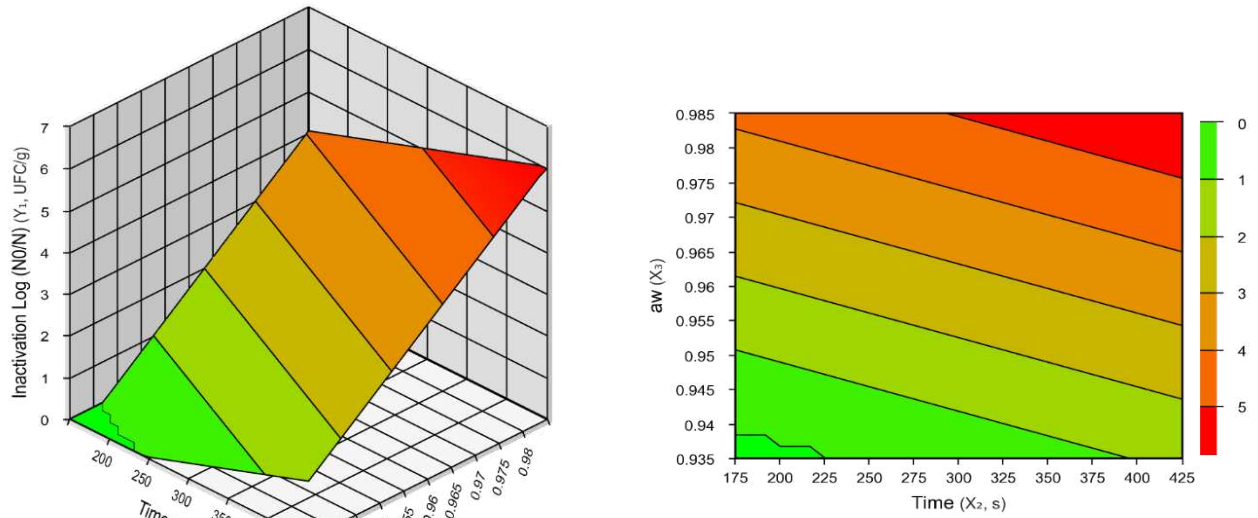
The response surface plots are shown in Figure 2. The surfaces were generated based on the polynomial equation developed (Equation 4) and show an overview of how the three factors influence the HPP-induced inactivation of *L. monocytogenes* ATCC 19111. The lack of curvature of the graphs is attributed to the lack of quadratic terms in the regression. The baroprotection of a_w is seen by the linear increase of inactivation of *L. monocytogenes* while a_w values increased. The same is observed with increasing pressure and holding time.


Inactivation Log (N0/N) (Y1, UFC/g)
Pressure (X1, MPa)
aw (X3)
aw (X3)
Pressure (X1, MPa)

(a)


Inactivation Log (N0/N) (Y1, UFC/g)
Pressure (X1, MPa)
Time (X2, s)
Time (X2, s)
Pressure (X1, MPa)

(b)


Inactivation Log (N0/N) (Y1, UFC/g)
Time (X2, s)
aw (X3)
aw (X3)
Time (X2, s)

(c)

Figure 2 - Response surface and contour plots of the inactivation of *L. monocytogenes* in a model meat emulsion with different values of a_w and submitted to HPP according to the model developed. (a) Effects of pressure and a_w; (b) Effects of pressure and time; (c) Effects of time and a_w. The factor not included in each plot was maintained at the central value of the CCRD (300s in the plot (a); a_w of 0.96 in the plot (b); and 500MPa in the plot (c)).

3.4 MODEL VALIDATION

The experimental validation of the model was conducted to validate the performance of the mathematical model created (Equation 4). To the experimental validation, three conditions were chosen analyzing the contour plots to obtain higher and lower inactivation. Table 4 shows the predicted and observed results obtained through the experimental validation. The experimental results represent the average of the Log (N_0/N) of the three samples. Although there was a difference between predicted and experimental results, it is possible to consider the results obtained in the experimental validation trials were satisfactory. In the three trials the inactivation was higher than the predicted results, thus indicating an underestimation of the model.

Table 4 - Results of predicted and obtained in three conditions chosen to experimental validation.

Pressure (MPa)	Time (s)	a_w	Predicted Results	Experimental Results Log(N/N_0)[a]
540	350	0.972	4.75	5.73 (0.25)
420	400	0.94	0.46	1.10 (0.05)
580	200	0.96	3.22	5.36 (0.22)

[a] Mean standard deviation

3.5 MODEL APPLICATION

With the increasing implementation of HPP in RTE meat products industries, the effect of intrinsic factors, since the parameters that characterize the matrices, such as a_w, interfere in the efficiency of the process in the inactivation of *L. monocytogenes* must be studied and modeled. The use of mathematical models may support food industries to set up processing

parameters and meet the logarithmic reduction of *L. monocytogenes* required by the legislation of different countries. According to Hereu et al., (2012) the mathematical equation of logarithmic reductions as a function of time and pressure of *L. monocytogenes* in mortadella proposed in their study (a_w: 0.976 (±0.00); pH: 6,11 (±0.26); lipids: 17.08% (±4.18); moisture: 63.26% (±3.10); and protein: 15.45% (±1.19)), processing parameters of 526MPa for 3min are required to comply with the legislation established by the European government of 4D for RTE meat products (AESAN, 2005; HEREU et al., 2012). If we apply the same parameters in the proposed equation of this study, it results in a reduction of 3.79 log and to meet the necessary reduction (4D), a pressure of 541MPa and time of 3min are necessary. The reduction response found is very close between the two equations, endorsing the results found in this work, and the hypothesis that may justify the small variation is that other antimicrobial ingredients were used in the composition of the product studied, such as sodium nitrite, which has a direct action on the membrane of microorganisms endorsing the relationship with other factors including other components of the product.

In the US, HPP has been recognized for a few years by the USDA (United States Department of Agriculture). The country's legislation requires validation of the application demonstrating that the process achieves sufficient logarithmic reduction. For RTE products the technology must guarantee a 5 log reduction of *L. monocytogenes* (FSIS, 2021). According to the results obtained in this work, locating the pressure and a_w of 600MPa and 0.97, respectively, on the contour plot of Fig. 2 (a), where time is set at 300s, it is possible to confirm the desired reduction would be achieved (5.07±0.02 CFU/g). However, from the industrial perspective, the shorter the processing time, the better. With a hypothetical case of the shortest processing time (180s) considered by the mathematical model proposed by this study, and respecting the maximum pressure of 600MPa that the available industrial equipment allows, the reduction of 5 log necessary to comply with the legislation, will be feasible only when the matrix characteristic of a_w is equal to or higher than 0.978.

It is important to highlight that the application of the mathematical model must be considered only within the ranges set by the experimental design of each variable. As previously mentioned, the number of studies evaluating meat emulsions with such intrinsic characteristics as the determined range of a_w used in this model is scarce, making comparisons between results difficult.

Even with the proven reduction of *L. monocytogenes* promoted by HPP, studies must be carried out to evaluate the behavior of the microorganism during the shelf-life of the emulsified meat product, since injured cells can recover.

4. CONCLUSION

The results obtained in the present study highlight the impact of a_w as a relevant physicochemical parameter as well as processing parameters, pressure and holding time, determining the inactivation of *L. monocytogenes* in meat matrix, as demonstrated in the mathematical model obtained. In the considered range, which represents real industrial conditions, the effect of the three variables is directly proportional to their increase. Therefore, we can conclude that, in these conditions, the lowered a_w protected *L. monocytogenes* of HP-inactivation. The reported data support the feasibility of high pressure in microbial inactivation but reinforce the need for a guided approach and its validation since intrinsic factors that characterize the product in combination with the technical parameters influence its effectiveness. However, the mechanisms that cause baroprotection are still not fully clear, more research should be applied for a better understanding. The mathematical model developed in this study is an indispensable tool for meat product industries, contributing to guaranteeing food safety and optimizing process efficiency.

ACKNOWLEDGMENTS

The authors are grateful to the State University of Campinas (UNICAMP) for the support given to the project.

REFERENCES

AESAN. Opinión del Comité científico de la AESA sobre una cuestión presentada por la Dirección Ejecutiva, en relación con la aplicación de altas presiones en carne y productos cárnicos (Ref. AESA-2003-007). **Revista del Comité Científico de la AESAN, 1,36–71.**, 2005.

AGUILAR, C. et al. Influence of high power ultrasound on natural microflora, pathogen and lactic acid bacteria in a raw meat emulsion. **Ultrasonics Sonochemistry**, v. 72, p. 105415, abr. 2021.

ALLENDE, A.; BOVER-CID, S.; FERNÁNDEZ, P. S. Challenges and opportunities related to the use of innovative modelling approaches and tools for microbiological food safety management. **Current Opinion in Food Science**, v. 45, p. 100839, jun. 2022.

AOAC. Association of Official Analytical Chemists. **Official Methods of Analysis (18th ed.) Whashington: AOAC**, 2007.

BAHRAMI, A. et al. Efficiency of novel processing technologies for the control of Listeria monocytogenes in food products. **Trends in Food Science and Technology**, v. 96, p. 61–78, 2020.

BALASUBRAMANIAM, V. M. (BALA); MARTÍNEZ-MONTEAGUDO, S. I.; GUPTA, R. Principles and Application of High Pressure–Based Technologies in the Food Industry. **Annual Review of Food Science and Technology**, v. 6, n. 1, p. 435–462, 10 abr. 2015.

BLACK, E. P. et al. Baroprotection of vegetative bacteria by milk constituents: A study of Listeria innocua. **International Dairy Journal**, v. 17, n. 2, p. 104–110, fev. 2007.

BOLUMAR, T. et al. High-pressure processing of meat: Molecular impacts and industrial applications. **Comprehensive Reviews in Food Science and Food Safety**, v. 20, n. 1, p. 332–368, 2021.

BOVER-CID, S. et al. Model for Listeria monocytogenes inactivation on dry-cured ham by high hydrostatic pressure processing. **Food Microbiology**, v. 28, n. 4, p. 804–809, jun. 2011.

BOVER-CID, S. et al. Modeling the protective effect of a w and fat content on the high pressure resistance of Listeria monocytogenes in dry-cured ham. **Food Research International**, v. 75, p. 194–199, set. 2015.

BUCHANAN, R. L. et al. A review of Listeria monocytogenes: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. **Food Control**, v. 75, p. 1–13, 2017.

DE OLIVEIRA, T. L. C. et al. Phenolic carvacrol as a natural additive to improve the preservative effects of high pressure processing of low-sodium sliced vacuum-packed turkey breast ham. **LWT - Food Science and Technology**, v. 64, n. 2, p. 1297–1308, 2015.

EFSA. **The European Union One Health 2018 Zoonoses Report: European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC).** Disponível em: <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5926>. Acesso em: 16 ago. 2020.

EFSA BIOHAZ PANEL. Listeria monocytogenes contamination of ready-to-eat foods and the risk for human health in the EU. **EFSA Journal**, v. 16, n. 1, jan. 2018.

EUROPEAN COMISSION. Regulamento (UE) 2015/2283, de 25 de novembro de 2015. **Jornal Oficial da União Europeia**, 2015.

FSIS. VERIFICATION ACTIVITIES FOR HIGH PRESSURE PROCESSING, IRRADIATION AND MICROWAVE TEMPERING. p. 1–9, 2021.

GEORGET, E. et al. Inactivation of microorganisms by high isostatic pressure processing in complex matrices: A review. **Innovative Food Science and Emerging Technologies**, v. 27, p. 1–14, 2015.

GONZÁLEZ-ANGULO, M. et al. Food and Beverage Commercial Applications of High Pressure Processing. In: **Innovative Food Processing Technologies**. [s.l.] Elsevier, 2021. v. 1p. 39–73.

HAYMAN, M. M. et al. Effect of water activity on inactivation of Listeria monocytogenes and lactate dehydrogenase during high pressure processing. **International Journal of Food Microbiology**, v. 124, n. 1, p. 21–26, 10 maio 2008.

HEALTH CANADA. **Foods treated with high pressure processing (HPP)**. Disponível em: <https://inspection.canada.ca/preventive-controls/high-pressure-processing/eng/1498504011314/1498504256677>. Acesso em: 2 fev. 2022.

HEREU, A. et al. Modeling the high pressure inactivation kinetics of Listeria monocytogenes on RTE cooked meat products. **Innovative Food Science & Emerging Technologies**, v. 16, p. 305–315, out. 2012.

HEREU, A. et al. Analysing and modelling the growth behaviour of Listeria monocytogenes on RTE cooked meat products after a high pressure treatment at 400MPa. **International Journal of Food Microbiology**, v. 186, p. 84–94, set. 2014.

HYGREEVA, D.; PANDEY, M. C. Novel approaches in improving the quality and safety aspects of processed meat products through high pressure processing technology - A review.

Trends in Food Science & Technology, v. 54, p. 175–185, ago. 2016.

KRISPIEN, K.; RÖDEL, W.; LEISTNER, L. Vorschlag zur Berechnung der Wasseraktivität (aw – Wert) von Fleischerzeugnissen aus den Gehalten von Wasser und Kochsalz. **Fleischwirtsch**, v. 59, n. 8, p. 1173–1177, 1979.

PÉREZ-BALTAR, A. et al. Listeria monocytogenes inactivation in deboned dry-cured hams by high pressure processing. **Meat Science**, v. 160, n. September 2019, p. 107960, fev. 2020.

POSSAS, A. et al. High hydrostatic pressure processing of sliced fermented sausages: A quantitative exposure assessment for Listeria monocytogenes. **Innovative Food Science & Emerging Technologies**, v. 52, n. January, p. 406–419, mar. 2019.

RITZ, M. et al. Inactivation of Salmonella Typhimurium and Listeria monocytogenes using high-pressure treatments: destruction or sublethal stress? **Letters in Applied Microbiology**, v. 42, n. 4, p. 357–362, abr. 2006.

RODRIGUES, M. I.; IEMMA, A. F. **Experimental design and process optimization**. 1th. ed. Boca Raton: CRC Press, 2015.

RUBIO, B. et al. Model for Listeria monocytogenes inactivation by high hydrostatic pressure processing in Spanish chorizo sausage. **Food Microbiology**, v. 69, p. 18–24, fev. 2018.

SALDAÑA, E. et al. Microstructure, texture profile and descriptive analysis of texture for traditional and light mortadella. **Food Structure**, v. 6, p. 13–20, out. 2015.

SANTHI, D.; KALAIKANNAN, A.; SURESHKUMAR, S. Factors influencing meat emulsion properties and product texture: A review. **Critical Reviews in Food Science and Nutrition**, v. 57, n. 10, p. 2021–2027, 3 jul. 2017.

SMELT, J. P. P. . Recent advances in the microbiology of high pressure processing. **Trends in Food Science & Technology**, v. 9, n. 4, p. 152–158, abr. 1998.

USDA/FSIS. **IFSAC Announces Priorities for 2022–2023**. Disponível em: <https://www.fsis.usda.gov/news-events/news-press-releases/constituent-update-january-14-2022>. Acesso em: 27 mar. 2022.

VORST, K. L.; TODD, E. C. D.; RYSER, E. T. Transfer of Listeria monocytogenes during slicing of turkey breast, bologna, and salami with simulated kitchen knives. **Journal of Food Protection**, v. 69, n. 12, p. 2939–2946, 2006.

ZHANG, X. et al. Review controlling Listeria monocytogenes in ready-to-eat meat and poultry products: An overview of outbreaks, current legislations, challenges, and future prospects. **Trends in Food Science & Technology**, v. 116, n. June, p. 24–35, out. 2021.

CAPÍTULO 3

EFFECT OF HIGH PRESSURE PROCESSING AND WATER ACTIVITY ON PRESSURE RESISTANT SPOILAGE LACTIC ACID BACTERIA IN A READY-TO-EAT MEAT EMULSION MODEL

Artigo a ser submetido

Effect of high pressure processing and water activity on pressure resistant spoilage lactic acid bacteria in a ready-to-eat meat emulsion model

ABSTRACT

The main use of High Pressure Processing (HPP) in food processing is microorganism inactivation, and studies demonstrated that the characteristics of matrix and microorganisms can interfere on it. As the behavior of lactic acid bacteria exposed to different water activity (a_w) levels in a meat product is still unclear, this study aimed to determine the effect of pressure, time, and a_w to inactivate a pressure resistant lactic acid bacteria (LAB) in a meat emulsion model through a response surface methodology. The meat emulsion model was designed with adjusted a_w (from 0.94 to 0.96) and was inoculated with a pressure resistant LAB and processed varying pressure (400-600MPa) and time (180-480s), following the Central Composite Rotational Design (CCRD). At studied conditions, according to the best fitting and most significant polynomial equation (R^2 of 89.73%), in a meat emulsion model, a_w had no influenced on HPP inactivation on LAB (p<0.05) and only pressure and holding time had significative impact on it. The information obtained in the present study stands out the matrix, microorganism and process effects at HPP efficiency. The answers obtained support food processors in product development, process optimization and food waste reduction.

Keywords: High Pressure Processing. Lactic Acid Bacteria. Water Activity. Meat products. Response surface methodology

1. INTRODUCTION

Consumers expect the food industry to deliver a variety of safe and quality products that address cultural differences and transcend geographic extensions. Such demands drive the search for technologies that reduce the negative changes caused by microorganisms and extend the shelf life of products. High Pressure Processing (HPP) is a promising technology used for the products conservation with increasing implementation in the food industry. González-Angulo et al. (2021) estimated the meat sector had one of the largest HPP food applications

with more than 400 thousand tons of products in 2019. The process consists of submitting the product in its final packaging at high pressure (400 to 600MPa) at certain period of time, and generally at refrigeration temperature The pressure itself is transmitted instantly and the product is compressed in all its area regardless of size and shape (BAHRAMI et al., 2020a; BAJOVIC; BOLUMAR; HEINZ, 2012). The HPP process can duplicate or even tripling the shelf life of meat products through the microbial reduction (GONZÁLEZ-ANGULO et al., 2021; KATSAROS; TAOUKIS, 2021; SLONGO et al., 2009).

The basic principle of microorganisms HPP inactivation is related to protein denaturation, which causes impacts on cell membrane, on biochemical functionality and on genetic mechanisms, destabilizing the cell (BAJOVIC; BOLUMAR; HEINZ, 2012; FARKAS; HOOVER, 2000; SOLADOYE; PIETRASIK, 2018). However, the effectiveness of microbial inactivation depends on several factors such as pressure, temperature, holding time, type of microorganism and its growth phase. It is also related to product characteristics such as a_w, pH, salt levels and presence of antimicrobials (GEORGET et al., 2015; PALOU et al., 1997; RUBIO et al., 2018).

The growth of ready-to-eat meat products consumption is significant. Considering the main intrinsic characteristics of them, such as high a_w, slight acidity and rich nutritional composition, so does its exposure during the packaging process, the product becomes vulnerable to contamination by microorganisms present in the environment and surfaces (HYGREEVA; PANDEY, 2016; TSALOUMI et al., 2021). The lactic acid bacteria (LAB) are identified as the biggest group of spoilages in meat products vacuum packed (NYCHAS et al., 2008; REMENANT et al., 2015). The deterioration caused by LAB leads the economic losses on food industries, and among the spoilage LAB, the major genera of LAB found in meat products are *Leuconostoc, Lactobacillus* and *Pediococcus* (IULIETTO et al., 2015; REMENANT et al., 2015). LAB are microorganisms that are difficult to control, as they support refrigeration temperature, environment with little oxygen and resist the presence of nitrite, salt and smoke (CAYRÉ; VIGNOLO; GARRO, 2003; NYCHAS et al., 2008). The metabolic activities of LAB result in food spoilage, appearing as acidic flavors, off-flavor and odor, slimeness, gas formation and greening causing consumer rejection (IULIETTO et al., 2015).

Knowing the behavior of microorganisms in different matrices under high pressure is necessary to design an effective and optimized process. However, quantifying the influence of matrix characteristics is one of the main scientific gaps in the high pressure field. Low values of a_w are known to protect microorganisms against pressure (GEORGET et al., 2015; RUBIO

et al., 2018; SMELT, 1998). Studies on meat products have shown the influence of a_w on pathogens such as *L. monocytogenes* in dry-cured ham (BOVER-CID et al., 2015) and Spanish chorizo (RUBIO et al., 2018); *Salmonella enterica* in dry-cured ham (BOVER-CID et al., 2017), *E. Coli* in fermented sausages (BALAMURUGAN et al., 2020); also in others no meat matrices as *L. monocytogenes* Scott A in cheese (MORALES et al., 2006). However, the number of studies conducted to understand the behavior of microorganism's deteriorations is limited and inexistent in matrices complex how emulsion meat.

To improve shelf life extension, mathematical approaches are built to describe the behavior of microorganisms in function of certain relevant processing and product design variables (ALLENDE; BOVER-CID; FERNÁNDEZ, 2022). In this context, the objective of this work was to evaluate the impact of pressure intensity, holding time and a_w in a meat emulsion model on the inactivation of a LAB through a response surface methodology. The availability of a validated mathematical model on LAB inactivation in complex matrices will greatly contribute to the food industry in the application of the optimized process condition in an efficient way, reducing processing costs and food waste.

2. MATERIAL AND METHODS

2.1 BACTERIAL ISOLATION, IDENTIFICATION, AND CULTURE PREPARATION

LAB was originally isolated from a spoiled meat emulsion product (mortadella) HP-processed (500MPa/180s/5°C) and kept at 4°C for 60 days. For isolation, an initial count was performed in commercial MRS Agar culture medium. Afterwards, a loop (10µL) was passed to the MRS plate by stretching in 4 fields to obtain individual colonies. After growth, a fully isolated colony was picked up and evaluated through microscopy. The selected colony was transferred to a BHI (Brain Heart Infusion) broth for a turbidity analysis. An aliquot of the turbidity was transferred to an MRS Agar plate and allowed to grow at 37°C for 24 h in a jar with a microaerophilic condition. Thus, the plate was washed using 2mL of BHI + 30% glycerin broth and frozen in microtubes at -20°C. The identification of the microorganism was done through biochemical and molecular methods. The taxonomical classification of the microorganism is *Latilactobacillus sakei*.

To prepare the inoculum, the stored culture was reactivated by transferring it to a MRS agar plate and subsequently incubated at 37°C for 24h in a jar with microaerophilic conditions. After 24 hours, the plate was washed with 2mL of BHI broth. With the LAB suspension, 100µL was inoculated into each of the 8 MRS agar plates and subsequently incubated at 37°C for 24h in a jar with microaerophilic conditions. After growth, each of the 8 plates were washed with 2mL of BHI and transferred to a Schott flask and subsequently incubated at 37°C for 24 hours in a jar with a microaerophilic. The cell suspension was seeded with a 3M™ Petrifilm™ lactic acid bacteria counting plate (AOAC PTM #041701) and incubated at 37±1°C for 48±2h for population confirmation.

2.2 EXPERIMENTAL DESIGN

The Central Composite Rotational Design (CCRD) was followed including three factors (pressure, time, and a_w) on the proposed inactivation of LAB. A 2^3 factorial design, including 6 axial points and 4 repetitions at the central point, totaling 18 trials was implemented (RODRIGUES; IEMMA, 2015). The experimental layout of CCRD is shown in Table 1.

Table 1 - Selected variables (factors) and the corresponding five levels used in the Central Composite Rotational Design.

Factors	Levels[a]				
	-1.68	-1	0	+1	+1.68
Pressure (MPa)	400	440	500	560	600
Time (s)	180	241	330	419	480
a_w	0.940	0.948	0.960	0.972	0.980

[a] Considering the CCRD for three factors, the scaled value of α for the coded ±1 values is 1.68 ($2^{3/4}$).

2.3 MEAT EMULSION MODEL

To represent the RTE meat product, a meat emulsion was designed to simulate the product without the interference of other ingredients in the sensitivity of the microorganism. The meat emulsion model with different values of a_w was prepared through the elaboration of a "base batter" composed of lean pork meat – 83%; pork fat – 16%; salt (sodium chloride) – 0.5%; sodium tripolyphosphate (E451i) - 0.35%; and sodium acid pyrophosphate (E450i) –

0.15%. The raw materials and ingredients were cut in a bowl chopper (model KRAMER GREBE, capacity 30Kg, Germany) until they reached a temperature of 5°C. Subsequently, a sample was taken (approximately 100g) and moisture analysis was performed in duplicate by rapid method (microwave) according to the methodology of AOAC 985.14 in the CEM equipment (Smart Turbo 5 model). From the "base batter" the different treatments were elaborated with the values of a_w proposed by the CCRD. Equation 1 proposed by Krispien; Rodel; Leistner, (1979) was used to calculate the amount of salt (sodium chloride) to be added in each treatment considering the average moisture value. The initial amount of salt added was discounted.

$$a_w = -8E\text{-}08x^6 + 4E\text{-}06x^5 - 9E\text{-}05x^4 + 0.0009x^3 - 0.0042x^2 + 0.0019x + 0.9917 \quad (1)$$

Where:

a_w = water activity

x = brine concentration of the product = (%NaCl*100)/(%NaCl + % Moisture).

The "base batter" was divided into 5 parts and each part was returned to the cutter to add the remaining salt previously calculated for each treatment. In the chopper, each part was emulsified until it reached 10 to 12°C. After, the product was stuffed (INCOMAF, capacity 15Kg) into plastic polyamide casings (Viskase ®, Brazil) with a diameter of 61-63mm and subjected to the cooking process in a chamber with appropriate staggered inter temperatures until reaching 72°C in the thermal center of the product (AGUILAR et al., 2021). Subsequently, the pieces were cooled in water and ice until reaching room temperature and stored in temperature-controlled chambers at 4±0.3°C for further treatment. To ensure a_w theoretical values were on target, the real a_w of the proposed method was measured in quintuplicate in AquaLab Model 3TE equipment (Decagon Devices, USA) at 25±0.3ºC (error ±0.005). Also, the physicochemical characterization of the product was performed in duplicate, where the pH was measured after initial dilution (10g of sample in 100g of distilled water) and homogenization followed by the introduction of meter electrodes in homogenized slurries for pH readings. The moisture, protein and fat contents were also determined according to the Association of Official Analytical Chemists (AOAC, 2007).

2.4 SAMPLE PREPARATION AND HIGH PRESSURE PROCESSING

Pieces were sliced, packed and sealed in plastic packaging compounded by nylon/polyethylene with permeability <5g/m^2.24h at 38°C (200x150mm Selovac, Brazil) simulating a commercial packaging composition of sliced products. The microbial analytical units were composed of one slice of 25±1g (±8mm). The slices were pasteurized (75°C x 15min) in a moist steam oven to eliminate the existing microbiota (decontamination was validated through the microbiological analysis of AOAC 990.12 mesophiles – Petrifilm 3M 48h (AOAC, 2007)). Then, in a laminar flow chamber, 300µl of the suspension (to final inoculum of ~10^7 CFU/g per slice) was spread on the sliced product and sit for 15min in the laminar flow chamber prior to vacuum packaging. The slices were dried by airflow inside the laminar flow hood and vacuum-sealed, simulating the most applied condition in the sliced market. The samples were stored at 4±0.3°C for 24h before HPP in order to fix the microorganism (DE OLIVEIRA et al., 2015).

After 24 hours of packaging, samples of each test proposed by the CCRD were submitted to HPP (QFP 2L-700, Avure Technologies®, USA), with a maximum operating pressure of 690 MPa, temperature control from 0 to 90°C, and pressurization chamber of 2 liters volume (inner vessel of 100x254mm). The unit is equipped with one intensifier (Intensifier, Flow International, Kent, WA). The system was operated using deionized water and a single operating cycle. Two thermocouples located at the top and middle of the treatment chamber monitored the fluid temperature, and another thermocouple monitored the temperature of the water jacket surrounding the pressure vessel. The mean pressurization rate was 5±0.30 MPa·s^{-1}. In all tests the initial water temperature was approximately 19±1°C, the high-pressure treatment was achieved in approximately 2 min and the decompression was instantaneous. After processing, all samples were immediately refrigerated for further analysis. Triplicate samples were performed for each combination of CCRD factors (N - number of survivals), as well as for the contaminated and unprocessed samples (positive control) (N_0 - initial count) and uncontaminated samples (negative control) of each of the 5 proposals from a_w. All samples were analyzed in duplicate, totaling 168 analyses.

2.5 MICROBIOLOGICAL DETERMINATIONS

After 24 hours post-HPP, samples were analyzed. For enumeration of the survivor LAB population, each sample was opened aseptically with sterile scissors, and 225ml of 0.1%

peptone water was added into the packaging and homogenized in a Stomacher device (Metroterm®, Brazil) with 490 strokes/ 2min at room temperature. A serial decimal dilution was performed (up to 10^{-6} in 0.1% peptone water) and plated in 3M™ Petrifilm™ Latic Acid Bacteria Count Plate (AOAC PTM #041701) and incubated at 37°C±1°C for 48h±2h. The detection limit of the aerobic plate count method used in the study is 10 CFU/g.

2.6 STATISTICAL ANALYSIS AND MATHEMATICAL MODELING

The inactivation of the isolated LAB was analyzed in terms of logarithmic reductions as Log (N_0 /N). To quantify the effect of the studied variables on the reduction of LAB, the Response Surface Methodology (RSM) was applied. The equation that best fitted the inactivation results was obtained through a linear regression where only statistically significant terms (p<0.05) were maintained, analyzed by the Protimiza Experiment Design Software (http://experimental-design.protimiza.com.br). Analysis of variance (ANOVA) of the models were performed to assess the significance and goodness of fit through the coefficient of determination (R^2), F test, and Lack of Fit test. Surface responses were designed to illustrate the effect of variables on microorganism inactivation. The significance of the differences was determined based on an analysis of variance using the Tukey test (p< 0.05) using Minitab (version 20) at a confidence interval of 95%.

3. RESULTS AND DISCUSSION

3.1 MEAT EMULSION MODEL CHARACTERISTICS

The meat emulsion model was designed to simulate a ready-to-eat cooked emulsified meat product in which other variables (such as spices and additives) did not interfere with the results of the proposed study. According to the equation used, the amount of salt (NaCl) to obtain the a_w of 0,94; 0,948; 0,96; 0,972 and 0,98 were 5,65%; 4,86%; 3,62%; 2,45%; and 1,72% respectively. Therefore, as expected, the 5 samples prepared for treatments with different a_w values (according to CCRD) had similar physical-chemical results. The average values of moisture were 61.48±1.13%, 16.51±2.42% of fat, 17.74±0.92 % of protein, and a pH of 6.1±0.05. The physicochemical results of the meat emulsion model are similar to RTE emulsified meat products typically found on the market (HEREU et al., 2014; SALDAÑA et al., 2015; SANTHI; KALAIKANNAN; SURESHKUMAR, 2017).

3.2 LACTIC ACID BACTERIA INACTIVATION

Table 3 presents the inactivation of *Latilactobacillus sakei* in the meat emulsion model expressed in log (N_0/N) for all combinations of pressure, holding time and a_w, according to the proposed in the CCRD. All the microbiological counting of negative control (uncontaminated samples) of each condition resulted in counts lower than 10 CFU/g and thus did not disturb measurement of the surviving inoculated bacteria. Under the conditions applied, total inactivation of the microorganism was not achieved in none of the experiments, even with the highest pressures and times applied.

High pressure treatments were effective for inactivating a pressure resistant LAB. The lower inactivation was found in trial 9 (400MPa/330s/0.96) achieving almost 1 log (0.99CFU/g). The most severe treatment used in this study inactivated 4.12 log CFU/g (trial 8, 560Mpa/419s/0.972). Differently, Oliveira et al., (2019) found 6 log reduction of *Leuconostoc mesenteroides* and *Lactobacillus sakei* in RTE sliced turkey breast with reduced salt (1.4%) in treatment conditions of pressure (600MPa) and time (30-180s) at 25°C. Both microorganisms had the same behavior in regard to pressure and holding time, however, the authors affirm that, when applying a lower pressure (<540MPa), the cocci morphology (*Lc. mesenteroides*) appears to be more baroresistant than bacilli form (*L. sakei*). Another study reported 6 log reductions of *Leuconostoc carnosum* M3 isolated from cooked ham (500 and 600MPa/10min/5°C) and also of *Lactobacillus sakei* DSM6333 isolated from vacuum-packaged pork meat (500MPa/10min/5°C) in a cooked ham model (VERCAMMEN et al., 2011). The difference between the LAB spoilage inactivation responses can be related to the resistance of the microorganism, to the matrix or processing parameters. This fact ensures the impact of the matrix, the genera and species of the microorganism and the processing parameters on the inactivation by HPP.

Interestingly, at the range of the variable studied, a_w does not affected the *L. sakei* ethality. The HP-lethality observed in trials assessing different a_w values does not enable to find clear effect of such factor. The comparison among treatments shows the difference within the answers when such physical-chemical parameter varied was less than 1log and there wasn't a statistic difference among tests according to the Tukey test. The treatments 13 and 14, which have the same pressure and the same time (500MPa/330s), but different values of a_w, being extremes of the CCRD (-α, 0.94 and +α, 0.98, respectively), has few differences in *L. sakei*

inactivation response (2.87 and 3.02, respectively). Observing the other CCRD trials, similar comparisons can be made (same pressure and time and different a_w) and they show the same response as treatments 1 and 5 (1.26 and 1.31 log CFU/g, respectively); treatments 2 and 6 (3.13 and 3.04 log CFU/g, respectively); treatments 4 and 8 (3.47 and 4.12 log CFU/g, respectively); treatments 13 and 15 (2.87 and 3.23 log CFU/g, respectively); and treatments 14 and 15 (3.02 and 3.23 log CFU/g, respectively).

Contrary to these findings, as cited previously, some authors have reported the lower a_w creates a protective effect on the microorganisms, especially pathogen, against HPP in a meat matrix (BOVER-CID et al., 2015; RUBIO et al., 2018). Bover-Cid et al. (2015), reported a clear linear baroprotection lowering a_w (0.86-0.96) of dry-cured ham (fat content 10-50%) in *L. monocytogenes* processed by HPP (347-852MPa/5min/15°C). Rubio et al. (2018) also reported a baroprotection on *L. monocytogenes* inactivation caused by low a_w (0.79-0.92) in Spanish chourizo sausage processed on HPP (349-600MPa/0-12.53min, at 18°C). On the other hand, in accordance with the present observations, but not on a meat matrix, a study conducted by Buerman; Worobo; Padilla-Zakour (2020) in a diluted apple juice concentrate to determine the effect of a_w and pH on fungi spoilage under high pressure reported that a_w affected fungi and yeasts tested with the exception of *Aspergillus nigger* S11-0041. Few changes were observed in the behavior of *A. nigger* in the a_w values of 0.94; 0.96; 0.98; and 1, when subjected to processing conditions of 450MPa, 1.5min, pH 4.6 (~5log CFU/g); 450MPa, 1.5min, pH 7.0 (~4.5log CFU/g); and 600MPa, 1.5min, pH 7.0 (~4log CFU/g) tending to almost same inactivation independent to the a_w. Thus, it is possible to conclude that the effect of a_w on microbial inactivation by high pressure is not homogeneous and depends on the matrix, microorganism and processing parameters. More studies should be conducted to understand the behavior of microorganisms in different matrices with different a_w values subjected to high pressure.

Inactivation levels of *L. sakei* after HPP processing in the meat emulsion model were impacted by pressure values. The results indicated that the degree of reduction of *L. sakei* levels increased when increasing the applied pressure. This effect can be evidenced comparing trials with the same time and a_w. The logarithmic reduction of trial 9 (lowest pressure/400MPa) was 0.99 log CFU/g, lower than the 2.86 log CFU/g found in trial 10 (higher pressure/600MPa). The same effect can be observed comparing trial 1 and 2; 3 and 4; 5 and 6; and 7 and 8 and according to Tukey's test, there are statistical differences ($p<0.05$) between the compared trials. The reason for this microbial behavior is that lower pressures retard microbial growth, while

higher pressures promote inactivation and reduced cell division. (BAJOVIC; BOLUMAR; HEINZ, 2012; BOLUMAR et al., 2021) due to the unfolding or denaturing of important cellular enzymes and proteins (GEORGET et al., 2015).

When pressure and a_w were maintained, it is possible to observe on most of experiments the longer holding time, increased the logarithmic reduction. However, interestingly, when comparing the tests that have the same pressure and the same a_w, only the tests that have a pressure higher than 500MPa and a_w higher than 0.96 have a statistical difference ($p<0.05$) between the tests with the lowest and highest holding time. For example, increasing the pressurization time from 241 to 419 seconds at a pressure of 560MPa and a_w of 0.972 (trials 6 and 8) resulted in higher inactivation (3.04 and 4.12 log CFU/g, respectively). The same effect can be observed comparing trials 11 and 12.

The logarithmic reductions of trials 15, 16, 17, and 18, which correspond to the central points of the CCRD (pressure of 500MPa, time of 330 seconds, and a_w of 0.96), were similar and there wasn't a statistical difference ($p<0.05$) between them, where the mean inactivation of *L. sakei* was 3.14 log CFU/g. The proximity of the results to the central point of the experimental design means high reliability in the experiment.

Table 2 - Results of inactivation of *L. sakei* after HPP according to combinations of factors proposed by CCRD.

Trial	Pressure (MPa)	Time(s)	a_w [a]	Inactivation log (N_0/N)[b,c]
1	440	241	0.948 (0.946)	1.26 (0.09)[d,e]
2	560	241	0.948 (0.946)	3.13 (0.12)[b,c]
3	440	419	0.948 (0.946)	1.85 (0.26)[d]
4	560	419	0.948 (0.946)	3.47 (0.26)[a,b,c]
5	440	241	0.972 (0.968)	1.31 (0.20)[d,e]
6	560	241	0.972 (0.968)	3.04 (0.35)[b,c]
7	440	419	0.972 (0.968)	1.60 (0.28)[d,e]
8	560	419	0.972 (0.968)	4.12 (0.26)[a]
9	400	330	0.96 (0.955)	0.99 (0.20)[e]
10	600	330	0.96 (0.955)	2.86 (0.22)[c]
11	500	180	0.96 (0.955)	1.14 (0.44)[d,e]
12	500	480	0.96 (0.955)	3.60 (0.09)[a,b]
13	500	330	0.94 (0.935)	2.87 (0.09)[c]
14	500	330	0.98 (0.977)	3.02 (0.26)[b,c]
15	500	330	0.96 (0.955)	3.23 (0.02)[b,c]
16	500	330	0.96 (0.955)	3.03 (0.16)[b,c]
17	500	330	0.96 (0.955)	3.03 (0.10)[b,c]
18	500	330	0.96 (0.955)	3.29 (0.11)[b,c]

[a] The column of a_w reports target theoretical values according to CCRD; in parenthesis, the actual measured value is reported.

[b] Mean (standard deviation) of the triplicate sample analysis.

[c] Values with different superscript letters are statistically different according to Tukey's test ($p < 0.05$).

3.3 REGRESSION MODELING OF HP-INACTIVATION DATA AND SURFACE PLOTS

A mathematical model to predict the interaction between a_w, pressure, and time in the inactivation of *L. sakei* in an emulsified meat product was developed. The results referring to the survivor's ratio log were applied in a multiple regression analysis to obtain the equation

and quantify the impacts of each of the statistically significant variables (p<0.05). This approach resulted a linear and quadratic terms model according to Equation 4.

$$\text{Log } (N_0/N) = 3.09 + 0.8.P - 0.4.P^2 + 0.47.t - 0.24.t^2 \quad (4)$$

where, Log (N_0/N) represents the logarithmic reduction of *L. sakei*; P, the pressure level (MPa); and t, the HPP processing time (seconds).

Among the variables studied in this work, a_w had no significant effect on *L. sakei* inactivation. On the other hand, pressure intensity and holding time contributed to explain the log reduction of *L. sakei* in meat emulsion model. The regression showed that there was no interaction between variables studied. Pressure and time were statistically significant (p<0.05) and it is presented as a second order term. At the range tested, pressure intensity was the variable with greater significance (p<0.05). The same result was found by Oliveira et al., (2019) to a CCDR designed to LAB spoilage flora *Leuconostoc mesenteroides* and *Lactobacillus sakei* in conditions of the variables pressure (400-600MPa) and time (30-180s) in RTE sliced turkey breast with reduced salt (1.4%).

According to ANOVA, the value of R^2 value obtained was 89.73% for the dependent variable indicating a good degree of correlation between the experimental data and the fitted model; the F value of regression was 28.4 (p<0.001) and the value obtained from the lack of fit of 8.6 (p<0.052) were higher than the respective tabulated F, indicating the lack of fit is not significant in relation to the pure error. The microbial reduction predicted by the mathematical model obtained in this study matches with the observed inactivation in the former work in comparable conditions. To exemplify this conclusion, a condition of 440MPa, 241s and a_w of 0.95 (trial 1, Table 3), the predicted log reduction values were 1.18±0.24CFU/g versus 1.26CFU/g observed and another condition of 500MPa, 330s and 0.96 (trial 16, Table 3), the predicted values were 3.09±0.14CFU/g and the observed 3.03CFU /g. Thus, it is possible to assume the model fits the experimental data and the correlation between the predicted and experimental values is satisfactory.

The response surface and contour plots (Figure 1) show an overview of how the two factors influence the HPP-induced inactivation of *L. sakei* in a meat emulsion model. The surfaces were generated based on the polynomial equation developed (Equation 4). The inactivation was described as a function of pressure and holding time. The increase of *L. sakei* inactivation was linked to the increase of both the pressure treatment and holding time as shown

by the steepest slope of pressure and time effects. The curvature of the graphs is attributed to the quadratic terms in the regression.

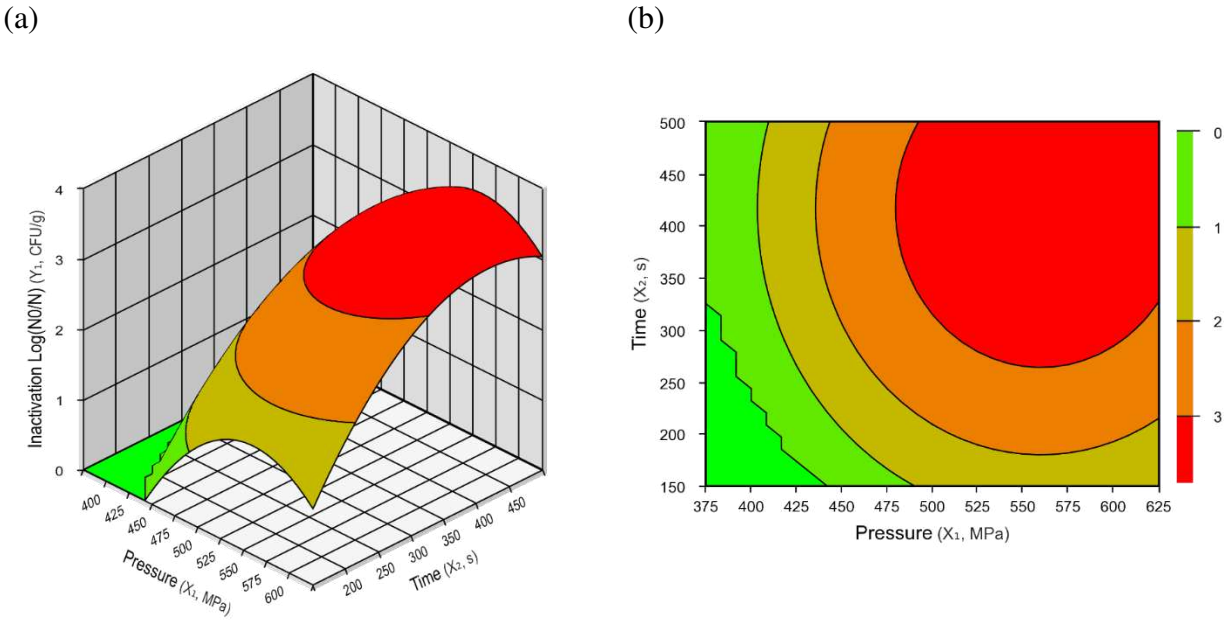


Figure 1 - Response surface graphs (a) and contour plots (b) of the effect of pressure and time on the inactivation of *L. sakei* in a model meat emulsion according to the model developed. The factor not included in the graph was maintained at the central value of the CCRD, a_w of 0.96.

4. CONCLUSION

The results clearly showed that HPP is an efficient technology to inactivate spoilage microorganisms such as *L. sakei*. However, the HP-inactivation of *L. sakei* in a meat emulsion matrix is strongly dependent on the processing parameters. With the results obtained in this study it is possible to conclude that, at the range used which represents real industrial conditions, the HP-inactivation of a pressure resistant lactic acid bacteria is totally independent of the a_w of a meat emulsion model. Also, the pressure intensity was the most significant influencing factor LAB inactivation followed by holding time. The effect of the significant variables is directly proportional to their increase. Thus, the interference of the physico-chemical parameters of the matrix processed by high pressure is dependent on the type of microorganism and the processing parameters. The developed mathematical model is an

indispensable tool for meat product industries, contributing to extend shelf life, reducing waste and optimizing process efficiency. Further studies are necessary to access the influence of a_w during the shelf life of a product processed by HPP as well as the effect of a_w in other microorganisms and matrix.

ACKNOWLEDGMENTS

The authors are grateful to the State University of Campinas (UNICAMP) for the support given to the project.

REFERENCES

AGUILAR, C. et al. Influence of high power ultrasound on natural microflora, pathogen and lactic acid bacteria in a raw meat emulsion. **Ultrasonics Sonochemistry**, v. 72, p. 105415, abr. 2021.

ALLENDE, A.; BOVER-CID, S.; FERNÁNDEZ, P. S. Challenges and opportunities related to the use of innovative modelling approaches and tools for microbiological food safety management. **Current Opinion in Food Science**, v. 45, p. 100839, jun. 2022.

AOAC. Association of Official Analytical Chemists. **Official Methods of Analysis (18th ed.) Whashington: AOAC**, 2007.

BAHRAMI, A. et al. Efficiency of novel processing technologies for the control of Listeria monocytogenes in food products. **Trends in Food Science and Technology**, v. 96, p. 61–78, 2020.

BAJOVIC, B.; BOLUMAR, T.; HEINZ, V. Quality considerations with high pressure processing of fresh and value added meat products. **Meat Science**, v. 92, n. 3, p. 280–289, nov. 2012.

BALAMURUGAN, S. et al. High pressure processing during drying of fermented sausages can enhance safety and reduce time required to produce a dry fermented product. **Food Control**, v. 113, n. March, p. 107224, 2020.

BOLUMAR, T. et al. High-pressure processing of meat: Molecular impacts and industrial

applications. **Comprehensive Reviews in Food Science and Food Safety**, v. 20, n. 1, p. 332–368, 2021.

BOVER-CID, S. et al. Modeling the protective effect of a w and fat content on the high pressure resistance of Listeria monocytogenes in dry-cured ham. **Food Research International**, v. 75, p. 194–199, set. 2015.

BOVER-CID, S. et al. Modelling the impact of water activity and fat content of dry-cured ham on the reduction of Salmonella enterica by high pressure processing. **Meat Science**, v. 123, p. 120–125, 2017.

BUERMAN, E. C.; WOROBO, R. W.; PADILLA-ZAKOUR, O. I. High pressure processing of spoilage fungi as affected by water activity in a diluted apple juice concentrate. **Food Control**, v. 107, n. July 2019, p. 106779, jan. 2020.

CAYRÉ, M. E.; VIGNOLO, G.; GARRO, O. Modeling lactic acid bacteria growth in vacuum-packaged cooked meat emulsions stored at three temperatures. **Food Microbiology**, v. 20, n. 5, p. 561–566, out. 2003.

DE OLIVEIRA, T. L. C. et al. Phenolic carvacrol as a natural additive to improve the preservative effects of high pressure processing of low-sodium sliced vacuum-packed turkey breast ham. **LWT - Food Science and Technology**, v. 64, n. 2, p. 1297–1308, 2015.

FARKAS, D. F.; HOOVER, D. G. High Pressure Processing. **Journal of Food Science**, v. 65, p. 47–64, 2000.

GEORGET, E. et al. Inactivation of microorganisms by high isostatic pressure processing in complex matrices: A review. **Innovative Food Science and Emerging Technologies**, v. 27, p. 1–14, 2015.

GONZÁLEZ-ANGULO, M. et al. Food and Beverage Commercial Applications of High Pressure Processing. In: **Innovative Food Processing Technologies**. [s.l.] Elsevier, 2021. v. 1p. 39–73.

HEREU, A. et al. Analysing and modelling the growth behaviour of Listeria monocytogenes on RTE cooked meat products after a high pressure treatment at 400MPa. **International Journal of Food Microbiology**, v. 186, p. 84–94, set. 2014.

IULIETTO, M. F. et al. Meat Spoilage: A Critical Review of a Neglected Alteration Due to Ropy Slime Producing Bacteria. **Italian Journal of Animal Science**, v. 14, n. 3, p. 4011, 17

jan. 2015.

KATSAROS, G.; TAOUKIS, P. Microbial Control by High Pressure Processing for Shelf-Life Extension of Packed Meat Products in the Cold Chain: Modeling and Case Studies. **Applied Sciences**, v. 11, n. 3, p. 1317, 1 fev. 2021.

KRISPIEN, K.; RÖDEL, W.; LEISTNER, L. Vorschlag zur Berechnung der Wasseraktivität (aw – Wert) von Fleischerzeugnissen aus den Gehalten von Wasser und Kochsalz. **Fleischwirtsch**, v. 59, n. 8, p. 1173–1177, 1979.

MORALES, P. et al. Effect of Cheese Water Activity and Carbohydrate Content on the Barotolerance of Listeria monocytogenes Scott A. **Journal of Food Protection**, v. 69, n. 6, p. 1328–1333, 1 jun. 2006.

NYCHAS, G.-J. E. et al. Meat spoilage during distribution. **Meat Science**, v. 78, n. 1–2, p. 77–89, jan. 2008.

OLIVEIRA, T. L. C. DE et al. Optimization of high pressure processing to reduce the safety risk of low-salt ready-to-eat sliced turkey breast supplemented with carvacrol. **British Food Journal**, v. 121, n. 11, p. 2592–2606, 23 out. 2019.

PALOU, E. et al. Kinetic Analysis ofZygosaccharomyces bailiiInactivation by High Hydrostatic Pressure1. **Lebensmittel-Wissenschaft und-Technologie**, v. 30, n. 7, p. 703–708, nov. 1997.

REMENANT, B. et al. Bacterial spoilers of food: Behavior, fitness and functional properties. **Food Microbiology**, v. 45, n. PA, p. 45–53, fev. 2015.

RODRIGUES, M. I.; IEMMA, A. F. **Experimental design and process optimization**. 1th. ed. Boca Raton: CRC Press, 2015.

RUBIO, B. et al. Model for Listeria monocytogenes inactivation by high hydrostatic pressure processing in Spanish chorizo sausage. **Food Microbiology**, v. 69, p. 18–24, fev. 2018.

SALDAÑA, E. et al. Microstructure, texture profile and descriptive analysis of texture for traditional and light mortadella. **Food Structure**, v. 6, p. 13–20, out. 2015.

SANTHI, D.; KALAIKANNAN, A.; SURESHKUMAR, S. Factors influencing meat emulsion properties and product texture: A review. **Critical Reviews in Food Science and Nutrition**, v. 57, n. 10, p. 2021–2027, 3 jul. 2017.

SLONGO, A. P. et al. Modeling the growth of lactic acid bacteria in sliced ham processed by high hydrostatic pressure. **LWT - Food Science and Technology**, v. 42, n. 1, p. 303–306, jan. 2009.

SMELT, J. P. P. . Recent advances in the microbiology of high pressure processing. **Trends in Food Science & Technology**, v. 9, n. 4, p. 152–158, abr. 1998.

SOLADOYE, O. P.; PIETRASIK, Z. Utilizing High Pressure Processing for Extended Shelf Life Meat Products. **Reference Module in Food Science**, p. 1–8, 2018.

VERCAMMEN, A. et al. Shelf-life extension of cooked ham model product by high hydrostatic pressure and natural preservatives. **Innovative Food Science & Emerging Technologies**, v. 12, n. 4, p. 407–415, out. 2011.

DISCUSSÃO GERAL

Os resultados obtidos nesse trabalho mostram que é possível observar que a interferência das características da matriz cárnea, o tipo de micro-organismo e os parâmetros de processamento por alta pressão isostática interferem na inativação microbiana.

A redução logarítmica de *L. monocytogenes* em uma matriz cárnea emulsionada quando submetido à alta pressão foi influenciada significativamente (p<0,05) pela baixa atividade de água e pelo aumento de pressão e tempo utilizados no processamento à alta pressão dentro da faixa estudada.

Por outro lado, nos parâmetros estudados, a atividade de água não influenciou na inativação de *Latilactobacillus sakei* resistente à pressão isolada de um produto cárneo, sendo apenas a intensidade de pressão e o tempo de processamento significativos (p<0,05).

Ao comparar os dados obtidos nesse trabalho, também foi possível observar que a resistência de *L. monocytogenes* à alta pressão foi menor quando comparada à resistência de *L. sakei* em condições semelhantes onde a inativação de *L. monocytogenes* foi de 5,18log UFC/g (tratamento 8, 560MPa/371s/0,972) e a de *L. sakei* de 4,12 log UFC/g (tratamento 8, 560MPa/419s/0,972). Esses dados endossam a interferência do micro-organismo na inativação pela alta pressão.

CONCLUSÕES GERAIS

Os resultados obtidos no presente estudo indicam que, na faixa dos parâmetros considerados que simulam condições reais de características de produto e processamento, a emergente tecnologia de alta pressão foi eficaz na a inativação microbiana. Ainda, foi observado que a atividade de água pode influenciar na inativação microbiana pela alta pressão, dependendo do micro-organismo, em uma matriz cárnea emulsionada.

Os modelos matemáticos criados considerando as variáveis que tiveram influência significativa em cada estudo realizado, servem de apoio à indústria de alimentos no desenvolvimento de produtos e otimização de processos (pressões mais baixas ou tempos menores de contenção), reduzindo custos de produção, garantindo o cumprimento de legislações e a segurança de alimentos além da redução do desperdício de alimentos e extensão da vida de prateleira.

Mais estudos considerando outros micro-organismos e outras matrizes devem ser realizados para aprofundar o entendimento do comportamento da atividade de água. Também, considerando o favorecimento da recuperação microbiana causada pela alta atividade de água, o comportamento dos micro-organismos sobreviventes após processamento à alta pressão em matrizes com diferentes atividades de água deve ser avaliado.

REFERÊNCIAS BIBLIOGRÁFICAS

BAHRAMI, A. et al. Efficiency of novel processing technologies for the control of Listeria monocytogenes in food products. **Trends in Food Science and Technology**, v. 96, p. 61–78, 2020.

BALAMURUGAN, S. et al. High pressure processing during drying of fermented sausages can enhance safety and reduce time required to produce a dry fermented product. **Food Control**, v. 113, n. March, p. 107224, 2020.

BOVER-CID, S. et al. Model for Listeria monocytogenes inactivation on dry-cured ham by high hydrostatic pressure processing. **Food Microbiology**, v. 28, n. 4, p. 804–809, jun. 2011.

BOVER-CID, S. et al. Modeling the protective effect of a w and fat content on the high pressure resistance of Listeria monocytogenes in dry-cured ham. **Food Research International**, v. 75, p. 194–199, set. 2015.

BUERMAN, E. C.; WOROBO, R. W.; PADILLA-ZAKOUR, O. I. High pressure processing of spoilage fungi as affected by water activity in a diluted apple juice concentrate. **Food Control**, v. 107, n. July 2019, p. 106779, jan. 2020.

BULLARD, B. R. et al. Effect of High Pressure Processing and Water Activity on the Survival of Listeria Monocytogenes on Ready-to-Eat Shelf-Stable Turkey-Based Meat Bars. **Meat and Muscle Biology**, v. 2, n. 2, p. 134–134, 1 jan. 2018.

CDC. National Enteric Disease Surveillance: The Listeria Iniciative. **Centers for Disease Control and Prevention**, 2016.

CDC. Outbreak of Listeria Infections Linked to Deli-Sliced Meats and Cheeses. **Centers for Disease Control and Prevention**, 2019.

CDC. Outbreak of Listeria Infections Linked to Deli Meats. **Centers for Disease Control and Prevention**, 2020.

DAMODARAN, S. .; PARKIN, K. L.; FENNEMA, O. R. Química de Alimentos de Fennema. In: ARTMED EDITORA S.A (Ed.). . **Química de Alimentos de Fennema**. 5th. ed. São Paulo: [s.n.]. p. 1104.

EFSA. **The European Union One Health 2018 Zoonoses Report: European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC)**.

Disponível em: <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5926>. Acesso em: 16 ago. 2020.

FAO. Global Food losses and Food waste. Save Food Congress. **Unep**, n. May, p. 1, 2011.

GANDHI, M.; CHIKINDAS, M. L. Listeria: A foodborne pathogen that knows how to survive. **International Journal of Food Microbiology**, v. 113, n. 1, p. 1–15, 2007.

GEORGET, E. et al. Inactivation of microorganisms by high isostatic pressure processing in complex matrices: A review. **Innovative Food Science and Emerging Technologies**, v. 27, p. 1–14, 2015.

HEREU, A. et al. Analysing and modelling the growth behaviour of Listeria monocytogenes on RTE cooked meat products after a high pressure treatment at 400MPa. **International Journal of Food Microbiology**, v. 186, p. 84–94, set. 2014.

HYGREEVA, D.; PANDEY, M. C. Novel approaches in improving the quality and safety aspects of processed meat products through high pressure processing technology - A review. **Trends in Food Science & Technology**, v. 54, p. 175–185, ago. 2016.

LEISTNER, L. Further Developments in the Utilization of Hurdle Technology for Food Preservation. In: **Water in Foods**. [s.l.] Elsevier, 1994. v. 22p. 421–432.

PÉREZ-BALTAR, A. et al. Impact of Water Activity on the Inactivation and Gene Expression of Listeria monocytogenes during Refrigerated Storage of Pressurized Dry-Cured Ham. **Foods**, v. 9, n. 8, p. 1092, 10 ago. 2020.

RUBIO, B. et al. Model for Listeria monocytogenes inactivation by high hydrostatic pressure processing in Spanish chorizo sausage. **Food Microbiology**, v. 69, p. 18–24, fev. 2018.

RUGNA, G. et al. Distribution, virulence, genotypic characteristics and antibiotic resistance of Listeria monocytogenes isolated over one-year monitoring from two pig slaughterhouses and processing plants and their fresh hams. **International Journal of Food Microbiology**, v. 336, n. October 2020, p. 108912, 2021.

THOMAS, M. K. et al. Economic cost of a listeria monocytogenes outbreak in Canada, 2008. **Foodborne Pathogens and Disease**, v. 12, n. 12, p. 966–971, 2015.

TSALOUMI, S. et al. Quantitative risk assessment of Listeria monocytogenes in ready-to-eat (RTE) cooked meat products sliced at retail stores in Greece. **Food Microbiology**, v. 99, n.

December 2020, p. 103800, out. 2021.

USDA/FSIS. **Quantitative assessment of the relative risk to public health from foodborne Listeria monocytogenes among selected categories of ready-to-eat foods.** Disponível em: <https://www.fda.gov/food/cfsan-risk-safety-assessments/quantitative-assessment-relative-risk-public-health-foodborne-listeria-monocytogenes-among-selected>. Acesso em: 7 out. 2020.

YORDANOV, D. G.; ANGELOVA, G. V. High Pressure Processing for Foods Preserving. **Biotechnology & Biotechnological Equipment**, v. 24, n. 3, p. 1940–1945, 15 jan. 2010.