THALES LEANDRO COUTINHO DE OLIVEIRA

HIGH PRESSURE PROCESSING OPTIMIZATION FOR LOW-SODIUM SLICED READY-TO-EAT TURKEY BREAST WITH ADDITIONAL NATURAL ANTIMICROBIAL HURDLE

OTIMIZAÇÃO DO PROCESSAMENTO A ALTA PRESSÃO PARA EMBUTIDO DE PERU FATIADO COM TEOR REDUZIDO DE SÓDIO COMBINADO A UMA BARREIRA ANTIMICROBIANA NATURAL

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Thesis presented to the Food Technology Department of the School of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Ph.D. in FOOD TECHNOLOGY.

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Orientador: PROF. DR. MARCELO CRISTIANINI

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ABSTRACT

The reduction of sodium chloride in processed food formulations represents a great challenge for modern industry, considering the relationship established between high sodium intake and the occurrence of arterial hypertension, the major risk factor associated with the occurrence of cardiovascular diseases and strokes. In meat products, in addition to acting on sensory quality and texture, salt plays a key role on the microbiological stability. A simple reduction can result in unstable products during their *shelf-life*. Therefore, for the success of salt reduction strategies implemented safely, it is necessary the application of complementary technologies or alternative preservatives. Non-thermal emerging technologies for microbial inactivation, such as High Pressure Processing (HPP), appear as feasible alternative for this purpose, eliminating the dependence of chemical preservatives considering the reduction of salt barrier. The addition of natural antimicrobial agents as additional hurdles (*Multi Hurdle Technology*) to the HPP, aiming to enhance the preservative effects, has been identified as a promising trend in modern literature. Given this exposed, after determination of low-sodium working formulation, this research aimed adjust the processing variables "pressure load" and "dwell time" (using low-processing temperatures of 25°C) for sliced vacuum-packaged ready-to-eat turkey breast. Post-processing microbial inactivation responses were evaluated (by logarithmic reductions) against inoculated populations of the target pathogen *Listeria monocytogenes* and spoilage lactic acid bacteria including *Leuconostoc mesenteroides* and *Lactobacillus sakei*; were also evaluated quality-attributes including color, water activity, pH, syneresis, texture and lipid oxidation. In addition, in order to use multiple barriers for preservation technology, was studied the effect of adding bioactive component, carvacrol at concentrations of 200ppm in the product formulation. Employing a simple reduction strategy, without adding salt substitutes, after evaluation of physicochemical aspects, sensory and microbiological, it was concluded that levels of 30% NaCl reduction (aproximatelly 25% less sodium) on the control made with 20g/kg, are viable; however, problems with microbiological stability were evidenced. After evaluation of different pressure ranges and processing time, following the performance criteria required for post-inactivation process *Listeria*

monocytogenes (4-5 log reduction), a value of 600MPa/180 seconds at 25°C was presented with an adequate treatment for studied product with low sodium content, promoting effective logarithmic reductions against the studied target pathogen and spoilage LAB. Instrumental changes (p<0.05) in some of the evaluated quality attributes could be revealed mainly in syneresis, lipid oxidation and texture; however, a sensory confirmation needs to be established. Additionally, data presented showed that consumers have not been able to differ low-sodium products processed by HPP (600MPa/180sec/25°C) and unpressurised; in fact, despite significant instrumental effects, these changes seems to be not identified by consumers.

The addition of natural barrier, such as carvacrol at acceptable sensory levels (200ppm), was able to potentiate the effects of post-process inactivation (logarithmic reductions) of the target microbial groups; combined benefit effects along the refrigerated product storage were also evidenced by reduced growth rates and increased lag phases, thus maximizing their potential for conservation. The combination of HPP and carvacrol represented a promising weapon against phenomena of sub-lethal injuries and cell recovery. It is suggested that the intensity of the HPP process (in terms of pressure load, process time and temperature) required to inactivate microorganisms in required levels, may be reduced in the presence of additional microbial barriers, ensuring the overall quality of the processed product under mild processing conditions. In addition, a number of industrial advantages can be highlighted such as reduced costs for the initial installation and maintenance equipment (low required pressure loads) and maximization of production by effectively processing cycles shortened (higher productivity cycles per hour).

Keywords: Carvacrol, multiple hurdle technology, *Listeria monocytogenes*, ready-to-eat, low-sodium, lactic acid bacteria, non-thermal technologies.

RESUMO GERAL

A redução do cloreto de sódio nas formulações de alimentos processados representa um grande desafio para indústria moderna, tendo em vista a relação estabelecida entre o consumo elevado de sódio e a ocorrência da hipertensão arterial, principal fator de risco associado à ocorrência de distúrbios do cérebro e cardiovasculares. Em produtos cárneos, além de atuar sobre aspectos sensoriais e de textura, o sal desempenha papel chave sobre sua estabilidade microbiológica. Uma simples redução pode resultar em produtos instáveis durante sua vida de prateleira. Logo, para que estratégias de redução de sal sejam implementadas de maneira segura, faz-se necessário a aplicação de tecnologias ou agentes de preservação complementares. Tecnologias emergentes não-térmicas de inativação microbiana como o processamento à Alta Pressão (HPP - *High Pressure Processing*) surgem como opções factíveis para tal propósito, eliminando a dependência de conservantes químicos em face à redução de sal. A adição de agentes antimicrobianos naturais como barreiras adicionais à HPP (*Tecnologia de Múltiplas Barreiras*), visando potencializar os efeitos conservadores, têm sido apontada como uma promissora tendência na literatura moderna. Diante deste exposto, após determinação da formulação de trabalho com teor reduzido de sódio, a pesquisa objetivou ajustar as variáveis de processo "carga de pressão" e "tempo de residência" (utilizando baixas temperaturas de 25°C) para o processamento de embutido de peito peru fatiado embalado a vácuo (tipo *"Blanquet"*). Foram avaliadas respostas de inativação microbiana pós-processo (reduções logarítmicas) para populações inoculadas do patógeno *Listeria monocytogenes* e bactérias ácido láticas deterioradoras incluindo as espécies *Leuconostoc mesenteroides* e *Lactobacillus sakei*; também foram avaliados os atributos de qualidade incluindo cor, atividade de água, pH, sinerese, textura e oxidação lipídica. Em adição, visando à utilização de tecnologia de múltiplas barreiras para conservação, foi estudado o efeito da adição do componente bioativo carvacrol em concentrações de 200ppm, na formulação do produto. Empregando estratégia de simples redução, sem a adição sais substitutos, após avaliação de aspectos físico-químicos, sensoriais e microbiológicos, concluiu-se que níveis de 30% de redução de sal (cerca de 25%

menos sódio) sobre o controle formulado com 20g/kg, são viáveis; entretanto, foi evidenciada redução da estabilidade microbiológica. Após avaliação de diferentes faixas de pressão e tempo de processo, seguindo os critérios exigidos de desempenho para inativação pós-processo de *Listeria monocytogenes* (4-5 reduções logarítmicas), um valor de 600MPa/180 segundos a 25°C se apresentou como um tratamento adequado para o produto com baixo teor de sódio estudado, promovendo reduções logarítmicas eficazes contra o patógeno-alvo estudado e a microbiota deteriorante. Alterações instrumentais (p<0.05) em alguns dos atributos de qualidade avaliados puderam ser destacadas principalmente sinerese, oxidação lipídica e textura; porém são necessários estudos mais amplos de análise sensorial. Adicionalmente, dados apresentados demonstraram que os consumidores não foram capazes de diferir o produto *low-sodium* processado por alta pressão isostática (600MPa/180sec/25°C) de um não pressurizado; de fato, apesar dos efeitos instrumentais significativos, estas alterações parecem não ser identificadas pelos consumidores.

A adição de uma barreira natural complementar, o carvacrol, em níveis sensorialmente aceitáveis, foi capaz de potencializar os efeitos de inativação pós-processo (reduções logarítimicas) dos grupos microbianos alvo; efeitos benéficos combinados ao longo da estocagem refrigerada do produto também foram evidenciados por meio de reduzidas taxas de crescimento e fase lag aumentada, maximizando assim o potencial de conservação. A utilização combinada de HPP e carvacrol representou uma alternativa promissora contra fenômenos de injúrias sub-letais e recuperação celular. Sugere-se que a intensidade do processo HPP (em termos de carga de pressão, tempo de processo e temperatura) necessária para inativar microrganismos em níveis requeridos, possa ser reduzida na presença de barreiras antimicrobianas adicionais, garantindo a qualidade total do produto processado em condições mais amenas. Em adição, uma série de vantagens industriais podem ser destacadas tais como a redução de custos para a instalação de equipamento inicial e de manutenção (equipamentos operando em cargas mais baixas); e maximização da produção de processamento por ciclos efetivamente encurtados (ciclos de produtividade mais elevados por hora).

Palavras-chave: Carvacrol, barreiras múltiplas, *Listeria monocytogenes*, reduzido teor de sódio; bactérias ácidas láticas, tecnologia emergente não-térmica.

SUMÁRIO

INTRODUÇÃO E JUSTIFICATIVAS

1. INTRODUÇÃO GERAL E JUSTIFICATIVA

A redução do cloreto de sódio nas formulações de alimentos processados representa um dos principais desafios tecnológicos para indústria alimentícia moderna. Estudos abordando os aspectos da redução de sal, consequentemente sódio, em alimentos processados adquirem grande relevância para saúde pública: este fato decorre da relação estabelecida entre a elevada ingestão do sódio e o desenvolvimento da hipertensão vascular, principal fator de risco associado à ocorrência de distúrbios cérebrais e cardiovasculares (HE & MaCGREGOR, 2009). Ingrediente de ampla aplicação na indústria alimentícia, o sal convencional (cloreto de sódio - *NaCl*), possui aproximadamente 40% de sódio (393mg/g) em sua composição, representando a principal fonte desse mineral nas dietas humanas modernas (RUUSUNEN & PUOLANNE, 2005; DOYLE & GLASS, 2010). Tendo o aditivo sal como ingrediente essencial em suas formulações, os produtos cárneos processados representam fontes expressivas de sódio na dieta. De acordo com Katz e Williams (2010) os produtos cárneos podem contribuir com até 80% do total diário ingerido. Categorias de carnes processadas como salames, bacon, salsichas, mortadelas e presuntos se destacam com níveis de sódio que podem atingir 1200mg/100g de produto (AASLYNG et al., 2014). Embutidos de carne de peru em suas formulações padrão contém cerca de 20g/kg de NaCl (até mais), e em uma porção de apenas 100g, chegam a superar 1000mg de sódio.

Com relação à magnitude do problema para órgãos de saúde pública, dados da Organização Mundial de Saúde (WHO, 2012), reportaram que 1 em cada 3 adultos no mundo sofre de aumento gradual de pressão e hipertensão. Agências mundiais de saúde apontam as doenças cardiovasculares (DCV´s) como as principais causas de morte no globo, estando os hábitos de uma dieta não saudável entre as principais causas de sua recorrência. Em 2030 espera-se que 25 milhões de pessoas morram de DCV's no planeta. No cenário atual, o consumo médio de sal supera 12g/dia (>4700mg de sódio). Para populações em geral, e seguindo recomendações da Organização Mundial de Saúde, este nível de ingestão deve ser reduzido para menos de 5g/dia restringindo a ingestão de sódio para menos de 2g/dia (WHO, 2011). Uma redução na média diária de sódio consumida seria viabilizada por meio de ações efetivas das indústrias

processadoras de alimentos, associadas a estratégias governamentais e campanhas educativas. Isto seria traduzido substancialmente em bem estar social, redução de mortalidade por acidentes vasculares cerebrais e doenças coronarianas, prevenção de milhões de mortes e economia de bilhões anuais gastos com medicamentos e sistemas de saúde pública (DICKINSON & HAVAS, 2007). Analisando os números do Ministério da Saúde do Brasil (MS/ANVISA) estima-se que 35% da nossa população acima de 40 anos (cerca de 17 milhões de pessoas) sofra de hipertensão, sendo que 75% desta fatia são dependentes do nosso Sistema Único de Saúde (SUS). Somente com medicamentos anti-hipertensivos, no ano de 2012, o MS estimou um gasto de mais de 100 milhões de reais.

Como medida de combate e prevenção o MS/ANVISA publicou no princípio de 2011, com reformulação no ano 2013 (inclusão de novas categorias), um termo de compromisso de adesão voluntária entre ministério e indústrias para a redução de sódio em alimentos processados, onde diversas categorias de alimentos, incluindo produtos cárneos, são incluídas no plano pactuado de metas para redução anual gradativa. Assim, a Associação das Indústrias Processadoras de Alimentos (ABIA) e o MS têm a expectativa de que sejam retiradas mais de 20 mil toneladas de sódio dos produtos alimentícios, que tiveram metas já pactuadas até 2020. Carnes processadas, dependendo de sua categoria e características intrínsecas, são contempladas com níveis de redução que vão de 16mg até 59mg para 2017 (REVISTA NACIONAL DA CARNE, 2013 e 2014).

Acredita-se que as metas iniciais previstas (2013-2017) do acordo ABIA-MS sejam alcançadas sem maiores problemas para indústria de processamento de carnes; no entanto, quando as metas de redução forem superiores, novas tecnologias precisarão ser estudadas e aplicadas. Estes fatos levam as indústrias a traçarem planos e estratégias de adequação em caráter emergencial. Seguindo os acordos de cooperação para redução de sódio na categoria de produtos cárneos, grandes *players* de mercado neste segmento já começam a adotar como estratégia a simples redução do teor de NaCl (na faixa de 10-30%). Em adição, sob outra perspectiva, a do marketing, alegações de redução de sódio podem desempenhar uma grande vantagem competitiva de mercado, onde alguns

produtos (das marcas Sadia® grupo BRF, Seara® grupo JBS) já fazem uso da rotulagem nutricional estratégica explicitando os níveis de redução de sódio que no mínimo devem se apresentar em 25% para garantia legal deste *claim* (reduzido, leve ou light). No entanto, os impactos decorrentes desta ação imediata e metas futuras de redução, ainda continuam obscuros no âmbito que concerne à qualidade e segurança destes produtos cárneos reformulados. Questões de vida de útil, redimento (CRA) e fatiabilidade estão entre os maiores dilemas que começam a ser relatados por processadores.

Apesar da imperativa necessidade de se reduzir o teor de sal e sódio em formulações de alimentos processados, a ação ainda representa um desafio tecnológico longe de ser equacionado. É preciso considerar que o sal adicionado em produtos cárneos é um ingrediente multifuncional chave, atuando simultaneamente: **a -** no desenvolvimento de sabor característico e percepção de salinidade; **b -** nas propriedades reológicas, via solubilização de proteínas miofibrilares, resultando na capacidade de retenção de água e perfil de textura característico; **c -** e atua, principalmente, como obstáculo microbiológico e agente conservante, retardando a deterioração microbiana e o crescimento de patógenos em produtos cárneos (RUUSUNEM & PUOLANNE, 2005; DESMOND, 2006). A redução da atividade da água devido à adição de sal e da presença de íons que exercem efeitos de pressão osmótica sobre os microrganismos aumentam a vida de prateleira da carne processada. Assim, quando o teor de sal em produtos cárneos é reduzido abaixo dos níveis tipicamente utilizados, o produto tem uma vida de útil mais curta ou pode não mais ser seguro, sem adição de outros conservantes complementares (MADRIL & SOFOS 1985). Para matrizes cárneas, incluindo presuntos, embutidos fatiados e derivados de carne de peru curados, pesquisas recentes continuam enfatizando os riscos da redução do sal adicionado sobre o crescimento de patógenos como *L. monocytogenes* e *Salmonella* spp. (STOLLEWERK et al., 2012; SULLIVAN et al., 2012; MYERS et al., 2013). Os efeitos negativos desta redução sobre o crescimento da microbiota deteriorante, implicando em menor vida de prateleira, e scores de qualidade reduzidos dos produtos ofertados, também são confirmados para estas categorias de produtos (NYCHAS et al., 2008; FULLADOSA et al., 2012). Como alternativa imediata e

com baixo aporte de custo para controle microbiológico, a indústria se vê forçada a intensificar a aplicação de conservantes químicos adicionais como sorbatos, lactatos, diacetatos, acidulantes dentre outros. No entanto, estas opções ainda são acompanhadas de aversão de mercados consumidores, os quais valorizam cada vez mais os conceitos de qualidade como "*Clean label"*, "*Friendly label"* e "*All natural"*. Em meio aos desafios tecnológicos de se reduzir o ingrediente multifuncional sal em produtos cárneos, além dos obstáculos microbiológicos, aspectos físico-químicos e sensoriais também devem ser observados. Dentre as diversas estratégias propostas além da simples redução (aplicada no atual momento pelas indústrias processadoras de carne), pode-se destacar a aplicação de realçadores e mascaradores, sal micronizado, e a redução parcial de NaCl acompanhada da adição de sais de cloreto substitutos como $MgCl_2$, $CaCl_2$, LiCl; e, sem dúvida, a ferramenta mais utilizada, o sal cloreto de potássio (KCl) (DESMOND, 2006). No entanto estas alternativas ainda enfrentam restrições por aspectos regulatórios e desenvolvimento de sabores indesejados como amargo ou metálico. No campo microbiológico, não é bem estabelecido se a adição destes sais substitutos é capaz de promover um efeito conservante equivalente ao do NaCl convencional.

Produtos embutidos de carne de peru se comportam como excelente meio para o crescimento microbiano, reunindo aspectos nutricionais favoráveis, fatores de crescimento, faixa de pH (6,0±0,5) e a_w mínima requerida (>0,96) pela maioria dos microrganismos patogênicos e deterioradores. Agências de saúde apontam as carnes e seus derivados processados como as categorias de alimentos entre as mais envolvidas com ocorrências de surtos e doenças transmitidas por alimentos DVA (CDC, 2008). A categoria dos produtos cárneos cozidos, como os itens de presuntaria, são produtos considerados prontos (*RTE's - Ready-to-Eat*), não sendo requerido de um processo térmico adicional pré-consumo (PUANGSOMBAT et al., 2011). Contaminações pré e pós-tratamento térmico (cozimento) sejam em ambientes de processo ou varejo em categorias RTE's tornam ainda mais elevados os riscos de ocorrência de DVA's. Os riscos de surtos alimentares são potencializados pela ausência de obstáculos, como o sal, que contribuem para estabilidade do produto; este poderia atuar como barreira para o

crescimento populacional de patógenos frente uma possível contaminação inicial, retardando que populações mínimas problemáticas para toxigênese ou infecção sejam atingidas.

Com relação às características de processo, estes gêneros são submetidos a uma etapa de cozimento monitorada (74°C no interior) que virtualmente garante a eliminação das formas microbianas vegetativas presentes. No entanto, as formas esporuladas termodúricas como *Clostridium* sp. toxigênicos, resistem ao tratamento térmico, sendo a germinação estimulada pela ativação sub-térmica dos esporos e o crescimento favorecido pela eliminação da microbiota competidora e extinção do obstáculo promovido pelo NaCl (LABBE, 2001). Além do problema relacionado aos microrganismos remanescentes do processo de cozimento aplicado aos presuntos, contaminações pós-processamento térmico em ambientes de produção e varejo são comuns. Na atualidade, com a busca por conveniência, produtos RTE's têm ganhado em popularidade, especialmente quando comercializados sob a forma pré-fatiada, em porções e re-embalada. No entanto esta modalidade de apresentação e manipulação favorecem as rotas de contaminação pós-processo aumentando consideravelmente os riscos de contaminação com patógenos emergentes relacionados à carne e produtos cárneos (*Slice Safety Risk*) como *Salmonella* sp., *L. monocytogenes*, *S. aureus*, *E. coli STECs* e *Campylobacter jejuni*. A microbiota deteriorante, predominantemente composta por bactérias ácido láticas e psicrotróficas, também representam um problema para a vida de prateleira na comercialização de RTE's fatiados e re-embalados à vácuo (PÉREZ-RODRÍGUEZ et al., 2007; KEGODE et al., 2008; SOFOS & GEORNARAS, 2010). A condição é ainda mais crítica com a redução dos níveis da barreira microbiológica proporcionada pelo sal. Na atualidade, o emergente patógeno psicrotrófico *L. monocytogenes* têm estado em evidência, em especial para rotas de contaminação pós-processo de cárneos RTE's (SOFOS & GEORNARAS, 2010). Devido a presença ubíqua deste patógeno nos ambientes de processo e varejo, formação de biofilmes, bem como habilidade de persistir em condições adversas por longos períodos, contaminações pós-processo de produtos cárneos RTE por *L. monocytogenes* representam um significante problema de saúde pública (SAMELIS & METAXOPOULOS, 1999; TOMPKIN,

2002), sendo apontada como a causa dos principais surtos de listeriose contabilizados (CDC 2000, 2002). Apesar dos esforços para erradicação do emergente patógeno em produtos RTE's, estes continuam encabeçando as listas de *recalls* e surtos alimentares. Em 2002, um surto recorde, com 15.000 toneladas de processado de peru RTE foi recolhido devido a contaminação por *L. monocytogenes*. O surto, atribuído a uma unidade de produção única, provocou 7 mortes e 47 internações (CDC, 2002). Pal et al. (2008) relataram o padrão de crescimento de *L. monocytogenes* inoculada em presunto e peito de peru fatiado RTE, sendo identificado o crescimento em condições refrigeradas, abuso de temperatura e diferentes atmosferas (embalagem a vácuo). Logo, a ausência de estudos detalhados descrevendo o comportamento de patógenos-alvo relacionados a rotas de contaminação pós-tratamento térmico em matrizes de expressivo consumo como os presuntos, reduzidas do agente conservante sal, faz desta reformulação um campo obscuro e altamente perigoso no que diz respeito à manutenção da almejada segurança alimentar.

Visando implementar de maneira segura estratégias de redução de sal, eliminando a dependência de conservantes químicos adicionais, a aplicação de tecnologias de preservação emergentes tais como o Processamento à Alta Pressão (*HPP - High Pressure Processing*) surgem como alternativas factíveis. Tratamentos HPP surgem como tendência inovadora para conservação de alimentos; por se tratar de uma tecnologia de conservação não-térmica, apresenta como principal vantagem a minimização dos efeitos do calor sobre a qualidade nutricional e sensorial dos produtos alimentícios. Na indústria de processamento de carnes, tratamentos com alta pressão são aplicados principalmente no pós-embalagem para extensão de vida de útil e garantia da segurança alimentar de cortes cárneos *in natura* e produtos cárneos RTE. Níveis de pressões aplicadas para pasteurização de carne e produtos cárneos variam de 400-600MPa com curtos períodos de processamento (3-7 minutos), a baixas temperaturas (T<35°C) (SIMONIN, DURANTON & LAMBALLERIE, 2012; BAJOVIC, BOLUMAR & HEINZ, 2012). A aplicação deste tratamento nos produtos RTE fatiados pode representar uma alternativa factível para eliminação de patógenos e redução da carga de microrganismos deterioradores adquiridos nas operações de manipulação e

fatiamento pós-processo, quando aplicados em produtos acabados em sua embalagem final. Resumidamente, o processo HPP (na sua forma isostática, também denominada Alta Pressão Hidrostática) consiste em submeter o produto a cargas de pressões elevadas (>100MPa até 600Mpa/6000bar/87000psi) em vasos apropriados, com ou sem elevação de temperatura, utilizando como meio pressurizador um fluido de baixa compressibilidade (geralmente água deionizada); a pressão é transmitida de maneira instantânea e igual por todo o conteúdo do produto. Como consequência, consegue-se a inativação de microrganismos sob as formas vegetativas e inativação enzimática por alterações de conformação protéica, retardando assim as principais reações responsáveis pela deterioração dos alimentos (CAMPOS et al., 2003; CRUZ et al., 2010; MATSER et al., 2004; HOGAN et al., 2005; RASTOGI et al., 2010;).

Como tecnologia inovadora, diversos estudos vêm sendo realizados abordando as variáveis de processo (carga, temperatura e tempo de residência) para tratamentos com HPP na inativação de microrganismos-alvo em carnes frescas e produtos cárneos. Tratamentos pós-processo para inativação de *L. monocytogenes* em presuntos fatiados geralmente são aplicados utilizando cargas de pressão de 600MPa, com tempos de residência inferiores a 10 minutos preconizando temperaturas abaixo de 30°C para minimização dos efeitos térmicos. Estudos demonstram que condições de processo próximas a esta garantem uma redução superior a 4 $\log_{10}$ UFC/g pós-processamento sobre as populações inoculadas (CHEN, 2007; JOFRÉ et al., 2008; JOFRÉ et al., 2009; BOVER-CID et al., 2011; HEREU et al., 2012; MEYERS et al., 2013). Para microrganismos esporulados, é bem estabelecido que cargas de pressões elevadas >600MPa somado ao efeito da temperatura >70°C são necessárias para efetividade de tratamento (CRAWFORD et al., 1996; REDDY et al., 2003; PAREDES-SABJA et al., 2007; WILSON et al., 2008; ZHU et al., 2008). Para extensão da vida de prateleira de presuntos fatiados estudos apontam tratamentos com cargas acima de 400MPa para efetividade contra microbiota deteriorante, aumentando significativamente o prazo de estocagem destes produtos (SLONGO et al., 2009; HAN et al., 2011; VERCAMMEN et al., 2011). Com relação aos mecanismos inibitórios de HPP sobre células microbianas a literatura atribui

efeitos combinados sobre estruturas de parede celular e membranas plasmáticas, organelas como vacúolos e ribossomos, materiais genéticos e complexos enzimáticos essenciais. Quando submetidas a cargas de pressão, alterações morfológicas como deformação, separação de parede da membrana, danos na membrana nuclear, mitocôndrias, regiões vacuolares, lisossomos (extravasamento enzimático) e deformação de citoesqueleto são identificados (MOR-MUR & YUSTE, 2005; RENDUELES et al., 2011).

Considerando situações de eliminação da barreira microbiológica proporcionada pelo sal em produtos cárneos, os resultados são ainda mais promissores; assim, não se faz necessária a adição de agentes conservantes químicos adicionais, indo de encontro às tendências contemporâneas de mercado. Myers et al. (2013) confirmaram a efetividade de segurança promovida por tratamentos com HPP (600Mpa/3min) em presunto fatiado RTE e peito de peru curado reduzidos de NaCl (1,8 e 2,4%) e $NaNO_2$ (100% redução) inoculados com *L. monocytogenes*. O tratamento a alta pressão resultou em reduções de aproximadamente 4 ciclos log_{10} UFC/g de *L. monocytogenes* (inóculo inicial de 10^5UFC/g) no pós-tratamento HPP. Numa avaliação ao longo do período de estocagem (inóculo inicial de 10^3 UFC/g), após tratamento, durante estocagem por 182 dias, não foram detectadas diferenças significativas para as populações do patógeno entre amostras dos produtos avaliados para os dois níveis de sal testados, indicando o efeito preservativo do tratamento aplicado. Fulladosa et al. (2012) reportaram o efeito do processamento HPP (600Mpa/6min/10°C) sobre bactérias láticas (LAB) e contagem total aeróbios mesófilos (CTP) em presuntos com 50% de redução de sal. Stollewerk et al. (2012) avaliaram os efeitos da substituição total do NaCl por KCl em presuntos fatiados submetidos a tratamentos com alta pressão (600MPa/5min) com temperatura inicial das amostras de 13°C, inoculados com *L. moncytogenes*. As amostras foram estocadas sobre refrigeração por 112 dias. Para amostras NaCl-*free* submetidas a HPP foram observadas populações inferiores ($p<0,05$) comparados ao ensaios não tratados durante todo o período de estocagem. Nesta mesma pesquisa os autores observaram efeito similar para contagem de bactérias ácido-láticas e cocos gram-positivos.

Apesar dos efeitos positivos bem estabelecidos relacionados à aplicação da tecnologia de HPP como ferramenta para conservação de produtos cárneos, alguns problemas e efeitos adversos, principalmente sobre atributos de qualidade, já começam a ser pontuados na literatura contemporânea: inicialmente, é preciso levar em conta a resistência e as injúrias sub-letais ocorridas durante a inativação microbiana pela aplicação de HPP, situação na qual células de microrganismos podem retomar seu crescimento durante a estocagem dependendo das condições intrínsecas e extrínsecas do produto avaliado (JUCK et al., 2012). A baroresistência apresentada pelas formas esporuladas também representam um importante desafio, uma vez que cargas de pressão acima de 600MPa, com efeito combinado de temperatura (>70°C), são necessárias para inativações efetivas (WILSON et al., 2008; ZHU, NAIM, MARCOTTE, RAMASWAMY & SHAO, 2008; ZHANG & MITTAL, 2008). Estas situações conduzem à percepção de que, dependendo dos objetivos do processamento HPP empregado, condições muito intensas de processo devem ser aplicadas (envolvendo variáveis de carga de pressão, tempo de processo e temperatura), o que pode conduzir aos efeitos indesejados. Neste campo, um vasto número de pesquisas tem emergido atualmente confirmando efeitos instrumentais significativos de tratamentos intensos sobre atributos de qualidade de carnes *in natura* e produtos cárneos pressurizados. Alterações de parâmetros de qualidade relacionados com modificações conformacionais e funcionalidade de proteínas como cor, textura e capacidade de retenção de água (sinérese) são relatadas (proteômica). Também são reportados efeitos sobre a estabilidade oxidativa, uma vez que HPP pode desencadear processos oxidativos por meio da disponibilização do ferro das heme proteínas e também ruptura de membranas expondo a estrutura de fosfolipídios a agentes e condições oxidantes (CHEFTEL & CULIOLI 1997; ORLIEN et al., 2000; BAJOVIC et al., 2012; MEDINA-MEZA et al., 2014). Diversos estudos avaliando atributos de qualidade de emutidos curados cozidos tratados com HPP sinalizam para efeitos indesejados sobre parâmetros de cor, textura e oxidação lipídica (VILLACÍS et al., 2008; CAVA et al., 2009; VENTANAS et al., 2010; CLARIANA et al., 2011; ALBA et al., 2012; BAK et al., 2012; CLARIANA et al., 2012; FERRINI et al., 2012). Logo, um modelo considerado ideal de tratamento deve ser aplicado

alcançando a produção de alimentos seguros minimamente tratados, apresentando assim seu padrão nutricional e sensorial-qualitativo ótimo. Assim, em situações de reformulação de produtos, como a redução de sal em matrizes cárneas processadas, a otimização e ajuste de variáveis de processo se faz fundamentalmente necessária, principalmente diante do papel de retenção de água e desenvolvimento de textura característica proporcionada pelo aditivo NaCl (DESMOND, 2006).

Como alternativa para o proposto entrave, a literatura atual direciona para possibilidade de aplicação de tratamentos combinados utilizando em conjunto aos processos HPP, agentes preservativos adicionais. Seguindo as tendências de mercado para valorização de aditivos naturais em alimentos, a aplicação de bioativos naturais tais como óleos essenciais e seus componentes majoritários, extratos, bacteriocinas, bacteriófagos entre outros, juntamente com HPP se mostram como alternativas viáveis (MARCOS et al., 2008; JOFRÉ et al., 2008; JOFRÉ et al., 2009; HEREU et al., 2011; VERCAMMEN et al., 2011; LIU et al., 2012). Em especial, óleos essenciais e extratos vegetais com propriedades antimicrobianas e antioxidantes (retardando fenômenos de oxidação que são engatilhados por HPP) são ferramentas promissoras (EVRENDILEK & BALASUBRAMANIAM, 2011; ESPINA et al., 2013; PATRIGNANI et al., 2013). Efeitos antimicrobianos sinérgicos podem ser pontuados: inicialmente as taxas de inativação (reduções log) bacteriana por HPP, bem como extensão de *shelf-life* podem ser notavelmente potencializados na presença de antimicrobianos naturais (teoria de múltiplas barreiras), o que pode representar uma alternativa para os fenômenos de baro-resistência, injúrias sub-letais e recuperação celular (VURMA et al., 2006; OGIHARA et al., 2009). Além disso, HPP combinado a barreiras adicionais pode tornar possíveis efeitos similares de conservação e segurança em tratamentos com condições mais brandas em termos de carga de pressão, tempo de processo e temperatura; isso, além de minimizar o impacto sobre os atributos de qualidade do produto, resulta em inúmeras possibilidades de benefícios de processo tais como custos reduzidos de instalação inicial (sistemas mais baratos uma vez que se necessita de cargas menores, por exemplo menor número de intensificadores), menor custo de manutenção de unidades de alta pressão (opera

com cargas de pressão mais leves e em tempos reduzidos) e maximização de volume processado por meio de ciclos de pressurização efetivos em tempos reduzidos (ciclos/h o que um importante parâmetro para produtividade). Destacando-se na lista dos benefícios da aplicação combinada, o efeito antioxidante de extratos naturais pode auxiliar retardando os processos oxidativos engatilhados por tratamentos HPP. Finalmente a aplicação combinada pode tornar efetiva a aplicação de extratos naturais em concentrações antimicrobianas sub-inibitórias, auxiliando a superar o principal entrave para uso efetivo destes compostos em matrizes alimentares: o grande impacto sensorial.

Sendo assim, este trabalho teve como objetivo avaliar o efeito combinado do processamento à alta pressão isostática (HPP) e utilização de agente fenólico antimicrobiano e antioxidante (carvacrol) na extensão de vida de prateleira de um produto embutido fatiado de carne de peito de peru (tipo *Blanquet*) formulado com teor reduzido de sal.

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OBJETIVOS

OBJETIVO GERAL

Por meio da avaliação de respostas de inativação de grupos microbianos-alvo bem como atributos de qualidade, a pesquisa objetivou ajustar as variáveis de processo “carga de pressão” e “tempo de residência” (*dwell time*), visando otimizar o tratamento a alta pressão isostática para embutido de peito de peru fatiado embalado a vácuo (tipo “*Blanquet*”) formulado com teor reduzido de sódio. Em adição, foi avaliado o efeito combinado do processamento HPP em conjunto à adição de uma barreira antimicrobiana natural, o carvacrol, adicionado em concentrações sub-inibitórias sensorialmente aceitáveis.

OBJETIVOS ESPECÍFICOS:

1) Ajustar o teor ideal de redução de sal (consequentemente sódio) por meio de estratégia de simples redução (sem adição de sal substituto) mediante avaliações microbiológicas, físico-químicas e sensoriais, durante vida de prateleira (60 dias) refrigerada do produto fatiado embalado à vácuo (***capítulo 2***).
2) Otimizar o processamento a alta pressão isostática para a formulação de trabalho reduzida de sódio, avaliando o efeito de diferentes faixas de pressão e tempo de processo na inativação de *Listeria monocytogenes* e bactérias láticas (*Leuconostoc mesenteroides* e *Lactobacillus sakei*), bem como os atributos de qualidade. Em adição, verificar nestas respostas os efeitos da incorporação na formulação do produto de uma barreira natural, o carvacrol, em concentrações sub-inibitórias sensorialmente aceitáveis (***artigo capítulo 3***).
3) Validar o tratamento otimizado (600MPa/180 segundos/25°C) por meio de avaliações físico-químicas e microbiológicas durante *shelf-life* (60 dias sob refrigeração) para a formulação de trabalho reduzida de sódio e também aquelas adicionadas do componente bioativo natural (***artigo capítulo 4***).

Capítulo 1: Review article - Natural antimicrobials as additional hurdles to preservation of foods by high pressure processing

RESUMO

O processamento de alimentos por Alta Pressão (HPP) surgiu como uma tecnologia não térmica inovadora para conservação de alimentos por garantir vantagens sensoriais e nutricionais quando comparada aos processos de conservação convencionalmente empregados. Apesar dos benefícios estabelecidos do HPP, a literatura atual tem destacado uma tendência promissora da aplicação combinada de HPP somados aos efeitos de agentes antimicrobianos obtidos de fontes naturais (NA's). A utilização de NA's coincide com as tendências contemporâneas de mercado, onde consumidores valorizam cada vez mais produtos saudáveis e conceitos de "*all natural"*, e "*clean* ou *friendly labels*". Efeitos sinérgicos benéficos da aplicação combinada de HPP e NA's podem ser destacados: inicialmente, os efeitos de inativação microbiana podem ser notavelmente potencializados na presença de NA's; isto pode representar uma arma promissora contra resistência bacteriana de formas vegetativas e esporuladas às altas pressões. Consequentemente, os fenômenos de injúrias sub-letais e recuperação celular podem ser minimizados. Em segundo lugar, HPP somado às barreiras NA's torna possível atingir requerimentos de segurança e extensão de vida de prateleira em condições de tratamento mais brandas (envolvendo carga de pressão, tempo de residência e temperatura de processo), assegurando custos reduzidos iniciais no dimensionamento e características operacionais de unidades HPP e também manutenção. Também são asseguradas vantagens de maximização de volume processado, por ciclos efetivos em tempos reduzidos; em adição, ciclos menos intensos resultam em manutenção das características frescas dos produtos a manutenção da qualidade global dos gêneros processados por alta pressão. A literatura atual tem mencionado extensivamente as alterações leves, porém, significantes em atributos de qualidade de alimentos engatilhadas por processos intensos HPP. Por fim, combinados a tratamentos HPP, alguns NA's podem ser aplicados em condições sub-inibitórias reduzindo seu efeito impacto organoléptico adverso (maior barreira para sua aplicação efetiva em matrizes alimentares). Uma abordagem multi-barreira com processos HPP combinado à NA's representa uma promissora alternativa para a otimização do processamento, qualidade e segurança

asseguradas eliminando a dependência de aditivos químicos adicionais. Esta revisão objetiva mostrar o atual status e barreiras de HPP, discutindo aspectos chave desta inovadora tecnologia de processamento de alimentos em combinação com compostos bioativos naturais.

Palavras-chave: Tecnologias Emergentes para o Processamento de Alimentos; Antimicrobianos Naturais; Segurança Alimentar; Bactérias deterioradoras; Tecnologia de Barreiras Múltiplas.

ABSTRACT

High Pressure food processing (HPP) has emerged as a promising non-thermal alternative for food conservation to assure nutritional and sensory advantages over conventionally employed preservation processes. Although clear benefits of HPP have been already evident, recent literature has highlighted a promising trend of the combined application of HPP plus antimicrobials obtained from natural sources (NA´s). The utilization of NA´s is consistent with current market trends, as consumers are requesting healthy products and value the *all-natural* and *clean-label* concepts. Outstanding synergistic benefits of joint HPP and NA´s application may be pointed out: Firstly the HP bacterial inactivation effects can be notably potentiated throughout the presence of NA´s: this can represents a hopeful weapon against pressure resistant vegetative and spore-forming strains; additionally, sub-lethal injury and cell recovery phenomena may be minimized. Secondly, combined HPP plus additional NA´s hurdles may become possible similar safety and shelf-life extension effects at mild HP treatments (slightly set up conditions involving holding time, pressure loads and temperature), assuring reduced costs at initial equipment installation and maintenance, and maximizing processing output by effective shortened cycles (higher productivity cycles per hour); in addition, less intense cycles results in increased fresh food maintenance and global quality of HP processed food. Actual literature has been extensively mentioned slighted but noticeable undesirable changes in the quality attributes of foods triggered by intense HPP required for safety purposes. Finally, combined with HPP, some NA´s can be applied in sub-inhibitory concentrations reducing their adverse organoleptic impact (major barrier of their effective applications in food matrices). HHP plus NA´s multi-hurdle approach is a promising alternative for HP processing optimization, food quality and safety assurance, eliminating dependence on additional chemical additives. This review aims to show the actual status and barriers of HPP by discussing key research results focused on this innovative food processing technology applied in combination with natural bioactive compounds.

***Keywords*:** Emerging food processing technologies; natural antimicrobials; food safety; spoilage bacteria; multi-hurdle technology.

Introduction

The food industry strives to ensure the safety of its products while maintaining quality. Generally, foods are thermally processed by being subjected to temperatures higher than 60°C for duration of seconds to several minutes to destroy deteriorative enzymes and vegetative microorganisms. During this period of treatment, a large amount of energy is transferred to the food, which can trigger unwanted reactions, leading to undesirable organoleptic and nutritional degradation effects (Tiwari *et al.,* 2009). In contrast, emerging innovative technologies, which mainly represent non-thermal alternative conservation methods, have recently received much attention from both academia and industry due to preservation of quality without compromising safety. A number of non-thermal preservation techniques are being developed to satisfy consumer demand regarding the nutritional and sensory aspects of foods. As highlights emerging conservation methods such as irradiation, pulsed electric fields (PFE), radio-frequency electric fields (RFEFs), microwaves, ultrasound, UV–light and High Pressure food Processing (HPP) represent the most promising alternatives (ERRC-USDA, 2010; Rajkovic, Smigic & Devlieghere, 2010). HPP treatments are a method of non-thermal food pasteurization that consists of subjecting food to intense pressure loads of 50-100 up to 1000 MPa, which are applied to inactivate enzymatic complexes and eliminate pathogenic microorganisms. HPP is currently being used to reduce microbial spoilage load and extend shelf life, to maintain higher natural/sensory quality, and to improve the safety of a wide spectrum of raw and processed food categories (Rendueles *et al.,* 2011; Simonin, Duranton & Lamballerie, 2012). Contrary to conventional thermal food processing, appropriate HPP application can promote the retention of freshness and the sensorial and nutritive value of food products. Small molecules, such as flavor compounds, vitamins and pigments, and essential amino acid bioavailability are typically minimally affected by pressure. HPP causes few changes in the characteristic flavor of foods; in fact, it is possible to keep many foods longer and in better conditions. This process thus allows the processing of foods with cleaner ingredients and fewer additives (Campos, Dosualdo & Cristianini, 2003). In addition, HPP represents a feasible alternative to maintaining safety and extending

shelf life at a time when chemical and harmful additives are being excluded from reformulations, such as low-sodium, *clean-label* and *all-natural* processed-food categories.

However, in the past 5-10 years, with the natural development and application of this innovative food processing technique, modern literature has begun to note the adverse effects of the high pressure loads that are currently applied to achieve satisfactory safety results. Usually, the application of HP-treatments consists of submitting food products to pressure loads higher than 50-100MPa and up to 1000MPa for several minutes and cycles, with or without a temperature increase. This intense processing is adopted due to the baroresistence and sub lethal injury of certain bacterial strains, which may recover their growth according to intrinsic food matrix characteristics and extrinsic storage factors. Nevertheless, this intense conventional set-up, involving time, temperature and pressure load, has been associated with sensory depreciation, negative effects on color and texture, and deteriorative reactions, such as lipid oxidation, in several food products categories (Cheftel & Culioli, 1997; Sun & Holley, 2010; Bajovic, Bolumar & Heinz, 2012). In fact, high pressures do not affect the covalent bounds of small molecules; in contrast, food quality properties related to structural, conformational or functional macromolecules are significantly affected. These anomalous effects on quality attributes, involving a wide range of food categories processed by high HP-technology loads, were found for several meat products and raw meat cuts, fish, fruits and juices, vegetables and dairy products (Zhu, Ramaswamy & Simpson, 2004; Oey, Lille, Loey & Hendrickx, 2008; Roeck *et al.,* 2009; Bajovic, Bolumar & Heinz, 2012; Evert-Arriagada, Hernández-Herrero, Juan, Guamis & Trujillo, 2012; Montiel, Alba, Bravo, Gaya & Medina, 2012; Wang *et al.,* 2013). Thus, despite the unquestionable utility and benefits of HPP for food preservation, complementary tools need to be investigated and established. Seeking simultaneous quality and safety *Multi-Hurdle Technology* appears to be a reasonable alternative (Leistner, 2000).

To overcome these reported adverse effects caused by the high-pressure loads required for safety assurance, a combination of mild HPP treatments with antimicrobial compounds and other preservation techniques has been proposed. In

addition, economical benefits of initial equipment design and maintenance costs related to mild required HP loads may be easily achieved. Combined HPP application with antimicrobial agents from natural sources is already evident. Although synthetic antimicrobials are approved in many countries, the use of natural preservatives has been a recent trend. The current consumer market demands products that have friendly labels and are naturally preserved; thus, natural and biopreservation concepts represent significant retail advantages for food manufacturers (Negi, 2012). The natural concept, as an additional hurdle in HPP, can support the natural preservation claims of HP equipment manufacturers. Antimicrobial compounds obtained from natural sources, such as plant-origin antimicrobial agents (essential oils (EOs), oleoresins and extracts) and animal/microbial-origin compounds (competitive cultures, antimicrobial peptides, active lipids, chitosan, lysozyme, lactoferrin, lactoperoxidase systems, bacteriocins, bacteriophages and organic acids), represent practicable alternatives (Tiwari *et al.,* 2009; Daglia, 2011). Additionally, some of these bioactive molecules can obstruct deteriorative food reactions; for example, phenolics may act against lipid oxidation triggered by HPP. Studies focusing on the synergistic effects of HPP that are associated with natural bioactive compounds represent promising and innovative research fields in the study of food safety assurance (Liu *et al.,* 2012; Bevilacqua, Corbo & Sinigaglia, 2012; Alba, Bravo & Medina, 2013; Marcos, Aymerich, Garriga & Arnau, 2013). This review provides a brief overview of HPP applied *in vitro* and in food matrices, combined with active antimicrobials originating from natural sources. Thus, despite the unquestionable utility and benefits of HP technology, we discuss viable alternative tools for improving the preservative effects of High Pressure and assuring the safety and quality of processed foods.

Relevant considerations in the HPP: an overview

HPP is a preservation method that consists of submitting a food product sample to elevated pressure loads (50-1000 MPa), with or without the addition of heat, to achieve microbial and enzymatic inactivation or to alter the food attributes to achieve industry/consumer-desired properties. HPP processes assure safety, retain food quality, maintain natural freshness and extend microbiological shelf life

(Ramaswamy, Balasubramaniam & Kaletunç, 2010). In addition to microbial and enzymatic inactivation, HP technology is currently applied for several additional purposes, including HP-assisted extraction in biotechnology, to obtain intracellular compounds, enzymatic activity; HP freezing and thawing; HP gelatinization; reducing particulate matter and stability; improving rheological properties; forming stable emulsions and homogenization; particularization; biological molecule alterations; new texture developments; and protein and polysaccharide modification (Campos, Dosualdo & Cristianini, 2003; Hogan, Kelly & Sun, 2005; Corrales, Toepfl, Butz, Knorr & Tauscher, 2008; Prasad et al., 2010; Kubo, Augusto & Cristianini, 2013; Tribist, Algusto & Cristianini, 2013).

The HPP process can be applied via two mainly different methods for food processing: as a High Isostatic Pressure processing (HHP) in an isostatic HP unit; and ultra-high-pressure processing, or Dynamic High Pressure Homogenization (UHP or HPH), in ultra-homogenizers. HPP can be conducted with a wide range of holding times and at hot, ambient or refrigerated temperatures, thereby eliminating thermally induced adverse effects (Rastogi, Raghavarao, Balasubramaniam, Niranjan & Knorr, 2007). In isostatic method (HHP), there are two general scientific principles of direct relevance to the use of high pressure in food processing: first, Le Chatelier's Principle, which states that any phenomenon accompanied by a decrease in volume will be enhanced by pressure; and second, the Isostatic Principle, in which pressure is transmitted in a uniform (isostatic) and quasi-instantaneous manner throughout the sample (Pauling, 1964; Olsson, 1995). Briefly, in the HHP process, the isostatic unit consists of a high-pressure vessel, a means to close off the vessel off, a system for pressure generation, a system for temperature and pressure control and a material handling system. In a typical HHP process, a product is packaged in a flexible container (usually a pouch or plastic bottle) and is loaded into a high-pressure chamber (vessel) filled with a pressure-transmitting, incompressible (hydraulic) fluid. The hydraulic fluid (normally water) in the chamber is pressurized with a pump, and this pressure is transmitted through the package and into the food itself. The pressure is applied for a specific time, which is usually 3-10 min. The processed product is then removed and stored/distributed in the conventional manner. The pressure is transmitted

uniformly (simultaneously in all directions, following the isostatic principle), and the food retains its shape, even at extreme pressures (Hogan, Kelly & Sun, 2005; Ramaswamy, Balasubramaniam, & Kaletunç, 2010). With respect to the antimicrobial effects of the HHP process, this method effectively inactivates pathogenic and spoilage organisms, especially by affecting morphology, cell walls, membranes (lipid crystallization and loss of permeability), key enzymes, essential biochemical reactions and genetic mechanisms (Mor-Mur & Youste, 2005). Recent studies involving HHP technology have evaluated numerous set-ups of pressure load, exposure holding times and applied temperatures against several vegetative or spore-forming foodborne pathogens and spoilage bacteria, including *Listeria monocytogenes* (Gudbjornsdottir, Jonsson, Hafsteinsson & Heinz, 2010; Bover-Cid, Belletti, Garriga & Aymerich, 2011; Hereu, Dalgaard, Garriga, Aymerich & Bover-Cid, 2012), *Salmonella* (Guan, Chen & Hoover, 2005; Maitland, Boyer, Eifert & Williams, 2011; Bover-Cid, Belletti, Garriga & Aymerich, 2012; Netoo & Chen, 2012), *Yersinia enterocolitica* (Chen & Hoover, 2003; Lamo-Castellví et al., 2005), toxigenic *Escherichia coli* (Del Olmo, Calzada & Nuñez, 2012; Alba, Bravo & Medina, 2013), *Campylobacter jejuni*, *Staphylococcus aureus* (Gervilla, Sendra, Ferragut & Guamis, 1999; Gao, Ju & Jiang, 2006), spore-forming *Clostridium* and *Bacillus* sp. (Zhu, Naim, Marcotte, Ramaswamy & Shao, 2008; Ramaswamy, Shao & Zhu, 2010; Aouadhi et al., 2013; Zimmermann, Schaffner & Aragão, 2013), lactic acid bacteria (LAB) (Slongo et al., 2009; Han et al., 2011; Vercammen et al., 2011) and *Pseudomonas* sp. (Del Olmo, Calzada & Nuñez, 2012). Most of these studies included food matrices, such as raw meat and processed meat products, ready-to-eat (RTE) sliced meat products, poultry, dairy products, juices, RTE meals, jams, fruits, vegetables and fish. However, the criteria and efficacy of ideal HP set-ups vary widely, as the inactivation effect of HHP treatments depends on bacterial cell morphology (Gram-negative bacteria are significantly weaker than Gram-positive cocci and rods), prior growth conditions, food sample composition (pH, water activity, buffering capacity and redox potential) and the growth stage of the bacteria (bacteria in the stationary phase are more resistant than those in the exponential phase) (Mor-Mur & Youste, 2005). Indeed, several books and major reviews focusing on HHP treatment design for specific foods and bacterial strains (pressure

loads, holding times and temperatures) are already found in the available literature (Farr, 1990; Hogan, Kelly & Sun, 2005; Palou, Lopez-Malo, Barbosa-Canovas & Swanson, 2007; Wilson et al., 2008; Rastogi, Raghavarao, Balasubramaniam, Niranjan & Knorr, 2007; Tewari & Juneja, 2007; Zhang & Mithal, 2008; Rendueles et al., 2011; Bajovic, Bolumar & Heinz, 2012). However, this review objective is not to describe results in this same research field; we want to concentrate our efforts on HPP and natural antimicrobials from a *Multi-Hurdle Technology* perspective.

Regarding UHP processing, an ultra-homogenizer is a single-acting, reciprocating multi-plunger pump with an adjustable valve, the homogenizing valve, which can create pressure to micronize and disturb various products. The passage of a sample at a very high pressure through a specially designed valve with an adjustable gap can disrupt and micronize dispersed particles and biologic structures down to the order of magnitude of micrometers or nanometers. Thus, UHP processing can promote stable dispersion and emulsion formation by particles micronization, biological cell lysis (bacteria, yeast, algae and plants) and consequently, partial or complete microbial inactivation. UHP technology combines the forces of high-velocity impact, high-frequency vibration, an instantaneous pressure drop, intense shear, cavitation and ultra-high pressures up to 300-400 MPa (Tribst, Franchi & Cristianini, 2008). Partial or complete yeast and bacterial inactivation was reached in liquid and semi-solid food samples using dynamic HPH: against *Lactobacillus plantarum* and *Saccharomyces cerevisiae* in orange juices at pressure loads >250 MPa (Campos & Cristianini, 2007); three UHP multi-passes at 150 MPa for the microbial inactivation of selected microbial strains (*Saccharomyces cerevisiae*, *Lactobacillus delbrueckii* and *Escherichia coli*) inoculated in commercial fruit juices (orange, red orange and pineapple) (Maresca, Donsì & Ferrari, 2011); repeated HPH passes at 100 MPa, allowing significant inactivation of the spoilage yeast *Saccharomyces cerevisiae* inoculated in apricot and carrot juices (Patrignani et al., 2009); UHP processing at 300 MPa against *Staphylococcus*-inoculated milk (Briñez, Roig-Sagués, Herrero & López, 2007); and UHP at 400 MPa against emerging pathogens, such as *Salmonella enterica* and *Listeria monocytogenes* in grape and orange juices (Velázquez-Estrada et al., 2011). Complete inactivation and a 5-log reduction in *E. coli* O157:H7 were

observed by Tahiri, Makhlouf, Paquin and Fliss (2006) in orange juice after five and three passes at 200 MPa/25°C, respectively. Maresca, Donsì and Ferrari (2011) confirmed the possibility to apply HPH multi-passes treatment for the pasteurization of low-acid and acid fruit juices (apple, orange e pineapple) by inactivating *S. cerevisiae*, *L. delbrueckii*, *E. coli* with processing set-up varying from 50-250MPa/1-5 passes. HPH (or UHP) was proven to be efficient in inactivating both pathogenic and spoilage microorganisms in liquid and semi-solid products due to the method ability to mechanically disrupt microbial cells. The process of mechanical disruption is induced by the rapid release of pressure energy accumulated in the fluid, which is compressed by up to 300-400MPa during passage through the homogenization valve. This passage consequently generates of intense fluid-mechanical stresses, such as elongation and shear stresses, turbulence and cavitation (Donsì, Annunziata & Ferrari, 2013; Dumay et al., 2013).

HP technology in the HHP or UHP form (UHP mainly for liquid and semi-solid samples) can be applied to a wide range of foods, including meat-based products (e.g., cooked and dry ham and sausages, sliced post-processed), fish, pre-cooked dishes, jams, fruits, dairy, beverages, RTE meals, vegetables and juices. Today, the main applications are in the production of jams, fruit juices, soups, oysters and, more recently, processed meats, such as hams. Currently, HPP is being used in the United States, Europe and Japan on a select set of high-value foods, either to extend shelf life or to improve food safety. In the USA, under the safety supervision of the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) but without particular regulation, several products are commercially produced using HPP, including cooked, RTE meats; avocado products (guacamole); tomato salsa; applesauce; orange juice; and oysters (Ramaswamy, Balasubramaniam & Kaletunç, 2010). On the American market, a registered trademark, Natural Choice Products®, may be found for Hormel Company, and Cargill Company has commercialized a hamburger called Fressure®. Infantis® entered the European market and includes HP-preserved sliced ham, mortadella, sausages and salami (Bajovic, Bolumar & Heinz, 2012). U.S. regulation of HPP application as a post-intervention process in RTE meat products from cattle and poultry, mainly for the control of *L. monocytogenes*, is

indicated by government agencies (USDA-FSIS, 2003). In European Union countries, however, national regulations for new products have been replaced, due to the application of the precautionary principle, by community regulation of novel foods and ingredients (Regulation 258/97/EC), which has been in force since 1997. This “Novel Foods” legislation establishes an evaluation and licensing system that is compulsory for new foods and new processes. HP-processed food products are novel foods because they fulfill two conditions: their history of human consumption has so far been negligible, and a new manufacturing process has produced these products (Hogan, Kelly & Sun, 2005). In fact, the HP food market movement generates more than 2 billion dollars annually, with meat products (entire or sliced) and RTE as the main category. On the commercial scale, a high-pressure vessel costs between $500.000,00 and $2.5 million, depending upon the equipment’s load capacity(liters), high-pressure peak and extent of automation. As the new processing technology has a limited market, pressure-processed products may cost 0.03-0.10 USD more per pound to produce than thermally processed products (Ramaswamy, Balasubramaniam & Kaletunç, 2010). One HP homogenization unit may vary from 13.000€ to thousands of € depending on pressure load and treated product flow rate. As demand for HPP equipment grows, the capital and operating costs will continue to decrease. Consumers and industry benefit from the increased shelf life quality and availability of value-added products and new types of foods may be reached, that are impossible to make using thermal processing methods.

Despite the evident potential and benefits of HP technology application to food processing, two items need to be noted: first, sub lethal high-pressure injures a fraction of the microbial population; and second, the evident baroresistence of certain bacterial strains and spore-forming bacteria results in severe conditions that are required for safety assurance. These injured microorganisms can recover and develop, which represents a risk to the safety and preservation of foods. The pressure inactivation of microorganisms is often characterized by survival curves with first-order kinetics, but with marked tailing of inactivation, indicating a residual, resistant bacteria fraction. Given these facts, the peak of high pressure loads required for effective shelf-life extension and safety may be conducive to anomalous negative effects on food quality attributes. Thus, additional tools for

improving HPP effects represent a promising research field in modern food safety and quality assurance.

Intense HPP loads: related effects on the quality attributes of foods

Conventional thermal sterilization processes involve extensive, slow heat penetration into the core (cold point) of the product and subsequent slow cooling. This thermal process induces changes in product quality to an extent dependent on the product being treated and the temperatures reached; these may include off-flavor generation, textural softening and destruction of colors and vitamins. As previously stated, in contrast to heating, HP treatment at “mild pressures” generally does not change the odor, flavor or other sensory characteristics of foods. Therefore, HPP offers to the food industry a technology that can achieve the food safety properties of heat-treated foods while meeting consumer demand for fresher-tasting foods. To select the most suitable processing conditions for a particular food product, all quality attributes must be taken into account (Polydera et al., 2003). Increasing treatment pressures will generally increase microbial inactivation in shorter times, but higher pressures may also cause greater levels of protein denaturation and other potentially detrimental changes in food quality that could affect the appearance and texture of food compared with the unprocessed product. Because covalent bonds are unaffected by pressure, many of the small molecules that contribute to the color, flavor or nutritional quality of a food are unchanged by pressure. However, if the organoleptic quality of a food depends upon structural or functional macromolecules, and especially polysaccharides and proteins, pressure may affect the food quality (Oey, Lille, Van Loey & Hendrickx, 2008; Patterson, Ledward, Leadley & Rogers, 2012). From this standpoint, animal products, and especially raw and processed meats, require major attention regarding HPP (Bajovic, Bolumar & Heinz, 2012). One of the main prerogatives of the application of HPP is better quality/freshness with equivalent safety compared with conventionally thermal processed food.

Several studies found in the available literature are related to the efficacy of HPP treatments in a wide range of food matrices in improving food safety and shelf life; however, noticeable to significant changes in quality attributes were

connected. Hartyáni et al. (2011) confirmed the impact of an HHP treatment of 600MPa/10 min on orange, grapefruit and tangerine juices, which were significant alterations in color (measured as the total color difference ∆E) and sensory evaluation. Zhang et al. (2011) showed that hydrostatic pressure loads of 600-900 MPa resulted in watermelon juice browning and alterations on dynamic viscosity. Ferrari, Maresca and Ciccarone (2010) confirmed significant color CIELAB (*L**, *a** and *b** color indexes) and polyphenol content differences in pomegranate juice after 400 MPa HHP treatment. Carrot texture depreciation was confirmed after 600 MPa of HHP (De Roeck, Mols, Duvetter, Loey, Hendrickx, 2010). Adverse effects have been reported for fruits and vegetables. For example, high-pressure-processed (600 MPa for 1 min) precut mango during storage at 3°C had a slightly reduced fresh flavor and an increased off-flavor and sweetness but an improved microbial status; however, color, texture and other sensory attributes changed (Boynton et al., 2002). High-pressure treatment above 100 MPa induced the browning of diced onions, and the rate of browning was found to increase with an increase in pressure (Butz et al., 1994). Stute et al. (1996) also illustrated that the application of high pressure at an ambient temperature (25°C) resulted in an undesirable softening of vegetables, such as carrots, potatoes and green beans, due to destruction of cell membranes and a loss of soluble pectin, along with partial liberation of cell liquor. Regarding dairy products, HPP at 500MPa has been found to alter the characteristic color and texture of goat cheeses (Capellas, Mor-mur & Guamis, 2001). Evert-Arriagada, Hernández-Herrero, Juan, Guamis and Trujillo (2012) affirmed that HHP treatments (300 and 400 MPa for 5 min at 6°C) modified the texture (more firm) and color (more yellow) compared with control fresh cheeses. Juan, Trujillo, Guamis, Buffa and Ferragut (2007) observed that moderate pressures (200 and 300 MPa) enhanced the firming of cheeses, which was demonstrated by an increase in fracture stress. The highest pressures applied, and especially 500 MPa, produced a weakening effect on the casein matrix, and these cheeses showed the highest deformability and the lowest fracturability and rigidity. In addition, a sensory panel found that this treatment produced the softest and less elastic and less crumbly cheeses. Studies focusing on HP technology in fisheries have noted several major effects. Gudbjornsdottir,

Jonsson, Hafsteinsson and Heinz (2010) revealed effects on the lightness of salmon fillets, which become lighter in color as a function of both time and the applied pressure range (700-900 MPa, <60 s). HP treatments of 260 MPa/3 min significantly altered the color (*L**, *a** and *b**) of oysters (Cruz-Romero, Kelly & Kerry, 2007). In raw and processed meat products, which represent one of the main commercialized HP-processed food categories, the effects are remarkable, affecting color, texture and sensory attributes. Importantly, HPP of meat and meat products can trigger lipid oxidation reactions (Cheftel & Culioli, 1997; Bajovic, Bolumar & Heinz, 2012). In special, HPP has been identified problematic for raw beef processing mainly due to denaturation of hemoglobin, breakdown of heme-group and release of iron (which in proportions trace the pro-oxidant system acts), and oxidation of myoglobin to metmyoglobin ferrous. Studies showed that treatments with pressures above 300MPa for fresh meat culminate in increased brightness L * and reduction of red index a * (> 400MPa), reduced proportion of oxymyoglobin front of metmyoglobin, and significant increase in heme iron content not (release of iron pirrolica structure) in minced beef pressurized (Carles, Venciana-Nogues & Cheftel, 1995). Chicken breast fillets treated with HP at 600MPa showed an increased cooking loss index and altered color with increasing *L**, *a** and *b** values, and the textural parameters showed increases in hardness, cohesiveness, gumminess and chewiness (Kruk et al., 2011). Knorr (2007) showed declining cohesiveness and *L** values at a pressure range of 500-600 MPa for pork, chicken and turkey meat. McArdle et al. (2010) evaluated beef at 200, 300 and 400 MPa and showed that lower pressure levels (200 MPa) minimally affected meat quality parameters and that increased pressure and temperature levels increased cooking loss and lipid oxidation and altered the color. HPP at 600MPa modified the color of dry-cured ham, increasing the *L** value, and altered sensory attributes, resulting in an increase in hardness, chewiness, brightness, odor intensity and saltiness (Clariana, Guerrero, Sárraga & Díaz, 2011). In addition, regarding processed meats, such as dry-cured and cooked hams, meat patties, mortadella and sausages, several studies revealed noticeable effects of elevated HP loads on quality attributes (Villacís, Rastogi & Balasubramaniam, 2008; Cava et al., 2009; Ventanas, Morcuende, Estévez & Ventanas, 2010; Alba, Montiel, Bravo,

Gaya & Medina, 2012; Ferrini, Composada, Arnau & Gou, 2012; Bak et al., 2012; Clariana, Guerrero, Sárraga & Garcia Regueiro, 2012). Regarding UHP, Patrignani, Tabanelli, Siroli, Gardini and Lanciotti (2013) confirmed the significant effect o HPH multi-passes at 100MPa against spoilage yeasts in apricot juice, but the effects on characteristic viscosity (MPa/s) were remarkable.

Despite the innumerous benefits of HPP as a non-thermal conservation process for promoting the safety and extending shelf-life for food products, conventional intense high pressure treatments set ups may cause slight but noticeable alterations in quality attributes, and mainly color, texture characteristics, oxidation and the sensory profile. These events may harm the products acceptance by the consumer market. Consequently, studies should focus efforts on mapping and optimizing HPP processes for specific each category of food product and target pathogen, ensuring safety without quality loss. Hurdle technology (Leistner & Gorris, 1995; Leistner, 2000) represents the most promising alternative of modern food safety trends.

Multi-hurdle approach: combining HPP and natural antimicrobials

Food preservation implies stressing microbial cells or putting microorganisms in a hostile environment to inhibit their growth, shorten their survival or cause their inactivation and death. A multi-hurdle approach or the *Hurdle Technology of Leistner*, to food preservation means that hurdles are deliberately combined to improve the microbial stability and quality of foods andtheir nutritional and economic properties. Thus, hurdle technology aims to improve the total quality of foods by applying an intelligent mixture of intrinsic and extrinsic hurdles. This multipurpose strategy combines the principles of homeostasis, metabolic exhaustion, stress reactions and multi-target preservation to achieve microbial stability and safety in foods coming from future lines, including healthful foods with less fat and/or salt, fewer chemicals, minimal processing, natural preservation and minimal packaging (Leistner & Gorris, 1995; Leistner, 2000; 2004). The commercial challenge of minimally processed, high-quality foods provides a strong motivation to study food preservation systems that combine

traditional microbial stress factors or hurdles while introducing “new” variables for bacteriostatic and bactericidal effects.

The sterilization of food by HPP requires elevated pressure (up to 1000 MPa) to inactivate barotolerant and spore-forming pathogens in certain foods. As mentioned above, high pressure loads may adversely alter the texture, color and flavor of many foods and additionally increase initial and maintenance costs, promote wear and shorten the life of equipment. The application of moderately high-pressure treatment (up to 400MPa) may cause sublethal injury on a fraction of population, compromising the safety of the food product due to the potential recovery of injured bacteria during storage (Earnshaw et al., 1995; Patterson et al., 1995). Many reported data indicate that the commercial pasteurization or sterilization of low-acid foods using high pressure is very difficult without using certain additional factors to enhance inactivation rates and promoting hurdle effects. The great resistance of bacterial endospores is one of the major food processing and safety challenges in the industry. Although most vegetative cells are inactivated at 600MPa, spores of certain species can survive at pressures under 1000MPa (Gould, 1995). Unless the pressure is higher than 800MPa, heat is required to inactivate of spore-forming bacteria in low-acid foods (Matser, Krebbers, Berg & Bartels, 2004). Intrinsic and extrinsic factors, such as heat, natural and chemical antimicrobials and additional intervention strategies (e.g., ultrasound and ionizing radiation), can be used in combination with high pressure. These approaches not only help to accelerate and optimize the rate of inactivation but also can be useful in reducing the severity of pressure levels and hence the cost of the process while eliminating the commercial problems associated with sublethal injury, endospore formation, survivor tails or spore outgrowth (Palou, Lopez-Malo, Barbosa-Canovas & Swanson, 2007). Therefore, a processing protocol based on a multi-hurdle approach or hurdle technology, combining moderately high pressures with another preservation method, can be used to manufacture safe food without quality loss and high processing costs. It is possible that this hurdle approach will be used in many of the commercial applications of HPP technology. The **Figure 1**, illustrate the synergistic hurdle concept of natural antimicrobials and HPP considering global processed food quality.

During the HP treatment of a selected sample, the microbial population load decreases at the first moment of the holding time process. With holding time running forward, a residual barorresistant population may survive due to sublethal pressure injuries. Thus, residual, sub lethally injured inoculums may restore their outgrowth, depending on the intrinsic and extrinsic factors of food during storage. The patterns of the inactivation of microorganisms during HPP do not follow first-order kinetics as in thermal inactivation but are generally non-linear curves marked by tailing or “shoulders”. Deviations from first-order kinetics, the occurrence of survivor “tails” in death kinetics and the possibility of cell recovery after pressurization have been observed. HPP often results in microbial inactivation patterns that leave a fraction of the remaining population viable, even after prolonged processing. HPP may be combined with other preservation methods to increase its efficacy and commercial feasibility. Thus, food processors often combine lethal treatments to assure food safety. Process combinations that act synergistically against vegetative and bacterial spores are the most desirable, and improvements in spore inactivation by HPP have been achieved when the pressure treatments were used in conjunction with additional physical and chemical hurdles, such as heat, chemical preservatives and natural compounds (Shearer et al., 2000; Lopez-Pedemonte et al., 2003; Clery-Barraud et al., 2004). In addition to potential bactericidal effects, a residual hurdle effect may be observed; slowing or preventing the outgrowth of a surviving, sub lethally injured bacterial population. Following the modern market trends for processed foods, natural antimicrobials may represents feasible alternatives for application with HPP in multi hurdle approach.

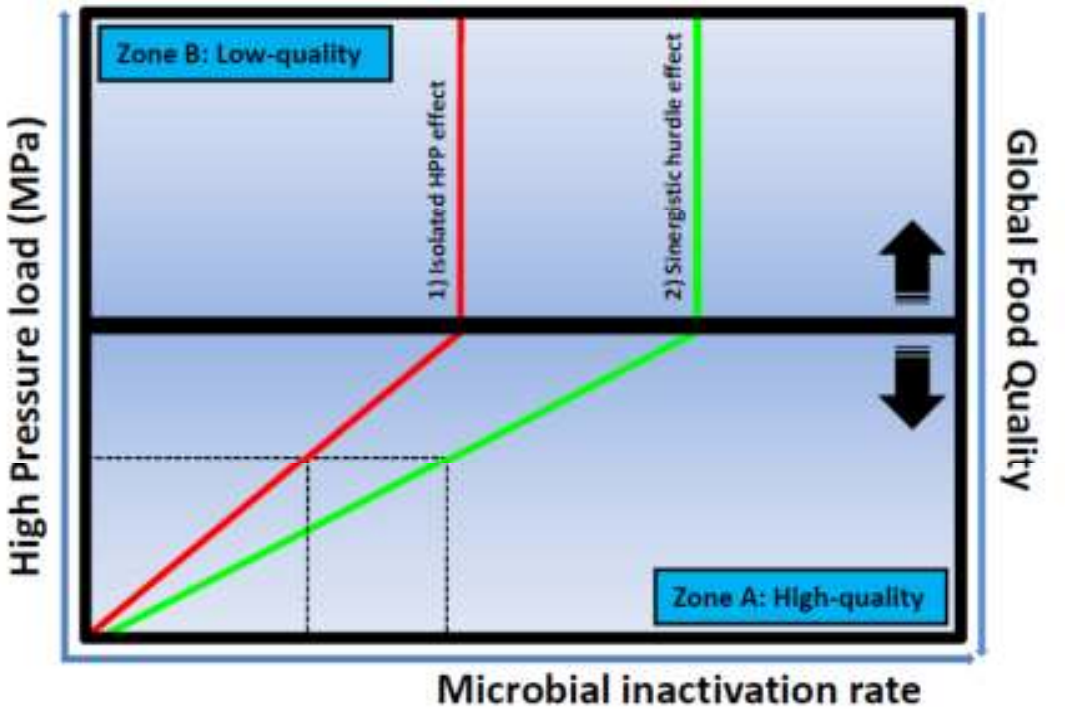

Figure 1. Graphical representation of the possible synergistic hurdle effect of High Pressure food Processing (HPP) and natural antimicrobials, achieving the same inactivation rates at mild HPP loads, considering the same processing time (holding time) and temperature. Zone A represents high-quality food, processed at mild HPP processing.

Synergistic effects of HPP and natural antimicrobials

In order to improve the inactivation rates of vegetative and spore-forming spoilage/pathogenic bacteria induced by HPP, and to promote outgrowth inhibition of sub lethally injured bacterial populations (hurdle effect), HPP has been proposed applied in combination with natural antimicrobials, such as plant-origin antimicrobial agents (EOs, oleoresins and vegetal extracts), organic acids and animal/microbial-origin compounds (competitive flora, antimicrobial peptides, active lipids, chitosan, lactoperoxidase systems, lysozyme, bacteriocins and bacteriophages). **Table 1** depicts the main recent results focusing on HPP (HHP or UHP technologies) treatments combined with natural bioactive compounds, several of which originated from distinct natural sources.

HPP and plant-origin antimicrobial compounds

Edible, medicinal and herbal plants and their derived extracts, essential oils (EOs and their hydrosols, i.e., byproducts of an essential-oil purification procedure) and isolated compounds contain a large number of secondary metabolites that are known to retard or inhibit the growth of gram-positive and gram-negative bacteria. Antimicrobial compounds are commonly found in the extracts and EO fractions of popular rosemary, sage, lemongrass basil, oregano, thyme, cilantro, coriander, cinnamon, marjoram and clove, among many other vegetal species. The major vegetal bioactive components with antimicrobial effects that are found in plants, herbs and spices are phenolic compounds, polyphenols, terpenes, aliphatic alcohols, aldehydes, ketones, acids and isoflavonoids. The main antimicrobial compounds include thymol, carvacrol, p-cymene, linalool, α-pinene, 1,8-cineole, camphor, terpinene, eugenol, citral, polyphenols, catechins, quercetin and kaempferol (Burt, 2004; Bakkali & Averbeck, 2008; Tiwari, 2009). These compounds main antimicrobial locations and mechanisms in the bacterial cell were proven to include degradation of the cell wall, damage to the cytoplasmic membrane and to membrane proteins, induction of the leakage of cell contents, the coagulation of cytoplasm and depletion of the proton motive force (Oliveira et al., 2011). References addressing HP food processing combined with natural vegetal antimicrobial compounds are listed in **Table 1** (Vurma, Chung, Shellhammer, Turek & Yousef, 2006; Gomez-Estaca, Montero, Gimenez & Gomez-Guillen, 2007; Ogihara, Yatuzuka, Horie, Furukawa &Yamasaki, 2009; Evrendilek & Balasubramaniam, 2011; Raouche, Mauricio-Iglesias, Peyron, Guillard & Gontard, 2011; Púlido, Árbol, Burgos & Gálvez, 2012; Bevilacqua, Corbo & Sinigaglia, 2012; Espina, García-Gonzalo, Laglaoui, Mackey & Pagán, 2013; Patrignani, Tabanelli, Siroli, Gardini & Lanciotti, 2013).Because HHP is believed to cause damage to the cell membrane, the common target is suggested to be the root of the observed synergism between natural compounds (Karatzas et al., 2001). Alternatively, microorganisms are baroresistant to selective chemical/natural antimicrobials due to their ability to exclude such agents from the cell, mainly by the action of the cell membrane and transport. However, if the membrane and active transport mechanisms become damaged, for example by HHP, this tolerance may be lost.

Evrendilek and Balasubramaniam (2011) tested the combined effects of HHP (pressure range of 0.1-600MPa at 25°C for up to 300s/5 min) and mint EO (0.05-0.1%) on *L. monocytogenes* and *L. innocua* inoculated in ayran yogurt. These authors observed that the HP treatment of ayran samples at 600 MPa for a treatment time of 300 s reduced *L. monocytogenes* and *L. innocua* levels by more than 5 log units at ambient temperature and that the addition of mint EO further enhanced the inactivation of both bacteria by more than 1 log CFU/ml. The combination of mint EO with HPP provided a reduction in pressure treatment severity of 100-300MPa or by 210 s to achieve the same amount of inactivation relative to HPP alone (5-log reduction at 600 MPa). These authors concluded that HPP combined with mint EO appeared to be a promising technique for preserving microbiologically safe ayran, with no significant impact on product quality. The inactivation rates achieved with *Escherichia coli* O157:H7 and *Listeria monocytogenes* by combining HHP and EOs or their chemical constituents (CCs) were studied by Espina, García-Gonzalo, Laglaoui, Mackey and Pagán (2013); HHP treatments (175-400 MPa for 20 min) were combined with 200 µl/l of each EO (*Citrus sinensis* L., *Citrus lemon* L., *Citrus reticulata L.*, *Thymus algeriensis* L., *Eucalyptus globulus* L., *Rosmarinus officinalis* L., *Mentha pulegium* L., *Juniperus phoenicea* L. and *Cyperus longus* L.) or each CC ((+)-limonene, α-pinene, β-pinene, p-cymene, thymol, carvacrol, borneol, linalool, terpinen-4-ol, 1,8-cineole, α-terpinyl acetate, camphor and (+)-pulegone), and the synergistic inactivation effects of the HHP-combined treatments were observed. Púlido, Árbol, Burgos and Gálvez (2012) confirmed that the same inactivation rates against *Staphylococcus aureus* at 600MPa/10min may be reached at 500MPa/5m in rice pudim (a mild HHP) when is proposed their combined application with nisin (500 IU/g), cinnamon oil (0.2%) and clove oil (0.25%).

The synergistic effects of vegetal antimicrobial compounds and HPH were also reported in the recent literature. Patrignani, Tabanelli, Siroli, Gardini and Lanciotti (2013) evaluated the combined effects of the aromatic compound citral, used at a concentration of 50 mg/L, and HPH multi-passes treatments (performed at 100MPa for 1-8 successive passes) on the inactivation of *S. cerevisiae* spoilage yeast. It was observed that the effect of HPH treatment can be notably potentiated

throughout the presence of the citral compound, increasing the time necessary to reach a spoilage threshold during storage.

One of the main compounds present in vegetal fractions and responsible for these fractions bacteriostatic (sub-minimal inhibitory concentration levels) and bactericidal effects are phenolics and polyphenols. Vegetal extracts rich in naturally occurring phenolics increase the lethality of high pressure against the microbial load in food. Phenolic compounds at levels innocuous to microorganisms may in fact sensitize the organisms to high-pressure treatment (Chung, Malone & Yousef, 2007). Combining naturally occurring phenolics with HPP is likely useful for eradicating pressure-resistant microorganisms in food (or conversely). Vurma, Chung, Shellhammer, Turek and Yousef (2006) demonstrated the sensibilization effects of a pré-HHP (400MPa/5 min) treatment with several phenolic compounds, including carvacrol, eugenol, thymol, catechins and rosemary extract, at 100 ppm in phosphate buffer on *L. monocytogenes.* Ogihara, Yatuzuka, Horie, Furukawa and Yamasaki (2009) performed studies evaluating a pressure range of 250-400 MPa combined with polyphenolic tannins and reported synergistic effects against *Salmonella*, which was not detected at viable counts after 250 MPa/30 min. In addition to antimicrobial effects, naturally occurring phenolics from vegetal sources can play an antioxidant role, preventing lipid oxidation in in vitro and food matrix studies (Oliveira et al., 2012). This classical hydrogen-donating antioxidant activity of phenolic and polyphenolic compounds can delay the oxidation processing triggered by high-pressure treatments conducted at elevated pressure loads or holding times (Chung, Malone & Yousef, 2007; Mariutti, Orlien, Bragagnolo & Skibsted, 2008). Alves, Bragagnolo, Silva, Skibsted and Orlien (2012) found that polyphenolic residue fractions of tomatoes may protect against the formation of secondary lipid oxidation products, measured as thiobarbituric acid-reactive substances in chicken breast meat pressurized in an isostatic press at up to 800MPa. Rosemary extract was found to be effective in inhibiting lipid oxidation in minced chicken breast and thigh muscle processed at 600 MPa for 10 min during subsequent heat treatment (Bragagnolo, Danielsen & Skibsted, 2007). Consequently, the compounds may act by preventing oxidation-coupled fading and color loss, which frequently occur in cooked, cured, and sliced meat products, one

of the major commercialized HP-processed food product categories (Sebranek, Sewalt, Robbins, House, 2005). Gomez-Estaca, Montero, Gimenez and Gomez-Guillen (2007) indicated that a combination of mild HHP processing (300MPa/20°C/15min) and edible films yielded the best results in terms of preventing oxidation and inhibiting microbial growth in cold-smoked sardines, thereby increasing shelf life (total plate count and sulfide-reducing bacteria); coating the muscle with films enriched with oregano or rosemary extracts increased the phenol content and antioxidant power when used in combination with HPP.

HPP and animal-origin antimicrobial compounds

There are numerous antimicrobial systems of animal origin, which have often evolved as host defense mechanisms (Tiwari et al., 2009). Several references depicted in **Table 1** reveal the potential application and the synergistic effects of HPP and natural antimicrobial compounds obtained from animal sources.Lysozyme is a bacteriolytic enzyme commercially sourced from hen's egg white, which is reported to inhibit bacteria and spore-forming bacterial species (Abdou, Higashiguchi, Aboueleinin, Kim, Ibrahim, 2007; Barbiroli et al., 2012). The synergistic effects of UHP homogenization treatments and lysozyme on *Lactobacillus brevis* were observed by Tribst, Frank and Cristianini (2008), who reported a 6-log reduction (next to high pressure loads of 200 MPa) for treatments with 150 MPa plus lysozyme at 50 mg/l. Lysozyme's antimicrobial activity relies on the enzyme's ability to hydrolyze the β-1,4 linkage between N-acetyl muramic acid and N-acetyl glucosamine, which are present in peptidoglycan (a bacterial cell wall component). Gram-positive bacteria are very susceptible to lysozyme because their cell wall is composed of 90% peptidoglycan, whereas in Gram-negative bacteria, peptidoglycan accounts for only 5-10% of the cell wall and lies beneath the outer membrane of the cell envelope (Losso, Nakai, & Charter, 2000). Nevertheless, lysozyme can be effective against Gram-negative bacteria in the presence of membrane destabilizing agents, such as detergents and chelators (Gill & Holley, 2000). Importantly, combined treatments that include High Pressure can sensitize gram-negative bacteria to lysozyme treatments. Nakimbugwe,

Masschalck, Atanassova, Zewdie-Bosüner and Michiels (2006) confirmed the synergistic effect of HHP treatments and lysozyme. At ambient pressure, none of the studied gram-negative bacteria (*Yersinia enterocolitica*, *Shigella flexneri*, *Escherichia coli* O157:H7, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) were sensitive to any biopreservative lysozymes. HHP treatment (130-300MPa/25°C/15 min) sensitized several gram-negative bacteria to one or more lysozymes. Diels, Taeye and Michiels (2005) have observed the sensitization of *E. coli* to lysozyme combined with HPH treatment in culture media and PBS buffer. Vannini, Lanciotti, Baldi and Guerzoni (2004) observed that addition of the enzyme lysozyme enhanced the instantaneous pressure efficacy against several food-relevant microorganisms, such as *Lactobacillus, Listeria*, *Salmonella, Staphylococcus and Bacillus*. Interestingly, the hypothesis formulated in this work is that the combination of HPH and lysozyme is associated with conformational modifications in the protein (enzyme), with a consequent enhancement in its activity.

Lactoferrin (bovine and activated lactoferrin - ALF) is an 80 kDa, iron-binding glycoprotein that can bind to two ferric ions per protein molecule. The antibacterial activity of lactoferrin is due to two different and unrelated mechanisms: one based on iron deprivation, which inhibits bacterial growth, and one related to the large cationic patches present on the lactoferrin surface (Jenssen & Hancock, 2009). These cationic patches allow direct interaction with the anionic lipid A, a component of the lipopolysaccharide (LPS) of Gram-negative bacteria, thus altering outer membrane permeability and resulting in the release of LPS. As a natural component of milk, lactoferrin satisfies all regulatory requirements and has been directly used in an antimicrobial spray for the treatment of beef carcasses (Taylor, Brock, Kruger, Berner, & Murphy, 2004).

Table 1. Synthesis of studies focusing on High Pressure food processing (HPP) combined to natural bioactive compounds to inactivate pathogens and spoilage bacteria in culture media, raw and processed foods. HHP[1] – High Hydrostatic Pressure; DHP[2] – Dynamic High Pressure (or HPH - High Pressure Homogenization)

Product or sample / Microorganism	Processing conditions setup	Main effects	References
Shiguella flexineri in ground beef and *Vibrio cholera*e in salmon and mussels	Inoculated foods were treated individually with HHP[1] (150-450 MPa for 5 and 9 min, 300 MPa for 13 min, and 550 MPa for 5 min), with phages (cocktail of 3*S. flexneri* or single *V. cholerae* phages, both applied at 10^9 PFU/mL or combinations thereof (HHP/VP, VP/HHP)	Stand-alone treatments with VP, HHP below 450 MPa (seafood) or 550 MPa (meat), and combined treatments of VP and HHP at 250 MPa for 5 min did not reduce bacterial counts below the detection limit. By contrast, complete inactivation of S. flexneri and V. cholerae, was achieved at 550 MPa for 5 min or, more energy-efficient, at 350 MPa for 5 min followed by addition of phages, thus, indicating a combination of HHP and VP as an efficacious hurdle technology for meat and seafood processing	Ahmadi, Anany, Walkling-Ribeiro & Griffiths, (2015)
Yogurt drink (ayran) *Listeria monocytogenes* and *Listeria innocua*	HHP[1] (0.1-600 MPa) at initial 25°C for up to 300 s (5 min) pressure holding time with mint essential oil (0,1 and 0,05%).	Reducing pressure load and by half (600 to 300MPa) and time to 210s the same amount inactivation were detected by synergistic application with mint essential oil.	Evrendilek & Balasubramaniam, (2011)
Liquid fruit juices: orange and apple juices *Listeriamonocytogenes* and *E. coli* O157:H7	HHP[1] treatments (175–400MPa for 5-20 min) were combined with several essential oils and isolated essential oils compounds.	Were observed synergistic inactivation effects of HHP and essential oils or isolated compounds and proposes their possible use in liquid food such as fruit juices.	Espina, García-Gonzalo, Laglaoui, Mackey & Pagán, (2013)
Phosphate buffer (pH 7.0) *Listeria monocytogenes*strains (Scott A, OSY-8578, and OSY-328)	Pré-HHP[1] treatments with naturally and chemical phenolic compounds at 100ppm/60min followed by HHP[1] 400MPa/5min.	The naturally occurring phenolic compounds including thymol, eugenol, carvacrol, cathechins and rosemary extracts improve log reductions for selected HHP[1] treatment.	Vurma, Chung, Shellhammer, Turek & Yousef, (2006)
Culture media broth (BHI)	HHP[1] 250MPa for 30min in combination with 30 food additives (FA) including	At 1% concentrations, all of the FAs tested showed significant synergistic effects, and 9 of 30 FAs showed	Ogihara, Yatuzuka, Horie, Furukawa &Yamasaki, (2009)

Salmonella enterica serotype Enteritidis	natural bioactive compounds at 1%.	strong synergistic effects. Citric acid, adipic acid, C8-sugarester, C10-sugarester, tannin, nisin, wasabi extract, e-polylysine, and protamine. These results indicate that some FAs are useful for increasing the inactivation ratio of *S. enteritidis* in HHP treatment.	
Apricot juice *Saccharomyces cerevisiae* yeast	Combined effects of an aroma compound citral, used at a concentration of 50 mg/l and High Pressure homogenization HPH[2] treatments (performed at 100MPa with 1–8 successive. passes).	The results showed that yeast cell viability decreased with the increases of passes at 100MPa. In addition, the effect of HPH treatment can be notably potentiated throughout the presence of citral, increasing the time necessary to reach a spoilage threshold during storage.	Patrignani, Tabanelli, Siroli, Gardini & Lanciotti, (2013)
Rice Pudim *Staphylococcus aureus*	Inactivation of *Staphylococcus aureus* strains by high hydrostatic pressure (HHP[1]) treatments applied singly or in combination with bacteriocins nisin, enterocin AS-48, and cinnamon and clove essential oil.	Treatments at 600 MPa for 10 min reduced initial populations of staphylococci (7.9 log CFU/g) below detectable levels of 1 log CFU/g in the puddings. Treatments at 500 MPa/5 min were tested and additional reductions of 0.87, 1.3 and 1.8 log cycles were recorded for the combined HHP treatments with nisin (500 IU/g), cinnamon oil (0.2%) and clove oil (0.25%), respectively. During refrigeration storage for one week, viable counts in puddings from combined treatments were significantly lower compared to the single HHP treatments. These results suggest mild HHP treatments are required for inactivation of *S. aureus* in puddings when HHP is applied in combination with selected natural antimicrobials.	Púlido, Árbol, Burgos & Gálvez, (2012)
Apple juice at 25°C for 8 days for an accelerated shelf life test	HPH[2] 0-60MPa performed in a laboratory medium combined with limonene (0-1800 ppm) and citrus	HPH[2] reduced by 2-4 $\log_{10}$ cfu/ml colony count of *S. bayanus*, whereas citrus extract was able to control yeast growth for 4-8 days.	Bevilacqua, Corbo & Sinigaglia, (2012)

Spoilage yeast *Saccharomyces bayanus*	extract (0-3ppm); thus, two combinations HPH[2] at 20MPa + 900 ppm of limonene and HPH[2] at 20 MPa + 2ppm of citrus extract were chosen for validation in apple juice.	Finally, consumer test pointed out panel acceptability for homogenized juice, containing citrus extract; the use of limonene was not advisable for its strong organoleptic impact. This paper proposes use of HPH + citrus extract or limonene to inhibit *Saccharomyces bayanus*.	
Pasteurized whole milk (simulating cold chain brake 25°C/48h) *Staphylococcus aureus*	HHP[1] at 600, 500, 400, 300 and 200MPa, combined to A mixture (1:1) of two bacteriophages, vB_SauS-phiIPLA35 (phiIPLA35)and vB_SauS-phiIPLA88 (phiIPLA88).	A synergistic effect between HHP and phages was observed. Compared to each single treatment, the combined treatment was able to reduce the initial *S. aureus* contamination below the detection limit (10 CFU/mL). 400MPa was found to be the most suitable pressure to be used in combination with these phages.	Tablá *et al*., (2012)
Culture media broth TSB-YE *Salmonella entérica* serovar Enteritidis	HHP[1] (0.1–550MPa for 10 min at 25°C) alone and in combination with nisin (200 IU/ml nisin) in culture broth.	An 8 $\log_{10}$ CFU/ml reduction was observed after a pressure treatment at 450-500MPa for the tested strain without nisin.When nisin was added, a similar reduction was obtained at 350MPa. Authors affirmed HHP facilitated penetration of nisin into the cell above 100MPa pressure.	Lee & Kaletunç, (2010)
Fermented sausages *Salmonella enterica*, *Listeria monocytogenes* and *Staphylococcus aureus*	HHP[1] 400 MPa/10 min at a starting temperature of 17°C combied to enterocins A and B 2000 AU/g	Combination of enterocins A e B and HHP[1] could synergistically reduce the counts of evaluated pathogens	Jofré, Aymerich & Garriga, (2009)
Sliced Cooked ham *Listeria monocytogenes*	HHP[1] 400 MPa/10 minand natural antimicrobials including enterocins at 2400 AU/g and lactate-diacetate 0,1%. During three months at 1°C and 6°C and evaluating cold break chain	The combination of low storage temperature (1 °C), high pressure processing (HPP) and addition of lactate–diacetate reduced the levels of *L. monocytogenes* during storage by 2.7 log CFU/g. The most effective treatment was the combination of HPP, enterocins and refrigeration at 1 °C, which reduced the population	Marcos, Jofre, Aymerich, Monfort & Garriga, (2008)

		of the pathogen to final counts of 4 MPN/g after three months of storage, even after the cold chain break.	
Sliced cooked ham *Salmonella* sp.	HHP[1] 400MPa/10min/17°C interleavers (11-11 cm and 133 µm thick) consisting of a perforated polypropylene layer added of 200 or 2000 AU/cm^2 of enterocins A and B, sarkacin K; 200 AU/cm^2; 200 AU/cm2 of nisin A; 1,8% potassium lactate; and 200 AU/g nisin plus 1.8% potassium lactate (NL) and distilled water (control, C).	*Salmonella* counts decreased from initial 10^4 CFU/g to <10 CFU/g, a value that was maintained for 3 months of storage at 6°C. However, the elimination of the pathogen could only be achieved by combining HHP and nisin-containing interleavers. Therefore, antimicrobial packaging, HHP and refrigerated storage appear as an effective combination of hurdles to obtain value added ready-to-eat products with a safe long-term storage.	Jofré, Aymerich & Garriga (2008)
Cooked chicken *Listeria monocytogenes*	HHP[1] at 600 MPa/2 min/20°C with antilisterial agent sodium lactate at 2% (w/w).	Pressure treatment alone was not sufficient to eliminate all of the *Listeria*. Numbers of survivors were initially below the level of detection (50 CFU/g) but increased during storage to reach >10^8 CFU/g by day 21.The addition of 2% sodium lactate in combination with pressure treatment was most effective at inhibiting the growth of *L. monocytogenes* and numbers remained below the limit of detection throughout the 105 day storage. The addition of antimicrobial agents, in combination with pressure could be used to give additional food safety assurance without increasing pressure hold time.	Patterson, Mackle & Linton, (2011)
Blood sausage (morcillas) Microbial groups evaluated: Total viable count, *Enterobacteria*, *Pseudomonas*, Lactic acid bacteria and *Clostridium*	HHP[1] 300, 500 and 600MPa were tested for 10 min, with organic acid salts (3% potassium lactate; 3% mixture potassium/sodium	The results suggest that an addition of potassium + sodium lactate and the application of 600MPa for 10 min increases the shelf-life of the morcillas by 15 days, compared to	Diez, Santos, Jaime & Rovira, (2008)

perfringens	lactate and 2,5% potassium lactate/sodium diacetate.	the others treatments.	
Refrigerated shelf-life of sliced cooked ham *Listeria monocytogenes*, *Salmonella*, S. aureus psychrotrophic bacteria, aerobic plate count (APC), lactic acid bacteria (LAB) and enterobacteriaceae	HHP[1], 200 MPa for 10 min, and 400 MPa for 10 min combined to enterocin LM-2 at 256 and 2560 AU/g.	A strong combined effect of both methods for inactivation of *Listeria monocytogenes* and *Salmonella enteritidis* was observed. Overall, from a microbiological and physicochemical point of view, the most effective treatment was achieved with a combination of 400 MPa HHP and 2560 AU/g enterocin, extending the shelf life to above 90 days and producing a better sensory profile during the whole storage.	Liu *et al.,* (2012)
Low-acid fermented sausages *Listeria monocytogenes* and *Staphylococcus aureus*	HHP[1] 600MPa for 5 min at an initial fluid temperature of 15°C with bioprotective poll of cultures (*Enterococcus* sp.)	The application of the selected HHP treatment at the end of ripening (day 21) produced an immediate reduction in the counts of *Enterobacteriaceae* to levels <1 log_{10}cfu/g and promoted a decrease of 1-log_{10} unit in the counts of *S. aureus*. *E. faecium* CTC8005, which reduced the counts of *L. monocytogenes* ca. 2 log_{10}cfu/g immediately after stuffing and in combination with HHP treatment promoted a further reduction of 1 log_{10}cfu/g in the pathogen counts. The combination of *E. faecium* CTC8005 and HHP was the most efficient antilisterial approach.	Rubio, Bover-cid, Martin, Garriga & Aymerich, (2013)
Dry-cured ham *Escherichia coli* O157:H7	HHP[1] 400, 500MPa with holding time of 10 min at approximatelly 12°C. This setup was combined with bacteriocins nisin and pediocin on the surface of dry-cured ham at 100 IU/g and 0.6%	Synergistic antimicrobial effect was registered when 400MPa and 500MPa for 10 min combined with nisin were applied. Changes in textural parameters caused by pressurization and biopreservatives were minor. Lightness (*L**) values were slightly affected. Redness (*a**) and yellowness (*b**) were less modified by HP and biopreservatives	Alba, Bravo & Medina (2013)

		and tended to diminish during refrigeration.	
Sliced fermented sausages without NaCl *Listeria monocytogenes*	HHP[1] 600MPa/5 min/12°C Antimicrobial packaging with PVOH films containing nisin at 450 AU/cm^2	HPP had no antimicrobial effect against *L. monocytogenes* at the studied conditions. Combination of HPP with antimicrobial packaging did not produce any extra protection against *L. monocytogenes* compared to antimicrobial packaging alone.	Marcos, Aymerich, Garriga & Arnau (2013)
Fresh chiken breast fillets Total viable counts, *Pseudomonas*, lactic acid bacteria (LAB), *Brochothrix thermosphacta*, coliforms and *Escherichia coli*	HHP[1] 300MPa/5min/20°C combined to commercial liquid antimicrobial edible coating consisting of lactic and acetic acid, sodium diacetate, pectin and water ("articoat-DLP") followed by modified atmosphere packaging (MAP - (30% CO_2/70% N_2)	The combination of 3 hurdles (MAP + HHP + coating) was the most efficient in extending the durability of chicken breast fillets, which maintained their sensory and microbiological quality for up to 28 days. At the time of rejection, total counts were 6.3 ± 0.7 log cfu/g, with LAB being dominant (100%). For MAP + coating and HHP-MAP fillets, the storage life was estimated to be two weeks while that of the untreated fillets (C-MAP) was estimated to be one week. Colour, tenderness and overall acceptability were the best maintained sensory attributes during storage for MAP + HHP + coating samples.	Rodríguez-Calleja, Cruz-Romero, O'Sullivan, García-López & Kerry, (2012)
Low-acid fermented sausages: *fuet* *Listeria monocytogenes*, *Salmonella enterica* and *Staphylococcus aureus*	400MPa for 10min at 17°C combined with enterocin AS-48 at 148 AU.g^{-1}	After pressurization (400 MPa) and storage *Listeria* counts remained below 5 CFU/g in all *fuets* containing AS-48 (pressurized or not). HHP alone had no anti-*Listeria* effect. HHP treatment significantly reduced *Salmonella* counts, with lowest levels in pressurized *fuets* with AS-48. *S. aureus* showed similar growth for all treatments and storage conditions. These results indicate that AS-48 can be applied alone to control *L. monocytogenes* and	Ananou et al., (2010)

		combined with HHP treatment to control *Salmonella* in *fuets.*	
Culture media PBS buffer *Escherichia coli*	High Pressure homogenization (HPH^2) at pressures ranging from 100 to 300MPa to sensibilize cells to lysozyme, nisin and lactoperoxidase	At above 150MPa, *E. coli* became sensitive to lysozyme and nisin when these compounds were added before the HPH processing	Diels, Taeye & Michiels, (2005)
Apple juice and apple cider *Escherichia coli* K-12strain	HPH^2 ranging from 50 to 350MPa were used. Two types of chitosan (regular and water soluble) with 2 levels of concentration 0.01% and 0.1% were investigated for synergistic effect with high-pressure homogenization for the bacterial inactivation.	High-pressure homogenization (HPH) induced significant inactivation in the range of 100 to 200 MPa. The homogenization pressure and the incremental quantity of chitosan (both types) acted synergistically with the pressure to give higher inactivation.	Harte *et al.,* (2009)
Roast Beef Meat spoilage Clostridial species (*Clostridium sporogenes*, *Clostridium perfringens*, *Clostridium tertium*, *Clostridium laramie*)	$HHP^1$345MPa for 5 min at 60°C combined to 5000 AU/g pediocin + nisin	The HHP treatment of roast beef samples inoculated with a mixture of clostridial spores could be stored for 42 days at 4°C. The HP in combination with bacteriocins, extended the shelf-life of roast beef up to 7 days at 25°C. The combined treatment of HHP and Bacteriocin controlled the growth *Clostridium laramie* spores and extended the shelf-life of roast beef for 84 days when stored at 4°C.	Kalchayanand, Dunne, Sikes & Ray, (2003)
UHT Milk *Clostridium botulinum* spores	HHP^1 ranging from 300.0–700.0MPa, temperature 30–70 °C, holding times 7.5–17.5 min, and the presence of nisin 0–333 IU/ml.	By analyzing the response surface plots and their corresponding contour plots the authors conclude that the optimum process parameters for a 6-log_{10} cycle reduction of *C. botulinum* spores were obtained as: pressure, 545.0 MPa; temperature, 51 °C; pressure holding time, 13.3 min; and nisin concentration, 129 IU/ml	Gao & Ju (2008)
Cooked ham	400 MPa for 10 min at	Use of HPP resulted in the reduction	Marcos, Jofre, Aymerich,

Listeria monocytogenes	17°C, in combination with alginate films containing enterocins 2000AU/cm^2	of 3,4 log units of *L. monocytogenes*. Combining antimicrobial films with HPP was effective to achieve a shelf-life of 60 days.	Monfort & Garriga, (2008)
Cooked ham *Carnobacterium divergens*, *Leuconostoc carnosum*, *Brochothrix thermosphacta*, *Listeria innocua* and *E. coli* O157:H7	HHP[1]100–700 MPa, processing time for 10 min at 5–40 °C were evaluated. Selected 600MPa/10°C/10min (for 5 log reduction of microbial groups) were combined with caprylic acid 0,15% and Purasal® (K-lactate + sodium diacetate) 2,5%	Without HP treatment, a plate count of 6 log CFU/g was reached after 40 days, both in presence and absence of antimicrobials. HP treatment delayed this initiation of spoilage to 59 days in absence of antimicrobials. However, microbial growth was completely suppressed during at least 84 days in the HP treated products containing caprylic acid or Purasal®. HP treatment increased drip loss but had no or little effect on colour and sensorial evaluation by a taste panel. However, the antimicrobials had a noticeable influence on the flavour and aroma at the concentrations used.	Vercammen et al., (2011)
Dry cured ham *Listeria monocytogenes*	HHP[1] 600MPa, for 5 min at 15°C and addition of nisin	Depending on the type of dry cured ham inactivation from 1.82 – 3.85 log units for L. monocytogenes by HPP was achieved. L. monocytogenes was more resistant to HPP treatment at low a_W. Nisin enhanced the high pressure inactivation.	Hereu, Bover-Cid, Garriga, &Aymerich (2011)
Saline solution 0,9% Endospores and vegetative cells of *C. sporogenes* and *C. beijerinckii*	HHP[1] Spore suspensions were exposed to 90°C and 90°C/600 MPa in the presence of 16 mg L^{-1}nisin or 6.4 mg L^{-1}reutericyclin for 0-60 min Dipicolinic acid (DPA) release and endospore permeability were evaluated.	Vegetative cells of *C. sporogenes* exhibited higher sensitivity to nisin relative to endospores. Nisin increased DPA release when endospores were treated at 90 °C; however, only C. sporogenes ATCC 7955 exhibited higher inactivation, suggesting strain or species specific effects. Reutericyclin did not enhance spore inactivation or DPA release. Use of nisin in combination	Hofstetter, Gebhardt, Ho, Gänzle & McMullen, (2013)

		with high pressure, thermal treatments enhanced inactivation of endospores of *Clostridium* spp. and may have application in foods.	
Smoked salmon *Listeria monocytogenes*	HHP processing at 250 and 450MPa for 10 min combined with the lactoperoxidase system (LPS)	A synergistic antimicrobial effect of 450 MPa and LPS against L. monocytogenes was registered, preventing the pathogen recovery; however noticeable quality attributes alteration were detected.	Montiel, Bravo, Alba, Gaya, & Medina (2012)
Phospahte buffer *Lactobacillus brevis*	ultra-high pressure homogenization ($UHPH^2$) combined to g 50 $mg.L^{-1}$ of lysozyme and pressure between 150 and 170 MPa	The combined effect showed a reduction ofabout 6 logarithmic cycles and the unaltered count of *L. brevis* after pressure treatment for a week, with the samples stored at room temperature (25 °C). Lysozyme added at MIC concentration to a suspension of *L. brevis* caused a reduction of 1 logarithmic cycle after two hours of contact. The UHPH treatment against *L. brevis* resulted in a reduction of 7 log cycles at 200 MPa. Lysozyme was resistant to UHPH (200 MPa), without loss of muramidase activity or significant loss of antimicrobial power.	Tribst, Frank & Cristianini, (2008)
Cucumber juice drinks natural microbial flora (TAB- total plate count and Yeasts and Moulds	HHP^1 at 400 MPa/4min and 500 MPa/2min and thermal pasteurization at 85 °C/15 s with 100 IU/mL nisin	Yeast and molds were completely inactivated by all treatments, and their levels were below the detection limit during storage. Nisin with HHP had a synergistic effect on the inactivation of total aerobic bacteria (TAB). The samples treated by 500 MPa/2 min with 100 IU/mL nisin exhibited a longer shelf life as compared with other treated samples.	Zhao *et al.,* (2013)
chicken filets *Escherichia coli* O157:H7 and	HHP^1 at 200, 300, 400, and 500MPa for 10 min at 10 °C combined to bovine lactoferrin at 0.5 mg/g.	Treatment at 400 MPa, by itself, lowered counts of inoculated *E. coli* O157:H7 by more than 5 log_{10} CFU/g and those of inoculated *P.*	Del Olmo, Calzada & Nuñez, (2012)

Pseudomonas fluorescens		*fluorescens* by more than 6 log_{10} CFU/g. A synergistic effect on *P. fluorescens* was observed when treatment at 300MPa was combined with lactoferrin, with a 2.3 log_{10} CFU/g additional reduction in counts on day 9, with respect to only300 MPa treatment. Additional reductions in *E. coli* O157:H7 counts achieved by combined treatments remained below 0.5 log_{10} CFU/g.	
Apple and carrot juices *Escherichia coli* and *Listeria innocua*	0 or 10 IU/ml nisin and subjected to 0 to 350 MPa high pressure homogenization (HPH^2).	As processing pressure increased, inactivation of *E. coli* increased, and >5 log reduction of cells was achieved following exposure to pressures in excess > 250 MPa. In contrast, little inactivation was observed for *L. innocua* with pressure 250 MPa and up to 350 MPa processing pressure was required to achieve an equivalent 5 log inactivation. The addition of 10 IU nisin, together with high pressure homogenization, did not exhibit significant additional *E. coli* inactivation, but interactions were observed with *L. innocua*.	Pathanibul, Taylor, Davidson & Harte (2009)
Skim milk Gram positive and Gram negative species (*Lactobacillus helveticus, Lactobacillus plantarum and Listeria monocytogenes* were the most pressure resistant species *Bacillus subtilis, Pseudomonas putida, Salmonella typhimurium, Staphylococcus aureus, Proteus vulgaris Salmonella enteritidis*)	HPH^2 up to 130Mpa combined to lyzozyme and lactoperoxidase system at 3.3 mg/100 ml, 30µg/ml respectively	The enzyme addition enhanced the instantaneous pressure efficacy on almost all the considered species as indicated by their instantaneous viability loss following the treatment the interaction of high pressure homogenization and lysozyme or lactoperoxidase is associated to conformational modifications of the two proteins with a consequent enhancement of their activity.	Vannini, Lanciotti, Baldi & Guerzoni, (2004)
Food model *Botrytis cinerea* fungi	Antimicrobial bio-sourced films based on poly (lactic acid) containing either	The combined effect of HP treatment and volatile antimicrobial packaging	Raouche, Mauricio-Iglesias, Peyron, Guillard & Gontard, (2011)

	carvacrol or allyl isothiocyanate plus HHP[1] up to 800MPa.	allowed the use of lower individual treatment intensities to inhibit *B. cinerea* growth. Combining such"hurdles" is of relevance in the context of development of low-cost and eco-friendly food technologies.	
Cold-smoked sardine (*Sardina pilchardus*) Total counts, sulphide-reducing bacteria	300 MPa/20°C/15 min plus gelatin-based functional edible films enriched by adding an extract of oregano (*Origanum vulgare*) or rosemary (*Rosmarinus officinalis*) or by adding chitosan.	The edible films with the added plant extracts lowered lipid oxidation levels (as measured by the peroxide and TBARS indices) and also, to a lesser extent, reduced microbial growth (total counts), whereas the gelatin–chitosan-based edible films lowered microbial counts (total counts, sulphide-reducing bacteria).	Gomez-Estaca, Montero, Gimenez & Gomez-Guillen, (2007)
Ready-to-eat (RTE) sliced dry-cured ham *Listeria monocytogenes*	HHP[1] 600MPa + nisin directly applied (200AU/cm^2) and nisin applied through active packaging, polyvinyl alcohol films with 200AU/cm^2. Counts of *L. monocytogenes* were periodically monitored throughout 60 days of storage at 8 °C.	The reduction of *L. monocytogenes* immediately after HHP and during storage was more evident in batches with nisin applied directly to the surface of the product. The effect of nisin applied through active packaging was lower than the direct application.	Hereu, Bover-Cid, Garriga & Aymerich, (2012)
Skim Milk and Banana juice Gram negative bactéria including *Escherichia coli* O157:H7, *Shigella flexneri*, *Yersinia enterocolitica* and *Salmonella typhimurium*	HHP[1] up to 350MPa combined to Lyzozyme	In combination with HP treatment lyzozyme were efficient on all bacteria in both milk and banana juice. Depending on the bacteria, inactivation levels in banana juice were increased from 0.4–2.7 log units by HP treatment alone to 3.6–6.5 log units in the presence of 224 UA/ml lyzozyme. Bacterial inactivation in milk was also enhanced by lyzozyme but by 0.5–2.1 log units.	Nakimbugwe, Masschalck, Anim & Michiels (2006)
phosphate buffer Two *Escherichia coli* strains, *Salmonella*	HHP[1] up to 400MPa e in the presence of bovine lactoferrin (500 mg/ml),	None of these compounds, at the indicated dosage, were bactericidal when applied at	Masschalck, Houdt & Michiels (2001)

enteritidis, *Salmonella typhimurium*, *Shigella sonnei*, *Shigella flexneri*, *Pseudomonas fluorescens* and *Staphylococcus aureus*	pepsin hydrolysate of lactoferrin (500 mg/ml), lactoferricin (20mg/ml) and nisin (100 IU/ml)	atmospheric pressure, except nisin, which caused a low level of inactivation of the bacteria. Under high pressure, lactoferrin, lactoferrin hydrolysate and lactoferricin displayed bactericidal activity against some of the test bacteria. The HP-sensitization to lactoferrin was an evident phenom.	
potassium phosphate buffer *Escherichia coli*	HHP[1] at 200-600MPa with lysozyme at 50 mg/ml and nisin 100 IU/ml	The natural antimicrobial peptides enhanced considerably the inactivation of the target bacteria under pressure A hypothetical mechanism of 'pressure-promoted uptake' is proposed to explain *E. coli* outer membrane permeabilization for lipophilic and cationic peptides like lysozyme and nisin under pressure.	Masschalck Garcia-Graells, Van Haver & Michiels, (2000)
low-acid fermented sausages (fuet and chorizo) *Enterobacteriaceae*	Lactobacillus sakei CTC6626 and Staphylococcus xylosus CTC6013 as starter culture combined to 400 MPa for 10 min at 17°C	Starter culture significantly reduced *Enterobacteriaceae* and *Enterococcu*s levels in the finished sausages. High pressure induced an additional reduction of *Enterobacteriaceae* in non-starter sausages.	Marcos, Aymerich, Guardia & Garriga, (2007)
Cooked ham Salmonella sp. Listeria monocytogenes and Staphylococcus aureus	HHP[1] 600MPa for 5 min at 10°C plus 800 AU/g nisin; 1.8% potassium lactate and) 800 AU/g nisin + 1.8% potassium lactate.	The application of an HPP reduced the levels of *Salmonella* and *L. monocytogenes* to levels below 10 CFU/g. These levels continued until the end of storage at both 1 and 6°C. HPP produced a reduction of less than 1 Log CFU/g to *S. aureus*. The combination of HPP, nisin and refrigeration at 6°C was necessary to decrease the levels of *S. aureus* by 2.4 Log CFU/g after 3-months of storage.	Jofre, Garriga & Teresa Aymerich, (2008)
Restructured hams	600MPa/6min/10°C plus potassium lactate	HP treatment at 600 MPa provided an additional reduction in the microbiological counts, increased	Fulladosa, Sala, Gou, Garriga & Arnau, (2012)

Aerobic mesophilic total counts, Lactic acid bacteria (LAB), *Enterobacteriaceae, Listeria monocytogenes, Staphylococcus aureus*, *Salmonella* spp.		pink color, brightness, hardness and saltiness and reduced pastiness and adhesiveness.	
meat model system *Escherichia coli, Salmonella entérica, Staphylococcus aureus, Listeria monocytogenes, Lactobacillus sakei, Leuconostoc carnosum*	400MPa/10min/ 17°C added of bacteriocins (enterocins A and B, sakacin K, pediocin AcH or nisin)	Samples including nisin displayed lower and signicantly diferent counts during the 4°C storage than the rest of the treatments. A greater inactivation of *Escherichia* in the presence of nisin was recorded; the number of survivors remained unchanged during storage at 4°C for 61 days.	Garriga, Aymerich, Costa, Monfort & Hugas (2002)

Del Olmo, Calzada and Nuñes (2012) confirmed the synergistic effects of HHP treatments and lactoferrin against *Escherichia coli* O157:H7- and *Pseudomonas fluorescens* inoculated in chicken filets. Masschalck, Van Houdt and Michiels (2001) tested the bactericidal activity of pressurization HHP in a range of 155-400MPa for 15 min at 20°C in phosphate buffer (against *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas* and *Staphylococcus*) with or without lactoferrin (500µg/ml) or lactoferricin (20µg/ml). These authors confirmed that these natural antimicrobial compounds have no bactericidal effect when applied at atmospheric pressure, contrary to the pronounced effects when combined with HP treatments.

The lactoperoxidase system, which is naturally active in milk, has strong antimicrobial effects against both bacteria and fungi. A wide range of both Gram-negative and Gram-positive bacteria are inhibited by lactoperoxidase. This effect is related to the lactoperoxidase enzyme's catalysis of the oxidation of SCN^- by H_2O_2 into short-lived intermediate products with antibacterial properties, such as the hypothiocyanite anion ($OSCN^-$) and hypothiocyanous (HOSCN) acid (which are reactive products that oxidize the sulfphydryl groups (−SH) of proteins in the bacterial cell membrane and of key enzymes) (Siragusa & Johnson, 1989; Naidu, 2000). The combined effects of High Pressure (HPH dynamic technology) and the lactoperoxidase system were observed by Diels, Taeye and Michiels (2005) for *Escherichia coli* in phosphate buffer and by Vannini, Lanciotti, Baldi and Guerzoni (2004) for several food-relevant microorganisms including *Lactobacillus*, *Listeria*, *Salmonella,Staphylococcus* and *Bacillus*. Montiel, Bravo, Alba, Gaya and Medina (2012) studied the effect of HHP processing at 250 and 450MPa for 10 min holding time combined with the biopreservative, active lactoperoxidase system (LPS) on the inactivation of *Listeria monocytogenes* in cold-smoked salmon for 35 days of storage at 5°C. These authors observed that HHP at 450MPa for 10 min in combination with the LPS in smoked salmon might be used as a hurdle technology approach against *L. monocytogenes*, increasing the safety and shelf life during refrigerated storage. This HHP set-up (450 MPa/10 min) may be considered as a milder process than conventional 600 MPa HHP loads (or above up to 1000MPa), as reflected by the favorable evaluated quality attributes of salmon samples throughout the entire storage time.

Chitosan, a natural biopolymer obtained from the exoskeletons of crustaceans and arthropods, is known for its unique polycationic nature and has been used as an active material due to its antibacterial activity (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, Roller, 2001). HPH (50-350 MPa) was combined with chitosan (0.01 or 0.1%) against *Escherichia coli* K-12 in low-acid apple and apple-cider juices. The authors observed that the homogenization pressure and the incremental quantity of chitosan (both types) acted synergistically with pressure to cause higher inactivation (Harte et al., 2009). The combined antimicrobial effects of water-soluble chitosan and HHP were also observed by Papineau, Hoover, Knorr and Farkas (1991).

HPP and microbial-origin antimicrobial compounds

Bacteria may produce many compounds that are active against other bacteria and that can be harnessed to inhibit the growth of potential spoilage or pathogenic microorganisms. These compounds include fermentation end products, such as organic acids, hydrogen peroxide and diacetyl, in addition to bacteriocins and other antagonistic compounds, such as reuterin (Tiwari et al., 2009). Bacteriophages and bioprotective cultures may be considered as microbial-origin active compounds that can exhibit competitive bacteriostatic effects. Bacteriocins are proteinaceous antibacterial compounds that constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides. These compounds are cationic peptides that display hydrophobic or amphiphilic properties, and in most cases, the target of their activity is the bacterial membrane. Bacteriocin production can be exploited by food processors to provide an additional barrier to undesirable bacterial growth in foods.

Bacteriocins are effective against Gram-positive microbial strains and less effective against gram-negative strains due to the LPS-based external membrane (Tiwari et al., 2009; Zacharof & Lovittb, 2012). Several studies have reported synergism between HPP in isostatic and dynamic forms and bacteriocins, mainly nisin, but including pediocin, enterocins and sarkacin (Garriga, Aymerich, Costa, Monfort & Hugas, 2002; Marcos, Jofre, Aymerich, Monfort & Garriga, 2008; Marcos, Jofre, Aymerich, Monfort & Garriga, 2008; Jofré, Aymerich & Garriga,

2008; Jofré, Aymerich & Garriga, 2009; Ananou et al., 2010; Lee & Kalentunç, 2010; Púlido, Árbol, Burgos & Gálvez, 2012; Liu et al., 2012; Alba, Bravo & Medina, 2013; Marcos, Aymerich, Garriga & Arnau, 2013; Hereu, Bover-Cid, Garriga & Aymerich 2012; Hofstetter, Gebhardt, Ho, Gänzle & McMullen, 2013). Importantly, HP treatments can further the diffusion of natural antimicrobials that are only effective against gram-positive bacteria, such as lysozyme and bacteriocins, across the outer membrane, promoting bactericidal effects against gram-negative bacterial strains. Lee and Kalentuç (2010) observed an 8 $\log_{10}$ CFU/ml reduction after pressure treatment at 450-500MPa of the *Salmonella enterica* serovar Enteritidis without nisin and in culture media TSB. When nisin was added (200 IU/ml), a similar reduction was achieved at 350MPa. The authors affirmed that HHP facilitated the penetration of nisin into the cell at pressures above 100 MPa. This combined approach could produce the same inactivation effect with a mild HHP set-up.

Púlido, Árbol, Burgos and Gálvez (2012) evaluated the combined effects of HHP (600MPa/10 min and 500 MPa/5 min) and nisin or enterocin against *Staphylococcus aureus* inoculated in rice pudding. Treatment with 600 MPa for 10 min reduced the initial populations of staphylococci (7.9 $\log_{10}$ CFU/g) to below detectable levels of 1 log CFU/g, whereas 500 MPa/5 min achieved a 2.9-log reduction in viable counts; this set 500MPa/5min was tested in combination with nisin (200 and 500 IU/g) or enterocin AS-48 (25 and 50 μg/g). It was observed that during refrigerated storage for one week, viable counts in puddings following combined treatments were significantly lower compared with the counts following single HHP treatments, including 600MPa pressure load. This finding suggests that the duration and intensity of HHP treatments required for the inactivation of *S. aureus* in puddings can be reduced when HHP is applied in combination with selected natural antimicrobials. Liu et al. (2012) confirmed the synergistic effects in sliced ham samples submitted to HHP (200-400 MPa/10 min) and enterocin LM-2 treatments against *Listeria monocytogenes*, *Salmonella*, *S. aureus*, and shelf life-indicative psychrotrophic bacteria, aerobic plate counts (APCs), LAB and Enterobacteriaceae. Among additional relevant results, the authors showed that 200MPa HHP and enterocin had no effect on physicochemical and sensory

characteristics; however, the final product possessed a comparatively short shelf life (30-40 days). HHP at 400MPa in combination with enterocin (256 or 2560 AU/g) could extend the shelf life by up to 70 or 90 days when a slight lipid oxidation was induced that affected the hardness, color, odor and overall acceptability of the ham. Overall, from a microbiological and physicochemical perspective, the most effective treatment was achieved with a combination of 400MPa HHP and 2560 AU/g enterocin, extending the shelf life to over 90 days and producing a better sensory profile during storage.

Dynamic HPH treatment combined with the bacteriocin nisin in apple and carrot juices was evaluated by Pathanibul, Taylor, Davidson and Harte (2009). Cells of *Escherichia coli* and *Listeria innocua* were inoculated in apple or carrot juice (approximately 7 $\log_{10}$ CFU/ml) containing 0 or 10 IU/ml nisin and subjected to 0-350 MPa HPH. The addition of 10 IU nisin to HPH did not cause significant additional *E. coli* inactivation, but significant marked interactions were observed with *L. innocua* inactivation.

Bacteriophages are viruses that infect bacteria and are regarded as natural antibacterial agents because these viruses can specifically infect and lyse undesirable pathogenic and spoilage strains. Among the advantages of using phages as biocontrol tools in food, phages' history of safe use and their high host specificity have been reported (García, Rodríguez, Rodríguez & Martínez, 2010). The combined effect of bacteriophages, vB_SauS-phi-IPLA35 (phiIPLA35) and vB_SauS-phi-IPLA88 (phiIPLA88) and high hydrostatic pressure against *Staphylococcus aureus* Sa9 was evaluated in pasteurized whole milk under a simulated cold-chain break (25°C for 48 h) by Tabla et al. (2012). The authors found that 400MPa was the most suitable pressure for use in combination with these phages and concluded that HP plus bacteriophages enabled milder hydrostatic pressure treatments. Therefore, phages can be regarded as a valuable hurdle in minimally processed foods.

The combined application of HHP and competitive cultures was studied in the recent literature by Rubio, Bover-cid, Martin, Garriga and Aymerich (2013). HHP treatment at 600 MPa/5 min at the end of ripening (day 21) was tested in association with bioprotective *Enterococcus* sp. strains against *Staphylococcus*

aureus and *Listeria monocytogenes*. These authors reported the isolated protective effect of *Enterococcus* cultures against the tested bacterial pathogens. After ripening (21 days), the HHP treatment was applied, and additional reductions were detected during the next week of storage, affirming that the combination of *E. faecium* and HHP was the most efficient antilisterial approach.

HPP combined with organic acids and their Sodium/Potassium salts

Organic acids (e.g., acetic, citric, lactic, malic, propionic and tartaric acids), including salts of natural lactic acid (potassium and sodium lactates and lactate diacetate), are frequently applied as additional hurdles in food preservation. Lactate is the salt form of lactic acid produced by bacterial groups (Mani-López, García & López-Malo, 2012). These acids are commonly applied in beef carcass preservation and to several raw meat cuts and meat products (Sofos & Geonaras, 2010), including bakery, dairy, seafood, beverages among others food categories. The combined effects of potassium, sodium lactates (Na-L and K-L) and sodium diacetates with HP treatments were extensively reported in the recent literature: HHP at 400MPa/10 min in sliced cooked ham was reported by Marcos, Jofre, Aymerich, Monfort and Garriga (2008) against *L. monocytogenes*; the synergistic effects of HHP 400MPa/10 min on *Salmonella* inoculated in sliced cooked ham were related by Jofré, Aymerich and Garriga (2008); Patterson, Mackle and Linton (2011) confirmed the synergistic effects of Na-L at 2% and HHP 600 MPa/2 min; Diez, Santos, Jaime and Rovira (2008) reported that Na-L, K-L and sodium diacetate were effective in increasing blood sausages shelf life by 15 days when combined with HHP at 600 MPa; and Fulladosa, Sala, Gou, Garriga and Arnau (2012) reported the combined effects of 600MPa/6 min/10°C plus potassium lactate against the aerobic total count and lactic acid bacteria groups in restructured hams.

Rodríguez-Calleja, Cruz-Romero, O'Sullivan, García-López and Kerry (2012) evaluated the combined effects of a mild HHP process of 300MPa/5 min/20°C and a commercial, antimicrobial edible liquid coating consisting of lactic and acetic acids, sodium diacetate, pectin and water ("articoat-DLP") on the shelf life of fresh chicken breast fillets. The tested combination was efficient in extending

the durability of chicken breast fillets, which maintained their sensory and microbiological quality for up to 28 days (compared with 1 and 2 weeks for control samples and isolated treatments, respectively). Due to the mild HPP characteristics, color, tenderness and overall acceptability sensory attributes were maintained during storage of the samples.

Vercammen et al. (2011) evaluated the shelf life of a packaged sliced cooked ham model product during storage (7°C) after treatment at 600 MPa (10°C, 10 min) in combination with caprylic acid and commercial lactate/diacetate blend (72.8% potassium lactate and 5.2% sodium diacetate as active ingredients). Without HP treatment, a plate count of 6 log CFU/g was reached after 40 days, both in the presence and in the absence of antimicrobials. HP treatment delayed this initiation of spoilage to 59 days in the absence of antimicrobials. However, microbial growth was completely suppressed for at least 84 days in the HP-treated products containing caprylic
acid or commercial lactate/diacetate blend, being maintained the quality. In consequence of regulatory approvals in wide locations, low-cost and the large application of the multifunctional lactates as additives in food industry their usage in combination with HPP represents promising fields.

Multi-hurdle approach to HPP against spore-forming bacterial strains

As mentioned above, the barotolerant characteristic of endospore-forming bacterial strains represents the key challenge in HP food processing. Bacterial strains that can produce endospores, such as *Bacillus* and *Clostridium*, represent the major targets. Hydrostatic pressures loads above 800 up to 1000 MPa are minimally required for significant inactivation endospores effects (Matser, Krebers, Berg & Bertel, 2004); however, food quality attributes and installation and maintenance costs are negatively affected. Alternatively, other processing methods applied in combination with HP can be effective for the elimination of bacterial spores, achieving a synergistic lethal or hurdle effect. In particular, HP treatment at elevated temperatures (e.g., HP treatment at up to 90°C) is very effective in the elimination of bacterial spores in foods. Bacterial endospores can be inactivated at pressures in the range of 500-700 MPa at 90-110°C (Farkas & Hoover, 2000; Zhu,

Naim, Marcotte, Ramaswamy, & Shao, 2008). However, the thermal degradation of food quality needs to be considered, braking down the non-thermal prerogatives employed for HP food processing. Therefore, if the hurdle concept could be applied to the optimization of high pressure for the treatment of low-acid foods, a combination of moderate treatments, including pressure, could lead to a food preservation method effective against bacterial spores.

A wide range of antimicrobial compounds have been studied in combination with HPP to enhance the efficacy of pressure induced inactivation of bacterial endospores. Application of one or more additional hurdles with HPP has the advantage of allowing less severe pressure treatments, thus allowing a higher probability of maintaining the nutritional and sensory qualities of the food (Black et al., 2007). The synergistic effects of HHP and bacteriocins against *Clostridium* spores were investigated by Gao and Ju (2008). These authors analyzed the response surface plots and the corresponding contour plots, the authors concluded that the optimum process parameters for a 6-$\log_{10}$ cycle reduction in *C. botulinum* spores were as follows: pressure, 545.0 MPa; temperature, 51°C; pressure holding time, 13.3 min; and nisin concentration, 129 IU/ml. This set-up may be considered as an effectively mild HHP process compared with other configurations applied in spore-forming bacteria inactivation without additional hurdles. Hofstetter, Gebhardt, Ho, Gänzle and McMullen (2013) confirmed the synergistic effects of bacteriocins and HP against the endospores of *C. sporogenes*, exposed to 90°C/600 MPa in the presence of 16 mg/l nisin or 6.4 mg/l reutericyclin for 0-60 min in a 0.9% saline solution. The nisin potentiated inactivation rates. The authors concluded that the use of nisin in combination with high pressure and thermal treatments enhanced inactivation of the endospores of *Clostridium* sp. and may have application in foods. Roberts and Hoover (2000) evaluated the effect of combinations of 400 MPa pressure, heat, exposure duration, acidity and nisin against B. coagulans spores. The sub lethal injury of spores by pressurization in combination with heat and acidity caused *B. coagulans* spores to become more sensitive to nisin. In conclusion, acidic foods could be protected from spore outgrowth by the combined treatment.

Lopez Pedemonte et al. (2003) have studied the combined effects of nisin or lysozyme with pressure to inactivate *B. cereus* in cheese, being observed that the compounds increased the sensitivity of spores to pressure. In a study by Kalchayanand, Dunne, Sikes and Ray (2003), nisin, pediocin, and treatment at 345 MPa for 5 min at 60°C were combined to inactivate spores of *C. laramie* or a mixture of spores from 4 clostridial species including *C. sporogenes, C. perfringens, C. tertium, and C. laramie*, inoculated into roast beef. Following HP treatment alone, samples inoculated with a mixture of clostridial spores could be stored for 42 d at 4°C without spoilage; the use of HPP in combination with either pediocin or nisin extended the shelf-life of the beef to 84 d at 4°C.

Conclusion and future perspectives

In contrast to conventional thermal food processing, appropriate HPP application can promote safety and extend shelf life, maintaining the freshness, sensorial and nutritive value of processed food products. Despite the innumerous benefits and potential application of HPP as a non-thermal conservation process, conventional pressure loads adopted for microbiological purposes in food processing (up to 1000MPa) may cause slight but noticeable/significant alterations in quality attributes, and mainly color, texture characteristics and the sensory profile. These noticeable quality-degradation events may harm products acceptance by the consumer market. Barotolerant spore-forming bacterial strains and sublethal injury on vegetative form represent major HPP industrial challenges. Feasible commercial sterilization using HPP technology is almost impossible without the additional hurdle effect. Consequently, studies should focus efforts on mapping and optimizing HPP processes for each specific category of food product and target pathogen, ensuring safety without quality loss. Hurdle technology represents the most promising modern food safety trend. Natural antimicrobials may act as additional hurdle or synergistically by permeabilization and sensibilization events against spoilage and pathogenic microbial cells, potentiating the bactericidal effects. Thus, mild HPP treatments may be safe, and low doses of additional antimicrobials are required for effectiveness. Optimized hurdle effects with HPP performance can reduce the initial and maintenance costs, time and

working pressure loads. It can be concluded that natural antimicrobials and biopreservation concepts may become important hurdles in minimally processed, safe and high-quality HP-processed foods.

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Capítulo 2 – testes preliminares:

Achieving current Brazilian low-sodium reformulation goals for RTE turkey breast and high pressure processing at 600MPa as complementary preservation technology

RESUMO

No que concerne o problema emergente da elevada ingestão de sódio, agências governamentais de saúde em todo o mundo estão se movendo para traçar planos de ação de redução por meio de novas regulamentações e pactos formais com as indústrias de processamento de alimentos. Seguindo esta tendência, na etapa inicial deste experimento, objetivou-se determinar o maior nível de redução de cloreto de sódio possível, sem a adição de sal substituto (simples redução), aplicando reduções de 20, 30 e 40% p/p sobre o controle formulado com 20g/kg de NaCl, por meio de avaliacões microbiológicas, físico-químicas e sensoriais de embutido de peito de peru fatiado embalado a vácuo durante 60 dias de estocagem refrigerada (4°C). Formulações com níveis de NaCl reduzidos em 40% foram descaracterizadas (p<0.05) em termos dos parâmetros físico-químcos de textura, cor, pH e atividade de água; além disso, foram observados escores de aceitação siginificativamente reduzidos nos atributos de aparência, sabor e aceitação global para estes níveis de redução. Após avaliação dos resultados obtidos na etapa 1, a formulação reduzida em 30% de sal adicionado (14 g/kg de NaCl) foi selecionada como o melhor formulação viável reduzida de sódio (28% menos Na^+ comparada ao controle); no entanto, a estabilidade microbiológica durante o armazenamento refrigerado desta formulação foi comprometida. Um comportamento diferente no crescimento (p<0.05) dos grupos-alvo de bactérias deteriorantes para produtos cárneos embalados a vácuo (principalmente bactérias ácido lácticas, e psicrotróficos) foi observado. Na etapa 2 do experimento, a estratégia de processamento a alta pressão (HPP) a 600MPa/180s em baixas temperaturas de 25°C (amplamente aplicado para fins microbiológicos em produtos cárneos cozidos fatiados embalados a vácuo) foi proposto como tecnologia de conservação complementar para superação das questões microbiológicas evidenciadas na etapa 1 da proposta reformulação. Os testes sensoriais “cegos” (*blind tests*) confirmaram o sucesso da ferramenta HPP como alternativa de conservação pós-processamento, sendo observado que os participantes do painel (consumidores frequentes do produto) não foram capazes de identificar amostras *low-sodium* tratadas com HPP de amostras não-tratadas do embutido de peito de peru fatiado (p<0.05). Os assessores apontaram textura

como o atributo mais relevante em sua decisão. A reformulação proposta alcança as metas atuais brasileiras pactuadas para esta categoria de produto cárneo pelas indústrias de processamento de alimentos e o governo; a ferramenta de conservação HPP representa uma alternativa viável para garantia de segurança e conservação em matrizes reduzidas de sal, não interferindo nas características sensoriais do produto processado.

Palavras-chave: reduzido teor de sódio; bactérias ácido láticas; psicrotróficos; Escala do Ideal, risco de fatiamento.

ABSTRACT

Concerning to emerging problem of human high sodium intake, governmental health agencies around the world are moving on to trace reduction action plans by newly regulations and formal pacts with food processing industries. Following this trend, in initial experiment step, this study was aimed determine the optimum level of sodium chloride reduction without salt-replacer addition, applying reductions of 20, 30 and 40% w/w, against the control formulated with 20g/kg of NaCl, by evaluation of microbiological, physico-chemical and sensory evaluation traits of sliced vacuum-packaged RTE turkey breast during 60 days of refrigerated (4°C) shelf-life. Product formulations with reduced salt content in levels of 40% were mischaracterized (p<0.05) in terms of quality-attributes mainly in texture, color, pH and water activity; additionally, significant reduced acceptance scores in attributes of appearance, taste and overall acceptability were observed at this reduction level. After data results (quality and sensorial) evaluation of step 1, formulation reduced in 30% of added salt (14g/kg NaCl) was selected as the best *low-sodium* formulation (28% less Na^+ than control); nevertheless, microbiological stability during refrigerated storage of this formulation was proved to be prejudiced. Different behavior in growth (p<0.05) of major target spoilage bacterial groups found in refrigerated storage of vacuum-packaged meat products (lactic acid bacteria - LAB, and psychrotrophic) were observed. In step 2 of experiment, high hydrostatic pressure treatment (HPP) by 600Mpa/180sec at low processing temperatures of 25°C (largely applied to meeting microbiological purposes in sliced cooked vacuum-packaged meat products) was proposed as complementary technology preservation to overcome these addressed microbiological barriers in salt reformulation. Blind sensorial tests supported the success of the HPP as post-processing alternative being observed that the panelists (regular product consumers) were not able to differentiate HHP treated of non-treated low-sodium sliced turkey breast ham (p<0.05).The panelists pointed texture as the attribute most relevant in their decision.The proposed reformulation achieves the Brazilian current goals established for this meat product category accorded by government and food processing industries. HPP conservation tool is a viable alternative to

safety and shelf-life maintenance in low-salt matrices, not interfering within sensory characteristics of the final processed product.

Keywords: low-sodium; lactic acid bacteria; psychrotrophic; *Just-About-Right* scale; *Slice-Safety-Risk*.

1 Introduction

High salt and sodium human intake with consequent blood pressure increase and cardiovascular diseases development represents one of the more impacting public health problems of modern society. Actually, world average salt intake exceeds 12g/day (>4700mg sodium), and following the recommendations of the World Health Organization, this level of intake should be reduced to less than 5g/day restricting sodium intake to less than 2000mg/dia (WHO, 2011). It has been estimated that approximately 75% of the sodium eaten is added during industrial manufacturing (Brown et al., 2009). Processed meats are considered one of the largest contributors for human daily sodium intake, followed by bakery, dairy and sauces categories; thereby reduce NaCl content in these processed foods category represents a great industrial challenge (Doyle & Glass, 2010). Yet, political and consumer awareness is increasing. Governmental health agencies around the world are moving on to trace reduction action plans by newly regulations and formal pacts with food processing industries. European Union and the United States have given increased attention to salt consumption, and both voluntary and regulatory initiatives have been undertaken to reduce the use of salt. The EU has set an annual reduction target of 4% for a 4-year period (Aaslyng et al., 2014). In Brazil, the Sanitary Surveillance National Agency (ANVISA) entered into agreement with food processors aiming voluntary sodium reduction plans for several categories (ANVISA, 2011). In the last year (ANVISA, 2014) published a goal plan that established annual reductions patterns according product category, which processed meats are comprised with reduction goals ranging from 16 to 59mg/100g until 2017 depending on their product category. The expectation with the agreement is to remove more than 20 tons of sodium in all processed food products that had the goals established for 2020.

However, despite the emerging requests to reduce this ingredient in processed food formulations, NaCl reduction in meat products is a complex challenge far from be solved. Salt is the most common and cheap ingredient used in meat processing and it has many functions besides flavoring; jointly with phosphates, they are responsible for miofibrillar protein extraction and activation, texture properties development and water biding properties; also, it works on

microbiological stability and safety during retail and shelf life (Ruusunem & Puolanne, 2005; Desmond, 2006). As a feasible solution for sodium reformulation, the food processing industry has been tested non-sodium based replacer chloride salts (mainly KCl and others such as MgCl and $CaCl_2$), physical salt state alteration, i.e. powder micronization, flavor enhancers among others (Horita et al., 2011; Campagnol et al., 2012; Galvão et al., 2014); nevertheless these alternatives still remain blinded mainly to adverse flavor effects and regulatory aspects. The immediate practicable reduction strategy seems to be NaCl simple reduction. Aiming achieve current ANVISA goals for low-sodium reformulation, major Brazilian meat processing market players have been adopting urgent strategies of simple salt reduction (without salt-replacer) around of 20-30%,sometimes combining with approved flavor enhancers (eg. PuraQArome PURAC®, IMP GMP and nucleotides CJ®); however, slicing properties and the microbiological stability problem, identified as shortened shelf-life and product recall intensification, begun to be already evident.

Considering low-sodium reformulation action for meat products, several points needs to be taking into account: firstly, they are a favorable food matrix for bacterial growth (pH, a_w, nutritional composition and growth factors), and the reduction of barrier of salt may represents a dangerous challenge; secondly, in nowadays cooked meat products, recognized as RTE (*Ready-to-Eat*), are extensively commercialized as a sliced form and post-thermal treatment manipulation results in enhanced risk *Slice Safety Risk* (Sofos & Geonaras, 2010). The salt barrier reduction in RTE may represent a great safety concern in low-sodium reformulation purposes. The core control target pathogen for RTE meat products is *Listeria monocytogenes,* an emergent psicrotolerant pathogen responsible for a foodborne disease with high-lethality rate called listeriosis; lactic acid bacteria and psychrotrophic groups represents main spoilage groups for this food category. As sodium chloride acts as preservation agent, their reduction may result in loss of safety and quality (Pal et al., 2008; Nychas et al., 2008; Fulladosa et al., 2012; Sullivan et al., 2012; Myers et al., 2013). High Pressure food processing is non-thermal post-processing preservation alternative widely applied for RTE sliced vacuum-packaged meat products. HPP consists of subjecting food

to intense pressure loads up to 1000MPa, using a compressible pressure-transmitting fluid; these intense pressure loads are applied aiming to eliminate pathogenic and spoilage microorganisms (Campos et al., 2003; Rendueles et al., 2011; Simonin et al., 2012). It has been established that HPP set up for meeting these microbiological purposes in meat products, uses pressures around 600MPa in the range time of 180-300 seconds at room temperatures (25-30°C) (Bajovic, Bolumar & Heinz, 2012). However, processing conditions needs to be adjusted and optimized for each product category, and low salt reformulation strongly affects HHP efficacy in terms of quality and safety. In addition, the impact of HHP in low-sodium formulations needs to make clear.

This study aimed to evaluate the effects of a simple sodium chloride reduction (without salt-replacer added) by the 20, 30 and 40% w/w against a control formulated with 20g/kg on physico-chemical, microbiological and sensory properties of sliced vacuum-packaged ready-to-eat turkey breast during 60 days refrigerated (4°C) storage. Afterwards, the effects of suitable HPP treatment at 600MPa/180sec/25°C on sensorial characteristics of low-sodium formulation proposed was studied.

2 Material and methods

2.1 Ready-to-eat turkey breast formulations

Batches (3kg) of RTE turkey breast was formulated with different concentrations of sodium chloride as follows: $\mathbf{F_c}$: 20g/kg – control; $\mathbf{F_1}$: 16g/kg - 20% reduction; $\mathbf{F_2}$: 14g/kg -30% reduction; $\mathbf{F_3}$:12g/kg - 40% reduction. These concentrations were selected aiming the Brazilian health government agency (ANVISA) goals for sodium in product categories. The formulation were achieved as follows (g/kg): ground skinless turkey breast 700.00, water 235.45, NaCl (12.00 to 20.00), cassava starch 20.00, soy protein (isolate) 10.00, sugar 5.00, spice/seasonings 0.30, phosphates 3.50, carrageenan 3.00, monosodium glutamate – MSG 2.00, sodium erythorbate 0.50, sodium nitrite ($NaNO_2$) 0.15 and carmine coloring 0.10. All additives were kindly provided by IBRAC® (Brazil). Frozen vacuum-packaged turkey breast meat (*Pectoralis major* and *Pectoralis minor*, 70±5% moisture, 3±1% fat, 20±2% protein, pH 5.9±0.2) was obtained within

72h of slaughtering from BRF® foods. After thawing, turkey breast skins were removed, and the raw material was subjected to a grinding process with 90% in a grinding disc of 35mm and the remaining 10% disk of 4mm. Then, all the ingredients/additives excepting the starch (cassava starch) and soy protein isolate, were added to 60% of the total water used and homogenized for preparation of brine (up to the formation of a single phase).The brine was added to the ground turkey breast meat and mixed for 20 minutes in an industrial blender (Jamar®, Brazil). Subsequently, the remaining ingredients and water were added with a further 10 minutes of mixing. The product was stuffed in plastic polyamide casings Visflex (Viskase®, Brazil) with a 76 mm diameter and subjected to the cooking process in a chamber with appropriate staggered internal temperatures reaching 74°C. Cooking procedure was proggramed as follows: initially 60°C/30min, by increasing 5°C/30min until chamber temperatre reach 80°C and core 74°C, measured by a thermocouple inserted into the core of the product. The cooked turkey breast was cooled in a water bath for 10min and stored in a controlled chamber at 4±1°C for further procedures. Basal formulation and processing follow recommendations of Galvão et al. (2014).

After 24hs of refrigeration period, the RTE turkey breast pieces were aseptically opened and sliced in a 4mm and 10mm (texture profile evaluation) thickness wide, being subsequently vacuum-packaged in 150x300mm Nylon-Poly 16μ (COEX:LDPE-PA-LDPE) with a permeability rate of PRO_2 of 50$cm^3 \cdot (m^2 \cdot day)^{-1}$. The analytical samples were stored under refrigeration (4±1°C), protected from light, without display, for 60 days, and analyzed at 0 (24hs post product slicing), 15, 30, 45 and 60 days for microbiological, physico-chemical and sensorial characteristics.

2.2 Experiment 1: reformulation studies to select optimum salt reduction level

2.2.1 Physico-chemical analysis

The sliced vacuum-packaged RTE turkey breast formulations (F_c, F_1, F_2 and F_3) were submitted to physico-chemical analysis including proximate composition and sodium content, pH, water activity, rheological behavior with Texture Profile

Analysis –TPA, lipid oxidation and objective CIELAB color indexes (*lightness, redness and yellowness*).

The moisture, protein, fat and ash contents were determined according to the Association of Official Analytical Chemists (AOAC, 2007). The sodium content was determined in triplicate for each formulation as described by AOAC (2007).

The water activity (a_w) was determined directly using an AquaLab water activity meter (Dacagon Devices, Inc., model 4TE, USA); cominuted samples were puted into capsules and the value was determined at 25±0.1°C. The pH measurements were obtained after initial dilution and homogenization of samples at a ratio of 1:10 (10g of sample in 100ml of distilled water), followed by the introduction of meter electrodes in homogenized slurries for pH readings.

Color measurements were taken with a colorimeter (Chroma meters CR300, Konica Minolta Sensing, Inc.) established at a 10° angle for the observer and illuminated at D65 to calculate color indices in the CIELAB system following the recommendations of Ramos and Gomide (2007). The color parameters lightness (*L**), redness (*a*)* and yellowness (*b**) were obtained from an average of 5 readings taken at different points in 3 turkey breast ham slices.

Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBAR) index according to Raharjo et al. (1992). Ten-gram portions of turkey breast ham samples were combined with 40ml of 5% trichloroacetic acid (TCA) and 1ml of 0.15% antioxidant BHT (2,6-di-tert-butyl-4-methylphenol (Sigma Aldrich) and refrigerated homogenized in ultrarrax for 5 min. Next, the homogenates were centrifuged (3000g for 5 min), and the supernatant was filtered through Whatman No. 1 filter paper. Two ml of filtrate was combined with 2ml of 0.08mol/L TBA reagent and heated in boiling water (100°C) for 5 min. The absorbance of the resulting solution was measured at 532nm, and the TBAR (thiobarbituric acid reactive substances) values were expressed as mg of malondialdehyde (MDA) per kg sample, calculated using 1,1,3,3-tetraethoxypropane (TEP) as the standard.

Texture was evaluated by TPA - Texture Profile Analysis, according to the recommendations of Bourne (1978), using a *TA.XT2i* Texturometer (Texture Analyzer, Stable Micro Systems, Inc., England) coupled to a microcomputer

equipped with Texture Expert Software. The TPA test consists of compressing the sample two times in cycles in a reciprocating motion that simulates the jaw action, and extract, from the resulting force-time curve, a number of different textural parameters. Ten standardized squared cubes (10x10mm) of 3 independent turkey breast ham slices of 10mm thickness were cut for the TPA tests. Next, the slices were compressed twice to 50% of their original height; the measurements were taken after the samples reached room temperature (±25°C). The deformation curve (force-time) was obtained with a compression velocity of 180mm/min, using a P-20 probe (20mm diameter). According to Bourne (2002), the texture parameters were determined from the force curves as follows: hardness (N) is the height of the force peak on the first compression cycle; cohesiveness is the ratio of positive areas under the first and second compressions (A1/A2); adhesiveness (N·mm) is the negative force area (called A3) for the first bite representing the work necessary to pull the compressing plunger away from the sample; springiness (mm) is defined as the distance that the sample recovers between the end of the first bite and the start of the second bite; chewiness (N·mm) is the energy required to masticate a solid food (product of hardness x cohesiveness x springiness).

2.2.2 Microbiological evaluation

Populations of target microbial groups including lactic acid bacteria (LAB), aerobic mesophilic, psychrotrophic and *Enterobacteriaceae* were monitored in sliced vacuum-packaged RTE turkey breast at 0 (24hs post-slicing), 15, 30, 45 and 60 days of refrigerated storage (4°C). 25g (slices 4mm) of RTE turkey breast were weighed and transferred into sterile stomacher bags (Baglight®), combined with 225mL of sterile peptone water 0.1% (w/v) and homogenized in a Stomacher (Metroterm®, Brazil) with 490 strokes/2min at room temperature. Stomached slurries were decimally serially diluted into peptone water and plated under the following conditions: psychrotrophic bacteria were determined in PCA - Plate Count Ágar at 7°C/10 days incubation; lactic acid bacteria in MRS – de Man Rogosa and Sharpe Agar at 32°C/48hs; *Enterobacteriaceae* in VRGB – Violet Red Bile Glucose Agar at 35°C/48hs and aerobic mesophilic bacteria in PCA at 37°C/48hs (Silva et al., 2007).

Aiming to evaluate microbial quality of different formulations of processed product, selected microbial populations, following the Brazilian microbiological requiriments (BRASIL, 2001), were monitored during 60 days (0 and 60 days) of the refrigerated shelf-life; this includes the following microbial groups enumeration and culture media/methods: total (35°C) and thermo tolerant (44,5°C) coliforms using Lauril Sulphate Tripotose broth (LST), Brilliant Green broth (VB) and *Escherichia coli* broth (EC); *Salmonella* sp., with pre-enrichment step in buffered peptone water, selective enrichment step in Tethrationate and Rapapport broth and growth in RAMBACH® Differential Selective Agar; sulphide reducing Clostridia in SPS agar under anaerobic atmosphere (ANAEROBAC® atmosphere generator); and *Staphylococcus* sp. in Baird Parker Agar base supplemented with egg yolk emulsion and added of potassium telluride solution (Silva et al., 2007).

2.2.3 Consumer behavior towards salt and sodium reduction

2.2.3.1 Acceptance test

Consumer acceptance test was carried out aiming to obtain the maximum reduction of added salt that not induced in rejection of reformulated product. The tests were conducted in appropriate individual booths (standardized), under artificial daylight-type illumination and with temperature control (between 22 and 24°C) and air circulation following recommendations of Meilgaard et al. (2007). Sliced samples (4mm) were placed in plates coded with random 3-digit numbers and monadic presented to consumers. The first order and carry over effects were prevented by balanced using a specific block design (MacFie et al., 1989). The consumers (n=60) were instructed to evaluate with respect to the degree of liking for appearance, aroma, taste, texture, and overall liking using a 9-point hybrid hedonic scale (9 - liked extremely; 8 - liked very much; 7 - liked moderately; 6-liked slightly; 5 - neither liked nor disliked; 4 - disliked slightly; 3 - disliked moderately; 2 - disliked very much; 1 - disliked extremely). Between tasting each sample, the participants were requested to eat a cream cracker biscuit and drink some spring water. The data were collected in specific sensorial sheet scores. Acceptance test was carried out after product processing and slicing with 24hs refrigeration period (total 72hs post-manufacture).

2.2.3.2 Penalty analysis and JAR scales for salty level (Just-About-Right)

Just-about-right (JAR) scales have been one of the first and simplest consumer-based approaches to get information about the optimum intensity of sensory attributes. In our study JAR scales were applied for evaluation of salt level reductions from 20 to 40% on saltiness and salty taste of reformulated sliced vacuum-packaged RTE turkey breast. For this purpose an ideal scale was applied (mutually with acceptance test), being panelists instructed to evaluate product with respect to the degree of salty taste, using a 9-point scale with verbally terms of extreme and central as follows: 1- extremely less salty than optimum, 5- optimum salty level, and 9 – extremely over salty than optimum (Morais et al., 2014; Ares et al., 2014). JAR scores were evaluated according to the penalty analysis, being considered significant when more than 20% consumers evaluated the sample above or below the Just Right (Drake et al., 2011; Narayanan et al., 2014).

2.3 Experiment 2: High pressure processing for selected low-sodium formulation

2.3.1 High Pressure Processing

According to results obtained at experiment step 1, the maximum of sodium chloride reduction level accepted, considering sensorial and physico-chemical aspects, was 30% of reduction (14g/kg of NaCl). This formulation showed sodium content 28% less than control formulation with 20g/kg NaCl. Microbiological aspects were significantly impacted in this formulation. Thus, an HHP treatment at 600MPa/180sec at room temperature (established preliminary as a treatment useful for sliced meat products to meet microbiological and preservation purposes) was applied. The low-sodium (30% NaCl simple reduction) sliced vacuum-packaged formulation were submitted to high pressure processing using the high hydrostatic pressure unit AVURE QFP 2L-700 (Avure Technologies® USA) with a 2L volume treatment chamber (inner vessel diameter 100x254mm), maximum vessel pressure of 690MPa (6900bar/100.000psi) and temperature control at 10 to 90°C. Pure demineralized water was used as a pressure-transmitting fluid. Two thermocouples located at the top and midway in the treatment chamber monitored the temperature of the pressure-transmitting fluid; another thermocouple monitored

the temperature of the water jacket surrounding the pressure vessel. Considering the adiabatic heating that occurred during the pressurization (approx. 3°C/100MPa for water), the initial sample and water (pressure-transmitting fluid) temperatures were controlled to reach 25±1°C during processing. After HPP the treated samples were immediately refrigerated for further sensorial analysis.

2.3.2 Blind discriminative sensory test (HHP x Non-HHP)

Aiming to evaluate the effect of HHP 600MPa/180sec/25°C on product acceptance, a triangle discriminative test was applied (n=37). This blind test is commonly used in determining whether shifts in processing or ingredients have significantly changed a product. The six possible order combinations were randomized across panelists. For samples A (HHP) and B (non-HHP), the six possible order combinations are: AAB, ABA, BAA, BBA, BAB, and ABB. Panelists received low-sodium formulated samples (30% NaCl reduction) HHP treated or non-treated being inquired to select the different sample. In addition to taste samples, the panelists were instructed to observed samples in packages and relate with their words the apparent differences among samples. Also, they were inquired what attribute most influenced the decision to select the different sample.

2.4 Experimental design and statistical analysis

The data were obtained from two independent experiments (batches/processing) and the means from replicate analyses results. The data obtained were subjected to normality tests (Shapiro-Wilk test) consequently to analysis of variance (ANOVA), and the comparison between means was determined by Scott–Knott test adopting a 5% significance level. The statistical analyses of data were carried out using statistical R software (2010). In addition, XLSTAT® software was used in penalty analysis and JAR test data evaluation.

3. Results and Discussion

3.1 Optimum salt and sodium reduction level

Mean values obtained in proximate composition determination of different evaluated formulations of RTE turkey breast are shown in Table 1.

Table 1. Proximate composition and sodium content of different evaluated RTE turkey breast formulations.

Formulations/ Sodium chloride levels	g/100g of RTE turkey breast				mg/100g
	Moisture	Protein	Fat (lipids)	Ash	Sodium
F_c - Control formulation	75.70(±0.14)[a]	21.10(±0.90)[a]	0.64(±0.04)[a]	2.91(±0.05)[a]	1005.12(±7.30)[a]
F_1 – 20% of NaCl reduction	75.56(±0.06)[a]	20.04(±0.55)[a]	0.76(±0.08)[a]	2.49(±0.07)[b]	868.93(±2.94)[b]
F_2 - 30% of NaCl reduction	75.21(±0.15)[b]	21.70(±0.13)[a]	0.68(±0.04)[a]	2.20(±0.04)[c]	722.63(±4.26)[c]
F_3 – 40% of NaCl reduction	75.71(±0.05)[a]	21.14(±0.59)[a]	0.59(±0.03)[a]	2.17(±0.03)[c]	713.33(±5.58)[c]

F_c (20g/kg of NaCl); **F_1** (16g/kg of NaCl); **F_2** (14g/kg of NaCl); **F_3** (12g/kg of NaCl). Mean values ± S.E (Standard Error).Values followed by the different letter within the same column, are significantly different according to Scott-Knott test ($p \leq 0.05$).

Besides slightly variations in moisture and ash content, the salt reduction reformulation strategy did not affect ($p>0.05$) protein and fat levels. The obtained proximate composition in terms of moisture and protein are in agreement with the results observed by Galvão et al. (2014) and Myers et al. (2013) that evaluated proximate composition of similar RTE turkey meat products. Evaluating sodium content, the proposed reformulation was effective in reducing sodium amounts ($p<0.05$). In control treatment, formulated with entire salt (20g/kg) the amount of sodium reached 1005mg/100g of sodium. Galvão et al. (2014) found similar sodium levels of 1048 mg/100g in their turkey ham product formulated with 20g/kg of NaCl. In formulations with 20, 30 and 40% w/w of NaCl reduction, sodium levels were reduced in 13.54% (868.93mg/100g), 28.10% (722.63mg/100g) and 29.05% (713.33mg/100g) respectively. The Brazilian goals to meet the agreement established between government ANVISA and ABIA (Brazilian Association of Food Industries) has been set on 37.5mg until 2017 (ANVISA, 2014) for this meat product category; thus, the selected working low-sodium formulation (30% of NaCl reduction, 14g/kg) showed 28% less sodium compared to control, being reached the future needs. In addition, following legislation for nutritional complementary regulation (INC) that means the declaration of particularly nutritional properties in

labels (RDC n°54, BRASIL, 2012), sodium reduction levels higher than 25% support label claims of “low-sodium” or “reduced levels of sodium” content

Considering consumer awareness about sodium is increasing, this may represents retail competitive advantages.

For meat products, color and color stability are some of the most important quality features for the consumer purchasing decision; hence, color stability during storage and retail display is important to the meat processing industries and the retailers. For cured cooked sliced meat products category, color stability has been tested due to enhanced fading phenomena conditions including oxidizing agents such as light, atmosphere and pH falling event (Fainer, 2006). Table 2 shows CIELAB color indexes of lightness (L^*), redness (a^*) and yellowness (b^*). Reducing salt in RTE turkey breast formulations resulted in increased lightness values ($p<0.05$) mainly in formulations above 30% of salt reduction. From 15 day of refrigerated storage L^* mean values in F_2 and F_3 formulation become higher, being observed an effect of time storage, mainly in formulations were salt reduction was higher. This represents an evident colour fading phenomena installation that was configured intense in low-salt added formulations. Exposure to light in combination with residual oxygen is critical to the color stability of cooked, cured meat products, since may cause photooxidation of nitrosylmyochromogen (hemochrome) to a gray–brown ferric myoglobin species (Moller et al., 2000; Moller et al., 2003). The proposed salt reduction strategies in all tested levels did not affect redness (a^*) color index that remained relatively stable during entire storage time. Yellowness indexes were significantly affected by salt reduction being observed higher b^* values in low-salt added formulations. Our results were consistent with Pietrasik and Gaudette (2014) that evaluated the effect of salt reduction (control - 2% salt; LS: low-salt 1.2% salt) on color of cooked cured ham; these authors related that ham treatments containing reduced amounts of salt (LS) were lighter and more yellow compared to hams containing 2% of salt. Reducing salt levels higher than 30% against control formulated with 20g/kg showed significantly changes in characteristic color of the sliced vacuum-packaged RTE turkey breast product; this was evidenced through higher lightness indexes with discoloration events affected by the time of refrigerated storage (fading). Added to this data, increased

yellowness values were observed in these low salt formulations. Correlating with sensorial data, the formulation F_3, with 12g/kg of NaCl (40% reduction), was the only treatment that showed reduced sensorial scores for appearance attribute (p<0.05) among other evaluated.

Table 3 shows the results for lipid oxidation indexes (TBARs), water activity and pH. Lipid oxidation rates during refrigerated storage were measured as thiobarbituric acid reactive substances (TBARs) indexes. As shown, the salt reduction levels up to 40% did not influenced (p>0.05) lipid oxidation until day 45 of refrigerated storage among formulations. However, evaluating the TBARs evolution along shelf-life period, a significant effect of time may be observed with lipid oxidation indexes increasing from 45 days of storage for all tested formulations. At the end of shelf-life, higher TBARs scores were registered for all evaluated formulations. Lipid oxidation represents one of the major causes of the progressive deterioration in the quality of meat products, limiting their storage and shelf-life. The deterioration in organoleptic characteristics, off-odors and off-flavors formation and the associated loss of nutritional value by the oxidative process are the main associated problems. Generally, it is well assumed that salt accelerate lipid oxidation in meat products (Romans, Costello, Carlson, Greaser, & Jones, 2000). The lipid oxidation behavior (monitored as TBARs) for raw turkey meat (including breasts, tights, grounded and mechanically deboned turkey meat) and processed turkey meats were extensively documented in available literature (Mielnik, et al., 2003; Govaris et al., 2007; Wang et al., 20120; Sikler et al., 2013; Contini et al., 2014); however, similar formulations of studied turkey meat product (RTE turkey breast) were not found for further precise comparisons. Karpinska-Tymoszczyk (2014) found TBARs levels ranging from 0.46 to 0.74 mg MDA/kg in cooked turkey meatballs (80% raw meat and 2% NaCl) during 90 days of storage, being identified a significant effect of time storage, as observed in our studies. Regardless of higher TBARs levels showed by the research (our results show maximum average value of 0.34 mg MDA/kg), the turkey meat product meatballs studied did not contain in their formulation, sodium nitrite and sodium erythorbate, recognized antioxidants in cooked cured meat products.

Table 2. Average values of objective color indexes in CIELAB system (*L*, a*, b**) of vacuum-packaged sliced RTE turkey breast stored under refrigeration (4°C) for 60 days.

Formulations / Sodium Chloride levels	*L* (Lightness)*				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c - Control formulation	66.17(±1.21)^{Ba}	66.09(±1.60)^{Ba}	65.98(±0.70)^{Ba}	69.70(±0.33)^{Ba}	67.35(±1.04)^{Ba}
F_1 (20% of NaCl reduction)	67.74(±0.74)^{Bb}	67.96(±1.06)^{Bb}	66.25(±1.39)^{Bb}	70.67(±0.33)^{Ba}	66.73(±1.18)^{Bb}
F_2 (30% of NaCl reduction)	73.58(±0.45)^{Aa}	73.92 (±0.16)^{Aa}	73.53(±0.58)^{Aa}	73.92(±0.06)^{Aa}	73.45(±0.20)^{Aa}
F_3 (40% of NaCl reduction)	69.67(±0.67)^{Bb}	69.40(±1.48)^{Bb}	70.27(±0.78)^{Ab}	72.88(±0.36)^{Aa}	70.51(±0.55)^{Ab}
	a* (Redness)				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c- Control formulation	4.40(±0.17)^{Aa}	3.98(±0.12)^{Bb}	4.50(±0.16)^{Aa}	4.13(±0.06)^{Bb}	4.18(±0.09)^{Bb}
F_1 (20% of NaCl reduction)	4.87(±0.07)^{Aa}	4.49(±0.13)^{Aa}	4.62(±0.12)^{Aa}	4.66(±0.07)^{Aa}	4.64(±0.09)^{Aa}
F_2 (30% of NaCl reduction)	4.63(±0.09) ^{Aa}	3.83(±0.03)^{Bb}	4.01(±0.13)^{Bb}	4.64(±0.01)^{Ab}	4.49(±0.06)^{Ab}
F_3 (40% of NaCl reduction)	4.60(±0.08)^{Aa}	4.51(±0.08)^{Aa}	4.68(±0.10)^{Aa}	4.75(±0.08)^{Aa}	4.78(±0.12)^{Aa}
	b* (Yellowness)				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c -Control formulation	7.28(±0.25)^{Ca}	7.84(±0.30)^{Ba}	7.46(±0.27)^{Ca}	7.12(±0.15)^{Ca}	7.18(±0.19)^{Ca}
F_1 (20% of NaCl reduction)	8.27(±0.12)^{Ba}	8.41(±0.26)^{Ba}	8.23(±0.18)^{Ba}	8.28(±0.13)^{Ba}	7.59(±0.13)^{Cb}
F_2 (30% of NaCl reduction)	9.61(±0.14)^{Aa}	9.51(±0.11)^{Aa}	9.60(±0.10)^{Aa}	9.61(±0.14)^{Aa}	9.82(±0.05)^{Aa}
F_3 (40% of NaCl reduction)	8.15(±0.15)^{Ba}	8.47(±0.23)^{Ba}	8.44(±0.23)^{Ba}	8.63(±0.20)^{Ba}	8.25(±0.16)^{Ba}

F_c (20g/kg of NaCl); **F_1** (16g/kg of NaCl); **F_2** (14g/kg of NaCl); **F_3** (12g/kg of NaCl).

Mean scores (5 reading on each slice/3 slices per sampling package) ± S.E (Standard Error).Values followed by the different small letter within the same line, and by the different capital letter within the same column, are significantly different ($p \leq 0.05$) according to Scott–Knott test.

Conditions: illuminant D_{65}/10°.

Table 3. Average values of pH, water activity and TBARs indexes of vacuum-packaged sliced RTE turkey breast stored under refrigeration (4°C) for 60 days.

Formulations / Sodium Chloride levels	*pH*				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c - Control formulation	6.34(±0.00)^{Aa}	6.40 (±0.01)^{Aa}	6.34 (±0.05)^{Aa}	6.10 (±0.07)^{Ab}	5.86(±0.14)^{Ac}
F_1 (20% of NaCl reduction)	6.33 (±0.01)^{Aa}	6.35 (±0.01)^{Ba}	6.22 (±0.06)^{Ba}	6.03 (±0.03)^{Ab}	5.90(±0.22)^{Ab}
F_2 (30% of NaCl reduction)	6.10(±0.01)^{Bb}	6.36 (±0.02)^{Ba}	5.85 (±0.01)^{Bc}	5.77 (±0.06)^{Bc}	5.42 (±0.05)^{Bd}
F_3 (40% of NaCl reduction)	6.29 (±0.01)^{Ba}	6.34 (±0.01)^{Ba}	6.01 (±0.03)^{Bb}	5.71 (±0.16)^{Bc}	5.38(±0.04)^{Bd}
	Water activity - a_w (25±0.1°C)				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c- Control formulation	0.974(±0.0026)^{Ba}	0.975(±0.0013)^{Ba}	0.975(±0.0005)^{Ca}	0.975(±0.0013)^{Ba}	0.976(±0.0005)^{Ba}
F_1 (20% of NaCl reduction)	0.975(±0.0035)^{Ba}	0.976(±0.0010)^{Ba}	0.977(±0.0005)^{Ba}	0.976(±0.0010)^{Ba}	0.976(±0.0008)^{Ba}
F_2 (30% of NaCl reduction)	0.978(±0.0001)^{Ab}	0.981(±0.0001)^{Aa}	0.978(±0.0001)^{Bb}	0.982(±0.0013)^{Aa}	0.977(±0.0021)^{Bb}
F_3 (40% of NaCl reduction)	0.980(±0.0013)^{Aa}	0.979(±0.0015)^{Aa}	0.981(±0.0010)^{Aa}	0.979(±0.0015)^{Aa}	0.983(±0.0042)^{Aa}
	TBARs (mg Malondialdehyde/kg)				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c – Control formulation	0.0044(±0.0034)^{Ab}	0.0092(±0.0025)^{Ab}	0.0420(±0.0031)^{Ab}	0.0550(±0.0414)^{Bb}	0.1988(±0.1244)^{Aa}
F_1 (20% of NaCl reduction)	0.0220(±0.0105)^{Ab}	0.0114(±0.0122)^{Ab}	0.0331(±0.0076)^{Ab}	0.0330(±0.0171)^{Bb}	0.2488(±0.0276)^{Aa}
F_2 (30% of NaCl reduction)	0.0389(±0.0207)^{Ab}	0.0102(±0.0057)^{Ab}	0.0289(±0.0068)^{Ab}	0.1428(±0.0203)^{Aa}	0.0535(±0.0250)^{Bb}
F_3 (40% of NaCl reduction)	0.0040(±0.0034)^{Ab}	0.0215(±0.0162)^{Ab}	0.0439(±0.0116)^{Ab}	0.0203(±0.0130)^{Bb}	0.3431(±0.1314)^{Aa}

F_c (20g/kg of NaCl); **F_1** (16g/kg of NaCl); **F_2** (14g/kg of NaCl); **F_3** (12g/kg of NaCl).
TBAR's - Thiobarbituric Acid Reactive Substances.
Mean scores ± S. E. (Standard Error). Values followed by the different small letter within the same line, and by the different capital letter within the same column, are significantly different ($p \leq 0.05$) according to Scott–Knott test.

The pH falling patterns for different low-sodium formulations during shelf-life of sliced vacuum-packaged RTE turkey breast were shown in Table 3. For this quality-attribute the salt reduction strategy significantly affected pH falling behavior being observed that in formulations with reductions higher than 30%, F_2 and F_3, it showed a rapid pH decay pattern that initiated already from 15 day of refrigerated storage. Mainly from 30 days of storage, in formulations with less sodium (12 and 14g/kg NaCl), it was observed lower average (p<0.05) pH values. The effect of time of storage was also significant with lower pH values next to 5.38 and 5.42 registered for 30 and 40% NaCl reduction at the end of product shelf-life; slightly salt reduction of 20% and in control sample final pH was higher (p<0.05). The more pronounced pH falling event in low-salt added formulation may be correlated to increased growth rates observed for spoilage LAB in these formulations (Table 4) mainly in F_2 and F_3 formulations. The main spoilage microbial group involved in a deterioration of sliced vacuum-pakaged cooked meat products were lactic acid bacteria (LAB); these are responsible for slime production, acidification, gas production, discoloration and sour-sweet odor in packaged samples (Cayré, Vignolo & Garro, 2003). In vacuum-packed cured cooked turkey breast, the LAB genus of *Lactobacillus* sp. and *Leuconostoc* sp. has been found to be the predominant spoilage flora (Samelis et al., 2000). Considering the sugar added to product formulation (5g/kg) it can act as immediately substrate for homo and heterofermentative LAB growth resulting in accumulated acids and pH decreasing.

The salt level reductions at 30 and 40% altered significantly water activity average value, being observed higher aw values during entire storage time for F_2 and F_3. A similar behavior was related by Pietrasik and Gaudette (2014) that observed aw values for control restructured cooked ham of 0.977 formulated with 2% NaCl and significant increased value to 0.981 for formulations with 1.2% (12g/kg). Water activity (aw = p/p_0) is a physico-chemical parameter correlated with dissolved solute content in solution and vapors pressure increase (p); when salt is added to water or aqueous system, water molecules, which are polar, associate with the salt ions (Na^+ and Cl^-) and surround them; as a consequence aw decreases and osmotic pressure increase. Bacterial cells tend to equalize water concentration inside and outside the cell wall by a process of osmosis (solvent flow

through the cell membrane). Generally when microrganisms are placed in solutions which have high osmotic pressure, such as concentrated salt brine, water inside the microbial cell moves out through the membrane causing a partial dehydration of the cell. This slows metabolic processes and interferes with multiplication of the micro-organisms; it explains slowly bacterial growth rates in salt added or preserved meat products. Most micro-organisms cease growth at aw<0.9 and the majority of bacteria that can cause superficial spoilage on meat are very sensitive to slight reductions in aw (Forsythe, 2002).The preservation and shelf life of processed meats is of vital importance when reducing the salt levels. Reducing NaCl levels below those typically used without any other preservative measure has been shown to reduce product shelf life.

The characteristic texture profile of processed meats, as related to both the adhesion of meat particles and water binding (holding) by the meat proteins, relies on extraction of myofibrillar proteins from the meat. In Table 4 it is possible to notice that the parameters of *Hardness*, *Chewiness* and *Cohesiveness* were affected (p<0.05) by salt reduction; *Adhesiveness* and *Springiness/Elasticity* did not differ significantly among evaluated sodium chloride levels (20, 30 and 40% of reduction). Between several significant variations the results evidenced that salt reduction in levels higher than 30%, mainly at 40% salt reductions levels (F_3) resulted in mischaracterized product in terms of texture. In restructured cooked meat products texture depends on structure and integrity of the protein matrix formed during cooking. Salt performs an important ionic strength increasing function in tumbled/massaged meat products by the extraction, activation and hydration of salt soluble proteins subsequent coagulation during cooking. Optimal binding is achieved with solubilized myofibrillar proteins (soluble in high-ionic-strength solutions greater than 0.3 M NaCl), demonstrating that 1.2% (-40% / <2%) salt in does not guarantee that desired characteristics and development of characteristic texture, by increasing the binding strength, promote an increase in *cohesiveness*and*hardness*.

Table 4. Texture Profile analysis (TPA) parameters of vacuum-packaged sliced RTE turkey breast stored under refrigeration (4°C) for 60 days.

Formulations / Sodium Chloride levels	*Hardness (N)*				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c - Control formulation	22.17(±3.11)^a	26.96(±2.53)^a	29.80(±3.12)^a	24.04(±3.59)^a	25.16(±2.64)^a
F_1 (20% of NaCl reduction)	17.31(±2.09)^b	25.02(±4.24)^a	23.05(±4.44)^b	20.34(±2.79)^b	24.30(±3.23)^a
F_2 (30% of NaCl reduction)	20.85(±3,26)^a	17.28(±3,79)^c	22.59(±4,27)^b	22.03(±5,14)^a	21.87(±3,49)^b
F_3 (40% of NaCl reduction)	20.39(±4.84)^a	21.06(±2.41)^b	21.94(±3.61)^b	17.61(±1.82)^b	19.23(±2.75)^c
	Cohesiveness				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c - Control formulation	0.56(±0.04)^a	0.57(±0.05)^a	0.55(±0.04)^a	0.58(±0.04)^a	0.54(±0.05)^a
F_1 (20% of NaCl reduction)	0.51(±0.05)^b	0.51(±0.04)^b	0.48(±0.04)^b	0.52(±0.04)^b	0.50(±0.03)^b
F_2 (30% of NaCl reduction)	0.57(±0.06)^a	0.52(±0.03)^b	0.49(±0.03)^b	0.52(±0.05)^b	0.56(±0.04)^a
F_3 (40% of NaCl reduction)	0.51(±0.04)^b	0.50(±0.04)^b	0.48(±0.06)^b	0.51(±0.04)^b	0.49(±0.02)^b
	Chewiness (N.mm)				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c - Control formulation	54.23(±8.33)^a	77.50(±16.91)^a	78.00(±14.52)^a	62.94(±8.99)^a	59.58(±8.38)^a
F_1 (20% of NaCl reduction)	40.67(±8.94)^b	56.62(±10.52)^b	53.27(±10.86)^b	48.56(±7.89)^c	56.23(±5.73)^a
F_2 (30% of NaCl reduction)	52.24(±8.48)^a	43.52(±8.77)^c	53.01(±7.80)^b	55.24(±15.30)^b	60.14(±10.29)^a
F_3 (40% of NaCl reduction)	47.96(±14.72)^b	44.55(±4.39)^c	51.82 (±10.48)^b	40.78(±5.54)^c	43.29(±5.86)^b

F_c (20g/kg of NaCl); **F_1** (16g/kg of NaCl); **F_2** (14g/kg of NaCl); **F_3** (12g/kg of NaCl).

Depicted only significant parameters of Hardness, Chewiness and Cohesiveness. Adhesiveness and Springiness/Elasticity did not differ significantly (p>0.05) among evaluated sodium chloride levels.

Mean scores ± S.E. (Standard Error).Values followed by the different letter within the same column, are significantly different (p≤0.05) according to Scott–Knott test.

The solubilized proteins assist in binding meat particles together and effectively promote cohesion during thermal processing. This was evidenced in altered textural attributes of *hardness*, *cohesiveness* and *chewiness* (p<0.05). This assists in the formation of the texture required to create a uniformly attractive cured product with desirable slicing characteristics (Desmond, 2006; Horita et al., 2014). Nevertheless, in sensorial acceptance test, the evaluated attribute texture was not significant (p>0.05) with mean scores ranging from 6.79 (for F_3) to 7.02 (for F_C).

Growth of target microbial populations including LAB, psychrotrophic, aerobic mesophilic and *Enterobacteriaceae*, are shown in Table 5. Additonaly, the growth curves behavior of LAB and psychrotrophic were depicted in Figure 1. For LAB, from day 15 of storage significant higher populations were found for low-salt added formulations. At day 30, LAB in formulations F_2 and F_3, reached more than 8 $\log_{10}$ CFU/g, being significantly higher of F_C and F_1 formulation populations; LAB counts next to 8 log cycles were reached in this salt added formulations only after 45 days of refrigerated storage. Lactic acid bacteria (LAB) are naturally found in many vacuum-packaged meat products stored under refrigeration, e.g. ham, causing spoilage and decreasing shelf-life. Low oxygen concentration, high water activity (normally between 0.96 and 0.98) and pH around 6.0 are some of the characteristics that favor the lactic acid bacterial growth (Cayre et al., 2005; Cayre, Vignolo & Garro, 2003). In vacuum-packed cured cooked turkey breast, the LAB genus of *Lactobacillus* sp. and *Leuconostoc* sp. has been found to be the predominant spoilage flora (Samelis et al., 2000). A significant effect of salt reduction was also observed for psychrotrophic bacteria, when problematic spoilage population higher than 7 $\log_{10}$ CFU/g were reached forward in F_1, F_2 and F_3 formulations at 30 days of storage. The main spoilage microbial groups involved in a deterioration of sliced vacuum-pakaged cooked meat products are lactic acid bacteria (LAB) and psychrotrophic (mainly *Pseudomonas*. sp, *Aeromonas* sp., *Weissella* sp., *Shewanella putrefasciens* and *Brochotrix thermosphacta)*; these are responsible for ropy slime production, acidification, gas production, discoloration and sour-sweet odor in packaged samples (Cayré, Vignolo & Garro, 2003).

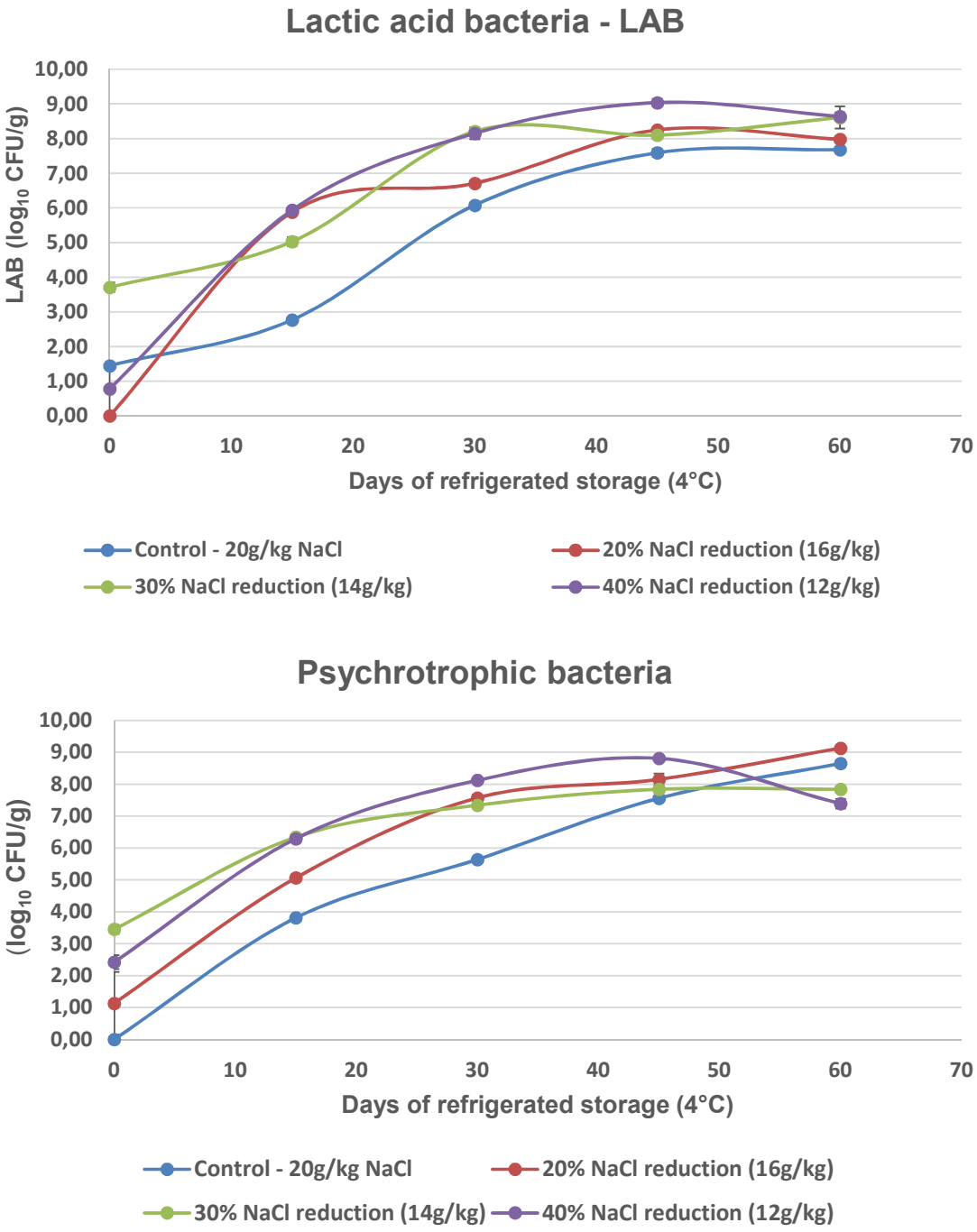

Figure 1. Growth behavior of lactic acid bacteria and psychrotrophic in different formulations of sliced vacuum-packaged RTE turkey breast during storage. Average populations ± S.E. (standard errors) represented by bars.

An interesting event as spoilage indicative thresholds were observed in all low-salt added formulations (mainly F_2 and F_3) that showed intense ropy slime formation and gumminess on turkey breast ham slices before 60 days of

refrigerated storage (4°C). This phenomenon, jointly acidification pH falling, may be attributed to early growth and problematic spoilage populations reached forward. For aerobic mesophilic populations and *Enterobacteriaceae* populations, the effects of the proposed reformulations were less pronounced evaluating these bacterial groups. Concerning salt reduction and microbial stability, Aaslyng et al. (2014) evaluated the growth behavior of LAB and total plate count during 28 days of refrigerated storage for low-sodium (3.1, 2.4 and 1.7%) reformulated cooked ham; these authors identified non-significant alterations for total count, but significant ($p<0.05$) higher LAB populations were observed. Duranton et al. (2012) registered a different growth behavior ($p<0.05$) for brined meat with salt levels of 0, 1.5 and 3% for LAB, aerobic mesophiles and *Enterobacteriaceae*. Table 6 shows the results for microbial growth monitored in Brazilian legal requiriments (BRASIL, 2001) obtained for the different evaluated low-salt formulations. Results confirm lower microbial stability of reformulated formulations against microbial specific target groups mainly for *Staphylococcus* sp. and thermotolerant coliforms.

In fact, salt simple reductions strategies, without non-sodium chloride based replacer salt addition result in more quickly bacterial growth: *Enterobacteriaceae* and aerobic mesophilic, and mainly for spoilage LAB and psychrotrophic problematic bacteria. High pressure processing (HPP) represents a feasible post-processing natural clean-label alternative (against chemical preservatives) aiming to overcome these addressed microbiological safety and quality points of salt reduction in processed meats. Considering available commercial HPP units and pressure loads required for vegetative microbial inactivation at applicable feasible holding times (industrial production outputs), HPP at 600MPa with holding times lower than 600sec were widely applied in sliced RTE vacuum-packed meat products as a post-processing cold pasteurization system (Grebol, 2002; Slongo et al., 2009 Han et al., 2011; Myers et al., 2013; Vercammen et al., 2011). However the impact of this established treatment (600MPa) using this set up, needs to be tested for low-sodium formulations, besides salt functions in processed meat matrices (as we stated in item below).

Table 5. Populations (log_{10} CFU/g) of target microbial groups monitored during refrigerated storage (4°C) for 60 days of low-sodium sliced vacuum-packaged RTE turkey breast.

Formulations / Sodium Chloride levels	***Lactic acid bacteria – LAB (log_{10} CFU/g)***				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c - Control formulation	*ND*(<1.00)^a	2.77 (±0.02)^b	6.08 (±0.01)^b	7.59 (±0.12)^c	7.68(±0.03)^b
F_1 – 20% of NaCl reduction	*ND*(<1.00)^a	5.88 (±0.03)^a	6.71 (±0.01)^b	8.25 (±0.07)^b	7.98(±0.08)^b
F_2 - 30% of NaCl reduction	*ND*(<1.00)^a	5.03(±0.13)^a	8.21 (±0.02)^a	8.10(±0.10)^b	8.61(±0.32)^a
F_3 – 40% of NaClreduction	*ND*(<1.00)^a	5.94 (±0.02)^a	8.15 (±0.17)^a	9.04 (±0.11)^a	8.64(±0.12)^a
	Aerobic Mesophilic Counts (log_{10} CFU/g)				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c - Control formulation	*ND*(<1.00)^b	3.97 (±0.07)^b	4.48 (±0.06)^a	6.94 (±0.04)^b	6.42(±0.18)^d
F_1 – 20% of NaClreduction	*ND*(<1.00)^b	3.94 (±0.13)^b	6.53 (±0.05)^a	6.76 (±0.19)^b	6.87(±0.03)^c
F_2 - 30% of NaCl reduction	3.54(±0.08)^a	5.24(±0.03)^a	5.38(±0.07)^a	7.26(±0.07)^b	7.27(±0.02)^b
F_3 – 40% of NaClreduction	*ND*(<1.00)^b	4.05 (±0.02)^b	5.50 (±0.11)^a	8.58 (±0.05)^a	7.89(±0.02)^a
	Psychrotrophic bacteria (log_{10} CFU/g)				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c - Control formulation	*ND*(<2.00)^b	3.81 (±0.03)^c	5.64 (±0.06)^b	7.56 (±0.03)^b	8.65(±0.11)^a
F_1 – 20% of NaCl reduction	*ND*(<2.00)^b	5.06 (±0.04)^b	7.57 (±0.10)^a	8.15 (±0.18)^b	9.13(±0.05)^a
F_2 - 30% of NaCl reduction	3.45 (±0.14)^a	6.34(±0.02)^a	7.34(±0.05)^a	7.84(±0.10)^b	7.84(±0.07)^b
F_3 – 40% of NaClreduction	2.42 (±0.22)^a	6.29 (±0.03)^a	8.12 (±0.02)^a	8.81 (±0.03)^a	7.39(±0.15)^a
	Enterobacteriaceae (log_{10} CFU/g)				
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
F_c - Control formulation	*ND*(<1.00)^b	1.49 (±0.25)^b	3.57 (±0.06)^a	5.45 (±0.03)^a	5.92(±0.08)^a
F_1 – 20% of NaClreduction	*ND*(<1.00)^b	2.17 (±0.11)^b	3.02 (±0.16)^b	6.01 (±0.13)^a	6.69(±0.12)^a
F_2 - 30% of NaCl reduction	2.59 (±0.05)^a	3.36(±0.02)^a	3.43(±0.05)^a	3.64(±0.20)^a	4.68(±0.22)^b
F_3 – 40% of NaClreduction	*ND*(<1.00)^b	2.26 (±0.12)^b	2.88 (±0.20)^b	6.37 (±0.08)^a	5.08(±0.11)^b

F_c (20g/kg of NaCl); **F_1** (16g/kg of NaCl); **F_2** (14g/kg of NaCl); **F_3** (12g/kg of NaCl). Mean microbial population counts (log_{10} UFC/g) ± S.E. (Standard Error). Values followed by the different letter within the same column, are significantly different ($p \leq 0.05$) according to Scott–Knott test. *ND* – not detected - below the detection limit (<1 or <2 log_{10} CFU/g).

Table 6. Microbiological evaluation according to Brazilian requirements

Treatments	*Staphylococcus* sp. (log_{10} CFU/g)		Legislation standards*
	Day 0	Day 60	
F_c - Control formulation	2.11	5.66	3x10^3 3.48 log_{10} CFU/g
F_1 (20% of NaCl reduction)	2.47	6.37	
F_2 (30% of NaCl reduction)	<2.00	5.80	
F_3 (40% of NaCl reduction)	<2.00	4.95	
Treatments	**Total Coliforms (35,0°C - NMP/g)**		**Legislation standards***
	Day 0	**Day 60**	
F_c - Control formulation	9.20	1.10x10^6	n/a
F_1 (20% of NaCl reduction)	<3.00	1.10x10^6	
F_2 (30% of NaCl reduction)	<3.00	1.10x10^5	
F_3 (40% of NaCl reduction)	<3.00	2.40x10^5	
Treatments	**Thermo stable Coliforms (45,0°C - NMP/g)**		**Legislation standards***
	Day 0	**Day 60**	
F_c - Control formulation	9.20	<3.00	10^3
F_1 (20% of NaCl reduction)	<3.00	4.30x10^4	
F_2 (30% of NaCl reduction)	<3.00	4.60x10^4	
F_3 (40% of NaCl reduction)	<3.00	2.30x10^4	
Treatments	**Sulphide reducing Clostridia - 46,0°C (CFU/g)**		**Legislation standards***
	Day 0	**Day 60**	
F_c - Control formulation	*<10*	*<10*	5x10^2
F_1 (20% of NaCl reduction)	*<10*	*<10*	
F_2 (30% of NaCl reduction)	*<10*	*<10*	
F_3 (40% of NaCl reduction)	*<10*	*<10*	
Treatments	***Salmonella* spp.**		**Legislation standards***
	Day 0	**Day 60**	
F_c - Control formulation	*Absence*	*Absence*	Absence in 25g
F_1 (20% of NaCl reduction)	*Absence*	*Absence*	
F_2 (30% of NaCl reduction)	*Absence*	*Absence*	
F_3 (40% of NaCl reduction)	*Absence*	*Absence*	

*National Health Surveillance Agency – ANVISA/Brazil. Technical Regulation on Microbiological Standards for Food. RDC n°12 de 02 de Janeiro de 2001. **F_c** (20g/kg of NaCl); **F_1** (16g/kg of NaCl); **F_2** (14g/kg of NaCl); **F_3** (12g/kg of NaCl).

Figure 2 shows the mean acceptance scores of evaluated sensory attributes appearance, flavor, taste, texture and overall acceptance. Variations in average of texture and flavor attributes were not significant. Mean scores ranged from 8 (liked very much) to 6 (liked slightly). For appearance, taste and overall acceptance significant lower mean scores were observed for formulation F_3 with 12g/kg of salt. Considering global acceptability, F_1 (7.17 $p>0.05$) and F_2 (7.18 $p>0.05$) formulations showed scores similar to control formulation F_c; However, reductions in level of 40% (F_3) result in significant reduced scores in overall acceptability. Generally, salt reduction results in lower consumer acceptance going according available working data (Galvão et al., 2014; Aaslyng et al., 2014; Ruusnen & Puolane, 2005), and exemplify the great industrial challenge to reduce this additive in meat products formulations.

Penalty analysis was performed using data from the just-about-right test for the salty flavor (Gaze et al., in press, Narayanan et al., 2014). Penalty analysis values ranged from -0.006 to 0.627 and were not significant ($p>0.05$) for evaluated minimal (16g/kg) and maximum (12g/kg) NaCl reduction levels tested in product reformulation; however, considering lacking of significance for penalty in low-sodium formulations, a minimal proposed salt reduction of 20% result in less salty taste intensity desired by consumers; this observation was strongly endorsed by high frequency of consumers that have chosen F_C as the ideal salty taste 67.8%. Slightly tested simple NaCl reduction of 20% just-about right ideal choices for salty taste frequency reduced to 50.85% being reached lower values of 38.98% of consumers selecting JAR at 40% of reduction (F_3). In Fact, according to Meullenet et al. (2007), a specific attribute is present in optimal levels in a product when a minimum of 70% of the answers are in the "JAR" group. Nevertheless, as stated above, an approved flavor enhancer for meat application may easily help to reduce this adverse addressed effect.

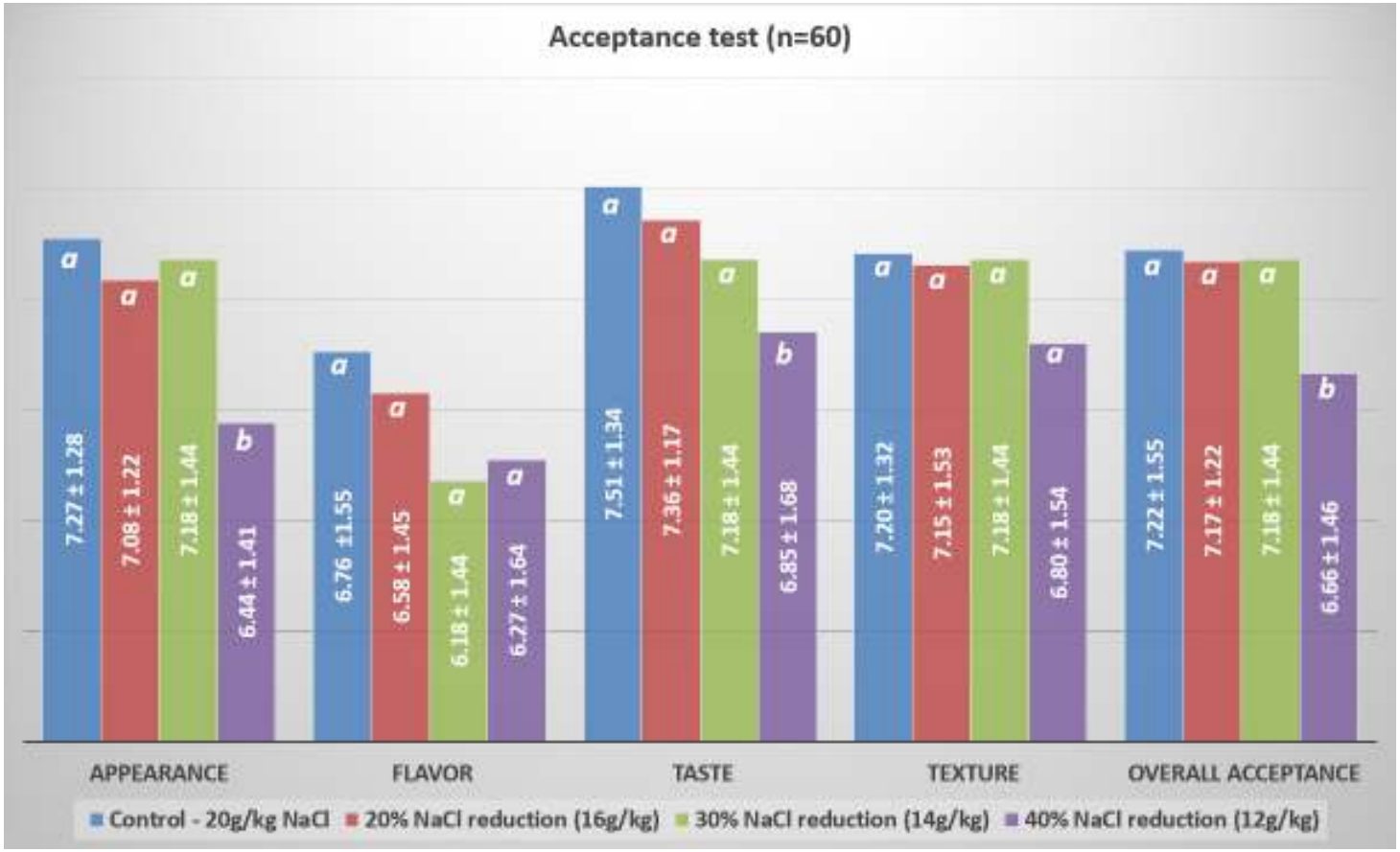

Figure 2. Mean acceptance scores for sensory tests of different low-sodium vacuum-packaged sliced RTE turkey breast formulations. Mean scores ± Standard deviation (s.d.). Values marked by the different leter, are significantly different ($p \leq 0.05$) according to Scott–Knott test. Flavor and texture parameters did not differ significantly. Hedonic scale of nine points (0 — completely disliked and 9 — completely liked). n=60 assessors. Consumer tests were applied after three days refrigerated storage of the sliced samples.

After data evaluation concerning physico-chemical and sensorial traits, it was concluded that a simple salt reduction strategy, with added salt level decreasing by 30% against control in sliced RTE turkey breast (formulated with 20g/kg) is feasible; in addition, it promoted an effective sodium reduction in more than 25%. However, decreased microbiological stability against spoilage groups represents an evident hurdle that needs to be overcomed.Thus the sensorial effects of HPP at 600MPa/180 seconds/25°C were tested in a blind test (Triangle-test) with RTE turkey breast formulated with 30% NaCl reduction. Statistically, consumers were not able to differ a HPP processed of non-processed product, being registered 13 correct judgments (correctness choice of different sample by assessors) and 24 misjudgments among 37 assessors. Labeled minimal correct judgments for significant difference among samples was 18 (p<0.05), 20 (p<0.01) and 22 (p<0.001). This confirms the possibility to apply HHP for processing low-sodium sliced RTE turkey breast as far as sensory evaluation is concerned. In addition, consumers did not reveal any marked alteration in packaged product when both conditions were visually compared. When consumers were inquired to write which attribute directed their decision in selecting different sample, high frequency of correct judgments may attributed to consumers that indicate texture as key factor.

4. Conclusions

Considering a feasible approach for current salt reduction goals in processed food formulations, a simple reduction, without any replacer non-sodium chloride based salts, represents an immediately feasible choice. Concerning physico-chemical and acceptance was concluded that a simple salt reduction strategy, with added salt level decreasing by 30% against control in RTE turkey breast (formulated with 20g/kg) is feasible; in addition to meeting legal targets, it promoted an effective sodium reduction in more than 25% less. This support label claim of “low-sodium” that may represents retail advantages in competitive market. Considering Just-About-Right for ideal salty taste determination, the proposed NaCl reformulations against control (starting already by 20% reduction) resulted in less than 70% of the answers are in the "JAR" group; flavor enhancer may help to

overcome this hurdle. Finally, decreased microbiological stability against spoilage groups represents an evident hurdle that needs to be overcome in 30% less NaCl formulations. HPP treatment at 600MPa for 180sec at room temperatures did not affect sensorial traits of low-sodium (-30%) formulations in blind tests, representing a feasible alternative in low-sodium reformulation trends aiming to assure quality and safety.

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Capítulo 3: High pressure processing optimization for low-sodium sliced ready-to-eat turkey breast as affected by an additional natural antimicrobial hurdle

RESUMO

Utilizando um DCCR (*Delineamento Central Composto Rotacional*), esta pesquisa objetivou otimizar o uso do processamento à alta pressão hidrostática (HPP) para embutido de peito de peru fatiado embalado a vácuo formulado com teor reduzido de sódio, pela avaliação dos efeitos de níveis de pressão e tempos de processo na inativação do patógeno alvo *Listeria monocytogenes* e a bactérias ácido láticas deteriorantes (*Leuconostoc* sp. and *Lactobacillus* sp.); em adição foram avaliados os efeitos de HPP sobre atributos de qualidade que incluíram pH, atividade de água, parâmetros de cor (*L**, *a** e *b**), perfil de textura e oxidação lipídica. Os efeitos combinados do componente bioativo carvacrol, como barreira antimicrobiana natural adicional a HPP, em níveis sub-inibitórios sensorialmente aceitáveis (200ppm), também foram estudados. Reduções logarítimicas decimais das populações microbianas alvo foram significativamente ($p \leq 0.05$) afetadas pelas variáveis do processo HPP avaliadas. Em temperaturas de processo reduzidas de 25°C, tratamentos a 600MPa por 180 segundos foram efetivos em reduzir populações dos deteriorantes e patógenos em mais de 5 ciclos logarítimicos 24hs pós-processo. As variáveis do processo HPP, na faixa avaliada, afetaram ($p \leq 0.05$) a sinérese, oxidação lipídica (índice TBARs) e o parâmetro de textura *dureza*. Com relação aos efeitos combinados do carvacrol com HPP, efeitos sinérgicos de inativação puderam ser detectados em algumas condições, promovendo um efetivo ganho de efeito nas variáveis de processo, sugerindo taxas de inativação equivalentes em condições de processo mais amenas. Melhorias evidentes nos efeitos de conservação foram confirmadas durante a validação do tratamento HPP otimizado (600MPa/180 segundos + carvacrol) durante estocagem refrigerada. Seguindo os critérios de performance requeridos para tratamento letais pós-processo para *Listeria*, o tratamento a 600MPa/180 segundo a 25°C parece ser aplicável para o produto reduzido de sódio estudado; contudo efeitos instrumentais significativos sobre atributos de qualidade foram detectados. No entanto, estes efeitos precisam ser confirmados sensorialmente com consumidores. Inativação bacteriana pós-HPP e efeitos de conservação durante o shelf-life podem ser potencializados na presença de barreiras naturais adicionais, e o uso do carvacrol representa uma promissora ferramenta contra injurias sub-letais e recuperação

celular; concluindo, requerimentos de segurança podem ser atingidos em condições HHP mais amenas, garantindo vantagens industriais e qualidade global do produto.

Palavras-chave: *Listeria monocytogenes*, bacterias acido láticas, teor reduzido de sódio, *Slice Safety Risk*, tecnologias emergentes não térmicas, carvacrol.

ABSTRACT

Using a CCRD (*Central Composite Rotatable Design*), this research aimed to optimize the use of high hydrostatic pressure processing (HPP) for low-sodium sliced vacuum-packaged RTE turkey breast by evaluating the effects of pressure load and dwell time on inactivation of target pathogen *Listeria* and spoilage microbiota (*Leuconostoc* sp. and *Lactobacillus* sp.); in addition, were evaluated the HHP effects on quality attributes, including pH measurements, water activity, color parameters (*L*,a** and *b**), texture profile and lipid oxidation. Combined effects of the essential oil compound carvacrol at sub-inhibitory sensory acceptable levels (200ppm) as a natural antimicrobial additional hurdle to HHP was also evaluated. The decimal logarithmic reductions of target microbial populations were significantly ($p \leq 0.05$) affected by the HPP processing variables. At low processing temperatures of 25°C, treatments with 600MPa/180 seconds were effective in reducing all of the pathogen and spoilage populations over more than 5 log cycles at 24h post-processing. The HPP variables in the range that was studied influenced ($p \leq 0.05$) syneresis, lipid oxidation (TBARs index) and the textural parameter of hardness. Regarding the combined application of carvacrol and HHP, combined post HHP inactivation effects may be detected in some of the evaluated setup conditions, promoting an effective weight increase in the processing variables and suggesting possible equal inactivation rates under milder processing conditions. Clear improvements in preservation effects were confirmed during the validation of the optimized HHP treatment (600MPa/180sec + carvacrol) along refrigerated shelf-life storage. Following the required performance criteria for *Listeria* post-lethality treatment, a treatment at 600MPa/180 seconds at 25°C appears to be suitable for the studied low-sodium product, promoting effective inactivation rates of target microbial groups; however, adverse significant instrumental effects were detected. Nevertheless, this needs to be supported by sensory results with consumers. Post-HPP bacterial inactivation and preservative effects during shelf-life can be potentiated through the presence of natural barriers, and the use of carvacrol represents a promising weapon against sub-lethal injury and cell recovery phenomena; concluding, safety requirements may be reached at

mild HHP set-up conditions, ensuring several industrial advantages and global product quality.

Keywords: *Listeria monocytogenes*, lactic acid bacteria, low-sodium, Slice Safety Risk, emerging non-thermal technologies, carvacrol.

1. Introduction

Salt reduction in processed food formulations represents a great challenge for the modern food industry because of the established relationship between high dietary sodium intake and hypertension, which is the primary risk factor associated with brain and cardiovascular disease (CVD) development (He & MacGregor, 2009). Sodium chloride represents the primary source of sodium (Na) in human diets, containing approximately 400mg/g of Na. Because salt is a key ingredient in their formulations, processed meats are one of the major dietary contributors of sodium (Desmond, 2006). Cognizant of the extent of the problem for public health agencies, the World Health Organization (WHO, 2012) reported that one in three adults in the world suffers from a gradual increase in blood pressure and hypertension, pointing out CVDs as the leading cause of death in the world. A reduction in the average daily sodium consumption would be possible through the effective action of the food processing industries. This reduction would translate into substantial social welfare with reduction of mortality from stroke and coronary heart disease, preventing millions of deaths and saving billions of dollars annually spent on medicines and public health systems (Dickinson & Havas, 2007). Recently, the average salt intake was found to exceed 12g/day totaling almost 5000mg of Na. Following the recommendations of the WHO, this level should be reduced to less than 5g/day, restricting sodium intake to less than 2000mg/day (WHO, 2011).

However, from the technological point of view, the salt reduction strategies remain far from being resolved. Beyond the effects on the sensory aspects and water-binding/texture development, salt plays a key role on the microbiological stability of cooked meat products. A simple reduction or replacement may result in shortened shelf-life and unsafe products during their storage (Doyle & Glass, 2010; Ruusunen & Puolanne, 2005). In addition to the microbial hurdle reduction of salt, post-thermal manipulation occurring in slicing operations for RTE (*Ready-to-eat*) meat products may result in an enhanced risk (*Sliced Safety Risk*) and increase spoilage rates. For refrigerated sliced vacuum-packed processed meats, including dry-cured/cooked ham, sausages, salamis and bolognas, pathogens such as psychrotrophic *Listeria monocytogenes* and spoilage flora mainly composed of

lactic acid bacteria (LAB), represent the core control targets (Sofos & Goenaras, 2010). For vacuum-packed cured cooked turkey breast, the LAB genus of *Lactobacillus* sp. and *Leuconostoc* sp. has been found to be the predominant spoilage flora (Samelis et al., 2000). Although many strains of LAB are often used as starters in cultured meats, LAB are described as spoilage bacteria because of the ropy slime production, acidification, gas production, discoloration and sour-sweet odor (Hu et al., 2009). Oliveira et al. (2015) reported that a simple salt reduction of 30% (without another chloride-salt substitution) in sliced vacuum-packaged turkey breast ham resulted in duplicated LAB growth rates against control with 20g/kg of NaCl added. The predicted shelf-life (time expended to reach 10^7 CFU·g^{-1}) for aerobic mesophilic populations, spoilage psychrotrophic bacteria and LAB was decreased by nearly half.

For the success of salt reduction strategies, technologies or alternative additional preservatives are obviously required. Emerging technologies for food processing such as High Pressure Processing (HPP) emerge as a feasible alternative, eliminating the dependence on chemical preservatives. HPP is a method of non-thermal food pasteurization that consists of subjecting food to intense pressure loads (using a compressible pressure-transmitting fluid) up to 1000MPa. These intense pressure loads are applied to eliminate pathogenic microorganisms and inactivate deteriorative enzymes. HPP is currently being used to reduce the microbial spoilage load and extend shelf-life, improving the safety of a wide spectrum of raw and processed food categories (Rendueles et al., 2011; Simonin et al., 2012). Contrary to conventional thermal food processing, appropriately adjusted HPP application can promote the retention of freshness and the sensorial and nutritive value of food products (Campos et al., 2003).

In the meat processing industry, treatments with HPP are mainly applied in post-packaging for extending shelf-life and assuring the food safety of raw cuts and RTE meat products. Pressure levels applied for target spoilage/pathogen inactivation purposes in meat and meat products range from 400 to 600MPa with short processing times of up to 5 to 7 min at low temperatures<30°C (Bajovic et al., 2012; Simonin et al., 2012). Nevertheless, the processing conditions, including pressure levels, holding time and temperature, should be optimized for each food

category or formulation because of the influence of intrinsic food factors on the extent of microbial inactivation and maintenance of quality/freshness.

Although the success of HPP technology as a non-thermal application for food preservation is evident, the recent literature has highlighted a promising trend consisting of the combined use of HPP plus natural antimicrobials (NAs) (Alba et al., 2013; Bevilacqua et al., 2012; Liu et al., 2012; Marcos et al., 2013). The current consumer market demands products that have friendly labels and are naturally preserved; therefore, NAs (extracts and plant essential oils, bacteriocins, lysozymes, among others) present a feasible additional hurdle alternative for HPP improvements. As expected, advances in HPP plus the combined application of NAs are pointed out as achieving enhanced inactivation rates with minimization of sub-lethal injury and cell recovery phenomena (shelf-life effect). Effective lethality rates may be reached at mild HP treatments (conditions involving holding time, pressure levels and temperature), assuring reduced costs at initial equipment installation and maintenance and maximizing processing output by effective shortened cycles (higher productivity in cycles per hour). In addition, less intense cycles result in increased global quality food maintenance. Current studies have highlighted the slight but significant alterations of food quality attributes (mainly lipid oxidation, color and texture) due to very intense HPP processing conditions (Evrendilek & Balasubramaniam, 2011; Medina-Meza et al., 2014; Púlido et al., 2012; Vercammen et al., 2011). Finally, combined with HPP, some NAs (mainly plant extracts) become effective at sub-inhibitory concentrations for reducing their adverse organoleptic impact (major barrier to their effective applications in food matrices).

This research was aimed to evaluate the effects of HP variables, pressure level and dwell time on the microbial inactivation rates of target pathogen (*Listeria monocytogenes*) and LAB spoilage flora (*Leuconostoc mesenteroides* and *Lactobacillus sakei*) and quality attributes for low-sodium sliced vacuum-packaged RTE turkey breast. In addition, were evaluated the effect of carvacrol (major compound of *Origanum vulgare* essential oil) at sub-inhibitory sensory acceptable levels as a natural additional antimicrobial hurdle to HHP in these responses and during 60 days of refrigerated storage.

2. Material and Methods

2.1 Experimental design and statistical analysis of data

The HPP processing optimization using the *Response Surface Methodology* (Rodrigues & Iemma, 2009) was applied to evaluate the effects of high pressure processing variables including pressure level (x_1) and holding time (x_2) on responses of target pathogen/spoilage inactivation (as decimal logarithmic reduction) and quality attributes of low-sodium sliced vacuum-packaged RTE turkey breast. These responses were evaluated using a *CCRD* (*Central Composite Rotatable Design*) including 2^2 factorial, 4 axial, and 3 central point repetitions totaling 11 assays as shown in Table 1. The pressure level values ranged from 400 to 600MPa and holding times from 30 to 180 seconds for microbiological inactivation purposes determined according to the previous evaluation of the available literature data. For quality attributes evaluation, pressure levels ranged from 200 to 650 MPa and holding times from 30 to 300 seconds, considering the maximum ranges conventionally employed for raw meat and meat product processing purposes. The high pressure processing temperature was held at 25±1°C to avoid unwanted adverse thermal effects.

For each experimental factor, the variance was partitioned into components (linear, quadratic and interaction) to assess the adequacy of the following second-order polynomial function and the relative relevance of these components:

$$Y = \beta_0 + \beta_1 x_1 + \beta_{11} x_1^2 + \beta_2 x_2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon,$$

Where Y is the estimated response, the β-parameters are constant regression coefficients of the fitted models, and x_i, x_j and x_{ij} represent the linear, quadratic and interaction effects of independent variables. The significance of the equation parameters and fitted model for each response variable was assessed by the *p*-levels and F_{ratio}-test (F_{ratio}= $MS_{regression}/MS_{residual}$). The fitted models shown were designed including only significant terms at the $p \leq 0.05$ level. The analysis of variance (ANOVA) of the models was performed to evaluate whether the fitted model was adequate: goodness of fit was evaluated using the adjusted determination coefficients (R_{sqr}), the significance of the *p*-LOF values of the Lack-of-Fit test. Response surfaces and contour plots were plotted using STATISTICA 7 (StatSoft, USA) to note the effects of the evaluated variable pressure load and

holding time on experimentally dependent parameters (responses). For each experimental value obtained (per assay 1-11), two independent repetitions (including raw materials collection and product processing) with required experimental replicates were evaluated.

Table 1. Codes and levels of variables evaluated according to the *CCDR* design for microbial inactivation and quality attributes in low-sodium sliced vacuum-packaged RTE turkey breast.

A – Design for microbial inactivation purposes					
Processing conditions / assays	**Variables codes**		**Pressure Load (MPa)**	**Holding time (seconds)**	**Come-up time (sec)**
	x_1	**x_2**			
Trial 1	-1.00	-1.00	430.0	52.0	88.0
Trial 2	+1.00	-1.00	570.0	52.0	110.0
Trial 3	-1.00	+1.00	430.0	158.0	88.0
Trial 4	+1.00	+1.00	570.0	158.0	111.0
Trial 5	-1.41	0.00	400.0	105.0	83.0
Trial 6	+1.41	0.00	600.0	105.0	113.0
Trial 7	0.00	-1.41	500.0	30.0	99.0
Trial 8	0.00	+1.41	500.0	180.0	99.0
Trial 9	0.00	0.00	500.0	105.0	98.0
Trial 10	0.00	0.00	500.0	105.0	99.0
Trial 11	0.00	0.00	500.0	105.0	98.0
B – Design for quality-attributes evaluation purposes					
Processing conditions / assays	**Variables codes**		**Pressure Load (MPa)**	**Holding time (seconds)**	**Come-up time (sec)**
	x_1	**x_2**			
Trial 1	-1.00	-1.00	265.0	70.0	61.0
Trial 2	+1.00	-1.00	584.0	70.0	114.0
Trial 3	-1.00	+1.00	265.0	260.0	64.0
Trial 4	+1.00	+1.00	584.0	260.0	103.0
Trial 5	-1.41	0.00	200.0	165.0	99.0
Trial 6	+1.41	0.00	650.0	165.0	114.0
Trial 7	0.00	-1.41	425.0	30.0	97.0
Trial 8	0.00	+1.41	425.0	300.0	95.0
Trial 9	0.00	0.00	425.0	165.0	90.0
Trial 10	0.00	0.00	425.0	165.0	87.0
Trial 11	0.00	0.00	425.0	165.0	90.0

* Processing temperature: samples, jacket and treatment chamber was held at 25±1°C.

**x_1 and x_2 represents variables codifications applied in *CCRD* design for models generation.

2.2 Selecting the natural antimicrobial compound employed: carvacrol

2.2.1 Screening of antimicrobial compounds and determination of Minimum Inhibitory Concentrations (MICs)

The antimicrobial effectiveness of several natural antimicrobial compounds including spices and essential oils and their major compounds was previous tested against *Listeria monocytogenes* ATCC 19117 (provided by the INCQS-FIOCRUZ collection). *In vitro* minimum inhibitory concentrations (MICs) were assessed by the broth microdilution method proposed by Oliveira et al. (2012a), testing the following compounds (commercialized by Sigma-Aldrich® and Ferquima®): essential oils of thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), clove (*Syzugium aromaticum*), cinnamon (*Cinnamomum zeylanicum*), and major compounds carvacrol, citral, cinnamaldehyde, eugenol and thymol. Briefly, the *L. monocytogenes* strain was cultured in TSB-YE 37°C/24h (Tryptic Soy Broth supplemented with yeast extract at 0.6%) and used as the initial inoculum.The following concentrations of the natural compounds were prepared in flat-bottomed sterilized 96-well polystyrene microplates: 10.00, 5.00, 2.50, 1.25, 0.65, 0.30, 0.15, 0.08% v/v. These working concentrations were obtained by homogenizing pure tested compounds with TSB containing 0.5% of TWEEN® 80 solution in a final volume of 150µL in each microplate well.Thus, 10µL of the standardized bacterial initial inoculum (10^7 CFU/mL) was added to wells, and three repetitions were performed. One column in the microplate was prepared without inoculum to assess the influence of absorbance derived from natural compounds + TSB in the observed results. The prepared microplates were capped and incubated at 37°C/24h. The absorbance was measured at 620nm in a microplate reader prior to incubation and after 24h. From the absorbance value obtained at 24h, the reading at the time 0 was subtracted. The MIC corresponded to the lowest concentrations of the compounds that resulted in the complete inhibition of the bacterial growth (no absorbance evolution compared to the control compound + TSB + TWEEN® without inoculum). The inhibition was confirmed by inoculating 10µL of the well solution in a test tube containing TSB-YE 37°C/24h. After evaluation of the results obtained for each antimicrobial essential oil or tested major compound, we concluded that the most effective compound identified against the *L.*

monocytogenes was the carvacrol. This compound was effective at 0.65% (MIC of 6500ppm), the lowest effective concentration obtained among all tested compounds. The carvacrol was selected as a natural antimicrobial to apply in combination with HPP.

2.2.2 Consumer test: determination of sensory acceptable level for carvacrol in low-sodium RTE turkey breast (sub-inhibitory)

The original research project was initially submitted and previously approved by the Ethics in Research Committee of the Brazilian platform. To determine the maximum acceptable level of the selected natural antimicrobial carvacrol in RTE turkey breast as indicated by consumers, a sensory test of "*Difference from Control"* was employed. This test is commonly utilized to determine if there is any significant difference between a control sample (standard) and other treatments. The tests were conducted in appropriate individual booths (standardized), under artificial daylight-type illumination and with temperature control (between 22 and 24°C) and air circulation following recommendations of Meilgaard et al. (2007). Briefly, the assessors received a standard RTE turkey breast sliced sample (without carvacrol) coded as P, and four samples of added carvacrol at levels of 1625ppm (MIC/4), 812ppm (MIC/8), 406ppm (MIC/16) and 203ppm (MIC/32). The standard (P) was included coded among the samples. The low-sodium RTE turkey breast was processed as described below in item 2.3. The team of assessors (40) was informed to taste the standard (P) samples (slices of turkey breast, measuring 4mm in thickness, on plastic plates coded with 3 digits) and the samples with carvacrol added, indicating the difference using an auxiliary scale (0- no difference and 9- extremely different). The obtained results were evaluated by an analysis of variance (ANOVA) and *Dunnett* mean test, comparing standard P and the samples with carvacrol. After evaluation of the results obtained, we concluded that the maximum carvacrol level with no significant (p>0.05) difference from the control (without carvacrol) was 203ppm, with the 406ppm and higher levels identified as different by panelists.

2.3 Low-sodium RTE turkey breast formulation and processing

The turkey-derived meat product used in conducting the studies was the RTE turkey breast. The composition of this product shows reduced levels of fat and high protein content associated with a healthy diet, so the sodium reduction studies in healthy foods become completely appropriate. In this current study, two different formulations were evaluated: one with salt/sodium reduction only named **F_{SR}** (14g/kg NaCl: salt reduction of 30% against control with 20g/kg; nearly a 25% Na^+ reduction) and another with salt/sodium reduction plus carvacrol addition at 200ppm named **F_{SRE}**. The NaCl reduction level (30%) had been determined in a previous research step conducted by Oliveira et al. (2015) evaluating sensorial, physicochemical and microbiological aspects. The formulation and processing according to Galvão et al. (2014) may be described as follows (g/kg): ground skinless turkey breast 700.0, water 241.45, NaCl 14.00, cassava starch 20.00, soy protein (isolate) 10.00, sugar 5.00, spice/seasonings 0.30, phosphates 3.50, carrageenan 3.00, monosodium glutamate – MSG 2.00, sodium erythorbate 0.50, sodium nitrite ($NaNO_2$) 0.15 and carmine coloring 0.10. All additives were kindly provided by IBRAC® (Brazil). Frozen vacuum-packaged turkey breast meat (*Pectoralis major* and *Pectoralis minor*, 70±5% moisture, 3±1% fat, 20±2% protein, pH 5.9±0.2) was obtained within 72h of slaughtering from BRF® foods. After thawing, turkey breast skins were removed, and the raw material was subjected to a grinding process with 90% in a grinding disc of 35mm and the remaining 10% disk of 4mm. Then, all the ingredients/additives excepting the starch (cassava starch) and soy protein isolate were added to 60% of the total water used and homogenized for preparation of brine (up to the formation of a single phase). The purified carvacrol (natural 99% Food grade/W224511 Sigma-Aldrich® FDA 21CFR172.515) was added to brine with seasonings in a stock solution containing pure carvacrol at 25.000ppm (carvacrol + polysorbate POE 80 and propylene glycol food grade – patent application pending). The brine is added to the ground turkey breast meat and mixed for 20 minutes in an industrial blender (Jamar®, Brazil). Subsequently, the remaining ingredients and water were added with a further 10 minutes of mixing. The product was stuffed in plastic polyamide casings Visflex (Viskase®, Brazil) with a 76 mm diameter and subjected to the cooking

process in a chamber with appropriate staggered internal temperatures reaching 74°C (measured by a thermopar inserted into the core of the product). The cooked turkey breast was cooled in a water bath for 10 minutes and stored in a controlled chamber at 4±1°C for further procedures.

2.3.1 Slicing, packaging and preparing analytical samples

After the RTE turkey breast processing, the stuffed pieces (500 ±50g) were stored in a controlled chamber at 4±1°C for 24h until slicing operations. The pieces were aseptically opened and sliced to a 4mm thickness (a 10mm thickness slice was used for texture analysis purposes) and subsequently vacuum-packaged in 150x300mm Nylon-Poly 16μ (COEX:LDPE-PA-LDPE) with a permeability rate of PRO_2 of $50cm^3 \cdot (m^2 \cdot day)^{-1}$. Prior tests were conducted to determine the adequate package film. The microbiological analytical units were composed of three slices of 4mm thickness with 10±1g.The physico-chemical analytical units were composed of three slices of 4mm and one 10mm-thickness slice. The samples were appropriately identified according to the HPP processing conditions (microbiological inactivation purposes or quality attribute evaluation). The inoculation procedures are described in detail in item 2.4, below.

2.4 Bacterial strains, inoculum preparation and storage

For the inactivation evaluation of target microorganisms in low-sodium sliced RTE turkey breast treated under different HPP conditions (pressure level x holding time), bacterial strains of *Listeria innocua* (ATCC 33090 serotype 6a type-strain) were used as a surrogate pathogen model for *L. monocytogenes* (mainly for processing plant pilot safety purposes). For LAB spoilage microflora, bacterial strains of *Leuconostoc mesenteroides* subsp. *mesenteroides* (ATCC 10830) (cocos morphology spoilage LAB) and *Lactobacillus sakei* (ATCC 15521) (bacilli morphology spoilage LAB) were tested. All of the strains were generously provided by Andre Tosello Tropical Collection (Brazil). *Lc. mesenteroides* and *Lb. sakei* were cultured in de ManRogosa and Sharpe broth (MRS) 32°C/48h and *L. innocua* on TSB-YE (plus 0.6% yeast extract) by 37°C/48h (HiMedia, Mumbai India). After the strains grew, the bacterial cells were pelleted by centrifugation (3000g/5min) in

microtubes (Eppendorfs) and covered by freezing culture media composed of (g/100g): glycerol 15.0, bacteriological peptone 0.50, yeast extract 0.30% and 0.50 NaCl, pH 7.2-7.4, and maintained under freezing temperatures (-20°C) throughout the experiments.

For work culture production prior to inoculations and HHP processing, an aliquot of the microtube was transferred to appropriate growth media (MRS broth for LAB and TSB-YE for *L. innocua*) and grown with two subcultures at an appropriate temperature of 32°C or 37°C. The overnight cultures were pelleted by centrifugation (3000g/5min), the cells washed 2x with saline solution 0.85% (w/v), and the pelleted cells resuspended in a 50mL volume of saline solution 0.85% (w/v) that was standardized by the *McFarland Standard Scale* to 10^7 CFU/mL. The cell suspensions were plated on MRS agar (LAB) or TSA-YE (*L. innocua*) for exact population quantification and confirmation to turkey ham slice inoculation.

2.5 Sample inoculation and high pressure processing (HHP)

The inoculation procedure may be briefly described as follows: after the aseptic slicing operation of low-sodium RTE turkey breast, the slices obtained (4mm/10±1g) were placed in an aseptic laminar flow chamber under UV-light for ten minutes on each side to eliminate the residual flora that might influence the tests. Thus, 1000µL of the standardized cell suspension (obtained as described in item 2.4) was spread on the product slice and let for 15 minutes (in the laminar flow chamber) prior to vacuum packaging, as described in item 2.3.1.

The vacuum-packaged sliced turkey breast ham samples (inoculated for microbial inactivation purposes, or not inoculated in quality attribute evaluation) were submitted to high pressure processing using the high hydrostatic pressure unit AVURE QFP 2L-700 (Avure Technologies® USA) with a 2L volume treatment chamber (inner vessel diameter 100x254mm), maximum vessel pressure of 690MPa (6900bar/100.000psi) and temperature control at 10 to 90°C. Pure demineralized water was used as a pressure-transmitting fluid. Two thermocouples located at the top and midway in the treatment chamber monitored the temperature of the pressure-transmitting fluid; another thermocouple monitored the temperature of the water jacket surrounding the pressure vessel. Considering the adiabatic

heating that occurred during the pressurization (approx. 3°C/100MPa for water as pressure-transmitting fluid), the initial sample and water (pressure-transmitting fluid) temperatures were controlled to reach 25±1°C by the processing final time. The different HHP setups evaluated are depicted in Table 1. The average pressurization rate was 5 $MPa \cdot s^{-1}$ (±0.30) among the evaluated setups. In this current study, 2 genuine repetitions were carried out for product processing, and HPP repetitions/variability was evaluated in the 3 central points of the *CCRD*.

2.6 Microbiological analysis

The microbiological count of the selected inoculated microorganisms in low-sodium sliced vacuum-packaged RTE turkey breast samples was carried out 24h after high pressure processing of the samples. After HPP, the samples were maintained under refrigeration (4±1°C) until the analysis procedure. For the enumeration of the survivor populations of *L. innocua*, *Lc. mesenteroides* and *Lb. sakei*, 10g (one slice) of HP-treated RTE turkey breast were weighed and transferred into sterile stomacher bags (Baglight®), combined with 90mL of sterile peptone water 0.1% (w/v) and homogenized in a Stomacher (Metroterm®, Brazil) with 490 strokes/2min at room temperature. Stomached slurries were decimally serially diluted in peptone water and plated under the following conditions: for *L. innocua,* 100µL of the sample dilutions was spread on Listeria selective differential Palcam agar (Himedia®, Mumbai, India) supplemented (polimixine B, ceftazidime and acriflavine) by incubation of the plates at 37°C/24-48h. The absence of growth (considered as 0 for the log reduction calculation purposes; <10 CFU/g) detected in some treatments was confirmed with pre-enrichment steps performed in Buffered Listeria Enrichment Broth (BLEB). For LAB enumeration, diluted sample aliquots of 1000µL were pour-plated on sterile Petri dishes and covered (with an over layer) with molten de ManRogosa and Sharpe agar MRS (HiMedia®, Mumbai, India) with the plates incubated at 32°C/48h before colony count. The population of target microorganisms was monitored by negative controls (samples without inoculum) and positive controls (inoculated but not HHP-treated – 0.1 MPa).

After incubation the survivor populations were calculated with the results expressed as a $\log_{10}$ CFU/g. For the different HPP assays evaluated (varying

pressure level and holding time), the bacterial inactivation rates were stated in terms of logarithmic reductions as $-\log_{10} (N/N_0)$ where N represents the survivor population after HPP processing and N_0 represents the initial population in inoculated non-HP-treated samples (control 0.1 MPa).

2.7 Physical and chemical analyses

The quality attributes of the HHP-processed low-sodium sliced vacuum-packaged RTE turkey breast (F_{SR} and F_{SRE}) that were evaluated included pH, aw, syneresis, lipid oxidation (TBARs), CIELAB (*L*, a**and *b**) color and Texture Profile Analysis (TPA). All of the physical and chemical parameters evaluated were determined after 7 days of refrigerated storage (4±1°C) after HHP processing of the samples.

2.7.1. Syneresis, pH and water activity (a_w)

The syneresis or the total amount of released liquid in sample packages was determined by weighing the released liquid after HHP processing under different conditions (pressure x holding time). The syneresis formula is described as follows:

$$\textit{Released liquid (syneresis) g/100g} = (W_{liquid}\,(g) / W_{sample}(g))*100$$

The water activity (aw) was determined directly using an AquaLab water activity meter (Dacagon Devices, Inc., model 4TE, USA) following manufacturer recommendations with the samples at 25±0.1°C. The pH measurements were obtained after initial dilution and homogenization of samples at a ratio of 1:10 (10g of sample in 100 ml of distilled water), followed by the introduction of meter electrodes in homogenized slurries for pH readings.

2.7.2 Color objective evaluation

Color measurements were taken with a colorimeter (Chroma meters CR300, Konica Minolta Sensing, Inc.) established at a 10° angle for the observer and illuminated at D65 to calculate color indices in the CIELAB system following the recommendations of Ramos and Gomide (2007). The color parameters lightness

(*L**), redness (*a**) and yellowness (*b**) were obtained from an average of 5 readings taken at different points in 3 HPP-treated RTE turkey breast slices.

2.7.3 Lipid Oxidation (TBARs)

The effect of HPP treatments on lipid oxidation was determined by the thiobarbituric acid reactive substances (TBAR) index according to Raharjo et al. (1992). Ten-gram portions of RTE turkey breast samples were combined with 40 ml of 5% trichloroacetic acid (TCA) and 1 ml of 0.15% antioxidant BHT (2,6-di-tert-butyl-4-methylphenol (Sigma Aldrich)and refrigerated homogenized in ultrarrax for 5 min. Next, the homogenates were centrifuged (3000g for 5 min), and the supernatant was filtered through Whatman No. 1 filter paper. Two ml of filtrate was combined with 2 ml of 0.08 mol/L TBA reagent and heated in boiling water (100°C) for 5 min. The absorbance of the resulting solution was measured at 532 nm, and the TBAR (thiobarbituric acid reactive substances) values were expressed as mg of malondialdehyde (MDA) per kg sample, calculated using 1,1,3,3-tetraethoxypropane (TEP) as the standard.

2.7.4 TPAs - Texture Profile Analyses

The HHP-treated RTE turkey breast texture was evaluated by TPA - Texture Profile Analysis, according to the recommendations of Bourne (1978), using a *TA.XT2i* Texturometer (Texture Analyzer, Stable Micro Systems, Inc., England) coupled to a microcomputer equipped with Texture Expert Software. The TPA test consists of compressing the sample two times in cycles in a reciprocating motion that simulates the jaw action, and extract, from the resulting force-time curve, a number of different textural parameters. Ten standardized squared cubes (10x10mm) of 3 independent HPP-treated turkey breast ham slices of 10mm thickness were cut for the TPA tests. Next, the slices were compressed twice to 50% of their original height; the measurements were taken after the samples reached room temperature (±25°C). The deformation curve (force-time) was obtained with a compression velocity of 180mm/min, using a P-20 probe (20mm diameter).

According to Bourne (2002), the texture parameters were determined from the force curves as follows: hardness (N) is the height of the force peak on the first compression cycle; cohesiveness is the ratio of positive areas under the first and second compressions (A1/A2); adhesiveness (N·mm) is the negative force area (called A3) for the first bite representing the work necessary to pull the compressing plunger away from the sample; springiness (mm) is defined as the distance that the sample recovers between the end of the first bite and the start of the second bite; chewiness (N·mm) is the energy required to masticate a solid food (product of hardness x cohesiveness x springiness).

2.8 Validation of the optimized HHP: a shelf-life study

After evaluation of data obtained in the HPP optimization in a previous research step as described above, the proposed selected HPP treatment (600MPa/180seconds/25°C) was validated in microbiological analyses during product refrigerated storage (60 days at 4°C). Were enumerated naturally occurring (without initial inoculums) spoilage microbial groups which included psychrotrophic (determined in PCA - Plate Count Ágar 7°C/10 days incubation) and lactic acid bacteria (in MRS Agar 32°C/48hs). In particular for *L. innocua*, high initial inoculums (~10^6 CFU/g) were applied, being enumerated the populations during refrigerated storage. In this shelf-life studies were evaluated the following formulations: F_{SR} (-30% NaCl, 14g/kg), F_{SRE} (-30% NaCl plus carvacrol 200ppm) and a control (entire amount of NaCl of 20g/kg used in conventional formulations) all in HPP- treated and non-HPP conditions.

3. Results and Discussion

3.1 HHP and inactivation of target microorganisms

Table 2 shows the regression coefficients that were estimated and the analysis of variance (ANOVA) for the obtained regression models for responses of target microrganisms inactivation in F_{SR} and F_{SRE} sliced RTE turkey breast formulations. As expected, the estimated *L. innocua* decimal logarithmic reduction was significantly ($p \leq 0.05$) affected by the evaluated pressure level and holding time variables. The more intense the processing in time and pressure level, the higher

were the observed log reduction rates. According to Bover-Cid et al. (2011), who evaluated *L. monocytogenes* HHP inactivation in dry-cured ham, pressure level and holding time were the most important factors determining the extent of HHP-induced *Listeria* inactivation. Despite the "*effects analysis of variables*" x_1 and x_2 against this dependent response, the pressure level seems to be have a greater impact than holding time. The sensitivity of the surrogate pathogen *L. innocua* in sliced low-sodium RTE turkey breast to pressure increase was higher than the sensitivity to an increase in the exposure time (in the range studied). Among several pathogens, *Listeria* spp. was found to be one of the most sensitive to applied pressure level changes, and thus, to obtain more efficient lethality treatments, an increase in pressure level may result in processing that shows exposure times considerably reduced (Bover-Cid et al., 2011). In meat processing food industries, these events may be reflected in adjusted processing conditions to maximize throughput production. However, an economic evaluation is appropriate to evaluate the cost of more powerful high pressure units. Pressure level and holding time ($p \leq 0.05$) affected the *L. innocua* log reductions linearly.

A quadratic effect was detected for pressure level in both formulations evaluated for the surrogate pathogen. Moreover, a quadratic influence of the holding time ($p \leq 0.05$) may be detected only for the F_{SR} formulation. The interaction terms were significant at the $p < 0.10$ level, excluding of the models that were fitted only with $p \leq 0.05$ regression coefficients. The ANOVA results indicated that the regression models developed for *L. innocua* inactivation (expressed as $\log_{10}N/N_0$) were adequate, with R^2 values higher than ≥0.90 for both F_{SR} and F_{SRE} formulations. The F_{ratio} values obtained were higher than critical *F-values* (5-6 times higher), corresponding to low probability *p*-values. The *p*-values obtained in the lack of fit test were not significant ($p > 0.05$). A model can be considered predictive if the coefficient of determination (R^2) is close to 1.00 and if the F_{ratio} is higher than the critical *F-value* (Gutarra et al., 2009; Rigueira et al., 2011).

Table 2. Regression coefficients and analysis of variance (ANOVA) for the regression models for responses of target microorganism inactivation in F_{SR} and F_{SRE} formulations after 24h HHP processing.

Parameter	***Listeria innocua* inactivation (log_{10} N/N$_0$)**					
	F_{SR}			**F_{SRE}**		
	R.C	**S.E**	***p*-level**	**R.C**	**S.E**	***p*-level**
Mean/Intercept	5.3951	0.1909	0.000001	5.6666	0.2741	0.000005
Pressure Load	1.5373	0.1169	0.000045	1.5661	0.1678	0.000238
Pressure Load^2	-0.5876	0.1392	0.008310	-0.7902	0.1998	0.010792
Holding time	0.7306	0.1169	0.001538	1.0042	0.1678	0.001869
Holding time^2	-0.4315	0.1392	0.026832	-0.4010	0.1998	0.101014
Pressure X Time	-0.3842	0.1653	0.067750	-0.5105	0.2374	0.084166
Regression ANOVA and goodness of fit						
	F_{SR}			**F_{SRE}**		
Regression (F_{ratio})	33.6822**			23.1154**		
Lack of Fit (*p*-value)	0.1306			0.2098		
R^2	0.9573			0.9083		
*Fitted model**	Log_{10} N/N$_0$ =5.39+1.53*p*-0.58p^2+0.73*ht*-0.43ht^2			Log_{10} N/N$_0$ =5.66+1.56*p*-0.79p^2+1.00*ht*		
***Leuconostoc mesenteroides* inactivation (log_{10} N/N$_0$)**						
	F_{SR}			**F_{SRE}**		
	R.C	**S.E**	***p*-level**	**R.C**	**S.E**	***p*-level**
Mean/Intercept	3.1266	0.2375	0.000045	3.1178	0.1846	0.000013
Pressure Load	1.7193	0.1454	0.000076	1.5950	0.1130	0.000032
Pressure Load^2	0.3916	0.1731	0.073137	0.4850	0.1345	0.015474
Holding time	0.3505	0.1454	0.060838	0.3573	0.1130	0.025078
Holding time^2	-0.1733	0.1731	0.362688	-0.0947	0.1345	0.512681
Pressure X Time	-0.2750	0.2057	0.238860	-0.2216	0.1598	0.224400
Regression ANOVA and goodness of fit						
Regression (F_{ratio})	60.5877**			70.7608**		
Lack of Fit (*p*-value)	0.0537			0.1024		
R^2	0.8706			0.9680		
*Fitted model**	Log_{10} N/N$_0$ =3.12+1.71*p*			Log_{10} N/N$_0$ =3.11+1.59*p*+0.48p^2+0.35*ht*		
***Lactobacillus sakei* inactivation (log_{10} N/N$_0$)**						
	F_{SR}			**F_{SRE}**		
	R.C	**S.E**	***p*-level**	**R.C**	**S.E**	***p*-level**
Mean/Intercept	4.800000	0.2386	0.000006	4.810000	0.4299	0.000100
Pressure Load	1.543505	0.1461	0.000131	1.335259	0.2632	0.003862
Pressure Load^2	-0.203200	0.1739	0.295415	-0.084375	0.3133	0.798501
Holding time	0.494992	0.1461	0.019527	0.656185	0.2632	0.055010
Holding time^2	-0.371575	0.1739	0.085742	-0.444375	0.3133	0.215389
Pressure X Time	-0.286175	0.2066	0.224782	-0.172500	0.3723	0.662634
Regression ANOVA and goodness of fit						
Regression (F_{ratio})	41.7017**			17.6185**		
Lack of Fit (*p*-value)	0.2769			0.1053		
R^2	0.9124			0.8149		
*Fitted model**	Log_{10} N/N$_0$ =4.80+1.54*p*+0.49*ht*			Log_{10} N/N$_0$ =4.81+1.33*p*+0.65*ht*		

R.C – Regression coefficients / S.E – Standard Error / F_{SR} (formulation with sodium reduction only) and F_{SRE} (sodium reduction formulation plus natural antimicrobial: carvacrol) *Codded reparametrized (model including only significant terms $p \leq 0.05$) with pressure named as (*p*) and holding time as (*ht*).

F_{ratio} = Regression$_{mean\ square}$ / Residual$_{mean\ square}$

Model adequacy**($F_{ratio} >> F_{critical}$) = $F_{0.05\ /\ 4,6}$ = 4.53/ $F_{0.05\ /\ 3,7}$= 4.35 / $F_{0.05\ /\ 1,9}$= 5.12 / $F_{0.05/2,8}$ = 4.46

The *L. innocua* experimental inactivation rates achieved in sliced vacuum-packaged RTE turkey breast in different HHP processes are shown in Table 3, in terms of logarithmic viability reduction (log_{10} N/N_0). Treatments with pressure levels of 400-430MPa at short times (<120sec) results in less than 2 log reduction in survivor (after 24h of HHP processing) population counts for both F_{SR} and F_{SRE}. At 430MPa with a longer holding time of 158seconds, the effect of variable "holding time" was expressed by experimental inactivation rates reaching 4.33 and 4.60 log reductions for low-sodium RTE turkey breast without carvacrol and with carvacrol added at 200ppm. At higher pressure levels of 500MPa with short holding times, the observed inativation was nearly 3 log cycle reduction for both formulations. At this same pressure level of 500MPa with a holding time increase to >105 sec, an inactivation increase resulted in reaching more than 5 log reductions for the F_{SR} formulation and more than 6 log reductions for the formulation with added carvacrol (F_{SRE}). In this case, the weight of the variable holding time was potentiated by the addition of carvacrol. Next to the limit of tested pressure level of 570 and 600MPa, a shortened treatment time of 52s was able to reduce the survivor population by more than 5 log reductions. Finally, the most intense evaluated binomial treatments of 570/158 and 600/105 MPa/seconds results in no surrogate pathogen *L. innocua* growth (>6 log reductions) in surface agar plate counts until after pre-enrichment steps. Accoding to the current literature, the primary site for pressure-induced microbial inactivation is the cell membrane, and the main effects include modifications in permeability and ion exchange (McClements et al., 2001). In addition, high pressure causes changes in cell morphology and biochemical reactions, protein denaturation and inhibition of genetic mechanisms. Other mechanisms of action that may be responsible for microbial inactivation include the denaturation of key enzymes and the disruption of ribosomes (Linton and Patterson, 2000). The HPP processing effectiveness and inactivation extent are affected by several factors including intrinsic bacterial strain and growth history, morphology and culture growth stage; media recovery and post-HPP analysis time; efficiency and properties of equipment construction (such as come-up rate $MPa \cdot s^{-1}$); and mainly the nature of the food matrix. Intrinsic factors such as pH, water activity (aw), formulation components, and natural antimicrobial hurdles are

perceived to be the most relevant. Thus, selected HPP processing setups should be optimized for each food category/formulation, target pathogen, and shelf-life extension objective because of the influence of intrinsic food factors on microbial log reduction and recovery.

Table 3. Experimental and predicted values (with the fitted models) for decimal logarithmic reductions in different experimental conditions evaluated in the *CCDR* design.

Trial	***Listeria innocua*** **(logarithmic reduction log_{10} N/N_0)**							
	F_{SR}				**F_{SRE}**			
	Experimental	**Predicted**	**Fit pure error (residuals)**	**Error (%)**	**Experimental**	**Predicted**	**Fit pure error (residuals)**	**Error (%)**
1	1.946	2.108	-0.162	-8.3	1.710	2.047	-0.337	-19.7
2	5.366	5.183	0.183	3.4	5.239	5.179	0.060	1.1
3	4.332	3.569	0.763	17.6	4.601	4.055	0.546	11.8
4	6.215	6.644	-0.429	-6.8	6.089	7.187	-1.099	-18.0
5	1.658	2.046	-0.388	-23.3	1.496	1.730	-0.234	-15.6
6	6.604	6.394	0.210	3.1	6.808	6.160	0.648	9.5
7	3.521	3.499	0.022	0.6	3.413	3.869	-0.456	-13.3
8	5.366	5.565	-0.200	-3.7	6.448	6.709	-0.261	-4.0
9	5.215	5.395	-0.180	-3.4	5.524	5.289	0.234	4.2
10	5.604	5.395	0.209	3.7	5.388	5.289	0.098	1.8
11	5.366	5.395	-0.029	-0.5	6.089	5.289	0.800	13.1
	Leuconostoc mesenteroides **(logarithmic reduction log_{10} N/N_0)**							
	Experimental	**Predicted**	**Fit pure error (residuals)**	**Error (%)**	**Experimental**	**Predicted**	**Fit pure error (residuals)**	**Error (%)**
1	1.010	1.566	-0.556	-55.0	1.501	1.589	-0.087	-5.8
2	5.510	5.005	0.505	9.1	5.166	4.779	0.387	7.5
3	1.930	1.566	0.364	18.8	2.171	2.303	-0.132	-6.0
4	5.330	5.005	0.325	6.1	4.950	5.493	-0.543	-10.0
5	1.740	0.854	0.886	50.9	1.915	1.798	0.116	6.0
6	5.880	5.717	0.163	2.7	6.381	6.310	0.071	1.1
7	1.950	3.285	-1.335	-68.4	2.138	2.523	-0.387	-17.0
8	3.410	3.285	0.125	3.6	3.839	3.534	0.303	7.9
9	3.250	3.285	-0.035	-1.0	3.067	3.028	0.039	1.2
10	3.090	3.285	-0.195	-6.3	3.145	3.026	0.117	3.7
11	3.040	3.285	-0.245	-8.0	3.139	3.028	0.111	3.5
	Lactobacillus sakei **(logarithmic reduction log_{10} N/N_0)**							
	Experimental	**Predicted**	**Fit pure error (residuals)**	**Error (%)**	**Experimental**	**Predicted**	**Fit pure error (residuals)**	**Error (%)**
1	1.944	2.343	-0.398	-20.4	2.640	2.434	0.205	7.8
2	5.367	5.430	-0.065	-1.2	4.840	5.104	-0.264	-5.5
3	3.940	3.333	0.607	15.4	4.740	3.746	0.993	21.0
4	6.215	6.420	-0.205	-3.3	6.250	6.416	-0.166	-2.7
5	1.900	2.199	-0.299	-15.7	1.840	2.537	-0.697	-37.9
6	6.604	6.565	0.039	0.6	6.770	6.313	0.456	6.7
7	3.521	3.682	-0.161	-4.6	2.970	3.497	-0.527	-17.8
8	4.310	5.082	-0.772	-17.9	4.200	5.353	-1.153	-27.5
9	4.590	4.382	0.208	4.5	5.070	4.425	0.644	12.7
10	5.170	4.382	0.788	15.2	4.830	4.425	0.404	8.4
11	4.640	4.382	0.258	5.6	4.530	4.425	0.104	2.3

*F_{SR} (formulation with sodium reduction only) and F_{SRE} (sodium reduction formulation plus natural antimicrobial: carvacrol at 200ppm).

L. innocua is a non-pathogenic strain with characteristics similar to the pathogenic *Listeria* species (*L.monocytogenes*) in terms of physiology, metabolism, growth and resistance (equaling resistance to low pH, drying, heating, and salt levels). *L. innocua* is therefore used as a surrogate microorganism for the foodborne *L. monocytogenes* in several studies of inactivation effectiveness including HPP (Fröhling et al., 2012; IFT, 2000; Saucedo-Reyes at al., 2009; Vercammen et al., 2011). Several HPP setup conditions were proposed for *Listeria* spp. control in RTE meat products being the most applied pressure levels of 600MPa and holding time in the range of 2-10min (Hereu et al., 2012; Myers et al., 2013a). Importantly, this post-processing non-thermal alternative has been planned for safety purposes in reformulated RTE, including low-sodium or low-nitrite formulations (Myers et al., 2013b). For RTE meat products (a meat product that in its final form is edible without additional preparation or thermal treatment to achieve food safety), recontamination during post-processing manipulation (e.g., during slicing) is assumed to be 10 CFU/g (i.e., 1 Log_{10} CFU/g) in the worst case (ICMSF, 2002). Taking into consideration the European microbiological criteria in relation to *L. monocytogenes* to a maximum tolerance of 100 CFU/g (equivalent to 2 Log_{10} CFU/g), the target performance criterion for RTE meat products has, in general, been set at 4 log reductions to ensure a maximum of 1 CFU/kg (3 log CFU/g) of *L. monocytogenes* after an in-package pasteurization treatment such as HPP (Hayman et al., 2004; Ordóñez et al., 2004). The US Food Safety and Inspection Services (FSIS/2003) recommend a post-lethality treatment in RTE applied to the final product or sealed package of product to reduce or eliminate the level of pathogens for control of *L. monocytogenes* resulting from contamination from post-lethality exposure in ≥2 log_{10} reductions.

The elevated coefficient of determination R^2 obtained for the fitted *L. innocua* inactivation models indicated a high degree of correlation between the experimental values and predicted values, as confirmed in Table 3. Thus, an efficient and validated discussion may be performed concerning predicted behavior values, mainly in 500-600MPa and holding time>105 seconds, the region of effective required inactivation and with lower errors. The models were used for

construction of the response surfaces and contour diagrams, showing the expected values for *L. innocua* log reductions (Figure 1).

Regarding the combination of HPP and carvacrol at sub-inhibitory concentrations of 200ppm, a positively inactivation combined effect (for *L. innocua*) may be suggested, but only at some regions of the evaluated range of the studied variables (Table 4). This observation may only be clearly supported in the pressure load range of 500-600MPa and treatment times >150 seconds. A minimal pressure disturbance of cells (including pressure load and holding time extent) is needed to potentiate inactivation effects. According to our results, minimum bacterial cell damage was required for the sub-inhibitory level of carvacrol effectiveness. The antibacterial effects of carvacrol, an aromatic ring linked to hydroxyl, ethyl and methyl radicals (2-methyl-5-(1-methylethyl) phenol) were dependent on its ability to disturb cell membrane permeability (Lambert et al., 2001). Carvacrol interacts with the cell membrane, dissolving the phospholipid bilayer, and is assumed to align between the fatty acid chains disturbing their physical structure. As a consequence, an expansion and destabilization of cell membranes increase their fluidity which in turn would increase passive permeability (Ultee et al., 2002). Because HPP is believed to cause damage to the cell membrane, the common target is suggested to be the root of the observed synergism (Karatzas et al., 2001). Microorganisms are baroresistant to selective chemical/natural antimicrobials due to their ability to exclude such agents from the cell, mainly by the action of the cell membrane and transport. However, if the membrane and active transport mechanisms become damaged, for example by HPP, this tolerance may be lost. Ait-Ouazzou et al. (2011) evaluated the effects of several essential oils and their major constituents including carvacrol at 2000ppm, combined with moderate heat and milder PEF treatment (30kv/cm/25pulses) against *L. monocytogenes* in fruit juices. The authors observed that the most EO constituents caused membrane permeability and sublethal injuries. Outstanding synergistic lethal effects were shown by the natural antimicrobial application and mild heat or moderate pulsed electric fields achieving 5 $\log_{10}$ cycles of cell inactivation.

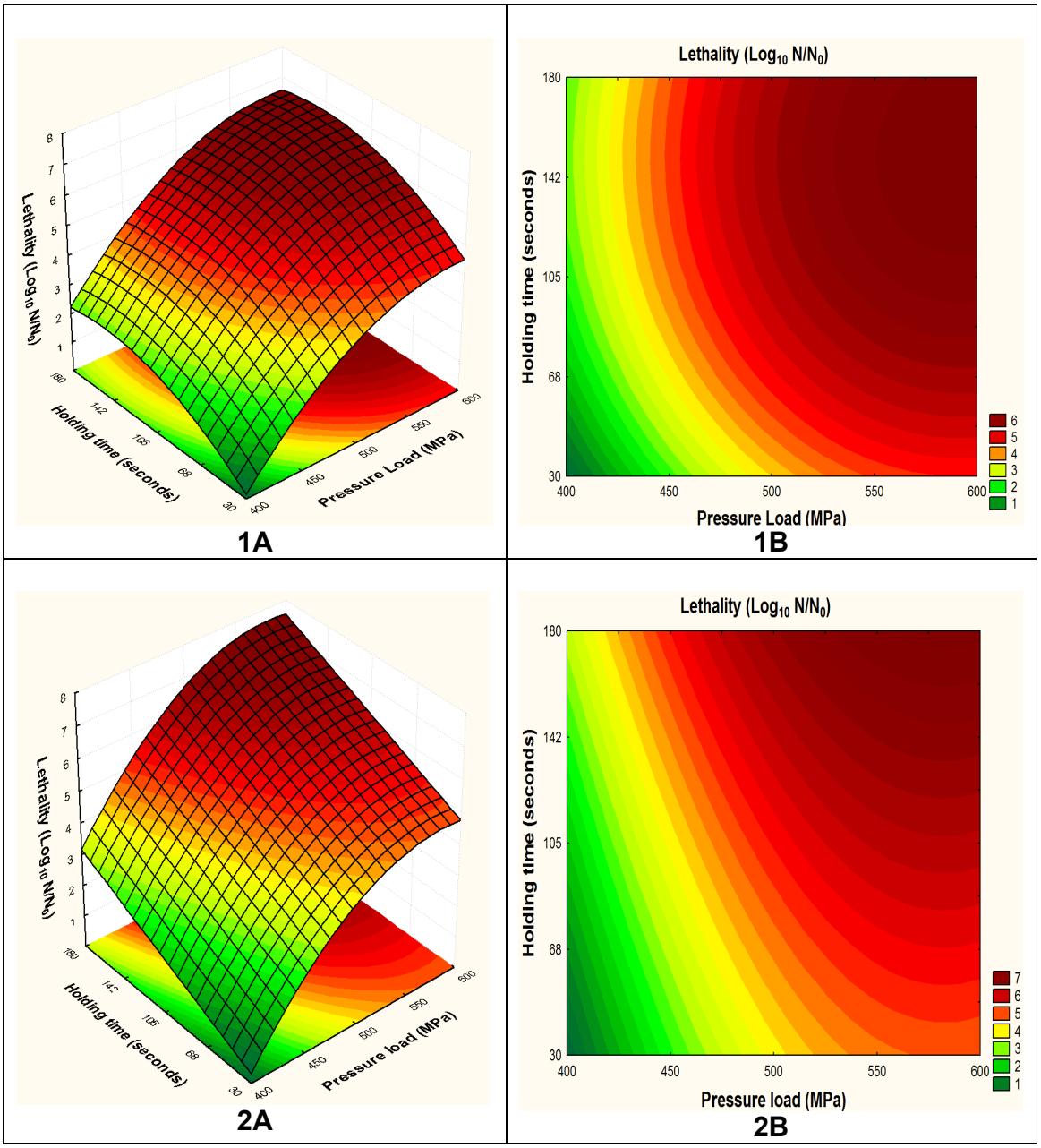

Figure 1. Fitted response surface and contourplots for the effects of variables (pressure level and holding time) on *Listeria innocua* inactivation ($log_{10}N/N_0$) after 24h of high pressure processing. 1A and 1B – **F_{SR}** formulation (sodium reduced); 2A and 2B **F_{SRE}** formulation (sodium reduced plus carvacrol). ($p \leq 0.05$). Processing temperature held at 25°C.

Evaluating the predicted lethality rates in F_{SR} and F_{SRE} turkey breast ham formulations (Table 4) of *L. innocua* for pressure and time ranging from 500-600MPa and time of 30, 60, 90, 120, 150 and 180 seconds, a 6 log reduction was reached at 500MPa and 150-180 seconds for the formulation with carvacrol added.

In these same treatments, only 5 log reductions may be observed for F_{SR}. The 6 log reduction performance was reached with 500MPa only for the F_{SRE} formulation. In the F_{SR} formulation, this inactivation performance may be reached only at 600MPa with holding times higher than 90sec, suggesting a significant decrease in required pressure that may be translated to a reduction in initial equipment installation and maintenance. However, a long holding time (significant variable) plus 1 minute was needed. Despite this long holding time, adequate moderation needs to be established. Evaluating 600MPa of pressure load, the 7 log reduction performance was reached only for the F_{SRE} formulation.

Lactic acid bacteria (LAB) are naturally found in many vacuum-packaged meat products stored under refrigeration, e.g., ham, initiating spoilage and diminishing shelf-life. Low oxygen concentration, high water activity (normally between 0.96 and 0.98) and pH of approximately 6.0 are some of the characteristics that favor the lactic acid bacterial growth (Cayré et al., 2003; Cayré et al., 2005). Table 2 has shown the regression coefficients obtained and the analysis of variance (ANOVA) for the regression models for *Lc. mesenetroides* and *Lb. sakei* inactivation in F_{SR} and F_{SRE} low-sodium RTE turkey breast formulations. For both the ANOVA results indicated that the regression models developed were satisfactory, with R^2 values >0.80 for F_{SR} and F_{SRE} formulations. The F_{ratio} values obtained were higher than the critical *F-values* (more than 5-10 times), corresponding to low probability *p*-values. By the "*effects analysis of variables*", the pressure levels variable appears to have the most impact over holding time for cocos and bacilli cell morphology. The elevated coefficient of determination R^2 obtained for the fitted lethality models indicated a high degree of correlation between the experimental values and predicted values, as confirmed in Table 3. Despite the significant relative error observed in some model regions, at applicable regions for required log reduction 500-600MPa and holding time >100sec, the error was low, being viable in some discussions of predicted values in these regions. The models were used for construction of the response surfaces and contour diagrams, showing the expected values (Figures 2 and 3).

Table 4. Predicted logarithmic reductions (24hs post-HHP) values obtained under different conditions of coded pressure level and holding time according to the codified fitted models.

***Listeria innocua* logarithmic reductions (log_{10} N/N_0)**							
Pressure level (MPa)	**Code X_1**	**Holding time (sec)**	**Code X_2**	**Predicted inactivation F_{SR}**	**Log reduction F_{SR}**	**Predicted inactivation F_{SRE}**	**Log reduction F_{SRE}**
500	0.00	30	-1.41	3.50	**>3**	3.87	**>3**
500	0.00	60	-0,85	4.46	**>4**	4.43	**>4**
500	0.00	90	-0.28	5.15	**>5**	5.00	**>5**
500	0.00	120	+0.28	5.56		5.57	
500	0.00	150	+0.85	5.70		6.14	**>6**
500	0.00	180	+1.41	5.56		6.70	
600	+1.41	30	-1.41	4.50	**>4**	4.75	**>4**
600	+1.41	60	-0.85	5.46	**>5**	5.30	**>5**
600	+1.41	90	-0.28	6.15	**>6**	5.87	
600	+1.41	120	+0.28	6.56		5.88	
600	+1.41	150	+0.85	6.70		7.01	**>7**
600	+1.41	180	+1.41	6.57		7.58	
***Leuconostoc mesenteroides* logarithmic reductions (log_{10} N/N_0)**							
Pressure level (MPa)	**Code X_1**	**Holding time (sec)**	**Code X_2**	**Predicted inactivation F_{SR}**	**Log reduction F_{SR}**	**Predicted inactivation F_{SRE}**	**Log reduction F_{SRE}**
500	0.00	30	-1.41	3.28	**>3**	2.52	**>2**
500	0.00	60	-0,85	3.28		2.72	
500	0.00	90	-0.28	3.28		2.93	
500	0.00	120	+0.28	3.28		3.13	**>3**
500	0.00	150	+0.85	3.28		3.33	
500	0.00	180	+1.41	3.28		3.53	
600	+1.41	30	-1.41	5.71	**>5**	5.79	**>5**
600	+1.41	60	-0.85	5.71		5.99	
600	+1.41	90	-0.28	5.71		6.20	**>6**
600	+1.41	120	+0.28	5.71		6.39	
600	+1.41	150	+0.85	5.71		6.60	
600	+1.41	180	+1.41	5.71		6.80	
***Lactobacillus sakei* logarithmic reductions (log_{10} N/N_0)**							
Pressure level (MPa)	**Code X_1**	**Holding time (sec)**	**Code X_2**	**Predicted inactivation F_{SR}**	**Log reduction F_{SR}**	**Predicted inactivation F_{SRE}**	**Log reduction F_{SRE}**
500	0.00	30	-1.41	3.68	**>3**	3.50	**>3**
500	0.00	60	-0,85	3.96		3.86	
500	0.00	90	-0.28	4.24	**>4**	4.24	**>4**
500	0.00	120	+0.28	4.52		4.60	
500	0.00	150	+0.85	4.80		4.98	
500	0.00	180	+1.41	5.07	**>5**	5.35	**>5**
600	+1.41	30	-1.41	5.86		5.38	
600	+1.41	60	-0.85	6.13	**>6**	5.75	**>6**
600	+1.41	90	-0.28	6.41		6.12	
600	+1.41	120	+0.28	6.69		6.49	
600	+1.41	150	+0.85	6.97		6.86	
600	+1.41	180	+1.41	7.25	**>7**	7.23	**>7**

*F_{SR} (formulation with sodium reduction only) and F_{SRE} (sodium reduction formulation plus natural antimicrobial: carvacrol at 200ppm)

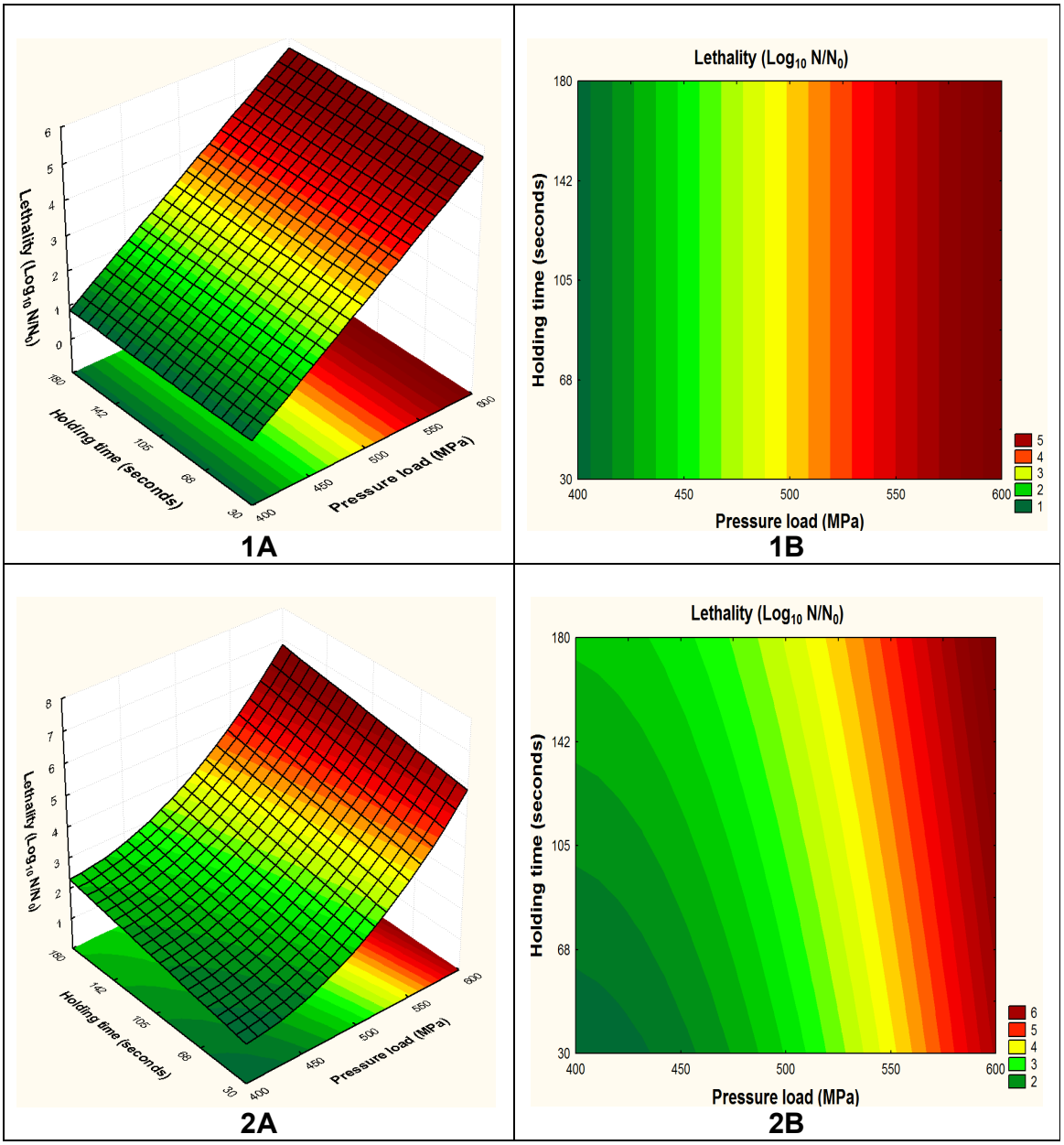

Figure 2. Fitted response surface and contourplots for the effects of variables (pressure leve and holding time) on spoilage LAB (*Leuconostoc mesenteroides*) inactivation ($log_{10}N/N_0$) after 24h of high pressure processing. 1A and 1B – **F_{SR}** formulation (sodium reduced); 2A and 2B **F_{SRE}** formulation (sodium reduced plus carvacrol). ($p \leq 0.05$). Processing temperature held at 25°C.

As shown in Table 2 and Figure 2, the "holding time" variable effect was significant ($p \leq 0.05$) only for the F_{SRE} formulation (for *Lc. mesenteroides*) indicating a synergistic effect of the carvacrol applied at 200ppm and the HHP treatments.

Evaluating the respective response surface and contourplots for *Lc. mesenteroides* lethality in the F_{SR} formulation (Figure 2 1A and 2B), the dependent

response was not affected by the holding time (important: at this short applicable studied range of 30-180sec), with this event confirmed in Table 2 by the *p*-level (p=0.060 >0.05) for the x_2 terms.

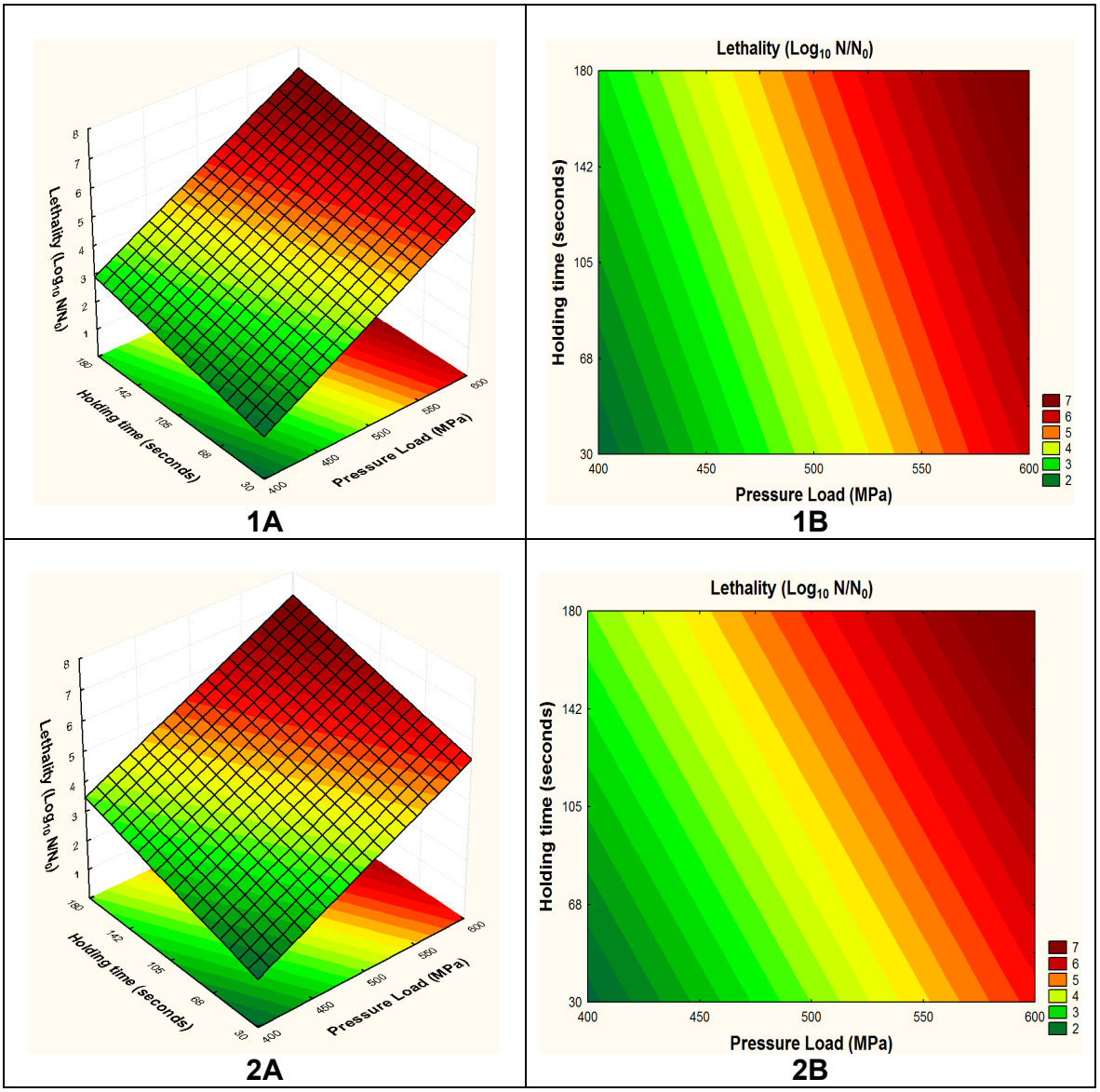

Figure 3. Fitted response surface and contourplots for the effects of variables (pressure load and holding time) on spoilage LAB (*Lactobacillus sakei*) inactivation ($log_{10}N/N_0$) after 24h of high pressure processing. 1A and 1B – $\mathbf{F_{SR}}$ formulation (sodium reduced); 2A and 2B $\mathbf{F_{SRE}}$ formulation (sodium reduced plus carvacrol) ($p \leq 0.05$). Processing temperature held at 25°C.

The known bareresistance of cocos morphology bacteria (when compared to bacilli) may answer this indicated event, mainly when the holding time range

evaluated was short (but inside industrial reality) as in this study. However, in the low-sodium RTE turkey breast formulation with carvacrol added, the variable holding time appeared to be significant (*p*-value of 0.025). This observation was evident in the contour analysis (Figure 2 - 2A and 2B) when the same lethality rates might be reached by increasing holding times or decreasing the applied pressure levels. Regarding the synergistic carvacrol and HHP inactivation effects, for *Lc. mesenteroides,* a >6 log reduction was reached only in the F_{SRE} cavacrol added formulation at pressure levels of 600MPa, with a maximum 5 logarithmic cycle reduction observed in formulations without carvacrol. Among LAB spoilage flora of vacuum-packaged ham, the cocos morphology appears to be more baroresistant than bacilli forms.

The HPP treatment was able to promote a pronounced decimal logarithmic reduction of the evaluated microbial populations after 24h post-processing of the surrogate pathogen *L. innocua* and spoilage LAB flora (*Lc. mesenteroides* and *Lb. sakei*). The HHP processing setup with a pressure load of 600MPa and a holding time >120 seconds promotes a >5 log reduction of all target microorganisms evaluated. The HHP processing represents a feasible alternative to maintain safety and extend shelf-life of unstable low-sodium RTE processed meats, mainly those RTE processed meats with post-processing manipulation (slicing operations). The additional natural barrier potentiates inactivation effects (in some setup adjustments), being active at sensory acceptable sub-inhibitory levels.

3.2 HHP and the effects on quality attributes

For the physico-chemical quality attributes evaluated, the responses of the *L* (lightness)*, *a* (redness)* and *b* (yellowness)* color parameters, water activity and pH were not significantly ($p \leq 0.05$) affected by a 200-650 pressure level range and holding times oscillating from 30-300 seconds for both the F_{SR} and F_{SRE} formulations of low-sodium sliced vacuum-packaged RTE turkey breast. The average experimental values for F_{SR} and F_{SRE} formulations for color parameters were, respectively: F_{SR} (72.31 for non-treated control 0.1MPa/71.19 for HHP processed) and F_{SRE} (69.80 for non-treated control 0.1MPa/70.79 for HHP processed) for the *L** parameter; F_{SR} (4.29 for non-treated control 0.1MPa/4.45 for

HHP processed) and F_{SRE} (4.31 for non-treated control 0.1MPa/4.39 for HHP processed) for *a** parameter; F_{SR} (9.13 for non-treated control 0.1MPa/8.65 for HHP processed) and F_{SRE} (8.08 for non-treated control 0.1MPa/8.63 for HHP processed) for yellowness (b*) parameter. The average pH measurements have varied as follows: F_{SR} (6.10 for non-treated control 0.1MPa/6.09 for HHP processed) and F_{SRE} (6.10 for non-treated control 0.1MPa/6.15 for HHP processed). Water activity for F_{SR} of 0.980 for non-treated control 0.1MPaa nd 0.980 for HHP processed, and F_{SRE} of 0.980 for non-treated control 0.1MPa/ and 0.978 for HHP processed.

Table 5 shows the regression coefficients and analysis of variance (ANOVA) obtained for the regression models for the significantly altered quality attributes responses of syneresis, lipid oxidation (TBAR) and *hardness* texture profile parameter in F_{SR} and F_{SRE} low-sodium sliced vacuum-packaged RTE turkey breast formulations. The dependent response called syneresis, or the amount of released liquid in the sample package after HPP processing, was significantly affected in the pressure and time ranges studied. In the F_{SR} formulation, a significant increase in exudates was observed (p<0.10), with the variable pressure load linearly affected (p-value 0.052). However, in the F_{SRE}, the amount of released liquid was affected by the linear term of pressure level and holding time (p≤0.05), with a significant increase observed when the pressure level and holding time were amplified. The average syneresis values observed in the control non-treated samples (0.1MPa) were 2.83 and 3.94 (g/100g) for F_{SR} and F_{SRE} formulations, respectively. In extreme conditions of the HPP studied range, syneresis values reached next to 6g/100g of product in both formulations. The syneresis is an important response in low-sodium meat products, mainly because salt affects protein hydration and water holding capacity. The significant effect of pressure levels and holding time must be considered in HPP processing. Vercammen et al. (2011) observed a statistically significant syneresis increase of approximately 2% for the unpressurized control to 4-5% immediately after HP (600MPa/10 min/10°C) treatment for a cooked ham model and these values were stable over 10 weeks of storage.

Table 5. Regression coefficients and analysis of variance (ANOVA) for the regression models for responses of significant quality attributes in F_{SR} and F_{SRE} formulations.

Parameter	Syneresis (g released liquid/100g)					
	F_{SR}			F_{SRE}		
	R.C	**S.E**	***p*-level**	**R.C**	**S.E**	***p*-level**
Mean/Intercept	3.917647	0.273021	0.000030	4.070588	0.289296	0.000033
Pressure Load	0.421871	0.167190	0.052961	0.604663	0.177157	0.018978
Pressure Load^2	0.195221	0.198996	0.371633	0.048529	0.210859	0.827094
Holding time	-0.047951	0.167190	0.785767	0.455805	0.177157	0.049860
Holding time^2	-0.112132	0.198996	0.597434	0.161765	0.210859	0.477622
Pressure X Time	-0.041176	0.236443	0.868579	-0.33970	0.250538	0.233149
Regression ANOVA and goodness of fit						
	F_{SR}			F_{SRE}		
Regression (F_{ratio})	-			9.8393**		
Lack of Fit (*p*-value)	-			0.1689		
R^2	-			0.7109		
*Fitted model**	-			*Syn.*(g/100g)= 4.07+0.60*p*+0.35*ht*		
TBARs mg malondialdehyde/kg (MDA/kg)						
	F_{SR}			F_{SRE}		
	R.C	**S.E**	***p*-level**	**R.C**	**S.E**	***p*-level**
Mean/Intercept	0.123365	0.022606	0.002809	0.064861	0.004989	0.000048
Pressure Load	0.011498	0.013843	0.444033	-0.00521	0.003055	0.148732
Pressure Load^2	-0.006933	0.016476	0.691369	-0.00083	0.003636	0.827473
Holding time	0.000973	0.013843	0.946714	0.008529	0.003055	0.038366
Holding time^2	-0.035781	0.016476	0.081975	-0.00432	0.003636	0.287487
Pressure X Time	0.011843	0.019577	0.571631	-0.01169	0.004321	0.042491
Regression ANOVA and goodness of fit						
Regression (F_{ratio})	-			6.4717**		
Lack of Fit (*p*-value)	-			0.4524		
R^2	-			0.6180		
*Fitted model**	-			*MDA/kg* = 0.064+0.008*ht*+0.004*ht.p*		
***Hardness* (N)**						
	F_{SR}			F_{SRE}		
	R.C	**S.E**	***p*-level**	**R.C**	**S.E**	***p*-level**
Mean/Intercept	21.46773	0.859568	0.000002	20.39378	0.392591	0.000000
Pressure Load	-0.38334	0.526376	0.499120	1.38592	0.240412	0.002206
Pressure Load^2	2.54318	0.626512	0.009736	0.76340	0.286148	0.044462
Holding time	0.13488	0.526376	0.807967	0.64786	0.240412	0.043052
Holding time^2	0.43355	0.626512	0.519749	-0.00916	0.286148	0.975711
Pressure X Time	0.57089	0.744408	0.477768	-0.78658	0.339994	0.068606
Regression ANOVA and goodness of fit						
Regression (F_{ratio})	21.9826**			10.8945**		
Lack of Fit (*p*-value)	0.9209			0.3763		
R^2	0.7095			0.8236		
*Fitted model**	*(N)* = 21,46+2.54p^2			*(N)* = 20.39+1.38*p*+0.76p^2+0.64*ht*		

R.C – Regression coefficients / S.E – Standard Error / F_{SR} (formulation with sodium reduction only) and F_{SRE} (sodium reduction formulation plus natural antimicrobial: Carvacrol)

*Codded reparametrized (model including only significant terms $p \leq 0.05$) with pressure named as (*p*) and holding time as (*ht*).

F_{ratio} = $\text{Regression}_{\text{mean square}}$ / $\text{Residual}_{\text{mean square}}$

Model adequacy**($F_{ratio} >> F_{critical}$) = $F_{0.05\,/\,2,8}$= 4.46 / $F_{0.05\,/\,1,9}$ =5.12 / $F_{0.05\,/\,3,7}$ = 4.35

The range evaluated for the variable pressure level and holding time significantly affected ($p \leq 0.05$) the lipid oxidation stability of the HPP-treated sliced vacuum-packaged RTE turkey breast. After 7 days of refrigerated storage post-HPP, the observed mean values for control non-treated samples (0.1MPa) were 0.059 and 0.058 mg MDA/kg for F_{SR} and F_{SRE} formulations, respectively. Therefore, after pressurization, the formulations without carvacrol suffered an intense oxidation process with an observed increase in TBAR values for the F_{SR} formulation. The triggered oxidation was observed in all studied pressure and time ranges. However, a different behavior may be seem in formulations with carvacrol added at 200ppm when the pressurization resulted in increased TBARs values, but these oxidation rates were dependent on HP processing intensity, mainly the holding time of the processing. According to Medina-Meza et al. (2014), high pressure changes the thermodynamic equilibrium of chemical reactions. As in lipid oxidation, the kinetics are accelerated in the presence of high hydrostatic pressure. A pressure of 300-400MPa appears to be a critical pressure for inducing marked changes in meat. The TBAR index variation among 11 processing conditions in the F_{SR} formulation was found not to be significant ($p > 0.05$). However, average values found in this formulation among all tested processes were higher than in F_{SRE} formulations. For the most intense HPP treatments of 600MPa/105sec, the TBAR index reached 0.152 mg MDA/kg in the F_{SR} formulation compared to 0.061mg MDA/kg for the F_{SRE} formulation for this same process. In the F_{SRE} formulation, the variable holding time affected ($p \leq 0.05$) the lipid oxidation, with longer treatments being more problematic for this answer. Importantly, the TBAR index increased in the F_{SRE} formulations, but the values were always (in all tested processes) lower than in formulations without carvacrol. This observation endorses the recognized antioxidant role of the phenolic compounds including carvacrol (5-isopropyl-2-methylphenol) against lipid oxidation processes (Ruberto and Baratta, 2000). The antioxidant activity of phenolic compounds is related to the hydroxyl groups linked to the aromatic ring, which is capable of donating hydrogen atoms with electrons and stabilizing free radicals (Oliveira et al., 2012b). According to Medina-Meza et al. (2014), the extent of post-HPP treatment oxidation can be reduced by applying protective strategies such as the addition of antioxidants and chelating agents.

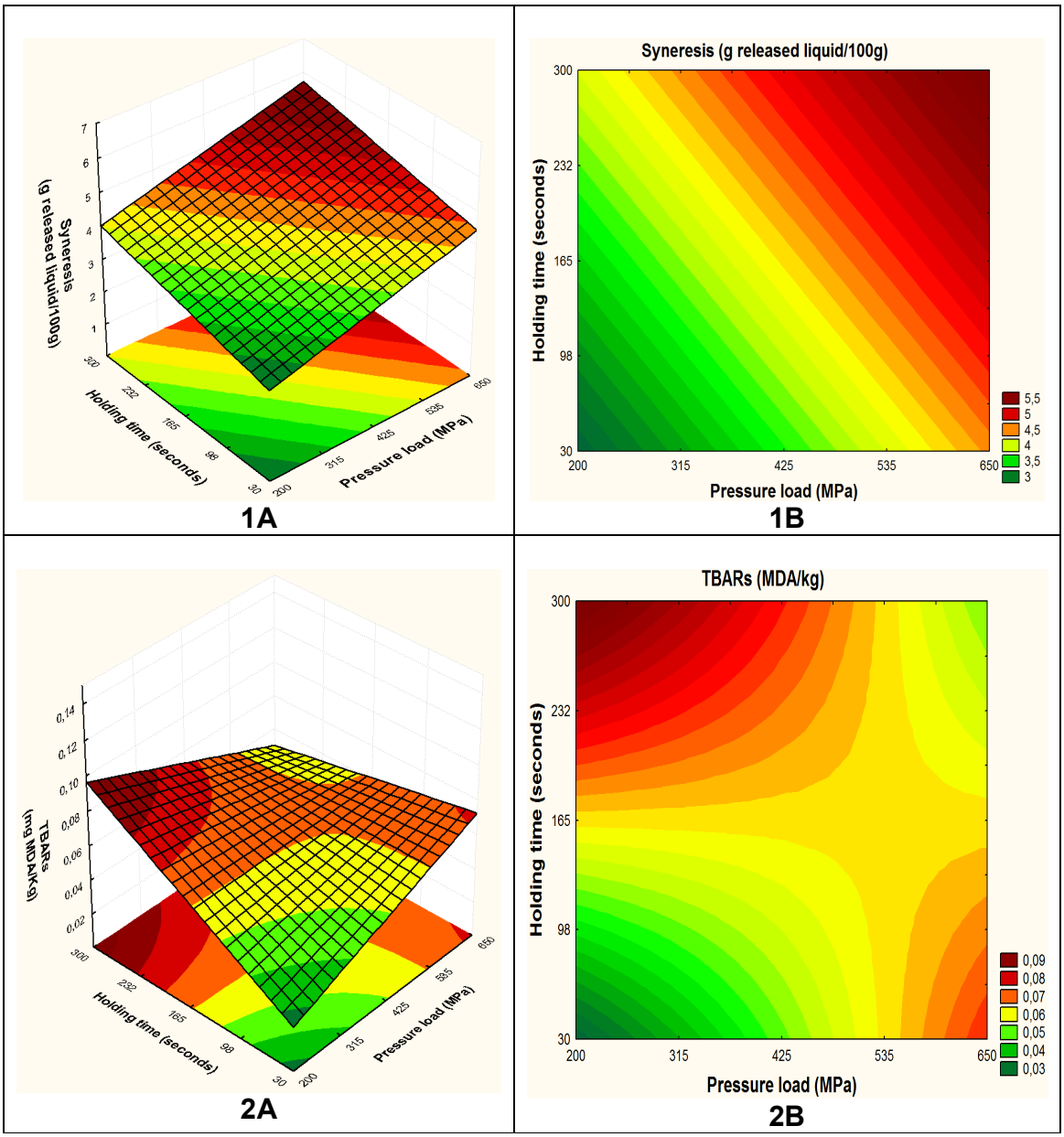

Figure 4. Fitted response surface and contourplots for the effects of variables (pressure load and holding time) on quality-attributes after 7 days at 4±2°Cafter high pressure processing.1A and 1B – **F_{SRE}** formulation (syneresis); 2A and 2B **F_{SRE}** formulation (TBARs) ($p \leq 0.05$). Processing temperature held at 25°C.

The evaluated pressure levels and holding time values did not disturb ($p \leq 0.05$) textural parameters of *cohesiveness*, *adhesiveness*, *springiness* and *chewiness*. However, the textural parameter *hardness* was significantly affected. The effect of the variable pressure level was expressed in quadratic and linear terms for both F_{SR} and F_{SRE} formulations. Despite the processing time, this variable

did not significantly affect the *hardness* response. The short (30-300 seconds) holding time range of the study did not affect the response. A significant decrease in *hardness* measurements compared with control treatments (27.34 and 24.27 N for F_{SR} and F_{SRE}, respectively for 0.1MPa), may be identified under mild pressure conditions (next to 425 MPa), with the extreme processing conditions of 600MPa leaded to a hardness increase probably due to the physical compression effects, that's may be correlated to the water syneresis values evolution. According to the review by Sun and Holley (2010), HHP textural effects depend upon the meat protein system integrity, the pressure and the duration of the pressure treatment for raw and processed meats.

Regarding the HHP on quality-attributes of RTE meat products, several studied have revealed significant effects in lipid oxidation, rheology measurements and CIELAB color indexes (Alba et al., 2012; Clariana et al., 2012; Liu et al., 2012; Mathias et al., 2010). Applicable 600MPa pressurelevel and holding time >120 seconds for microbiological purposes appears to be problematic for syneresis results, showing a 50% increase for in-package released liquid. Under this HP setup conditions, the lipid oxidation indexes were higher in HHP-treated samples compared with controls. However, the impact on consumer acceptance of the alteration of these quality-attributes is expected to be minimal.

3.3 HPP and combined effects during refrigerated storage

During the validation of the selected HHP treatment (600MPa/180sec/25°C) target naturally occurring microbial populations of LAB, psychrotrophic bacteria and inoculated *L. innocua* were enumerated (60 days/4°C) as shown in Figures 5, 6 and 7. Initially, as depicted in results the carvacrol application alone (without HPP) at the 200ppm sub-inhibitory (MIC/32) concentration was not able to control the growth of microbial populations. This event was similarly detected in above described previous step of HHP optimization, being not detected a population reduction (inoculums of *L. innocua*, and *Lc. mesenteroides* and *Lb. sakei*) 24hs post-inoculation for controls non-HPP treated (0.1MPa in F_{SRE}).

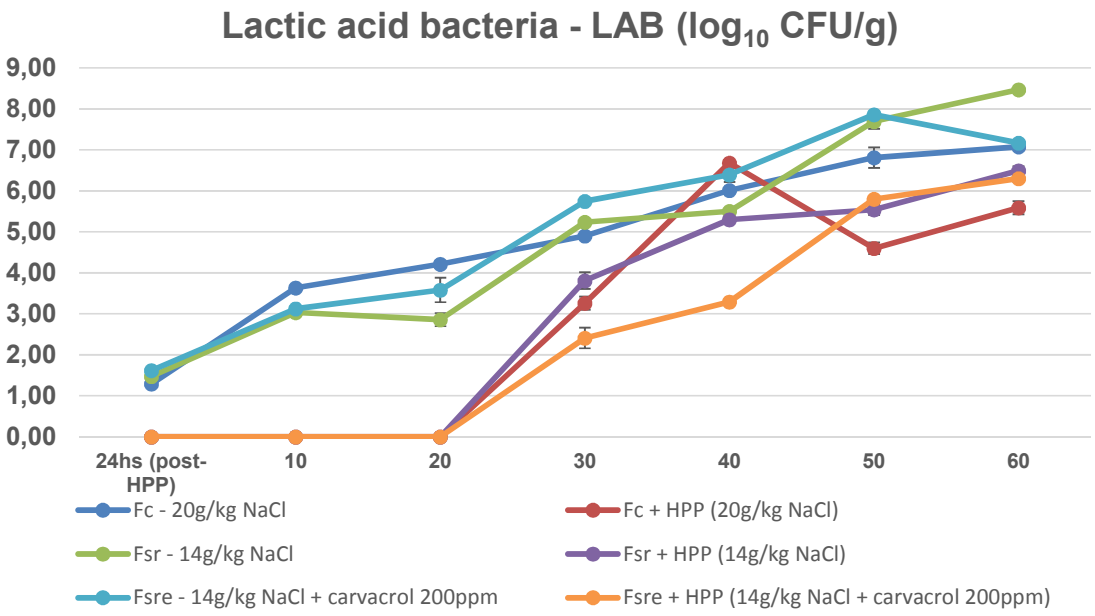

Figure 5. Shelf-life evaluation during 60 days under refrigeration (4°C) of sliced vacuum-packaged RTE turkey breast formulations pressurized at HPP optimized (600MPa/180seconds/25°C) or non presurized. Average population values ± S. E. (standard error).

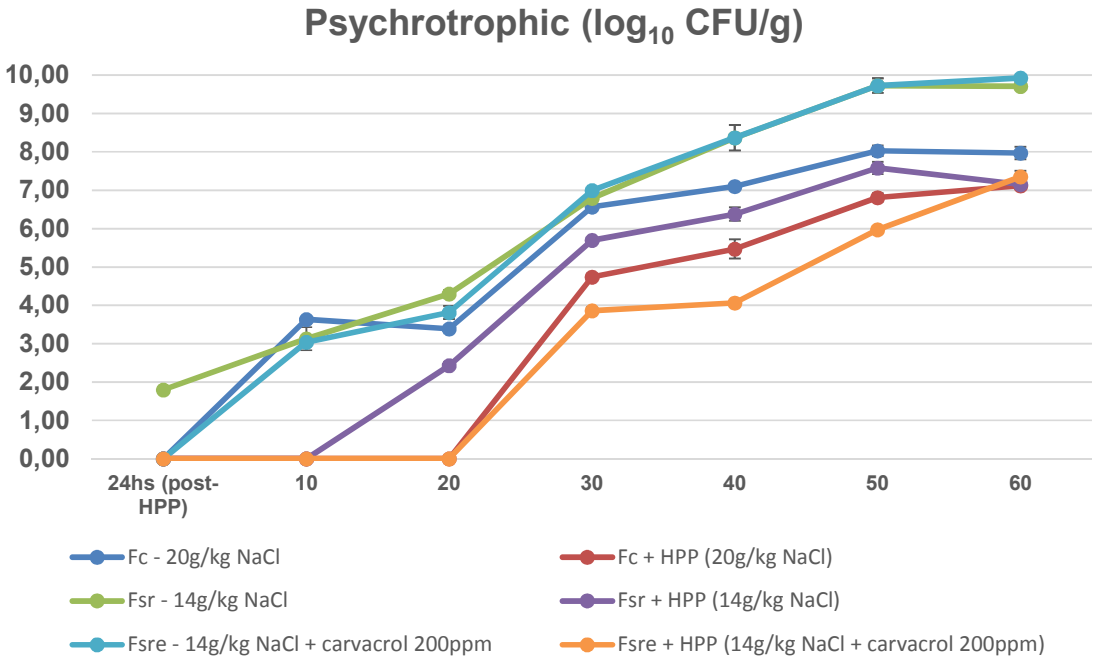

Figure 6. Shelf-life evaluation during 60 days under refrigeration (4°C) of sliced vacuum-packaged RTE turkey breast formulations pressurized at HPP optimized (600MPa/180seconds/25°C) or non presurized. Average population values ± S. E. (standard error).

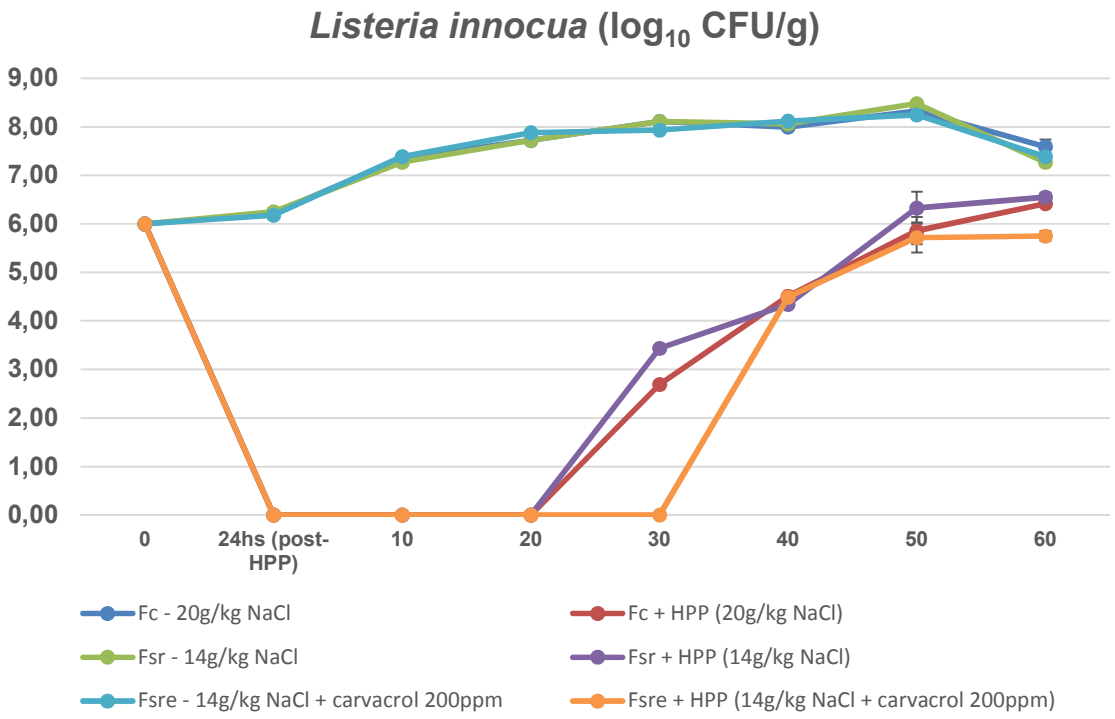

Figure 7. Shelf-life evaluation during 60 days under refrigeration (4°C) of sliced vacuum-packaged RTE turkey breast formulations pressurized at HHP optimized (600MPa/180seconds/25°C) or non presurized. Average population values ± S. E. (standard error).

The antimicrobial effect of carvacrol observed *in vitro* was at the level of 6500ppm, the sub-inhibitory tested concentration being ineffective. Several studies showed that the *in vitro* MIC carvacrol ranged from 0.1-5 µl/ml with different pathogen and spoilage strains (Burt, 2004; Silva-Angulo et al., 2014). However, in food matrices, the required concentration for significant effects varies significantly. The powerful antimicrobial activity of natural antimicrobials was demonstrated to be minimized when applied in food matrices (compared to the *in vitro* effects), mainly due to the food component interactions and the bacterial protection effects (Gutierrez et al., 2008). Thus, evaluating our sensorial results, the limit of carvacrol application to low-sodium turkey breast ham was 200ppm, and this point needs to be considered.

The carvacrol is a very common essential oil constituent existing in significant concentrations (usually a major compound) in many spices and aromatic plants including oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*). As a

GRAS (Generally Recognized as Safe) status food additive, carvacrol is used as a flavoring agent in baked goods, sweets, beverages and chewing gum (Fenaroli, 2002). Carvacrol has been registered by FDA (21 CFR 172.515), and the European Commission (EU Regulation 1334/2008 & 178/2002) for use as flavoring in foodstuffs because carvacrol is considered to present no risk to the health of consumers. Nevertheless, despite carvacrol having shown a strong antimicrobial activity against many spoiling and pathogenic bacteria, its application in food preservation, as with most natural extracts, may be limited due to the high concentrations required. These high concentrations are usually associated with an undesirable flavor and sensory developments (Ait-Ouazzou et al., 2013).

The selected HPP treatment effectiveness was proved evaluating the population LAB and psychrotrophic bacteria growth during shelf-life; lower counts or no growth were detected among pressurized samples until 20 days of refrigerated storage. After outgrowth, these populations maintained reduced ($p \leq 0.05$) along entire evaluated storage time. Concerning the combined application of carvacrol and HHP, the preservation improved effects was powerfully supported in the shelf-life study being observed lower populations of psychrotrophic and LAB among all evaluated treatments in F_{SRE} plus HHP conditions. In particular, for pressurized formulations, when the bacterial growth starts up after 20 days of storage, in the F_{SRE} plus HHP conditions lower ($p \leq 0.05$) spoilage microbial populations were detected. The shelf-life extending effects and preservative improve effects of combined treatments were mightily confirmed. The main spoilage groups of vacuum-packaged processed meats including lactic acid bacteria and psychrotrophic (mainly composed by *Pseudomonas sp., Brochothrix thermosphacta, Shewanella and Serratia)*, being their outgrowth reduced during refrigerated storage by HPP and in combined conditions. For *L. innocua* pressurized F_{SRE} formulation outgrowth were detected only after 30 days of storage, being started at day 20 for another pressurized formulation. Slongo et al. (2009) evaluated the effect of HHP processing at 400MPa/15min in naturally occurring LAB microflora in the sliced vacuum-packaged ham. Under these processing setup conditions, a lag phase extension may be observed in more than 40 days, and the total shelf-life of the treated product under refrigerated storage

increased up to 85 days. Vercammen et al. (2011) evaluated the inactivation rates of HHP processing by varying pressure load (100-700MPa), temperature (5, 25 and 40) for 10 minutes against spoiling cooked ham flora including *Lc. carnosum* and *Lb. sakei*, identifying a suitable treatment of 600MPa/10°C/10min for a 5D inactivation performancecriterion. Thus, the combined effects of HHP (600MPa/10°C/10min) and natural preservatives including caprylic acid (0.15%) or a mixture of potassium lactate and sodium diacetate (2.5% - Purasal) were evaluated against total LAB flora during refrigerated storage. These authors observed that the HHP treatment alone delayed spoilage to 59 days. However, microbial growth was completely suppressed during at least 84 days in the samples treated with physical HHP treatment and natural barriers.

4. Conclusions

Following the required performance criteria for *Listeria* post-lethality treatments (4-5 log reductions), a value of 600MPa/180 seconds at 25°C appears to be a suitable treatment for the studied low-sodium product, promoting effective inactivation rates with slight quality-attribute alteration. The HPP processing setup with a pressure level of 600MPa and a holding time of 180 seconds promoted >5 log reduction of all target pathogen and spoilage microbial groups evaluated. Significant instrumental alterations of quality-attributes were observed; however, further sensory confirmation thus needs to be performed to connect these data. The HPP processing represents a feasible alternative to maintain safety and extend shelf-life of unstable low-sodium RTE processed meats mainly with post-processing manipulation such as slicing (*Slice Safety Risk*). The addition of an additional natural barrier promotes higher inactivation rates and improvements of preservation effects, being effective at sensory acceptable sub-inhibitory levels. HP bacterial inactivation effects can be noticeably potentiated through the presence of NAs, representing a promising weapon against sub-lethal injury and cell recovery phenomena. The intensity of pressures required to inactivate microorganisms may be reduced in the presence of antimicrobial compounds, since moderate pressurization or short exposures can cause sublethal injury to bacterial cells, making them more susceptible to antibacterial compounds such as plant derived

volatiles. Combined HPP plus additional NA hurdles may become possible with similar safety and shelf-life extension effects with mild HP treatments (setup conditions involving holding time, pressure loads and temperature), thereby assuring the food industry of reduced costs for initial equipment installation and maintenance and maximizing processing output by effectively shortened cycles (higher productivity cycles per hour). Less intense cycles results in increased maintenance of food freshness and global quality of HP-processed food.

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Capítulo 4: Phenolic carvacrol as a natural additive to improve preservative effects of high pressure processing for low-sodium sliced ready-to-eat turkey breast

RESUMO

A alta pressão (HPP) é uma promissora tecnologia emergente não térmica amplamente aplicada no processamento de alimentos. Entretanto, apesar dos benefícios já estabelecidos para efeitos de conservação, a literatura moderna tem destacado o sucesso da aplicação combinada de HPP a agentes bioativos antimicrobianos naturais, numa abordagem de múltiplas barreiras. Esta pesquisa objetivou avaliar as melhorias nos efeitos de conservação por HPP (600MPa/180sec/25°C), sobre aspectos físico-químicos e microbiológicos, resultantes da adição do fenólico bioativo carvacrol em níveis sensorialmente aceitáveis (200ppm), durante 60 dias de estocagem refrigerada (4°C), de embutido de peru fatiado embalado a vácuo formulado com teor reduzido de sódio. Com relação aos atributos de qualidade, a adição do carvacrol foi capaz de reduzir (p<0.05) os índices de oxidação lipídica em amostras de embutido de peru pressurizadas ao longo da estocagem. Modelos de crescimento primário ajustados mostraram um efeito significativo de extensão de vida de prateleira por meio de reduções das taxas de crescimento de bactérias deteriorantes (bactérias láticas e psicrotróficos) e extensão de fase lag para o patógeno-alvo *Listeria,* devido à adição da barreira natural. Melhorias dos efeitos de conservação em amostras combaixo teor de sódio (30% de redução de NaCl) também foram detectadas. Efeitos de conservação foram notavelmente potencializados, uma vez que níveis de populações problemáticas de microrganismos deteriorantes foram atingidas tardiamente pela adição do composto bioativo natural; em adição, reações adversas de oxidação lipídica aceleradas por conseqüência de processos HPP, foram desaceleradas.

***Palavras-chave**:* Barreiras multiplas, Tecnologias emergentes, *Listeria monocytogenes*, deterioração, oxidação lipídica.

ABSTRACT

High pressure processing (HPP) is a promising emerging non-thermal technology widely applied in food processing. However, despite established advantages in conservation effects, current literature has been highlighted the success of a combined application of HPP plus natural antimicrobials in multi-hurdle perspective. This research aimed to evaluate the effects of HPP (600MPa/180sec at 25°C) combined with natural phenolic bioactive carvacrol (200ppm) on microbiological and physico-chemical characterisitics of a low-sodium sliced vacuum-packaged RTE turkey breast during 60 days of refrigerated (4°C) storage. Concerning quality attributes, carvacrol was able to reduce TBARs indexes (p<0.05) in pressurized samples that was triggered by HPP in ready-to-eat turkey breast. Adjusted primary growth models showed a significant shelf-life extension by reduced growth rates and maximized lag phases of *Listeria* and major spoilage groups of lactic acid and psychrotrphic bacteria due to additional natural hurdle. Improvements against preservation effects in samples with low sodium (30% NaCl reduction) were also detected. Shelf-life were remarkably potentiated, since problematic populations of spoilage microorganisms were achieved delayed, due to addition of the natural bioactive compound; in addition, adverse unwanted reactions of lipid oxidation triggered as a consequence of HPP, may be slowed.

***Keywords**:* Multi-hurdle approach, Emerging Technologies, *Listeria monocytogenes*, spoilage, Ready-to-eat, lipid oxidation.

1. Introduction

High pressure processing (HPP) emerged as a promising non-thermal food preservation technique being extensively applied in modern food processing industries mainly due their benefits of nutritional and sensory quality aspects maintenance. Briefly, HPP is a method that consists of subjecting food to intense pressure loads up to 1000MPa, using a compressible pressure-transmitting fluid; these intense pressure loads are applied aiming eliminate pathogenic and spoilage microorganisms and inactivate deterioration enzymes, preventing main food degradation process mechanisms. It has been widely applied in food processing of ready meals, fruits, juices and smoothies, sauces, dairy products and raw and ready-to-eat meat products (RTE's) (Campos, Dosualdo, & Cristianini, 2003; Rendueles et al., 2011; Simonin, Duranton, & Lamballerie, 2012). The main proposals of HPP application have been set on pathogen elimination; spoilage bacteria inactivation and shelf-life extension; clear and friendly labels development without chemicals, innovative fresh products and especially for safety and quality of "low-sodium" reformulated products (Avure® Technologies, 2014).

Processed meats are recognized as one of the major sodium sources of modern human diets; it shows elevated levels of a sodium chloride (~2.0% w/w) as multifunctional ingredient in their formulations that acts on sensory, physico-chemical and especially as a preservation agent. High sodium intake has been linked with blood pressure increase and cardiovascular diseases and stroke development. Thus, sodium reduction innovative alternatives and strategies, such as HPP, represent an emerging focus regarding public health agencies around the world (Ruusunen & Puolanne, 2005; Desmond, 2006; Doyle & Glass, 2010). As a tool for sodium chloride reduction in sliced RTE meat products, HPP has been applied for safety, controlling emerging pathogens such as *Listeria monocytogenes* and shelf-life extension against spoilage bacteria (Duranton et al., 2012; Myers et al., 2013); usually HPP set up applied for meeting these microbiological purposes in meat products uses pressures around 600MPa in a range time of 180-600 seconds at room temperatures (25-30°C) (Bajovic, Bolumar & Heinz, 2012; Avure® Technologies & NC-Hyperbaric® factsheets 2014). However, despite the clearly effectiveness in microbial inactivation post-HP processing, variable intrinsic

baroresistence of microrganisms combined to food matrix characteristics (composition, pH and a_w, salt) may result in sub-lethal injuries and recovery phenomena inquiring food safety and quality; it is also well documented for example, that when HPP is used for improving safety and prolonging the shelf life of meat and meat products, a large number of sub-lethally injured micro-organisms can revive and become fully functional in a favorable environment during subsequent storage (Han et al., 2011). To overcome these problems, the intensity of the treatment applied could be easily increased. However, this in turn could be economically not feasible in the case of HPP treatments, or detrimental to the quality of the product in the case comparing of *e.g*. heat treatment (Karatzas et al., 2001). For sliced vacuum-packaged low-sodium (30% salt reduction) ready-to-eat turkey breast HPP processed at 600MPa/180sec/25°C, aiming microbiological inactivation purposes, Oliveira (2015) registered required effective log reductions of target pathogen and spoilage microbial populations post-HP processing; nevertheless, marked significant effects in quality-attributes including lipid oxidation, liquid exudates in samples packages (measured as syneresis) and textural parameter of *hardness* were also detected. In addition, bacterial outgrowths for target evaluated groups were observed during refrigerated shelf-life evaluation. Considering the intensification of the high pressure processing variables (pressure load, holding time and temperature) noticeable instrumental and sensory adverse effects may be reached.

A possible alternative to improve preservative effects of HPP is the employment of an additional hurdle that could act synergistically or additively against microorganisms. Natural bioactive compounds such as essential oils, extracts and their isolated compounds may represent feasible alternatives, additionally assuring HPP natural claims. A suitable compound for combined use with HPP is carvacrol (5-isopropyl-2-methylphenol), a phenolic bioactive plant-derived flavour compound present as a major constituent in several spices (oregano, thyme, savory) essential oils fractions; carvacrol is widely known for its antimicrobial and antioxidant activity (Ultee et al.1998). Its hydrophobic nature allows for accumulation in the bacterial cytoplasmic membrane, where it can elicit several toxic effects that may eventually lead to cell death (Sikkema et al. 1994;

Ultee et al. 2002). Additionally, considering bacterial membranes as main HPP active site, a synergistically or additively conservation effects may be expected (Ait-Ouazzou et al., 2013). Antioxidant effects were also attributed to carvacrol; this effect is related to the hydroxyl groups linked to the aromatic ring, which is capable of donating hydrogen atoms with electrons and stabilizing free radicals (Oliveira et al., 2012a). According to Medina-Meza, Barnaba and Barbosa-Cánovas (2014) the extent of post-HPP treatment oxidation can be reduced by applying protective strategies such as the addition of antioxidants.

This research aimed to evaluate the high pressure processing preservation improvements regarding microbiological and physico-chemical aspects resultant of the addition of natural phenolic bioactive carvacrol at sensory acceptable levels (200ppm) in low-sodium sliced vacuum-packaged RTE turkey breast processed at applicable usual HPP conditions for sliced meat products (600MPa/180sec/25°C).

2. Material and Methods

2.1 Carvacrol as natural additional HPP hurdle

2.1.1 Minimal Inhibitory Concentration (MIC)

The antimicrobial effectiveness of carvacrol (natural 99% Food grade/W224511 Sigma-Aldrich®) was previous tested against *Listeria monocytogenes* ATCC 19117 (provided by the INCQS-FIOCRUZ collection). *In vitro* minimum inhibitory concentration (MIC) was assessed by the broth microdilution method proposed by Oliveira et al. (2012b). Briefly, the *L. monocytogenes* strain was cultured in TSB-YE 37°C/24h (Tryptic Soy Broth supplemented with yeast extract at 0.6%) and used as the initial inoculum. The following concentrations of the natural compound were prepared in flat-bottomed sterilized 96-well polystyrene microplates: 10.00, 5.00, 2.50, 1.25, 0.65, 0.30, 0.15, 0.08% v/v. These working concentrations were obtained by homogenizing pure carvacrol with TSB containing 0.5% of TWEEN® 80 solution in a final volume of 150µL in each microplate well. Thus, 10µL of the standardized bacterial initial inoculum (10^7 CFU/mL) was added to wells, and three repetitions were performed. One column in the microplate was prepared without inoculum to assess the influence of absorbance derived from carvacrol + TSB in the observed results. The

prepared microplates were capped and incubated at 37°C/24h. The absorbance was measured at 620nm in a microplate reader prior to incubation and after 24h. From the absorbance value obtained at 24h, the reading at the time 0 was subtracted. The MIC corresponded to the lowest concentrations of the compounds that resulted in the complete inhibition of the bacterial growth (no absorbance evolution compared to the control compound + TSB + TWEEN® without inoculum). The inhibition was confirmed by inoculating 10µL of the well solution in a test tube containing TSB-YE 37°C/24h.

2.1.2 Sensory evaluation studies

The original research project was initially submitted and previously approved by the Ethics in Research Committee of the Brazilian platform. To determine the maximum acceptable level of the selected natural antimicrobial carvacrol in RTE turkey breast as indicated by consumers, a sensory test of "*Difference from Control"* was employed. This test is commonly utilized to determine if there is any significant difference between a control sample (standard) and other treatments. The tests were conducted in appropriate individual booths (standardized), under artificial daylight-type illumination and with temperature control (between 22 and 24°C) and air circulation following recommendations of Meilgaard et al. (2007). Briefly, the assessors received a standard turkey breast ham sliced sample (without carvacrol) coded as P, and four samples of added carvacrol at levels of 1625ppm (MIC/4), 812ppm (MIC/8), 406ppm (MIC/16) and 203ppm (MIC/32). The standard (P) was included coded among the samples; however, during processing a strong adverse impact were detected in MIC/4 and MIC/8 levels being tested only 406 and 203 against control formulation. The low-sodium turkey breast ham was processed as described below in item 2.2. The team of assessors (40) was informed to taste the standard (P) sample (slices of turkey breast ham, measuring 4mm in thickness, on plastic plates coded with 3 digits) and the samples with added carvacrol, indicating the difference using an auxiliary scale (0-no difference and 9-extremely different). The obtained results were evaluated by an analysis of variance (ANOVA) and *Dunnett* mean test, comparing standard P and the samples with carvacrol.

2.2 RTE turkey breast formulations and processing

Three different RTE turkey breast formulations were evaluated, being submitted to HPP processing or non-HPP conditions: one control with entire traditionally employed amount of *NaCl* salt 20g/kg (±1005mg/100g of sodium) codded as **F_C**; one with *NaCl* reduction only named **F_{SR}** with 14g/kg NaCl (±722mg/100g of sodium, with a reduction of 30% compared to control sample with 20g/kg - nearly a 25% sodium reduction) and another with salt/sodium reduction (30% NaCl less) plus carvacrol addition at 200ppm (after 2.1.1 and 2.1.2 data analysis) named **F_{SRE}**. The NaCl reduction level (30% NaCl reduction) had been determined in a previous research step conducted by Oliveira (2015) evaluating sensorial, physicochemical and microbiological aspects. The different RTE turkey breast formulations (Table 1) and all processing characteristics were executed according to Galvão et al. (2014).

Table 1. RTE Turkey breast formulations

Raw material/Ingredient	**g/kg**	**F_c**	**F_{sr}**	**F_{sre}**
Skinless turkey breast	700.00	700.00	700.00	700.00
Water/ice	235.45	235.45	241.45	241.45
Sodium Chloride (*NaCl*)	**20.00**	**20.00**	**14.00**	**14.00**
Cassava Starch	20.00	20.00	20.00	20.00
Soy Protein (Isolate)	10.00	10.00	10.00	10.00
Sugar	5.00	5.00	5.00	5.00
Spice/Seasonings	0.30	0.30	0.30	0.30
Phosphates	3.50	3.50	3.50	3.50
Carrageenan	3.00	3.00	3.00	3.00
Monosodium glutamate – GMS	2.00	2.00	2.00	2.00
Sodium erythorbate	0.50	0.50	0.50	0.50
Sodium nitrite (*$NaNO_2$*)	0.15	0.15	0.15	0.15
Carmine coloring	0.10	0.10	0.10	0.10
Carvacrol solution (carvacrol + polysorbate POE 80 and propylene glycol food grade)	**mg/kg ou ppm**	**-**	**-**	**200.00**
TOTAL		1000.00		

***Fc–** Control formulation (20g/kg of *NaCl* – 1005mg Na/100g) ****Fsr–** Sodium reduced (14g/kg of *NaCl* – 722mg Na/100g; 30% of salt reduction) *****Fsre** - Sodium reduced (14g/kg of *NaCl*) plus carvacrol 200ppm.
Carvacrol-natural 99% Food grade/W224511 Sigma-Aldrich® FDA 21CFR172.515 and European Commission (EU Regulation 1334/2008 & 178/2002).

Frozen vacuum-packaged deboned raw turkey breast meat (*Pectoralis major* and *Pectoralis minor*, 70±5% moisture, 3±1% fat, 20±2% protein, pH 5.9±0.2) was obtained within 72h of slaughtering from BRF® Brasil foods. After thawing, turkey breast skins were removed, and the raw material was subjected to a grinding process with 90% in a grinding disc of 35mm and the remaining 10% disk of 4mm. Then, all the ingredients/additives excepting the starch (cassava starch) and soy protein isolate were added to 60% of the total water used and homogenized for preparation of brine (up to the formation of a single phase). In F_{SRE} the purified carvacrol (natural 99% Food grade/W224511 Sigma-Aldrich® FDA 21CFR172.515) was added to brine with seasonings in a stock solution containing pure carvacrol at 25.000ppm (carvacrol + polysorbate POE 80 and propylene glycol food grade), to a final concentration of 200ppm. The brine was added to the ground turkey breast meat and mixed for 20 minutes in an industrial blender (Jamar®, Brazil). Subsequently, the remaining ingredients (starch/soy) and water were added with a further 10 minutes of mixing. The product was embedded in plastic polyamide Visflex casing (Viskase®) with a 76 mm diameter and subjected to the cooking process in a chamber with appropriate staggered internal temperatures reaching 74°C (measured by a thermocouple inserted into the core of the product). The cooked RTE turkey breast pieces were cooled in a water bath for 10min and stored in a controlled chamber at 4±1°C for further procedures.

2.2.1 Slicing, packaging and storage

After the RTE turkey breast processing, the stuffed pieces (500 ±50g) were stored in a controlled chamber at 4±1°C for 24h until slicing operations. The pieces were aseptically opened in laminar flow chamber and sliced into a 4mm thickness (a 10mm thickness slice was used for texture analysis purposes) and subsequently vacuum-packaged in 150x300mm Nylon-Poly 16μ (COEX:LDPE-PA-LDPE) with a permeability rate of PRO_2 of $50cm^3 \cdot (m^2 \cdot day)^{-1}$. The microbiological analytical units were composed of three slices of 4mm thickness with 10±1g. The physic chemical analytical units were composed of three slices of 4mm and one 10mm-thickness slice. The samples were stored under refrigeration temperature 4±1°C during shelf-life of 60 days for analysis.

2.3 High pressure processing

The vacuum-packaged sliced RTE turkey breast samples were submitted to high pressure processing using the high hydrostatic pressure unit AVURE QFP 2L-700 (Avure Technologies® USA) with a 2L volume treatment chamber (inner vessel diameter 100x254mm), maximum vessel pressure of 690MPa (6900bar/100.000psi) and temperature control at 10 to 90°C. Pure demineralized water was used as a pressure-transmitting fluid. Two thermocouples located at the top and midway in the treatment chamber monitored the temperature of the pressure-transmitting fluid; another thermocouple monitored the temperature of the water jacket surrounding the pressure vessel. The average pressurization rate was 5±0.30 $MPa \cdot s^{-1}$. Considering the adiabatic heating that occurred during the pressurization (approx. 3°C/100MPa for water), the initial sample and water (pressure-transmitting fluid) temperatures were controlled to reach 25±1°C by the processing final time. After processing, the analytical units were immediately refrigerated for further analysis. A typical processing set up applied for sliced cooked cured vacuum-packed meat products at 600MPa/180seconds/25°C was used in experiments.

2.4 Microbiological evaluations

2.4.1 Spoilage target microbial groups: lactic acid and psychrotrophic bacteria

The main spoilage microbial groups involved in a deterioration of sliced vacuum-pakaged cooked meat products were lactic acid bacteria (LAB) and psychotrophic groups. These are responsible for ropy slime production, acidification, gas production, discoloration and sour-sweet odor in packaged samples (Cayré, Vignolo & Garro, 2003). Thus, naturally occurring (without initial inoculums) spoilage microbial groups of total psychrotrphic and lactic acid bacteria were enumerated in different evaluated treatments under HPP or non-HPP conditions. For the enumeration of the target spoilage microbial groups, 10g (one slice) of RTE turkey breast were weighed and transferred into sterile stomacher bags (Baglight®), combined with 90mL of sterile peptone water 0.1% (w/v) and homogenized in a Stomacher (Metroterm®, Brazil) with 490 strokes/2min at room temperature. Stomached slurries were decimally serially diluted in peptone water

and spread-plated in PCA - Plate Count Ágar 7°C/10 days of incubation for psychrotrophic bacteria; for lactic acid bacteria enumeration, diluted sample aliquots of 1000µL were pour-plated on sterile Petri dishes and covered (with an over layer) with molten de Man Rogosa and Sharpe agar MRS (HiMedia®, Mumbai, India) with the plates incubated at 32°C/48h before colony count.

These target spoilage populations were monitored during 60 days of refrigerated storage (4±1°C), being counted 24hs post-HPP (0) and every 10 days up to 60 days of shelf-life. The outgrowing population of LAB and psychotrophic were expressed as a $\log_{10}$ CFU/g of product.

2.4.2 Target pathogen: Listeria inoculation and enumeration procedures

Listeria innocua (ATCC 33090 serotype 6a type-strain) was used as a surrogate pathogen model for *L. monocytogenes* (mainly for processing plant pilot safety purposes). The strain was provided by Andre Tosello Tropical Collection (Brazil). *L. innocua* was cultured on TSB-YE (plus 0.6% yeast extract) by 37°C/48h (HiMedia, Mumbai India). After the strain grew, the bacterial cells were pelleted by centrifugation (3000g/5min) in microtubes (Eppendorfs) and covered by freezing culture media composed of (g/100g): glycerol 15.0, bacteriological peptone 0.50, yeast extract 0.30% and 0.50 NaCl, pH 7.2-7.4, and maintained under freezing temperatures (-20°C) throughout the experiments.

For work culture production prior to inoculations and HPP processing, an aliquot of the microtube was transferred to TSB-YE and grown with two subcultures at 37°C. The overnight cultures were pelleted by centrifugation (3000g/5min), the cells washed 2x with saline solution 0.85% (w/v), and the pelleted cells resuspended in a 50mL volume of saline solution 0.85% (w/v) that was standardized by the *McFarland Standard Scale* to 10^7 CFU/mL. The cell suspension was plated on TSA-YE for exact population quantification and confirmation prior to turkey ham slice inoculation. The inoculation procedure may be briefly described as follows: after the aseptic slicing operation of low-sodium RTE turkey breast, the slices obtained (4mm/10±1g) were placed in an aseptic laminar flow chamber under UV-light for ten minutes on each side to eliminate the residual flora that might influence the tests. Thus, 1000µL of the standardized cell

suspension (to final inoculum of ~10^6 CFU/g on slice) was spread on the product slice and let for 15 minutes (in the laminar flow chamber) prior to vacuum packaging.

For the enumeration of the survivor populations of *L. innocua*, 10g (one slice) of the samples were weighed and transferred into sterile stomacher bags (Baglight®), combined with 90mL of sterile peptone water 0.1% (w/v) and homogenized in a Stomacher (Metroterm®, Brazil) with 490 strokes/2min at room temperature. Stomached slurries were decimally serially diluted in peptone water and 100µL was spread on Listeria selective differential Palcam agar (Himedia®, Mumbai, India) supplemented (polymixyn B, ceftazidime and acriflavine) by incubation of the plates at 37°C/24-48h. The absence of growth (considered as 0 for the log reduction calculation purposes/detection limit <10 CFU/g) detected in some treatments was confirmed with pre-enrichment steps performed in Buffered Listeria Enrichment Broth (BLEB).

The survivor *L. innocua* populations were monitored during 60 days of refrigerated storage (4±1°C), being counted 24hs post HPP and and every 10 days up to 60 days of shelf-life, being expressed as a $\log_{10}$ CFU/g of product.

2.4.3 Microbiological analysis according Brazilian legislation requirements

Aiming assure the sanitary quality of different formulations of processed product, selected microbial populations following the Brazilian microbiological requirements (BRASIL, 2001) were monitored during 60 days (0, 30 and 60) of the refrigerated shelf-life; this includes the following microbial groups enumeration culture media/methods: total (35°C) and thermo tolerant (44,5°C) coliforms using Lauril Sulphate Tripotose broth (LST), Brilliant Green broth (VB) and *Escherichia coli* broth (EC); *Salmonella* sp., with pre-enrichment step in buffered peptone water, selective enrichment step in Tethrationate and Rapapport broth and growth in RAMBACH® Differential Selective Agar; Sulphide reducing Clostridia in SPS agar under anaerobic atmosphere; and *Staphylococcus* sp. in Baird Parker Agar base supplemented with egg yolk emulsion and added of potassium telluride solution (Silva et al., 2007).

2.5 Evaluation of quality-attributes during refrigerated shelf-life

2.5.1 Lipid oxidation (TBARs) and CIELAB color indexes

The effect of treatments on lipid oxidation was determined by the thiobarbituric acid reactive substances (TBAR) index according to Raharjo et al. (1992). Ten-gram portions of turkey breast ham samples were combined with 40 ml of 5% trichloroacetic acid (TCA) and 1 ml of 0.15% antioxidant BHT (2,6-di-tert-butyl-4-methylphenol (Sigma Aldrich) and refrigerated homogenized in ultrarrax for 5 min. Next, the homogenates were centrifuged (3000g for 5 min), and the supernatant was filtered through Whatman No. 1 filter paper. Two ml of filtrate was combined with 2 ml of 0.08 mol/L TBA reagent and heated in boiling water (100°C) for 5 min. The absorbance of the resulting solution was measured at 532 nm, and the TBAR (thiobarbituric acid reactive substances) values were expressed as mg of malondialdehyde (MDA) per kg sample, calculated using 1,1,3,3-tetraethoxypropane (TEP) as the standard.

Color measurements were taken with a colorimeter (Chroma meters CR300, Konica Minolta Sensing, Inc.) established at a 10° angle for the observer and illuminated at D65 to calculate color indices in the CIELAB system following the recommendations of Ramos and Gomide (2007). The color parameters lightness (*L**), redness (*a**) and yellowness (*b**) were obtained from an average of 5 readings taken at different points of 3 turkey breast ham slices of analytical units.

2.5.2 Syneresis and pH

The pH measurements and falling curves were obtained after initial dilution and homogenization of samples at a ratio of 1:10 (10g of sample in 100 ml of distilled water), followed by the introduction of meter electrodes in homogenized slurries for pH readings.

The syneresis or the total amount of released liquid in sample packages was determined by weighing the released liquid after processing and during the refrigerated shelf-life (0, 30 and 60 days of refrigerated storage). The syneresis formula was described as follows (Eq.(1)):

$$(1)\ \text{Released liquid (Syneresis) g/100g} = (W_{liquid}\,(g) / W_{sample}\,(g))*100$$

Where, W_{liquid} represents exudate into the package (g) and W_{sample} the weight of turkey breast ham packaged.

2.5.3 Texture Profile Analyses (TPA)

The texture was evaluated by TPA - Texture Profile Analysis, according to the recommendations of Bourne (1978), using a *TA.XT2i* Texturometer (Texture Analyzer, Stable Micro Systems, Inc., England) coupled to a microcomputer equipped with Texture Expert Software. The TPA test consists of compressing the sample two times in cycles in a reciprocating motion that simulates the jaw action, and extract, from the resulting force-time curve, a number of different textural parameters. Ten standardized squared cubes (10x10mm) of 3 independent turkey breast ham slices of 10mm thickness were cut for the TPA tests. Next, the cubes were compressed twice to 50% of their original height; the measurements were taken after the samples reached room temperature (±25°C). The deformation curve (force-time) was obtained with a compression velocity of 180mm/min, using a P-20 probe (20mm diameter).

According to Bourne (2002), the texture parameters were determined from the force curves as follows: hardness (N) is the height of the force peak on the first compression cycle; cohesiveness is the ratio of positive areas under the first and second compressions (A1/A2); adhesiveness (N·mm) is the negative force area (called A3) for the first bite representing the work necessary to pull the compressing plunger away from the sample; springiness (mm) is defined as the distance that the sample recovers between the end of the first bite and the start of the second bite; chewiness (N·mm) is the energy required to masticate a solid food (product of hardness x cohesiveness x springiness).

2.6 Experimental design and analysis of data

The whole experiment was conducted in two genuine repetitions including raw material collection, product formulation/processing and obtaining required experimental replicates (by analytical samples). Collected data were subjected to normality tests (Shapiro-Wilk test), consequently analysis of variance (ANOVA) to verify the effects against evaluated answers. The obtained means were compared

with a Scott-Knott test, adopting a 5% significance level. The statistical analyses and plots were performed using Statistical R-Software (2010). In addition, primary growth models were fitted aiming access the effect of carvacrol addition in the growth parameters of target spoilage groups (LAB and Psychrothophic) and *L. innocua* in HPP processed sliced turkey breast ham. For this purposes, R-package *NLSTools - Tools for nonlinear regression diagnostics* (Baty & Delignette-Muller, 2012) was used being growth curves fitted with Baraniy & Roberts (1994) model (Eq. (2)):

(2) $Log_{10}N \sim Log_{10}Nmax + log_{10}((-1 + exp(\mu max * lag) + exp(\mu max *t))/(exp(\mu max * t) - 1 + exp(\mu max * lag) * 10\text{^}(LOG_{10}Nmax - LOG_{10}N_0)))$

(3) $RSS_{residuals} = \sum (y_{observed} - y_{predicted})^2$ / (4) $RSE = \sqrt{}$ RSS / degrees of freedom

To evaluate the performance of the models the following statistical indicators were used: residual sum of squares (RSS (Eq. (3))) and residual standard error (RSE (Eq. (4))). As the lowest RSS and RSE values the better is the goodness-of-fit of model. The growth parameters estimated include lag phase (λ days), maximum specific growth rate μmax (day^{-1}), N_0 (initial population) and N_{max} (maximum population).

3. Results and Discussion

3.1 Carvacrol as natural additional hurdle to HPP

Table 2 shows the absorbances obtained for the different evaluated concentrations of pure natural antimicrobial carvacrol in minimal inhibitory concentration determination against *L. monocytogenes*. After incubation *Listeria* cell density increased into micro wells containing 800, 1500 and 3000ppm being the growth of the inoculated bacteria confirmed with carvacrol at these levels after TSB-YE inoculated tubes incubation 24hs/37°C. Besides, up to 6500ppm (0.65%) the absorbance evolution was not observed and the absence of growth was confirmed in test tube. This concentration was selected as minimal inhibitory concentration (MIC). Carvacrol was tested against *L. monocytogenes* for several

authors being in vitro MIC values ranging from 0.375 to 5.0μL.ml^{-1} (Burt, 2004). Carvacrol MIC concentration of 6500ppm, and their sub-inhibitory concentrations of 406ppm (MIC/16) and 203ppm (MIC/32) were applied in RTE turkey breast formulation to assess the maximum sensory acceptable level of the natural compound; MIC, MIC/2, MIC/4, MIC/8 were removed from sensorial tests due to strong impacted flavor detected during processing. The results obtained in *Difference from control* test, were depicted in Table 3.

Table 2. Absorbance profile in *Minimal Inhibitory Concentration* (MIC) determination with pure carvacrol as antimicrobial agent against *L. monocytogens* ATCC 19117.

	***L. monocytogenes* (Abs 620nm)**					
	Before incubation (37°C/24hs)			**24hs after incubation**		
Carvacrol level (ppm)	**Absorbance**	**Control column**	**Growth confirmation**	**Absorbance**	**Control column**	**Growth confirmation**
100.000	2.08(±0.13)	1.89	ND	0.84(±0.06)	0.56	ND
50.000	2.81(±0.03)	2.70	ND	1.16(±0.06)	1.08	ND
25.000	2.96(±0.07)	2.32	ND	2.98(±0.07)	2.68	ND
12.500	1.93(±0.17)	1.29	ND	1.79(±0.31)	0.86	ND
6.500	0.43(±0.17)	0.24	ND	0.63(±0.22)	0.78	ND
3000	0.09(±0.02)	0.09	ND	1.01(±0.51)	0.13	+
1500	0.09(±0.01)	0.10	ND	1.36(±0.20)	0.17	+
800	0.10(±0.01)	0.11	ND	1.55(±0.01)	0.13	+

*ND – growth not detected / Average absorbance values ± S.D (Standard deviation).

Regarding the obtained sensory results after an analysis of variance (ANOVA), were observed that Fc>>Ft being evidencied significant difference between control (sample without cravacrol) and carvacrol added RTE turkey breast formulations. Additionally was observed that the maximum of carvacrol level additon with no significant difference from the control (without carvacrol) was 203ppm, with the 406ppm and higher levels identified as different by panelists. Thus, level of 200ppm was selected as sensory-acceptable level and applied in F_{SRE} product formulation. As a GRAS (Generally Recognized as Safe) status food additive, carvacrol is used as a flavoring agent in baked goods, sweets, beverages and chewing gum (Fenaroli, 2002).

Table 3. ANOVA and Difference from control test.

Source of variation	*DF*	*SS*	*MSS*	F_c	F_t
Sample	2	270.75	135.75	33.60	3.10*
Assessors	39	218.00	5.59	1.38	
Residue	78	315.25	4.04		
Total	119	804.00			
Formulation**	**Mean Score**	***LSD***			
Control	2.72	0.88 (p<0.05)[a]		Control x 203ppm = 0.85[ns]	
203ppm carvacrol	3.57	1.16 (p<0.01)[b]		Control x 406ppm = 3.53[ab]	
406ppm carvacrol	6.25				

*F_c>>F_t (p<0.05). DF: Degrees of freedom; SS: Sum of Squares; MSS: Mean sum of squares. LSD – Least significant difference. Critical values of monocaudal *Dunnett* test 3/78 (3 - number of samples: 78 DF residue): 5%: 1.95 / 1%: 2.60.

Carvacrol has been registered by FDA (21 CFR 172.515), and the European Commission (EU Regulation 1334/2008 & 178/2002) for use as flavoring in foodstuffs because carvacrol is considered to present no risk to the health of consumers. Nevertheless, despite carvacrol having shown a strong antimicrobial activity against many spoiling and pathogenic bacteria, its application in food preservation, as with most natural extracts, may be limited due to the high concentrations required. High concentrations are usually associated with an undesirable flavor and sensory developments (Ait-Ouazzou et al., 2013). Thus, evaluating our sensorial results, the limit of carvacrol application to low-sodium turkey breast ham was 200ppm (MIC/32).

3.2 Effects against quality-attributes

The CIELAB color indexes *lightness* (*L**), yellowness (*b**) and redness (*a**) of different evaluated formulations HPP or non-pressurized were shown in Table 4. Despite minor variations in *lightness* values (ranging from 69.0-73.0), *redness* values (from 4.5-5.2) and *yellowness* values (ranging from 7.9-10.0) a constant trend for the effects of different formulations (salt reduction or carvacrol addition) and high pressure processing were not found. Significant effects of variations sources along 60 days of refrigerated storage were not detected for the evaluated

color parameters (time storage effect $p>0.05$). Generally, studies on cured meat products reported an increase in lightness and a decrease in redness when cured cooked meat products are pressurized (Bajovic, Bolumar, & Heinz, 2012).

However, color data reported in the current literature concerning RTE meat products high pressure processed diverge significantly: Mathias et al. (2009) evaluated the effects of HPP for L^*, a^* and b^* color parameters of cooked turkey ham processed at 200 and 400MPa 5, 10 and 15 min at room temperature; these authors observed that treatments did not alter lightness parameter during entire 65 days of refrigerated storage, and pressure loads higher than 300MPa are capable to reduce ($p<0.05$) redness index of the cooked turkey ham samples only at 45 and 65 day evaluation. The color of meat depends on the optical properties of the meat surface as well as on the myoglobin content and state in the muscle. Overall, high pressure causes dramatic changes in sarcoplasmatic protein conformation, consequently the color of fresh meat and thus makes difficult the commercialization of HPP fresh meats since they lack the typical color of fresh meat from the consumer's perspective (Cheftel & Culioli, 1997). In contrast, the color of cured meat products is mainly created due to the presence of nitrosyl-myoglobin, resulting from the reaction of nitric oxide (from sodium nitrite or sodium nitrate added) with myoglobin showing a different behavior (Ferrini et al., 2012; Bak et al., 2012). The term-stabilized nitrosyl-hemocromo pigment was more resistant to adverse processing conditions (Honikel, 2008), making HPP a suitable post-processing alternative for cooked cured meat products. Also, the addition of the coloring carmim agent may help to control color variation phenomena, including recurrent fading. Studies indicate that HPP provokes drastic changes in fresh meat color, while the minimal changes in cured meat products are acceptable as demonstrated for our data with a cured cooked turkey meat product. Thus, the treatment with 600MPa/180seg/25°C did not negatively affect the color of cured cooked RTE turkey breast, being color indexes relatively stable during entire storage period. Effects of salt reduction and carvacrol addition in the product formulation were not statistically ($p>0.05$) as far as color is concerned.

Table 4. CIELAB color indexes *lightness* (*L**), *yellowness* (*b**) and *redness* (*a**) of RTE turkey breast.

Lightness (L*) – days of refrigerated storage (4°C)							
Treatment	**24hs pos-HPP**	**10**	**20**	**30**	**40**	**50**	**60**
F_C	71.20(±0.55)^{Ba}	71.21(±0.86)^{Aa}	71.30(±0.22)^{Aa}	69.80(±1.20)^{Aa}	71.70(±0.46)^{Aa}	72.00(±0.48)^{Ba}	71.29(±0.56)^{Aa}
F_C + HPP	70.73(±0.59)^{Bb}	71.58(±0.55)^{Aa}	69.77(±0.21)^{Ab}	69.09(±0.90)^{Ab}	72.83(±0.52)^{Aa}	70.68(±0.65)^{Bb}	69.91(±0.38)^{Ab}
F_{SR}	72.94(±0.51)^{Aa}	71.68(±0.88)^{Aa}	72.19(±0.75)^{Aa}	70.61(±1.64)^{Aa}	72.21(±0.47)^{Aa}	73.38(±0.28)^{Aa}	70.64(±1.13)^{Aa}
F_{SR} + HPP	72.77(±0.27)^{Aa}	72.76(±0.34)^{Aa}	72.48(±0.57)^{Aa}	70.12(±1.22)^{Ab}	71.26(±0.41)^{Ab}	72.69(±0.34)^{Aa}	72.43(±0.70)^{Aa}
F_{SRE}	72.39(±0.53)^{Aa}	73.09(±0.62)^{Aa}	71.08(±0.67)^{Aa}	71.55(±0.44)^{Aa}	72.24(±0.28)^{Aa}	73.13(±0.41)^{Aa}	72.11(±0.66)^{Aa}
F_{SRE} + HPP	71.55(±0.48)^{Ba}	71.57(±1.09)^{Aa}	69.77(±0.74)^{Aa}	69.09(±0.53)^{Aa}	71.12(±0.47)^{Aa}	73.12(±0.37)^{Aa}	70.54(±0.57)^{Aa}
Redness (a*) – days of refrigerated storage (4°C)							
	24hs pos-HPP	**10 dias**	**20 dias**	**30 dias**	**40 dias**	**50 dias**	**60 dias**
F_C	4.69±(0.17)^{Ba}	4.43(±0.14)^{Ba}	4.68(±0.07)^{Aa}	4.49(±0.02)^{Aa}	5.00(±0.13)^{Aa}	4.72(±0.11)^{Aa}	4.64(±0.11)^{Aa}
F_C + HPP	4.58±(0.15)^{Ba}	4.76(±0.17)^{Ba}	4.99(±0.14)^{Aa}	4.53(±0.28)^{Aa}	5.11(±0.05)^{Aa}	4.69(±0.13)^{Aa}	4.79(±0.15)^{Aa}
F_{SR}	5.20±(0.10)^{Aa}	4.79(±0.23)^{Ba}	5.08(±0.16)^{Aa}	5.15(±0.19)^{Aa}	5.12(±0.16)^{Aa}	4.80(±0.10)^{Aa}	4.94(±0.13)^{Aa}
F_{SR} + HPP	4.77±(0.12)^{Ba}	5.05(±0.15)^{Aa}	5.13(±0.18)^{Aa}	4.77(±0.09)^{Aa}	5.22(±0.19)^{Aa}	4.91(±0.10)^{Aa}	5.18(±0.11)^{Aa}
F_{SRE}	5.11±(0.11)^{Aa}	5.14(±0.06)^{Aa}	5.08(±0.11)^{Aa}	4.95(±0.06)^{Aa}	4.47(±0.12)^{Bb}	4.93(±0.09)^{Aa}	5.15(±0.13)^{Aa}
F_{SRE} + HPP	5.31±(0.19)^{Aa}	4.96(±0.09)^{Aa}	5.15(±0.21)^{Aa}	4.74(±0.17)^{Ab}	4.74(±0.17)^{Bb}	4.53(±0.13)^{Ab}	4.99(±0.12)^{Aa}
Yellowness (b*) – days of refrigerated storage (4°C)							
	24hs pos-HPP	**10 dias**	**20 dias**	**30 dias**	**40 dias**	**50 dias**	**60 dias**
F_C	7.94(±0.26)^{Bb}	8.93(±0.20)^{Aa}	7.61(±0.26)^{Bb}	8.24(±0.47)^{Bb}	9.12(±0.36)^{Aa}	8.37(±0.16)^{Bb}	8.80(±0.17)^{Ba}
F_C + HPP	7.64(±0.10)^{Bb}	8.46(±0.10)^{Aa}	7.79(±0.27)^{Bb}	8.09(±0.24)^{Bb}	9.04(±0.11)^{Aa}	9.14(±0.11)^{Aa}	8.80(±0.31)^{Ba}
F_{SR}	9.15(±0.19)^{Aa}	9.15(±0.36)^{Aa}	8.68(±0.29)^{Aa}	9.16(±0.31)^{Aa}	8.95(±0.15)^{Aa}	9.54(±0.18)^{Aa}	9.08(±0.24)^{Ba}
F_{SR} + HPP	8.67(±0.20)^{Ab}	9.03(±0.12)^{Aa}	8.80(±0.08)^{Ab}	9.22(±0.06)^{Aa}	8.38(±0.49)^{Ab}	9.59(±0.11)^{Aa}	9.36(±0.16)^{Aa}
F_{SRE}	8.77(±0.22)^{Ab}	9.59(±0.19)^{Aa}	8.62(±0.15)^{Ab}	9.04(±0.15)^{Ab}	8.30(±0.39)^{Ab}	9.41(±0.22)^{Aa}	9.63(±0.11)^{Aa}
F_{SRE} + HPP	8.96(±0.13)^{Ab}	8.98(±0.22)^{Ab}	9.38(±0.27)^{Aa}	9.29(±0.34)^{Aa}	8.18(±0.49)^{Ab}	9.98(±0.29)^{Aa}	9.92(±0.19)^{Aa}

Mean CIELAB color indexes of lightness (*L**), redness (*a**), and yellowness (*b**) obtained for 5 reading in 3 sample slices. Mean values ± S.E. HPP processing at 600MPa/180seconds/25°C. Values followed by different capital letter (AB) within the same column, and values followed by different small letter (ab) within the same line and are significantly different according Scott-Knott test (p<0.05). Fc – Control (2% NaCl: ±1005mg Na/100g); Fc + HPP – Control plus HHP; F_{SR} (30% NaCl less: ±720mg Na/100g); F_{SR} + HPP (30% NaCl less + HPP); F_{SRE} (30% NaCl less + carvacrol 200ppm); F_{SRE} + HPP (30% NaCl less + carvacrol 200ppm + HPP).

Figure 1 shows the average syneresis values for the different evaluated formulations F_C, F_{SR}, F_{SRE} under HPP or non-pressurized conditions.

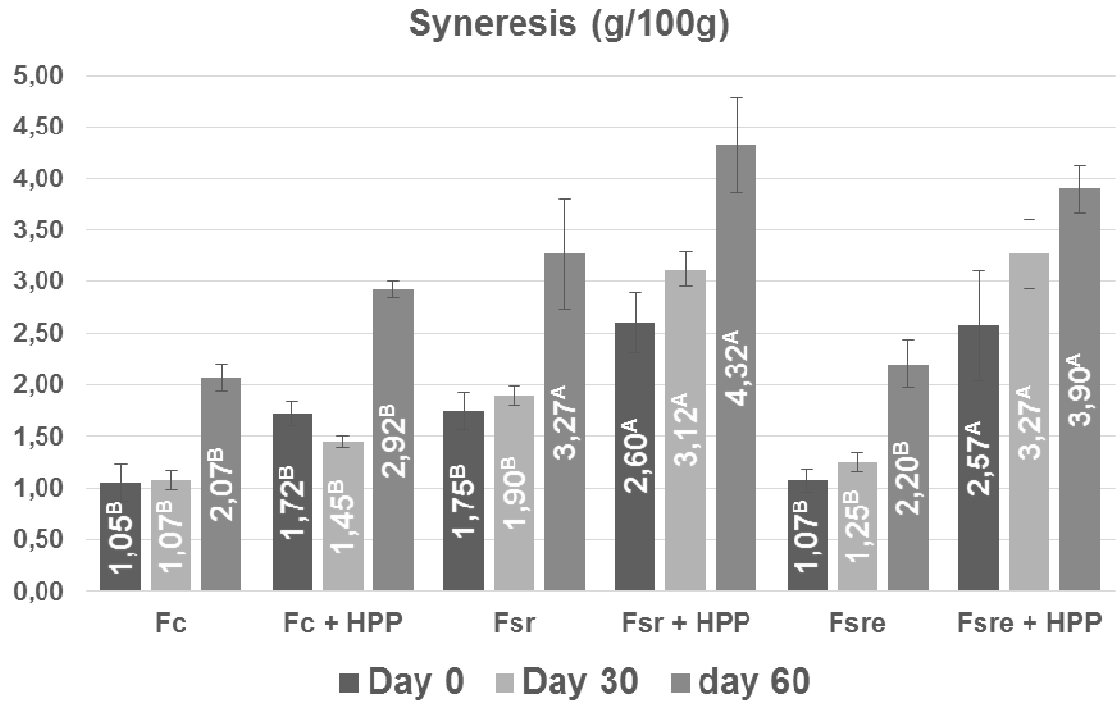

Figure 1. Average syneresis values (g/100) during refrigerated shelf-life (60 days at 4°C) of sliced vacuum-packaged RTE turkey breast. HPP processing at 600MPa/180seconds/25°C. Mean values ± Standard Error (bars). Fc – Control (2% NaCl: ±1005mg Na/100g); Fc + HPP – Control plus HHP; F_{SR} (30% NaCl less: ±720mg Na/100g); F_{SR} + HPP (30% NaCl less + HPP); F_{SRE} (30% NaCl less + carvacrol 200ppm); F_{SRE} + HPP (30% NaCl less + carvacrol 200ppm + HPP). AB (p<0.05) Scott-Knott test among diferent treatments.

An evident significant effect of the HP processing may be detected; at pressurized conditions the syneresis values were significantly (p<0.05) higher than non-pressurized samples during all storage time; an exception may be noticed in formulations with no sodium chloride reduction (20g/kg) where the pressurization did not influence (p<0.05) the amount of released liquid. On F_c + HPP treatment, the observed average value was higher (p<0.05) only at the end (60 days) of refrigerated storage. The salt reduction reformulation influenced the product behavior when it was high pressure processed. According to Desmond (2006), apart from it flavouring characteristics, one of salt's main functions in processed

meats is the solubilisation of the functional myofibrillar proteins in meat. This activates the proteins to increase hydration and water-binding capacity; thus, reducing salt levels below that typical applied may influence syneresis answer decorrent to mechanical effects of pressurization.

Vercammen et al. (2011) evaluated the syneresis in 600MPa/10min pressurized conditions of cooked ham being reported that in general, a statistically significant increase of syneresis was observed from approximately 2% for the unpressurized control to 4–5% immediately after HP treatment. Overall, syneresis remained relatively stable over the 10 week storage period. On the contrary our results showed an increase effect observed with evolution of the time storage, with higher values being registered at the end of 60 days of storage (p<0.05). Despite significant increases in syneresis values due to HPP, processing optimization reducing the treatment intensity (pressure loads, holding time and temperature) may be an effective alternative for minimization of this phenomenon; furthermore, aiming conservation effect, microbiological criteria of the treatment need to be taking into account; for this specific addressed point, addition of an antimicrobial additional hurdle may act synergistically making safety slightly adjusted HPP treatments (Evrendilek & Balasubramaniam,2011; Pulido et al., 2012). In addition, consumer behavior and repugnance of released liquid in product package needs to be more evaluated.

Decaying pH average values during the refrigerated storage are shown in Table 5. pH average values varied slightly among all of the different evaluated treatments until 30 days of storage. From 40 days, registered pH values were lower (p<0.05) in non-HPP treated low-sodium formulations F_{SR} and F_{SRE}; at the end of storage time at 60 days, these treatment/formulations pH reached 5.37 and 5.89 for F_{SR} and F_{SRE} respectively, pH values drop in pressurized samples also occurred at the end of storage time. Pressure treatment of meat and meat products is known to produce a small increase in pH, which may result from a decrease in acidic groups due to conformational changes of proteins associated with denaturation (Poulteret al., 1985).

Table 5. TBARs indexes and pH values for different evaluated treatments during refrigerated shelf-life (60 days at 4°C) of low-sodium sliced-vacuum packaged RTE turkey breast.

TBARs mg malondialdehyde/kg during refrigerated storage (60 days at 4°C)							
Treatments	**24hs post-HPP**	**10 days**	**20 days**	**30 days**	**40 days**	**50 days**	**60 days**
F_C	0.032(±0.01)^{Ab}	0.006(±0.01)^{Bb}	0.024(±0.01)^{Bb}	0.002(±0.01)^{Cb}	0.049 (±0.01)^{Ba}	0.049 (±0.01)^{Ba}	0.080 (±0.01)^{Ba}
F_C + HPP	0.081(±0.02)^{Aa}	0.114(±0.03)^{Aa}	0.042(±0.01)^{Aa}	0.020(±0.01)^{Ba}	0.069 (±0.01)^{Aa}	0.108 (±0.01)^{Aa}	0.173 (±0.04)^{Aa}
F_{SR}	0.036(±0.01)^{Ab}	0.032(±0.01)^{Bb}	0.041(±0.02)^{Ab}	0.010(±0.01)^{Cb}	0.035 (±0.02)^{Bb}	0.062 (±0.01)^{Ba}	0.070 (±0.01)^{Ba}
F_{SR} + HPP	0.069(±0.02)^{Ab}	0.057(±0.02)^{Bb}	0.055(±0.01)^{Ab}	0.023(±0.01)^{Bb}	0.066 (±0.02)^{Ab}	0.143 (±0.03)^{Aa}	0.148 (±0.03)^{Aa}
F_{SRE}	0.038(±0.01)^{Aa}	0.022(±0.01)^{Bb}	0.055(±0.03)^{Aa}	0.005(±0.01)^{Cb}	0.040 (±0.02)^{Ba}	0.064 (±0.02)^{Ba}	0.068 (±0.01)^{Ba}
F_{SRE} + HPP	0.046(±0.01)^{Ab}	0.006(±0.01)^{Bb}	0.044(±0.01)^{Ab}	0.041(±0.01)^{Ab}	0.040 (±0.01)^{Bb}	0.091 (±0.02)^{Ba}	0.071 (±0.03)^{Ba}
pH falling curves during refrigerated storage (60 days at 4°C)							
Treatments	**24hs post-HPP**	**10 days**	**20 days**	**30 days**	**40 days**	**50 days**	**60 days**
F_C	6.46 (±0.03)^{Aa}	6.06 (±0.01)^{Ac}	6.21 (±0.07)^{Ab}	6.21 (±0.01)^{Ab}	6.11(±0.02)^{Ac}	6.19(±0.11)^{Ab}	5.85(±0.07)^{Ac}
F_C + HPP	6.35 (±0.01)^{Ba}	6.05 (±0.01)^{Ab}	6.01 (±0.01)^{Bb}	6.24 (±0.01)^{Ab}	6.10(±0.06)^{Ab}	6.12(±0.07)^{Ab}	5.94(±0.03)^{Ac}
F_{SR}	6.32 (±0.01)^{Ba}	6.15 (±0.03)^{Aa}	5.99 (±0.01)^{Ba}	6.23 (±0.01)^{Aa}	5.42(±0.15)^{Bb}	5.35(±0.13)^{Bb}	5.37(±0.02)^{Bb}
F_{SR} + HPP	6.30 (±0.02)^{Ba}	6.03 (±0.01)^{Aa}	6.02 (±0.01)^{Ba}	6.23 (±0.08)^{Aa}	6.17(±0.12)^{Aa}	6.20(±0.04)^{Aa}	5.98(±0.02)^{Aa}
F_{SRE}	6.29 (±0.02)^{Ba}	6.24 (±0.12)^{Aa}	6.04 (±0.02)^{Ba}	5.96 (±0.01)^{Ba}	5.55(±0.14)^{Bb}	5.80(±0.03)^{Bb}	5.89(±0.11)^{Ab}
F_{SRE} + HPP	6.27 (±0.01)^{Ba}	6.06 (±0.01)^{Aa}	6.04 (±0.01)^{Ba}	6.23 (±0.07)^{Aa}	6.25(±0.04)^{Aa}	5.90(±0.04)^{Ab}	5.91(±0.07)^{Ab}

Average values ± Standard error. Mean values followed by different capital letter in the same column and mean values followed by different small letter in the same line differ significantly (p<0.05) according Scott-Knott test. HPP processing at 600MPa/180seconds/25°C. Fc – Control (2% NaCl: ±1005mg Na/100g); Fc + HPP – Control plus HHP; F_{SR} (30% NaCl less: ±720mg Na/100g); F_{SR} + HPP (30% NaCl less + HPP); F_{SRE} (30% NaCl less + carvacrol 200ppm); F_{SRE} + HPP (30% NaCl less + carvacrol 200ppm + HPP).

However, a similar behavior in pH falling curve pattern were detected by Ham et al. (2010) evaluating sliced vacuum-packed cooked ham; these authors reported that initial pH values were above 6.38 for pressurized and non-pressurized samples and HPP samples were characterized by a drop in pH values from above 6.38 (day 0) to just above 5 (day 30). In the case of HPP samples, a slow decrease of pH was observed during the first 30 days, however during the following 60 days the pH decreased reaching values around 5.20 at the end of the study (90 days). Our observed pH results were in agreement with the findings registered by Cayre, Garro and Vignolo (2005) that registered pH falling pattern of cooked meat emulsion with initial pH next to 6.50 reaching 5.40 at the end of refrigerated storage (52 days). Coincidently, non-pressurized samples showed a rapid increase in total acid lactic bacteria population being reached more than 6 log_{10} cycles at 40 days of storage; at the end of shelf-life, higher LAB counts were observed for F_{SR} and F_{SRE} formulations. HPP processing was effective in slowing LAB growth rates and preventing pH decay event. Homo and heterofermentative LAB are main spoilage group of vacuum-packaged cooked meat products being responsible for conversion of added sugar added in formulation into lactate (lactic acid) leading to an acidification that indicate spoilage threshold (Forsythe, 2002).

The effects of pressurization (600MPa/180sec/25°C) and different product formulation (salt reduction or 200ppm carvacrol addition) against lipid oxidation measured as TBARs index during refrigerated shelf-life of the product are shown in Table 5. Initially, pressurization affected lipid oxidation rates already in early 24hs post-HP processing being identified higher TBARs scores for pressurized samples. For F_{SRE} + HPP treatment the observed average value was close to non-pressurized samples at this same period of analysis. At 10 days of storage F_C + HPP formulation reached 0.114 mg MDA/kg (p<0.05), the highest value among all tested conditions. Significant effect of time storage on oxidation answer was observed, and from 50 days of refrigerated storage the TBARs index increased; pressurized samples of F_C and F_{SR} showed TBARs scores of 0.108 and 0.143 mg MDA/kg respectively, being significantly (p<0.05) higher than all other evaluated conditions. High pressure changes the thermodynamic equilibrium of chemical reactions and it has been show that lipid oxidation in raw meat and meat products

is accelerated when processed by high hydrostatic pressure (Medina-Meza, Barnaba & Bárbosa-Canovas, 2014). The mechanisms by which HPP induces lipid oxidation are not fully understood. Generally, it has been suggested that HPP triggers lipid oxidation by two mechanisms: increased accessibility for iron from hemoproteins and membrane disruption. The release of iron from hemoproteins can promote lipid oxidation (Bajovic, Bolumar & Heinz, 2012). Besides enzymatic, hydrolytic and photo-oxidation mechanisms, autoxidation is recognized as a major oxidative degradation of fats in foods (Ramalho & Jorge, 2006). Briefly, autoxidation mechanism may be detailed in initiation, propagation and termination steps: initially fat acids radicals' formation occurred (hydrogen sequester) due to high energy conditions (generally heat); production of these radicals is driven catalytically by trace levels of iron, nickel and copper (Schaich, 1992). The resulting carbon-centered alkyl free radicals (R•) react with oxygen to form peroxyl radicals (RO_2•) and other oxygenated compounds. In this propagation process, RO_2• reacts with more RH to from lipid hydroperoxides (RO_2H), which are the fundamental primary products of autoxidation (Frankel, 1984). The primary products of autoxidation are peroxides or hydroperoxides, but these compounds are frequently unstable frequently combining (more stable) or and undergo scission to form lower molecular-weight secondary oxidation products such as aldehydes, ketones and epoxide and volatile or non volatile compounds (Ramalho & Jorge, 2006). All of which contribute to flavor and structural deterioration of foods.

For cooked ham from turkey or pork, high pressure processing has been identified to accelerate lipid oxidation process (Mathias et al., 2010; Liu et al., 2009). At 50 and 60 days of storage, for F_{SRE} + HPP treatment the registered TBARs values were lower (p<0.05) than other pressurized samples and closer to the non-pressurized samples 0.091±0.02 and 0.071±0.03 mg MDA/Kg. Carvacrol added at 200ppm was able to decrease lipid oxidation rates in HP processed sliced vacuum-packaged RTE turkey breast; thus a time storage effect (p<0.05) may be also detected. Natural antioxidants such as essential oils, isolated natural compounds and plant extracts have been considered a promising emerging tool in preventing triggered lipid oxidation in raw and processed meat (Karre, Lopez & Getty, 2013; Shah, Don Bosco & Mir, 2014). Carvacrol is a phenolic natural

ocourring compound that is capable to prevent oxidation as a primary antioxidant: phenolic are primary antioxidants that promote the removal or inactivation of free radicals in systems formed during the initiation or propagation of the reaction, by donating hydrogen atoms to these molecules and blocking the chain reaction. The compound structure is stabilized by resonance promoted by aromatic ring (Oliveira et al., 2012a). Success of natural antioxidants in preventing oxidation process triggered by high pressure treatments in meat products has been confirmed in several works (Bragagnolo et al., 2007; Mariutti et al., 2008; Alves et al., 2011).

The effects of sodium reduction, carvacrol and pressurization on the textural properties, such as hardness, cohesiveness, springiness, adhesiveness and chewiness are shown in Table 6. The different process conditions did not affect evaluated textural parameters except for hardness and chewiness, that was significantly affected (p<0.05). At 24hs post-HPP, higher hardness and chewiness values were detected for F_C formulation. Established pressurization effects may be described for raw meat (Sun & Holey, 2010) and their effects on cooked meat products are not fully understood. Generally published literature affirms that pressure treatments can improve the cohesion between meat particles in re-structured meat products thus altering characteristic texture (MacFarlene 1985; Suzuki et al., 2006). However, the high pressure intensity of 600MPa/180sec did not affect evaluated control and low-sodium pressurized formulations. Regarding High Pressure processing and salt reduction, Crehan et al. (2000) investigated the application of 150 and 300 MPa pressure on frankfurter quality at 1.5% and 2.5% NaCl. Among the combination of different pressure/salt levels used, the 300 MPa/1.5% (pressure/NaCl) treatments appeared to be the best because juiciness, hardness, springiness, cohesiveness, gumminess, and chewiness scores were the highest. Their results suggested that high pressure (300 MPa) treatment can be used to improve the sensory properties of frankfurters processed with lower salt levels (1.5%) when applied in pre-cooking processing step.

Table 6. Textural parameters obtained in TPA tests (Texture profile Analysis) of sliced vacuum-packaged RTE turkey breast during 60 days of refrigerated storage (4°C).

Treatments		F_C	F_C + HPP	F_{SR}	F_{SR} + HPP	F_{SRE}	F_{SRE} + HPP
Hardness (N)	**24hs post-HPP**	24.85±1.64^a	20.56±0.87^b	20.84±0.94^b	20.31±1.34^b	20.58±0.83^b	19.65±0.81^b
	30 days	22.67±0.88^a	22.88±1.04^a	17.28±1.09^b	21.84±1.17^a	22.49±1.11^a	19.91±0.74^b
	60 days	25.44±1.50^a	24.42±1.19^a	23.07±1.06^a	24.08±1.16^a	22.42±0.79^a	20.91±0.46^a
Cohesiveness	**24hs post-HPP**	0.59±0.01^a	0.58±0.02^a	0.56±0.02^a	0.54±0.03^a	0.53±0.02^a	0.59±0.02^a
	30 days	0.51±0.01^a	0.56±0.01^a	0.51±0.01^a	0.52±0.01^a	0.50±0.01^a	0.57±0.01^a
	60 days	0.57±0.01^a	0.60±0.02^a	0.52±0.02^a	0.48±0.01^a	0.49±0.01^a	0.53±0.01^a
Adhesiveness (N.mm)	**24hs post-HPP**	0.13±0.02^a	0.16±0.01^a	0.15±0.02^a	0.18±0.02^a	0.14±0.02^a	0.12±0.02^a
	30 days	0.13±0.02^a	0.13±0.01^a	0.08±0.01^a	0.12±0.02^a	0.12±0.01^a	0.10±0.01^a
	60 days	0.12±0.02^a	0.13±0.01^a	0.09±0.01^a	0.10±0.02^a	0.10±0.01^a	0.13±0.02^a
Springiness or Elasticity (mm)	**24hs post-HPP**	4.76±0.11^a	4.75±0.08^a	4.45±0.10^a	4.38±0.15^a	4.55±0.07^a	4.63±0.17^a
	30 days	4.52±0.43^a	5.03±0.05^a	4.86±0.06^a	4.80±0.05^a	4.88±0.07^a	4.96±0.07^a
	60 days	4.98±0.13^a	5.12±0.09^a	4.89±0.06^a	5.05±0.04^a	4.88±0.08^a	5.23±0.05^a
Chewiness (N.mm)	**24hs post-HPP**	69.95±4.87^a	55.62±2.09^b	52.24±2.45^b	47.90±3.24^b	49.72±2.30^b	55.01±2.78^b
	30 days	58.65±3.29^a	64.91±2.84^a	43.51±2.53^b	54.74±3.58^a	55.17±2.44^a	56.94±2.30^a
	60 days	72.49±4.57^a	74.23 ±2.65^a	58.96±3.40^b	58.68±2.81^b	54.67±3.08^b	58.91±1.67^b

Average values ± Standard error. Values followed by the different letter within the line are significantly different according Scott-Knott test (p<0.05). HPP processing at 600MPa/180seconds/25°C. Fc – Control (2% NaCl: ±1005mg Na/100g); Fc + HPP – Control plus HHP; F_{SR} (30% NaCl less: ±720mg Na/100g); F_{SR} + HPP (30% NaCl less + HPP); F_{SRE} (30% NaCl less + carvacrol 200ppm); F_{SRE} + HPP (30% NaCl less + carvacrol 200ppm + HPP).

3.3 Microbiological effects along refrigerated storage

Growth patterns (average populations expressed as $\log_{10}$ CFU/g) of target spoilage populations (LAB and Psychrotrphic), and inoculated pathogen *Listeria innnocua* in sliced vacuum-packaged RTE turkey breast during 60 days of refrigerated storage are shown in Table 7. Table 8 shows the estimated growth parameters (with Baranyi & Roberts primary growth model) lag phase λ, maximum specific growth rate μmax, initial population N_0 and maximum reached population N_{max}.

Lactic acid bacteria (LAB) are naturally found in many vacuum-packaged meat products stored under refrigeration, e.g. ham, causing spoilage and decreasing shelf-life. Low oxygen concentration, high water activity (normally between 0.96 and 0.98) and pH around 6.0 are some of the characteristics that favor the lactic acid bacterial growth. (Cayre et al., 2005; Cayre, Vignolo, & Garro, 2003). In vacuum-packed cured cooked turkey breast, the LAB genus of *Lactobacillus* sp. and *Leuconostoc* sp. has been found to be the predominant spoilage flora (Samelis et al., 2000). For LAB, the absence of growth was registered for all evaluated HPP pressurized formulations up to 20 days of storage (4°C). For non-pressurized samples F_C, F_{SR} and F_{SRE} the populations after 24hs of storage were 1.29±0.06, 1.47±0.04 and 1.62±0.06 $\log_{10}$ CFU/g respectively; the populations in these non-pressurized samples did not differ significantly (p>0.05) up to 30 days of storage, reaching next to 5 $\log_{10}$ CFU/g. For pressurized formulations, outgrowth starts up from day 20, being estimated phase lag raging from 18-22 days (p>0.05) as showed in Table 8. At 40 days of storage the F_{SR} + HPP population showed a mean population close to non-pressurized conditions showing the effect of salt reduction in shelf-life stability for spoilage LAB. According to Karatzas (2001), the intrinsic factors of food matrix such as salt content influences directly the recovery and outgrowth of pressurized microbiota. At 40 days F_C + HPP and F_{SRE} + HPP treatments showed average populations lower (p<0.05) than the other treatments (F_C, F_{SR}, F_{SR} + HPP, F_{SRE}). At 50 days of storage, non-HPP treated treatments reached LAB populations of 7 $\log_{10}$ indicating spoilage thereshold; LAB levels of 10^7 CFU/g have been pointed by some authors as the safety limit for cooked meat products (Ruiz-Capillas, Carballo, & Colmenero,

2007). Shelf-life extension effect of pressurization at 600MPa/180sec/25°C was evident with problematic LAB populations reach delayed. The combined application was able to reduce specific growth rates in low-sodium formulations next to RTE turkey breast formulations with entire amount of 20g/kg of salt, being observed values of 1.15 (p<0.05) for pressurized F_{SR} and next of 0.40 for F_C and F_{SRE} pressurized conditions. Figure 2 shows the fitted growth curve for F_C, F_{SR} and F_{SRE} pressurized conditions. Cayré, Vignolo and Garro (2003) found specific growth rates ranging from 0.40-0.60 $days^{-1}$ in cooked meat product stored under refrigeration temperatures. Slongo et al. (2009) modeled the growth of LAB in sliced vacuum-packaged cooked pork ham, being registered growth rates of 0.21 day^{-1} and lag phase of 55 days in most intense tested pressurization conditions; then again, in non-pressurized control conditions growth rates close to 1.00 day^{-1} and lag phase of 11 days were confirmed. Despite combined application of natural antimicrobials in synergistic effects with HPP, some reported data may be found in current literature: Liu et al. (2009) evaluated the combined application of HPP (200 and 400MPa) and bacteriocins (enterocin LM 2 from *Enterococcus*) against spoilage LAB of ready-to-eat sliced vacuum-packed cooked ham; as stated in our studies, in control samples 8 log_{10} CFU/g of LAB were reached after 50 days of storage. The combined application of HPP at 400MPa and enterocin was able to retard LAB growth until 20 days, being detected LAB populations of 4 log_{10} at 50 days of storage. Vercammen et al. (2011) evaluated the inactivation rates of HHP processing by varying pressure load (100-700MPa), temperature (5, 25 and 40) for 10 minutes against spoiling cooked ham flora including *Leuconostoc carnosum* and *Lactobacillus sakei*, identifying a suitable treatment of 600MPa/10°C/10min for a 5D inactivation performance criterion. Thus, the combined effects of HHP (600MPa/10°C/10min) and natural preservatives including caprylic acid (0.15%) or a mixture of potassium lactate and sodium diacetate (2.5% - Purasal) were evaluated against total LAB flora during refrigerated storage. These authors observed that the HHP treatment alone delayed spoilage to 59 days. However, microbial growth was completely suppressed during at least 84 days in the samples treated with physical HHP treatment and additional natural barriers.

Table 7. Growth of target spoilage populations LAB and Psychrotrophic, and inoculated pathogen *Listeria innnocua* (expressed as log_{10} CFU/g) in sliced vacuum-packaged RTE turkey breast during 60 days of refrigerated storage (4°C).

Treatments	Lactic acid bacteria - LAB						
	24h/post-HPP	**10 days**	**20 days**	**30 days**	**40 days**	**50 days**	**60 days**
F_C	*ND*(<1.00)^a	3.63±0.03^a	4.21±0.02^a	4.90±0.06^a	6.01±0.06^a	6.81±0.25^a	7.08±0.04^a
F_C + HPP	*ND*(<1.00)^a	*ND*(<1.00)^b	*ND*(<1.00)^b	3.26±0.16^b	3.68±0.02^b	4.60±0.14^b	5.59±0.16^a
F_{SR}	*ND*(<1.00)^a	3.03±0.04^a	2.86±0.16^a	5.24±0.03^a	5.50±0.05^a	7.69±0.18^a	8.47±0.01^a
F_{SR} + HPP	*ND*(<1.00)^a	*ND*(<1.00)^b	*ND*(<1.00)^b	3.81±0.20^b	5.30±0.05^a	5.54±0.13^b	6.49±0.11^a
F_{SRE}	*ND*(<1.00)^a	3.12±0.03^a	3.58±0.30^a	5.75±0.03^a	6.39±0.17^a	7.86±0.02^a	7.17±0.01^a
F_{SRE} + HPP	*ND*(<1.00)^a	*ND*(<1.00)^b	*ND*(<1.00)^b	2.41±0.25^b	3.29±0.06^b	5.80±0.07^b	6.30±0.08^a
	Psychrotrophic bacteria						
	24h/post-HPP	**10 days**	**20 days**	**30 days**	**40 days**	**50 days**	**60 days**
F_C	*ND*(<2.00)^a	3.63±0.03^a	3.39±0.05^a	6.57±0.03^a	7.10±0.07^a	8.03±0.14^a	7.97±0.16^b
F_C + HPP	*ND*(<2.00)^a	*ND*(<2.00)^b	*ND*(<2.00)^b	4.74±0.09^b	5.47±0.25^b	6.81±0.11^b	7.12±0.08^b
F_{SR}	*ND*(<2.00)^a	3.13±0.30^a	4.30±0.10^a	6.79±0.05^a	8.37±0.33^a	9.73±0.19^a	9.71±0.08^a
F_{SR} + HPP	*ND*(<2.00)^a	*ND*(<2.00)^b	2.43±0.09^a	5.70±0.07^a	6.38±0.18^a	7.58±0.16^b	7.15±0.11^b
F_{SRE}	*ND*(<2.00)^a	3.04±0.07^a	3.81±0.17^a	7.00±0.00^a	8.37±0.33^a	9.73±0.19^a	9.93±0.05^a
F_{SRE} + HPP	*ND*(<2.00)^a	*ND*(<2.00)^b	*ND*(<2.00)^b	3.86±0.09^b	4.06±0.07^b	5.97±0.04^b	7.35±0.16^b
	***Listeria innocua* (initial inoculum 10^6 CFU/g)**						
	24h/post-HPP	**10 days**	**20 days**	**30 days**	**40 days**	**50 days**	**60 days**
F_C	6.22±0.02^a	7.36±0.04^a	7.72±0.02^a	8.11±0.01^a	8.00±0.01^a	8.33±0.04^a	7.59±0.15^a
F_C + HPP	*ND*(<2.00)^b	*ND*(<2.00)^b	*ND*(<2.00)^b	2.69±0.03^b	4.51±0.02^b	5.86±0.28^b	6.42±0.08^b
F_{SR}	6.25±0.07^a	7.27±0.07^a	7.72±0.03^a	8.11±0.01^a	8.06±0.05^a	8.48±0.01^a	7.27±0.07^a
F_{SR} + HPP	*ND*(<2.00)^b	*ND*(<2.00)^b	*ND*(<2.00)^b	3.44±0.05^b	4.34±0.02^b	6.33±0.33^b	6.55±0.09^b
F_{SRE}	6.18±0.08^a	7.39±0.03^a	7.88±0.03^a	7.93±0.02^a	8.12±0.01^a	8.25±0.02^a	7.39±0.03^a
F_{SRE} + HPP	*ND*(<2.00)^b	*ND*(<2.00)^b	*ND*(<2.00)^b	*ND*(<2.00)^c	4.49±0.02^b	5.72±0.31^b	5.75±0.10^b

Average populations ± Standard error. Mean values followed by different capital letter in the same column differ significantly ($p<0.05$) according Scott-Knott test. HPP processing at 600MPa/180seconds/25°C. Fc – Control (2% NaCl: ±1005mg Na/100g); Fc + HPP – Control plus HHP; F_{SR} (30% NaCl less: ±720mg Na/100g); F_{SR} + HPP (30% NaCl less + HPP); F_{SRE} (30% NaCl less + carvacrol 200ppm); F_{SRE} + HPP (30% NaCl less + carvacrol 200ppm + HPP). ND – not detected - below the detection limit (<1 or <2 log_{10} CFU/g).

Table 8. Estimated primary growth model parameters for the different pressurized formulations of sliced vacuum-packaged RTE turkey breast during 60 days of refrigerated storage.

Formulations / processing conditions	Lactic acid bacteria - LAB				
	μmax (Growth rate day^{-1})	**Lag λ (days)**	**$Log_{10}N_0$ CFU/g**	**$Log_{10}N_{max}$ CFU/g**	**RSS-RSE**
F_C + HPP	0.4058±0.05^b	18.2602±2.86^a	0.00±0.00^a	5.23±0.40^a	1.850 - 0.7856
F_{SR} + HPP	1.1518±0.02^a	22.6118±0.11^a	0.00±0.00^a	5.99±0.26^a	0.778 - 0.5092
F_{SRE} + HPP	0.4347±0.01^b	19.6605±0.84^a	0.00±0.00^a	6.37±0.08^a	0.629 - 0.4580
	Psychrotrphic bacteria				
	μmax (Growth rate day^{-1})	**Lag λ (days)**	**$Log_{10}N_0$ CFU/g**	**$Log_{10}N_{max}$ CFU/g**	**RSS-RSE**
F_C + HPP	1.1406±0.10^a	20.3628±0.35^a	0.00±0.00^a	6.7113±0.03^c	1.1200-0.6107
F_{SR} + HPP	0.8653±0.04^b	12.8721±0.34^b	0.00±0.00^a	7.1761±0.08^b	0.7220-0.490
F_{SRE} + HPP	0.5080±0.03^c	16.7134±0.21^a	0.00±0.00^a	7.6216±0.22^a	2.470 - 0.9082
	Listeria innocua				
	μmax (Growth rate day^{-1})	**Lag λ (days)**	**$Log_{10}N_0$ CFU/g**	**$Log_{10}N_{max}$ CFU/g**	**RSS-RSE**
F_C + HPP	0.5511±0.03^b	19.2623±0.29^b	0.00±0.00^a	6.33±0.12^a	0.353 - 0.3430
F_{SR} + HPP	0.4956±0.02^b	17.5746±0.26^b	0.00±0.00^a	6.52±0.15^a	1.210 – 0.6356
F_{SRE} + HPP	1.2658±0.12^a	31.5711±0.30^a	0.00±0.00^a	5.49±0.07^b	0.0045-0.0122

Estimated primary growth model parameters ± Standard error. Values followed by the different small letter within the same column are significantly different ($p \leq 0.05$) according to Skott-knott. RSS/RSE goodness of fit parameters (Residual Sum of Squares and Residual Standad Error. Sliced vacuum-packaged RTE turkey breast processed at 600MPa/180seconds/25°C. Fc + HPP – Control plus HHP; F_{SR} + HPP (-30% NaCl + HHP); F_{SRE} + HPP (-30% NaCl + carvacrol 200ppm + HHP). 0.00 represents <1 log_{10} cfu/g (detection limit <10 or 100 CFU/g).

Model Formula: LOG10N ~ LOG10Nmax + log10((-1 + exp(mumax * lag) + exp(mumax *t))/(exp(mumax * t) - 1 + exp(mumax * lag) * 10^(LOG10Nmax - LOG10N0)))

Besides LAB spoilage group, psychrotrophic bacteria are one of major important deteriorative agents in spoilage threshold of sliced vacuum-packed RTE meat products. This bacterial group is mainly composed by the following strains of *Pseudomonas, Aeromonas*, *Brochothrix thermosphacta, Shewanella, Weissella viridescens* and *Serratia*; jointly they are able to produce several spoilage indicative products that results in ropy slime (mainly exo-polysaccharides matrix from *Pseudomonas*), acidification, gas production, discoloration, lactic acid, ethanol and small amounts of short chainfatty acids causing off-odours. Psychrotrophic bacteria growths are presented in Tables 7 and 8. In non pressurized low-sodium formulations a rapid psychotrophic population increases was detected, reaching around 7 $\log_{10}$ in F_C, F_{SR} and F_{SRE} populations. At the end of storage period F_{SR} and F_{SRE} populations were higher among evaluated treatments (p<0.05). Observing pressurized samples, in F_{SR} formulation the growth starts up from day 20 of storage, and in formulation added of carvacrol at 200ppm growth was not detected; this combined effect HPP + carvacrol is confirmed with a significant lag phase extension, compared to F_{SR} + HPP which was predicted 12 days. For pressurized F_C and F_{SRE} average lag phase values found were close to 20 days, when lower (p<0.05) populations were detected at 30 days of refrigerated storage. Concerning natural antimicrobials and HPP controlling psychrotrphic bacteria, Liu et al. (2009) showed that combined application of 400MPa and bacteriocins delayed psychrotrophic growth compared with control treatments, proving a significant increase in time to reach 10^4 CFU/g, indicated by these authors as a problematic number. Figure 3 shows the fitted growth curves fitted for different pressurized formulations in psychrotrophic determination. In our studies the time required (predicted) for LAB and psychrotrophic populations (main spoilage groups) reach 5 $\log_{10}$ CFU/g in HPP conditions were respectively: 48, 33 and 46 days for LAB in F_C, F_{SR} and F_{SRE}; 26, 39 and 40 days in F_C, F_{SR} and F_{SRE} respectively.

L. innocua populations and growth parameters are shown in Tables 7 and 8. Figure 4 illustrate the fitted growth curves in estimation of growth parameters under pressurized conditions.

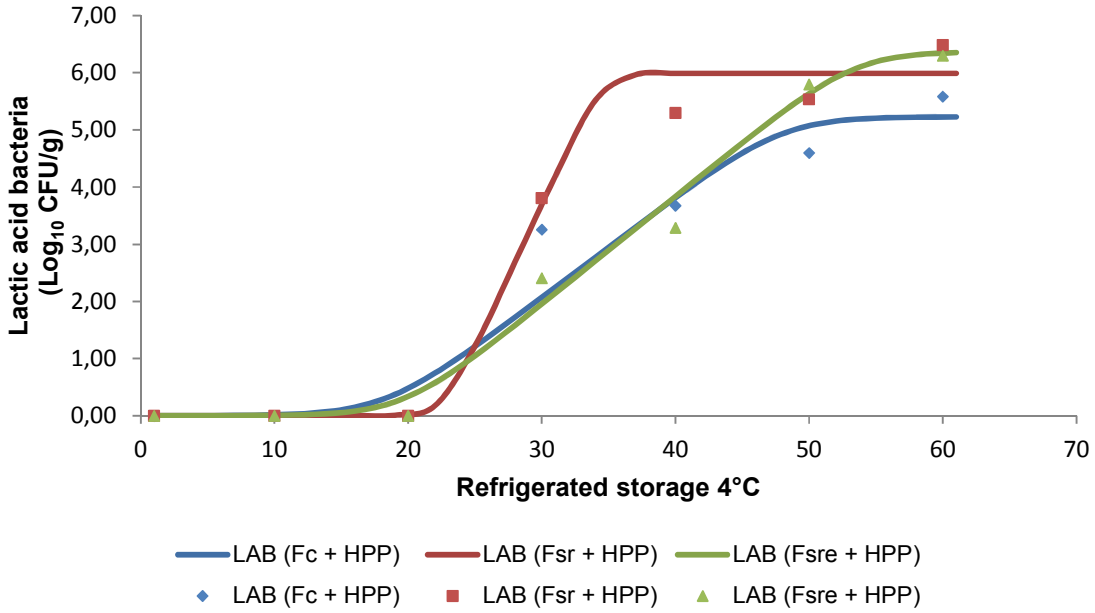

Figure 2. Fitted growth curves for Lactic acid bacteria (LAB) during 60 days of refrigerated storage (4°C) for HP processed sliced vacuum-packaged RTE turkey breast processed at 600MPa/180seconds/25°C. F_C + HPP – Control plus HPP; F_{SR} + HPP (-30% NaCl + HPP); F_{SRE} + HPP (-30% NaCl + carvacrol 200ppm + HPP). Curves were fitted with model of Baranyi and Roberts (1994) with four parameters (lag phase, $LOG_{10}N_0$, μmax, $LOG_{10}N_{max}$). RSS and RSE were used as goodness of fit for models.

In non-pressurized conditions *L. innocua* populations showed an initial count of 6 log_{10} CFU/g reaching next to 8 log_{10} CFU/g at the end of 60 days of refrigerated storage. For HPP treated F_C, F_{SR} and F_{SRE} the outgrowth was delayed starting from 20 days of refrigerated storage for F_C + HPP and F_{SR} + HPP. When the growth takes place in pressurized F_{SR} and F_C, F_{SR} showed higher *L. innocua* populations at 30 days of storage. High pressure processing has been applied successfully for *Listeria monocytogenes* control in low-sodium reformulated RTE meat products (Myers et al., 2013). In F_{SRE} plus HPP bacterial growth starts only from 30 days as confirmed in Table 8, that presents an estimated lag phase of 30 days compared to 17-19 days (p<0.05) of F_C and F_{SR} pressurized samples.

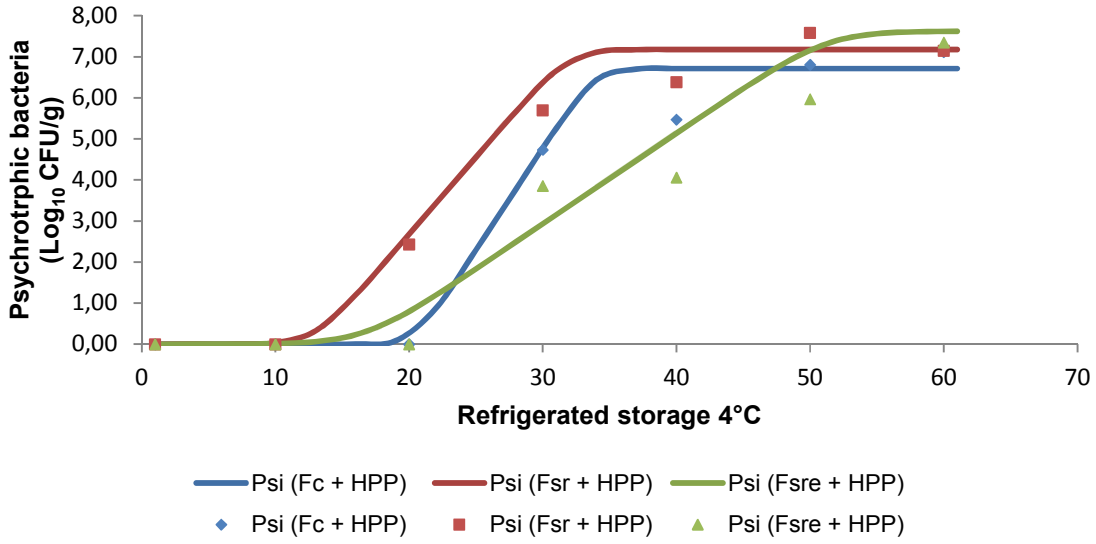

Figure 3. Fitted growth curves for Psychrotrophic bacteria during 60 days of refrigerated storage (4°C) for HP processed sliced vacuum-packaged RTE turkey breast processed at 600MPa/180seconds/25°C. F_C + HPP – Control plus HPP; F_{SR} + HPP (-30% NaCl + HPP); F_{SRE} + HPP (-30% NaCl + carvacrol 200ppm + HPP). Curves were fitted with model of Baranyi and Roberts (1994) with four parameters (lag phase, $LOG_{10}N_0$, μmax, $LOG_{10}N_{max}$). RSS and RSE were used as goodness of fit for models.

Despite an increase in maximum specific growth rate for F_{SRE} + HPP, the maximum population reached needs to take into account and a significant reduced (5.49 $Log_{10}N_{max}$) estimated maximum population was predicted for this formulation. An evident combined effect in *Listeria* control was registered due to carvacrol addition combined with high pressure processing. The antibacterial effects of carvacrol, an aromatic ring linked to hydroxyl, ethyl and methyl radicals (2-methyl-5-(1-methylethyl) phenol) were dependent on its ability to disturb cell membrane permeability (Lambert et al., 2001). Carvacrol interacts with the cell membrane, dissolving the phospholipid bilayer, and is assumed to align between the fatty acid chains disturbing their physical structure. As a consequence, an expansion and destabilization of cell membranes increase their fluidity which in turn would increase passive permeability (Ultee et al., 2002). Because HHP is believed to cause damage to the cell membrane, the common target is suggested to be the root of the observed synergism (Karatzas et al., 2001).

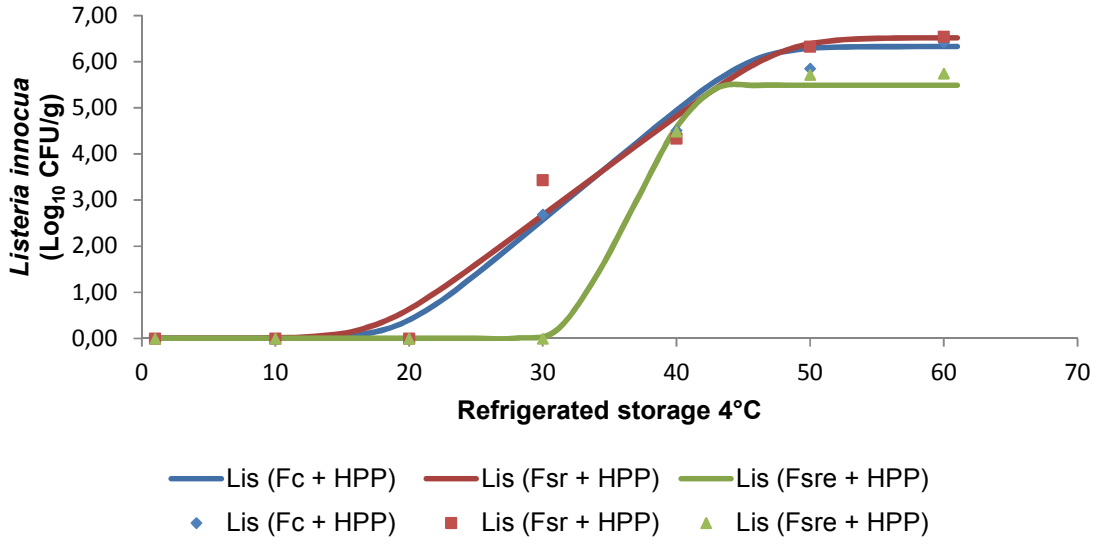

Figure 4. Fitted growth curves for *Listeria innocua* during 60 days of refrigerated storage (4°C) for HP processed sliced vacuum-packaged RTE turkey breast processed at 600MPa/180seconds/25°C. F_C + HPP – Control plus HPP; F_{SR} + HPP (-30% NaCl + HPP); F_{SRE} + HPP (-30% NaCl + carvacrol 200ppm + HPP). Curves were fitted with model of Baranyi and Roberts (1994) with four parameters (lag phase, $LOG_{10}N_0$, μmax, $LOG_{10}N_{max}$). RSS and RSE were used as goodness of fit for models.

Alternatively, microorganisms are baroresistant to selective chemical/natural antimicrobials due to their ability to exclude such agents from the cell, mainly by the action of the cell membrane and transport. However, if the membrane and active transport mechanisms become damaged, for example by HPP, this tolerance may be lost. This emerging problem of *Listeria monocytogenes* outgrowth patterns has been described in details by Valdramidis, Patterson and Linton (2015) in cured meat model formulated with varying levels of sodium chloride processed at 600MPa/3min/20°C; they found that the content of added salt highly influences the recovery indexes of the pathogen. *L. monocytogenes* is psychrotrophic, any cells that eventually survive against pressure treatment could potentially recover and grow during this extended storage period. Koseki, Mizuno, and Yamamoto (2007) found that counts of *L. monocytogenes* increased in sliced cooked ham during refrigerated storage after being apparently inactivated (<10 CFU/g) by HPP.

Table 9. Microbiological evaluation according to Brazilian requirements (for human consumption) of different treatments during refrigerated storage (4C°) shelf-life of sliced vacuum-packaged RTE turkey breast. Indicative samples.

Treatments	*Staphylococcus* sp. (CFU/g)			Regulatory standards*
	24hs	30 days	60	
F_C	$<10^2$	$1.0x10^2$	$4.30x10^3$	$3x10^3$
F_C + HPP	$<10^2$	$<10^2$	$6.50x10^2$	
F_{SR}	$<10^2$	$7.50x10^2$	$1.21x10^5$	
F_{SR} + HPP	$<10^2$	$<10^2$	$3.95x10^3$	
F_{SRE}	$<10^2$	$1.0x10^2$	$4.55x10^5$	
F_{SRE} + HPP	$<10^2$	$<10^2$	$1.45x10^3$	
Treatments	**Total Coliform (35,0°C - MPN/g)**			**Regulatory standards***
	24hs	**30 days**	**60**	
F_C	< 3.00	3.00	$1.10x10^5$	n/a
F_C + HPP	< 3.00	< 3.00	$9.30x10^3$	
F_{SR}	< 3.00	9.20	$1.10x10^5$	
F_{SR} + HPP	< 3.00	< 3.00	$2.40x10^3$	
F_{SRE}	< 3.00	< 3.00	$1.10x10^5$	
F_{SRE} + HPP	< 3.00	3.00	$7.50x10^3$	
Treatments	**Thermo stable Coliforms (45,0°C - MPN/g)**			**Regulatory standards***
	24hs	**30 days**	**60**	
F_C	< 3.00	< 3.00	3.60	10^3
F_C + HPP	< 3.00	< 3.00	< 3.00	
F_{SR}	< 3.00	9.20	$4.60x10^4$	
F_{SR} + HPP	< 3.00	< 3.00	$2.40x10^2$	
F_{SRE}	< 3.00	7.20	$4.60x10^4$	
F_{SRE} + HPP	< 3.00	< 3.00	$2.40x10^2$	
Treatments	**Sulphide reducing Clostridia (CFU/g)**			**Regulatory standards***
	24hs	**30 days**	**60**	
F_C	*<10*	*<10*	*<10*	$5x10^2$
F_C + HPP	*<10*	*<10*	*<10*	
F_{SR}	*<10*	*<10*	*<10*	
F_{SR} + HPP	*<10*	*<10*	*<10*	
F_{SRE}	*<10*	*<10*	*<10*	
F_{SRE} + HPP	*<10*	*<10*	*<10*	
Treatments	***Salmonella* sp.**			**Regulatory standards***
	24hs	**30 days**	**60**	
F_C	*absence*	*absence*	*absence*	Absence in 25g
F_C + HPP	*absence*	*absence*	*absence*	
F_{SR}	*absence*	*absence*	*absence*	
F_{SR} + HPP	*absence*	*absence*	*absence*	
F_{SRE}	*absence*	*absence*	*absence*	
F_{SRE} + HPP	*absence*	*absence*	*absence*	

*National Health Surveillance Agency – ANVISA/Brazil. Technical Regulation on Microbiological Standards for Food. RDC n°12 de 02 de Janeiro de 2001.

Natural antimicrobials have been appointed to help control the growth of *L. monocytogenes* during refrigerated storage of HPP processed meat products such as bacteriocin nisin in fermented sausages (Marcos et al., 2013); sodium lactate in cooked chicken (Patterson, Mackle & Linton, 2011); eneterocin and lactate-diacetate in cooked ham (Marcos et al., 2008). Results presented in Table 9 confirmed the promising preservation effects of HPP evaluated conditions. The HPP products were suitable for human consumption during entire storage time. On the other hand, non-HPP treated F_C, F_{SR} and F_{SRE} became inappropriate showing populations higher than established legal requirements (BRASIL, 2001) for *Staphyloccus* and Coliforms (45°C) at the end of storage period (60 days).

4. Conclusions

Addition of carvacrol in low-sodium sliced vacuum-packaged RTE turkey breast notably improves conservation effects of HPP processing at applicable set ups of 600MPa/180sec at room temperature; this combined treatments were able to reduce growth rates of spoilage groups and extend lag-phase of target pathogen *Listeria*. In addition, triggered lipid oxidation events may be delayed by carvacrol addition. Preservative effects during shelf-life can be potentiated through the presence of natural barriers, and the use of carvacrol represents a promising technology against sub-lethal injury and cell recovery phenomena.

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CONCLUSÕES GERAIS

I) A de redução de sódio em embutido de peito de peru (tipo *Blanquet*) fatiado embalado a vácuo utilizando como estratégia a simples redução do cloreto de sódio adicionado (sem a adição de sais substitutos) permite reduções em níveis de 30% sobre o controle formulado com 20g/kg, sem que o produto seja descaracterizado em termos físico-químicos e tenha sua aceitação prejudicada. No entanto, foi evidenciado que reduções do NaCl adicionado nestas proporções implicam em questionamentos quanto a segurança microbiológica e estabalidade frente ao crescimento de grupos microbianos deterioradores incluindo as bactérias ácido láticas e psicrotróficas; o potencial de conservação durante vida de prateleira, sem dúvida, é prejudicado. Reduções de 30% do *NaCl* adicionado resultam em 28% de redução do sódio, cumprindo assim as metas do acordo de cooperação entre o órgão de saúde pública nacional e as índústrias de alimentos, além de garantir as alegações de rótulo para o produto de acordo a legislação vigente para rotulagem nutricional “teor reduzido de sódio”.

II) O processamento utilizando alta pressão hidrostática representou uma alternativa viável para garantia de segurança e estabilidade do produto “low-sodium reformulado. Seguindo os critérios exigidos de desempenho para inativação pós-processo de *Listeria monocytogenes* (4-5 reduções de log), um valor de 600MPa/180 segundos a 25°C, parece ser um tratamento adequado para o produto com baixo teor de sódio estudado, promovendo reduções logarítmicas eficazes. Um eficiente efeito sobre a microbiota deterioradora do tratamento HPP nesta intensidade também foi confirmado. No entanto, ligeiras alterações em alguns dos atributos de qualidade avaliados puderam ser destacadas (principalmente sinerese, oxidação lipídica e textura); estes efeitos foram significativos em avaliações instrumentais, porém uma confirmação sensorial precisa ser fundamentada. Adicionalmente, dados apresentados demostraram que os consumidores não foram capazes de diferir o produto low-sodium processado por HPP de um não processado. Assim, o processamento HPP representa uma alternativa viável para manter a segurança e estender o prazo de validade de produtos cárneos RTE formulados com baixo teor de sódio; principalmente aqueles comercializados na modalidade fatiada re-embalada (manipulação de pós-processamento), os quais apresentam risco potencializado (*Slice Safety Risk*).

III) A adição de uma barreira natural complementar, como o carvacrol, em níveis sensorialmente aceitáveis, foi capaz de potencializar os efeitos de inativação (reduções logarítmicas) dos grupos microbianos alvo; isto representa uma arma promissora contra fenômenos de injúrias sub-letais e recuperação de celular. Sugere-se que a intensidade do processo HPP (em termos de carga de pressão, tempo de processo e temperatura) necessária para inativar microrganismos em níveis requeridos pode ser reduzida na presença de barreiras antimicrobianas adicionais. Efeitos combinados durante a estocagem refrigerada do produto processado também foram evidenciadas por meio de reduzidas taxas de crescimento e fase lag aumentada.

Assim, combinando HPP a obstáculos naturais adicionais, pode tornar-se possíveis efeitos semelhantes de segurança e de extensão de vida de prateleira com tratamentos HPP mais amenos, assegurando a manutenção da qualidade total do produto processado. Em adição a prospecção de uma série de vantagens industriais pode ser destacada em virtude da aplicação combinada tais como a redução de custos para a instalação de equipamento inicial e de manutenção (operação em pressões mais baixas); e maximização da produção de por ciclos efetivos mais curtos (ciclos/hora medida de efetividade de equipamento). É comprovado que ciclos muito intensos podem resultar em alteração das características naturais; logo ciclos menos intensos contribuem para potencialização da manutenção de frescor e qualidade global de alimentos pressurizados.

ANEXOS

UNIVERSIDADE FEDERAL DE LAVRAS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITOS DO PROCESSAMENTO POR ALTA PRESSÃO HIDROSTÁTICA SOBRE OS ATRIBUTOS DE QUALIDADE DE BLANQUET DE PERU COM TEOR REDUZIDO DE SÓDIO

Pesquisador: EDUARDO MENDES RAMOS

Área Temática:

Versão: 2

CAAE: 16684013.4.0000.5148

Instituição Proponente: Universidade Federal de Lavras

Patrocinador Principal: FUNDACAO DE DESENVOLVIMENTO DA PESQUISA

DADOS DO PARECER

Número do Parecer: 320.062
Data da Relatoria: 28/05/2013

Apresentação do Projeto:

Ok

Objetivo da Pesquisa:

Ok

Avaliação dos Riscos e Benefícios:

Ok

Comentários e Considerações sobre a Pesquisa:

Ok

Considerações sobre os Termos de apresentação obrigatória:

Todas as solicitações foram atendidas.

Recomendações:

Foram atendidas.

Conclusões ou Pendências e Lista de Inadequações:

Não há mais pendências.

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Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Considerações Finais a critério do CEP:

LAVRAS, 28 de Junho de 2013

Assinador por:
Joziana Muniz de Paiva Barçante
(Coordenador)