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TESE

Conservação de Queijo Coalho por Combinação de Alta Pressão Hidrostática e Embalagem Ativa Antimicrobiana

Sheyla Moreira Gonçalves

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CONSERVAÇÃO DE QUEIJO COALHO POR COMBINAÇÃO DE ALTA PRESSÃO HIDROSTÁTICA E EMBALAGEM ATIVA ANTIMICROBIANA

SHEYLA MOREIRA GONÇALVES

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Amauri Rosenthal

e Co-orientação das Doutoras
Nathália Ramos de Melo da Conceição e Janine Passos Lima da Silva

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AMAURI ROSENTHAL (Dr) EMBRAPA (orientador)

ELISA HELENA DA ROCHA FERREIRA (Dra) UFRRJ

KARINA MARIA OLBRICH DOS SANTOS (Dra) EMBRAPA

FRANCYS KLEY VIEIRA MOREIRA (Dr) UFSCAR

CAROLINE CORRÊA DE SOUZA COELHO (Dra) UNIRIO

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ASSINANTE EXTERNO
CPF: 025.072.978-40

(Assinado digitalmente em 13/10/2020 12:13)
CAROLINE CORRÊA DE SOUZA COELHO
ASSINANTE EXTERNO
CPF: 131.073.747-92

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FRANCYS KLEY VIEIRA MOREIRA
ASSINANTE EXTERNO
CPF: 017.584.875-00

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À minha mãe Anita Pereira Gonçalves, que considero mãe e pai, a mulher que me deu a vida e ainda me dá, todos os dias. A responsável pelos valores que possuo. Mãe e amiga, para sempre.

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“Determinação, coragem e autoconfiança são fatores decisivos para o sucesso. Se estamos possuídos por uma inabalável determinação, conseguiremos superar qualquer obstáculo. Independentemente das circunstâncias, devemos ser sempre humildes, recatados e despidos de orgulho”.

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RESUMO GERAL

GONÇALVES, Sheyla Moreira. **Conservação de Queijo Coalho por Combinação de Alta Pressão Hidrostática e Embalagem Ativa Antimicrobiana.** 2020. 109p Tese (Doutorado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Departamento de Ciência e Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2020.

Esta tese teve por finalidade avaliar os efeitos do processamento de alta pressão hidrostática (APH) (200, 300 ou 400 MPa/5 ou 10 min) sobre as propriedades funcionais do filme de acetato de celulose (AC), com ou sem incorporação de óleo essencial de orégano (OEO), bem como avaliar os efeitos da APH (300 MPa/5 min ou 400 MPa/10 min) e/ou filme antimicrobiano (FA) sobre a textura, parâmetros de cor e inativação de *Staphylococcus aureus*, *Escherichia coli* e *Listeria monocytogenes* em queijo coalho (QC) armazenado durante 21 dias/5 ^{0}C. Após o processamento com APH, filme de AC sem OEO apresentou redução da resistência à tração (RT) e no módulo de Young's (MY) e aumento do alongamento na ruptura (AR), luminosidade, coloração amarela/vermelha e opacidade. Os filmes pressurizados apresentaram redução da afinidade pela água, detectada através das análises de absorção de umidade (AU), ângulo de contato e taxa de transmissão ao vapor d'água (TTVA). Delaminações causadas pelo processamento com APH foram detectadas por microscopia eletrônica de varredura (MEV), exceto para os filmes tratados com 200 MPa/10 min e 300 MPa/10 min. Análises de difração de raio-X (DRX) e calorimetria diferencial de varredura (CDV) mostraram predominância de estrutura amorfa para todos os filmes. Além disso, o tratamento com APH provocou aumento da resistência mecânica durante a selagem a 250 ^{o}C. Para o filme de AC incorporado com OEO, o processamento com APH causou alterações ópticas, redução da RT, MY, TTVA, AU e aumento do AR e ângulo de contato, além de delaminações observadas em imagens de MEV. Curvas de DRX detectaram predominância de estrutura amorfa em todos os filmes, o que pode ter impedido a detecção de parâmetros térmicos para a maioria das amostras, em análise de CDV. O processamento do FA com APH não prejudicou a eficiência do OEO avaliada em meios de cultura inoculados (ágar, caldo BHI e micro-atmosfera), apresentando inibição total de *Staphylococcus aureus*, *Escherichia coli* e *Listeria monocytogenes*, para todos os filmes. No QC armazenado, a combinação do FA e 400 MPa/10 min foi a mais eficiente contra as três bactérias estudadas, no tempo zero e ao longo do armazenamento, enquanto o tratamento somente com APH não foi capaz de impedir a multiplicação das bactérias nas amostras de QC. Portanto, somente o FA ou a combinação de FA e APH foram eficientes no controle do crescimento microbiano em QC. Quanto aos parâmetros de cor, o processamento com APH e/ou embalagem com FA causou aumento da luminosidade e coloração amarela/vermelha nas amostras de QC aos 21 dias de armazenamento sob refrigeração (5 ^{o}C). Para os parâmetros de textura do QC, a presença do FA não causou mudanças significativas, enquanto o tratamento com 400 MPa/10 min causou as maiores reduções na dureza e coesividade e o aumento da gomosidade e mastigabilidade. A elasticidade do QC não foi afetada por nenhuma das condições estudadas. Em conclusão, este estudo demonstrou que a combinação da embalagem antimicrobiana e alta pressão hidrostática é promissora para aplicação em queijo coalho, sem prejudicar as propriedades funcionais do filme de acetato de celulose.

Palavras-chave: Combinação de tecnologias, Propriedades físicas, Produto lácteo.

GENERAL ABSTRACT

GONÇALVES, Sheyla Moreira. **Conservation of coalho cheese by combining high hydrostatic pressure and antimicrobial active packaging.** 2020. 109p. Thesis (Doctorate in Food Science and Technology). Instituto de Tecnologia, Departamento de Ciência e Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2020.

This thesis aimed to evaluate the effects of high hydrostatic pressure (HHP) processing (200, 300 or 400 MPa/5 or 10 min) on the functional properties of cellulose acetate (CA) film, with or without incorporation oregano essential oil (OEO), as well as to evaluate the effects of HHP (300 MPa/5 min or 400 MPa/10 min) and/or antimicrobial film (AF) on the texture, color parameters and inactivation of *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes* in coalho cheese (CC) stored for 21 days/5ºC. After processing with HHP, CA film without OEO showed a reduction in tensile strength (TS) and in Young's module (YM) and an increase in elongation at break (EB), luminosity, yellow/red color and opacity. The pressurized films showed reduced affinity for water, detected through the analysis of moisture absorption (MA), contact angle and water vapor transmission rate (WVTR). Delaminations caused by HHP processing were detected by scanning electron microscopy (SEM), except for films treated with 200 MPa/10 min and 300 MPa/10 min. X-ray diffraction (XRD) and differential scanning calorimetry (DSC) analyzes showed predominance of amorphous structure for all films. In addition, treatment with HHP caused an increase in mechanical strength during sealing at 250 ºC. For the CA film incorporated with OEO, processing with HHP caused optical changes, reduced TS, YM, WVTR, MA and increased EB and contact angle, in addition to delamination observed in SEM images. XRD curves detected a predominance of amorphous structure in all films, which may have prevented the detection of thermal parameters for most samples, in DSC analysis. The processing of AF with HHP did not affect the efficiency of the OEO evaluated in culture media (agar, BHI broth and micro-atmosphere), showing total inhibition of *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*, for all films. In the stored CC, the combination of AF and 400 MPa/10 min was the most efficient against the three bacteria studied, at zero time and throughout storage, while treatment only with HHP was not able to prevent the multiplication of bacteria in the samples of CC. Therefore, only the AF or the combination of AF and HHP were effective in controlling microbial growth in CC. As for color parameters, processing with HHP and/or packaging with AF caused an increase in brightness and yellow / red color in the CC samples at 21 days of storage under refrigeration (5 ºC). For the texture parameters of CC, the presence of FA did not cause significant changes, while the treatment with 400 MPa / 10 min caused the greatest reductions in hardness and cohesiveness and an increase in gumminess and chewiness. The springiness of the CC was not affected by any of the conditions studied.

Keywords: Combination of technologies, Physical properties, Dairy product.

LISTA DE FIGURAS

LISTA DE ABREVIAÇÕES E SIGLAS

± a*	red/green chromaticity
AAP	antimicrobial active packaging
AC	acetato de celulose
APH	alta pressão hidrostática
AR	alongamento na ruptura
ATR	attenuated total reflection
AU	absorção de umidade
± b*	yellow/blue chromaticity
C*	Chroma
CA	cellulose acetate
CC	coalho cheese
CDV	calorimetria diferencial de varredura
ADN	ácido desoxirribonucléico
DRX	difração de raio-X
DS	degree of substitution
DSC	differential scanning calorimetry
EA	embalagem ativa
EAA	embalagem ativa antimicrobiana
EB	elongation at break
EPEC	*Escherichia coli* enteropatogênica clássica
ETEC	*Escherichia coli* enterotoxigênica
EIEC	*Escherichia coli* enteroinvasiva
EHEC	*Escherichia coli* enterohemorrágica
FEEC	*Escherichia coli* enteropatogênica facultativa
EO	essential oils
FA	filme antimicrobiano
FTIR	fourier transform infrared reflection
g/cm^{-3}	grama por centímetro cúbico
h^{o}	hue angle
HHP	high hydrostatic pressure
IgG	imunoglobulina G
L*	brightness
Log/UFC	logaritmo de unidades formadoras de colônia
Kg	kilograma
Kgf	kilograma força
kV	kilovolts
mA	miliampère
MA	moisture absorption
MEV	microscopia eletrônica de varredura
min	minutos
mm	milímetro
MPa	megapascal
MRHST	maximum resistance of the heat seal to traction
MY	módulo de Young's
N	newton
NaCl	cloreto de sódio
OE	óleos essenciais
OEO	óleo essencial de orégano/oregano essential oil
pH	potencial de hidrogênio
QC	queijo coalho

RH	relative humidity
RT	resistência à tração
s	segundos
SEM	scanning electron microscopy
Tg	glass transition temperature
TS	tensile strength
TTVA	taxa de transmissão ao vapor d’água
YM	Young's modulus
w/v	weight/volume
WVTR	water vapor transmission rate
XRD	X-ray diffraction
ΔE*	color total difference
µl	microlitros

Sumário

1 INTRODUÇÃO GERAL

No mercado consumidor moderno, verifica-se uma crescente demanda por produtos alimentícios tradicionais. Tal comportamento pode estar relacionado com a tendência dos consumidores em estar mais próximos às tradições regionais do país. O queijo coalho (QC) é um produto tradicional da região Nordeste do Brasil que tem atraído a atenção e o paladar dos consumidores nacionais e internacionais (FONTENELE et al., 2017). Trata-se de um produto ainda considerado artesanal e suas características dependem da matéria-prima, da microbiota envolvida e da tecnologia de processamento aplicada (BEZERRA, et al., 2016).

A prática de produção artesanal de QC representa uma fonte expressiva de renda e trabalho para uma considerável parcela da população de pequenos e médios produtores rurais nordestinos (SILVA et al., 2012). Porém, a manipulação durante o processamento e/ou a qualidade microbiológica do leite empregado podem facilitar a veiculação de agentes patogênicos aos consumidores (SOUSA et al., 2006). Dentre os agentes patogênicos envolvidos em surtos alimentares e contaminações de QC no Brasil, *Listeria monocytogenes*, *Escherichia coli* e *Staphylococcus aureus* são citados (RUWER et al., 2011).

O crescente conhecimento dos consumidores sobre as doenças transmitidas por alimentos (DTAs), somado ao risco microbiológico associado ao QC comercializados em regiões do Nordeste brasileiro, pode representar um entrave ao consumo do produto (CAVALCANTE, 2005). Além disso, é cada vez maior o interesse dos consumidores por produtos mais saudáveis, seguros, com maior qualidade nutricional e sensorial. Portanto, a busca por práticas tecnológicas que possam minimizar os prejuízos econômicos e à saúde, sem causar danos à qualidade dos alimentos, torna-se relevante. Dentre as tecnologias emergentes e promissoras visando atender o mercado alimentício, destaca-se a alta pressão hidrostática (APH), método não térmico de preservação (FELLOWS, 2006; GAVA e FRIAS, 2009) que pode ser aplicado individualmente (COSTABEL et al., 2016), ou em combinação com outros métodos de conservação (ARQUÉS et al., 2005), como barreiras adicionais.

A tecnologia de APH apresenta vantagens, como aumento da validade comercial, manutenção das propriedades nutricionais e sensoriais, sem causar danos ao ambiente (OLIVEIRA et al., 2015; HU et al., 2017). Outra tecnologia emergente que tem atraído a atenção da indústria alimentar consiste na utilização de embalagem ativa antimicrobiana (EAA). Esta é capaz não só de proteger, mas também de interagir com o produto, modificando de maneira desejável características sensoriais e promovendo inibição do crescimento microbiano, estendendo a validade comercial do alimento (CORRADINI et al., 2013). Diante da crescente demanda por produtos mais saudáveis e seguros, o uso de compostos naturais como substituintes aos sintéticos têm ganhado a atenção dos consumidores e das indústrias de alimentos. Dessa maneira, compostos antimicrobianos naturais, como os óleos essenciais (OE), dentre eles o de orégano (OEO), têm sido utilizados como aditivos em materiais poliméricos (HOSSEINI et al., 2015) para elaboração de embalagem ativa antimicrobiana (EAA).

É preocupante as informações acerca da degradação dos recursos naturais e dos impactos gerados ao meio ambiente devido à utilização de embalagens plásticas não biodegradáveis. Logo, existe grande interesse em alternativas naturais biodegradáveis para produção de embalagens para alimentos (APPENDINI e HOTCHKISS, 2002; ARANCIBIA et al., 2014). Contudo, de acordo com a aplicação desejada, estas embalagens precisam se adequar conforme as exigências em resistência mecânica, barreira a umidade, aspectos visuais, dentre outros (BENAVIDES et al., 2012).

Estudos têm demonstrado que a APH (RAMOS et al., 2015) e EAA (BERISTAIN-BAUZA et al., 2017), quando utilizadas individualmente, têm efeitos positivos sobre a

extensão da validade comercial dos alimentos (ARANCIBIA et al., 2014). No entanto, muitas vezes não são suficientes para eliminar de forma efetiva os micro-organismos deteriorantes ou patogênicos (EVELYN e SILVA, 2015), havendo necessidade da combinação de outras tecnologias, como barreiras, para obter melhor eficiência. Pesquisas relatam efeito sinérgico antimicrobiano na combinação de APH e EAA em salames (MARCOS et al., 2013) e presunto cozido (JOFRÉ et al., 2008).

A incorporação de aditivos (naturais ou sintéticos) em materiais poliméricos é um artifício para adequação de propriedades funcionais desejáveis, porém pode alterar as características originais desses materiais (MANO e MENDES, 2013). Além disso, a embalagem para acondicionar um alimento a ser pressurizado precisa ser adequada, uma vez que a APH pode causar alterações em sua estrutura polimérica (MENSITIERI et al., 2013). Portanto, investigações a respeito das propriedades da embalagem após a incorporação de aditivo ou pressurização torna-se necessária, uma vez que irá direcionar sua aplicação.

O desenvolvimento de tecnologias que, além de serem utilizadas para conter o desenvolvimento microbiano em alimentos, possam ser usadas em combinação às tecnologias individuais de preservação, representam importante avanço na minimização dos prejuízos econômicos e perigos de interesse em saúde pública. Portanto, o objetivo específico inicial do presente estudo consistia em avaliar a eficiência individual ou combinada de APH e EAA na conservação de QC, considerando a eficiência de ambas as tecnologias no controle do crescimento de *S. aureus, E. coli* e *L. monocytogenes,* durante o armazenamento refrigerado de QC. Porém, a falta de informações sobre a capacidade da EAA em suportar a compressão e descompressão causadas pela APH, nos motivou a realizar estudos prévios, afim de conhecer, dentre as condições de APH utilizadas (200 MPa/ 5 ou 10 min; 300 MPa/5 ou 10 min; 400 MPa/5 ou 10 min), quais causariam menores danos à funcionalidade do filme de acetato de celulose. Logo, o objetivo geral deste estudo foi adaptar o filme de acetato de celulose incorporado com óleo essencial de orégano a duas condições de APH, que causassem modificações mínimas em sua estrutura e funcionalidade, para ser usado posteriormente como uma embalagem que garantisse a conservação do queijo coalho. Nos estudos prévios, gerou-se então, resultados sobre as possíveis alterações causadas pela APH sobre as propriedades da embalagem, incorporada ou não com OEO.

A presente tese está organizada da seguinte forma: No Capítulo 1 encontra-se uma revisão bibliográfica referente aos aspectos teóricos e práticos de relevância ao tema proposto. No Capítulo 2, um estudo acerca dos possíveis efeitos causados pela alta pressão hidrostática (200 MPa/ 5 ou 10 min; 300 MPa/5 ou 10 min; 400 MPa/5 ou 10 min) sobre as propriedades do filme de acetato puro (sem óleo essencial de orégano). No Capítulo 3, um estudo acerca dos possíveis efeitos causados pela alta pressão hidrostática (200 MPa/ 5 ou 10 min; 300 MPa/5 ou 10 min; 400 MPa/5 ou 10 min) sobre as propriedades do filme de acetato incorporados com óleo essencial de orégano. Após esses dois estudos, duas condições de alta pressão hidrostática foram definidas, levando em consideração as maiores resistências mecânicas apresentadas pelos filmes tratados com as diferentes pressões e tempos. As condições de 300 MPa/5 min e 400 MPa/10 min foram definidas para dar continuidade aos dois próximos estudos. No Capítulo 4, o estudo está dividido em: Análise da composição do óleo essencial de orégano; Avaliação da eficiência dos filmes tratados com alta pressão (300 MPa/5 min ou 400 MPa/10 min) contra o crescimento de *S. aureus, L. monocytogenes* e *E. coli*, em meios de cultura; Avaliação da eficiência da embalagem antimicrobiana, da alta pressão hidrostática (300 MPa/5 min ou 400 MPa/10 min) ou combinação de embalagem antimicrobiana e alta pressão hidrostática contra o crescimento de *S. aureus, L. monocytogenes* e *E. coli* em queijo coalho, armazenado por 21 dias a 5 ^{o}C. No Capítulo 5, o estudo traz informações sobre os efeitos da utilização da embalagem antimicrobiana, da alta

pressão hidrostática (300 MPa/5 min ou 400 MPa/10 min) ou combinação de embalagem antimicrobiana e alta pressão hidrostática sobre os parâmetros de cor e textura do queijo coalho, armazenado por 21 dias a 5 ºC.

CAPÍTULO I

REVISÃO BIBLIOGRÁFICA

1 Queijo Coalho

De acordo com a Instrução Normativa n° 30, de 26 de Junho de 2001, do Ministério da Agricultura, Pecuária e Abastecimento, QC é o "queijo que se obtém por coagulação do leite por meio do coalho ou outras enzimas coagulantes apropriadas, complementada ou não pela ação de bactérias lácteas selecionadas, e comercializado normalmente com até 10 (dez) dias de fabricação" (BRASIL, 2001). Trata-se de um alimento de média a alta umidade, teor de gordura variável entre 35% e 60%, produzido com massa semi-cozida ou cozida, podendo ser adicionado de outros ingredientes, como condimentos (NASSU et al., 2006).

O QC é uma variedade de queijo tradicional da região nordeste do Brasil de importância socioeconômica e nutricional, com comercialização e consumo crescente em todo o País. Ainda considerado artesanal, o QC tem suas características particulares definidas pelo método de processamento empregado, o tipo de leite e a microbiota envolvida. A microbiota, em particular, produz e define os compostos específicos que caracterizam o aroma e sabor do queijo (FONTENELE et al., 2017).

A produção e comercialização do QC é a principal fonte de renda para uma parcela de pequenos e médios produtores rurais nordestinos (BRUNO et al., 2017) e o seu fabrico tem sido realizado por mais de 100 anos (CAVALCANTE, 2005). O consumo de QC nas demais regiões brasileiras tem crescido nos últimos anos (SILVA et al., 2012), principalmente no período compreendido entre 2006 a 2013 (DE CARVALHO et al., 2015). Suas principais características são o sabor ligeiramente salgado e ácido, aparência úmida e textura semelhante à borracha (SILVA et al., 2012). O QC pode ser consumido de forma *in natura*, assado ou frito, e como ingrediente de diversos pratos culinários (BEZERRA et al., 2016). Estudos sobre a qualidade e autenticidade de QC foram realizados (FONTENELE et al., 2017), assim como sua capacidade de incorporar micro-organismos probióticos (BEZERRA et al., 2017).

O Regulamento Técnico de Identidade e Qualidade de Queijo de Coalho (BRASIL, 2001) determina que "o leite a ser utilizado deverá ser higienizado por meios mecânicos adequados e submetido à pasteurização ou tratamento térmico equivalente". Porém, ainda é encontrado QC produzido com leite cru e manipulado em condições higiênicas inadequadas (RUWER et al., 2011).

Estudos sobre a qualidade de QC destacaram a presença de contaminação do produto por coliformes termotolerantes, microrganismos indicadores de contaminação fecal, *Staphylococcus aureus*, *Salmonella* spp. e *Listeria monocytogenes* (BORGES et al., 2003; SANTANA et al., 2008; DE FREITAS et al., 2013).

Listeria monocytogenes, bactéria gram-positiva com capacidade de se desenvolver em ambientes com diferentes condições de temperaturas (principalmente em ambientes refrigerados), pH, concentração de sal ou ambientes secos. Sua capacidade de resistir a desinfetantes e formar biofilmes (CARPENTIER e CERF, 2011) a torna um micro-organismo de grande preocupação em alimentos prontos para consumo (HENRIQUES e FRAQUEZA, 2017).

Staphylococcus aureus, bactéria gram-positiva que representa um problema de saúde pública através do consumo de queijos, uma vez que produz enterotoxinas nos alimentos (SEYYED et al., 2017). De acordo com Mehli et al. (2017), a alta incidência de *S. aureus* produtor de enterotoxina é motivo de preocupação com a segurança de alimentos na fabricação de queijos artesanais, utilizando-se ou não leite pasteurizado.

Escherichia coli, bactéria gram-negativa comumente encontrada em alimentos e derivados lácteos. Sua presença indica contaminação por micro-organismos de origem fecal e a possível presença de outros agentes patogênicos. Dentre as linhagens que causam enfermidades de origem alimentar citam-se: enteropatógenas clássicas (EPEC),

enterotoxigênicas (ETEC) (ÁLVAREZ-SUÁRE et al., 2016), enteroinvasivas (EIEC), enterohemorrágicas (EHEC) e enteropatogênicas facultativas (FEEC) (OMBARAK et al., 2016).

2 Embalagem para Alimentos

2.1 Embalagem Primária

Embalagem primária "é o material que está em contato direto com os produtos, destinado a contê-los, desde a sua fabricação até a sua entrega ao consumidor, com a finalidade de protegê-los de agente externos, de alterações e de contaminações, assim como de adulterações" (BRASIL, 2001).

2.2 Polímeros Naturais

Polímeros são macromoléculas caracterizadas pela presença de unidades químicas repetidas denominadas "meros", unidas geralmente por ligações covalentes. As propriedades dos materiais poliméricos são definidas de acordo com tamanho da cadeia (massa molar), da estrutura química e suas interações intra e intermoleculares. Os polímeros podem ser naturais, sintéticos ou semissintéticos (MENDES e MANO, 2004; CANEVAROLO JR, 2006) e a regulação de sua utilização para o desenvolvimento de embalagens para contato com alimentos é determinada pela Agência Nacional de Vigilância Sanitária (ANVISA) (BRASIL, 1999).

Dentre os polímeros utilizados pela indústria, os que compõem os plásticos são os mais conhecidos. Os plásticos se tornam fluidos em algum estágio de seu processamento (podendo assim ser moldados) através da ação isolada ou conjunta do calor e pressão. Os plásticos mais importantes industrialmente são de origem sintética (MENDES e MANO, 2004; MANO e MENDES, 2013). Porém, diante da crescente conscientização ecológica mundial, os polímeros naturais vêm ganhando gradativamente importância industrial (VARGHESE et al., 2020). A maioria das embalagens alimentares são produzidas com polímeros derivados do petróleo ou não-biodegradáveis, no entanto, a dificuldade de eliminação de tais compostos tem gerado grave problema ambiental. Dessa maneira, para redução do impacto gerado ao meio ambiente, torna-se necessária, dentre outras medidas, a substituição destes materiais por polímeros biodegradáveis de fontes renováveis (ANDRADE-MOLINA et al., 2013).

Acetato de celulose (AC) (Figura 1) é um polímero biodegradável abundante, obtido através da acetilação da celulose por anidrido acético ou ácido acético, utilizando ácido sulfúrico como catalisador (CERQUEIRA et al., 2010).

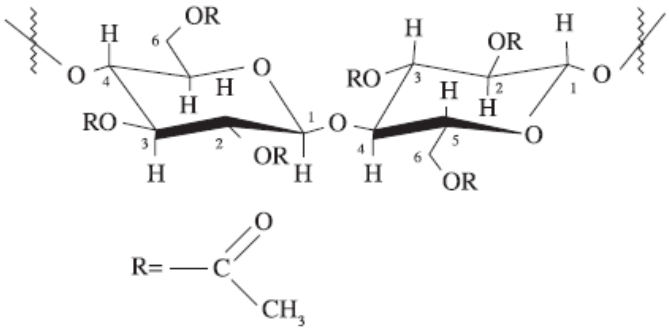

Figura 1. Estrutura do acetato de celulose.
Fonte: Cerqueira et al., 2010.

AC apresenta custo baixo, maciez, conforto, brilho e sensação natural, o que permite sua utilização na produção de fita adesiva transparente, cabos de ferramentas, armações de óculos, têxteis e materiais relacionados. É um material termoplástico, com boa resistência ao impacto, transparente e boas propriedades elétricas (PARK et al., 2004; CANEVAROLO JR, 2006). O AC é sensível à absorção de água, alterando todas as suas propriedades mecânicas. Apresenta, também, baixa permeabilidade ao vapor de água e aos gases, que decresce com o aumento do grau de acetilação (MILES e BRISTON, 1975; CANEVAROLO JR, 2006).Várias pesquisas relatam o desenvolvimento e caracterização de embalagens para alimentos à base de acetato de celulose (RODRÍGUEZ et al., 2012; BRUNA et al., 2014; BAO et al., 2015; MOHAN et al., 2015; POLA et al., 2016; DANNENBERG, 2017) e o uso de derivados de celulose para reforço de outros materiais poliméricos (PEREDA et al., 2011a, 2011b).

2.3 Embalagem Ativa

A crescente procura dos consumidores por alimentos mais saudáveis, seguros, práticos e com características sensoriais mais próximos ao alimento *in natura*, é notável. Dessa maneira, as embalagens ativas (EA) são desenvolvidas com intuito de, não somente de proteger o alimento, como também modificar suas condições de forma desejável, sendo importantes para preservar o frescor e obter maior validade comercial dos produtos (GÓMEZ-ESTACA et al., 2014; TEIXEIRA et al., 2014).

O conceito de EA permite a interação da embalagem com o produto (Resolução (EC) nº 1935/2004). As EA alteram as condições dos alimentos, seja por absorção e remoção de compostos indesejáveis, ou por liberação de substâncias desejáveis para a superfície do produto ou para o espaço entre o produto e a embalagem. Logo, são obtidos efeitos satisfatórios sobre a validade comercial, além de assegurar a qualidade do alimento (CORRADINI et al., 2013; BARBOSA-PEREIRA et al., 2014). EA antimicrobianas, antioxidantes e aromatizantes (DAINELLI et al., 2008) são classificadas na sua maioria como sistemas migratórios (liberação ativa), onde há a migração intencional de compostos não-voláteis ou voláteis da embalagem para o alimento.

A garantia da qualidade e segurança do alimento está alinhada ao controle da contaminação microbiológica, causadora de consideráveis prejuízos econômicos e à saúde dos consumidores. Dessa maneira, pesquisas objetivando o desenvolvimento de embalagens, com incorporação ou imobilização de compostos antimicrobianos, têm sido promissoras (SILVESTRE et al., 2011; CORRADINI et al., 2013). Além disso, a conscientização sobre a limitação dos recursos naturais, impactos ao ambiente e saúde causados pelo uso de materiais e conservantes sintéticos é crescente. Tal tendência tem gerado interesse na produção de embalagens biodegradáveis contendo componentes ativos naturais (DAINELLI et al., 2008).

Dentre os polímeros naturais utilizados para produção de EA para contato com alimentos destacam-se: derivados de celulose (OSORIO et al., 2011; CHEN et al., 2014; MUPPALLA et al., 2014; AZZAOUI et al., 2015; DASHIPOUR et al., 2015; HASSAN et al., 2015; DANNENBERG, 2017; GONÇALVES et al., 2019), proteínas (YOSHIDA e ANTUNES, 2009; GALUS e KADZIŃSKA, 2016; BERISTAIN-BAUZA et al., 2017; DÍAZ et al., 2017; KAEWPRACHU et al., 2017), quitosana (AIDER, 2010; KANATT et al., 2012), carragena (CHANG et al., 2014), amidos (MACHADO et al., 2012; LÓPEZ-CÓRDOBA et al., 2017), alginatos (BENAVIDES et al., 2012; TAVASSOLI-KAFRANI et al., 2016), pectina (AZEREDO et al., 2016; MANRICH et al., 2017; NISAR et al., 2017).

2.4 Aditivos: Compostos Antimicrobianos

Para elaboração de sistemas ativos, geralmente torna-se necessária a incorporação ou imobilização de substâncias com funções ativas (DAINELLI et al., 2008). A conscientização a respeito do efeito nocivo dos conservantes sintéticos, associado à alterações no paradigma das embalagens primárias para alimentos, tem gerado interesse entre os pesquisadores para uso de produtos naturais (BARBOSA-PEREIRA et al., 2014). A utilização de compostos antimicrobianos naturais em alimentos tem ganhado muita atenção por parte dos consumidores e da indústria alimentícia, que busca alternativas para melhorar a segurança e a qualidade dos alimentos (BERISTAIN-BAUZA et al., 2017).

Antimicrobianos naturais podem ser obtidos a partir de diferentes fontes, incluindo: vegetal (XIA et al., 2011; SAGDIC et al., 2012; KOŁODZIEJCZYK et al., 2013; OLIVEIRA et al., 2013; SUAREZ-QUIROZ et al., 2013; YE et al., 2013; BADAWY e ABDELGALEIL, 2014; JOBIM et al., 2014; KALLEL et al., 2014; MOLVA e BAYSAL, 2015), animal (BARBIROLI et al., 2012; LI et al., 2012; ARANCIBIA et al., 2014; LEE e MIN, 2014; HAROUNA et al., 2015), bacteriana (CAO-HOANG et al., 2010; DIEZ et al., 2012; ESPITIA et al., 2013; GARDE et al., 2014), algas (XU et al., 2003; OH et al., 2008; VIJAYABASKAR et al., 2012; AL-SAIF et al., 2014) e fúngicas (YALTIRAK et al., 2009; BEATTIE et al., 2010; NOWACKA et al., 2014; REN et al., 2014).

A utilização de antimicrobianos naturais em alimentos ainda é limitada devido aos efeitos indesejáveis sobre características sensoriais (NEGI, 2012). Portanto, mais pesquisas são necessárias para determinar níveis de segurança para aplicação de antimicrobianos em alimentos, sem alterar indevidamente quaisquer características do produto (GYAWALI e IBRAHIM, 2014). As plantas são fontes valiosas de moléculas biologicamente ativas com propriedades antimicrobianas. O foco atual em conservantes naturais está em um pequeno número de agentes antimicrobianos que têm sido usados por muitos anos, e há uma necessidade de expandir esta lista para a sua aplicação em alimentos, garantindo a segurança e qualidade dos produtos (NEGI, 2012). Neste contexto, os óleos essenciais e extratos de ervas medicinais e especiarias têm ganhado importância. No entanto, investigação sobre o isolamento dos fitoquímicos com atividade antimicrobiana e o impacto destes extratos em atributos sensoriais são necessários (NEGI, 2012; KUMAR et al., 2014).

Os óleos essenciais (OE) são substâncias voláteis, aromáticas, extraídas de vegetais, principalmente por hidrodestilação (ELGNDI et al., 2017). Eles podem estar presentes em botões, flores, folhas, caules, ramos, sementes, frutos, raízes, madeira ou cascas. Os OE possuem uma mistura de compostos como terpenos, álcoois, acetonas, fenóis, ácidos, aldeídos e ésteres (CALO et al., 2015). Estes óleos são usados na indústria alimentícia e farmacêutica (TIAN et al., 2014). Geralmente, a ação antimicrobiana de OE se dá através da inibição da síntese de proteínas e alterações na membrana citoplasmática, interrupção da força motriz de prótons e do fluxo de elétrons (SUNG et al., 2013; CALO et al., 2015). Dentre os OE incorporados em embalagens de alimentos incluem-se: óleo de cravo, manjericão, orégano, alho, canela, alecrim, funcho doce (SUNG et al., 2013).

Origanum vulgare, popular orégano, é uma planta medicinal, aromática, herbácea, perene, pertencente à família Lamiaceae, que cresce em toda a área do Mediterrâneo e regiões montanhosas do sul da Europa (LUKAS et al., 2015). Os tricomas glandulares dessas plantas secretam OE como mecanismo de defesa contra bactérias, fungos e outros predadores. Esse OE é constituído por compostos voláteis complexos como: carvacrol, timol e γ-terpineno (Figura 2), que possuem ampla atividade antimicrobiana e antifúngica (MASTRO et al., 2017). *Origanum vulgare* é uma das especiarias mais comercializadas, sendo seu OE

amplamente utilizado como antimicrobiano natural e acentuador de sabor e aroma em alimentos (HOSSEINI et al., 2015; LUKAS et al., 2015).

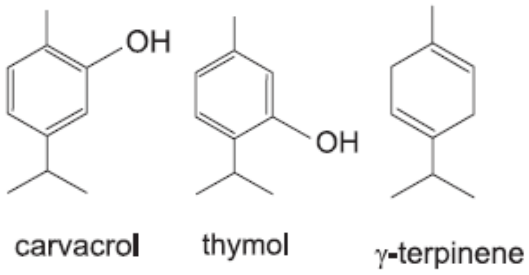

Figura 2. Estrutura química do composto volátil presente no OE de orégano.
Fonte: Burt, 2004.

Diante da crescente busca por produtos naturais como substituintes aos sintéticos, o OE de orégano vem sendo utilizado como agente ativo para incorporação em materiais para embalagens de alimentos. Estudos comprovam a atividade antimicrobiana do OE de orégano sobre diferentes microrganismos-alvo, além de relatar sua capacidade de incorporação em embalagens ativas (JOUKI et al., 2014; PAVELKOVA et al., 2014; TEIXEIRA et al., 2014; WU et al., 2014).

2.5 Propriedades dos Materiais Aditivados

As embalagens ativas antimicrobianas são desenvolvidas no intuito de ser uma alternativa de embalagem para alimentos, mas, para isso, precisam se apresentar versáteis, resistentes, além de ter aspectos atrativos aos olhos dos consumidores. Tais propriedades funcionais estão diretamente relacionadas com a estrutura química do polímero e do aditivo, além do método de processamento ao qual é submetido o material (AZZAOUI et al., 2015; AZEREDO et al., 2016).

A capacidade dos polímeros em aceitar uma variedade de aditivos é fundamental para definição de suas propriedades (CANEVAROLO JR, 2006). Portanto, o efeito da incorporação de compostos antimicrobianos ou plastificantes sobre as propriedades funcionais dos filmes depende da característica de cada composto adicionado e de sua interação com a matriz polimérica (CORRADINI et al., 2013; TAWAKKAL et al., 2014; HOSSEINI et al., 2015; LIU et al., 2017).

A morfologia das cadeias do polímero deve ser considerada, uma vez que a presença de ramificações tem efeitos significativos sobre as propriedades físicas dos materiais poliméricos. Polímeros ramificados possuem menor solubilidade que os de cadeias lineares. Durante o processo de polimerização, pode ocorrer a reação denominada reticulação (cadeias interligadas por ligações covalentes ou ligações cruzadas), a qual promove uma maior rigidez e estabilidade dimensional em termoplásticos (CANEVAROLO JR, 2006).

A mobilidade da cadeia polimérica determina as características físicas da embalagem, sendo esta diretamente proporcional à temperatura (MANO e MENDES, 2013). Segundo o comportamento térmico, os polímeros possuem dois tipos de temperaturas de transição importantes, a temperatura de transição vítrea (Tg: temperatura que permite mobilidade dos segmentos de cadeias poliméricas em regiões amorfas, adquirindo mobilidade) e temperatura de fusão cristalina (Tm: temperatura para desarranjo da estrutura regular de empacotamento permitindo a fusão) (CANEVAROLO JR, 2006).

A cristalinidade de um material polimérico está diretamente relacionada com o arranjo das cadeias. Os polímeros são formados por duas fases estruturais: cristalina e amorfa. A fase cristalina é caracterizada por arranjo perfeito das cadeias, enquanto a fase amorfa apresenta arranjo espacial de longo alcance desordenada. É praticamente impossível a existência de polímeros totalmente cristalinos porque apenas parte da molécula adota a conformação ordenada necessária, por isso são preponderantemente apenas semicristalinos ou amorfos. Em semicristalinos, a fase cristalina é constituída por zonas ordenadas imersas na matriz amorfa (CARRASQUEIRO, 2004; CANEVAROLO JR, 2006).

O conhecimento das características químicas e estruturais dos polímeros é importante para prever influência de compostos incorporados. Além disso, o peso molecular inicial do material polimérico deve ser considerado, uma vez que pode determinar as propriedades mecânicas e aplicação do produto final polimérico (CANEVAROLO JR, 2006). As propriedades mecânicas dos polímeros podem ser avaliadas de forma estática ou dinâmica, em que na maioria das análises atinge-se a ruptura do material. Parâmetros como resistência à tração, módulo de elasticidade (Young's), alongamento na ruptura e perfuração são determinados no limite da resistência destrutiva do polímero (MENDES e MANO, 2004; CANEVAROLO JR, 2006).

3 Alta Pressão Hidrostática

O processo de Alta Pressão Hidrostática (APH) é uma tecnologia não térmica com uso crescente pela indústria de alimentos na busca de suprir os anseios dos consumidores modernos, que demandam alimentos seguros, com qualidade nutricional e sensorial (AYMERICH et al., 2008). Trata-se de uma operação onde o alimento (líquido ou sólido, a granel ou mais comumente embalado) é submetido a uma determinada pressão, distribuída de maneira uniforme pelo produto, o que garante que o alimento mantenha sua forma inicial mesmo frente a pressões elevadas extremas (STRATAKOS et al., 2015; PRAKASAM et al., 2018; CAVA et al., 2020).

Os primeiros vestígios de estudos envolvendo a utilização da APH foram registrados no ano de 1895, porém, em 1990, produtos conservados pela tecnologia passaram a ser comercializados, inicialmente no Japão. Compreenderam molhos para saladas, iogurtes e geleias tratados a 400 MPa. Pouco depois, sucos de frutas, doces em massa, abacate, presunto e alguns mariscos tratados com APH passaram a ser comercializados nos EUA e Europa (FELLOWS, 2006; GAVA et al., 2009). Além destes, vegetais, frutas, frutos do mar, leite e derivados, cortes de carnes frescas, sucos, vitaminas e massas são algumas das principais aplicações do tratamento de HHP (ROSENTHAL et al., 2017).

Diferente dos métodos térmicos que adotam sistema fatorial bidimensional (tempo e temperatura), a tecnologia de alta pressão utiliza sistema fatorial tridimensional (pressão, tempo e temperatura) e que, independentemente do tamanho e formato do alimento, tal processo evita que o produto sofra deformação ou aquecimento indesejado. Isso ocorre devido ao processo permitir pouca variação de temperatura diante do aumento da pressão. A pressurização no processo pode ser gerada e transmitida por dois métodos: direto e indireto. No método de compressão direta, o meio de transmissão (geralmente água) será comprimido e bombeado ao tanque através de um pistão com diâmetro considerável, gerando pressão de maneira rápida. No método de compressão indireta, o meio de transmissão será bombeado da câmara reservatória para o interior do tanque através de um intensificador de pressão. Esse último é normalmente empregado em nível de processamento industrial que, ao contrário do método direto, pode alcançar níveis mais elevados de pressão, assim como abranger maiores volumes (STRATAKOS et al., 2015; PRAKASAM et al., 2018).

O equipamento de APH por método direto (Figura 3) é dotado de vaso de alta pressão, sistema de geração e, comumente, também de intensificação de pressão, e sistemas de manuseio de materiais e controle de tempo e temperatura. O meio de transmissão de pressão no interior do vaso é geralmente água ou álcool, que transfere a pressão gerada para o produto quase que instantaneamente (STRATAKOS et al., 2015; PRAKASAM et al., 2018). Normalmente, são usadas pressões que variam de 100 a 900 MPa, o que possibilita otimização do processo, de acordo com o alimento a ser tratado (STRATAKOS et al., 2015).

Figura 3: Equipamento de alta pressão hidrostática, Embrapa Agroindústria de Alimentos.

O processo é normalmente utilizado para redução da contaminação e/ou viabilidade microbiana (GOUVEA et al., 2020), ativação (TELES et al., 2021) ou inativação enzimática, o que contribui para manutenção da segurança do produto e aumento de sua vida útil. São diversos os benefícios promovidos pela APH (aumento da validade comercial, manutenção das propriedades nutricionais e sensoriais), dependendo dos parâmetros utilizados, e se, mais eventualmente, o método for utilizado em combinação com outra tecnologia (OKPALA et al., 2010; HU et al., 2017). No entanto, o uso desta tecnologia ainda é limitado, devido aos custos elevados para manutenção dos equipamentos (HU et al., 2017).

3.1 Efeitos da APH sobre os Alimentos

A faixa de pressão normalmente utilizada para alimentos não interfere nas ligações covalentes, que sustentam a estrutura primária de proteínas e ácidos graxos (PRAKASAM et al., 2018). Porém, as ligações de hidrogênio, ligações iônicas e interações hidrofóbicas, que sustentam as estruturas secundária e terciária, são afetadas. Dessa maneira, ocorrem alterações da conformação das estruturas proteicas que dependem do tipo da molécula, pressão utilizada, temperatura e tempo de tratamento (AYMERICH et al., 2008). Substâncias como vitaminas, compostos aromáticos, aminoácidos e outros compostos de baixo peso molecular são pouco afetados. Assim sendo, as propriedades sensoriais e nutricionais são minimamenmte modificadas (MARTINS, 2004).

De acordo com o objetivo do estudo, a APH pode ser utilizada com a finalidade de inibir ou melhorar a atividade de certas enzimas (ALBUQUERQUE et al., 2016), melhorar a digestibilidade de alguns alimentos (HU et al., 2017), aumentar a disponibilidade de

nutrientes, além de poder melhorar as propriedades funcionais e tecnológicas de certos produtos e ingredientes (RENDUELES et al., 2011; HU et al., 2017).

Estudos sobre o comportamento proteico diante da APH evidenciaram aumento da quantidade de peptídeos antioxidantes em hidrolisado de linhaça (PERREAULT et al., 2017) e aumento da digestibilidade, capacidade antioxidante e anti-hipertensiva da α-caseína (HU et al., 2017). Em estudo realizado por Ramos et al. (2015), foi relatado que a aplicação de 600 MPa a 20 ºC durante 15 min em leite bovino cru desnatado preservou a atividade enzimática de lactoperoxidase, enquanto provocou desnaturação de 80%, 70%, 44% e 7% de b-lactoglobulina, lactoferrina, IgG e a-lactalbumina, respectivamente.

É crescente a utilização da APH como tecnologia modificadora das propriedades funcionais dos alimentos, com a finalidade de ampliar as aplicações industriais. Khan et al. (2014) ressaltaram o crescente interesse das indústrias de alimentos em agregar funcionalidade e suplementação a certos produtos através da modificação da estrutura protéica vegetal, afim de obter uma funcionalidade melhorada e, assim, ampliar seu campo de aplicação industrial. No entanto, as alterações na estrutura química e bioatividade de tais moléculas dependem fortemente de sua estrutura inicial e do trinômio tempo, temperatura e pressão utilizados no processamento. Estudos relataram que, para certas moléculas protéicas, os tratamentos por APH com ciclos múltiplos podem causar graves danos estruturais quando comparados com tratamento de ciclo único e mesmo tempo de processamento (HU et al., 2017).

A utilização de APH apresenta, assim, ótimas perspectivas para a modificação das estruturas protéicas de certos alimentos, com a finalidade de obter aumento de bioatividade molecular, além de proporcionar ao mercado consumidor um alimento com melhoria de propriedades nutracêuticas e farmacêuticas (HU et al., 2017).

3.2 Efeitos da APH sobre Micro-organismos

Os micro-organismos gram-positivos e gram-negativos possuem diferenças, tanto em composição química quanto em propriedades estruturais de sua membrana celular, o que resulta em diferenças de resistência ao processo de APH. Segundo relatos, as bactérias gram-positivas (*Listeria monocytogenes* e *Staphylococcus aureus*) geralmente são mais resistentes quando comparadas aos micro-organismos gram-negativos (*Escherichia coli*) (BRUSCHI et al., 2017; HU et al., 2017). Isso se deve, aparentemente, à presença de dupla camada fosfolipídica na parede celular das bactérias gram-positivas, o que proporciona, consequentemente, maior rigidez, conferindo menor flexibilidade diante da aplicação da APH (HU et al., 2017). Bruschi et al. (2017) relataram que cepas de *L. monocytogenes* resistentes à pediocina apresentaram maior resistência após tratamento com 400 MPa. Este relato é preocupante, pois a sobrevivência de tal micro-organismo nas condições de tratamento apresentadas pode representar riscos à saúde de consumidores.

Alterações causadas em eventos envolvidos na síntese proteica e destruição da membrana celular são os principais efeitos da APH sobre os micro-organismos. Com a membrana alterada, ocorre a permeação de material citoplasmático, levando à morte microbiana. Os esporos apresentam grande resistência à inativação por APH (RENDUELES et al., 2011). Dentre as espécies formadoras de esporos com potencial patogênico significativo, os gêneros *Bacillus* e *Clostridium* têm sido fortemente investigados com variações de resistência entre as cepas (PINA-PÉREZ et al., 2012; SYED et al., 2012; LENZ e VOGEL, 2014).

De acordo com Yang et al. (2012), a inativação de agentes patogênicos como *Salmonella, E. coli, Shigella* e *S. aureus* ocorre principalmente devido a alterações que levam à ruptura da parede celular, a danos causados na membrana celular e degradação do ácido

desoxirribonucléico (ADN) cromossômico. Segundo Gayan et al. (2017), a APH pode induzir alterações em ribossomos e proteínas, impactando na homeostase protéica, comprometendo o desenvolvimento e sobrevivência microbiana.

Estudos têm mostrado a eficiência da APH na eliminação de protozoários com potencial de transmissão através de leite contaminado como *Toxoplasma gondii* (LINDSAY et al., 2006) e *Cryptosporidium parvum* (SCHUWARTZ et al., 2011). Segundo relatos, dependendo da estrutura, os vírus podem apresentar grande resistência à pressão, sendo os envelopados menos resistentes quando comparados aos vírus nus. Combinações de pressão (300 e 400 MPa) e tempo (600 s) foram relatadas como eficazes na inativação de dose de infecção do vírus da hepatite A (GROVE et al., 2008).

Conforme anteriormente considerado, as modificações na estrutura celular de cada micro-organismo dependem de fatores relacionados ao processo como temperatura, tempo e nível de pressão aplicada. Além destes, outros fatores também são determinantes como presença de substâncias nocivas ao micro-organismo, composição da matriz alimentar envolvida, condições de armazenamento pós tratamento com APH, entre outros (MOTA et al., 2013; HUANG et al., 2014). Fatores relacionados aos micro-organismos como composição da membrana, protéinas da fase estacionária e/ou proteínas de estresse podem modificar a resistência à pressão, de acordo com o relatado por Hayman et al. (2007).

3.3 Efeitos da AHP sobre os Materiais Poliméricos

A embalagem usada para acondicionar o alimento a ser pressurizado deve ter capacidade de suportar redução de volume. Isso ocorre porque, durante a elevação da pressão, o volume do alimento reduz e, logo depois, expande durante a descompressão. A APH pode levar a mudanças de conformação em estruturas poliméricas, principalmente em protéinas, ocasionando, no caso de alimentos, formação de gel, amaciamento de carnes ou mudanças de textura (FELLOWS, 2006; GAVA et al., 2009).

Trabalhos acerca dos efeitos da APH em filmes poliméricos sintéticos (polietileno de baixa densidade, copolímero de etileno álcool vinílico, polietileno de alta densidade, polipropileno isotático, polietileno linear de baixa densidade, poliamida, poliamida biorientada) têm evidenciado alterações reversíveis e irreversíveis. Em resposta às condições de altas pressões, podem ocorrer mudanças nas fases cristalina e amorfa, o que reflete diretamente nas propriedades funcionais das embalagens poliméricos (MENSITIERI et al., 2013). Logo, é de fundamental importância prever os possíveis efeitos causados pela APH sobre as estruturas da embalagem, assim como na estrutura dos aditivos utilizados (FRALDI et al., 2014).

Mudanças extremas na estrutura química das embalagens podem impossibilitar sua aplicação. Além disso, o tratamento de EAA com APH pode afetar positivamente ou negativamente a estrutura e/ou migração do composto ativo, podendo inviabilizar o uso da tecnologia para conservação do alimento (MENSITIERI et al., 2013; FRALDI et al., 2014; LAVOINE et al., 2016).

Vale ressaltar a deficiência em informações acerca das possíveis alterações provocadas pela APH sobre as estruturas e propriedades funcionais de polímeros biodegradáveis. Isto deve-se, em grande parte, à falta de estudos de correlação. Martins (2014) evidenciou tal deficiência ao estudar as possíveis alterações em tripas suínas tratadas com APH e informou que a APH (540 MPa/390 s) contribuiu para manutenção das propriedades mecânicas, aumento da luminosidade, da cor amarela e redução de cor vermelha.

3.4 APH em Leite e Derivados

A indústria de produtos lácteos torna-se cada vez mais atraente aos olhos dos consumidores, uma vez que, além dos produtos habituais de consumo como leite, queijos, iogurtes e bebidas à base de leite, destaca-se a procura por produtos lácteos com redução de calorias ou com propriedades funcionais que influenciam diretamente nas atividades metabólicas, contribuindo para melhor funcionamento do organismo. Dessa maneira, pesquisadores e indústria de laticínios têm voltado sua atenção para alternativas tecnológicas que, além de promover alterações desejáveis, possam contribuir para melhoria da qualidade e segurança dos produtos lácteos. De acordo com o disposto em pesquisas, o uso da APH para processamento de produtos lácteos contribui para a oferta de alimentos contendo componentes ativos com propriedades antioxidantes, anti-envelhecimento, anti-inflamatórias, imunes e antimicrobianas (BLEOANCĂ et al., 2016; BURNS et al., 2015; MENG et al., 2017; MOTA et al., 2015).

A APH é um processo com elevado potencial para tratamento de leite e derivados (OKPALA et al., 2010). Desde a utilização pioneira da APH em 1899 para tratamento de leite e após várias melhorias dos equipamentos e processo, essa tecnologia vem sendo usada com diferentes propósitos, seja para modificar as propriedades sensoriais e físico-químicas, como para alcançar a inocuidade de derivados lácteos (AYMERICH et al., 2008). Estudos relatam que a APH revela ser um método eficaz para inativação de micro-organismos patogênicos transmitidos por leite e derivados (LÓPEZ-PEDEMONTE et al., 2006; LOPEZ-PEDEMONTE et al., 2007).

A APH tem sido reportada como método para melhoria do coalho e/ou da coagulação ácida do leite, sem causar alterações na qualidade sensorial e nutricional do alimento. Dessa maneira, o processamento por APH configura como tecnologia emergente com inúmeras aplicações para a indústria de laticínios, favorecendo a produção de alimentos microbiologicamente seguros, com qualidade e maior validade comercial (SERRA et al., 2007; OKPALA et al., 2010).

Para produção de queijo e fermentados lácteos são necessárias condições ideais para que ocorra a ação da quimosina sobre a caseína no processo de coagulação. Portanto, quaisquer alterações na estrutura da caseína poderá comprometer o rendimento e produção de queijo. O tratamento térmico do leite como a pasteurização, pode causar danos na membrana dos glóbulos de gordura assim como a redução do seu tamanho, o que favorece a aderência em sua superfície de micelas de caseína e proteínas do soro, modificando assim a composição e contribuindo para efeitos negativos nas propriedades físicas de alguns queijos e iogurtes. Dessa maneira, Garcia-Amezquita et al. (2009) aplicaram APH (400 MPa por 15 e 20 min; 500 MPa por 5 e 10 min) em leite como alternativa viável para substituição da pasteurização e observaram que o tratamento com tal tecnologia não-térmica promoveu aumento dos glóbulos de gordura, o que mostra ser uma ferramenta útil para produção de queijos crus, além de contribuir para obtenção de alimento seguro.

Na busca do estabelecimento de condições ótimas (tempo, pressão e temperatura) para aumentar a atividade de coagulação do leite, Leite Júnior et al. (2017), aplicaram diferentes pressões sobre o coalho bovino (280 MPa/20 min a 25 °C) e pepsina porcina (50 MPa/5 min a 20 °C) em condições de ativação. Os resultados demonstraram que as condições sob as quais as enzimas foram submetidas provocaram alterações em suas estruturas secundárias, evidenciando sensibilidade de tais enzimas diante das pressões aplicadas. Porém, tais alterações no coalho bovino foram fundamentais para melhorar seu desempenho, provocando aceleração da coagulação do leite e produção de coágulos mais firmes e consistentes, o que pode levar a um maior rendimento na produção de queijos.

É crescente a preocupação dos consumidores a respeito do potencial alergênico atrelado ao consumo de leite e derivados. Portanto, é relatado a eficiência da APH para redução da alergenicidade em leite bovino. Alguns estudos tem demonstrado que o tratamento pela tecnologia de alta pressão pode ser eficaz na diminuição da alergenicidade sem alterar a qualidade sensorial e valor nutricional do leite e derivados, o que representa um ponto positivo na demanda e aceitação do consumidor (AMBROSI et al., 2016; MENG et al., 2017). Outra questão bastante apreciada pelos consumidores e que tem atraído o interesse industrial é a produção de alimentos funcionais, porém a viabilidade dos micro-organismos probióticos durante o armazenamento ainda é algo a ser melhorada, a despeito de resultados positivos obtidos com queijo Minas Frescal. Estudos também relatam a eficiência do uso da APH sobre a viabilidade de bactérias probióticas microencapsuladas para uso em leites fermentados e homogeneizadas em leite para produção de queijo crescenza (BURNS et al., 2008; PATRIGNANI et al., 2017).

O processamento por APH tem sido utilizado em fabrico de queijos com finalidades distintas. Giannoglou et al. (2016) trataram culturas iniciadoras com APH e relataram que o processamento pode acelerar a proteólise e, consequentemente, a maturação de queijos em salmoura. O mesmo foi observado por Costabel et al. (2016), tratando queijo Reggianito Argentino, após 1 dia de fabrico, com 400 MPa durante 10 min. Cultura de *Lactobacillus paracasei*, tratada com APH a 50 MPa favoreceu a aceleração da maturação de queijo probiótico Caciotta (BURNS et al., 2015). No entanto, para alguns queijos a maturação excessiva pode gerar sabor amargo e aromas desagradáveis e, nesse sentido, Calzada et al. (2014) demonstraram que APH pode também ser eficiente para a conservação do sabor e prevenção do excesso de maturação em queijo Brie. Da mesma forma, Evert-Arriagada et al. (2013) observaram que APH favoreceu a manutenção das características aromáticas de queijos frescos durante vida útil estendida.

Nos últimos anos a APH tem sido utilizada como objeto de estudo para a inativação de diferentes micro-organismos em queijos. Letalidade máxima para dois sorotipos de *Salmonella* (*Salmonella enteritidis* e *Salmonella typhimurium*) foi observada em queijo tratado a 300 e 400 MPa e produzido com coalhada tratada com esses mesmos níveis de pressão (DE LAMO-CASTELLVÍ et al., 2007). Para queijo fresco contaminado com *Listeria innocua*, Hnosko et al. (2012) demonstraram que somente tratamentos com pressões de 500, 550 ou 600 MPa durante pelo menos 15, 3 ou 1 min, respectivamente, foram capazes de causar reduções acima 5 ciclos logarítmicos na população microbiana. No entanto, tais condições aplicadas não seriam recomendadas para a inocuidade de queijo fresco, uma vez que a inativação microbiana não foi total nem permanente.

Lopez-Pedemonte et al. (2007) relataram variação significativa na sensibilidade à pressão entre diferentes linhagens de *L. monocytogenes*, evidenciando o potencial da APH para melhorar a segurança do queijo. Contagens de *Listeria* spp foram reduzidas (2.66 log/ufc g^{-1}) em queijo de cabra fresco tratado (600 MPa/7 min) nos dias 1 e 30 de armazenamento e analisados no 60º dia (DELGADO et al., 2012).

3.5 Combinação de AHP com outras Tecnologias para Conservação de Leite e Derivados

De acordo com Leistner (1994), a prevenção é a melhor forma de controlar a contaminação dos alimentos por micro-organismos indesejáveis. Logo, a aplicação de tecnologias que visem prevenir o desenvolvimento microbiano nos produtos alimentícios e manter sua qualidade inicial é o principal objetivo para oferta de alimentos seguros (AYMERICH et al., 2008). Uma estratégia eficaz para o controle do crescimento microbiano nos alimentos é a utilização da combinação de tecnologias de barreiras. LI et al. (2016) observaram efeito sinérgico da APH em uso combinado com dióxido de carbono e nisina

contra desenvolvimento *in vitro* de *Escherichia coli* e *Staphylococcus aureus.* Da mesma forma, Feyaerts et al. (2015) demonstraram que a combinação de APH e antimicrobianos naturais reativos a tiol pode ser utilizada como eficiente ferramenta inativadora de bactérias gram-positivas e gram-negativas.

A inativação de esporos bacterianos somente com APH pode ser limitada. Desse modo, a associação de tal tecnologia com tratamento térmico (SCURRAH et al., 2006; EVELYN e SILVA, 2015) em leite mostrou-se eficaz para obtenção de sinergismo na inativação de esporos de várias espécies de *Bacillus*. Em queijo frescal, o parâmetro cinético z_p (variação de pressão para decréscimo logarítmico do tempo de redução decimal D_p à temperatura constante) de maior inativação para *L. monocytgenes* com APH foi de 55,99 MPa, enquanto na combinação de APH e extrato de tomilho reduziu para 33,29 MPa (BLEOANCĂ et al., 2016). Além disso, Arqués et al. (2005) também observaram ação sinérgica de APH e bactérias láticas produtoras de bacteriocina (BP-LAB) para a inativação de *L. monocytogenes* em queijo produzido com leite cru. Os autores sugerem que a APH tornou as bactérias-alvo mais sensíveis à ação das bacteriocinas, sendo portanto uma combinação promissora para garantir a qualidade de queijos frescais.

Sob o ponto de vista microbiológico, o uso combinado de APH com bacteriófagos em leite bovino vem sendo reportado com efeito sinérgico contra o desenvolvimento de *S. aureus* (TABLA et al., 2012). A associação de APH com antimicrobianos naturais vem sendo bastante explorada com o propósito de garantir a inocuidade de diversos alimentos. Dessa forma, Gao and Ju (2008) utilizaram combinação de APH, nisina e tratamento térmico em leite UHT e relataram as condições ótimas de pressão, tempo, temperatura e concentração de nisina para a redução de seis ciclos logarítmicos de esporos de *C. Botulinum*. Além destes, o efeito sinérgico entre APH e nisina foi observado para inativação de *Escherichia coli, Pseudomonas fluorescens, Listeria innocua* e *Lactobacillus viridescens* em leite desnatado (BLACK, KELLY e FITZGERALD, 2005). O mesmo foi relatado para *Escherichia coli O157: H7, Shigella flexneri, Yersinia enterocolitica* e *Salmonella Typhimurium* em leite tratado com APH e lisozimas (NAKIMBUGWE et al., 2006), assim como para *Listeria monocytogenes* em leite tratado com APH, lisozima e lactoferrina (IUCCI et al., 2007).

A associação de óleos essenciais e APH são relevantes e tem sido relatada como eficiente para inativação de *Listeria monocytogenes* Scott A em leite semi-desnatado, o que demonstra o potencial de tais tratamentos combinados para a conservação de alimentos (Karatzas, Kets, Smid, & Bennik, 2001). Outros antimicrobianos naturais foram utilizados em associação com APH, com efeito sinérgico para inativação de bactérias gram-negativas em leite desnatado (Nakimbugwe, Masschalck, Anim, & Michiels, 2006). O efeito sinérgico do sistema Lactoperoxidase-Tiocianato-Peróxido de Hidrogênio (LP), para melhorar a eficiência da APH para inativação de bactérias gram-positivas e gram-negativas em leite desnatado, foi demonstrado por García-Graells et al. (2003) e García-graells, Valckx, and Michiels (2000), porém a capacidade do sistema LP em potencializar o efeito bactericida da APH foi dependente da concentração de células presentes no leite.

Além da vertente microbiológica, combinação de tecnologias de processamento são cada vez mais usadas no intuito de preservar ou melhorar a qualidade dos alimentos. Uma das principais características indesejáveis na produção de iogurtes é a baixa viscosidade e firmeza do gel, o que pode levar à sinerese durante a etapa de agitação. Nesse sentido, a obtenção de iogurte com géis mais compactos e com elevado grau de reticulação foi alcançada através do tratamento do leite pela combinação da APH (676 MPa/5 min) e tratamento térmico (85 ºC/30 min) (PENNA et al., 2007).

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CAPÍTULO II

EFFECTS OF HIGH HYDROSTATIC PRESSURE PROCESSING ON STRUCTURE AND FUNCTIONAL PROPERTIES OF BIODEGRADABLE FILM

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Sheyla Moreira Gonçalves[a*], Davy William Hidalgo Chávez[a], Léa Mariza de Oliveira[d], Claire Isabel Grígoli de Luca Sarantópoulos[d], Carlos Wanderley Piler de Carvalho[a,c], Nathália Ramos de Melo[a,b], Amauri Rosenthal[a,c]

[a]Department of Food Science and Technology, Rodovia 465 - Km 7, UFRRJ, Seropédica, RJ 23891-360, Brazil.
[b]Department of Agribusiness Engineering, Av. dos Trabalhadores 420 - Vila Sta. Cecília, UFF, Volta Redonda, RJ 27255-125, Brazil.
[c]Embrapa Food Technology, Av. das Américas, 29501, Guaratiba, Rio de Janeiro, RJ 23020-470, Brazil
[d]Packaging Technology Center Cetea, Food Technology Institute Ital, Campinas, São Paulo, Brazil

[*]Corresponding author: sheylapa1@hotmail.com

ABSTRACT

Effects of high hydrostatic pressure (HHP) processing (200-400 MPa/5 or 10 min) on functional properties of cellulose acetate (CA) films were investigated. As for mechanical properties, HHP caused a reduction in tensile strength (TS), Young's modulus (YM) and an increase in elongation at break (EB). The pressurized films were more luminous, yellowish, reddish and opaque. Less affinity for water was detected for pressurized films through analyses of contact angle and moisture absorption, in addition to reducing the water vapor transmission rate (WVTR). Scanning electron microscopy (SEM) showed the occurrence of delamination for most films, except those treated with 200 MPa/10 min and 300 MPa/10 min. All films showed a predominance of amorphous structure in X-ray diffraction analysis (XRD). That is alignment with the results of differential scanning calorimetry (DSC), which presented values for glass transition temperature (Tg), water adsorption and melting temperature characteristic of materials with low crystallinity. Films treated with HHP had better mechanical resistance during the sealing at 250 ºC. In overall the results confirmed the minimal influence of HHP on the functional properties of the CA film and contributed to the scientific and technological knowledge for its potential application in foods processed by HHP.

Keywords: Emerging technology; Cellulose acetate; Packaging materials; Heat seal strength; Physicochemical properties.

1. Introduction

Among the polymers used to make food packaging, non-biodegradable plastics derived from petroleum is the best-known and has the broadest use. However, in view of the growing global environmental awareness, natural polymers are gradually gaining industrial importance (Siracusa et al., 2008). Cellulose acetate (CA) is a biodegradable and abundant polymer, synthesized industrially through the acetylation of cellulose with acetic anhydride and acetic acid with sulfuric acid as a catalyst (Cerqueira et al., 2010). For the production of CA, the hydroxyl groups of the glycosidic bound units of the cellulose chain are replaced by acetyl groups, thus generating a cellulose ester. In this way, different types of CA can be elaborated according to the degree of substitution (number of acetyls linked to hydroxyls). CA has a specific weight of 1.32 g/cm^{-3} and decomposes at a temperature of 240 ºC. Its properties vary according to the degree of acetylation, quantity and quality of additives used, but it is usually a rigid and compact material. It can be used in different applications such as in the production of transparent adhesive tape, tool handles, eyeglass frames, films with controlled release of active substances, textiles, composites and polymeric membranes. It is a thermoplastic

material with good impact resistance, transparent and good water vapor barrier properties (Miles and Briston, 1975; Canevarolo Jr, 2006).

For achieving specific functional properties CA films have been made with the addition of plasticizers (Gonçalves et., 2019; Liu et al., 2019; Richardson et a., 2014) and antimicrobial compounds (Dannenberg et al., 2017; Gonçalves et al., 2020; Zizovic et al., 2018), thus fulfilling its potential for preparing packaging for direct contact with food. According to a literature study, polymers that have amorphous and crystalline segments have difficulty in segregation during film making, since the amorphous region has greater miscibility power when compared to the crystalline region, favoring greater resistance to traction (Rana et al., 1999; Rana et al., 2000). In addition, the morphology of the polymer chains directly reflect the compatibility between the polymer matrix and the incorporated compounds (Song et al., 2020), with a consequent influence on the mechanical and thermal properties (Hasa et al., 2020). However, according to Gonçalves et al. (2019) CA film is predominantly amorphous, which suggests less segregation between the chains, greater miscibility power and greater uniformity of mechanical properties.

High hydrostatic pressure (HHP) is a non-thermal technology that is increasingly used by the food industry in order to meet the desires of consumers who demand safe, nutritious and closer to natural foods. It is a process where the food is subjected to a certain pressure distributed evenly to the product for a set time, which ensures that the food maintains its initial shape even in the face of extreme pressures (Oliveira et al., 2015; Stratakos et al., 2015). The packaging used to pack the food to be pressurized must have the capacity to withstand the change in volume since during the pressure increase the volume of the food reduces and, soon after, expands during decompression (Fellows, 2006). In addition, during volume reduction high pressure can favor chemical reactions and changes in molecular and structural conformation of the organic and inorganic polymeric matrixes (Galazka et al., 2000; Gross and Jaenicke, 1994). Such characteristics justify the widespread use of HHP for structural modification of proteins, enzymes or polysaccharides (Fellows, 2006; Molinaro et al., 2015). Studies on the effects of HHP on synthetic polymeric films have shown both reversible and irreversible changes. In response to high pressure conditions, changes in the crystalline and amorphous phases can occur, which directly reflects on the functional properties of polymeric packaging (Mensitieri et al., 2013). Therefore, it is of fundamental importance to predict the possible effects caused by HHP on the properties of the packaging, since extreme changes in the structure can make its application impossible (Fraldi et al., 2014).

According to Martins (2014), there was a notable lack of studies on the possible changes caused by HHP on the structures and functional properties of biodegradable polymers, which it is still verified in the literature. Furthermore, no study has investigated the effect of HHP on functional properties of CA films according to our knowledge. Therefore, this study aimed to submit a CA film to HHP processing at different pressure and time levels and evaluate the structural and functional changes in the film. The films were characterized in terms of visual appearance, barrier properties (water vapor), thermal properties (DSC), mechanical properties, heat sealing, contact angle, moisture absorption, chemical interactions based on FTIR and DRX analyses and morphological changes by SEM.

2. Materials and methods

2.1. Materials

Cellulose acetate (CA) and Acetone (p.a.) was purchased from Sigma-Aldrich, Brazil.

2.2. Preparation of films and application of HHP

By means of the casting method (Melo, 2003), the gels formed by the solubilization of CA in acetone (1:10 w / v) were poured into a glass plate, spread with the aid of a glass stick and dried under controlled conditions (temperature of 25 ± 2 °C and 75% humidity) for 10 min. After drying, the films were detached from the plates, packed in a vacuum in sterile bags with identification stripe (Nasco Whirl-Pak) and subjected to HHP processing (200, 300 or 400 MPa for 5 or 10 min) in Stansted Fluid Power, model S-FL-850-9-W. The films were preconditioned at 75% relative humidity, for a maximum of 5 days, to carry out the analyses. The control film was made without HHP.

2.3. Visual aspects

Samples of 4 x 4 cm were evaluated using the Minolta CM-5-ID colorimeter for the degree of brightness L*, red/green chromaticity (± a*), yellow/blue chromaticity (± b*), total difference (ΔE*), Chroma (intensity or saturation of color (C*)) and Opacity (Romani et al., 2017). Hue angle (h^o) locates the color in polar coordinates and is expressed in degrees: 0° for red (+a*), 90° for yellow (+b*), 180° for green (-a*), and 270° for blue (-b*).

2.4. Mechanical analyses

The TA.XTplus texturometer (Stable Micro Systems, Surrey, England), operating according to ASTM standard method D 882-82, was used to determine tensile strength (TS) (MPa), elongation at break (EB) (%) and Young's modulus (YM) (MPa). Specimens of 5 x 2 cm films were fixed in a texturometer with initial separation of 25 mm, operated with a 30 kg cell, with a force of 0.049 N and a velocity of 1 mm/s. With the help of Exponent Texture TEE32 (Stable Micro Systems), the TS was obtained through the relation of the maximum force (N) by the sample area (mm). The YM was calculated from the linear region of the stress versus strain curve. The EB was calculated according to Eq. (1).

$$EB = \frac{L}{Ci} \times 100 \qquad \text{Eq (1)}$$

In which,
EB: elongation at rupture expressed in %;
L: Deformation expressed in mm;
Ci: Initial sample length in mm.

2.5. Contact angle

The wettability of the films was obtained using a contact angle meter (model CAM 101, KSV Instruments, Finland), equipped with diffuse light, DMK 21AF04 camera (1 photo/s) and syringe with distilled water (Nascimento et al., 2012). Three drops of distilled water (2 µl) were deposited on the surface of each film (20 mm long and 10 mm wide), fixed on a glass slide using double-sided tape. The image of each drop was taken by digital camera at 1s intervals for 30 s. The contact angle was calculated from the average of the right and left angles of the drop, as a function of the analysis time (30 s).

2.6. Moisture absorption

Samples of 20 mm x 20 mm were oven dried at 100 ºC/2 h, maintained at 0 % relative humidity (RH) for 24 h and then weighed. Then, the samples were conditioned in a desiccator containing saturated NaCl solution with 75% RH for 7 days, and the final weighing was performed (Alizadeh-Sania et al., 2018). RH was calculated according to Eq. (2).

$$MA = \frac{Wt - W0}{W0} \times 100 \qquad \text{Eq (2)}$$

Where,
MA: relative humidity in %;
W0: initial weight;
Wt: final weight after 7 days.

2.7. Water vapor transmission rate (WVTR)

Water vapor transmission rate (WVTR) was determined gravimetrically, according to the (ASTM E96-95) with modifications, under a relative humidity gradient of 75%. The tests were conducted in triplicate.

2.8. Fourier transform infrared attenuated total reflection (FTIR-ATR) spectroscopy

The changes in the chemical structure of CA films as a result of the action of HHP were characterized using Perkin Elmer (Spectrum 100) under attenuated total reflection (ATR) mode. The spectrum was recorded in the wavelength range of 650 – 4000 cm^{-1}, 4 cm^{-1} resolution and 32 scans.

2.9. Scanning electron microscopy (SEM)

Images of the surfaces and fracture regions of the films were obtained with the help of the Scanning Electron Microscope (Carl Zeiss, model EVO MA 10. Specimens of 1 x 0.5 cm were fixed in "stub", covered with gold (Au) (metallizer EMITEC K550X) with current of 25

mA/2 min and observed in the SEM in low vacuum, using an acceleration voltage of 5000 kV, 450 of filament current, and scans of 5000 and 500x.

2.10. X-ray diffraction (XRD)

XRD curves were obtained using a Phaser D2 Bruker Diffractometer (Bruker, Germany), operated at 30 kV and 10 mA. After conditioning at 75% relative humidity, two specimens of each film with 2 cm of diameter were fixed in specific support and analyzed in the range of 2 to 29º and ω-2θ (Candido et al., 2017).

2.11. Differential scanning calorimetry (DSC)

DSC thermograms were obtained using a Q200 DSC (TA Instruments, New Castle, USA). All films were preconditioned to 75% relative humidity for 48 h. Then, 3.5 mg samples were sealed in aluminum capsules and heated to 20-250 °C at a rate of 10 °C/min. and cooled at a rate of 20 °C/min. The glass transition temperature (Tg) was determined by a second heating curve of 20-250 °C with a heating rate of 10 °C/min.

2.12. Heat sealing tensile strength

The sealing curves were determined according to the ASTM F 2029-08 standard in two stages: heat sealing of the specimens and determination of the tensile strength of the heat sealing. The heat seals of the specimens were made in a Brugger heat sealer operating with two heated jaws with a smooth profile and 5 mm wide with Teflon coating operating with a force of 300 N (pressure = 4 bar) and contact time of 1 s. The temperatures of the two sealing jaws were set in the range of 240 °C to 260 °C. The specimens were prepared in a controlled temperature environment (23 °C) after conditioning the samples in an environment at 23 °C ± 2 °C and 50 ± 5% relative humidity for at least 48 hours. The resistance of the heat seal to traction until the occurrence of failure was determined according to the ASTM F 88 / F 88M-09 standard. Specimens 25.4 mm wide were inserted in an Instron universal testing machine model 5966-E2 operating with a 100 N load cell at a speed of 300 mm/min. The distance between the fixing claws was 25 mm. The test was carried out in at 23 °C ± 2 °C and 50 ± 5% relative humidity after conditioning the specimens in that same environment for at least 24 hours.

2.13. Statistical analyses

Statistical analyses were performed using software R, version 3.2.4 (R Foundation for Computational Statistics, Vienna, Austria) and FactoMineR version 1.32. The Tukey multi comparative test was used to obtain differences between samples after ANOVA test both (Tukey and ANOVA tests) with a significance level of 5%. In addition, possible correlations between treatments and/or variables were investigated with the help of Pearson's Correlation. The stronges of Pearson’s correlation was evaluated following the rule proposed by Teles et al. (2019).

3. Results and discussion

3.1. Visual aspects

According to Table 1, HHP increased Luminosity (L*) and total difference (ΔE*). HHP processing resulted in films with lower color saturation (C*) and less intense red (+a*) and yellow (+b*) colors, as compared to the control film (CAP0T0). For hue angle (h^o), the color of all films was between 0^o (red) and 90^o (yellow), but HHP caused an increase in the mean values for the CAP200T10 film and reduction of the values for the CAP400T5 and CAP400T10 films.

Table 1
Visual appearance of the cellulose acetate films treated with high hydrostatic pressure (200, 300 or 400 MPa/5 or 10 min) in comparison to non-pressurized control.

Samples	L*	a*	b*	ΔE*	C*	h°	Opacity
CA P0T0	97.13 ± 0.2^{b}	0.04 ± 0^{a}	0.32 ± 0.07^{a}	2.88 ± 0.18^{c}	0.33 ± 0.06^{a}	82.83 ± 1.6^{b}	92.81 ± 0.49^{b}
CA P200T5	97.20 ± 0.02^{ab}	0.01 ± 0^{c}	0.17 ± 0.01^{b}	3.35 ± 0.05^{b}	0.16 ± 0.01^{b}	85.85 ± 0.99^{a}	92.97 ± 0.05^{ab}
CA P200T10	97.24 ± 0.02^{ab}	0.02 ± 0^{b}	0.14 ± 0.01^{bc}	3.4 ± 0.03^{ab}	0.14 ± 0.01^{bc}	82.65 ± 0.52^{b}	93.06 ± 0.04^{ab}
CA P300T5	97.22 ± 0.02^{ab}	0.02 ± 0^{b}	0.14 ± 0.01^{bc}	3.42 ± 0.05^{ab}	0.14 ± 0.01^{bc}	81.89 ± 1.04^{b}	93.02 ± 0.05^{ab}
CA P300T10	97.22 ± 0.05^{ab}	0.02 ± 0^{b}	0.12 ± 0.01^{c}	3.5 ± 0.05^{a}	0.12 ± 0.01^{c}	81.61 ± 0.81^{b}	93.01 ± 0.11^{ab}
CA P400T5	97.24 ± 0.02^{ab}	0.02 ± 0^{b}	0.12 ± 0.01^{c}	3.48 ± 0.03^{a}	0.12 ± 0.01^{c}	79.76 ± 1.38^{c}	93.06 ± 0.05^{ab}
CA P400T10	97.25 ± 0^{a}	0.02 ± 0^{b}	0.11 ± 0.01^{c}	3.50 ± 0.03^{a}	0.11 ± 0.01^{c}	79.65 ± 1.21^{c}	93.09 ± 0.01^{a}

P: Pressure level (MPa) and T: Treatment time (min.); CAP0T0: control film without high pressure treatment; *Mean values followed by the same letters in the same column do not differ ($p > 0.05$) by the Tukey test at the 5% level of significance. The results are expressed as mean ($n = 4$) ± standard deviation.

HHP treatments caused an increase in the opacity of all films, being the film CAP400T10 the most opaque. This effect may be justified by the possible closure of spaces in the polymeric chemical structure due to the applied pressure (Yoo et al., 2013) preventing passage of light beams through the sample. Most treatments lasting 10 min caused a slight increase in L*, ΔE*, opacity, and reduction of b*, C*, and h^o, when compared to 5 min processes.

3.2. Mechanical analysis

The mechanical properties of the CA film were influenced by both the pressure and the time of the process (Table 2). A reduction of tensile strength (TS) and Young's modulus (YM) was observed for all HHP conditions, especially the film with 300 MPa/5 min (CAP300T5), which presented the least resistance and rigidity. Structural changes in polymeric films can occur in different HHP conditions, which can be reflected in the mechanical functions of the packaging. Such effect may happen due to changes in

crystallinity, film delamination, or plastification caused by HHP (Marangoni Júnior et al., 2019). Therefore, the results reveal that the HHP conditions may have caused modifications in the chemical structure of the CA film, leading to a certain fragility to the material.

Table 2
Mechanical properties, contact angle, and moisture absorption of cellulose acetate films treated with high hydrostatic pressure (200, 300 and 400 MPa/5 and 10 min) in comparison to non-pressurized control.

Samples	TS (MPa)	YM (MPa)	EB (%)	Contact angle (°)	Moisture absorption (%)	WVTR ($g.m^{-2}.day^{-1}$)
CAP0T0	40.9 ± 1.2^{a}	1894.9 ± 31.5^{a}	4.2 ± 0.1^{c}	57.46 ± 1.4^{c}	0.06 ± 0.0^{ab}	232.56 ± 2.29^{a}
CAP200T5	34.5 ± 1.5^{bcd}	1342.1 ± 69.1^{b}	5.5 ± 0.3^{b}	61.65 ± 0.2^{b}	0.05 ± 0.0^{abc}	205.57 ± 4.48^{b}
CAP200T10	34.9 ± 1.1^{bc}	1347.1 ± 58.4^{b}	5.5 ± 0.3^{b}	58.61 ± 0.3^{c}	0.05 ± 0.0^{abc}	197.84 ± 1.86^{c}
CAP300T5	28.9 ± 1.3^{e}	1104.4 ± 155.8^{c}	6.3 ± 0.4^{a}	65.85 ± 0.6^{a}	0.03 ± 0.0^{c}	192.35 ± 1.13^{d}
CAP300T10	36.6 ± 1.3^{b}	1483.1 ± 92.4^{b}	5.3 ± 0.3^{b}	58.23 ± 1.8^{c}	0.07 ± 0.0^{a}	185.78 ± 1.77^{e}
CAP400T5	32.4 ± 0.7^{d}	1131.3 ± 66.5^{c}	6.2 ± 0.3^{a}	59.68 ± 0.7^{bc}	0.04 ± 0.0^{c}	182.53 ± 0.68^{ef}
CAP400T10	32.6 ± 1.4^{cd}	1189.3 ± 37.1^{c}	5.8 ± 0.3^{ab}	65.76 ± 1.4^{a}	0.04 ± 0.0^{bc}	177.36 ± 2.26^{f}

P: Pressure level (MPa) and T: Treatment time (min.); CAP0T0: control film without high pressure treatment; *Mean values followed by the same letters in the same column do not differ ($p > 0.05$) by the Tukey test at the 5% level of significance. The results are expressed as mean ($n = 4$) ± standard deviation.

The increase in elongation at break (EB) of the films treated with HHP (Table 2) are in agreement with the other mechanical properties, and the highest EB was presented by the film with lower TS (CAP300T5). TS and EB are usually the mechanical parameters most used to characterize food packaging (Khaneghah et al., 2018).

3.3. *Contact angle, moisture absorption (MA) and water vapor transmission rate (WVTR)*

According to Table 2, only the CAP200T10 and CAP300T10 films did not differ from the control film (CAP0T0) for the contact angle. The other HHP conditions presented an increase in the contact angle, indicating a lower affinity of the films surface for the water and, consequently, a lower degree of wettability. The HHP conditions may have caused changes in the intermolecular forces of the CA film (CAHHP) in such a way that the balance of these forces was greater than the forces of interaction between water and surface of the films. When the surface tension is greater than the attraction force of water to the film surface, the contact will be restricted and so that the droplet will become more spherical with greater contact angle. In this sense, when deposited on the surface of the film, the water droplet contracted, forming a more spherical droplet (Sarafraz and Arjomandi, 2019).

The results in Table 2 show that the highest values for moisture absorption (MA) were observed for the films that presented the lowest values for contact angle (CAP0T0, CAP200T10 and CAP300T10). On the contrary, in the same way the films with the lowest MA capacity were those with higher contact angle CAP300T5, CAP400T5 and CAP400T10. Therefore, the lower affinity for water by the film surfaces, caused by the HHP conditions,

may have prevented the water molecules from being absorbed in the polymer matrix. The CAP200T5 film despite having a higher contact angle showed a higher moisture absorption capacity. This phenomenon may be associated to the greater delamination inside the film which, in turn, may have allowed the passage of water through the spaces in the polymer matrix.

The treatment with HHP caused a reduction in the WVTR of all films (Table 2), which is in agreement with the results for the analysis of contact angle and moisture absorption, thus demonstrating a reduction in the affinity of the films for water. Molinaro et al. (2015) reported a reduction in WVTR in gelatin films treated with HHP (600 MPa/30 min) and attributed the results to a possible change in the stability of hydrogen bonds. The authors suggested that the greater structural compactness generated by HHP may explain the reduction in the passage of water molecules through the polymeric network.

3.4. Fourier transform infrared attenuated total reflection (FTIR-ATR) spectroscopy

FTIR spectra (Fig. 1) show that the treatment of films with HHP did not cause detectable changes in their chemical structures. For CA production, cellulose-free hydroxyl groups are replaced by acetyl groups through the cellulose esterification process with acetic anhydride. The presence of a band at 1736 cm^{-1}, which may be associated with vibration of C=O in carboxylic acid, is characteristic of acetyl groups. According to Liu et al. (2019), the presence of this peak indicates cellulose acetylation. The 1376 cm^{-1} band can be associated with vibration of the C-H bond, which in turn can also characterize bands of acetyl groups. In addition to these, the presence of bands at 1223, 1163, 1121 and 1034 cm^{-1} may be associated with the vibration of the ester group (C-O-C) of the acetate (Liu et al., 2019).

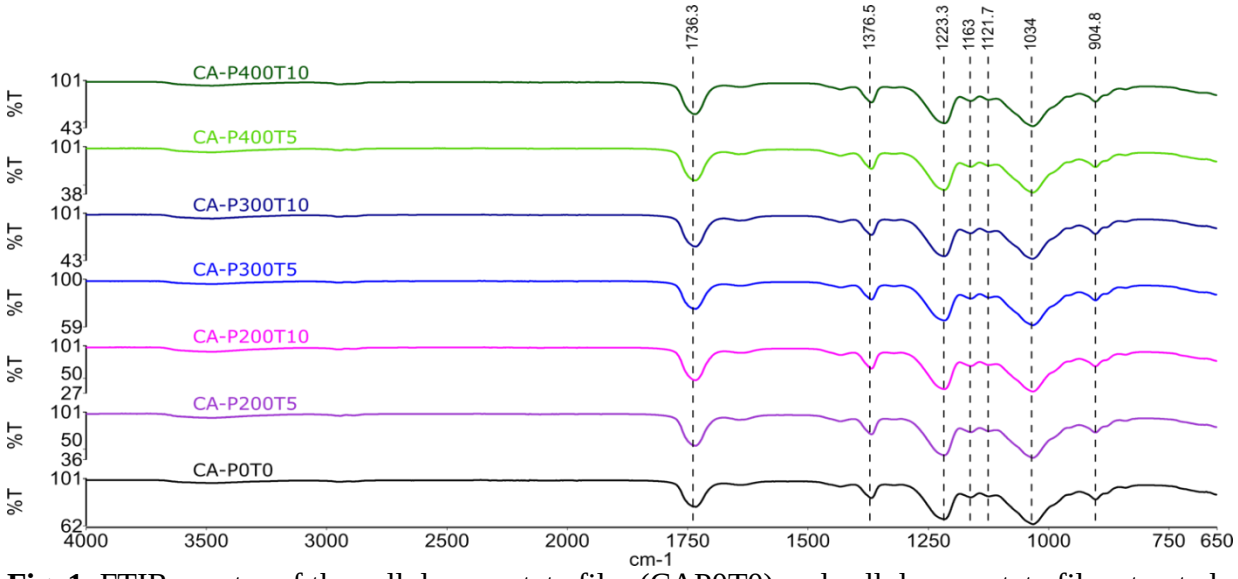

Fig. 1. FTIR spectra of the cellulose acetate film (CAP0T0) and cellulose acetate films treated with high hydrostatic pressure (CAP200T5, CAP200T10, CAP300T5, CAP300T10, CAP400T5 and CAP400T10), in which P indicates pressure (MPa) and T is the time (min).

3.5. Scanning electron microscopy (SEM)

SEM images (Fig. 2) for the surfaces of all CA films, treated or not with HHP, were homogeneous and smooth. Therefore, it is noted that all HHP processing conditions did not cause changes in the surface of the CA films. The fracture region of the film without HHP treatment (CA-P0T0 500 and 5000 x) also proved to be smooth and uniform. However, most of the CA films resulting from HHP treatment presented delamination in the fracture region, except for the CAP200T10 and CAP300T10 films, which was more homogeneous. Therefore, the lower means for TS (Table 2) presented by CA films processed by HHP may be associated with changes in their fracture regions. However, among the pressurized films the CAP200T10 and CAP300T10 films presented the highest TS (Table 2), which, in turn, can be justified by the apparent delamination within the free fracture region (Marangoni Júnior et al., 2019).

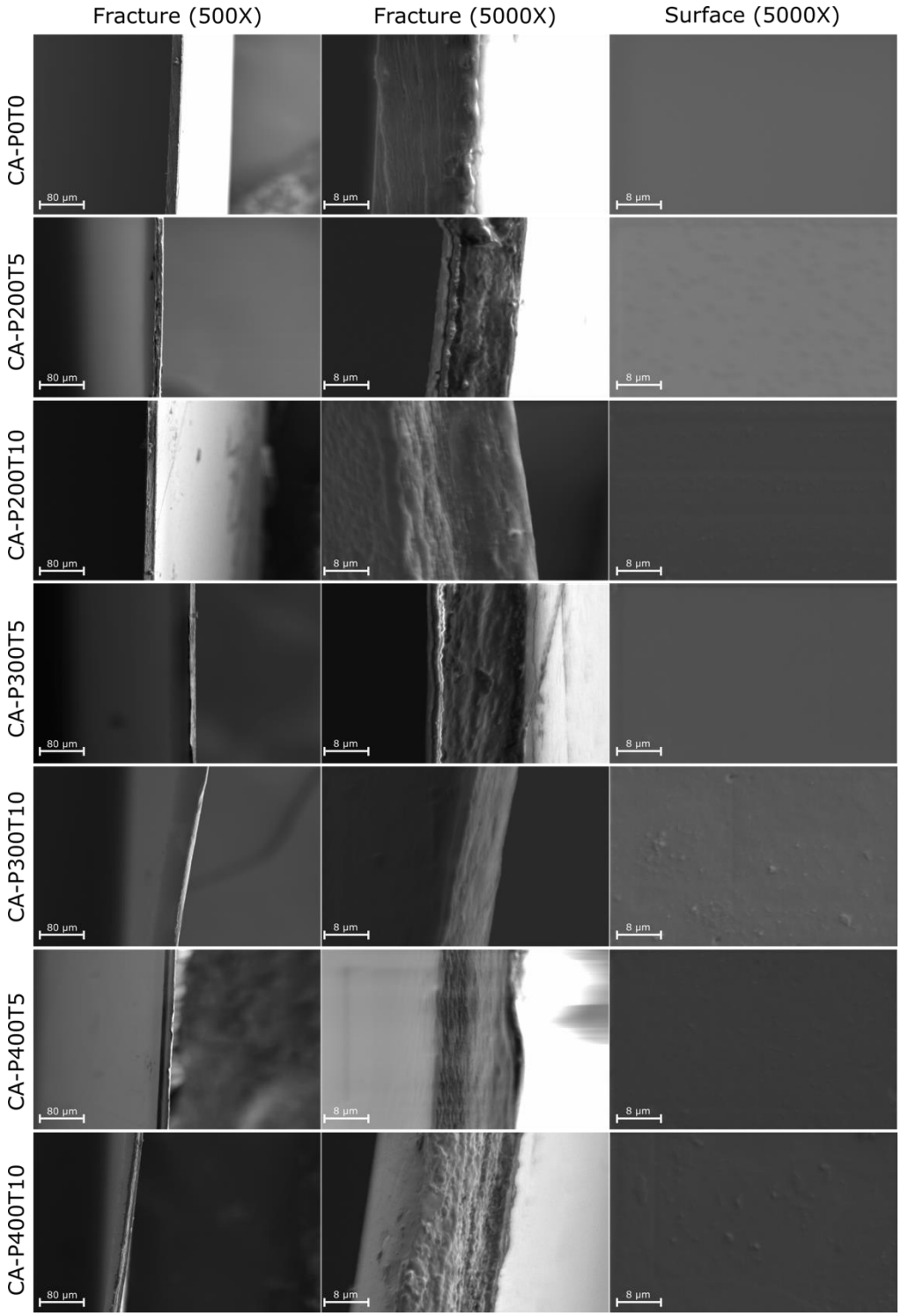

Fig. 2. SEM of the surface and fracture region of the cellulose acetate film (CAP0T0) and cellulose acetate films treated with high hydrostatic pressure (CAP200T5, CAP200T10, CAP300T5, CAP300T10, CAP400T5 and CAP400T10), in which P indicates pressure (MPa) and T is the time (min).

3.6. X-ray diffraction (XRD)

The Diffractograms revealed low crystallinity for all CA films (Fig. 3), caused by cellulose acetylation. Small peaks can be observed in the region of 2θ = 8.7º and 23º.

Crystalline diffraction of the CA usually occurs around 2θ = 8, 10, and 13º (Chen et al., 2016). Peaks in the region 2θ = 8 º of CA are associated with the presence of the acetyl group in the cellulose chain, which, in turn, cause rupture in the cellulose fibrillar microstructure during the acetylation process (Wan Daud et al., 2015). In this study, Fig. 3 shows that the changes caused by HHP in the crystalline structure of the CA film were minimal being verified mainly in the region 2θ = 8.7º, resulting in slight reduction for the treatments at 300 MPa and 400 MPa. Furthermore, peak 2θ = 23º showed a slight reduction only for CAP300T5 and CAP400T5. Castãnón-Rodríguez et al. (2013) processed sugarcane bagasse with HPP and verified the presence of a characteristic cellulose peak between 22 and 23º. The authors reported that the XRD standards of the samples treated with HHP indicated modification of cellulose crystalline structure. However, the study suggested that the cellulose amorphous region may be more susceptible to treatment with HHP when compared to the crystalline region.

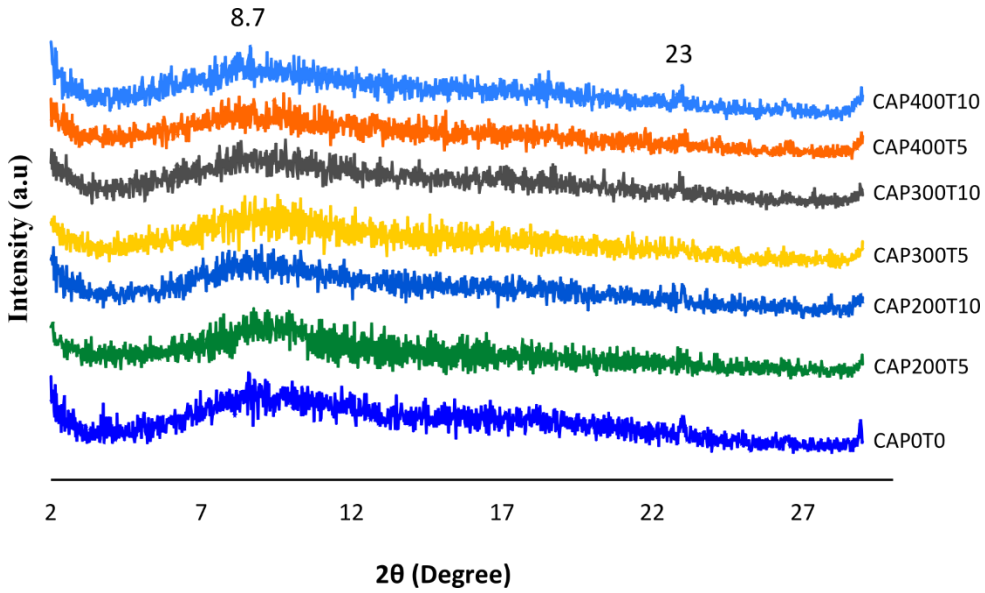

Fig. 3. DRX Diffractograms of the CA film (CAP0T0), CA films with HHP treatment (CAP200T5, CAP200T10, CAP300T5, CAP300T10, CAP400T5, CAP400T10), in which P indicates pressure (MPa) and T is the time (min).

3.7. Differential scanning calorimetry (DSC)

Fig. 4 shows changes caused by HHP on CA films regarding water adsorption capacity, glass transition temperature (Tg) and melting temperature. The endothermic peaks (EP) of the first curves correspond to water desorption events (Kendouli et al., 2014). The first endothermic peaks occurred between 100 and 150 ºC and their variation depended on the moisture retention capacity of each film. According to the degree of substitution (DS) of CA these peaks can occur at different temperatures (De Freitas et al., 2017). However, although all films had the same DS, treatment with HHP caused a slight increase in the temperature of the second endothermic peaks of all films, except for CAP200T5, as shown in Fig. 4.

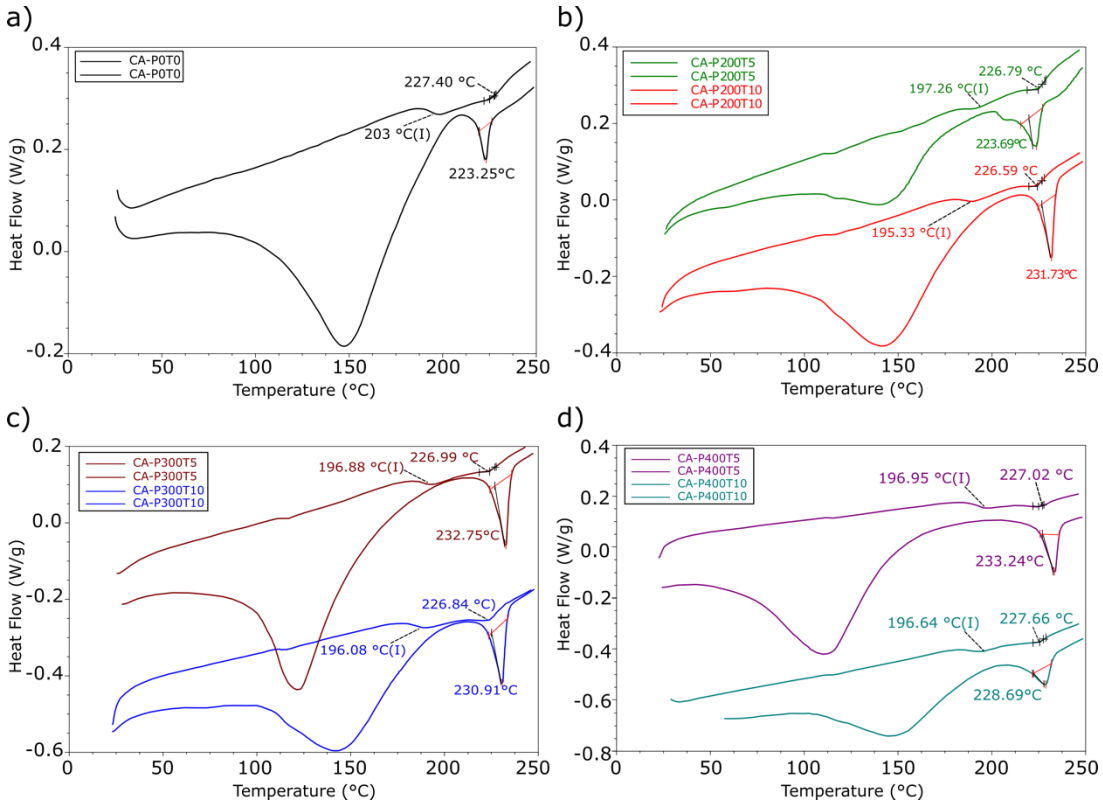

Fig. 4. DSC thermograms of the CA film (a: CAP0T0) and CA films with HHP treatment (b: CAP200T5, CAP200T10; c: CAP300T5, CAP300T10; d: CAP400T5, CAP400T10), in which P indicates pressure (MPa) and T is the time (min).

The results for DSC (Fig. 4) showed that Tg (first endothermic peak in second heating curve) of control CA film was 203 °C (I) (CAP0T0). Previous study showed that Tg of CA with a substitution degree of 1.48° located at around 223 °C (De Freitas et al., 2017). When submitted to the different HHP conditions CA films had a slight reduction in Tg ranging from 197.26 to 195.33 °C (I). The second peaks of the second heating curve represent the melting temperature of the films. The processing with HPP caused minimal changes in the melting temperature values, presenting temperatures in a narrow range between 227.66 (CAP400T10) to 226.59 °C (CAP200T10). XRD results obtained for Tg and melting temperature for CA films are in agreement with thermal properties of low crystallinity polymeric materials (Kendouli et al., 2014; De Freitas et al., 2017).

3.8. Heat sealing tensile strength

Resistance to mechanical stress during normal or adverse conditions is of fundamental importance to direct the application of packaging (Marangoni Júnior et al., 2020a). Therefore, thermal sealability and resistance to mechanical stress during heat sealing have been studied for different types of films (Hernandez-Lzquierdo and Krochta, 2009; Cho et al., 2010; Marangoni Júnior et al., 2020b). According to Fig. 5 the sealing was restricted to the temperature range evaluated since it did not occur at 230 °C but instead melted at 270 °C. Therefore, the sealing of all films came out at 240, 250 and 260 °C. For the films treated with HHP the highest seal strength was verified at 250 °C (Fig. 5), thus achieving the highest means of 0.635 and 0.571 Kgf/25.4 mm for CA-P200T5 and CA-P400T10, respectively. The

control film (CAP0T0) showed the highest seal strength at 240 °C followed by a slight reduction at higher temperatures.

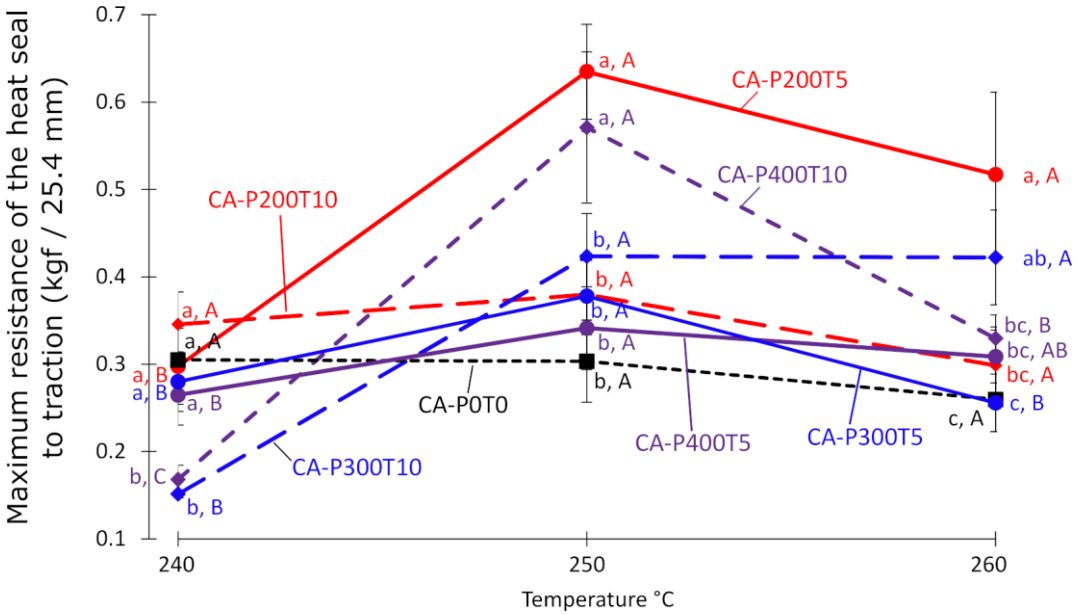

Fig. 5. Sealing curves of the CA film (CAP0T0) and CA films with HHP, where P indicates pressure (MPa) and T is the time (min).

CA-P300T10 and CA-P400T10 films presented the lowest resistances and statistical differences for sealing at 240 °C comparing to the film without high pressure processing (CA-P0T0). Concerning sealing at 260 °C, CA-P0T0 and CA-P300T5 films resulted in the lowest values for seal strength, while CA-P200T5 conditions provided the highest film seal strength at that temperature and the greatest resistant for sealing both at 250 and 260 °C. In addition, such film also presented the third highest seal resistance at 240 °C, suggesting in overall positively influence of 200 MPa/5 min processing to improve film heat sealing resistance. According to the data presented for XRD and DSC, all films were notably amorphous. Therefore, it is believed that the greater susceptibility of the amorphous domains to compression and decompression during HHP treatment (Castãnón-Rodríguez et al., 2013) favored changes in the structure of the CA film, thus positively influencing the resistance of most films to sealing at 250 and 260 °C.

3.9. Pearson's correlation

In Table 3, strong or very strong positive correlations (Teles et al., 2019) occurred when the variables showed similar behavior in the face of the same HHP conditions (positive values highlighted in bold). Positive correlation was observed in: ΔE * with L *; b * with a *; C * with a * and b *; Opacity with L * and ΔE *; YM with b *, C * and TS; EB with L *, ΔE * and opacity; MA with TS and YM; WVTR with b *, C * and YM. In addition to these, strong or very strong negative correlations (negative values highlighted in bold) were observed (b * with L *; ΔE * with a * and b *; C * with L * and ΔE *; Opacity with b * and C *; YM with L *, ΔE * and opacity; EB with b *, C *, TS and YM; Contact angle with TS; MA with contact angle; WVTR with L *, ΔE *, opacity and EB. In the negative correlations, the same HHP condition was able to cause opposite responses in the different variables. Table 3 also shows that the variables h^o and heat sealing tensile strength (MRHST) did not show a

strong correlation for either positive or negative. Therefore, it is concluded that the different HHP conditions caused changes in AC films for the studied variables.

Table 3
Pearson's correlation for dependent variables for cellulose acetate films treated with high hydrostatic pressure.

Variables	L*	a*	b*	ΔE*	C*	h°	Opacity	TS	YM	EB	Contact angle	MA	WVTR	MRHST 240
a*	-0.701													
b*	**-0.965**	**0.784**												
ΔE*	**0.947**	**-0.803**	**-0.998**											
C*	**-0.958**	**0.820**	**0.998**	**-0.998**										
h°	-0.490	-0.196	0.414	-0.386	0.364									
Opacity	**0.999**	-0.697	**-0.961**	**0.941**	**-0.953**	-0.495								
TS	-0.730	0.635	0.740	-0.730	0.743	0.306	-0.746							
YM	**-0.866**	0.740	**0.862**	**-0.851**	**0.866**	0.365	**-0.876**	**0.958**						
EB	**0.828**	-0.700	**-0.842**	**0.835**	**-0.844**	-0.381	**0.836**	**-0.965**	**-0.989**					
Contact angle	0.436	-0.409	-0.442	0.433	-0.447	-0.218	0.469	**-0.792**	-0.687	0.645				
MA	-0.443	0.295	0.355	-0.326	0.355	0.288	-0.472	**0.847**	**0.765**	-0.754	**-0.781**			
WVTR	**-0.936**	0.630	**0.961**	**-0.956**	**0.948**	0.624	**-0.935**	0.709	**0.829**	**-0.813**	-0.479	0.372		
MRHST 240	-0.328	0.136	0.447	-0.471	0.429	0.529	-0.325	0.089	0.154	-0.146	-0.251	-0.218	0.601	
MRHST 250	0.295	-0.694	-0.359	0.381	-0.401	0.338	0.305	-0.228	-0.307	0.201	0.483	-0.086	-0.287	-0.296
MRHST 260	0.086	-0.660	-0.241	0.290	-0.288	0.548	0.071	0.093	-0.032	-0.012	-0.072	0.384	-0.117	-0.247

**Values in bold are different from 0 with a significance level alpha=0.05*

4. Conclusions

Processing by HHP resulted in more luminous and opaque CA films, with lower color saturation and less intensity of red and yellow colors. In addition to these characteristics, HHP caused reduction of TS, YM, Tg, MA, WVTR besides increase of EB and contact angle. Fracture regions observed by SEM showed that most HHP treatment conditions evaluated caused delamination or slight porosity of CA films. Improvement in resistance to mechanical stress during heat sealing (250 ºC) was shown by all pressurized films. Therefore, this study brought a promising approach on the use of AC films to pack food to be pressurized, without impairing its functional properties.

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CAPÍTULO III

IMPACT OF HIGH HYDROSTATIC PRESSURE TREATMENT IN CELLULOSE ACETATE-BASED PACKAGING INCORPORATED WITH ESSENTIAL OIL

Artigo a ser submetido

Sheyla Moreira Gonçalves[a], Davy William Hidalgo Chávez[a], Léa Mariza de Oliveira[d], Claire Isabel Grígoli de Luca Sarantópoulos[d], Carlos Wanderley Piler de Carvalho[a,c], Amauri Rosenthal[a,c*], Nathália Ramos de Melo[a,b]

[a]Department of Food Science and Technology, Rodovia 465 - Km 7, UFRRJ, Seropédica, RJ 23891-360, Brazil.
[b]Department of Agribusiness Engineering, Av. dos Trabalhadores 420 - Vila Sta. Cecília, UFF, Volta Redonda, RJ 27255-125, Brazil.
[c]Embrapa Food Technology, Av. das Américas, 29501, Guaratiba, Rio de Janeiro, RJ 23020-470, Brazil.
[d]Packaging Technology Center Cetea, Food Technology Institute Ital, Campinas, São Paulo, Brazil.

[*]Corresponding author: sheylapa1@hotmail.com

Abstract

Cellulose acetate films incorporated with oregano essential oil (OEO) were evaluated for possible functional changes caused by treatment with high hydrostatic pressure (HHP) (200, 300 or 400 MPa for 5 or 10 min). The films were analyzed by differential scanning calorimetry (DSC), x-ray diffraction (XRD), *Fourier transform infrared attenuated total reflection (FTIR-ATR) spectroscopy*, scanning electron microscopy (SEM), contact angle, moisture absorption, water vapor transmission rate (WVTR), mechanical and optical analysis. The treatments with HHP caused a reduction in tensile strength, Young's modulus, moisture absorption, WVTR and increased elongation at break and contact angle, in addition to optical changes. SEM images showed delamination for most films, while XRD showed that all films were mostly amorphous, which reflected in the non-detection of thermal parameters for most films. However, the results showed that the treatments with HHP caused minimal changes in the functional properties of the films, being able to withstand the conditions of HHP explored in this study, which, industrially, is desirable. Thus, the films in this study, which are incorporated with an antimicrobial compound, could be used as a technology associated with HHP for food preservation.

KEYWORDS: Packaging; Non-thermal process; Functional properties.

1. INTRODUCTION

The food industry has been looking for technologies to offer quality, practicality, secure, free of synthetic preservatives and with low environmental impact. Thus, there is a growing interest in the production of antimicrobial active packaging (AAP) with biodegradable polymers[1] added with natural compounds. AAP is an innovative technology used to extend the commercial validity of several types of food, in which the packaging interacts with the product and the environment, favoring the maintenance of quality, microbiological safety and extended validity[1,2].

Traditionally, most food packaging is produced with petroleum derivative polymers. However, some limitations such as the environmental impact generated by the disposal, health problems caused by the incineration of these packages, in addition to the reduction of petroleum non-renewable resources, have motivated the development of eco-friendly packaging, based on biodegradable polymers[2]. The ability of cellulose acetate (CA) to produce films is remarkable, as it is a biodegradable, non-edible, abundant, thermoplastic polymer, with good impact resistance, transparency, obtained through the acetylation of cellulose. Recent research on the use of CA to design innovative packaging has shown its

great potential to add active functionality[3,4,5]. CA films can carry and allow the migration of antimicrobial additives, such as essential oils (EO)[2]. The application potential of EO as antimicrobial agents in the food industry has been the focus of numerous studies[6,7,8], since they are considered safe for use in food[9,10]. It is well established in the literature that CA films incorporated with certain types of essential oil, such as oregano[4] or pink pepper[11], presented great potential for controlling microbial growth, in addition to showing functional properties desirable for the intended application. *Origanum vulgare* is one of the most commercialized spices, and its EO is widely used as a natural antimicrobial, enhancing flavor and aroma in foods[12]. The oregano essential oil (OEO) is composed of a mixture of bioactive phenolic compounds such as carvacrol, thymol and γ-terpinene, and has been considered one of the most interesting EO proposed for active packaging[13]. However, the application of polymeric films in foods depends on their functional properties and these are directly related to the chemical structure of the polymer, the additive and the processing method[2]. In addition, due to its volatile characteristic, OEO can easily evaporate or decompose when exposed to certain conditions of heat, light or pressure[14].

High hydrostatic pressure (HHP) is a non-thermal technology increasingly used by the food industry to reduce microbial development, enzyme inactivation, with minimal modification of the sensory and nutritional properties of products. However, the inactivation of some microorganisms with HHP alone can be limited[15]. Thus, the use of the combination of technologies to form barriers has been explored, as it is an effective strategy for the control of microbial growth, in addition to preserving or improving the quality of food[16,17,18].

The combination of HHP and AAP can be promising for food preservation[19,20], however, HHP can cause reversible or irreversible conformation changes in polymeric structures. The package containing the product to be pressurized must be able to withstand changes in the volume of the food caused by the compression and decompression steps during pressurization. In addition, the efficiency of HHP depends on the physicochemical and mechanical properties of the packages containing the foods to be pressurized[21]. Depending on the conditions of the HHP, changes in the crystalline and amorphous phases may occur, which directly reflects on the functional properties of polymeric packaging[22]. Therefore, it is of fundamental importance to predict the possible effects caused by HHP, since extreme changes in the chemical structure can positively or negatively affect the packaging, which may make the use of technology for food preservation unfeasible[23].

It is worth mentioning the lack of studies on the possible changes caused by HHP on the structures and functional properties of biodegradable polymers. Therefore, this study aimed to evaluate the possible changes caused by the application of different HHP conditions in CA films with EO on the optical properties, water vapor permeability, moisture absorption, thermal properties (DSC), chemical structure and morphological (XRD and SEM) and mechanical properties.

2 EXPERIMENTAL

2.1 Materials

Cellulose acetate (CA) and Acetone (p.a.) were purchased from Sigma-Aldrich, Brazil; oregano essential oil (*Origanum vulgare*) was acquired from a local company (Ferquima, São Paulo, Brazil).

2.2 Preparation of films and application of HHP

The minimum inhibitory concentration of OEO for incorporation in the CA film and the w / v ratio of CA and ideal acetone for film formation were defined in previous works. By means of the casting method[24], the gels (CA in acetone (1:10 w/v) and OEO (50% w/v)) were spread into a glass plate and dried to room temperature (25 ± 2°C) for 10 min. After dried, the films were detached from the plates and subjected to conditions of, 300 MPa/5 min or 400 MPa/10 min in HHP equipment, laboratory model (Stansted Fluid Power, model S-FL-850-9-W, located at Embrapa Agroindústria de Alimentos, Rio de Janeiro). The films, with or without HHP were preconditioned (75% relative humidity and 25 ± 2°C) for analysis. The films produced were: Film without HHP (CAEOP0T0); Film treated with 200 MPa/5 min (CAEOP200T5); Film treated with 200 MPa/10 min (CAEOP200T10); Film treated with 300 MPa/5 min (CAEOP300T5); Film treated with 300 MPa/10 min (CAEOP300T10); Film treated with 400 MPa/5 min (CAEOP400T5) and Film treated with 400 MPa/10 min (CAEOP400T10). All films had a thickness equal to 0.06 mm.

2.3 Optical aspects

The parameters in the CIELab color space were evaluated using the Minolta CM-5-ID colorimeter[10]. Samples of 4 x 4 cm were evaluated using the Minolta CM-5-ID colorimeter for the degree of brightness L* (0 = black and 100 = white), red/green chromaticity (± a*: -80 to 0= green; 0 to +100 = red), yellow/blue chromaticity (± b*: -100 to 0= blue; 0 to +70 = yellow) total difference (ΔE*), Chroma (intensity or saturation of color (C= a*2 + b*2) 1/2*) and Opacity[10]. Hue angle = arctan (b*/a*) locates the color in polar coordinates and is expressed in degrees: 0°for red (+a*), 90° for yellow (+b*), 180° for green (-a*) and 270° for blue (-b*).

2.4 Mechanical analyses

Mechanical properties were determined using TA.XTplus texturometer (Stable Micro Systems, Surrey, England) operated with a 30 kg cell, force of 0.049 N and speed of 1 mm/s, according to ASTM D-882-82[11]. Specimens of 5 x 2.5 cm films were fixed with initial separation of 25 mm. The parameters analyzed are tensile strength (TS) (MPa), elongation at break (EB) (%) and Young's modulus (YM) (Mpa). The TS was obtained through the relation of the maximum force (N) by the sample area (mm). The YM was calculated from the linear region of the stress versus strain curve. The EB was calculated dividing film elongation at break (mm) by the initial gauge length of the specimen (mm).

2.5 Contact angle, Moisture absorption and Water vapor transmission rate

Contact angles were measured using a contact angle meter (model CAM 101, KSV Instruments, Finland), equipped with diffuse light, DMK 21AF04 camera and lens with the adjustable viewing angle[12]. Three droplet of a similar volume, of distilled water (2 µl) were deposited on the surface of each film (20 x 10 mm). The measurements were made with 1s

intervals for 30 seconds, immediately after each droplet. The results were calculated from the average of the right and left angles of the droplet, as a function of the analysis time.

Moisture absorption (MA) was measured according to method described by Alizadeh-Sania et al.[13], with some modifications. The samples (20 x 20 mm) were oven dried (100 ºC/2 h) and maintained at 0% relative humidity (RH) for 24h. Then, these were weighed and conditioned in a desiccator containing saturated NaCl solution with 75% RH for 7 days. The final weighing was performed and MA was calculated according to Eq. (2).

$$\%MA = \frac{Wt - W0}{W0} \times 100 \qquad \text{Eq (2)}$$

Where,
MA: moisture absorption in %;
W0: initial weight;
Wt: final weight after 7 days.

Water vapor transmission rate (WVTR) experiments were conducted gravimetrically, according to the ASTM E96-00[14], with modifications. The tests were conducted in triplicate, under a relative humidity of 75% and 25 ± 2°C.

2.6 Fourier transform infrared attenuated total reflection (FTIR-ATR) spectroscopy

Interactions between the polymeric matrix and OEO, after HHP treatment were conducted using Perkin Elmer (Spectrum 100), under attenuated total reflection (ATR) mode. The spectra of the films were collected over the wavelength range of 650–4000 cm^{-1}, 4 cm^{-1} resolutions and 32 scans. Measurements were conducted in triplicate, at random locations of each film.

2.7 Morphology and crystallinity

The microstructural of the films were obtained with the help of the Scanning Electron Microscope (SEM) (Carl Zeiss, model EVO MA 10). Prior to visualization, specimens were covered with gold (Au) (metallizer EMITEC K550X) with current of 25 mA/2 min. Then, were observed in the SEM in low vacuum, with acceleration voltage of 5000 kV, 450 of filament current, and scans of 5000 and 500x.

The crystallinity of the films was obtained using a Phaser D2 Bruker Diffractometer (Bruker, Germany), operated at 30 kV and 10 mA, according to the method described by Candido et al.[15]. Prior to analysis, the specimens were conditioned at 75% relative humidity, for 48 h. Then, two specimens of each film with 2 cm of diameter were analyzed in the range of 2 to 29º and ω-2θ.

2.8 Differential scanning calorimetry (DSC)

The specimens were conditioned for 48 h at 23 ºC and 75% relative humidity. Thermal properties were obtained using a Q200 DSC (TA Instruments, New Castle, USA), according to the ASTM D 3418–15[16] methodology, at a heating rate of 20 °C/min in the range from 20 to 250 °C. The glass transition temperature (Tg) and melting temperature was determined by a second heating curve of 20-250 °C with a heating rate of 10 °C/min.

2.9 Statistical analyses

Statistical analyses were performed using software R, version 3.2.4 (R Foundation for Computational Statistics, Vienna, Austria) and FactoMineR version 1.32. The Tukey multi comparative test was used to obtain differences between samples after ANOVA test both (Tukey and ANOVA tests) with a significance level of 5%.

3 RESULTS AND DISCUSSION

3.1 Optical aspects

Among the factors that influence the acceptance of the product by the consumer, the color and transparency of the packaging are of great importance. The attributes that define the appearance of the packaging can be influenced by several factors, such as the additives incorporated into the polymeric material as well as the method of preparing the packaging [17,18]. The results (Table 1) show that the majority of HHP conditions caused an increase in luminosity (L*).

TABLE 1 Visual appearance of film without (CAEOP0T0) and with high hydrostatic pressure (CAEOP200T5, CAEOP200T10, CAEOP300T5, CAEOP300T10, CAEOP400T5 and CAEOP400T10).

Samples	L*	a*	b*	ΔE*	C*	h°	Opacity
CAEO P0T0	97.04 ± 0.01^{abc}	0.01 ± 0^{a}	0.18 ± 0.01^{c}	3.47 ± 0.03^{a}	0.18 ± 0.01^{c}	86.32 ± 0.77^{d}	92.57 ± 0.02^{abc}
CAEO P200T5	97.03 ± 0.03^{bc}	0 ± 0^{b}	0.2 ± 0.01^{b}	3.41 ± 0.04^{b}	0.2 ± 0.01^{b}	90.5 ± 0.34^{c}	92.54 ± 0.06^{bc}
CAEO P200T10	97.05 ± 0.01^{ab}	-0.01 ± 0^{c}	0.21 ± 0^{ab}	3.36 ± 0.02^{cd}	0.21 ± 0^{ab}	92.22 ± 0.94^{ab}	92.59 ± 0.02^{ab}
CAEO P300T5	97.05 ± 0.02^{a}	-0.01 ± 0^{c}	0.22 ± 0.01^{a}	3.33 ± 0.04^{d}	0.22 ± 0.01^{a}	93.26 ± 1.05^{a}	92.62 ± 0.03^{a}
CAEO P300T10	97.06 ± 0.02^{a}	-0.01 ± 0^{c}	0.28 ± 0.01^{a}	3.33 ± 0.03^{d}	0.22 ± 0.01^{a}	91.84 ± 0.57^{b}	92.62 ± 0.03^{a}
CAEO P400T5	97.05 ± 0.02^{a}	-0.01 ± 0^{c}	0.20 ± 0.01^{b}	3.37 ± 0.03^{bc}	0.20 ± 0.01^{b}	91.37 ± 0.91^{bc}	92.61 ± 0.04^{a}
CAEO P400T10	97.02 ± 0.02^{c}	-0.01 ± 0^{c}	0.21 ± 0.01^{ab}	3.39 ± 0.03^{bc}	0.21 ± 0.01^{ab}	91.50 ± 0.66^{bc}	92.53 ± 0.04^{c}

*Mean values followed by the same letters in the same column do not differ ($p > 0.05$) by the Tukey test at the 5% level of significance. P: Pressure level (MPa) and T: Treatment time (min). The results are expressed as mean ($n = 4$) ± standard deviation.

The parameters b*, C* and hº increased slightly in view of all HHP conditions, revealing more yellowish films, with minimum saturation (C*) and location (hº) between yellow (90º) and green (180º). Hue angle (h°) represents the qualitative attribute of the color. The saturation coordinates (C*) represent the quantitative attribute for the color intensity, that is, the higher the C*, the greater the perception by the human eye. Therefore, more neutral colors (close to zero) have low saturation and, therefore, less bright for human perception[19]. For parameter a*, except the CAEOP200T5 film, all HHP conditions caused the appearance of a greenish color in the films. Lee et al.[20] reported that hydroxypropyl methylcellulose films showed an increase in green color when added with oregano essential oil nanoemulsions. In this study, Table 1 shows that the film with OEO, without HHP (CAEOP0T0, had a red color, close to the gray scale. When subjected to 200 MPa / 5 min, the film was completely gray (a*= 0), followed by a slight greenish tint (a*=-0.01) for the other HHP conditions. For ΔE*, which represents the three parameters in the CIE L*a*b* color space, a slight reduction is noted under all HHP conditions. For opacity, Table 1 shows minimal changes, with a slight

increase presented only by films treated with 300 MPa/5 min, 300 MPa/10 min and 400 MPa/5 min.

3.2 Mechanical analysis

Table 2 shows that the film without HHP (CAEOP0T0) presented TS equal to 19.7 MPa, while Assis et al.[21] reported TS equal to 67.48 MPa for the pure CA film. Therefore, in comparison with the data released by the authors, it is concluded that, in this study, the addition of OE to the CA film reduced TS. Changes in the mechanical properties of polymeric films can be attributed to the presence of EO, which in turn can penetrate the polymeric network, reduce how to reduce intermolecules, leading to plasticization and consequent reduction of TS[22].

TABLE 2 Mechanical properties, contact angle, moisture absorption and water vapor transmission rate of film without (CAEOP0T0) and with high hydrostatic pressure (CAEOP200T5, CAEOP200T10, CAEOP300T5, CAEOP300T10, CAEOP400T5 and CAEOP400T10).

Samples	TS (MPa)	YM (Mpa)	EB (%)	Contact angle (°)	MA (%)	WVTR (g.m^{-2}.day^{-1})
CAEOP0T0	19.7 ± 1.3^{a}	713.3 ± 77.4^{a}	17.7 ± 1.5^{b}	59.82 ± 1.8^{d}	0.023 ± 0.0^{a}	197.28 ± 6.9^{a}
CAEOP200T5	17.7 ± 0.9^{bc}	620.5 ± 49.7^{abc}	17.5 ± 1.1^{b}	65.20 ± 3.7^{bc}	0.012 ± 0.1^{bc}	166.31 ± 9.0^{b}
CAEOP200T10	18.9 ± 0.4^{ab}	684.6 ± 55.5^{a}	17.8 ± 1.2^{b}	70.06 ± 0.3^{ab}	0.017 ± 0.0^{ab}	160.08 ± 7.7^{b}
CAEOP300T5	19.1 ± 0.9^{ab}	685.3 ± 59.4^{a}	17.6 ± 1.3^{b}	66.46 ± 0.7^{ab}	0.011 ± 0.1^{bcd}	166.51 ± 5.2^{b}
CAEOP300T10	15.1 ± 1.0^{d}	516.7 ± 61.6^{c}	25.6 ± 1.4^{a}	69.88 ± 1.7^{ab}	0 ± 0.0^{d}	161.43 ± 6.8^{b}
CAEOP400T5	15.9 ± 0.7^{cd}	561.5 ± 50.5^{bc}	24.3 ± 3.2^{a}	70.49 ± 0.4^{a}	0.01 ± 0.0^{bcd}	166.06 ± 4.8^{b}
CAEOP400T10	18.1 ± 1.3^{ab}	665.9 ± 70.2^{ab}	17.9 ± 0.9^{b}	61.56 ± 0.3^{cd}	0.003 ± 0.0^{cd}	166.63 ± 4.2^{b}

*Mean values followed by the same letters in the same column do not differ ($p > 0.05$) by the Tukey test at the 5% level of significance. P: Pressure level (MPa) and T: Treatment time (min). The results are expressed as mean (n = 4) ± standard deviation.

Table 2 shows that TS and YM showed a reduction in face of all HHP conditions applied, mainly the films CAEOP300T10 and CAEOP400T5, which were less resistant and more flexible. For EB, only the films CAEOP300T10 and CAEOP400T5 differed statistically from the film without HHP (CAEOP0T0), revealing greater stretching capacity. It is believed that the HHP conditions used in this study, favored the molecular mobility of the polymer matrix, increasing the flexibility of the films. Industrially, selecting the ideal packaging to contain pressurized foods involves, among others, the ability to mechanically support HHP processing. In addition, flexible polymeric materials are the most suitable[23]. In this study, none of the HHP conditions used caused significant changes in the mechanical properties of the films, which allows for the suitability of the packaging to contain pressurized foods[24].

3.3 Contact angle, Moisture absorption (MA) and Water vapor transmission rate (WVTR)

The suitability of the packaging for a given application requires prior knowledge about its hydrophilic or hydrophobic nature. Table 2 shows that the HHP conditions contributed to the increase in the contact angle in all films, in addition to causing a reduction in moisture absorption (MA), especially in the CAEOP300T10 film, which showed zero absorption. It is

believed that HHP may have caused changes in the intermolecular forces of the polymeric structure, with a consequent reduction in the affinity of the water droplets on the surface of the films, making surface wettability and MA difficult. For the contact angle, if the surface is less hydrophilic, the smaller its contact with water, the droplet will not spread, becoming more spherical, forming a greater angle between the drop and the surface[25]. In addition, the MA of the polymeric film depends on its structure and constituents, where the attraction for moisture must be greater than the surface tension of the water, to obtain good wettability and absorption of the droplets[26,27].

The treatment with HHP caused a reduction in WVTR for all films, with no statistical difference between treatments. It is known that the hydrophilic nature of the CA film can hinder its application in foods that will be exposed in a high humidity environment[28], as well as the moisture absorbed by the polymeric materials can cause changes in their mechanical properties, glass transition temperature (Tg) and thermal conductivity[29,30,31]. Several strategies have been used to improve barrier properties and reduce the wettability of hydrophilic polymeric materials, such as the production of multilayer packaging[32] or with the incorporation of hydrophobic compounds, such as EO[2]. Therefore, we can conclude that in this study, in addition to the presence of EO, the HHP conditions contributed to reducing the affinity of the films for moisture, with a consequent increase in the water vapor barrier property.

3.4 Fourier transform infrared attenuated total reflection (FTIR-ATR) spectroscopy

FTIR spectra shows that there was no apparent difference between the films. Band that characterizes the cellulosic hydroxyl (OH) elongation was found at 3373.85 cm-1. In addition to this, another band was present at 2932.23 cm^{-1} representing the CH elongation and bands at 1232.29 and 1044.26 cm^{-1} representing the sterile carbonyl elongation, characteristic of CA[33]. Cellulose acetate is an ester, so the band 1742.37 cm^{-1} represents the elongation of the carbonyls of the esters present in the CA chains[34]. According to the literature, bands found between 1400 and 1600 cm^{-1} can be associated with the presence of OE of oregano[34], however, Gonçalves et al.[33] found bands in the same range in pure CA film. Therefore, in this study, we can predict that such bands may have overlapped and it was not possible to notice differences, when compared with the spectra disclosed in other surveys.

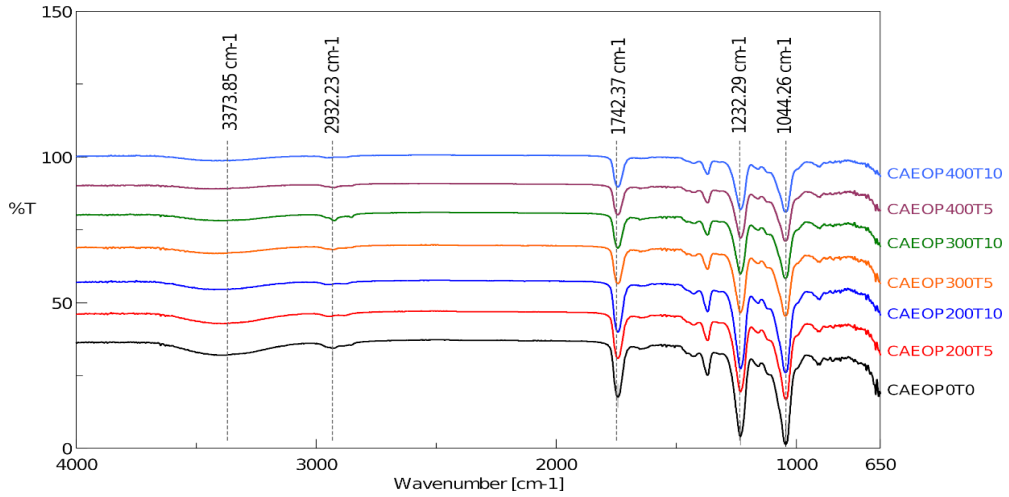

FIGURE 1 FTIR spectra of films without (CAEOP0T0) and with HHP (CAEOP200T5, CAEOP200T10, CAEOP300T5, CAEOP300T10, CAEOP400T5 and CAEOP400T10). P: Pressure level (MPa) and T: treatment time (min).

3.5 Morphology and crystallinity

Figure 2 shows that the control film (CAEOP0T0-S) exhibited an uneven appearance due to the incorporation of EO of oregano, as in Gonçalves et al.[33], pure CA film showed homogeneous surface. Lee et al.[20] also reported that hydroxypropyl methylcellulose films exhibited greater irregularities in the face of higher concentrations of oregano EO.

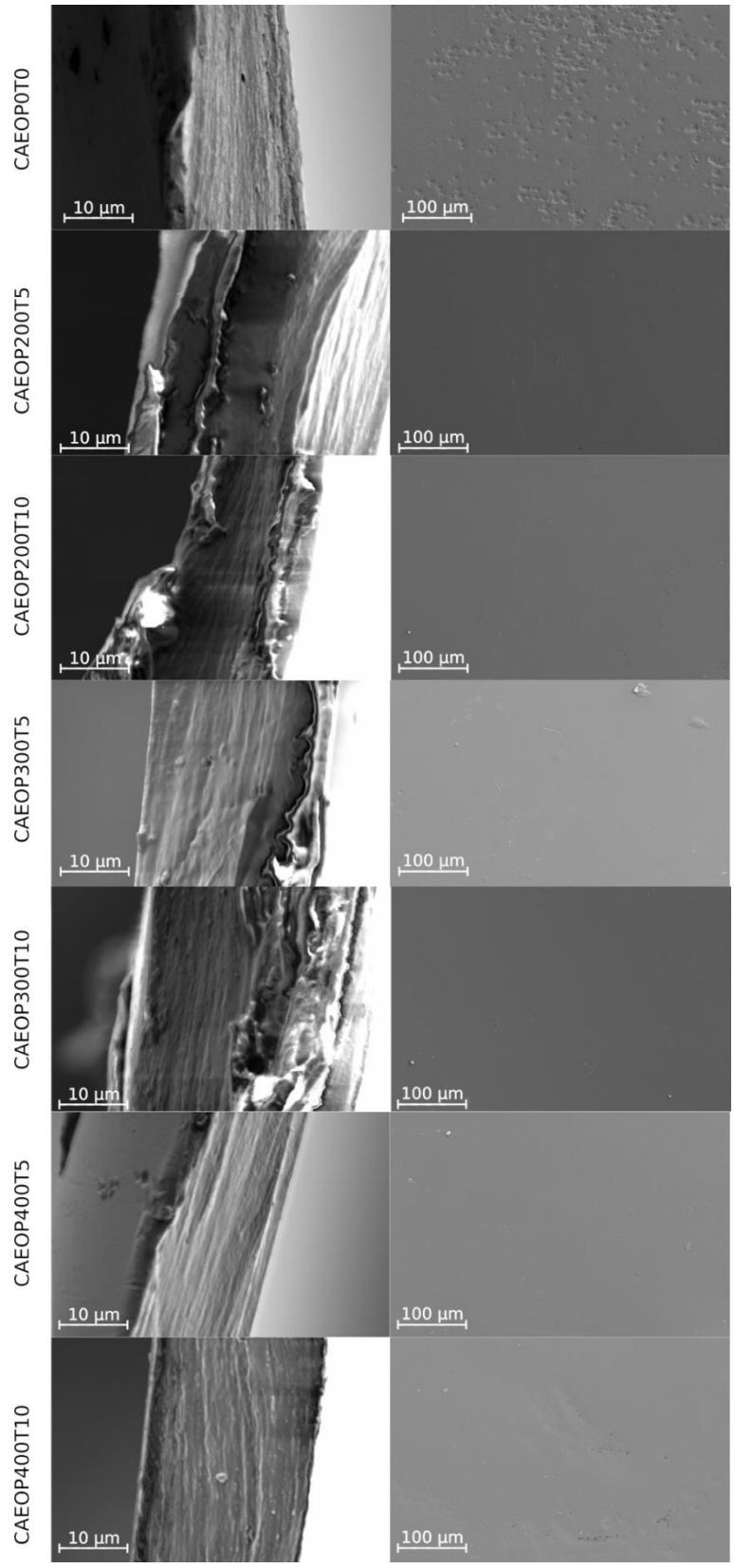

FIGURE 2 SEM of the surface (right column) and cross section (left column) of films without (CAEOP0T0) and with HHP (CAEOP200T5, CAEOP200T10, CAEOP300T5, CAEOP300T10, CAEOP400T5 and CAEOP400T10). P: Pressure level (MPa) and T: treatment time (min).

After treatment with HHP, the irregularities displayed by the surface of the control sample disappeared in all pressurized films. Therefore, it is believed that the compression strength of the HHP processing contributed to the rearrangement of the polymer chains, making the surface more homogeneous. For the transverse region, the film without HHP (CAEOP0T0) exhibited the presence of some droplets of EO in cavities, as reported by Lee et al.[20], while delamination was observed in all films submitted to treatment with HHP, except CAEOP400T5. Most films presented simple delamination, while the CAEOP400T10 film presented spongy aspect delamination. Depending on the polymeric material, HHP can cause microscopic changes leading to defects in the packaging layer or the appearance of delamination[23].

The diffractograms (Figure 3) were similar for all films, revealing amorphous materials, without evident peaks of crystallinity. Gonçalves et al.[33] found small peaks at 2θ = 8.8° and 23° in pure CA films and associated the finding with changes caused by the presence of the acetyl group, after the cellulose acetylation process for producing CA. However, in this study, the presence of EO caused the disappearance of these peaks, reducing the crystallinity of the material. Chen et al.[33] also observed a reduction in crystallinity in a cellulose nanofiber film after the addition of oregano EO. Therefore, it is believed that the interaction of the EO of oregano with the CA matrix, through hydrogen bonds, may have contributed to the appearance of a mostly amorphous structure. In addition, the treatment with HHP did not cause significant noticeable changes in the diffractograms, similar to that reported by Schauwecker et al.[36].

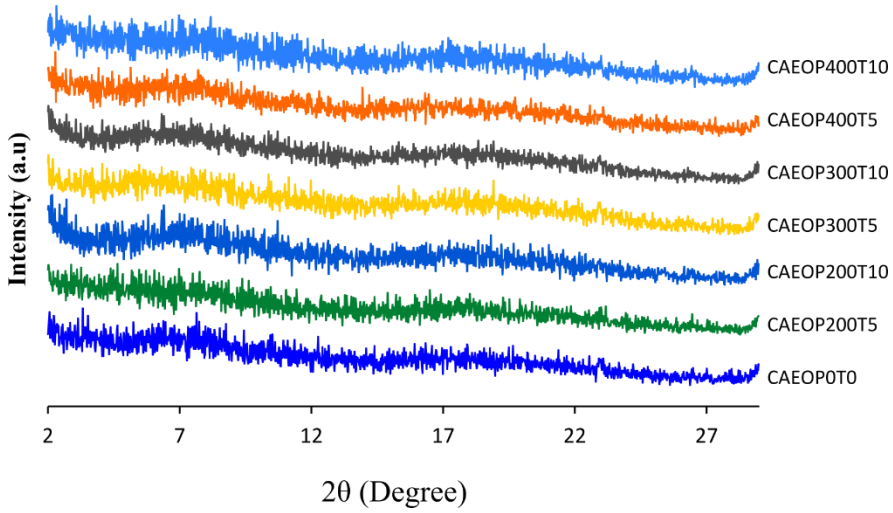

FIGURE 3 DRX diffractograms of films without (CAEOP0T0) and with HHP (CAEOP200T5, CAEOP200T10, CAEOP300T5, CAEOP300T10, CAEOP400T5 and CAEOP400T10). P: Pressure level (MPa) and T: treatment time (min).

3.6 Differential scanning calorimetry (DSC)

DSC curves (Figure 4) show the changes caused by the treatment with HHP on the moisture adsorption capacity, glass transition temperature (Tg) and melting temperature (Tm) of the films. In Figure 4a, the first heating cycle presents endothermic peaks at 59.10, 116.15 and 221.54 ºC, which in turn represent the moisture adsorption temperatures for the control film (CAEOP0T0). The moisture adsorption temperature of the AC film depends on its moisture holding capacity and the degree of substitution. In addition, adsorption peaks normally occur between 100 and 150 ºC[37,38]. Gonçalves et al.[33] reported the occurrence of the adsorption peak in pure CA film at 148.16 ºC, therefore, it is believed that the addition of the

EO of oregano has contributed to the appearance of several adsorption peaks, outside the expected range.

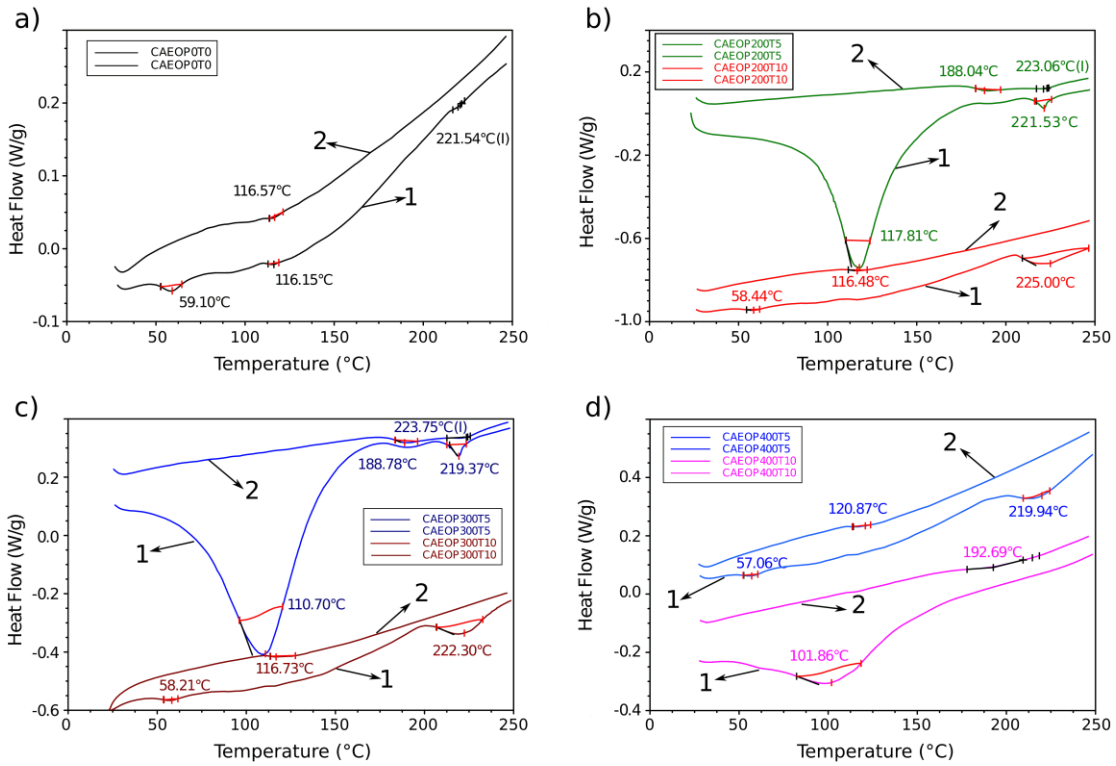

FIGURE 4 Thermograms of films without (CAEOP0T0) and with HHP (CAEOP200T5, CAEOP200T10, CAEOP300T5, CAEOP300T10, CAEOP400T5 and CAEOP400T10). P: Pressure level (MPa) and T: treatment time (min).

In the second heating curve, the first endothermic peak represents Tg while the second peak represents Tm. According to Figure 4, only the films CAEOP200T5, CAEOP300T5 and CAEOP400T10 showed slight endothermic peaks for Tg and, among these, only the film CAEOP400T10 did not show a peak for Tm. Pure CA film analyzed by Gonçalves et al.[33] presented Tg at 229.02 ºC, therefore, the EO of oregano (film CAEOP0T0) must have penetrated the amorphous regions of the CA, increasing the mobility of the polymer chains, by reducing intermolecular forces, with a consequent reduction in Tg[39] and making its detection impossible. In addition, treatment with HHP can cause changes in the amorphous or crystalline regions of polymeric materials[24] and the reduction in Tg can be caused by the reduction in the size of the crystals[40]. The effect of HHP on the thermal properties of polymeric packaging has been studied and compared using XRD and DSC techniques. These analyzes provide information on polymeric morphological parameters, such as degree of crystallinity, crystal size, melting temperature and glass transition temperature[7,41,42].

4 CONCLUSIONS

The treatment with different HHP conditions caused a slight increase in brightness and opacity, in addition to the appearance of a slightly greenish color, for most films. All treatments caused an increase in color saturation, Hue angle and red color. The mechanical properties showed a slight reduction in TS, YM and increase in EB. The treatment with HHP caused a reduction in the affinity of the films for moisture, detected by increasing the contact angle, reducing the absorption of moisture and the rate of transmission to water vapor. The

morphological analysis (SEM) indicated that delamination occurred after most HHP treatments, while the XRD analysis showed mostly amorphous structures for all films. The results obtained in this study showed that the treatments with HHP caused minimal changes in the functional properties of the films, therefore, they can be used as biodegradable, non-edible packaging to package products to be pressurized.

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CONFLICTS OF INTEREST

The authors declare no competing financial interest.

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CAPÍTULO IV

ANTIMICROBIAL PACKAGING AND HIGH HYDROSTATIC PRESSURE: COMBINED EFFECT IN IMPROVING THE SAFETY OF COALHO CHEESE

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Sheyla Moreira Gonçalves[a,*], Fabíola Santos Gouveia[a], Davy William Hidalgo Chávez[a], Amauri Rosenthal[a,b], Janine Passos Lima da Silva[b], Nathália Ramos de Melo[ac]

[a]*Department of Food Science and Technology, Rodovia 465 - Km 7, UFRRJ, Seropédica, RJ 23891-360, Brazil.*
[b]*Embrapa Food Technology, Av. das Américas, 29501, Guaratiba, Rio de Janeiro, RJ 23020-470, Brazil*
[c]*Department of Agribusiness Engineering, Av. dos Trabalhadores 420 - Vila Sta. Cecília, UFF, Volta Redonda, RJ 27255-125, Brazil.*

[*]Corresponding author: sheylapa1@hotmail.com

ABSTRACT

Active cellulose acetate (CA) films incorporated with oregano essential oil (AF) were previously subjected to high hydrostatic pressure (HHP) treatment (300 MPa/5 min – FHP1 or 400 MPa/10 min – FHP2) and investigated for possible changes in their antimicrobial efficiency. In parallel, the efficiency of the antimicrobial films (AF), HHP (300 MPa/5 min or 400 MPa/10 min), or a combination of AF and HHP, was tested on coalho cheese, experimentally contaminated with *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus*, stored for 21 days under refrigeration. Investigations *in* culture media (agar, BHI broth, and micro-atmosphere) detected antimicrobial efficiency for all films, with or without HHP, against the three bacteria. However, the data indicated that the treatment with 300 MPa/5 min may have impaired the migration of OEO from FHP1, justifying its lower efficiency in solid medium and BHI broth. In *cheese samples*, the combination of AF and 400 MPa/10 min (CAFP2) caused greater reductions in counts for the three microorganisms, at zero time throughout the entire coalho cheese storage. Only AF or combination (AF and HHP) were able to control microbial multiplication during the 21 days. Therefore, the results confirm that the individual use of HHP (300 MPa/5 min or 400 MPa/10 min) at the level evaluated can allow bacterial multiplication during storage and that the combination of antimicrobial packaging and HHP has greater potential to ensure a safer coalho cheese.

Keywords: Food safety; Foodborne pathogens; Natural antimicrobial; Non-thermal technology; Active packaging.

1. INTRODUCTION

The search for technological practices that can minimize economic and health losses, without damaging the quality of food, has become relevant. Among the non-thermal technologies aimed at serving the food market, the high hydrostatic pressure (HHP) (FELLOWS, 2006; GAVA & FRIAS, 2009) that can be applied individually (COSTABEL et al., 2016) or in combination with other conservation methods (ARQUÉS et al., 2005). Another emerging technology that has attracted the attention of the food industry includes active antimicrobial packaging (AAP) incorporated with natural antimicrobial compounds, with oregano essential oil (OEO) being a broad-spectrum antimicrobial against gram-positive and gram-negative bacteria (HOSSEINI et al., 2015; Artiga-Artigas et al., 2017).

Cellulose acetate (CA) is a biodegradable, thermoplastic and non-edible cellulose ester, with good barrier properties to oils and fats, as well as reduced moisture and oxygen permeability. In addition, CA is a flexible, transparent polymer with moderate mechanical resistance. It is obtained through the acetylation of native cellulose, in which the hydroxyl groups are replaced by acetyl groups (Wan Daud and Djuned, 2015). CA has been widely used for the

development of antimicrobial packaging by adding essential oils (Pola et al., 2016; Carvalho et al., 2017; Dannenberg et al., 2017).

Studies have shown that HHP (RAMOS et al., 2015) and AAP (BERISTAIN-BAUZA et al., 2017), when used individually, have positive effects on the food life span (ARANCIBIA et al., 2014). However, they are often not enough to effectively eliminate deteriorating or pathogenic microorganisms (EVELYN & SILVA, 2015), which demands a combination of technologies to obtain greater efficiency. Research reports a synergistic antimicrobial effect when combining HHP and AAP in salami (MARCOS et al., 2013) and cooked ham (JOFRÉ et al., 2008).

The antimicrobial efficiency of AAP depends, among other factors, on the efficiency of the antimicrobial compound after incorporation into the polymeric matrix. Therefore, the exposure of AAP to HHP conditions can positively or negatively affect the structure and/or migration of the active compound, which can make the use of technology for food preservation unviable (MENSITIERI et al., 2013; FRALDI et al., 2014; LAVOINE et al., 2016). The use of essential oils has been limited for packaging foods preserved by thermal processes, due to their volatile nature and thermal sensitivity (Fabra et al., 2016). In the case of HHP, in spite of being essentially a non-thermal process, it can significantly change volatile compounds (Mastello et al., 2018). Al-Bandak et al. (2011) reported that the antioxidant capacity of *Manjorona syriaca* extract was reduced after HHP treatment. Moreover, carvacrol, one of the volatile compounds of *Manjorona syriaca*, which is highly responsible for its antioxidant activity, is also a major component of OEO. Accordingly, the understanding of the HHP effect on the OEO antimicrobial capacity incorporated in the CA film may be used to predict the effects of HHP on the intrinsic properties of the packaging, being necessary a complementary and individualized assessment in the food matrix, aiming at its application in each food product. Therefore, investigations regarding the antimicrobial properties of the packaging after pressurization become necessary, since they will guide its application.

Cheese is an important vehicle for foodborne pathogenic bacteria, particularly artisanal cheeses, such as coalho cheese (FONTENELE et al., 2017), which, in turn, depends on the raw material, the microbiota involved, and the processing technology applied (BEZERRA, et al., 2016) to define its characteristics. According to the Technical Regulation for Identity and Quality of Coalho Cheese (Brasil, 2001), it is a semi-hard cheese with a slightly acidic flavor, uniform yellowish-white color, and a thin crust. Among the specificities and steps of production of artisanal coalho cheese, three of them are considered essential for the development of its characteristics: the use of raw cow milk, cooking the curd, and adding salt directly to the curd. The whey is separated after stirring and resting the pasta, being reincorporated to it after heating (85-100 °C). The curd is then cooked at different temperatures according to the type of coalho cheese, which could be either semi-cooked (up to 45 °C) or cooked (45 to 60 °C) cheese, followed by, salting, hanging, pressing, and storage (Carvalho, 2007; Cavalcante et al., 2007). The absence of good manufacturing practices in cheese processing, and/or the microbiological quality of the milk used as raw material, can facilitate the transmission of pathogens such as *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* (RUWER et al., 2011), often isolated from coalho cheese and involved in foodborne diseases outbreaks in Brazil (SILVA et al., 2015).

HHP has been successfully applied in cheese to increase shef life or accelerate the ripening period (Martinez-Rodriguez et al., 2012). However, treatments with high pressure levels or

long holding time have caused undesirable effects in cheeses, such as changes in texture (Tomasula et al., 2014) and loss of disintegration properties (Hnosko et al., 2012). Therefore, the combination of technologies, such as barriers in food production can help to minimize damage to the product and storage wastes, in addition to increasing safety in the cheese consumption. Furthermore, combining HHP and APP can reduce the pressure level required and, consequently, the processing and maintenance costs. The use of high-pressure levels over time can led to equipment wear, resulting in high maintenance costs (Augusto et al., 2018) and shortening in useful life, making the process less economically viable (O'Reilly et al., 2001). Therefore, this work aimed to investigate the possible changes caused by HHP on the antimicrobial properties of film AF and, in parallel, to evaluate the efficiency of individual use or combination of HPP and AF in improving the safety of coalho cheese.

2. MATERIALS AND METHODS

2.1 Materials

-Preparation of antimicrobial films: cellulose acetate (CA) (Sigma-Aldrich, Brazil), in powder, with a degree of substitution of 1.48°; acetone PA (Cap-Lab, São Paulo, Brazil), and oregano essential oil (Origanum vulgare) (Ferquima, São Paulo, Brazil).

-Cultivation of microorganisms: Brain-Heart Infusion Broth (BHI - Merck, São Paulo, Brazil), Peptone water 0.1% (BD Bacto™ peptone, Interlab, São Paulo, Brazil). Agars: *Staphylococcus* selective agar (Baird-Parker - Merck, São Paulo, Brazil), *Listeria* selective agar base (Oxford - Oxoid®), with a selective supplement for *Listeria monocytogenes* (Sigma®), BBL™ (Levine Eosin Methylene blue - BD), and Trypticase Soy Agar (TSA-BD Difco™).

2.1.1 Microbial cultures

- *Listeria monocytogenes* (ATCC 19111) maintained on trypticase soy agar (TSA) at 4 °C, reactivated in brain-heart infusion broth (BHI) at 37 °C for 24 h.

- *Staphylococcus aureus* (ATCC 14458) maintained on trypticase soy agar (TSA) at 4 °C, reactivated in brain-heart infusion broth (BHI) at 37 °C for 24 h.

- *Escherichia coli* (ATCC 8739) maintained on trypticase soy agar (TSA) at 4 °C, reactivated in brain-heart infusion broth (BHI) at 37 °C for 24 h.

2.2 Inoculum

To inoculate each of the microbial cultures in the coalho cheese, the cultures were resuspended in 0.1% peptone water, centrifuged at 40.000 rpm/10 min twice, and diluted in peptone water to the desired concentration, in CFU/mL (Lee et al., 2015).

2.3 Chemical composition of the EO

The composition analysis was performed according to Gao et al. (2020), with modifications. The extraction of volatile compounds occurred in Headspace Solid Phase Microextraction (HS-SPME), with a 50/30 mm carboxen/divinylbenzene/ polydimethylsiloxane fiber. The fiber was exposed to the headspace, above 0.1 mL of the OEO in a 5 mL tube, at 40 °C/40 min. The fiber was then taken for injection of the volatile compounds in gas chromatography-

mass spectrometry (GC-MS) (Agilent 7890A FID-MSD detector Agilent 5975C) with an HP-5ms column (30 m x 250 μm x 0.25 μm). Hydrogen was used as a carrier gas, with a flow rate of 0.1 mL/min and a division rate (splitless). The initial oven temperature was 70 °C, increasing to 180 °C with a heating rate of 4 °C/min, followed by an increase to 250 °C with a heating rate of 8 °C/min. The temperature of 250 °C was then maintained for 5 min. The temperatures of the FID detector and injector were 280 °C and 250 °C, respectively. Mass spectrometry was used to detect EO components. Component identification occurred in three associated libraries (Adams, Library 01, and NIST 11). The study was carried out in triplicates.

2.4 Preparation of films and application of HHP

The films were prepared using the casting method (Gonçalves et al., 2019), in which CA was solubilized in acetone (1:10 w/v), for 12 h, until the gel was formed. Then, OEO (50% w/v) was added under slight agitation. After 30 min of rest, the film-forming solution was deposited on a glass plate and spread at a predetermined height. The evaporation of the solvent (acetone) and drying of the films occurred under controlled conditions (temperature of 25 ± 2 °C and 75% humidity) for 10 min. The films were then detached from the glass plates, vacuum packed, and subjected to HHP processing (300 MPa/5 min or 400 MPa/10 min) in a Stansted Fluid Power, model S-FL-850-9-W, located at Embrapa Food Agroindustry, Rio de Janeiro. The films produced and used to control the growth of *L. monocytogenes*, *E. coli* or *S. aureus*, *in* culture media *were:* Antimicrobial film without HHP (AF); Film treated with 300 MPa/5 min (FHP1); Film treated with 400 MPa/10 min (FHP2).The treatments were preconditioned, for a maximum of 5 days, to carry out the analyses.

2.5 Antimicrobial activity in agar

Samples of the films, into disk of 10 mm diameter, were taken aseptically and placed in the center of plates containing agar contaminated with 10^7 CFU/mL of each bacterium (Hafsa et al., 2016). The plates were incubated at 37 °C or 10 °C, for 24 h. Inhibition zones were measured on the basis of the average diameter (cm) of the clear zone directly on the Petri plates, considering the coverage area by the films. The study was carried out in triplicates with 3 repetitions.

2.6 Antimicrobial activity in broth

Samples of the films with 4 cm^2 were immersed in tubes containing BHI broth, inoculated with 10^3 CFU/mL of each bacterium (Dannenberg et al., 2017, with modifications). Control tubes (without film) were also prepared. The tubes were incubated at 37 °C, or 10 °C, for 2 h. Aliquots of 0.1 mL, containing specific agar for each bacterium were collected at time 0 and every 20 minutes, for quantification in plates. The plates were incubated at 37 °C for 24 h and counting was performed. The study was carried out in duplicates, with 3 repetitions.

2.7 Antimicrobial activity in micro-atmosphere

According to the methodology proposed by Ghabraie et al. (2016), the plates containing agar, inoculated with 10^3 CFU/mL of each bacterium, were completely covered with antimicrobials films (90 mm diameter). The plates were sealed with parafilm and incubated at 37 °C or 10 °C, for 24 h. The percentage inhibition data were obtained by comparing the control, the micro-atmosphere (plates with parafilm only) and the antimicrobial systems in micro-

atmosphere (micro-atmosphere and antimicrobial film). The study was carried out in triplicates with 3 repetitions.

2.8 *Cheese Production*

According to Cavalcante et al. (2007), standardized pasteurized milk with 3.3% fat was heated to 35 °C, followed by the addition of lactic acid (100 μL/L), calcium chloride (500 μL/L), and coagulant (chymosin) (1 mL/L). Coagulation occurred at 35 °C for 45 min, followed by cutting the curd and stirring at 45 °C for 30 min, partial draining (about 95%), salting the dough (1.2% w/v), total draining, cutting the dough and shaping, first pressing (15 min), second pressing (after 15 h), and maturation in a cold chamber (10 to 12 °C) for 20 days.

2.9 *Cheese sampling, contamination, and in cheese samples antimicrobial efficiency*

Samples of 10 g of coalho cheese were exposed to ultraviolet light for 15 min in laminar flow and, subsequently, dipped in a suspension of 0.1% peptone water containing 10^7 CFU/mL of each microorganism to the final contamination of 10^5 CFU/g. After being removed from the suspension, the samples remained for 5 min to dry the inoculum, and then the following treatments were assembled:

- Control Cheese (CC): Contaminated cheeses samples, packed in sterile bags with identification stripe (Nasco Whirl-Pak (118 mL)), aseptically sealed in laminar flow and stored at 5 °C for 21 days.

-CAF: Contaminated cheeses samples, covered by AF, packed in sterile bags, aseptically sealed and stored at 5 °C for 21 days.

-CP1 and CP2: Contaminated cheeses samples, packed in sterile bags, aseptically sealed in laminar flow, submitted to HHP processing (300 MPa/5 min or 400 MPa/10 min) and stored at 5 °C for 21 days.

-CAFP1 and CAFP2: Contaminated cheeses samples, covered by AF, packed in sterile bags, aseptically sealed in laminar flow, subjected to processing with HHP (300 MPa/5 min or 400 MPa/10 min) and stored at 5 °C for 21 days.

The coalho cheese evaluation during storage proceeded at times 0, 7, 14, and 21 days. Each sample was homogenized in 0.1% peptone water, inoculated into plates containing Oxford agar, with selective supplement (*L. monocytogenes*), Baird-Parker with egg yolk (*S. aureus*), BBL Levine (*E. coli*), and incubated at 37 °C for 24 h. The study was carried out in triplicates with 2 repetitions.

2.10 Statistical analysis

Data were statistically analyzed by ANOVA (in triplicates), and Tukey multi comparative test was performed to detect the differences between samples with a significance level of 5%. All statistical analyses were carried out using software R, version 3.2.4 (R Foundation for Statistical Computing, Viena, Áustria), and FactoMineR, version 1.32.

3. Results and discussion

3.1 Chemical composition of OEO

Among the 23 components found in the OEO (Table 1), 83.9% of oxygenated monoterpenes, 12% of monoterpenes, 2.7% of sesquiterpenes and 0.8% of oxygenated sesquiterpenes stand out. Among the components of OEO, carvacrol, thymol, ρ-cymene, and terpinene are the most found components, in percentage (Cui et al., 2019). In this study, carvacrol was the major component, followed by p-cymene, linalool, γ-terpinene, and thymol. According to a literature study, the antimicrobial efficiency of OEO can vary, mainly due to the levels of thymol and carvacrol present, the quantity of the components, and the interaction between them. The possible synergistic effect between some components of OEO, such as carvacrol, p-cymene, and γ-terpinene, has been reported (Silva et al., 2010).

Table 1. Chemical composition of OEO.

IRL lit	Components	Percentage (%)
1298	Carvacrol	73.9
1024	p-cymene	6.4
1098	Linalool	3.0
1054	γ-terpinene	2.8
1417	trans- Caryophyllene	2.3
1289	Thymol	2.1
1165	Borneol	1.2
1026	1.8-cineole	1.2
1024	Limonene	0.9
1141	Camphor	0.9
1186	α-terpineol	0.8
1582	Oxide caryophyllene	0.8
1174	Terpinen-4-ol	0.7
1014	α-terpinene	0.5
974	β-pinene	0.5
1452	α-humulene	0.5
988	Myrcene	0.4
974	1-octen-3-ol	0.3
1241	Carvacrol methyl ether	0.2
932	α-pinene	0.2
946	Camphene	0.2
924	α-thujene	0.2

3.2. Antimicrobial activity of films on Agar

Only the condition of incubation at 37 °C allowed the multiplication of microorganisms. In Table 2, it is observed that, for *L. monocytogenes* and *S. aureus*, F and FHP2 showed the same antimicrobial efficiency, while FHP1 was less efficient. Therefore, the condition of 300 MPa/5 min must have caused a change in the film structure, which may have hindered the migration of the OEO to the contaminated agar. According to Mensitieri et al. (2013), certain HHP conditions can cause reversible or irreversible changes in the polymer matrix, which directly reflects on the functional properties of the packaging.

Table 2. Diameters of areas of inhibition of the antimicrobial films without HHP (AF) and antimicrobial films treated with HHP: 300 MPa/5 min (FHP1) or 400 MPa/10 min (FHP2), on agar at 37 °C, for *L. monocytogenes*, *S. aureus* or *E. coli*.

Treatment	*L. monocytogenes* Inhibition zone (cm)	*S. aureus* Inhibition zone (cm)	*E. coli* Inhibition zone (cm)
AF	2.5 ± 0.1^{a}	2.9 ± 0.1^{a}	1.9 ± 0.1^{a}
FHP1	2.1 ± 0.1^{b}	2.0 ± 0.2^{b}	1.8 ± 0.1^{a}
FHP2	2.4 ± 0.1^{a}	2.9 ± 0.1^{a}	1.9 ± 0.1^{a}

*Means followed by the same letters in the same column do not differ from each other ($p > 0.05$) by the Tukey test at the 5% level of significance. The results are expressed as mean ($n = 6$) ± standard deviation. Inhibition zones, expressed in cm, being considered the coverage area by the films (1 cm).

The smallest zones of inhibition were observed for *Escherichia coli*, with no statistical difference ($p<0.05$) for the three films (Table 2). According to reports, gram-positive bacteria (*L. monocytogenes* and *S. aureus*) are generally more sensitive to EO when compared to gram-negative microorganisms (*E. coli*). As stated in the literature, this is due to the fact that gram-negative microorganisms have cell walls with an external membrane, which, in turn, makes it difficult for hydrophobic substances to penetrate, such as EO (Burt, 2004; Gutiérrez et al., 2009).

3.3 Antimicrobial activity in broth

In BHI broth, it is noted that the control treatment (Control) (tubes without films) for *L. monocytogenes* (Figure 1a) showed a constant count during the 2 h of incubation at 37 °C. However, when subjected to incubation at 10 °C (Figure 1b-control), *L. monocytogenes* exhibited a slight numerical reduction from 3.40 to 3.22 log CFU/mL. In addition, the visual appearance of the tubes, after 24 h of incubation showed that tubes with *L. monocytogenes* at 37 °C had evident turbidity, while the tube at 10 °C was less turbid. Also, counts of *L. monocytogenes* after 24 h of incubation at 37 °C revealed an excessive number of cells to enumerate (greater than 300 colonies per plate), while *L. monocytogenes* at 10 °C showed a count reduction from 3.4 to 1.36 log CFU/mL.

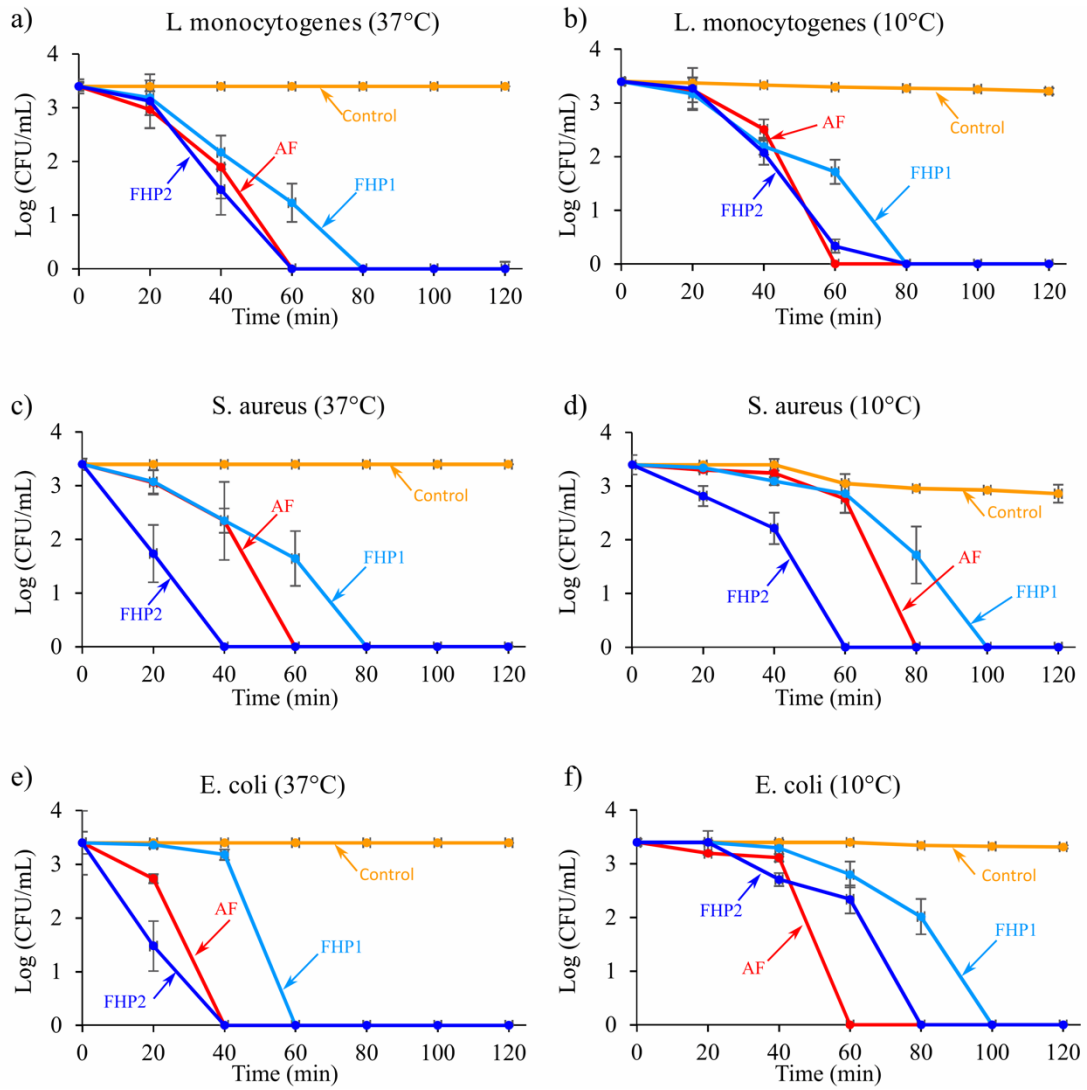

Figure 1. Efficiency of antimicrobial films without HHP (AF) and antimicrobial films treated with HHP: 300 MPa/5 min (FHP1) or 400 MPa/10 min (FHP2), in BHI broth, for *L. monocytogenes* (37 °C - 1a; 10 °C - 1b), *S. aureus* (37 °C - 1c; 10 °C - 1d) and *E. coli* (37 °C - 1e; 10 °C - 1f). The results are expressed as the mean (n = 6) ± standard deviation.

Studies report that *L. monocytogenes* stands out as a cause of foodborne diseases (FBD), due to its ability to survive and proliferate in foods preserved under refrigerated conditions (Gao et al., 2020). However, the ability of microorganisms to multiply depends as much on factors related to the food itself or enrichment medium (intrinsic properties), as on environmental factors (extrinsic properties) (Wei et al., 2019). The generation time of *L. monocytogenes* varies according to the food and its temperature, and at lower temperatures, the generation time is longer (Stratakos et al., 2020). Therefore, the behavior of *L. monocytogenes* in the studied conditions revealed that, at low temperature (10 °C), the generation time was longer, resulting in a smaller number of cells after 24 h of incubation. The same was observed for *S. aureus* and *E. coli* (Figures 2d and 2f-Control), which showed reductions of 0.54 and 0.10 log CFU/mL, after 2 h of incubation at 10 °C, respectively. After 24 h of incubation at 10 °C, reductions of 2.00 and 2.67 log CFU/mL were found for *E. coli* and *S. aureus*, respectively. *S. aureus* is a mesophilic bacteria that can grow at temperatures from 7 °C to 47.8 °C, though their optimal growth occurs in 37 °C and production of toxins in the range of 40 °C to 45 °C. *E. coli* prefers higher temperatures for

optimal growth (37 °C to 42 °C) (Franco & Landgraf, 2008), which justifies the reduction in the number of colonies in the incubated tube at 10 °C.

Figure 1 shows that all films caused total inhibition of the three bacteria in up to 100 min of incubation at 37 °C or 10 °C. However, it is observed that the film FHP1 reached a delayed total elimination for all bacteria tested, when compared to films AF or FHP2. Therefore, it is believed that processing with HHP (300MPa/5 min) reduced the migration of OEO from the film to the BHI broth, resulting in delayed microbial inhibition.

For *L. monocytogenes*, at 37 ° C (Figure 1a) and 10 ° C (Figure 1b), it is noted that, at 40 min of incubation, the FHP2 film showed greater reduction, when compared to the AF film. However, both reached total elimination at 60 min of incubation. For *S. aureus*, the FHP2 film was notably more effective than AF and FHP1 for incubation at 37 °C or 10 °C (Figures 1c and 1d, respectively). For *E. coli*, the FHP2 film was more efficient only for incubation at 37 °C (Figure 1e), followed by the AF and FHP1 film, while for incubation at 10 °C (Figure 1f), the AF film was more efficient, followed by the FHP2 and FHP1 films. As the release of volatile OEO compounds depends on temperature (Dorneles et al., 2019), the incubation condition at 10 °C may have hindered the migration of sufficient OEO from the film (FHP2) to eliminate *E. coli* cells with the same speed observed for *L. monocytogenes* and *S. aureus* at 10 °C (60 min) (Figures 1b and 1d). This may have happened because gram-negative bacteria such as *E. coli* are generally more resistant to EO (Burt, 2004; Gutiérrez et al., 2009), so they may need a greater amount of EO for their elimination. However, even with some resistance to OEO, at 37 °C (Figure 1e), the release of antimicrobial compounds by the FHP2 film was sufficient to cause a faster elimination of viable *E. coli* cells. Therefore, it seemed that the treatment of the film at 400 MPa/10 min (FHP2) caused changes in the polymeric matrix, facilitating the exit of OEO to the BHI broth, mainly in the experiment carried out at 37 °C, which exhibited greater speed in the complete elimination of bacteria.

Comparing the antimicrobial efficiency of films with OEO, between the different temperatures, it is observed that the reduction in the number of viable bacteria was faster at 37 °C (Figures 1a, 1c, and 1e), when compared with the incubation at 10 °C (Figures 1b, 1d, and 1f). This difference is believed to be directly related to the amount of OEO released into the BHI broth since the EO antimicrobial compounds are volatile and have a greater release at higher temperatures (Dorneles et al., 2019).

3.4 Antimicrobial activity in micro-atmosphere

Only microorganisms incubated at 37 °C were able to present viable cells after 24 h of incubation. For the Control treatment (Figures 2A, 2B and 2C) (plates without micro-atmosphere or films), the counts after 24 h of incubation, at 37 °C, revealed values equal to 3.40 log CFU/mL, for the three (3) bacteria. For the micro-atmosphere condition (without antimicrobial films) (Figure 2A1, 2B1 and 2C1), compared to the control treatment, the final counts revealed 0.40, 0.04 and 0.04 log CFU/mL with reductions of 3.00, 3.36 and 3.36 log CFU/mL for *L. monocytogenes*, *E. coli* and *S. aureus*, respectively. The three bacteria studied are considered facultative anaerobic, which means they can grow in environments without oxygen (Franco & Landgraf, 2008). However, there is a slight reduction in viable cells due to the absence of oxygen. In the micro-atmosphere condition associated with antimicrobial films (AF, FHP1, or FHP2) (Figures 2A2, 2B2 and 2C2), there was total elimination (3.40 log CFU/mL) of viable cells for all bacteria. Chen & Liu (2016) reported reductions of 93.40 and

100% of viable *E. coli* and *S. aureus* cells, respectively, after using a micro-atmosphere condition associated with cellulose sulfate film with 1.2% mustard essential oil.

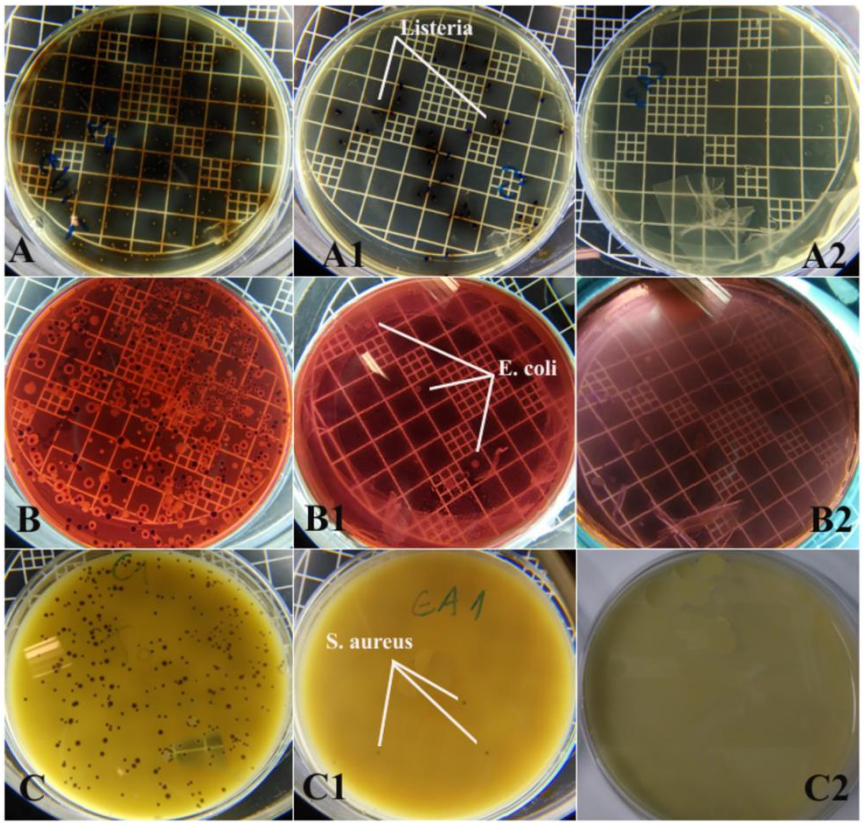

Figure 2. Microbial growth of the Control treatment (Figures 2A, 2B and 2C); micro-atmosphere treatment (Figures 2A1, 2B1 and 2C1); treatment in a micro-atmosphere condition and covered by antimicrobial films (AF, FHP1 or FHP2) (Figures 2A2, 2B2 and 2C2), for *L. monocytogenes*, *E. coli* or *S. aureus*, respectively.

3.5 In cheese antimicrobial activity

Figure 3 shows the behavior of the three bacteria in coalho cheese packed with antimicrobial film (CAF), processed with HHP (CP1 and CP2) or under the combination of AF and HHP (CAFP1 and CAFP2), stored for 21 days at 5 °C. Table 3 shows that the zero time counts for *L. monocytogenes* in Control (CC) and CAF cheeses, were 5.57 and 5.58 log CFU/mL, respectively. When submitted to HHP conditions, the cheeses showed a reduction of 0.25 and 2.30 log CFU/mL for CP1 and CP2 (Table 3), respectively, at time zero. In a study of the antimicrobial effect of HHP for different strains of *L. monocytogenes* in cheese, López-Pedemonte et al. (2007) observed that the treatment with 300 MPa/10 min caused a reduction of only 0.70 log CFU/g, while more significant reductions were observed for 400, 500, and 600 MPa/10 min. Batty et al., (2019) reported reductions of >5 log for *L. monocytogenes* in camembert-type cheese treated whit 450 and 550 MPa/10 min at 25 ºC. According to Martínez-Rodríguez et al. (2012), for most bacteria, reductions greater than 5 log in cheese are achieved with higher pressures and temperatures and longer hold times. In cheeses subjected to the combination of HHP and AF (Table 3), the counts revealed reductions of 0.30 and 2.68 log CFU/mL for CAFP1 and CAFP2, respectively, at time zero. Therefore, according to Figure 3a and Table 3, the processing of coalho cheese with 400 MPa/10 min

(CP2) and the combination of AF with 400 MPa/10 min (CAFP2) caused higher reductions for *L. monocytogenes*, in time zero.

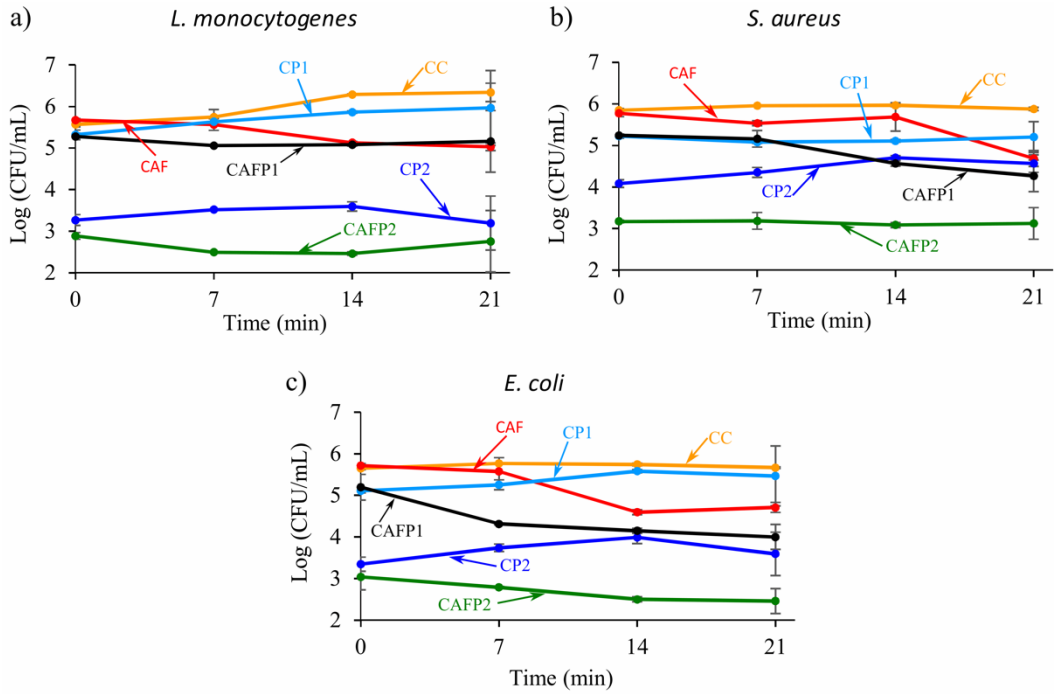

Figure 3. Efficiency of antimicrobial films (AF), HHP or combination of AF and HHP in coalho cheese, for *L. monocytogenes* (3a), *S. aureus* (3b) and *E. coli* (3c). The results are expressed as the mean (n = 6) ± standard deviation. CC = control cheese (without film or HHP); CAF = cheese packed with antimicrobial film; CP1 = cheese treated with 300 MPa/5 min; CP2 = cheese treated with 400 MPa/10 min; CAFP1 = cheese packed with film and treated with 300 MPa/5 min; CAFP2 = cheese packed with film and treated with 400 MPa/10 min.

Table 3. Counts at times 0 and 21 days of storage at 5 °C: CC = control cheese; CAF = cheese packed with antimicrobial film; CP1 = cheese treated with 300 MPa/5 min; CP2 = cheese treated with 400 MPa/10 min; CAFP1 = cheese packed with film and treated with 300 MPa/5 min; CAFP2 = cheese packed with film and treated with 400 MPa/10 min, for *L. monocytogenes*, *S. aureus*, and *E. coli*.

	L. monocytogenes log CFU/mL		***S. aureus*** log CFU/mL		***E. coli*** log CFU/mL	
Treatments	**Time 0**	**Time 21**	**Time 0**	**Time 21**	**Time 0**	**Time 21**
C	5.57 ± 0.04 ^{a}	6.34 ± 0.04 ^{a}	5.85 ± 0.01 ^{a}	5.88 ± 0.12^{a}	5.65 ± 0.01 ^{a}	5.67 ± 0.10^{a}
CF	5.58 ± 0.04 ^{a}	5.03 ± 0.04 ^{c}	5.77 ± 0.03 ^{a}	4.69 ± 0.20 ^{c}	5.66 ± 0.05 ^{a}	4.71 ± 0.00^{b}
CHP1	5.32 ± 0.05 ^{b}	5.96± 0.12 ^{b}	5.23 ± 0.05 ^{b}	5.20 ± 0.04 ^{b}	5.11 ± 0.03 ^{b}	5.47 ± 0.18^{a}
CHP2	3.27 ± 0.09^{c}	3.19 ± 0.00 ^{d}	4.08 ± 0.04 ^{c}	4.56 ± 0.01 ^{c}	3.35± 0.03 ^{c}	3.60 ± 0.03^{d}
CFHP1	5.28 ± 0.04 ^{b}	5.16 ± 0.01^{c}	5.24 ± 0.11 ^{b}	4.27 ± 0.05 ^{d}	5.20 ± 0.06 ^{b}	4.00 ± 0.06^{c}
CFHP2	2.89 ± 0.19 ^{d}	2.46 ± 0.24 ^{e}	3.17 ± 0.08 ^{d}	3.09 ± 0.09 ^{e}	3.08 ± 0.26 ^{d}	2.52 ± 0.21^{e}

*Means followed by the same letters in the same column do not differ from each other ($p > 0.05$) by the Tukey test at the 5% level of significance. The results are expressed as mean (n = 6) ± standard deviation.

At 21 days of storage, the counts showed a reduction of 0.55, 0.08, 0.12 and 0.43 log CFU/mL of *L. monocytogenes*, in the CAF, CP2, CAFP1 and CAFP2 cheeses (Table 3), respectively, when compared to the counts in the zero time. Therefore, all of these treatments were able to control the growth of *L. monocytogenes* during the storage period. However, CP1 cheese showed an increase of 0.64 log CFU/mL in counts on day 21 (Table 3), compared to time zero. Evert-Arriagada et al. (2018) reported that cheese treated with 400 MPa showed an increase of 3.45 logarithmic units of *L. monocytogenes* CECT 4031, during cold storage for 15 days. According to Figure 3a, the highest reduction observed at 21 days, compared to control cheese, was found for the combination CAFP2, followed by CP2, CAFP1, CAF and CP1.

For *S. aureus* (Table 3), the zero time counts for CC and CAF cheeses were 5.85 and 5.77 log CFU/mL, which means the brief contact of the antimicrobial film caused a reduction of 0.08 log CFU/mL of the bacteria in the cheese. Also, reductions of 0.62, 1.77, 0.61 and 2.68 log CFU/mL were observed at time zero for CP1, CP2, CAFP1 and CAFP2, respectively. Note that the highest reductions, at time zero, were caused by the combination CAFP2 and processing with 400 MPa/10 min (CP2). However, as reported for *L. monocytogenes*, the treatment of cheese with 300 MPa/5 min (CP1) was not able to reduce the counts of *S. aureus* during storage, presenting a number of viable cells on day 21 (Table 3) equal to time zero (5.20 log CFU/mL). Also, the treatment with 400 MPa/10 min (CP2) was not able to control the growth of *S. aureus*, presenting an increase of 0.48 log CFU/mL in the counts of day 21. For the other treatments, reductions in 1.08, 0.97 and 0.08 log CFU/mL were verified for CAF, CAFP1 and CAFP2, respectively, at 21 days of storage. Therefore, according to Figure 3b, at 21 days of storage, the highest antimicrobial efficiency for *S. aureus* was found for the combination CAFP2, followed by the treatments CAFP1, CP2, CAF and CP1.

For *E. coli*, at time zero (Table 3), the highest reductions were observed for the treatments CAFP2 (2.57 log CFU/mL) and CP2 (2.30 log CFU/mL), followed by CP1 (0.54 log CFU/mL) and CAFP1 (0.45 log CFU/mL). As reported for *S. aureus*, the treatments CP1 and CP2 were not sufficient to control microbial growth during storage, presenting, at time 21, increases of 0.36 and 0.25 log CFU/mL, respectively. For the other treatments, reductions of 0.95 (CAF), 1.2 (CAFP1) and 0.56 (CAFP2) log CFU/mL were observed at 21 days of storage. According to Figure 3c, at time 21, the highest reduction in log CFU/mL was presented by the cheese submitted to the combination CAFP2, followed by CP2, CAFP1, CAF and CP1.

Therefore, for the three bacteria, the combination of the antimicrobial film with 400 MPa/10 min (CAFP2) was the most efficient over the 21 days of storage. Comparing the action of the technologies, individually, we observed that the antimicrobial film was more efficient than 300 MPa/5 min (CP1), for the three microorganisms. As for the combinations, the CAFP2 treatment was the most efficient at zero time for the three microorganisms, in addition to controlling growth throughout the storage period, as illustrated in Figure 3. However, for cheese packed with films and processed at 300 MPa/5 min (CAFP1), the highest reductions occurred during storage. Arqués et al. (2005) used the combination of HHP and bacteriocins for the inactivation of *L. monocytogenes* in cheese produced with raw milk and suggested that HHP made the target bacteria more sensitive to the action of bacteriocins, thus being a promising combination to guarantee the quality of fresh cheeses. Therefore, according to the data obtained in this study, HHP caused considerable reductions in time zero, while OEO, present in the antimicrobial film, kept the counts reduced during storage.

The intrinsic factors of the food or of the growth medium (Pola et al., 2016), besides the microbial behavior itself, result in a different action of the essential oils. Some of the factors that influence the effectiveness of the essential oil are: type of culture medium (solid or liquid), food matrix and atmosphere involved, particularly micro-atmosphere (Ghabraie et al., 2016). Dannemberg et al. (2017) reported that *L. monocytogenes* and *S. aureus* were sensitivity to CA film incorporated with pink pepper essential oil, in all media tested (agar, BHI broth, micro-atmosphere, and mozzarella cheese), while *E. coli* was resistant in agar and *S. Typhimurium* in agar and micro-atmosphere. In addition, the authors observed a greater reduction in gram-positive bacteria during the storage of the mozzarella cheese packed with the antimicrobial film. In the present study, all bacteria were sensitive to all methods evaluated (agar, BHI broth, micro-atmosphere, and coalho cheese), except for samples treated only with HPP and analyzed during the storage period. Therefore, the studies reported showed a vast potential application of the active antimicrobial packaging combined with HHP, as an additional barrier. Thus, under the conditions tested, it was necessary to combine the two technologies to obtain greater antimicrobial efficiency in situ, ensuring a safer product along with the extended shelf life.

4. Conclusion

All films (processed or not with HHP) conferred antimicrobial activity *in vitro* against the three bacteria tested, demonstrating the antimicrobial capacity of OEO, by contact on agar or by diffusion in broth. However, the films submitted to 300 MPa/5 min showed a delayed total bacterial elimination, for the three bacteria, in agar or BHI broth. In a micro-atmosphere system, all films were efficient for the three bacteria, with a 3.40 log CFU/mL reduction after 24 h of incubation.

In experimentally contaminated coalho cheese, the combination of the 400 MPa/10 min antimicrobial film was the most efficient against the growth of the three pathogenic bacteria. The individual use of HHP was the only condition that was unable to prevent microbial growth in the coalho cheese during storage, despite having caused notable reductions in zero time. Therefore, the presence of the antimicrobial film was essential to reduce bacteria during storage.

The results indicate, therefore, that the CA films incorporated with OEO can be used to pack food to be pressurized, without damage to its antimicrobial efficiency. Besides, according to the data of this research, the combination of antimicrobial packaging and HHP has proved promising to ensure the safety of coalho cheese.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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CAPÍTULO V

EFFECTS OF THE COMBINATION OF HIGH HYDROSTATIC PRESSURE AND ACTIVE FILM ON MAINTAINING THE QUALITY OF COALHO CHEESE

Artigo a ser submetido

Sheyla Moreira Gonçalves[1]*, Nathália Ramos de Melo[1,3], Davy William Hidalgo Chávez[1], Carlos Wanderlei Piler de Carvalho[1,2], Amauri Rosenthal[1,2]

[1]Department of Food Science and Technology, Rodovia 465 - Km 7, UFRRJ, Seropédica, RJ 23891-360, Brazil.
[2]Embrapa Food Technology, Av. das Américas, 29501, Guaratiba, Rio de Janeiro, RJ 23020-470, Brazil
[3]Department of Agribusiness Engineering, Av. dos Trabalhadores 420 - Vila Sta. Cecília, UFF, Volta Redonda, RJ 27255-125, Brazil.

*Corresponding author: sheylapa1@hotmail.com

ABSTRACT

Coalho cheese (CC) (matured for 10 days at 10 ºC) was investigated for the effect of individual or combined use of high hydrostatic pressure (HHP) (300 MPa/5 min or 400 MPa/10 min) and active film with oregano essential oil (50 %) on the parameters color (L*, a* and b*) and texture (hardness, cohesiveness, gumminess, chewiness and springiness). CC samples were stored for 21 days at 5 ºC and analyzed at zero, 7, 14 and 21 days. The treatment with HHP and/or the presence of the active film significantly influenced the color of the CC samples, for most treatments, presenting a redder and more yellow color, at 21 days, when compared with the control sample (samples without film and without HHP). The comparison over storage showed that the CC samples treated with HHP and/or packed with active film showed a reduction in L*, an increase in a* and b*, in 21 days, when compared to time zero. Reduction of hardness and cohesiveness, gumminess and chewiness were more evident for samples treated with 400 MPa/10 min (with or without the active film), while springiness did not show significant difference for all treatments. In addition, the results showed that the presence of the active film did not significantly affect the texture parameters of the CC samples. Therefore, the data suggest that the samples maintained acceptable color and texture parameters throughout storage, so the combination of HHP levels explored in this study, associated with the active film, can be considered to ensure a safer coalho cheese, as shown in a previous chapter, without any damage to its appearance and texture.

Key words: Non-thermal technology; cellulose acetate; oregano essential oil; traditional cheese; texture analysis.

INTRODUCTION

Coalho cheese (CC) is defined according to the Brazilian legislation (Brazil, 2001) as semi-hard and springy cheese, with compact and soft texture, yellowish-white color, slightly acid flavor, with thin crust and the presence of a few glazes. It is a traditional product from the northeast of Brazil and its local production is usually handcrafted with raw milk, which can be consumed fresh or cured (FAO, 1990). However, CC production has expanded to other regions, such as the southeast of Brazil, but by using pasteurized milk as raw material (Souza et al., 2014), with semi-cooked or cooked curds (Soares et al., 2017), being consumed normally roasted in the form of barbecue skewers. CC can be produced with the coagulation of cow's milk (Lima et al., 2018) or goat (Bezerra et al., 2016), with or without the addition of lactic acid bacteria, following by aging of about 10 days at 10-12 ºC after the production (Costa et al., 2020). Artisanal products such as CC usually have problems with post-production surface contamination. In addition, the health deficiencies of dairy herds

associated with poor processing conditions of CC, contributes to the risks of contamination by deteriorating and pathogenic microorganisms. In the literature, some studies report the potential of CC to carry microorganisms such as *Mycobacterium avium* subspecies paratuberculosis (Faria et al., 2014), Staphylococcus aureus (Aragão et al., 2019), *Escherichia coli, Listeria monocytogenes* and *Salmonella* spp (Azevêdo et al., 2014).

Active biodegradable packaging containing essential oils has been used as an important strategy to contain and preserve food, with a competitive advantage in reducing environmental impacts and replacing synthetic preservatives in food products (Costa et al., 2018). Cellulose acetate (CA) is a biodegradable, non-edible polymer derived from native cellulose through acetylation reaction with acetic acid and acetic anhydride (Wan Daud and Djuned, 2015). CA packaging incorporated with essential oil has been successfully suggested for cheese application (Oliveira et al., 2011; Dannenberg et al., 2017). The use of oregano essential oil (OEO) in cheese has evidenced its effects on quality (Asensio et al., 2015) and antimicrobial efficiency against several microorganisms of importance in dairy products (Govaris et al., 2011; Bedoya-Serna et al., 2018; Diniz-Silva et al., 2020). However, changes in the appearance and/or texture of cheese can be caused by the presence of the packaging or the essential oil. In this sense, Oliveira et al. (2011) reported that coalho cheese packed with cellulosic film incorporated with *Cymbopogon citratus* exhibited changes in color parameters and increased hardness, after 25 days stored under refrigeration.

HHP treatment has been adopted by the food industry as an alternative to thermal methods for microbial inactivation, without impairing the nutritional quality of products (Tokusoglu, 2015), in addition to increasing commercial validity. The pioneering application of HHP in dairy products occurred more than a century ago in milk (Okpala et al., 2010) for increasing microbial stability. In cheeses, HHP processing has been used to accelerate proteolysis during ripening (Martínez-Rodríguez et al., 2012; Costabel et al., 2016), to slow down lipolysis (Saldo et al., 2003), to improve sensory properties with reduced calcium, moisture and fat (Ozturk et al., 2018), as well as for assessing physical-chemical changes (Okpala et al., 2010; Avila et al., 2017) and microbial inactivation (Komora et al., 2020).

Research has shown that, depending on the type of cheese or raw material used for its production, treatment with HHP can cause different changes in the color (Queiroga et al., 2013) and texture (Salazar et al., 2020) of the product. In addition, cheeses treated with higher pressures (600 MPa) can undergo degrading changes in their structure (Hnosko et al., 2012), while lighter pressures (200-400 MPa) have shown a satisfactory effect on the activation of primary and secondary proteolysis (Nunez et al., 2020). Studies have shown that the treatment of different types of cheese with pressures of 300 and 400 MPa (Rynne et al., 2008; Koca et al., 2011; Avila et al., 2017) did not cause undesirable changes in their physical-chemical and/or texture parameters. In addition to the pressure level and processing time, it is of fundamental importance to know the ideal cheese ripening time for HHP application. Batty et al. (2019) applied 550 MPa/10 min to camembert cheese and found that processing with HHP at 3 and 11 days of production impaired the formation of the rind and characteristic texture after the final ripening, in addition to showing degradation of its appearance.

HHP and active antimicrobial packaging are technologies potentially used to control microbial growth in cheeses. However, the difficulty in inactivating bacterial spores with

HHP alone has motivated the association with natural antimicrobials, as demonstrated by López-Pedemonte et al. (2006) for the control of *Bacillus* spp in CC. In addition to microbiological control, the combination of processing technologies is increasingly used in order to preserve or improve the quality of food (Augusto et al., 2018). According to the literature, higher pressures are necessary to eliminate certain microorganisms, however, fresh or semi-hard cheeses are reported to be negatively affected by high pressures, whether in texture, appearance or sensory parameters (Hnosko et al., 2012; Batty et al., 2019; Salazar et al., 2020). Therefore, through the combination of technologies, it is intended to obtain the beneficial effects of HHP by using lower pressures instead.

Both the packaging and the treatment with HHP can cause changes in the optical properties and texture of cheeses (Oliveira et al., 2011; Costabel et al., 2016), directly affecting their characteristics and product acceptance. For CC, there are no records in the literature on the ideal pressure levels or use of the combination of HHP and active packaging for its conservation. Therefore, the objective of this study was to evaluate changes in the color and texture of CC stored at 5 ºC for 21 days, packed with CA film incorporated with OEO and/or treated with HHP (300 MPa/5 min or 400 MPa/10 min).

MATERIALS AND METHODS

Materials

-Elaboration of the film: cellulose acetate (AC) (Sigma-Aldrich, Brazil), acetone PA (Cap-Lab, São Paulo, Brazil) and oregano essential oil (*Origanum vulgare*) (Ferquima, São Paulo, Brazil).
-Elaboration of the cheese: Calcium chloride and lactic acid (Rica Nata, Minas Gerais, Brazil), microbial chymosin coagulant (Chr. Hansen, São Paulo, Brazil), standardized pasteurized milk 3.3% fat (Cooperatva Mista de Valença, Rio de Janeiro, Brazil).

Development of films

The film was produced by the casting method, according to Gonçalves et al. (2020), by dissolving the CA in acetone (1:10 w/v) for 12 h, followed by the addition of OEO (50% w/v). The filmogenic solution was spread on a glass plate, followed by drying (evaporation of acetone) under controlled conditions (temperature of 25 ± 2 °C and 75% humidity) for 10 min. The dry film was detached, vacuum packed, pre-conditioned (temperature of 25 ± 2 °C and 75% humidity) for up to 3 days and used to package the CC samples.

Cheese manufacture

According to procedure based on Costa et al. (2020) with certain adjustments, standardized pasteurized bovine milk with 3.3% fat was heated to 35 ºC, added with calcium chloride (500 µL/L), lactic acid (100 µL/L) and chymosin coagulant (1mL/L), followed by coagulation at 35 ºC/40 min. After cutting the curd, the stirring was carried out at 45 ºC/30 min, alternating 3 stirring steps and 3 intervals of 5 min. In the initial syneresis, 95% of the

whey was removed, followed by the addition of salt to the mass (1.2% w/v), curd draining by bag hanging, pressing and maturation in a cold chamber (10 °C/10 days).

Cheese treatments with HHP

After CC ripening (Figure 1A), 12 x 3 cm samples were taken for control and treatments were as follows: coalho cheese control (CCC); coalho cheese packed with the active film (CF); cheese treated with 300 MPa/5 min (CHP1); cheese treated with 400 MPa/10 min (CHP2); cheese packed with the film and treated with 300 MPa/5 min (CFHP1); cheese packed with the film and treated with 400 MPa/10 min (CFHP2). The sample sizes were defined according to the cylinder capacity of the HHP equipment. All CC samples were packed in sterile bags (Nasco Whirl-Pak (118 mL) (Figure 1B) and sealed aseptically in laminar flow.

HHP processing of the samples were carried out in a lab unit (Stansted Fluid Power, model S-FL-850-9-W). All treatments were stored at 5 ºC for 21 days and analyzed at 0, 7, 14 and 21 days.

Figure 1. Ripened coalho cheese (A); Sampling 12 x 3 cm stored at 5 ºC / 21 days (B).

Color analysis

The color change in the stored CC samples was determined in Hunter Lab Colorimeter (Kônica Minolta, Japan). 2 x 2 cm samples were subjected to 6 readings in reflectance mode, on days 0, 7, 14 and 21 of storage. Through the standard illuminant D65 (Commission International d 'Eclair age) (CIE, 1996), the CIE coordinates L* a* b* were obtained, where L* corresponds to the brightness/luminosity, a* to the red (+a*) or green (-a*) component, b* to the yellow (+b*) or blue (-b*) component. The measurements were performed in triplicate, for each sampling time, using the external part of the cheese sample, immediately after removal from the packaging. For analysis at zero time of the cheese sample packed with the

film (CF), the readings were taken after 2 hours of contact between the sample and the surface of the film.

Texture analysis

Cylindrical samples of 2 cm in diameter and 2 cm in height (Figure 2A) were submitted to texture profile analysis (TPA) in a TA.XT plus texturometer (Stable Micro Systems, Surrey, England) (de Moraes et al., 2018, with modifications) (Figure 2B), operating with a 30 kg cell, 5 g contact force, P35 cylindrical probe, speed of 1.0 mm/s and compression distance of 40 mm (equivalent to 50% compression). The parameters hardness, gumminess, chewiness and springiness were obtained with the aid of Texture Expert 1.20 for Windows (Stable Micro Systems, Surrey, England). The experiment was conducted in triplicate for each sampling time.

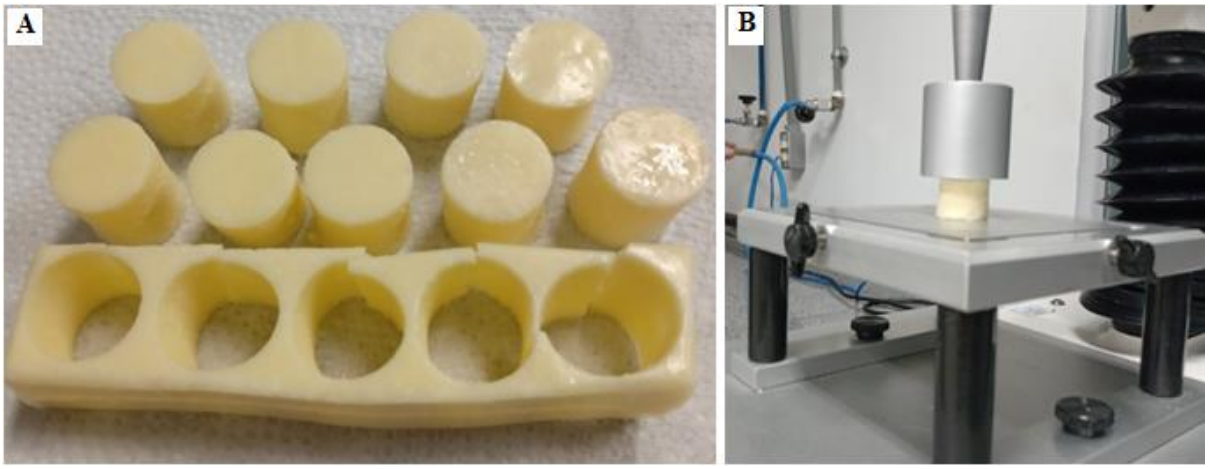

Figure 2. Sampling of coalho cheese for texture analysis (A); Texture profile analysis (TPA) (B).

Statistical analyses

Statistical analyzes were performed using software R, version 3.2.4 (R Foundation for Computational Statistics, Vienna, Austria) and FactoMineR version 1.32. The Tukey multi comparative test was used to obtain differences between samples after ANOVA test, both (Tukey and ANOVA tests) with a significance level of 5%.

RESULTS AND DISCUSSION

Color analysis

The visual aspect of the coalho cheese samples stored at 5 ºC for 21 days is shown in Figure 3. For the color coordinate a*, Figure 3a shows that all treatments differed statistically ($P < 0.05$) from the control sample, showing greater red color along the storage at 5 °C. At time zero, more reddish color was exhibited by the cheese samples treated only with HHP (CHP1 and CHP2) or from combination of F and HHP (CFHP1 and CFHP2), when compared to cheese sample packed with film (CF). Okpala et al. (2010) also observed that treatment with HHP caused an increase in the redness of fresh cheese. The application of HHP in

cheeses made with milk from different animal species has shown that color is significantly affected, both by the pressure level and by the processing time (Martinez-Rodriguez et al. 2012). Furthermore, the origin of the milk directly influences the a* coordinate, since cheeses made with goat's milk tend to have higher values for the green component (-a*), when compared to cheeses made with cow's milk (Queiroga et al., 2013), mostly reddish (Okpala et al., 2010).

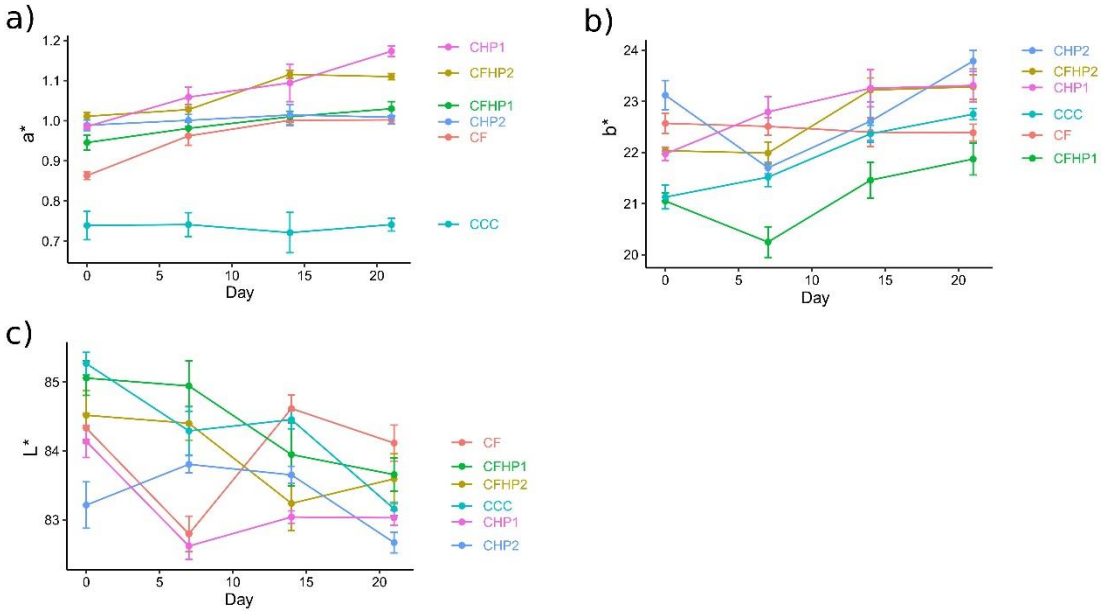

Figure 3. Color parameters of CC control (CCC) samples, CC packed with film (CF), CC treated with 300 MPa/5 min (CHP1), CC treated with 400 MPa/10 min (CHP2), CC packed with film and treated with 300 MPa/5 min (CFHP1) and CC packed with film and treated with 400 MPa/10 min (CFHP2), stored at 5 ºC for 21 days.

The CF sample (Figure 3a) also showed a difference of a* for the control sample, at time zero, which may be due to the essential oil present in the packaging (Ksouda et al., 2019). At 21 days, the CHP1 sample exhibited the greatest red color, followed by the CFHP2 sample. Comparing each treatment during the storage, it is observed that all samples, except CCC and CHP2 (Table 1), showed an increase in red color as storage time increased. According to the literature, the larger diameter of the fat globules in cow's milk contributes to a more reddish appearance of the cheese (Queiroga et al., 2013). However, treatment with HHP can cause changes in the diameter of fat globules (Kanno et al., 1998) and the size of the casein micelles (Tokusoglu, 2015), which have a direct effect on the color of dairy products. Kanno et al. (1998) observed that only treatments above 400 MPa/10 min caused an increase in the size of fat globules in cow's milk. Kiełczewska et al. (2020) reported that goat milk treated with 200-400 MPa exhibited an increase in the size of fat globules and that, during storage, milk treated with 300 MPa exhibited an increase in globules when compared to milk treated with 400 MPa. Therefore, this information corroborates the results in the present study, in which the cheese sample treated with 300 MPa/5 min (CHP1) or 400 MPa/10 min (CHP2) showed the same color, at time zero (Table 1). However, at 21 days, the CHP1 sample exhibited a more reddish color when compared to the CHP2 sample. For samples packed with film (CF) or submitted to a combination of F and HHP (CFHP1 and CFHP2), the

presence of OEO may also have contributed to the increase in reddish color during storage. Oliveira et al. (2017) reported variation in a* (green color) during storage of coalho cheese packed with CA film incorporated with *Cymbopogon citratus* essential oil, for 25 days, with red coloration being observed only in samples with 15 or 20 days of storage. In addition to Oliveira et al. (2017), Queiroga et al. (2013) also reported a predominance of green color for coalho cheese made with cow's milk, stored for 28 days, although the authors state that the green color is more attributed to goat's milk.

Table 1. Color parameters at times 0 and 21 days of storage at 5 °C: CC control (CCC) samples, CC packed with film (CF), CC treated with 300 MPa/5 min (CHP1), CC treated with 400 MPa/10 min (CHP2), CC packed with film and treated with 300 MPa/5 min (CFHP1) and CC packed with film and treated with 400 MPa/10 min (CFHP2).

Treatments	Days	*a**	*b**	*L**
CCC	0	0.71 ± 0.1 ^{a}	21.13 ± 0.7 ^{b}	85.27 ± 0.5 ^{a}
	21	0.74 ± 0.1 ^{a}	22.75 ± 0.3 ^{a}	83.16 ± 0.3 ^{c}
CF	0	0.86 ± 0.0 ^{b}	22.57 ± 0.6 ^{a}	84.33 ± 0.6 ^{a}
	21	1.00 ± 0.0 ^{a}	22.39 ± 0.5 ^{a}	84.11 ± 0.8 ^{a}
CHP1	0	0.99 ± 0.0 ^{c}	21.97 ± 0.4 ^{b}	84.14 ± 0.7 ^{a}
	21	1.17 ± 0.0 ^{a}	23.31 ± 0.9 ^{a}	83.03 ± 0.3 ^{b}
CHP2	0	0.99 ± 0.0 ^{a}	23.12 ± 0.9 ^{ab}	83.21 ± 1.1 ^{ab}
	21	1.01 ± 0.1 ^{a}	23.79 ± 0.6 ^{a}	82.67 ± 0.5 ^{b}
CFHP1	0	0.95 ± 0.1 ^{b}	21.05 ± 0.5 ^{ab}	85.06 ± 0.7 ^{a}
	21	1.03 ± 0.1 ^{a}	21.87 ± 0.9 ^{a}	83.66 ± 0.7 ^{b}
CFHP2	0	1.01 ± 0.0 ^{b}	22.03 ± 0.2 ^{b}	84.52 ± 1.1 ^{a}
	21	1.11 ± 0.0 ^{a}	23.28 ± 0.7 ^{a}	83.59 ± 1.1 ^{a}

*Means followed by the same letters in the same column do not differ from each other ($p > 0.05$) by the Tukey test at the 5% level of significance. The results are expressed as mean ($n = 6$) ± standard deviation.

For the b* coordinate, Figure 3b shows that at time zero all samples, with the exception of CFHP1, differed statistically ($P < 0.05$) from the control, with the highest values for yellow coloring (+b*) being displayed by the CHP2 and CF samples. At time 21, the CHP2 sample exhibited the greatest yellow color, while the lowest value was presented by the CFHP1 sample. Comparing each treatment during storage (Table 1), only the CF sample did not show an increase in yellow color. According to visual observation during the study, the sample pressurized with 400 MPa/10 min (CHP2) was more yellow when compared to the other treatments. Higher values for b* in cheese were attributed to the higher degree of proteolysis (Queiroga et al., 2013) or composition of fat globules (Michalski et al., 2003) altered during storage or through treatment with HHP. Koca et al. (2011) reported an increase in yellow color in cheeses treated with 400MPa/5 or 15 min and also visually observed the most pronounced yellowing. In addition, the authors highlighted that the different results found in the literature are due to the type of cheese and the different structural changes caused by the same treatment with HHP. It is also worth mentioning that, for samples packed with the film, the presence of OEO may have contributed to the different degrees of yellowing (Ksouda et al., 2019).

Studies report that the a* and b* values of milk and dairy products may be directly related to β-carotene present and fat content in milk (Iturmendi et al., 2020), and that the treatment of milk with HHP produced a turn to yellow-green color (Needs et al., 2000). However, in this study, the presence of the packaging or treatment with HHP caused an increase in red color in all samples of coalho cheese stored under refrigeration. In addition, the increase in the yellow color of the cheese samples was more evident at time zero, except for the sample treated with 400 MPa / 10 min (CHP2).

Figure 3c shows that all treatments showed an increase in luminosity (L*) at time zero, when compared to the cheese sample control (CCC). The lowest luminosities were exhibited by samples treated only with HHP (CHP2 and CHP1), at time zero and at 21 days of storage. These results are in line with those reported by Evert-Arriagada et al. (2014) and Juan et al. (2008), that detected lower luminosity in fresh cow's milk cheese treated with 500 MPa/5 min and in sheep's milk cheese treated with 300 MPa/10 min, respectively. As mentioned earlier, the structural changes caused by treatment with HHP depend mainly on the type of cheese (Koca et al., 2011) and on the milk composition (Queiroga et al., 2013) used for its production. Therefore, changes in the protein network or in the fat globules can change the amount of light scattered or absorbed by the sample, positively or negatively affecting the luminosity (Michalski et al., 2003). The greatest luminosity at 21 days of storage was shown by the sample stored only with the film (CF), which may be associated with the presence of OEO which can cause changes in the perception of brightness. In addition, treatment with HHP can also cause changes in the dispersion of OEO on the surface of the samples, reducing the diffraction of light (Kiełczewska et al., 2020) and consequently increasing the intensity of perception of luminosity. This information can justify the higher luminosity values at 21 days (Table 1) for samples treated with HHP and packed with films (CFHP1 and CFHP2), when compared to stored samples only treated with HHP (CHP1 and CHP2) and with control sample (CCC). By evaluating individual treatments during the storage (Table 1), only CF and CFHP2 samples did not show a reduction in luminosity at 21 days.

Texture analysis

The primary texture (hardness, cohesiveness and springiness) and secondary (gumminess and chewiness) parameters, for coalho cheeses stored at 5 ºC for 21 days, are shown in Figure 4. By definition, hardness is the force capable of causing deformation in the food when applied at a given distance. Cohesiveness is the degree of deformation of the sample before breaking, during the application of mechanical force. Springiness is the necessary resistance for the food to return to its original shape, after removing the partial compression. Chewiness is the effort required to give the food adequate consistency for later swallowing. Gumminess is the energy required to disintegrate semi-solid food into an ideal consistency for swallowing (Szczesniak, 1963; Bourne, 2002).

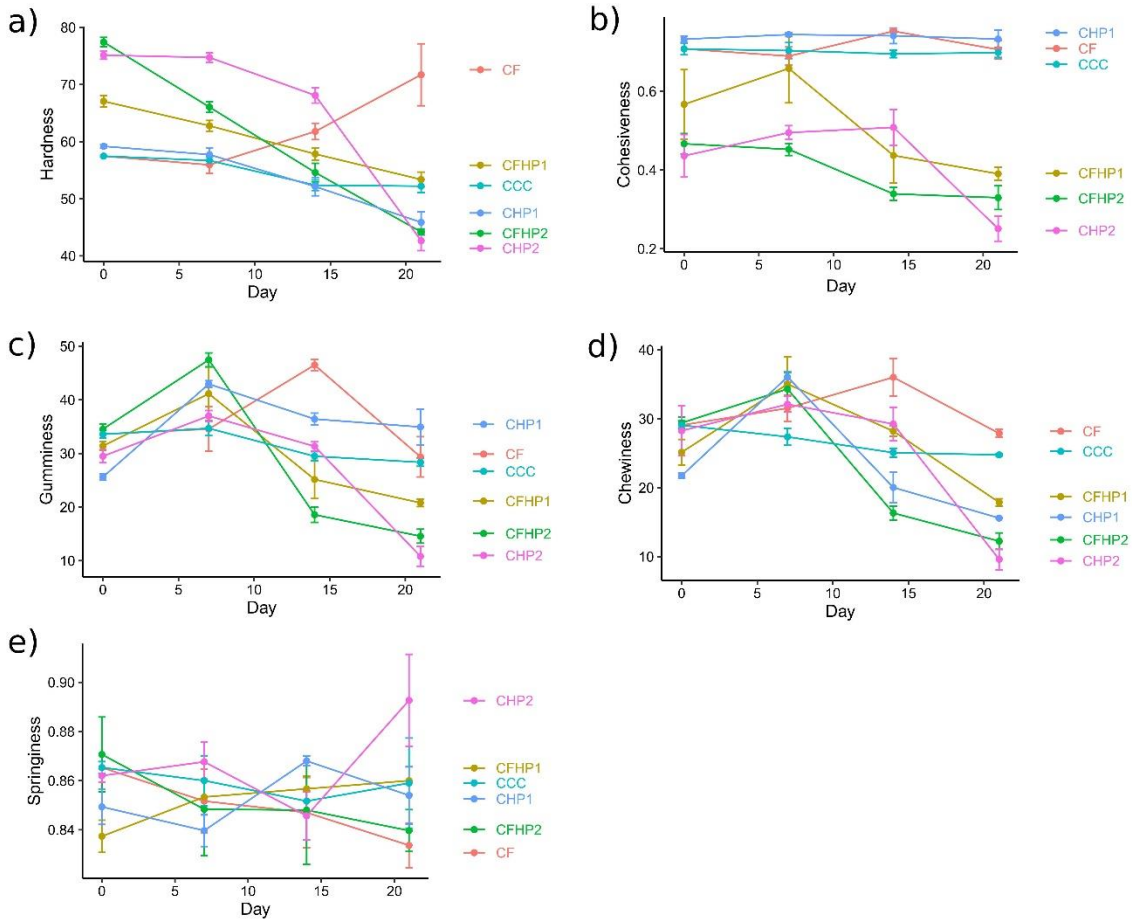

Figure 4. Texture parameters of CC control (CCC), CC packed with film (CF), CC treated with 300 MPa/5 min (CHP1), CC treated with 400 MPa/10 min (CHP2), CC packed with film and treated with 300 MPa/5 min (CFHP1) and CC packed with film and treated with 400 MPa/10 min (CFHP2), stored at 5 ºC for 21 days.

For hardness, Figure 4a shows that at time zero, CCC, CHP1 and CF showed the lowest hardness, while the highest values were exhibited by the CHP2 and CFHP2 samples. At 21 days, only the CF sample showed a statistical difference ($P < 0.05$) from the other treatments, showing greater hardness. Oliveira et al. (2017) reported that coalho cheese packed with CA film exhibited increased hardness at 20 and 25 days of storage. The authors associated this finding with the higher content of insoluble calcium retained during coagulation. Ozturk et al. (2018) also mentioned the importance of calcium solubilization as a determinant of cheese softening. In addition, the lack of uniformity inherent in coalho cheese, as reported by Oliveira et al. (2017), could also explain different texture results. According to Fox et al. (2017), the texture changes caused by HHP in cheese are usually due to intensification of proteolysis and not by microstructural changes. However, Koca et al. (2011) found that structural changes in Turkish cheese casein, induced by treatment with HHP, led to a reduction in the properties of hardness and cohesiveness, thus showing a certain correlation between structural changes and texture of the cheese. The increase in cheese softness was also associated with greater solubilization of insoluble calcium (Ozturk et al., 2018) or weakening of the hydrophobic interactions of the matrix protein and internal redistribution of moisture, caused by treatment with HHP (Avila et al., 2006). However, depending on the type of cheese

and its inherent intrinsic characteristics, the same HHP condition can cause both positive or negative changes in textural properties (Koca et al., 2011). Therefore, it is likely that the treatment with 400 MPa/10 min caused structural changes in the CHP2 and CFHP2 samples, favoring the increase of hardness, at zero time. However, at 21 days, these samples showed hardness values similar to other samples (except for CF), with continuous reduction for CFHP2 along the storage and reduction from day 14 ownwards for CHP2, proving that the expected reduction in hardness for treatment with 400 MPa/10 min was achieved only after certain storage period. On the other hand, the presence of the film was not decisive to cause a difference in the hardness of the pressurized samples (CFHP1 and CFHP2), which favors the use of the combination of technologies (F and HHP), without prejudice to the cheese texture. Comparing the hardness of the samples on days 0 and 21, Table 2 shows that, with the exception of CF, all samples showed a reduction in hardness at the end of the storage.

Table 2. Texture parameters at times 0 and 21 days of storage at 5 °C: CC control (CCC), CC packed with film (CF), CC treated with 300 MPa/5 min (CHP1), CC treated with 400 MPa/10 min (CHP2), CC packed with film and treated with 300 MPa/5 min (CFHP1) and CC packed with film and treated with 400 MPa/10 min (CFHP2).

Treatments	Days	Hardness	Cohesiveness	Gumminess	Chewiness	Springiness
CCC	0	57.46 ± 0.2 ^{a}	0.71 ± 0.0 ^{a}	33.59 ± 1.3 ^{ab}	29.08 ± 1.1 ^{a}	0.86 ± 0.0 ^{a}
	21	52.21 ± 1.9 ^{b}	0.70 ± 0.0 ^{a}	28.36 ± 1.3 ^{c}	24.77 ± 0.3 ^{b}	0.81 ± 0.0 ^{a}
CF	0	57.46 ± 0.2 ^{b}	0.71 ± 0.0 ^{a}	33.59 ± 1.3 ^{ab}	29.08 ± 1.1 ^{ab}	0.86 ± 0.0 ^{a}
	21	71.71 ± 9.4 ^{a}	0.71 ± 0.0 ^{a}	29.37 ± 6.5 ^{b}	27.89 ± 1.0 ^{b}	0.83 ± 0.0 ^{a}
CHP1	0	59.18 ± 0.6 ^{a}	0.73 ± 0.0 ^{a}	25.61 ± 1.0 ^{b}	21.73 ± 0.6 ^{b}	0.85 ± 0.0 ^{a}
	21	45.87 ± 3.2 ^{c}	0.73 ± 0.0 ^{a}	34.93 ± 5.8 ^{a}	15.59 ± 0.1 ^{c}	0.85 ± 0.0 ^{a}
CHP2	0	75.14 ± 1.2 ^{a}	0.44 ± 0.1 ^{a}	29.49 ± 2.1 ^{b}	28.27 ± 6.3 ^{a}	0.86 ± 0.0 ^{a}
	21	42.66 ± 3.0 ^{c}	0.25 ± 0.0 ^{b}	10.81 ± 3.2 ^{c}	9.61 ± 2.6 ^{b}	0.89 ± 0.0 ^{a}
CFHP1	0	67.06 ± 1.7 ^{a}	0.57 ± 0.1 ^{a}	31.39 ± 1.4 ^{ab}	25.14 ± 3.2 ^{ab}	0.83 ± 0.0 ^{a}
	21	53.37 ± 2.2 ^{b}	0.39 ± 0.0 ^{a}	20.79 ± 1.2 ^{b}	17.87 ± 0.9 ^{b}	0.86 ± 0.0 ^{a}
CFHP2	0	77.43 ± 1.4 ^{a}	0.47 ± 0.0 ^{a}	34.54 ± 1.7 ^{b}	29.41 ± 1.4 ^{a}	0.87 ± 0.0 ^{a}
	21	44.19 ± 0.9 ^{d}	0.33 ± 0.1 ^{b}	14.56 ± 2.3 ^{c}	12.25 ± 2.1 ^{b}	0.84 ± 0.0 ^{a}

*Means followed by the same letters in the same column do not differ from each other ($p > 0.05$) by the Tukey test at the 5% level of significance. The results are expressed as mean ($n = 6$) ± standard deviation.

In Figure 4b, the CCC, CF and CHP1 samples showed the highest cohesiveness, while CHP2 and CFHP2 showed the lowest values, at time zero. In other words, the greatest deformation before breaking was demonstrated by the samples with the greatest cohesiveness. At 21 days, CHP2 and CFHP2 exhibited the least deformations, which corroborates the results for hardness, in which less hard cheeses tend to be less cohesive. On the other hand, the CCC, CF and CHP1 samples did not show statistical difference ($P> 0.05$) during the entire storage, reaching 21 days with the same deformation behavior (Table 2). According to Koca et al. (2011), contradictory results found in the literature, on the effect of HHP on the texture of different cheeses, prove that less hard cheeses can be more cohesive, which reinforces the fact that each treatment with HHP can induce different textures. In addition, during cheese storage, mechanisms of proteolysis, lipolysis, glycolysis, pH changes and the degree of

calcium solubilization are specific to each type of cheese and each HHP treatment applied (Salazar et al., 2020). As for samples packed with film, in agreement with Oliveira et al. (2017), it is noted the cohesiveness did not show significant statistical changes due to the presence of the packaging.

For gumminess (Figure 4c), at time zero, the CHP1 sample required less energy to disintegrate, while CCC and CFHP2 presented the highest requirements to achieve the optimal state for swallowing. At 21 days, the CHP2 and CFHP2 samples became less gummy, while CHP1 exhibited greater gumminess, and this treatment, in turn, was the only one that showed an increase in the parameter compared to day zero (Table 2). In addition, it is noted that with the exception of the CCC and CF samples, all treatments showed an increase in gumminess on day 7 (Figure 4c), while CF showed an increase on day 14. Chewiness (Figure 4d) was the same for all samples over time zero, that is, according to the sensory definition, the effort required to provide ideal consistency to swallow the cheese samples was similar. At 21 days, CHP2 and CFHP2 samples required less effort for chewing, while the highest values were displayed by CF and CCC. Queiroba et al. (2013) observed that, although the storage time influenced the hardness of three types of cheese, chewiness and cohesion did not show any significant difference. Comparing the chewiness of the samples on days 0 and 21, Table 2 shows a reduction in the values for all treatments. As for springiness, Figure 4e and Table 2 shows that all treatments showed no statistical difference ($P > 0.05$) at time zero and throughout storage.

CONCLUSIONS

The present study demonstrated that samples of coalho cheese packed with active cellulose acetate film incorporated with oregano essential oil and/or treated with HHP (300 MPa/5 min or 400 MPa/10 min), stored under refrigeration for 21 days, exhibited increased red and yellow coloration and reduced luminosity for most treatments. Changes in texture parameters were more evident for samples treated with 400 MPa/10 min (CHP2 and CFHP2), which showed a reduction in hardness, cohesion, gumminess and chewiness. The springiness showed no difference between the treatments during the product storage period. Regarding the use of the packaging, only the color parameters were influenced by the presence of the essential oil. Therefore, the results of this study indicated that treatment with HHP did not cause degrading effects on the texture of coalho cheese, nor did it significantly affect its appearance. Therefore, it is concluded that the combination of active film and HHP can be considered for preserving coalho cheese, since it caused minimal changes in color and texture, without compromising its acceptance by consumers who appreciate the characteristics that are intrinsic to the product.

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CONFLICTS OF INTEREST

The authors declare no competing financial interest.

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2 CONCLUSÕES GERAIS

Esta pesquisa permitiu as seguintes conclusões quanto:

1-Avaliação dos efeitos da alta pressão hidrostática (APH) sobre as propriedades funcionais do filme de acetato de celulose, com ou sem incorporação de óleo essencial de orégano.

*O processamento com APH causou alterações das propriedades funcionais de todos os filmes, no entanto, tais mudanças não inviabilizam sua aplicação para embalar produtos alimentícios. Dentre as alterações, destaca-se a redução da resistência à tração, módulo de Young's, aumento da coloração amarela/vermelha, redução da afinidade pela água e presença de delaminações na estrutura dos filmes.

*A eficiência antimicrobiana do óleo essencial incorporado ao filme não foi afetada pelo processamento com APH, sendo avaliada sobre os micro-organismos *Staphylococcus aureus*, *Escherichia coli* e *Listeria monocytogenes*.

2-Avaliação da eficiência antimicrobiana do filme ativo (FA), da APH e da combinação destes tratamentos (FA e APH) contra *Staphylococcus aureus*, *Escherichia coli* e *Listeria monocytogenes* em queijo coalho.

*Somente a aplicação de APH não foi capaz de controlar o crescimento das três bactérias em amostras de QC armazenadas durante os 21 dias a 5 ^{o}C.

*A combinação de FA e 400 MPa/10 min foi o método mais eficiente para o controle do crescimento bacteriano, no tempo zero e ao longo do armazenamento.

3-Avaliação do efeito da APH, combinada ou não com FA, sobre a textura e cor do QC armazenado durante 21 dias a 5 ^{o}C.

Os parâmetros de cor (a e b*) e luminosidade foram afetados pelo processamento com APH e/ou presença do FA, tendo as amostras de QC apresentado coloração mais avermelhada/amarelada e maior luminosidade aos 21 dias de armazenamento.

*O FA não causou mudanças na textura do QC, enquanto o processamento a 400 MPa/10 min ou combinação de FA e 400 MPa/10 min causaram as maiores reduções da dureza e coesividade e aumento de gomosidade e mastigabilidade.

Diante dos resultados gerados e da avaliação visual do filme com óleo essencial de orégano, concluímos que sua aplicação no alimento deverá ser sob a forma de embalagem primária, sob possível proteção de uma embalagem secundária. Isso se faz necessário para garantir a permanência do óleo essencial na embalagem, já que este possui natureza volátil e sua exposição direta às condições do ambiente de armazenamento poderia prejudicar a eficiência antimicrobiana. Além disso, para conter alimentos a serem pressurizados, também há necessidade de uma embalagem secundária, já que o contato direto do filme antimicrobiano com o meio de transmissão da pressão (água ou álcool) pode induzir à saída do óleo essencial para o meio.

3 CONSIDERAÇÕES FINAIS

O interesse científico pela utilização de tecnologias não-térmicas, como embalagem antimicrobiana e APH para o controle do crescimento de micro-organismos deteriorantes e de importância em saúde pública em alimentos, tem atraído atenção especial de pesquisadores e indústria. No entanto, até o presente momento, não há registro de estudos sobre a utilização de APH em queijo coalho ou as possíveis alterações de textura e cor causadas pelo processamento. Também são escassos os estudos envolvendo a utilização de embalagem antimicrobiana contendo óleo essencial, neste tipo de queijo. Portanto, estudos referentes aos efeitos da aplicação de embalagem contendo óleo essencial e/ou utilização de APH frente as alterações causadas nas propriedades texturais e cor do queijo coalho e sua preservação contra os micro-organismos patogênicos são de grande relevância. Também é notável o interesse de estudiosos sobre o comportamento das embalagens diante de certas condições de uso, como por exemplo, quando são utilizadas para embalar alimentos a serem pressurizados. Apesar de promissores, os estudos obtidos até o momento acerca das alterações causadas pela APH sobre as propriedades funcionais de polímeros naturais são escassos. Portanto, é necessário intensificar esforços que permitam o conhecimento aprofundado dos benefícios da utilização de APH para a garantia de segurança de diferentes tipos de produtos alimentícios, sem prejuízos de suas características próprias, além de preservar a embalagem que o contém. Além disso, especificamente em relação ao presente estudo, pesquisas adicionais sobre os efeitos de ambas as tecnologias de preservação sobre a maturação e parâmetros sensoriais do queijo coalho também são necessários, considerando trabalhos futuros a serem executados pelo Grupo de Pesquisa responsável pela presente tese. Pesquisas sobre a cinética de migração do óleo essencial para o queijo coalho precisam ser realizadas, uma vez que a eficiência antimicrobiana do filme neste estudo foi comprovada apenas a nível de superfície do alimento. Além disso, pretende-se avaliar em estudos futuros os efeitos da APH sobre o potencial de migração do óleo e os impactos sobre os parâmetros sensoriais do queijo. Somado a estes, o estudo do efeito da APH sobre as bactérias láticas presentes no queijo coalho torna-se necessário, já que a presença destes micro-organismos é de fundamental importância para o processo de maturação e desenvolvimento de características sensoriais próprias do produto.