**GERALDO DE SOUSA CÂNDIDO**

# ANTIMICROBIAL AND ANTIOXIDANT BIONANOCOMPOSITES AND THEIR POTENTIAL APPLICATIOS IN FOOD PACKAGING

**LAVRAS-MG**

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Tese apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-Graduação em Agroquímica, área de concentração em Química/Bioquímica, para obtenção do título de Doutor.

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# BIONANOCOMPÓSITOS ANTIMICROBIANOS E ANTIOXIDANTES E AS SUAS POTENCIAIS APLICAÇÕES EM EMBALAGENS DE ALIMENTOS

Tese apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-Graduação em Agroquímica, área de concentração em Química/Bioquímica, para obtenção do título de Doutor.

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**2023**

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## RESUMO GERAL

A busca de alternativas para a eliminação de problemas ambientais provocados pelos resíduos sólidos oriundos de polímeros sintéticos tem despertado o interesse da indústria de alimentos e por pesquisas associadas a embalagens biopoliméricas. Atrelado à demanda ambiental a sociedade busca cada vez mais por alimentos naturais e saudáveis. Neste sentido a utilização de bionanocompósitos torna-se uma possibilidade viável para o desenvolvimento de novas embalagens ativas. Este trabalho teve como objetivo o desenvolvimento de embalagens ativas com ação antimicrobiana e antioxidante para aplicação em alimentos com alto teor de gorduras e umidade. Foram produzidos bionacompósitos de biopolímero de pectina contendo nanemulsão de óleo da semente de pracaxi e acetato de celulose com híbrido de nanorgila com óleo essencial de óleo de cravo. Foi investigado o efeito da nanoemulsão nas propriedades de barreiras ao vapor de água e energia de ativação, sorção de umidade, ângulo de contato propriedades antioxidantes e a sua aplicação em amostras de manteiga. Já os bionanocompósitos de acetato de celulose foram avaliados as propriedades barreiras contra o vapor de água, propriedades opticas e mecânicas, atividade antioxidante e antimicrobiana e avaliada a eficiência em amostras de presunto cozido. Os resultados demonstraram que a incorporação das nanoemulões de óleo de semente de pracaxi acarretaram em um aumento na hidrofilicidade e um aumento na quantidade de sitios ativos na superficie. No entanto a nanoemulsão atua como uma barreira fisica contra a permeabilidade. A atividade antioxidante medida atráves de compostos fenólicos totais (TPC), DPPH, fosfomolibdênio e beta caroteno/ácido linoleíco foi constatado que adição de 0,4% m/m de óleo e pracaxi leva um aumento de 36%, 56%, 3,25% e 80%, respectivamente. A presença da atividade antioxidante principalmente os compostos fenólicos acarretou uma proteção da manteiga contra a oxidação nos 60 dias de armazenamento. A incorporação do hibrido 30% m/m de nanorgila e óleo essencial de cravo no acetato de celulose provocou uma redução na permeabilidade que passou de 1,923±0,175 para 0,544±0,024. També houve uma redução nas propriedades mecânicas de 68,69% na resistencia a tração e 107,95% no alongamento a ruptura. Foi observado uma inibição contra as cepas das bacetrias de *Escherichia Coli* , *Listeria monocytogenens* e *Lactobacillus sakie*. Os compostos fenólicos totais (TPC) variou de 1,64±0,10 mg GAEq para 103.77±0.25mg GAEq e atividade antioxidante pelo método de DPPH alterou de 2.79% para 88.70% e um aumento de aproximadamente de 889 vezes na atividade antioxidante por fosfolibdênio. Além disso a sua aplicação em amostras de presunto cozido foi observado uma menor carga microbiologica do presunto (psicotróficos, mesófilos e bacterias lácteas), manteve o pH estável durante todo o período de armazenamento e a cor avermelhada foi preservada. Assim, a utilização de embalagens ativas a partir de bionanocompósitos é uma alternativa favorável para aumentar a vida útil dos produtos alimentícios.



**Palavras-chave:** Atividade antioxidante. Nanoemulsão. Biopolímeros. Atividade antimicrobiana. Embalagens ativas. Presunto. Manteiga;

## GENERAL ABSTRACT

The search for alternatives to eliminate environmental problems caused by solid waste from synthetic polymers has raised the interest of the food industry and for research associated with biopolymer packaging. Linked to the environmental demand, society is increasingly searching for natural and healthy foods. In this sense the use of bionanocomposites becomes a viable possibility for the development of new active packages. This work aimed to develop active packaging with antimicrobial and antioxidant action for application in foods with high fat and moisture content. Bionicomposites of pectin biopolymer containing pracaxi seed oil nanemulsion and cellulose acetate were produced with a hybrid of nanoclay with essential oil of clove oil. The effect of the nanoemulsion on the water vapor barrier properties and activation energy, moisture sorption, contact angle, antioxidant properties and its application in butter samples was investigated. As for the cellulose acetate bionanocomposites, the water vapor barrier properties, optical and mechanical properties, antioxidant and antimicrobial activity were evaluated and the efficiency in cooked ham samples was evaluated. The results showed that the incorporation of the pracaxi seed oil nanoemulsions resulted in an increase in hydrophilicity and an increase in the amount of active sites on the surface. However, the nanoemulsion acts as a physical barrier against permeability. The antioxidant activity measured through total phenolic compounds (TPC), DPPH, phosphomolybdenum and beta carotene/linoleic acid was found that the addition of 0.4% m/m of pracaxi oil leads to an increase of 36%, 56%, 3.25% and 80%, respectively. The presence of antioxidant activity, mainly phenolic compounds, resulted in butter protection against oxidation during the 60 days of storage. The incorporation of 30% w/w hybrid of nanoclay and clove essential oil in cellulose acetate caused a reduction in permeability from 1.923±0.175 to 0.544±0.024. There was also a reduction in the mechanical properties of 68.69% in tensile strength and 107.95% in elongation at break. An inhibition against *Escherichia coli* , *Listeria monocytogenens* and *Lactobacillus sakie* strains was observed. The total phenolic compounds (TPC) varied from 1.64±0.10 mg GAEq to 103.77±0.25mg GAEq and antioxidant activity by DPPH method changed from 2.79% to 88.70% and an approximately 889 fold increase in antioxidant activity by phospholibdenium. In addition, when applied to samples of cooked ham, a lower microbiological load of ham (psychotrophs, mesophiles and lactic bacteria) was observed, the pH was kept stable during the whole storage period and the reddish color was preserved. Thus the use of active packaging from bionanocomposites is a favorable alternative to increase the shelf life of food products.



**Keywords**: Antioxidant activity. Nanoemulsion. Biopolymers. Antimicrobial activity. Active packaging. Ham. Butter

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# PRIMEIRA PARTE

## 1INTRODUÇÃO

Com o crescimento populacional mundial observa-se um aumento significativo nos problemas ambientais relacionados ao acúmulo de resíduos sólidos em aterros sanitários e gradual acumulação nos oceanos. Grande parte desse problema está associado às embalagens não biodegradáveis tornando-se uma preocupação constante no mundo todo (ZHONG et al., 2020; PERERA et al., 2021). Dentre essas embalagens, as utilizadas em produtos alimentícios tem se destacado levando ao desenvolvimento de pesquisas que buscam alternativas como a substituição das embalagens por polímeros biodegradáveis como os polissacarídeos (AMIRI et al., 2019).

Outro grande desafio da indústria de alimentos se refere a conservação dos alimentos. Aproximadamente um terço da produção de alimentos voltada para o consumo humano é desperdiçada a cada ano em todo o mundo (MATHEUS et al., 2021; VARGHESE; SIENGCHIN; PARAMESWARANPILLAI, 2020). Entre as causas do exorbitante desperdício de alimentos encontra-se a oxidação lipídica e a deterioração provocada por microrganismos (PERERA et al., 2021). Neste sentido, a comunidade acadêmica vem estudando nas últimas décadas novas embalagens alimentícias biodegradáveis elaboradas a partir de matérias-primas renováveis. Além disso, são abordadas embalagens que possam contribuir para a conservação dos mais variados produtos alimentícios.

Biopolímeros são materiais que não apresentam toxicidade, são amplamente disponíveis na natureza, biodegradáveis e permeáveis ao oxigênio. Essas características permitem que eles sejam utilizados como material sustentável na produção de embalagens para alimentos (MOHAMED; EL-SAKHAWY; EL-SAKHAWY, 2020). Dentro da classe dos biopolímeros destacam-se os polissacarídeos que são macromoléculas produzidas pelas plantas, animais e microrganismos e incluem a pectina, amido, celulose, alginato e quitosana (SHARMA; JAFARI; SHARMA, 2020). No entanto, esses materiais apresentam propriedades mecânicas e de barreira limitadas para a aplicação como embalagens de alimentos. Assim, a incorporação de outros compostos em escala nanométrica apresenta o potencial de modificar as propriedades de filmes biopoliméricos e com isso agregar valor na sua aplicação como embalagens alimentícias (AZMANA et al., 2021).

Bionanocompósitos são materiais compostos por duas fases, a fase matriz constituída pelo biopolímero e a fase dispersa composta por diferentes nanoestruturas como nanoemulsões, nanopartículas metálicas e nanoargilas. Geralmente, as embalagens produzidas

com bionanocompósitos apresentam propriedades mecânicas e térmicas adequadas para sua aplicação nos mais diferentes produtos alimentícios. Além disso, elas são biodegradáveis e dependendo de sua composição podem apresentar ação antimicrobiana, antioxidante e barreira a gases (SHARMA; JAFARI; SHARMA, 2020).

Vários estudos de bionanocompósitos contendo nanoemulsões e nanoargilas em sua formulação têm sido reportados como embalagens ativas. Esses estudos indicam como fase dispersa nanoemulsões produzidas a partir de óleo de copaíba (NORCINO et al., 2020), óleo de *Origanum majorana* (ALMASI; AZIZI; AMJADI, 2020), óleo essencial de canela (GHANI et al., 2018), óleo de soja (GUTIÉRREZ-JARA et al., 2020), óleo essencial de *Zataria multiflora* (AMIRI et al., 2019) e óleo essencial de orégano (LEE et al., 2019). E bionacompósitos nanoreforçados com nanoargilas e incorporados compostos ativos como o óleo essencial de canela (IAMAREERAT et al., 2018), o óleo essencial de *Carum copticum* (ASDAGH; PIRSA, 2020), óleo essencial de tomilho (ISSA; IBRAHIM; TAHERGORABI, 2017) e o óleo essencial de cravo (ECHEVERRÍA et al., 2018). Em todos estes trabalhos a incorporação da nanoemulsão e ou nanoargila com óleo impactaram drasticamente em diversas propriedades dos bionanocompósitos.

Dessa forma as embalagens ativas surgem como uma abordagem inovadora onde oscompostos funcionais desses filmes podem interagir com o alimento embalado, atuando como um agente antioxidante e/ou antimicrobiano (MATHEUS et al., 2021). Vários ingredientes naturais como o extrato de casca de kiwi (HAN; SONG, 2021), óleos essenciais de *Carum copticum* (ASDAGH; PIRSA, 2020), óleo essencial *Ferulago angulata* (SHOKRI et al., 2020), extrato de casca de amendoim, resíduo de pimenta rosa (SERRANO-LEÓN et al., 2018) e da *Acca sellowiana* (SGANZERLA et al., 2020), foram recentemente incorporados em filmes biopoliméricos para garantir uma determinada ação antioxidante e antimicrobiana em aplicações como embalagens alimentícias.

Nesse contexto, este estudo visou o desenvolvimento de embalagens ativas com potencial antioxidante e ou antimicrobiano a partir de bionanocompósitos. Assim foram elaborados bionanocompósitos de pectina e nanoemulsão de óleo da semente de pracaxi e foi avaliada a sua aplicação em amostras de manteiga. Também foram produzidos bionanocompósitos de acetato de celulose incorporados diferentes concentrações de híbrido de nanoargila e óleo essencial de cravo e foi investigado a sua eficiência em amostras de presunto cozido. Buscando contribuir para a segurança alimentar dos consumidores e reduzir o impacto ambiental dos resíduos sólidos urbanos gerados pela indústria de alimentos.

# 2 REFERENCIAL TEÓRICO

## 2.1 Problemas ambientais provados por polímeros sintéticos

Devido às excelentes propriedades como baixo custo, resiliência e estabilidade os polímeros sintéticos estão presentes e são úteis em todas associedades modernas. Entretanto essas mesmas propriedades tornam-se esses materiais resistentes a degradação, levando em seu acúmulo, ao invés de decomposição, em aterros e ecossistemas como oceanos e costas. No ano de 2020, a produção de polímeros sintéticos foi de 367 milhões de toneladas. E segundo uma projeção das Nações Unidas a produção anual deverá dobrar até 2035 chegando a aproximadamente a 800 milhões de toneladas. Lamentavelmente, cálculos recentes indicam que 76% da produção total de plástico é tratada como lixo. Destes, 9% são reciclados, 12% são incinerados e 79% são aterrados ou lançados no meio ambiente. Este descarte inadequando desses materiais podem provocar danos severos ao ecossistema (ALI et al., 2021; ZHOU et al., 2023). Além de todos os danos ambientais provocados por esses resíduos aproxidamente 90% são sintetizados a partir de fontes não renováveis que são os combustíveis fósseis (ZHOU et al., 2023).

Além do acumulo desses resíduos nos aterros aproximadamente 3% são lançados nos oceanos onde podem ficar fultuando ou sedimentar no fundo do mar causando aglomerados que trazem grandes risco para a fauna marinha. Outro problema é que esses resíduospodem liberar compostos químicos na água do mar, que podem ser aditivos ou oligômeros que fazem parte da estrutura do polímero. A taxa de degradação tanto no oceano como em aterro sanaitário é extremamente baixa podendo permanecer acumulado por muitos anos. Embora a abordagem de “reutilizar, reduzir e reciclar” ofereça uma solução para essa problemática ela não será suficiente para conter o uso excessivo desses materiais . Assim, os polímeros biodegradáveis provinientes de fontes renováveis apresentam a melhor alternativa no cenário atual para a eleminação dos prolemas ambientais causados pelos polímeros sintéticos(RAI et al., 2021; ROMERA-CASTILLO et al., 2023).

## 2.2 Biopolímeros

Biopolímeros são macromoléculas orgânicas derivados de diferentes unidades monoméricas de ligação covalente. E podem ser encontrados em plantas, animais ou microorganismos (SAMROT et al., 2020; SIVAKANTHAN et al., 2020). Normalmente as unidades de nonoméricas presentes nos biopolímeros são derivadas de ácidos nucléicos,

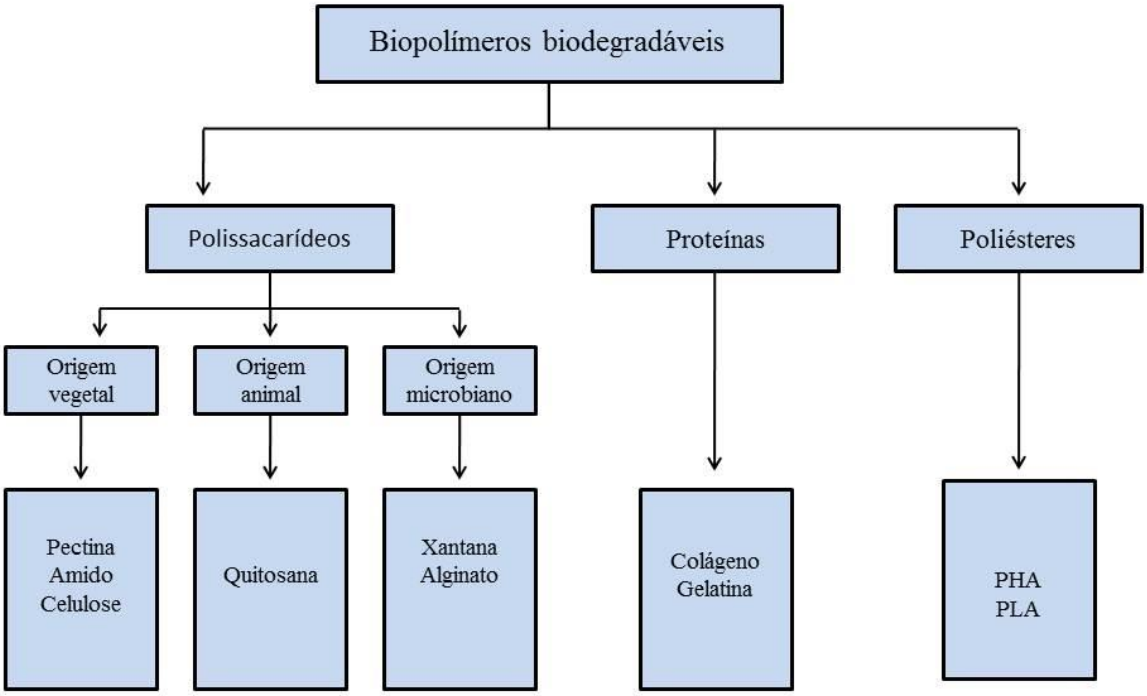
proteínas de aminoácidos ou sacarídeos derivados de açúcares (GEORGE et al., 2020). Os biopolímeros podem ser classificados de acordo com os diferentes tipos, processos de síntese ou pela suas fontes (Figura 1).

Os biopolímeros possuem diversas vantagens em aplicações envolvendo aplicações como embalagens alimentícias, como biodegradabilidade, biodisponibilidade, biocompatibilidade e não toxicidade. Além das aplicações como embalagens de alimentos suas excelentes propriedades tornam-se fortes candidatos para aplicações na medicina, farmacologia e em indústrias, incluindo embalagens, cosméticos, absorventes, eletrônicos, agricultura, tratamento de água, tecidos de vestuário, plásticos e biossensores (AL-TAYYAR; YOUSSEF; AL-HINDI, 2020; SIVAKANTHAN et al., 2020).

Esta classe de materiais apresenta elevada barreira ao oxigênio. Essa característica se deve ao elevado número de ligações de hidrogênio intermacromoleculares que atuam como uma rede molecular a permeação do oxigênio (SAHRAEE et al., 2019). No entanto, na maioria dos casos filmes formados por biopolímeros apresentam elevada rigidez e baixa flexibilidade. Dessa maneira as embalagens oriundas desses materiais apresentam restrições para a sua aplicação na indústria alimentícia (MOHAMED; EL-SAKHAWY; EL-SAKHAWY, 2020).

Para aumentar a flexibilidade de filmes biopoliméricos torna-se necessário a utilização de plastificantes que possuem a função de reduzir a temperatura de transição vítrea dos mesmos de forma a aumentar a mobilidade e a mobilidade molecular a temperatura ambiente e assim melhorar também flexibilidade dos filmes biopoliméricos. Alguns dos plastificantes rotineiramente empregados na formulação de filmes biopoliméricos são compostos de baixa massa molar como o glicerol, sorbitol, polietilenoglicol e xilitol (MOHAMED; EL-SAKHAWY; EL-SAKHAWY, 2020; DOMÍNGUEZ et al., 2018).

Figura 1: Classificação dos biopolímeros

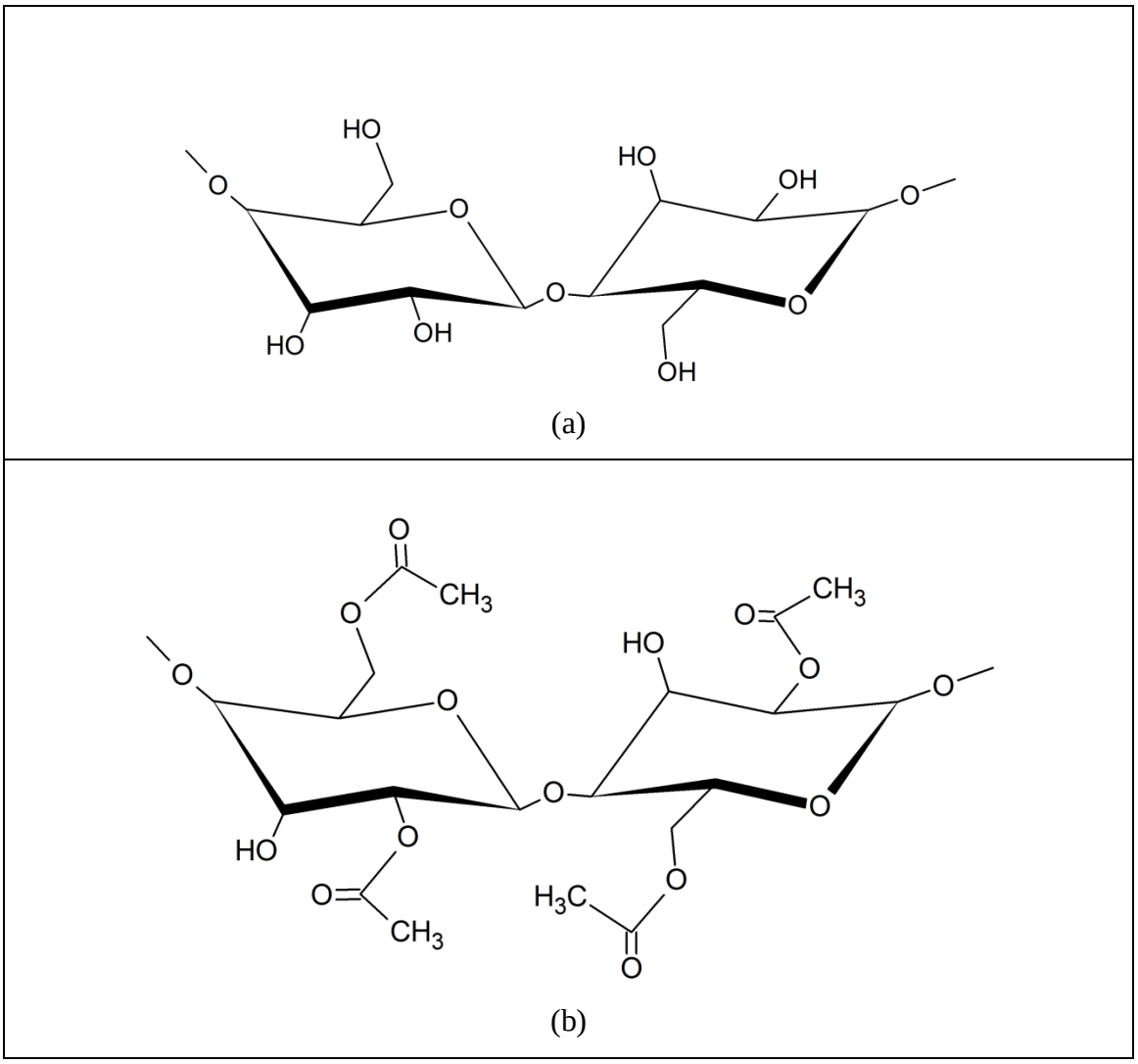



Fonte: Do autor (2023)

Devido a sua abundância natural,biodegradabilidade, permeabilidade seletiva ao dióxido de carbono e oxigênio os polissacarídeos são os biopolímeros mais utilizados em embalagens ativas para alimentos (AZMANA et al., 2021; MOHAMED; EL-SAKHAWY; EL-SAKHAWY, 2020). Adicionalmente, os polissacarídeos e seus derivados demonstram ser excelentes matrizes para difusão lenta de antioxidantese antimicrobianos (RHIM; PARK; HA, 2013).

A celulose (Figura 2a) é o polissacarídeo mais abundante na natureza, sendo encontrado na parede celular das plantas. A sua estrutura é composta por uma cadeia linear de unidades D-glicose, que são formadas por meio de condensação de ligações β (1→4) – glicosídicas(XIONG CHANG et al., 2021). Os derivados da celulose comumente utilizados para a fabricação de filmes poliméricos são o acetato de celulose, metilcelulose, hidroxipropilmetilcelulose e carboximetilcelulose. O acetato de celulose (Figura 2b) é obtido através da acetilação da celulose e tem características biodegradável, inodora e insípida (MOHAMED; EL-SAKHAWY; EL-SAKHAWY, 2020).

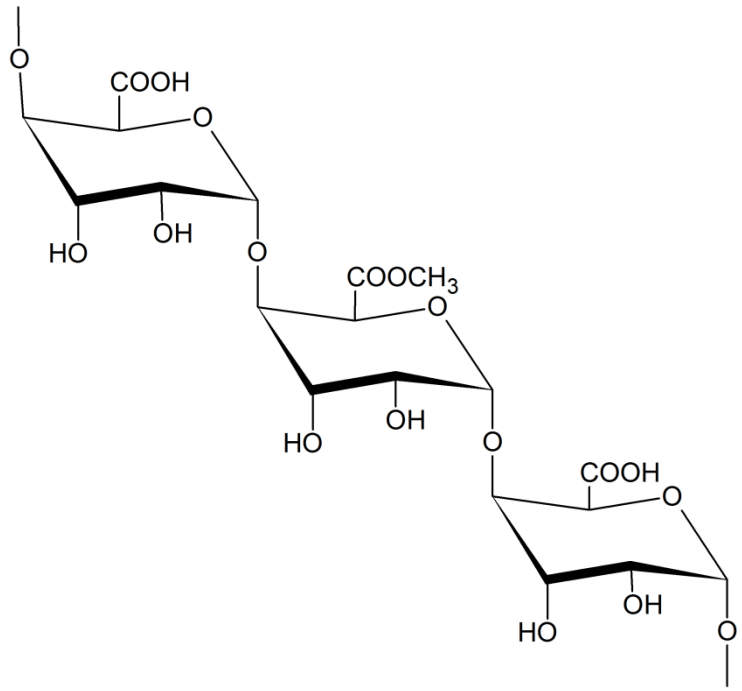
Figura 2: Repetição da estrutura da celulose (a) e estrutura do acetato de celulose (b)




Fonte: Do autor (2023)

A pectina é um polissacarídeo aniônico, extremamente complexo, encontrado principalmente em cascas de fruta, principalmente da laranja e maça. Que compreende em diversas estruturas sendo a principal cadeia lineares com ligações glicosídicas de α-(1→4) de ácido galacturônico (Figura 3) com aproximadamente 65% da cadeia em domínio homogalacturonana. Existem também resíduos de ramnogalacturonanas I e II, que consistem de unidades de ácido galacturônico alternadas com unidades específicas de ramnose (SIVAKANTHAN et al., 2020; REHMAN et al., 2019; CHAN et al., 2017). Durante a obtenção da pectina os monômeros do ácido galacturônico os seus grupos funcionais de ácidos carboxílicos podem estar esterificados ou não pelo álcool metílico a quantidade de grupamentos químicos ésteres determinará o grau de metoxilação da pectina (ESPITIA et al., 2014).

Figura 3: Repetição da estrutura da pectina




Fonte: Do autor (2023)

## 2.3Bionanocompósitos

Bionanocompósitos são materiais compostos por duas fases, sendo a fase matriz constituída pelo biopolímero e a fase dispersa composta por diferentes nanoestruturas como nanoemulsões, nanopartículas metálicas e nanoargilas com pelo menos uma de suas dimensões de até 100 nm (SHARMA; JAFARI; SHARMA, 2020; AZMANA et al., 2021 ;YOUSSEF; EL-SAYED, 2018). A fase dispersa engloba diversas nanoestruturas e dentre elas as nanoemulsões e nanoargilas tem ganhado destaque no estudo com embalagens ativas. Os bionanocompositos são econômicos, biodegradáveise fáceis de preparar, dessa maneira o interesse em todo mundo tem sido cada vez maior para a sua utilização (AZMANA et al., 2021). E podem ser produzidos por dois processos distintos a evaporação do solvente e a extrusão (DOMÍNGUEZ et al., 2018).

Quando comparado aos filmes dos biopolímeros puros, os bionancompósitos podem apresentar aumento significativo em suas propriedades de barreira, mecânicas, antioxidantes e antimicrobianas (AZMANA et al. 2021; Youssef & El-Sayed, 2018). Estes aspectos fazem com que estes materiais apresentam grande aplicabilidade para embalagens de alimentos (SHARMA; JAFARI; SHARMA, 2020).

### 2.3.1 Emprego de nanoemulsões em bionanocompósitos

O emprego das nanoemulsões tem sido amplamente utilizado em estudos que envolvem bionanocompósitos para embalagens de alimentos e tem apresentado resultados promissores (Tabela 2). Devido ao caráter hidrofóbico as nanoemulsões podem acarretar na diminuição da permeabilidade ao vapor de água em filmes hidrofílicos. Além disso, a incorporação dessas nanoestruturas pode acarretar maior flexibilidade e resistência a tração dos filmes associado a umaumento em suaação antioxidante antimicrobiana (AL-TAYYAR; YOUSSEF; AL-HINDI, 2020; ASGHER et al., 2020). Outro aspecto interessante do emprego de nanoemulsões na indústria de alimentos se deve a redução das características organolépticas em relação à adição do óleo essencial puro (PRAKASH et al., 2018). Devido ao tamanho das partículas as nanoelmulsões oferecem estabilidade física para substâncias ativas encapsuladas (AL-TAYYAR; YOUSSEF; AL-HINDI, 2020).

Para a preparação das nanoemulsões são utilizados óleos vegetais fixos e óleos essenciais. As emulsões são elaboradas a partir líquido imiscível disperso em outro líquido imiscível na forma de gotículas sendo necessária a utilização de surfactante que tem a função dereduzir a tensão interfacial entre as fases. As emulsões óleo em água são quando um surfactante utilizado é solúvel em água, a fase contínua é água e a dispersa é o óleo. Já as emulsões água em óleo são quando é usado um surfactante solúvel em óleo, a fase contínua é óleo, e a dispersa é a água (PRAKASH et al., 2018; BARRADAS; DE HOLANDA E SILVA, 2021; YUKUYAMA et al., 2016). No entanto, para se tornar uma nanoemulsão, a emulsão deve passar pelo sonicador para a quebra das gotículas uma vez que as nanoemulsões apresentem geralmente um tamanho menor que 100 nm (BARRADAS; DE HOLANDA E SILVA, 2021; AL-TAYYAR; YOUSSEF; AL-HINDI, 2020).

Tabela 1. Estudos sobre uso de nanoemulsões em embalagens de alimentos

| Biopolímero | Nanoemulsão | Propriedades melhoradas | Referência |
|---|---|---|---|
| Pectina | Nanoemulsões de óleo de copaíba | Aumento nas propriedades antimicrobianas, diminuição na permeabilidade ao vapor de água, aumento na extensibilidade e diminuição na rigidez. | (NORCINO et al., 2020) |
| Pectina | Nanoemulsões de óleo de *Origanum* | Aumento nas propriedades antioxidantes e antimicrobianas, | (ALMASI; AZIZI; |

| | | | |
|---|---|---|---|
| | *majorana L*. | diminuição na sorção, diminuição napermeabilidade ao vapor de águae aumento nas propriedades mecânicas. | AMJADI, 2020) |
| Isolado protéico de soja | Nanoemulsões de óleo essencial de canela | Redução na permeabilidade, redução na solubilidade e aumento na atividade antioxidante e antimicrobiana | (GHANI et al., 2018) |
| Alginato | Nanoemulsões de óleo de soja | Redução napermeabilidade, redução na sorção de umidade, impermeabilidade ao oxigênio e aumento nas propriedades mecânicas. | (GUTIÉRREZ-JARA et al., 2020) |
| Amido | Nanoemulsões de óleo essencial de *Zataria multiflora* | Aumento nas propriedades mecânicas, aumento nas propriedades antioxidantes e redução na peroxidação lipídica de hambúrgueres de carne moída. | (AMIRI et al., 2019) |
| Hidroxipropil-metilcelulose | Nanoemulsões de óleo essencial de orégano | Aumento nas propriedades mecânicas, diminuição na permeabilidade ao vapor de água e propriedades antioxidantes e antimicrobianas | (LEE et al., 2019) |
| Goma *Cordia dicotoma* | Nanoemulsões de óleo essencial de *Salvia mirzayanii* | Aumento nas propriedades mecânicas, diminuição no teor de umidade, diminuição na solubilidade em água, diminuição na permeabilidade ao vapor de água e aumento nas propriedades antioxidantes e antibacterianas | (HASHEMINYA; DEHGHANNYA, 2021) |

Fonte: Do autor (2023)

**2.3.1.1 Óleo de pracaxi**

A árvore de *Pentaclethra macroloba* pertencente à família *Fabaceae* é naturalmente encontrada na floresta amazônica. Produz um fruto na forma de uma vagem (Figura 4) com dimensão de 20 a 25 cm e contém aproximadamente de 3 a 8 sementes. Essas sementes são comestíveis e fornece um percentual de 45% a 48% de óleo vegetal. Esse óleo é rico em ácidos graxos monoinsaturados com uma maior concentração de ácido oleíco com teores que variam de 47,3 a 53,5%, seguido do ácido behênico, ácido linoléico e com um menor percentual o ácido lignocérico (TEIXEIRA et al., 2020). Também Pires *et al.* (2022) identificaram o composto ácido oleíco e hederagenin.

O óleo de pracaxi é muito utilizado por comunidades ribeirinhas como cicatrizante, picadas de cobra e insetos, em cosméticos e também em frituras de alimentos. No entanto a literatura é carente em estudos com as propriedades químicas e bioatividade do óleo de pracaxi (TEIXEIRA et al., 2020). Estudos com atividade antimicrobiana do óleo de pracaxi não inibiu cepas de *Staphylococcus aureus* (GUIMARÃES et al., 2016). Já a atividade antioxidante e a presença de compostos fenólicos foi relatado e que a extração realizada em menores pressão há um aumento de compostos bioátivos e os valores encontrados para o DPPH foram maiores que relatado por óleos fixos como: óleo de milho, óleo de semente de uva, óleo de soja, linho e semelhante ao óleo de farelo de arroz e óleo de girassol (TEIXEIRA et al., 2020).

Figura 4: *Pentacletrha macroloba*: árvore e sementes

Fonte: Pires *et al.* (2022)

**2.3.1.2 Óleo essencial do cravo da índia (*Syzygium aromaticum*)**

A planta *Syzygium aromaticum* (Figura 5) é aromática e amplamente cultivada em países tropicais e subtropicais pertencente à família *Myrtaceae* en que contem

aproximadamente de 15 – 20% em peso de óleo essencial. Os óleos essenciais do cravo possuem uma grande quantidade de compostos bioativos que incluem os compostos fenólicos que conferem as propriedades antioxidantes, antimicrobianas e antifúngicas. É reconhecido como seguro (GRAS por este motivo é amplamente utilizado em perfumaria, cosméticos, saneantes, medicamentos e alimentos (HARO-GONZÁLEZ et al., 2021).

Há diversos compostos químicos que já foram identificado sendo o componente majoritário o eugenol, representando pelo menos 50%. Os 10-40% restantes são compostos como acetato de eugenol, β-cariofileno e α-humuleno. Menos de 10% correspondem a componentes menores ou traços, como dietilftalato, óxido de cariofileno, cadineno, α-copaeno, 4-(2-propenil)-fenol, chavicol e α-cubebene, entre outros (HARO-GONZÁLEZ et al., 2021).

Alguns trabalhos de identificação de compostos químicos no óleo de cravo relatam teores de eugenol de 56,06%, seguido por cariofileno 39,63% e α-cariofileno 4,31% (RADÜNZ et al., 2019); dezesseis compostos foram identificados sendo os com maiores proporções o eugenol (65,33%), β-cariofileno(14,78%), acetato de eugenol (14,55%) e α-humuleno (1,43%) (DAS et al., 2021). O eugenol também foi o componente majoritário do óleo essencial estudado (78,72%), seguido do β-cariofileno (8,82%) e acetato de eugenol (8,74%) (SELLES et al., 2020) e a análise GC-MS revelou a presença de vários metabolitos secundários, com o eugenol (66,01%), cariofileno (19,88%), óxido de cariofileno (5,80%), fenol, 2-metoxi-4-(2-propenil)-acetato (4,55%) e humuleno (3,75%) (G. AL-HASHIMI et al., 2020).

Diversos estudos têm mostrado que o óleo essencial de cravo possui excelente atividade antioxidante. A atividade antioxidante medida por diferentes métodos têm sido reportada na literatura; ensaio de DPPH e o ensaio do poder redutor (SELLES et al., 2020); DPPH e radicais hidroxila e óxido nítrico (RADÜNZ et al., 2019); DPPH , poder redutor, quelação de metais e inibição da peroxidação lipídica (DAS et al., 2021) e inibição do radical ABTS (TELES et al., 2021).

Estudos com óleo essencial de cravo têm demonstrado um potencial antimicrobiano sobre diversas bactérias; o efeito inibitório foi relatado, contra *Salmonella, Listeria monocytogenes* , *Staphylococcus aureus* e *Escherichia coli* (RADÜNZ et al., 2019); suscetibilidade ao óleo das cepas de *Shigella dysenteriae* e *Staphylococcus aureus* (DAS et al., 2021) e inibição de cepas de *Escherichia coli*, *Staphylococcus aureus* e *Pseudomonas aeruginosa* (TELES et al., 2021)

Figura 3: Cravo da índia Syzygium aromaticum L: planta, flor e botão

Fonte: Souza (2019)

### 2.3.2 Emprego de nanoargilas em bionanocompósitos

A nanoargila é composta por argilominerais, que são filosilicatos hidratados pertencentes à família das esmectitas, onde a montmorilonita, a hectorita e a saponita são as mais utilizadas para a produção de embalagens alimentícias (DA SILVA SCUDELER et al., 2020). É um mineral de ocorrência natural sem toxinas e apresenta diversas aplicações em embalagens de alimentos, em cosméticos e na medicina (IAMAREERAT et al., 2018). As aplicações da nanoargila como nanocompósitos em embalagens de alimentos é devido ao seu baixo custo, eficácia e a sua alta estabilidade e tem apresentado resultados satisfatórios (Tabela 3) (ISSA; IBRAHIM; TAHERGORABI, 2017). A nanoargila é um composto que pode ser potencialmente usado na liberação controlada de agentes antimicrobianos e antioxidantes em materiais de embalagens ativas (ISSA; IBRAHIM; TAHERGORABI, 2017 ;DA SILVA SCUDELER et al., 2020).

Tabela 2. Estudos sobre uso de nanoargilas incorporadas com óleos essenciais em embalagens de alimentos

| Biopolímero | Nanoestrutura | Efeito | Referência |
|---|---|---|---|
| Amido de mandioca | Nanoargila e óleo essencial de canela | Maior ação antimicrobiana, aumento no alongamento na ruptura dos filmes e aumento na vida útil de almôndegas de porco | (IAMAREERAT et al., 2018) |
| Pectina | Nanoargila e óleo essencial de *Carum copticum* | Maior flexibilidade e resistência a tração do filme e maior ação antimicrobiana e antioxidante | (ASDAGH; PIRSA, 2020). |
| Amido de batata-doce | Nanoargila e óleo essencial de tomilho | Maior ação antimicrobiana reduzindo o crescimento de *Escherichia coli* e *Salmonella* | (ISSA; IBRAHIM; TAHERGORABI, 2017) |
| Isolado proteíco de soja | Nanoargila e óleo essencial de cravo | Maior ação antioxidante e antimicrobiana e aumento na vida útil de filés de atum rabilho ( *Thunnus thynnus* ) | (ECHEVERRÍA et al., 2018) |

Fonte: Do autor (2023)

## 2.4 Embalagens ativas

Embalagem é um sistema que apresenta como função a proteção de produtos para sua distribuição, armazenamento, venda e uso. A embalagem tem como principais objetivos a contenção, proteção, preservação, transporte, informação e comercialização de produtos como eletrônicos, medicamentos, alimentos dentre outros. Na indústria de alimentos as embalagens são essenciais para a proteção do alimento contra a contaminação por fontes externas (QIAN et al., 2021; MOHAMED; EL-SAKHAWY; EL-SAKHAWY, 2020). As embalagens necessitam do controle de diversas propriedades para a sua aplicação em alimentos. Essas propriedades de interesse incluem por exemplo barreira a gases e a luz, estabilidade térmica e limite de resistência a tração.

A embalagem ativa foi uma inovação no campo de embalagens de alimentos e pode ser caracterizada como uma interação positiva existente entre ambiente, embalagem e

produto. No caso para as embalagens ativas além dos atributos da embalagem tradicional devem apresentar características funcionais como a atividade antimicrobiana ou antioxidante (QIAN et al., 2021; KUAI et al., 2021). Com o objetivo de prolongar a vida útil do produto alimentício, garantir a sua segurança e preservar suas características físicas, químicas e microbiológicas (QIAN et al., 2021; PERERA et al., 2021 ; AHMED et al., 2017). As embalagens ativas ainda apresentam diversas vantagens em relação às embalagens convencionais como a redução na quantidade de aditivos sintéticos adicionados diretamente nos alimentos, redução de resíduos sólidos provienientes dos polímeros sintéticos e proteção dos alimentos contra a deterioração por microrganismos e também reações de oxidação (SIVAKANTHAN et al., 2020). A tabela 1, traz alguns estudos recentes de elaboração de embalagens ativas.

Atualmente, existem dois tipos de sistemas de embalagem ativa: sistemas de eliminação ativa, também chamada de absorvedores, que tem como função remover substâncias indesejáveis dos alimentos ou de seus arredores, como a umidade, $CO_2$, $O_2$, etileno ou odor. E os sistemas de liberação ativa, caracterizada por um mecanismo de liberação controlada que possui a finalidade de adicionar compostos ativos aos alimentos embalados ou ao espaço próximo ao produto, como compostos antimicrobianos e antioxidantes, sabores, etileno, $CO_2$ ou etanol (PERERA et al., 2021;DOMÍNGUEZ et al., 2018).

Tabela 3. Estudos sobre embalagens ativas

| Tipos de embalagens ativas | Biopolímero | Composto natural utilizado | Referência |
|---|---|---|---|
| Antioxidante / antimicrobiano | Pectina | Óleo essencial de cravo-da-índia | (NISAR et al., 2018) |
| Antioxidante / antimicrobiano | Pectina / Nanoargila | Óleos essenciais de *Carum copticum* e β-caroteno | (ASDAGH; PIRSA, 2020) |
| Antioxidante | Amido | Extrato de cascas de girassol | (MENZEL et al., 2019) |
| Antimicrobiano | Pectina | Purê de caqui ( *Diospyros kaki L.*) | (MATHEUS et al., 2021) |
| Antioxidante / | Pectina/gluco | Polifenol de chá | (LEI et al., 2019) |

| | | | |
|---|---|---|---|
| antimicrobiano | manano | | |
| Antioxidante | Quitosana | Extrato da folha de manga | (RAMBABU et al., 2019) |
| Antioxidante/ Antimicrobiano | Quitosana | Extrato de raiz de cebolinha chinesa | (RIAZ et al., 2020) |
| Antioxidante | Amido e xantana | Óleo de gengibre e óleo de cominho preto | (ARSHAD et al., 2020) |
| Antioxidante/ Antimicrobiano | Quitosana | Extratos de casca de amendoim e resíduo de pimenta rosa | (SERRANO-LEÓN et al., 2018) |

Fonte: Do autor (2023)

Diversos estudos têm mostrado resultados promissores na aplicação dessas embalagens ativas em alimentos. Filmes ativos (com propriedades antioxidantes) de pectina de casca de melancia e extrato de casca de kiwi foram empregados como embalagem em coxas de frango e mostraram menores valores de teores de peróxidos e substâncias reativas ao ácido tiobarbitúrico (TBARS) quando comparado com as amostras que não foram embaladas pelos filmes com ação antioxidante mostrando ser eficientes para retardar a oxidação de lipídios durante 9 dias de armazenamento (HAN; SONG, 2021).

Bionanocompósitos ativos de pectina, nanoargila e óleos essenciais de *Carum copticum* e β-caroteno foram testados como embalagem para manteiga e promoveram uma maior estabilidade oxidativa, e menor crescimento microbiano, aumentando assim a vida útil da manteiga (ASDAGH; PIRSA, 2020).

Em outro estudo embalagens ativas de quitosana com nanoemulsões de óleo essencial *Ferulago angulata* foram aplicadas em filés de peixe (truta arco-íris) apresentando resultados positivos no potencial antimicrobiano nas espécies deteriorantes testadas e redução na peroxidação lipídica (SHOKRI et al., 2020).

Extratos da casca do amendoim e resíduo de pimenta rosa foram utilizados para a incorporação no biopolímero de quitosana e foram empregados para embalar carnes de frango e os resultados mostraram que o uso da embalagem promoveu a estabilidade oxidativa em carnes de frango medida por meio dos teores de peróxidos e pela oxidação secundária o TBARS e houve redução das contagens de microrganismos psicrotróficos (SERRANO-LEÓN et al., 2018).

Estudos com aplicação de filmes em frutos, filmes de pectina cítrica e amido

funcionalizados com resíduos feijoa (*Acca sellowiana)* e foram aplicados para a conservação de pós-colheita da maçã e os resultados indicaram atividade antimicrobiana contra as bactérias *Escherichia coli, Salmonella* e *Pseudomas aeruginosa.* As embalagens produzidas mantiveram a qualidade das maçãs durante o armazenamento (SGANZERLA et al., 2020).

Estudos com filmes de amido de mandioca incorporados com nanoergila e óleo essencial de canela inibiram significativamente o crescimento das bactérias *Escherichia coli , Salmonella* e *Staphylococcus aureus* em almôndegas de carne suína, em comparação ao material de embalagem convencional (IAMAREERAT et al., 2018).

### 2.4.1Embalagens antimicrobianas

A presença de microrganismos indesejáveis nos alimentos pode provocar alterações em sua cor (descoloração), sabor desagradável e o desenvolvimento de mudanças na textura que refletem diretamente na redução da vida útil desses alimentos (QIAN et al., 2021) ou até mesmo riscos a saúde humana (AHMED et al., 2017; PERERA et al., 2021)

Para minimizar os danos causados pelos microrganismos alimentos diversos compostos ativos têm sido estudados para a elaboração de embalagens ativas antimicrobianas. Estas substâncias podem controlar o número de microrganismos, com a redução na taxa de crescimento e fase latente dos microrganismos patogênicos, contribuindo assim para prolongar a vida útil do produto e reduzir o risco de crescimento destes organismos nas superfícies dos alimentos (VARGHESE; SIENGCHIN; PARAMESWARANPILLAI, 2020 ; NISAR et al., 2018; QIAN et al., 2021).

A eficácia de embalagem ativa antimicrobiana depende de diversos fatores como a composição e a quantidade de substâncias ativas incorporadas ao biopolímero, a natureza e o tipo de biopolímero utilizado como matriz, a taxa de liberação e retenção dos compostos ativos, condições de armazenamento dos filmes poliméricos e os microrganismos utilizados para a avaliação (VARGHESE; SIENGCHIN; PARAMESWARANPILLAI, 2020).

Os agentes antimicrobianos são adicionados na matriz polimérica por meio da incorporação direta ou na forma de filmes multicamadas para realizar a liberação controlada na superfície do alimento (AHMED et al., 2017; ZHONG et al., 2020). No entanto a incorporação direta dos agentes antimicrobianos nos filmes de embalagem é o mais adequado para atingir melhores resultados. Os componentes não voláteis dos agentes antimicrobianos incorporados na matriz podem ser liberados na superfície do alimento por meio da migração para o alimento e os componentes voláteis por difusão ou evaporação no espaço circundante ao alimento (AHMED et al., 2017).

A eficiência dos agentes antimicrobianos depende especialmente da sua capacidade de difundir-se na superfície do alimento, onde ocorre a maior parte da deterioração. Quando o agente antimicrobiano é incorporado no filme polimérico os compostos que apresentam atividade antimicrobiana se difunde progressivamente na superfície estendendo a sua atividade para todas as partes do alimento (VARGHESE; SIENGCHIN; PARAMESWARANPILLAI, 2020).

### 2.4.2 Embalagens antioxidantes

Uma maneira eficiente para prolongar o tempo de prateleira de alguns alimentos são as embalagens ativas antioxidantes. O alto nível de oxigênio na embalagem pode afetar diretamente o crescimento de microorganismos e acelerar o processo de oxidação de lipídios diminuindo assim o valor nutricional do produto, provocando também alterações significativas na sua cor, textura e sabor. Assim são utilizadas as embalagens que reduzem a concentração de oxigênio e com isso limitam a oxidação dos lipídios (QIAN et al., 2021 ; ASGHER et al., 2020). Esse tipo de material é desenvolvido pela integração de produto ativo na matriz do polímero ou incorporado na superfície do polímero (DOMÍNGUEZ et al., 2018). Os compostos naturais geralmente mais utilizados são os extratos de plantas, óleos essenciais ou óleos vegetais fixos e tocoferóis (QIAN et al., 2021 ; ASGHER et al., 2020).

O principal mecanismo de ação dos compostos antioxidantes nas embalagens ativas envolve a desativação de centros reativos como radicais livres presentes no meio ou no alimento (QIAN et al., 2021; DOMÍNGUEZ et al., 2018). O mecanismo de migração dos antioxidantes na embalagem ativa abrange sua difusão até a superfície da matriz polimérica, a lixiviação ou volatilização dos agentes antioxidantes presentes na superfície da embalagem para o meio externo e a difusão para o interior do alimento (KUAI et al., 2021). A avaliação da migração de compostos ativos engloba mecanismos complexos que dependem de diversos fatores como a natureza química do composto antioxidante empregado, a matriz polimérica e os aspectos do alimento a ser embalado (DOMÍNGUEZ et al., 2018).

## 2.5 Agentes antimicrobianos e antioxidantes

### 2.5.1 Agentes antimicrobianos

O crescimento de microrganismos constitui a principal causa da deterioração de alimentos. Por isso os agentes antimicrobianos são os princípios ativos mais estudados atualmente (VILELA et al., 2018).

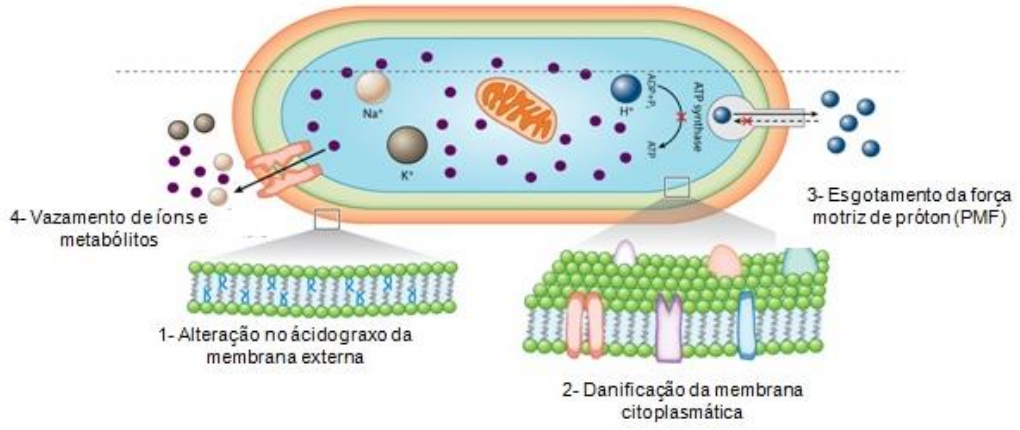
Os agentes antimicrobianos utilizados em embalagens para a preservação de alimentos

podem ser produzidos via rota sintética ou extraídos de fontes naturais (ASGHER et al., 2020). Dentre os agentes antimicrobianos extraídos naturalmente destacam-se os óleos essências ou vegetais e os extratos de plantas. Isso se deve ao fato que as plantas produzem os metabólitos secundários (terpenos e compostos fenólicos) em resposta a fatores ambientais como o ataque de herbívoros e o estresse abiótico (ÁLVAREZ-MARTÍNEZ et al., 2021). E estas substâncias podem inibir ou retardar o ataque de predadores como bactérias e fungos (ZANETTI et al., 2018).

Em geral, os extratos vegetais apresentam como compostos fenólicos principalmente os flavonóides e seus derivados. Esses compostos são responsáveis pela atividade antimicrobiana dos extratos vegetais. Os polifenóis possuem a capacidade de atuar mutuamente com as bicamadas lipídicas e provocar danos a membrana plasmática dos microrganismos pela aglomeração de grupos hidroxilas (ASGHER et al., 2020 ;ÁLVAREZ-MARTÍNEZ et al., 2021).

Os óleos essenciais são formados por uma mistura complexa de compostos voláteis de baixa massa molar. Essas substâncias podem ser obtidas de diferentes partes das plantas como casca, flores e frutos, folhas, raízes e caules (PATEIRO et al., 2021). Seus principais componentes são os terpenos e os compostos fenólicos. Os compostos fenólicos apresentam na sua estrutura química vários grupos benzênicos característicos, tendo como substituintes grupamentos hidroxilas (OH). E a sua ação antimicrobiana está associada à presença destes compostos e proporcional a quantidade de compostos presente no óleo (ÁLVAREZ-MARTÍNEZ et al., 2021). Existem diversos mecanismos de ação dos óleos contra microrganismos sendo o principal mecanismo de ação proposto é odano irreversível da parede e membrana citoplasmática bacteriana (Figura 6) (VALDIVIESO-UGARTE et al., 2019). De modo geral, as bactérias gram-negativas são mais resistentes do que as gram-positivas contra a ação antimicrobiana dos óleos essenciais (ZANETTI et al., 2018).

Figura 4: Mecanismos de ação comuns propostos e locais alvos dos óleos essenciais em células bacterianas




Fonte: Adaptado de Rao et al. (2019)

**2.4.2 Agentes antioxidantes**

Os processos oxidativos nos alimentos ocorrem nos ácidos graxos insaturados na presença do oxigênio singleto ($O_2^{\cdot}$) e são formados os radicais livres. Esses, são suscetíveis ao ataque do oxigênio atmosférico e são convertidos em compostos da oxidação primária que são os peróxidos e hidroperóxidos. Os peróxidos e hidroperóxidos se combinam formando compostos estáveis como aldeídos, cetonas, álcoois, compostos epóxi e ácidos orgânicos que são os compostos secundários da oxidação e que conferem ao alimento o sabor desagradável, o conhecido ranço (NOR ADILAH et al., 2020 ;ASDAGH; PIRSA, 2020 ;AHMED et al., 2017).

Os antioxidantes são capazes de transferir átomos de hidrogênio, principalmente pela presença de compostos fenólicos e estabilizar os radicais livres formados durante a oxidação. Isso influenciará diretamente a produção de hidroperóxidos e peróxidos e a limitação dos compostos secundários da oxidação (NOR ADILAH et al., 2020 ; AHMED et al., 2017).

Os compostos antioxidantes usados em embalagens ativas podem ser divididos em antioxidantes sintéticos, como hidroxianisolbutilado (BHA) e hidroxitoluenobutilado (BHT) (KUAI et al., 2021). E antioxidantes naturais que são provenientes dos óleos essenciais ou vegetais e os extratos de plantas e a sua alta atividade antioxidante ocorre principalmente pela presença de compostos fenólicos (SAHRAEE et al., 2019).

Nos últimos anos, surgiu a preocupação que os antioxidantes sintéticos sejam potencialmente prejudiciais para a saúde humana por ser cancerígeno, fato que levou a indústria de alimentos e pesquisadores a buscar outras substâncias naturais (óleos e extratos

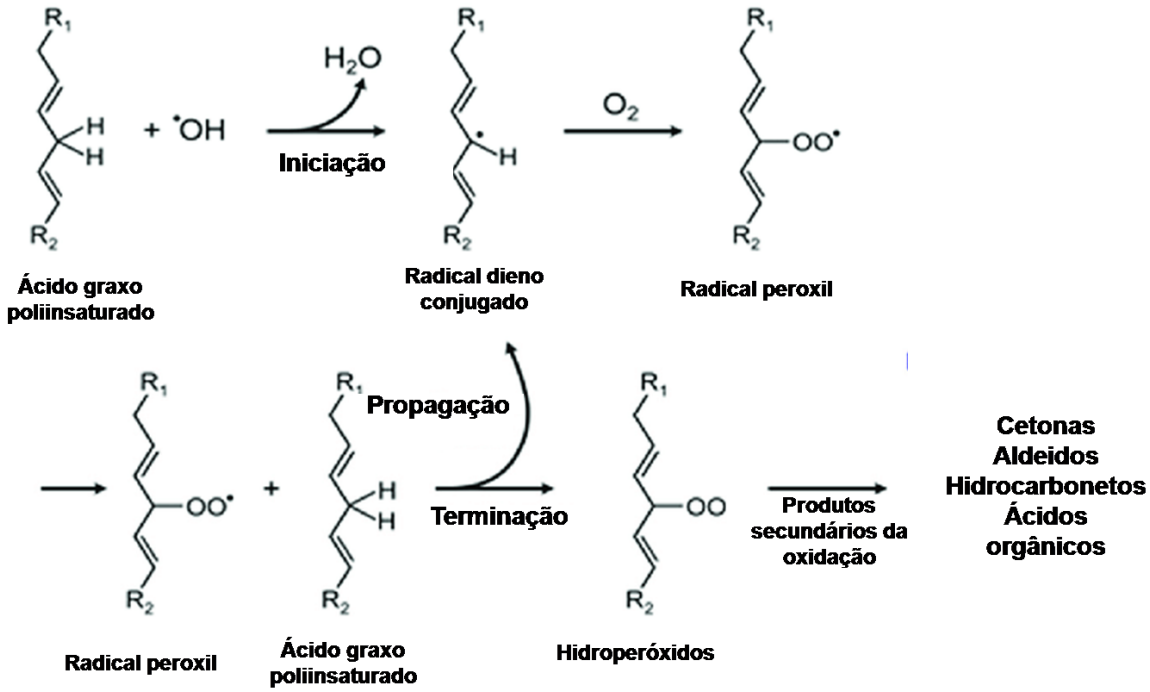
de plantas) que tenha potencial para a inclusão nas embalagens (VILELA et al., 2018). É essencial que o antioxidante utilizado nas embalagens ativas deve ser de baixo custo, não apresentar toxicidade, exibir forte permeabilidade e possuir boa estabilidade nas condições em que será aplicado. E ainda não pode afetar diversas propriedades do alimento como seu sabor, textura e cor (KUAI et al., 2021).

A atividade antioxidante dos compostos em alimentos e ou em filmes pode ser analisada por diferentes métodos devido aos variados modos de ação que o processo de oxidação pode apresentar. O DPPH **(**2,2-difenil-1-picril-hidrazil) é uma molécula de radical livre que apresenta coloração roxa quando dissolvido em metanol ou etanol. Ao reagir com compostos antioxidantes, o radical livre DPPH recebe átomos de hidrogênio dessas moléculas, ocasionando a redução da solução e mudança da cor roxa para amarela (WATHONI et al., 2019 ;NISAR et al., 2018 ;NISAR et al., 2018).

A atividade antioxidante avaliada pelo sistema β-Caroteno/Ácido linoléico é testada quanto à capacidade de proteção do β-caroteno contra a descoloração em um sistema contendo ácido linoléico. A presença do antioxidante na amostra inibe a formação de radicais livres que são gerados durante a peroxidação do ácido linoléico e isso faz com que o sistema mantenha a coloração inicial (JRIDI et al., 2017 ;JRIDI et al., 2020).

A análise antioxidante pelo método fosfomolibdênio ocorre por meio da formação de um complexo de fosfomolibdênio V pela presença do antioxidante. A solução em questão apresenta coloração verde devido a redução de $Mo^{+6}$ para $Mo^{+5}$e absorbância é medida em 695 nm e é comparada com uma curva padrão com diferentes concentrações de ácido ascórbico(HIFNEY et al., 2016).

Figura 5: Mecanismo de formação dos produtos secundários da oxidação




Fonte: Adaptado de Achyuthan et al. (2017)

## 2.5 Produtos alimentícios utilizados

### 2.5.1 Presunto

O presunto cozido é definido como um produto cárneo preparado exclusivamente por carne suína, salmoura, pode ser prensado e é realizado um tratamento térmico. E ainda podem ser adicionados ingredientes opcionais como: açúcares, aromatizantes, proteínas cárneas e não cárneas, amidos e aditivos(DIAS et al., 2020). Mas para ter um produto de qualidade é essencial que as carnes utilizadas como matérias-primas sejam de qualidade e que o produto final apresente a cor vermelha cereja da carne fresca. É essencial o monitoramento da cor da carne em todas as etapas do processamento incluindo o produto final(DIAS et al., 2020; HALAGARDA; WÓJCIAK, 2022).

Muitos grupos de microrganismos são responsáveis pela deterioração dos produtos cárneos incluindo o presunto cozido em condições apropriadas. O principal grupo bacteriano associado à deterioração do presunto é o das bactérias do ácido lático (BAL) (VESELÁ et al., 2022). Já as bactérias patogênicas mais comuns encontradas no presunto incluem cepas de *Salmonella, Listeria monocytogenes* , *Bacillus cereus* , *Clostridium botulinum* e *Escherichia coli*. *Listeria monocytogenes* é uma das maiores responsáveis por infecção bacteriana e mais agressiva, causando a listeriose que resulta em altas taxas de mortalidade. *Escherichia coli*

verotoxigênica também é um patógeno perigoso (CHO; HA, 2019; GRACIA-VALLÉS et al., 2022).

O crescimento de microrganismos em amostras no presunto é limitado por diversos fatores que incluem as condições microaerofílicas do produto, concentração de cloreto de sódio e nitrito de sódio e pela atividade de água (GRACIA-VALLÉS et al., 2022; VESELÁ et al., 2022). O presunto cozido é produto cárneo muito vulnerável a contaminação por microrganismos por apresentarem baixo teor de sal, pH próximo do neutro ( $\approx 6,0$), alta atividade de água (>0,9) e manuseio pós-processamento (CORRÊA et al., 2021). A contaminação microbiológica cruzada é principal fator e pode ser causado pelo contato direto com pessoal, aerossóis, utensílios e equipamentos contaminados, principalmente durante as etapas de desossa, compressão e fatiamento (GRACIA-VALLÉS et al., 2022).No entanto o fatiamento e a embalagem utilizada para o presunto cozido são fontes de contaminação dos patógenos de origem alimentar (CHO; HA, 2019).

A auto-oxidação ocorre com reações entre os ácidos graxos com o oxigênio e como resultado há a formação dos compostos primários como os hidroperóxidos e estes são quebrados em compostos secundários da oxidação como as cetonas, aldeídos entre outros e que causam o sabor estranho. Essas reações de oxidação são catalisadas pelaluz, calor, oxigênio, fotossensibilizadores e íons de metais de transição como ferro e cobre (SHAHIDI; HOSSAIN, 2022).

### 2.5.2 Manteiga

A manteiga é dos produtos lácteos mais antigos que são produzidos. É definido como um produto gorduroso derivado exclusivamente do leite, através do processo de batedura da nata pasteurizada. É caracterizada como uma emulsão do tipo água em óleo. A concentração mínima de gordura é de 80g/100g. A estrutura da manteiga consiste em gotas de água menores que 10 μm de diâmetro e são recobertas por cristais de gorduras que separam as gotas de água uma das outras impedindo o processo de coalescência (PĂDUREȚ, 2021; SOUNDOUS et al., 2019).

Além do seu alto teor de gordura a manteiga possui grandes quantidades de vitamina A, vitamina E, colesterol e em menores quantidades cálcio, fósforo, vitamina D e vitamina K e também um baixo teor de proteínas. A cor da manteiga é dada pela presença de caroteno (licopeno), vitamina A e outros pigmentos lipossolúveis. A sua fração lipídica é composta por triacilgliceróis (98%) e pequenas quantidades de monoacilgliceróis e diacilgliceróis,

glicolipídeos, éter lipídios, ácidos graxos livres, fosfolipídios e esteróis (PĂDUREŢ, 2021).

Devido ao seu alto teor de gorduras a manteiga é um produto alimentício propício a oxidação lipídica. Há grande variedade de produtos de degradação secundária de diferentes classes de compostos, como alcanos, álcoois, ésteres, aldeídos e cetonas que são formados durante as reações de oxidação. Esses compostos são responsáveis por provocar alterações sensoriais significativas. Além das alterações sensoriais a oxidação lipídica está associada à perda de valor nutricional dos alimentos porque as funções essenciais dos ácidos graxos ômega-3 e ômega-6 são reduzidas (GREBENTEUCH et al., 2021).

A manteiga é produto alimentício que contêm uma quantidade normalmente alta de gordura e baixos níveis de água e, portanto, têm baixa atividade água (aw). Essa baixa atividade água faz com que a maioria das bactérias não possa crescer causando doenças, deterioração ou produzir toxinas. Entretanto, o fato que elas não se reproduzem não significa que possam sobreviver em alimentos com baixa atividade água. Bactérias patogênicas como *Salmonella enterica* , *Listeria monocytogenes* e *Cronobacter* podem permanecer viáveis em alimentos com baixa atividade água por meses ou até anos e causar doenças quando consumidas (LIU et al., 2022).

## 3 CONSIDERAÇÕES FINAIS

Os resultados obtidos neste estudo demonstram que a combinação de nanoestruturas (nanoemulsão e nanoargila), com biopolímeros (pectina e acetato de celulose) para filmes com o proposito de aplicação em produtos alimentícios foram capazes de provocar alterações nas propriedades de barreiras com reduções na permeabilidade ao vapor de água. Também foi verificado um aumento nas propriedades antioxidantes e antimicrobianas.

A aplicação dessas embalagens nos produtos alimentícios foi eficiente para a redução na oxidação tanto da manteiga como do presunto. Também foi observada uma redução nas contagens de bactérias do ácido lático, psicotróficos e mesófilos nas amostras de presunto.
Dessa maneira a utilização dos bionacompósitos produzidos são promissores para a utilização como embalagens ativas. Que tem o propósito de garantir uma maior qualidade nos produtos alimentícios e prolongar a vida de prateleira dos mesmos.

## REFERÊNCIAS

ACHYUTHAN, K. et al. Volatile Metabolites Emission by In Vivo Microalgae—An Overlooked Opportunity? **Metabolites**, v. 7, p. 39, 31 jul. 2017.

AHMED, I. et al. A comprehensive review on the application of active packaging technologies to muscle foods. **Food Control**, v. 82, dez. 2017.

AL-TAYYAR, N. A.; YOUSSEF, A. M.; AL-HINDI, R. R. Edible coatings and antimicrobial nanoemulsions for enhancing shelf life and reducing foodborne pathogens of fruits and vegetables: A review. **Sustainable Materials and Technologies**, V.26, dez. 2020.

ALI, S. S. et al. Plastic wastes biodegradation: Mechanisms, challenges and future prospects. **Science of The Total Environment**, v. 780, p. 146590, ago. 2021.

ALMASI, H.; AZIZI, S.; AMJADI, S. Development and characterization of pectin films activated by nanoemulsion and Pickering emulsion stabilized marjoram (Origanum majorana L.) essential oil. **Food Hydrocolloids**, v. 99, n. June 2019, p. 105338, 2020.

ÁLVAREZ-MARTÍNEZ, F. J. et al. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action.**Phytomedicine**, V.90,set. 2021.

AMIRI, E. et al. Combined antioxidant and sensory effects of corn starch films with nanoemulsion of Zataria multiflora essential oil fortified with cinnamaldehyde on fresh ground beef patties. **Meat Science**, v. 153, p. 66–74, 1 jul. 2019.

ARSHAD, R. et al. Exploring the potential of active edible coating on the shelf stability of dairy products. **Pakistan Journal of Agricultural Sciences**, v. 57, n. 1, p. 237–244, 1 jan. 2020.

ASDAGH, A.; PIRSA, S. Bacterial and oxidative control of local butter with smart/active film based on pectin/nanoclay/Carum copticum essential oils/β-carotene. **International Journal of Biological Macromolecules**, v. 165, p. 156–168, 15 dez. 2020.

ASGHER, M. et al. Bio-based active food packaging materials: Sustainable alternative to conventional petrochemical-based packaging materials.**Food Research International**, V.137, 1 nov. 2020.

AZMANA, M. et al. A review on chitosan and chitosan-based bionanocomposites: Promising material for combatting global issues and its applications International.**Journal of Biological Macromolecules, V.185, p.** 832-848, ago. 2021.

BARRADAS, T. N.; DE HOLANDA E SILVA, K. G. Nanoemulsions of essential oils to improve solubility, stability and permeability: a review. **Environmental Chemistry Letters**.v.19, p.1153–1171, abr. 2021.

CHAN, S. Y. et al. Pectin as a rheology modifier: Origin, structure, commercial production and rheology.**Carbohydrate Polymers,** v.161, p.118-139, abr. 2017.

CHO, G.-L.; HA, J.-W. Application of X-ray for inactivation of foodborne pathogens in ready-to-eat sliced ham and mechanism of the bactericidal action. **Food Control**, v. 96, p. 343–350, fev. 2019.

CORRÊA, J. A. F. et al. Combined application of phenolic acids and essential oil components against Salmonella Enteritidis and Listeria monocytogenes in vitro and in ready-to-eat cooked ham. **LWT**, v. 149, p. 111881, set. 2021.

DA SILVA SCUDELER, C. G. et al. Development and characterization of Nile tilapia (Oreochromis niloticus) protein isolate-based biopolymer films incorporated with essential oils and nanoclay. **Food Packaging and Shelf Life**, v. 25, 1 set. 2020.

DAS, M. et al. In vitro evaluation of antioxidant and antibacterial properties of supercritical CO2 extracted essential oil from clove bud (Syzygium aromaticum). **Journal of Plant Biochemistry and Biotechnology**, v. 30, n. 2, p. 387–391, 23 jun. 2021.

DIAS, S. et al. Application of Natural Pigments in Ordinary Cooked Ham. **Molecules**, v. 25, n. 9, p. 2241, 10 maio 2020.

DOMÍNGUEZ, R. et al. Active packaging films with natural antioxidants to be used in meat industry: A review. **Food Research International**, v. 113, p. 93–101, 1 nov. 2018.

ECHEVERRÍA, I. et al. Active nanocomposite films based on soy proteins-montmorillonite-clove essential oil for the preservation of refrigerated bluefin tuna (Thunnus thynnus) fillets. **International Journal of Food Microbiology**, v. 266, p. 142–149, 2 fev. 2018.

ESPITIA, P. J. P. et al. Edible films from pectin: Physical-mechanical and antimicrobial properties - A review.**Food Hydrocolloids**, mar. 2014.

G. AL-HASHIMI, A. et al. Development of a Millet Starch Edible Film Containing Clove Essential Oil. **Foods**, v. 9, n. 2, p. 184, 13 fev. 2020.

GEORGE, A. et al. A comprehensive review on chemical properties and applications of biopolymers and their composites. **International Journal of Biological Macromolecules**, v. 154, jul. 2020.

GHANI, S. et al. The preparation, characterization and in vitro application evaluation of soluble soybean polysaccharide films incorporated with cinnamon essential oil nanoemulsions. **International Journal of Biological Macromolecules**, v. 112, p. 197–202, 1 jun. 2018.

GRACIA-VALLÉS, N. et al. Developing ethyl lauroyl arginate antimicrobial films to combat Listeria monocytogenes in cured ham. **Food Control**, v. 141, p. 109164, nov. 2022.

GREBENTEUCH, S. et al. The Formation of Methyl Ketones during Lipid Oxidation at Elevated Temperatures. **Molecules**, v. 26, n. 4, p. 1104, 19 fev. 2021.

GUIMARÃES, A. L. et al. Antimicrobial Activity of Copaiba (Copaifera officinalis) and Pracaxi (Pentaclethra macroloba) Oils against Staphylococcus Aureus: Importance in Compounding for Wound Care. **International journal of pharmaceutical compounding**, v.

20, n. 1, p. 58–62, 2016.

GUTIÉRREZ-JARA, C. et al. Physical, mechanical and transport properties of emulsified films based on alginate with soybean oil: Effects of soybean oil concentration, number of passes and degree of surface crosslinking. **Food Hydrocolloids**, v. 109, 1 dez. 2020.

HALAGARDA, M.; WÓJCIAK, K. M. Health and safety aspects of traditional European meat products. A review. **Meat Science**, v. 184, p. 108623, fev. 2022.

HAN, H. S.; SONG, K. BIN. Antioxidant properties of watermelon (Citrullus lanatus) rind pectin films containing kiwifruit (Actinidia chinensis) peel extract and their application as chicken thigh packaging. **Food Packaging and Shelf Life**, v. 28, 1 jun. 2021.

HARO-GONZÁLEZ, J. N. et al. Clove Essential Oil (Syzygium aromaticum L. Myrtaceae): Extraction, Chemical Composition, Food Applications, and Essential Bioactivity for Human Health. **Molecules**, v. 26, n. 21, p. 6387, 22 out. 2021.

HASHEMINYA, S. M.; DEHGHANNYA, J. Development and characterization of novel edible films based on Cordia dichotoma gum incorporated with Salvia mirzayanii essential oil nanoemulsion. **Carbohydrate Polymers**, v. 257, 1 abr. 2021.

HIFNEY, A. F. et al. Industrial optimization of fucoidan extraction from Sargassum sp. and its potential antioxidant and emulsifying activities. **Food Hydrocolloids**, v. 54, p. 77–88, 1 mar. 2016.

IAMAREERAT, B. et al. Reinforced cassava starch based edible film incorporated with essential oil and sodium bentonite nanoclay as food packaging material. **Journal of Food Science and Technology**, v. 55, n. 5, p. 1953–1959, 1 maio 2018.

ISSA, A.; IBRAHIM, S. A.; TAHERGORABI, R. Impact of sweet potato starch-based nanocomposite films activated with thyme essential oil on the shelf-life of baby spinach leaves. **Foods**, v. 6, n. 6, p. 1–13, 1 jun. 2017.

JRIDI, M. et al. Wound healing activity of cuttlefish gelatin gels and films enriched by henna (Lawsonia inermis) extract. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 512, p. 71–79, 1 jan. 2017.

JRIDI, M. et al. Physicochemical, antioxidant and antibacterial properties of fish gelatin-based edible films enriched with orange peel pectin: Wrapping application. **Food Hydrocolloids**, v. 103, 1 jun. 2020.

KUAI, L. et al. Controlled release of antioxidants from active food packaging: A review. **Food Hydrocolloids,** v. 120, nov. 2021.

LEE, J. Y. et al. Antibacterial and antioxidant properties of hydroxypropyl methylcellulose-based active composite films incorporating oregano essential oil nanoemulsions. **LWT**, v. 106, p. 164–171, 1 jun. 2019.

LEI, Y. et al. Investigation of the structural and physical properties, antioxidant and antimicrobial activity of pectin-konjac glucomannan composite edible films incorporated with

tea polyphenol. **Food Hydrocolloids**, v. 94, set. 2019.

LIU, S. et al. Recent development in low-moisture foods: Microbial safety and thermal process. **Food Research International**, v. 155, p. 111072, maio 2022.

MATHEUS, J. R. V. et al. Antibacterial films made with persimmon ( Diospyros kaki L.), pectin, and glycerol: An experimental design approach . **Journal of Food Science**, 24 ago. 2021.

MENZEL, C. et al. Antioxidant starch films containing sunflower hull extracts. **Carbohydrate Polymers**, v. 214, p. 142–151, 15 jun. 2019.

MOHAMED, S. A. A.; EL-SAKHAWY, M.; EL-SAKHAWY, M. A. M. Polysaccharides, Protein and Lipid -Based Natural Edible Films in Food Packaging: A Review. **Carbohydrate Polymers,** v. 238,jun. 2020.

NISAR, T. et al. Characterization of citrus pectin films integrated with clove bud essential oil: Physical, thermal, barrier, antioxidant and antibacterial properties. **International Journal of Biological Macromolecules**, v. 106, p. 670–680, jan. 2018.

NOR ADILAH, A. et al. Development of polyethylene films coated with gelatin and mango peel extract and the effect on the quality of margarine. **Food Packaging and Shelf Life**, v. 26, 1 dez. 2020.

NORCINO, L. B. et al. Pectin films loaded with copaiba oil nanoemulsions for potential use as bio-based active packaging. **Food Hydrocolloids**, v. 106, 1 set. 2020.

PĂDUREŢ, S. The Effect of Fat Content and Fatty Acids Composition on Color and Textural Properties of Butter. **Molecules**, v. 26, n. 15, p. 4565, 28 jul. 2021.

PATEIRO, M. et al. Application of essential oils as antimicrobial agents against spoilage and pathogenic microorganisms in meat products. **International Journal of Food Microbiology**, v. 337, 16 jan. 2021.

PERERA, K. Y. et al. Seaweed Polysaccharide in Food Contact Materials (Active Packaging, Intelligent Packaging, Edible Films, and Coatings). **Foods**, v. 10, n. 9, p. 2088, 3 set. 2021.

PIRES, C. L. et al. Pracaxi oil affects xenobiotic metabolisms, cellular proliferation, and oxidative stress without cytotogenotoxic effects in HepG2/C3A cells. **Toxicology in Vitro**, v. 83, p. 105392, set. 2022.

PRAKASH, A. et al. Essential oil based nanoemulsions to improve the microbial quality of minimally processed fruits and vegetables: A review.**Food Research International,** v.111, p. 509-523, set. 2018.

QIAN, M. et al.A review of active packaging in bakery products: Applications and future trends. **Trends in Food Science and Technology,** v. 114, p. 459-471, ago. 2021.

RADÜNZ, M. et al. Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (Syzygium aromaticum, L.) essential oil. **Food Chemistry**, v. 276, p.

180–186, mar. 2019.

RAI, P. et al. Recent advances in the sustainable design and applications of biodegradable polymers. **Bioresource Technology**, v. 325, p. 124739, abr. 2021.

RAMBABU, K. et al. Mango leaf extract incorporated chitosan antioxidant film for active food packaging. **International Journal of Biological Macromolecules**, v. 126, p. 1234–1243, 1 abr. 2019.

RAO, J.; CHEN, B.; MCCLEMENTS, D. J. Improving the Efficacy of Essential Oils as Antimicrobials in Foods: Mechanisms of Action. **Annual Review of Food Science and Technology**, v. 10, n. 1, p. 365–387, 25 mar. 2019.

REHMAN, A. et al. Pectin polymers as wall materials for the nano-encapsulation of bioactive compounds. **Trends in Food Science & Technology**, v. 90, ago. 2019.

RHIM, J.-W.; PARK, H.-M.; HA, C.-S. Bio-nanocomposites for food packaging applications. **Progress in Polymer Science**, v. 38, n. 10–11, out. 2013.

RIAZ, A. et al. Chitosan-based biodegradable active food packaging film containing Chinese chive (Allium tuberosum) root extract for food application. **International Journal of Biological Macromolecules**, v. 150, p. 595–604, 1 maio 2020.

ROMERA-CASTILLO, C. et al. Abiotic plastic leaching contributes to ocean acidification. **Science of The Total Environment**, v. 854, p. 158683, jan. 2023.

SAHRAEE, S. et al. Protection of foods against oxidative deterioration using edible films and coatings: A review. **Food Bioscience**, v. 32, dez. 2019.

SAMROT, A. V. et al. Production, characterization and application of nanocarriers made of polysaccharides, proteins, bio-polyesters and other biopolymers: A review.**International Journal of Biological Macromolecules,** v.165, p. 3088-3105, dez. 2020.

SELLES, S. M. A. et al. Chemical composition, in-vitro antibacterial and antioxidant activities of Syzygium aromaticum essential oil. **Journal of Food Measurement and Characterization**, v. 14, n. 4, p. 2352–2358, 13 ago. 2020.

SERRANO-LEÓN, J. S. et al. Chitosan active films containing agro-industrial residue extracts for shelf life extension of chicken restructured product. **Food Research International**, v. 108, jun. 2018.

SGANZERLA, W. G. et al. Bioactive food packaging based on starch, citric pectin and functionalized with Acca sellowiana waste by-product: Characterization and application in the postharvest conservation of apple. **International Journal of Biological Macromolecules**, v. 147, mar. 2020.

SHAHIDI, F.; HOSSAIN, A. Role of Lipids in Food Flavor Generation. **Molecules**, v. 27, n. 15, p. 5014, 6 ago. 2022.

SHARMA, R.; JAFARI, S. M.; SHARMA, S. Antimicrobial bio-nanocomposites and their

potential applications in food packaging. **Food Control**, v. 112, jun. 2020.

SHOKRI, S. et al. Application an edible active coating based on chitosan- Ferulago angulata essential oil nanoemulsion to shelf life extension of Rainbow trout fillets stored at 4 °C. **International Journal of Biological Macromolecules**, v. 153, p. 846–854, 15 jun. 2020.

SIVAKANTHAN, S. et al. Antioxidant and antimicrobial applications of biopolymers: A review. **Food Research International, v. 136,** 1 out. 2020.

SOUNDOUS, E. et al. Overview of the local production process of raw milk butter in Wallonia (Belgium). **International Journal of Dairy Technology**, p. 1471– 0307.12608, 29 maio 2019.

SOUZA, K. S. S. DE. **Perfil glicêmico, expressão de marcadores inflamatórios e metabolismo hepático de camundongos alimentados com bebida láctea fermentada adicionada de óleo essencial de Syzygium aromaticum.**Dissertação (Mestrado em Produção Animal) - Universidade Federal de Minas Gerais (UFMG), Montes Claros, 2019.

TEIXEIRA, G. L. et al. Composition, thermal behavior and antioxidant activity of pracaxi (Pentaclethra macroloba) seed oil obtained by supercritical CO2. **Biocatalysis and Agricultural Biotechnology**, v. 24, 1 mar. 2020.

TELES, A. M. et al. GC-MS Characterization of Antibacterial, Antioxidant, and Antitrypanosomal Activity of Syzygium aromaticum Essential Oil and Eugenol. **Evidence-Based Complementary and Alternative Medicine**, v. 2021, p. 1–12, 20 fev. 2021.

VALDIVIESO-UGARTE, M. et al. Antimicrobial, Antioxidant, and Immunomodulatory Properties of Essential Oils: A Systematic Review. **Nutrients**, v. 11, n. 11, 15 nov. 2019.

VARGHESE, S. A.; SIENGCHIN, S.; PARAMESWARANPILLAI, J. Essential oils as antimicrobial agents in biopolymer-based food packaging - A comprehensive review. **Food Bioscience**, v. 38, 1 dez. 2020.

VESELÁ, H. et al. The Pork Meat or the Environment of the Production Facility? The Effect of Individual Technological Steps on the Bacterial Contamination in Cooked Hams. **Microorganisms**, v. 10, n. 6, p. 1106, 27 maio 2022.

VILELA, C. et al. A concise guide to active agents for active food packaging.**Trends in Food Science and Technology**, v. 80, p. 212-222, out. 2018.

WATHONI, N. et al. Characterization and antioxidant activity of pectin from Indonesian mangosteen (Garcinia mangostana L.) rind. **Heliyon**, v. 5, n. 8, 1 ago. 2019.

XIONG CHANG, X. et al. A review on the properties and applications of chitosan, cellulose and deep eutectic solvent in green chemistry. **Journal of Industrial and Engineering Chemistry**, ago. 2021.

YOUSSEF, A. M.; EL-SAYED, S. M. Bionanocomposites materials for food packaging applications: Concepts and future outlook.**Carbohydrate Polymers,** v. 193, p. 19-27, 1 ago. 2018.

YUKUYAMA, M. N. et al. Nanoemulsion: Process selection and application in cosmetics - A review**. International Journal of Cosmetic Science, v.38, p.** 13-24, 1 fev. 2016.

ZANETTI, M. et al. Use of encapsulated natural compounds as antimicrobial additives in food packaging: A brief review. **Trends in Food Science & Technology**, v. 81, nov. 2018.

ZHONG, Y. et al. Biodegradable polymers and green-based antimicrobial packaging materials: A mini-review.**Advanced Industrial and Engineering Polymer Research,** v. 3, p. 27-35, 1 jan. 2020.

ZHOU, X. et al. Upcycling of real-world HDPE plastic wastes into high-purity methane and hierarchical porous carbon materials: Influence of plastics additives. **Journal of Environmental Chemical Engineering**, v. 11, n. 2, p. 109327, abr. 2023.

## SEGUNDA PARTE – ARTIGOS

## ARTIGO 1 - Bionanocomposites of pectin and pracaxi oil nanoemulsion as active packaging for butter

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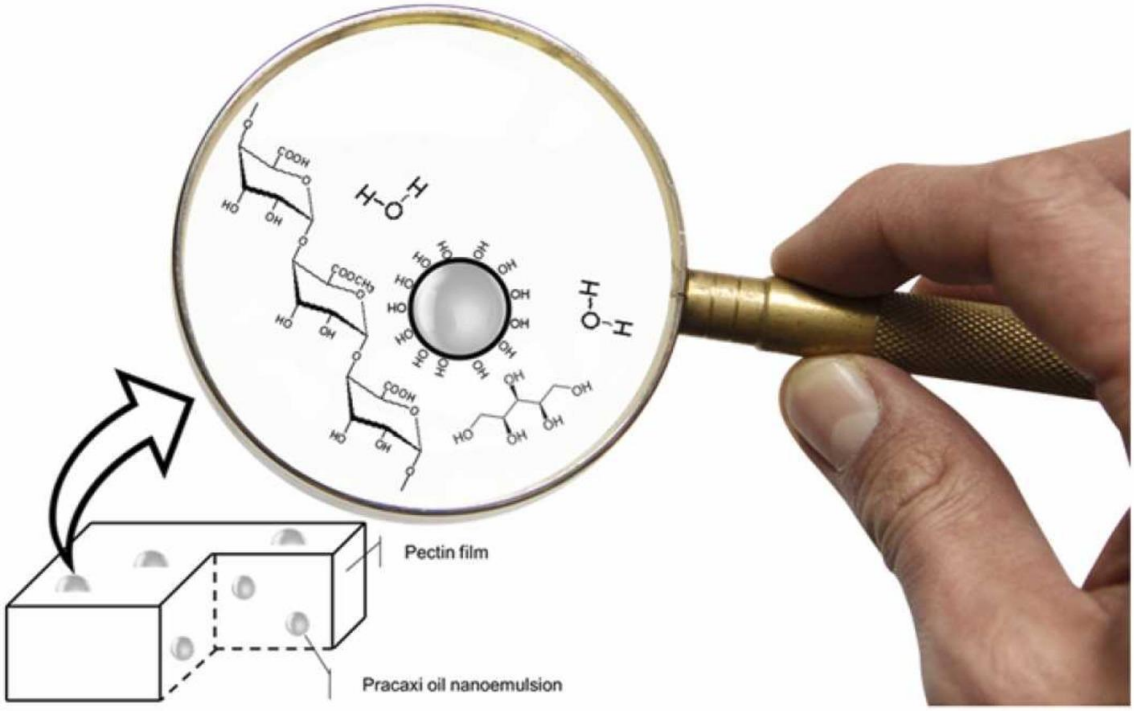
**Bionanocomposites of pectin and pracaxi oil nanoemulsion as active packaging for butter**

### GRAPHICAL ABSTRACT




### ABSTRACT

A new active packaging with antioxidant action was designed and developed. It consisted of a bionanocomposite in which different concentrations of a pracaxi oil nanoemulsion were incorporated into a plasticized xylitol pectin matrix. The water vapor barrier properties and the thermodynamic parameters associated with water adsorption were investigated, where the

experimental data pointed out that the addition of the pracaxi oil nanoemulsionincreased the activation energy of water vapor permeation in the films and consequently reduced the permeability of the bionanocomposites. In addition, the nanoemulsion increased the number of active sites on the surface of the films, which favored water adsorption on the surface of the films. Also, the results showed that, in comparison with the pectin film, the bionanocomposite containing the pracaxi oil nanoemulsion efficiently improved the stability of butter samples against oxidation processes and was a promising strategy for extending the shelf life of butter.

**Keywords:** Edible film; biopolymer; natural antioxidant; water vapor permeability; sorption isotherms.

## 1. INTRODUCTION

The development of novel food packaging from biopolymers (Kanmani & Rhim, 2014) is a field of great scientific and technological interest that is largely driven by consumer demands for fresher, safer and more nutritious foods (Grunert, 2005). Aspects associated with food safety require that the nutritional quality of food be preserved for longer periods of time. Due to these standards, in recent decades, several researchers have proposed new active packaging that can meet these challenges (Rhim et al., 2013).

Pectin (PEC) is a polysaccharide found in the cell wall and intercellular regions of plants and fruits (Espitia et al., 2014). It can be extracted from citrus and apple fruit residues. They are anionic, amorphous, nontoxic biopolymers that are easily solubilized in water. Moreover, this biopolymer is widely employed in the production of bionanocomposites with potential applications in active packaging (Shivangi et al., 2021). Pectin films have a good oxygen barrier, have good hardness and adhesiveness, however they are fragile, rigid, sensitive to water and with high permeability to water vapor (Rodsamran & Sothornvit, 2019). However, the use of nanoemulsions can contribute to improving the characteristics of pectin films. In studies of pectin films with copaiba oil nanoemulsions, there was a decrease in water vapor permeability, an increase in extensibility and a decrease in stiffness in addition to an increase in antimicrobial properties (Norcino et al., 2020). The use of *Origanum majorana L*. oil nanoemulsion in films also resulted in a decrease in water sorption, a decrease in water vapor permeability, an increase in mechanical properties and also an increase in antioxidant and antimicrobial properties (Almasi et al., 2020).

Active packaging, in particular, packaging with antioxidant activity, includes active agents that interact with food, either by stabilizing reactive chemical species, such as free radicals present in the medium, or by releasing antioxidant compounds to slow degradation due to lipid oxidation (Kuai et al., 2021). Lipid oxidation in food involves the loss of quality in products such as meats (Li & Liu, 2012), nuts (Buransompob et al., 2003) and butter (Ozturk & Cakmakci, 2006). This loss of quality associated with lipid oxidation reactions leads to unpleasant odor and flavor, reduced nutritional value, and changes in food texture and color. The sum of all these factors leads to a significant reduction in the shelf life of the product.

Antioxidant agents can be incorporated into active packaging through different routes, such as in the form of additives in the polymer matrix, in multilayer films, or through functionalization of the packaging surface that is in direct contact with the food (Gómez-

Estaca et al., 2014).

In recent decades, antioxidant compounds of natural origin, such as ginger (Alexandre et al., 2016) and clove (Nisar et al., 2018) essential oils and green tea plant extracts (Siripatrawan & Noipha, 2012), have been successfully applied to prevent lipid oxidation in fish and poultry meat, oils and mushrooms. However, many of these natural products present challenges due to their immiscibility with the polymer matrix, high volatility, and low thermal stability. Thus, the incorporation of natural antioxidant agents that have lower volatility, such as vegetable oils (Dhavamani et al., 2014), into biopolymeric films shows a promising alternative in the development of active packaging.

Pracaxi oil is extracted from the seed of the *Pentaclethra macroloba* tree, a plant found naturally in the Brazilian Amazon rainforest. The seeds are edible and are able to provide 45-48% oil. This oil is rich in monounsaturated fatty acids, with the main component being oleic acid (47.3 – 53.5%), also presenting an expressive content of behenic acid (16.1 – 25.5%), followed by linoleic acid (11.7 – 13.1%) and lignoceric acid (12.5%) (Teixeira et al., 2020).

In this study, we describe the incorporation of pracaxi oil nanoemulsions for the formation of bionanocomposites, which present high antioxidant activity and thermal stability associated with good interaction with the pectin matrix. The bionanocomposites were evaluated in terms of their barrier properties and butter preservation efficiency.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Highly methoxylated citrus pectin (PEC) with a poly(galacturonic acid) content of 84%, degree of methoxylation (MD) of 8.4% and average molecular weight (Mw) of $1.3x10^5$ g $mol^{-1}$ was kindly provided by CP Kelco (Limeira, SP, Brazil). Pracaxi oil, extracted from seeds of Pracaxi (Pentaclethara filamentosa – Benth), was purchased from GRAN OILS (São Paulo, Brazil). Xylitol and Tween 80 were purchased from Synth (Diadema, SP, Brazil). Deionized water ($\rho$> 18.2 MΩ cm) was obtained from a reverse osmosis system (Hidrotek, RO20, China).

### 2.2 Preparation of Nanoemulsions

Emulsions were prepared following the method described by Noori et al. (2018), with modifications. Direct oil-in-water (O/W) emulsions were prepared by adding pracaxioil at 0,

0.1, 0.2, 0.3, and 0.4% wt. in deionized water and Tween 80 (20% v/v oil). Then, both the oil and aqueous phases were mixed via mechanical stirring (Ultraturrax, IKA T10, USA) at 14500 rpm for 10 min at room temperature to obtain the coarse emulsion. Then, ultrasonic emulsification was used to convert the coarse emulsion into a nanoemulsion.

An ultrasonic tip sonicator (Ultronique, Desruptor, Indaiatuba-SP) operating at 750 W (30% amplitude) was used for nanoemulsification. The probe of the device, which had a diameter of 4 mm, was immersed in the coarse emulsion at a depth of 25 mm and carried out for 20 min (four cycles of 5 minutes). The obtained nanoemulsions were stored in an amber glass vial with a screw-on lid and kept in the dark.

### 2.3 Production of Bionanocomposites

Pectin (3% by mass) was dissolved in the previously prepared nanoemulsions, and the mixture was thoroughly homogenized using a magnetic stirrer for 5 h. Xylitol was then added at 30% by mass (dry basis) and stirred for another 1 h. Finally, 50 mL of the PEC and PEC/nanoemulsion formulations were poured into Petri dishes (15 cm diameter). The plates were dried in a circulating air oven at 37 °C for 24 h. The dried films were removed from the plates and stored under ambient conditions (approximately 25 °C and 53% relative humidity) for 3 days before characterization.

### 2.4 Scanning Electron Microscopy (SEM)

Morphological characterization was performed using a JSM-6510 microscope (JEOL Ltd., Japan) at 5 kV and a working distance between 2 and 3 mm. Cross-sectional views of the polymer samples were prepared by freeze fracturing in a liquid nitrogen bath (-165 °C for 3 minutes). All samples were coated with gold (~5 nm) in an argon atmosphere. The samples were prepared by powder deposition with conductive graphite ink (SPI Supplies, USA). All images were recorded in secondary electron mode.

### 2.5 Water Sorption Isotherms

Environments of constant relative humidity were established inside desiccators using concentrated salt solutions. The salts used were LiCl, NaCl, $MgCl_2$, KCl, and $K_2SO_4$, as recommended by ASTM E104-02 (ASTM, 2012), to cover water activities ranging from 0.112 and 0.985. Table S1 shows the water activity for all saturated salt solutions at different

temperatures. All salts used were of analytical grade.

The film samples were cut into approximately 2cm$^2$ pieces, weighed, placed in petri dishes, and then stored in desiccators containing saturated salt solutions. The desiccators were kept inside a BOD (Bio-Oxygen Demand) incubatorand/or refrigerator at a constant temperature. The film samples were equilibrated in desiccators for 4 days before being weighed. Equilibration was considered successful when the difference between two consecutive weighings of the samples was less than 1 mg. Adsorption tests were performed in four repetitions for each sample. The moisture sorption was determined at 5, 35 and 45 °C.

The GAB (Guggenheim-Anderson-De Boer) model was fitted to the experimental data of equilibrium moisture content ($M$) as a function of water activity ($a_w$), according to Equation 1 (Tian et al., 2020).

$$M = \frac{X_m C K a_w}{(1-K a_w)(1-K a_w+C K a_w)} \quad (1)$$

where$X_m$ is the moisture content of the monolayer and $C$ and $K$ are constants.

The net isosteric heat of sorption ($q_{st}$), also known as differential enthalpy, can be defined as the difference between the integral isosteric heat of sorption ($Q_{st}$) and the latent heat of vaporization of water ($\Delta H_{vap}$) (Equation 2) and can be obtained from the Clausius-Clapeyron equation (Equation 3) at a constant $M$(Tian et al., 2020).

$$q_{st} = Q_{st} - \Delta H_{vap} \quad (2)$$

$$q_{st} = -R\left[\frac{\partial(\ln a_w)}{\partial(1/T)}\right]_M \quad (3)$$

where $R$ is the universal gas constant and $T$ is the absolute temperature.

The differential entropy ($S_d$) can be obtained by rearranging the van't Hoff Gibbs-Helmholtz equations, as shown in Equations 4 and 5 (Tian et al., 2020).

$$S_d = \frac{\Delta H-\Delta G}{T} = \frac{q_{st}-RT\ln a_w}{T} \quad (4)$$

$$\ln a_w = -\frac{q_{st}}{RT} + \frac{S_d}{R} \quad (5)$$

By plotting the values of $\ln a_w$ as a function of $1/T$ at a given $M$, the net isosteric heat of sorption ($q_{st}$) and differential entropy ($S_d$) can be calculated from the slope and intercept of the linear regression of the points.

**2.6 Water Vapor Permeability**

The determination of water vapor permeability (WVP) was performed according to the methodology described in ASTM E96 (ASTM, 2009). Glass vials of 40 mL and openings in a 14 mm diameter lid, with 75% of their volume containing silica previously dried for 24 h at 150 °C, were used. The bionanocomposite samples were cut with areas corresponding to the lid opening and placed between the lids and the vials. The fractions containing the sample and the silica were placed in a desiccator maintained at controlled temperature and relative humidity (saturated solution of $MgCl_2$, NaCl and $K_2SO_4$). To study the influence of temperature on WVP, the samples were stored at temperatures of 8, 25, 35 and 45 °C, in which the flasks were weighed every 24 h for a period of seven days.

WVP was calculated from the slope ($G$) of a linear regression of weight gain versus time, as shown in Equation 6.

$$WVP = \frac{Gx}{A\Delta p} \quad (6)$$

where $x$ is the film thickness, $A$ is the area of the exposed film and $\Delta p$ is the differential partial pressure of water vapor across the film.

This method uses Fick's first law and Henry's law to calculate the WVP and assumes that the solubility and diffusivity of the film are constant. The measured WVP values were corrected for the distance between the water level and the position of the film according to the procedures described in Bertuzzi et al. (2007). The activation energy for the water vapor permeation of the bionanocomposites was evaluated at different relative humidities (30-90%) by employing the Arrhenius equation, as shown in Equation 7.

$$WVP = WVP_o e^{\left(-E_a/RT\right)} \quad (7)$$

where $WVP$ is the water vapor permeability, $WVP_o$ is a constant, $E_a$ is the activation energy of permeation, $R$ is the gas constant and $T$ is the absolute temperature. With this equation, it is possible to evaluate the effect of relative humidity on the activation energy of the permeation process of water molecules by bionanocomposites.

**2.7 Wettability**

For wettability analysis, the static contact angle (CA) and discharge angle (ShA) of liquids on surfaces were measured using a contact angle analyzer (model Krüss DSA25B, Hamburg, Germany). Distilled water (WA), glycerol (GLY), 1-bromonaphthalene (BNL), and diiodomethane (DIM) were measured to examine the wettability of surfaces by liquids with

different surface energy components. The surface energy components of the different liquids used in this study are listed in Table S2.

For the CA measurement, 2.5 ± 0.5 µL liquid droplets were placed on a surface, and the CA was measured within 0.5 s after the droplets settled. At least 10 measurements at room temperature (approximately 25 ºC) were taken and the average value was obtained. The surface energy with its dispersive and polar components for solid surfaces was estimated using the Owens-Wendt model (Owens & Wendt, 1969). With the surface energy components of liquids known and by measuring the contact angle on the surface of the bionanocomposites, the surface energy components of solid substrates can be calculated, as shown in Equations 8 to 11 (Owens & Wendt, 1969). For the calculation, the contact angles of WA, GLY, BNL, and DIM were measured on the surfaces of the bionanocomposites.

$$\gamma_{SL} = \gamma_S + \gamma_L - 2\sqrt{\gamma_S^d . \gamma_L^d} - 2\sqrt{\gamma_S^p . \gamma_L^p} \quad (8)$$

$$\gamma_{SL} = \gamma_{SL} + \gamma_L cos\theta \quad (9)$$

$$\gamma_S(1 + cos\theta) = 2\sqrt{\gamma_S^d . \gamma_L^d} - 2\sqrt{\gamma_S^p . \gamma_L^p} \quad (10)$$

$$\gamma_S = \gamma_S^d + \gamma_S^p \quad (11)$$

where $\theta$ is the contact angle of a liquid on a solid surface, $\gamma_{SL}$ is the interfacial energy between a solid and liquid, $\gamma_S$ is the surface energy of solids, $\gamma_S^d$ is the dispersive surface energy component of a solid, $\gamma_S^p$ is the polar surface energy component of solids, $\gamma_L$ is the surface energy of a liquid, $\gamma_L^d$ is the dispersive surface energy component of a liquid, and $\gamma_L^p$ is the polar surface energy component of a liquid.

## 2.8 Antioxidant activity and compounds of pracaxi oil and bionanocomposites

### 2.8.1. Preparation of pracaxi oil extract

The oil extraction was performed according to the methodology proposed by Arranz et al. (2008) with minor modifications. Each oil sample (2.5 g) was added to 5 mL of methanol, and the mixture was shaken vigorously for 20 min and centrifuged at 2500 g for 10 min at 4 °C, and the supernatant was recovered. Another 5 mL of methanol was added, the same process was repeated, and the methanolic fractions were combined, frozen and stored protected from light for further analysis.

### 2.8.2 Preparation of the extract of bionanocomposites

The extract was prepared according to a procedure described by Martins et al. (2012) and used for the determination of total phenolic compound (TPC) content and antioxidant activities by the DPPH, β-carotene/linolenic acid and phosphomolybdenum methods. In a container, 100 mg of the bionanocomposites were cut into small pieces, and 2 mL of methanol-water (80:20, v/v) was added and stirred for 3 h at room temperature to obtain the extract.

### 2.8.3 Antioxidant activity by the DPPH method

The DPPH radical scavenging activityfor the oil and bionanocompositeswasdetermined according to the methodology described by Rufino et al. (2007). For the oil, 0.1 mL aliquots of oil extract and its dilutions were transferred to test tubes along with 3.9 mL of DPPH solution (0.06 mM) in triplicate. As for the bionanocomposites, 0.5 mL of the bionanocompositeextract was added to 2 mL of DPPH (0.06 mM) solution. In both cases, a methanol/acetone control solution was used. After 60 minutes of reaction in the dark, the remaining DPPH was determined by absorbance at 515 nm using a UV-VIS spectrophotometer (Nova Instruments). The antioxidant activity of the oil was expressed as IC50 mg $mL^{-1}$, which was the mass of the sample required to reduce the initial concentration of the DPPH radical by 50%. The percentage of antioxidant activityby the DPPH radical scavenging activity assay ($\%AA_{DPPH}$) in the bionanocomposites and the oil was calculated according to the following equation:

$$\%AA_{DPPH} = 100 \times \left[1 - \left(\frac{A_{sample}}{A_{control}}\right)\right] \quad (12)$$

where $A_{sample}$ is the absorbance of the sample solution and $A_{control}$is the absorbance of the DPPH solution without the sample.

### 2.8.4. Antioxidant activity by the β-Carotene/Linolenic acid method

The evaluation of antioxidant activity by the β-carotene bleaching assay was performed according to the methodologies presented by Lopes-Lutz et al.(2008) and Jridi et al.(2017) with some modifications. In a round bottom flask, 60 mg of linoleic acid, 600 mg of Tween 20, 6 mg of β-carotene, and 30 mL of chloroform were added. After homogenization, the chloroform was completely evaporated using a rotary evaporator at 50 °C. After the chloroform was removed, the residue was dissolved in 150 mL of saturated oxygen solution

(Emulsion A). Then, 0.4 mL of pracaxi oil extracts and bionanocomposites were mixed with 4mL of Emulsion A, and for the control, only Emulsion A was used. The reading was performed on a UV-VIS spectrophotometer (Nova Instruments) at 470 nm at intervals of 0 and 2 hours of incubation at room temperature without light. The percentage of antioxidant activity by theβ-carotene bleaching assay ($\%AA_{\beta-carotene}$) was calculated using the equation:

$$\%AA_{\beta-carotene} = 100 \times \left[\frac{(A_o - A_t)}{(A_{oo} - A_{ot})}\right] \quad (13)$$

where $A_o$ is the absorbance of the solution with the sample at the start of the incubation, $A_t$ is the absorbance of the solution with the sample after 60 minutes, $A_{oo}$ is the absorbance of the solution without the sample at the start of the incubation, and $A_{ot}$ is the absorbance of the solution without the sample after 60 minutes.

### 2.8.5. Antioxidant activity by the Phosphomolybdenum method

The evaluation of antioxidant activity by the reduction of a phosphomolybdenum complex was performed as proposed by Merino et al. (2015) and Hifney et al.(2016) with some modifications, where a 0.1 mL fraction of the oil and bionanocomposite extracts was added to 3.0 mL of the reagent (4 mM ammonium molybdate, 28 mM sodium phosphate, 0.6 M sulfuric acid). The tubes were hermetically sealed and placed in a 95 °C water bath for 90 minutes. After heating, readings were taken on a UV-VIS spectrophotometer (Nova Instruments) at 695 nm. The standard curve was created from different concentrations of ascorbic acid, and the results were expressed as mg AAE (Ascorbic Acid Equivalent) $g^{-1}$ of sample.

### 2.8.6. Total phenolic compounds (TPC)

TPC was determined by the Folin-Ciocalteu method as described by Nisar et al.(2018). Then, 0.5 mL of the oil and bionanocomposite extracts, 2.5 mL of Folin-Ciocalteu reagent (10%) and 2.0 mL of sodium carbonate solution ($Na_2CO_3$) were added to a test tube. The mixture was homogenized, and after it rested for 2 hours, readings were taken on a UV-VIS spectrophotometer (Nova Instruments) at 720 nm. The standard curve was used with different concentrations of gallic acid, and the results were expressed as mg GAE (Gallic Acid Equivalent) 100 $g^{-1}$ of sample.

### 2.9 Preparation, packaging and storage of butter samples

The efficiency of the bionanocomposites was investigated for butter storage and evaluated by the formation of thiobarbituric acid reactive substances (TBARS). Butter with a high-fat content (65%) was purchased from a local store, cut into equal slices of 70 mm x 40 mm x 20 mm and completely wrappedby the bionanocomposites films. PVC films, with a thickness of approximately 0.15 mm and water vapor permeability of approximately 3 x $10^{-18}$ g $m^{-1}$ $s^{-1}$ $pa^{-1}$ (do Lago et al., 2021), were used as a control. The samples were stored in a refrigerator at a temperature below 8 °C and 30% relative humidityfor 60 days. Lipid oxidation was monitored by thiobarbituric acid reactive substances (TBARS) after 0, 30 and 60 days of storage.

#### 2.9.1 Thiobarbituric acid reactive substances (TBARS)

TBARS determination was used according to the methodology described by Bellinazo et al.(2019) with some modifications. Butter samples (1.5 g) were weighed into 15 mL tubes, and 1.5 mL of chloroform was added. After stirring, 5 mL of TBARS solution (100 mL of stock solution consisting of 15% TCA, 0.375% TBA and 0.25 M HCl + 3 mL of 2% BHT solution in ethanol) was added. The tubes were then boiled for 15 minutes in a water bath, cooled for 15 minutes on ice, and centrifuged for 10 minutes at 5000 RPM (Centrifuge, SOLAB). The optical density of the supernatant was measured at 535 nm on a UV-VIS spectrophotometer (Nova Instruments). The concentration of the TBARS solution was calculated using a standard curve prepared with 1, 1, 3,3 tetraethoxypropane, and the results are expressed as mg malondialdehyde (MDA) $g^{-1}$ butter.

### 2.10. Statistical Analysis

The experimental design used was completely casualized with 5 treatments: one control film (3 % wt. pectin) and 4 nanobiocomposites (3 % wt. pectin with 0.1 – 0.4 % wt. pracaxi oil nanoemulsions).

Each test was conducted in at least 3 replicates and the results were subjected to analysis of variance (ANOVA), and means were compared by the Tukey test at a 95% confidence level ($p<0.05$) using SISVAR software, version 5.4.

## 3. RESULTS AND DISCUSSION

### 3.1. Scanning Electron Microscopy (SEM)

All the obtained bionanocomposites were homogeneous with smooth surfaces and translucency (Figure 1-right). To investigate the effect of the added pracaxi oil nanoemulsion on the morphology of the pectin films, SEM images of the fracture surface of all films produced were obtained (Figure 1-left). The images show that the pure pectin film (Figure 1a) presents a uniform and smooth cryogenic fracture surface with evidence that there was a good dispersion of xylitol in the biopolymer matrix. The film containing 0.1% wt. pracaxi oil in the nanoemulsion also presented a smooth and homogeneous fracture surface, indicating that no agglomeration of the nanoemulsion occurred during solvent evaporation. However, films containing between 0.2 and 0.4% wt. pracaxi oil in the nanoemulsion showed a rough fracture surface and the presence of pores. It can also be observed that with increasing concentration of pracaxi oil nanoemulsion, there was an increase in the amount and size of pores present in the bionanocomposites. The presence of pores on the cryogenic fracture surface can be associated with phase separation processes between the nanoemulsion and the pectin matrix during the solvent evaporation process. As already reported, aggregation of the nanoemulsion occurs in the biopolymer films (Almasi et al., 2020; Galus, 2018; Hasheminya & Dehghannya, 2021). The different morphologies observed on the fracture surface of the bionanocomposites can be attributed to the coalescence of the nanoemulsion that occurs due to differences in the interfacial tension between the pectin-nanoemulsionofpracaxi oil and the difference in viscosity between the continuous phase (pectin), that has viscosity values of approximately $10^2$ m Pa s, and the discontinuous phase (nanoemulsion), that has viscosity values in the order of 1 mPa s and is a water-like solution due to the low concentration of pracaxi oil. Also, in Figure S1, there are the viscosity values for film-forming solutions (0 – 0.4 %wt. pracaxi oil), which shows an increase in viscosity in the solution with 0.4 % wt. pracaxi oil, which may be attributed to a higher concentration of nanoemulsion droplets and that may have caused the rougher surface with pore presence found in the bionanocomposite with 0.4 % wt. pracaxi oil.

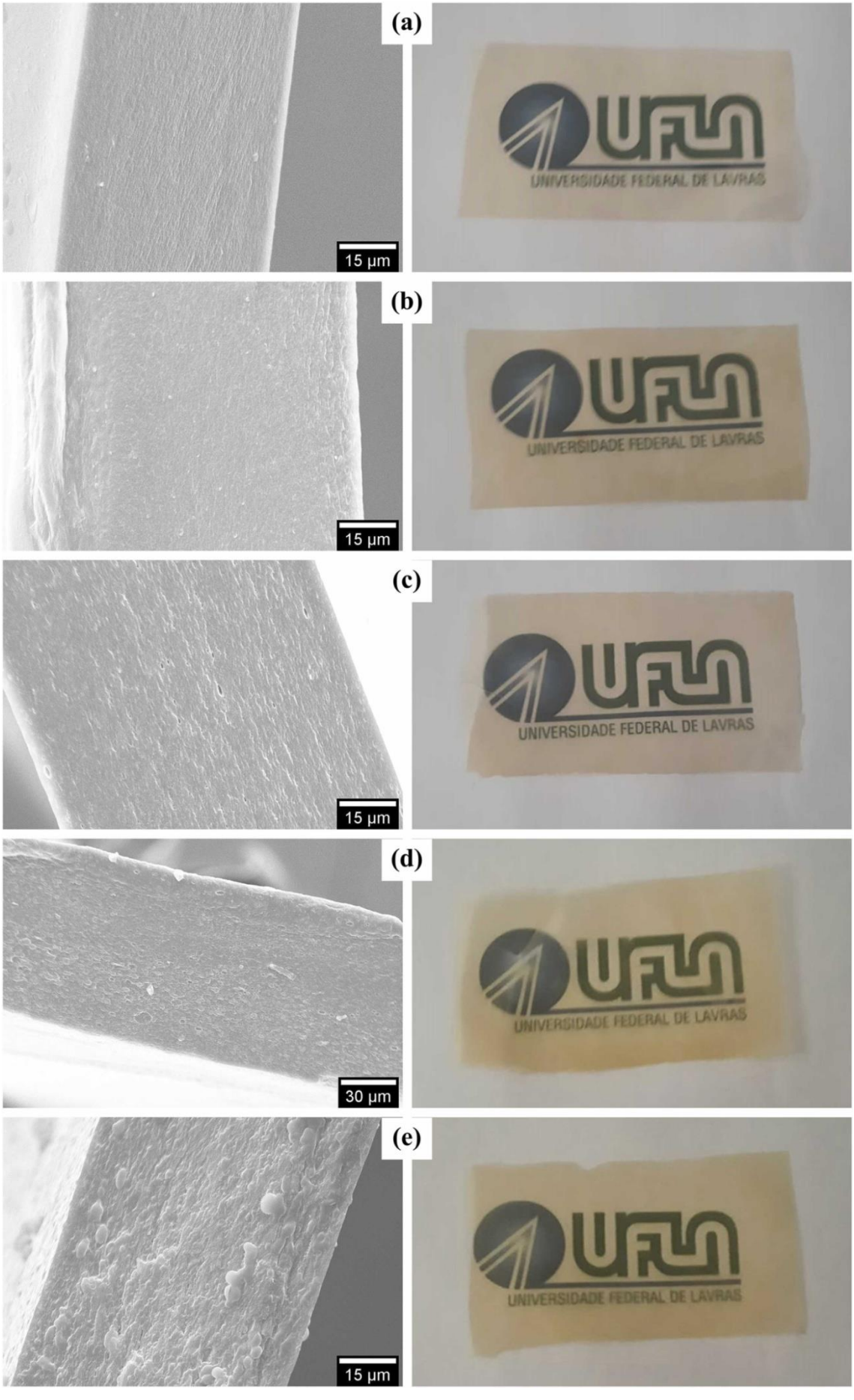

(a)
15 µm
UNIVERSIDADE FEDERAL DE LAVRAS
(b)
15 µm
UNIVERSIDADE FEDERAL DE LAVRAS
(c)
15 µm
UNIVERSIDADE FEDERAL DE LAVRAS
(d)
30 µm
UNIVERSIDADE FEDERAL DE LAVRAS
(e)
15 µm
UNIVERSIDADE FEDERAL DE LAVRAS

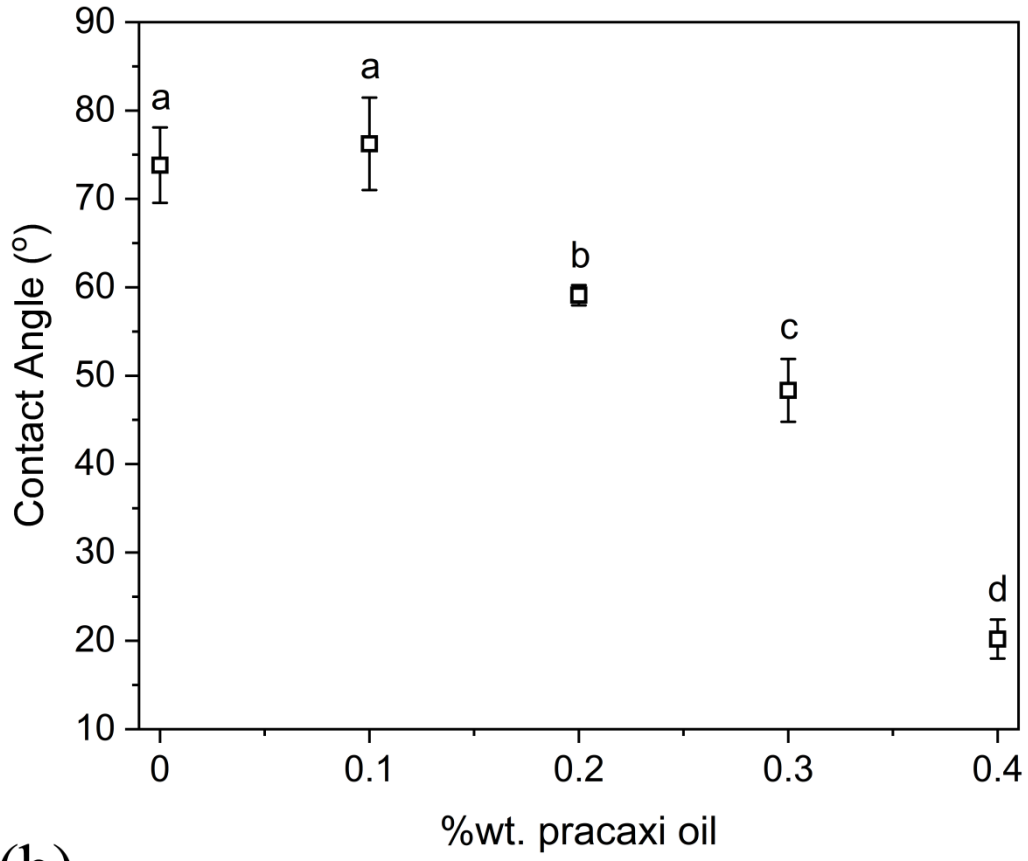
**Figure 1.** Cross-sectional SEM images on the left and images of bionanocomposites on the right: (a) 3% wt. pectin; (b) 3% wt. pectin - 0.1% wt. pracaxi oil; (c) 3% wt. pectin - 0.2% wt. pracaxi oil; (d) 3% wt. pectin - 0.3% wt. pracaxi oil; (e) 3% wt. pectin - 0.4% wt. pracaxi oil.

### 3.2. Contact angle and surface energy

The contact angle between various liquids (Table 2) and the surface of the bionanocomposites was determined and the surface energy and its dispersive and polar components were calculated using Owens-Wendt theory (Owens & Wendt, 1969). Figure 2b shows that the contact angle of the pectin films was 73.82±4.26°. According to previously reported work, contact angle values below 90° indicate hydrophilic biopolymer films (Ma et al., 2007). The addition of 0.1% wt. pracaxi oil in the nanoemulsion to pectin films did not significantly change the contact angle of the film (76.24±5.23°). However, the addition of higher concentrations of the pracaxi oil nanoemulsion led to a drastic reduction in the contact angle of the bionanocomposites, which became 59.11±1.14° (0.2% wt. pracaxi oil), 48.33±3.55°(0.3% wt. pracaxi oil) and 20.20±2.20° (0.4% wt. pracaxi oil). Thus, it can be concluded that the addition of pracaxi oil nanoemulsions to pectin films leads to an increase in their hydrophilicity. This reduction probably occurs due to the presence of pracaxi oil nanoemulsions on the surface of the films and the polar character of Tween 80 present on the surface of the nanoemulsion particles (Galus & Kadzińska, 2016). Similar results were reported in works using films of milk protein isolate and canola oil (Galus, 2018)and almond oil (Galus & Kadzińska, 2016).

The surface energy results (Figure 2a) show that the increase in the concentration of the pracaxi oil nanoemulsion leads to a considerable increase in the surface energy of the pectin films and an increase in the polar component of this surface energy. The increase in the polar component is due to the presence of the polar groups found in the Tween 80 molecules that coat the pracaxi oil particles. Large variations in the ratio between the polar and dispersive components lead to less interaction between the disperse and matrix phases (Harikrishnan et al., 2017). The surface energy results justify the phase separation evidenced by scanning electron microscopy for the bionanocomposites with concentrations between 0.2 and 0.4% wt. pracaxi oil in the nanoemulsion.

(a)


Contact Angle (°)
90
80
70
60
50
40
30
20
10
a
a
b
c
d
0
0.1
0.2
0.3
0.4
%wt. pracaxi oil

(b)

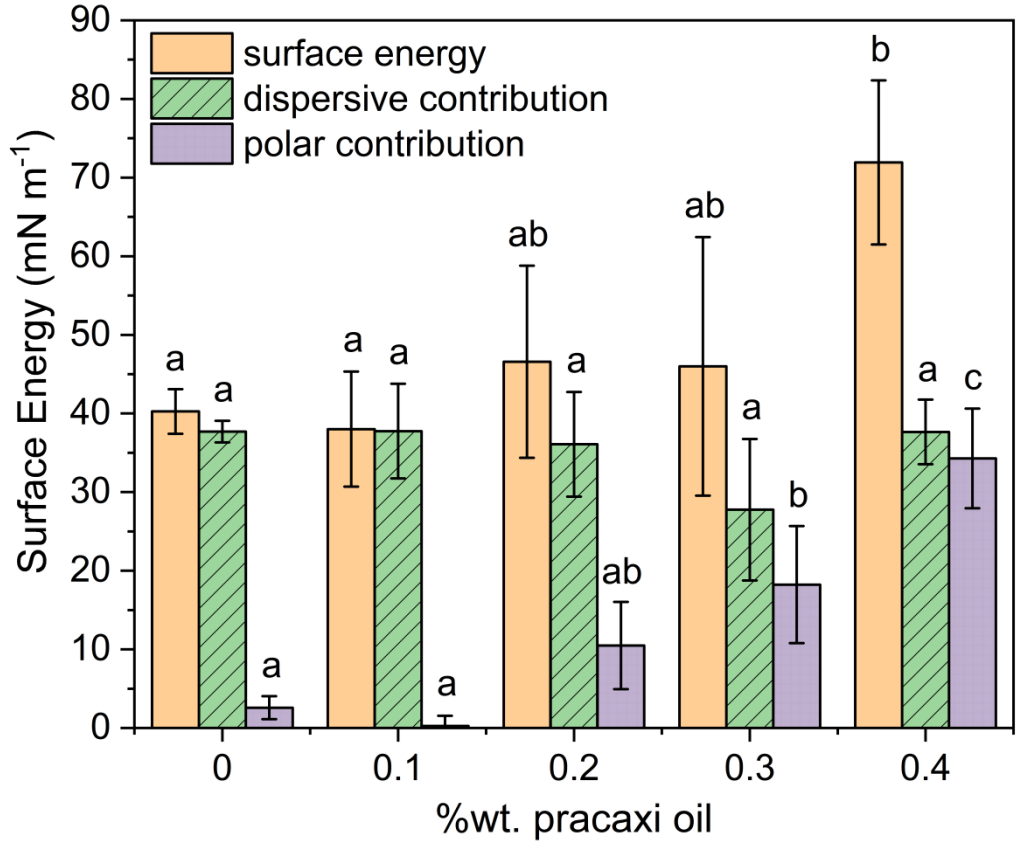

Surface Energy (mN m-1)
surface energy
dispersive contribution
polar contribution
a
a
a
a
a
a
ab
a
ab
ab
a
b
b
a
c
0
0.1
0.2
0.3
0.4
%wt. pracaxi oil

**Figure 2.** Effect of pracaxi oil nanoemulsion on contact angle (a) and surface energy (b) of bionanocomposites.

### 3.3 Water vapor permeability (WVP)

The effect of temperature on the water vapor permeability of the bionanocomposites is shown in Table 1. In the temperature range between 25 and 45 °C, the incorporation of pracaxi oil nanoemulsiondid not significantly affect the water vapor permeability of the biopolymer films. However, at 8 °C, it was possible to notice a drastic reduction in the water vapor permeability of the bionanocomposites, which ranged from $6.8x10^{-13}$ $gm^{-1}s^{-1}Pa^{-1}$ for pure pectin to $4.1x10^{-13}$ $gm^{-1}s^{-1}Pa^{-1}$ for the films containing 0.4% wt. pracaxi oil. This result is in agreement with the works of Gutiérrez-Jara et al. (2020) and Nisar et al. (2018), who indicated a reduction in WVP in alginate films due to the addition of a soybean oil nanoemulsion and in pectin films with the addition of a clove oil nanoemulsion, respectively, and may be related to hydrogen and covalent interactions between the pectin and functional groups of the pracaxi oil and its phenolics compounds, that may have limited the availability of hydrogen groups to form bonds with water and, consequently, decreased the WVP (Rodsamran & Sothornvit, 2019). This reduction in water vapor permeability indicates that at this temperature, the pracaxi oil nanoemulsion dispersed in the pectin matrix acts as a barrier for the diffusion of water molecules (Gutiérrez-Jara et al., 2020).

**Table 1.** Effect of temperature on the water vapor permeability of pectin bionanocomposites and pracaxi oil nanoemulsions.

| WVP ($x10^{-13}$ $gm^{-1}s^{-1}Pa^{-1}$) – RH=75% | | | | |
|---|---|---|---|---|
| Bionanocomposites | 8ºC | 25ºC | 35ºC | 45ºC |
| 3% wt. pectin | $6.8\pm0.9^{aC}$ | $8.6\pm0.4^{aC}$ | $6.1\pm0.2^{aC}$ | $2.3\pm0.1^{aD}$ |
| 3% wt. pectin – 0.1% wt. pracaxi oil | $3.7\pm0.4^{bD}$ | $8.9\pm1.2^{aC}$ | $6.7\pm0.4^{aC}$ | $2.4\pm0.1^{aD}$ |
| 3% wt. pectin – 0.2% wt. pracaxi oil | $4.9\pm0.3^{bD}$ | $8.1\pm0.3^{aC}$ | $7.2\pm0.6^{aC}$ | $2.7\pm0.2^{aD}$ |
| 3% wt. pectin – 0.3% wt. pracaxioil | $4.5\pm0.3^{bD}$ | $8.5\pm1.1^{aC}$ | $7.0\pm0,6^{aC}$ | $2.9\pm0.7^{aD}$ |
| 3% wt. pectin- 0.4% wt. pracaxi oil | $4.1\pm0.5^{bC}$ | $7.3\pm0.2^{aC}$ | $6.6\pm0.7^{aC}$ | $2.6\pm0.2^{aD}$ |

Means ± standard deviation of three replicates followed by the same lowercase letters (comparison between samples at the same temperature) in the same column and uppercase letters (comparison of

the same sample between different temperatures) in the same row do not differ statistically by Tukey's test at 5% ($p<0.05$).

The WVP of pectin and pectin bionanocomposites with pracaxi oil nanoemulsions decreases with increasing temperature. This fact can be explained by water sorption being the predominant factor for water vapor transmission. A nonthermal increase in diffusion, with the increase in the humidity gradient, causes a greater amount of water to be sorbed onto the pectin bionanocomposites, which plasticizes the polymer, favoring the elevation of WVP at lower temperatures (Spatafora Salazar et al., 2019). WVP is a property of great importance in food film studies, as it is essential in the chemical reactions that lead to food spoilage (Zhou et al., 2021).

The highest water vapor permeability results were found in the bionanocomposites, regardless of the oil concentration, at the highest relative humidity used. These results can be explained by the fact that by increasing the relative humidity, there is an increase in water vapor pressure, which causes a greater interaction through hydrogen bonds of the water molecules with hydrophilic groups in the films. These interactions cause the absorbed water molecules to plasticize the pectin polymer matrix and cause the absorbed water vapor to swell (Spatafora Salazar et al., 2019; Galus, 2018).

The activation energy of water vapor permeation consists of the energy required for water molecules to pass through the polymeric film (Chinma et al., 2015). Figure 3 displays the relationship between the activation energy and relative humidity in pectin bionanocomposites and pracaxi oil nanoemulsions. The values indicate that the activation energy of the permeation process was between -80 and 0 kJ $mol^{-1}$ for all films ($R^2>0.940$). It was possible to observe an increase in the activation energy with the increase in the concentration of the pracaxi oil nanoemulsion present in the bionanocomposites, which can be attributed to the fact that the nanoemulsion particles act as a physical barrier to the permeation of water molecules. In all bionanocomposites, an increase in activation energy was observed with increasing relative humidity. The bionanocomposites with the presence of pracaxi oil presented higher activation energy due to a lower permeation, since the pracaxi oil caused reductions in the WVP values. The low activation energy in the bionanocomposite without the presence of oil may be related to a higher free volume that requires less energy for the induction of segmental chain movement, increasing permeation (Spatafora Salazar et al., 2019).

As reported earlier, an increase in relative humidity causes greater plasticization in the

polymer chains, resulting in a swollen chain with a higher free volume, which is responsible for the higher activation energy (Spatafora Salazar et al., 2019).

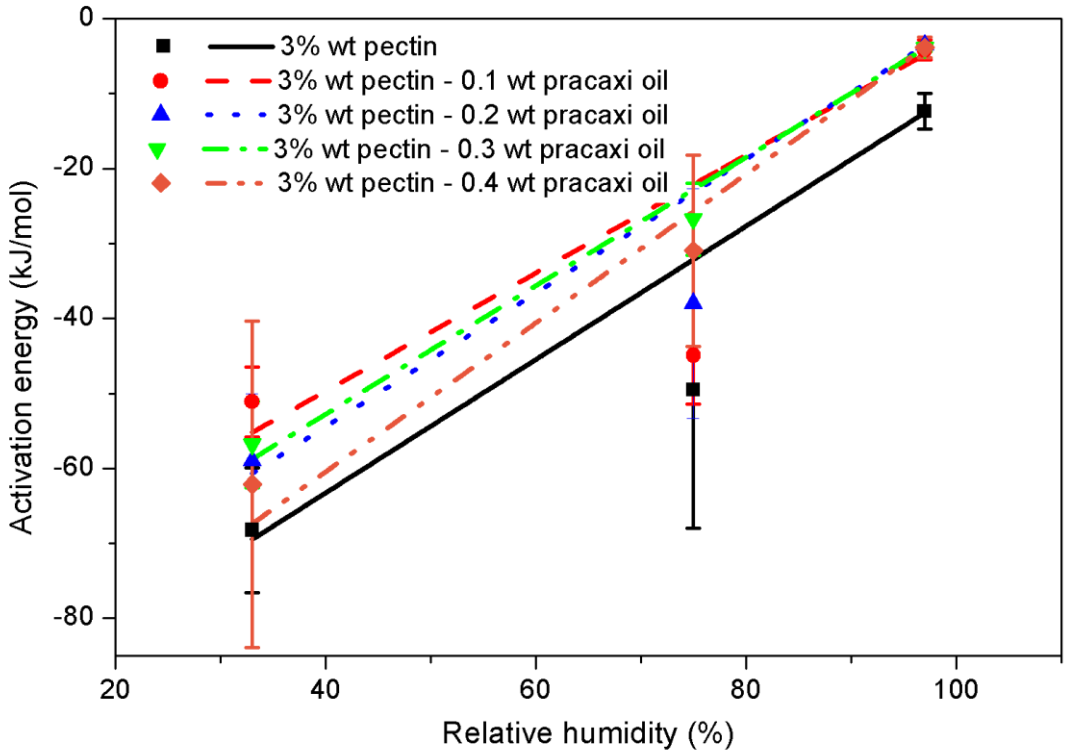



**Figure 3.** Activation energy for water vapor permeation as a function of humidity gradients for pectin-based films with and without pracaxi oil nanoemulsion.

### 3.4 Moisture sorption

#### 3.4.1. Sorption isotherms

The isotherms of moisture sorption in the bionanocomposites of pectin and pracaxi oil nanoemulsions are presented in Figure 4 and show a sigmoidal shape with the characteristic behavior of moisture-sensitive polymers (Ciannamea et al., 2018). In all samples and experimental conditions, the equilibrium moisture ($M$) of the films increased with increasing water activity ($a_w$). Figure 4 shows that the reduction in temperature leads to a greater increase in the equilibrium mass. This lower amount of water adsorbed on the surface of the films at higher temperatures is due to two factors that encompass a higher activation energy of the adsorption process and a reduction in the binding energy between the water molecules and the active sites on the surface of the films (Esquerdo et al., 2019).

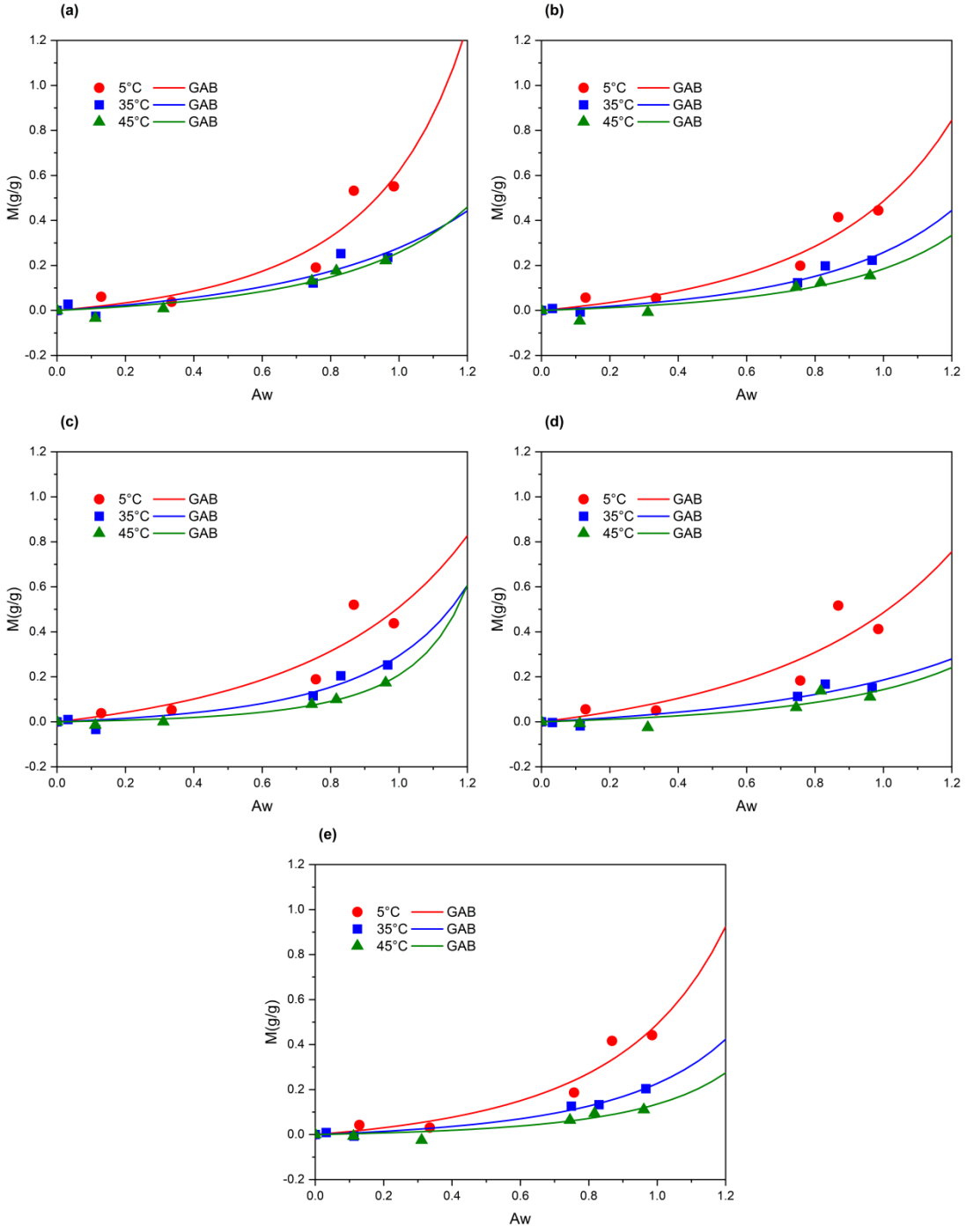



**Figure 4.** Experimental data and the fit of the GAB model at 5, 35 and 45 °C: (a) 3% wt. pectin; (b) 3% wt. pectin - 0.1% wt. pracaxi oil; (c) 3% wt. pectin - 0.2% wt. pracaxi oil; (d) 3% wt. pectin - 0.3% wt. pracaxi oil; (e) 3% wt. pectin - 0.4% wt. pracaxi oil.

For all samples, a slight increase in the amount of water adsorbed on the surface of the films ($M$) was observed for water activity ($a_w$) values between 0 and 0.6. However, for higher values of water activity, a large variation in the amount of water adsorbed on the surface of

the films was observed. This change in the behavior of the bionanocomposites with water activity can be explained by a change in the interactions between water molecules and the surface of the bionanocomposites (Gutiérrez-Jara et al., 2020). At higher relative humidity,more water molecules are available to participate in chemical and physical reactions (Lara et al., 2020).

### 3.4.2 GAB model

The GAB model has been the most commonly used model to describe the behavior of biopolymers under different environmental conditions (Karkhanis et al., 2021).The content of the adsorbed water monolayer on the surface of the bionanocomposites ($X_m$) is described in Table 3. All these parameters were obtained by fitting the experimental data of the sorption isotherms to the GAB model and showed $R^2$ values ranging from 0.88 to 0.99. The content of the adsorbed water monolayer on the surface of the films ($X_m$) is a measure of the number of active sites present on the surface of the bionanocomposites that are available for adsorption (Esquerdo et al., 2019; Gutiérrez-Jara et al., 2020).

Table 2 shows that with the formulations with 0.2, 0.3 and 0.4% wt. pracaxi oil, a significant increase in temperature caused a reduction in the content of the water monolayer adsorbed on the surface of the films ($X_m$). This reduction in $X_m$ is explained by changes in the structure of the bionanocomposites. Increasing the temperature leads to a reduction in the number of active sites available on the surface of the bionanocomposites where water is adsorbed. Furthermore, the increase in temperature leads to the breakdown of the intermolecular interactions between the water molecules and the active sites of the bionanocomposites, bringing the adsorbed water into the vapor phase.

**Table 2.** Estimated $X_m$ parameter of the GAB model for the adsorption isotherms of pectin bionanocomposites with pracaxi oil at 5, 35 and 45 °C.

| | Bionanocomposites (% wt. pracaxi oil) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.1 | 0.2 | 0.3 | 0.4 |
| 5°C | 1.60±0.30$^{a}$ | 1.07±0.51$^{a}$ | 1.83±0.22$^{a}$ | 1.88±0.16$^{a}$ | 1.39±0.37$^{a}$ |
| 35°C | 1.17±0.23$^{a}$ | 0.91±0.27$^{a}$ | 0.82±0.33$^{b}$ | 1.11±0.14$^{ab}$ | 0.64±0.13$^{ab}$ |
| 45°C | 0.86±0.29$^{a}$ | 0.82±0.06$^{a}$ | 0.38±0.23$^{b}$ | 0.85±0.10$^{b}$ | 0.48±0.26$^{b}$ |

Means ± standard deviation of three repetitions followed by the same lowercase letters (comparison between samples at the same temperature) in the same column do not differ statistically by Tukey's test at 5% ($p<0.05$).

The values of $C$ remained in a range of values between 0.09 – 0.25, showing no significant differences in relation to temperature and the addition of pracaxi oil, indicating that the variables do not influence the enthalpy of sorption.

The $K$ values for all samples at all temperatures showed no significant differences and remained constant in the range 0.43 – 0.67, which indicates that the binding energy of water molecules in the multilayer is not affected by the film composition and temperature.

### 3.4.3 Net isosteric heat of sorption ($q_{st}$)

Figure 5a presents the effect of equilibrium moisture ($M$) on the isosteric heat of sorption ($q_{st}$) of the bionanocomposites. This thermodynamic parameter represents the amount of energy required to evaporate the water adsorbed on the surface of the bionanocomposite (Tian et al., 2020). The isosteric heat is a good parameter for estimating the amount of energy required to remove the water molecules from the surface of the bionanocomposites and allows for some deductions about the surface microstructure of these materials.

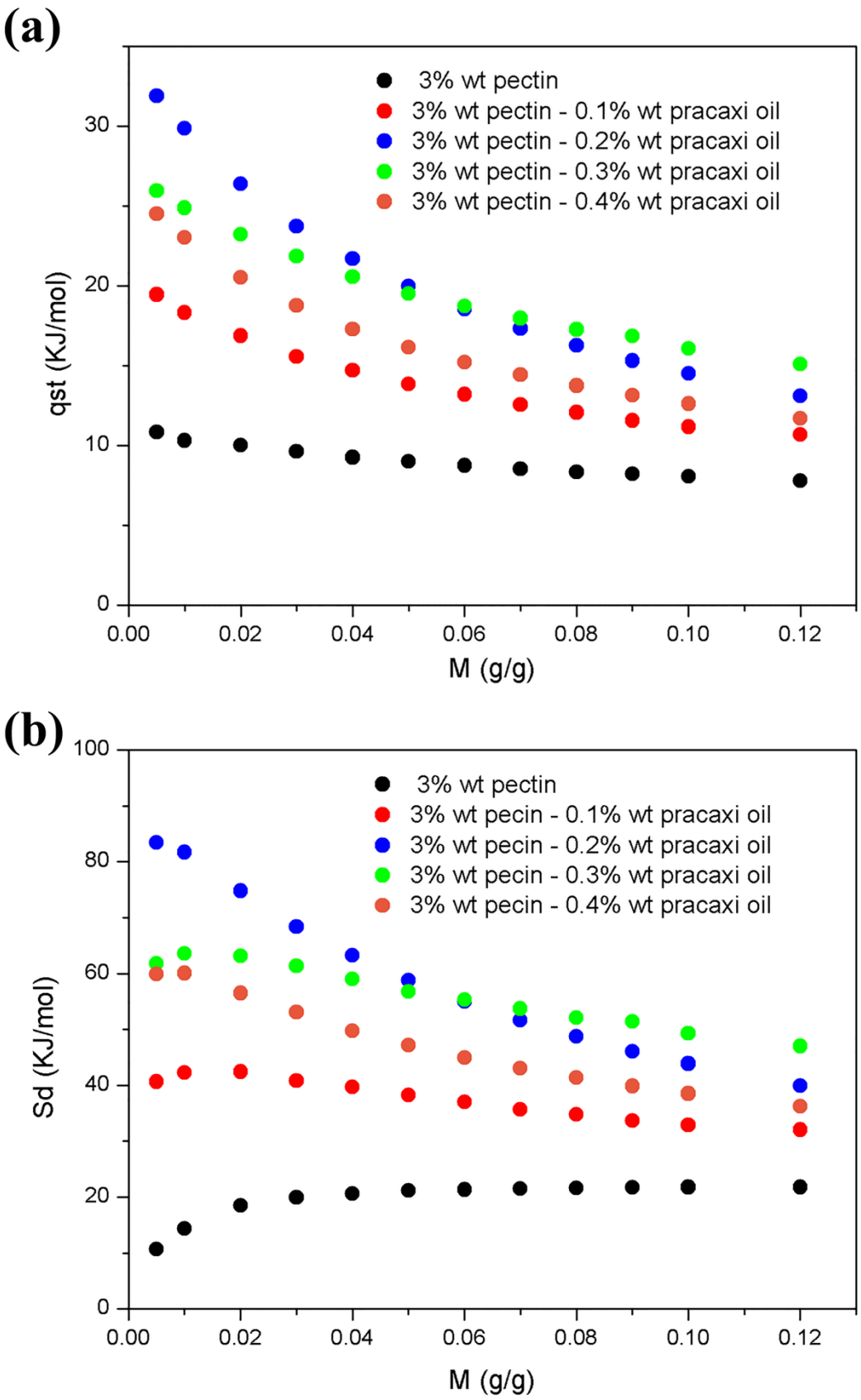



**Figure 5.** Values of net isosteric heat of sorption (a) and differential entropy (b) of pectin and pectin bionanocomposites with pracaxi oil nanoemulsion.

In all samples, a reduction inisosteric heat ($q_{st}$) was observed with increasing equilibrium moisture ($M$). This behavior indicates that the smaller the amount of water adsorbed on the surface of the bionanocomposites is, the greater the amount of energy required for the water molecules to transition to the vapor state. This reduction in $q_{st}$ at higher values of equilibrium moisture is related to the saturation of active sites available on the surface of bionanocomposites (Lara et al., 2020). The incorporation of nanoemulsions into pectin films resulted in an increase in the isosteric heat of the films at all equilibrium humidity values. This result indicates that the presence of the nanoemulsion of pracaxi oil in pectin films also leads to an increase in the amount of energy required for the adsorbed water to evaporate. This increase in isosteric heat due to the presence of the nanoemulsion of pracaxi oil in the bionanocomposites is related to higher binding energy between the water molecules and the active sites.

### 3.4.4 Differential entropy ($S_d$)

Figure 5b shows the behavior of the differential entropy ($S_d$) of the surface of the bionanocomposites as a function of equilibrium moisture content ($M$). Differential entropy is a thermodynamic quantity with a direct relationship to the number of active sites available on the surface (Tian et al., 2020). The incorporation of the pracaxi oil nanoemulsion leads to an increase in differential entropy ($S_d$), which represents the increase in the number of active sites available on the surface of the bionanocomposites.

The incorporation of the pracaxi oil nanoemulsion into the pectin polymer matrix leads to an increased exposure of hydroxyl groups on the surface of the bionanocomposites. The thermodynamic properties ($q_{st}$ and $S_d$) were demonstrated with the increase in the water-active site binding energy and the increase in the number of active sites. This result also corroborated the increase in contact angle and surface energy. However, the permeability results show that the presence of the nanoemulsion leads to an increase in the activation energy for permeation and a decrease in WVP values. This increase occurs because the nanoemulsion is a physical barrier, and although it favors the adsorption of water molecules on the surface of the films, it prevents their diffusion inside the bionanocomposites.

## 3.5. Antioxidant activity

The antioxidant activity and total phenolic compounds present in the pracaxi oil and bionanocomposites were determined by traditional spectrophotometric techniques based on

the elimination of DPPH radicals and the protection of β-carotene and phosphomolybdenum, and the results are shown in Table 3.

**Table 3.** Total phenolic compounds and antioxidant activity by DPPH, β-carotene/linoleic acid and phosphomolybdenum in pracaxi oiland bionanocomposites.

| Parameter | Pracaxi oil | Bionanocomposites (% wt. pracaxi oil) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 0.1 | 0.2 | 0.3 | 0.4 |
| TPC(mg GAE 100 $g^{-1}$) | 56.7±1.3 | 60.9±0.1 $^{d}$ | 69.8±1.3 $^{c}$ | 76.8±0.2 $^{b}$ | 78.7±0.7 $^{b}$ | 83.2±1.4$^{a}$ |
| DPPH (IC50 mg $mL^{-1}$) | 19.8±2.4 | - | - | - | - | - |
| DPPH ($\%AA_{DPPH}$) | 50.3±0.1 | 23.3±1.3 $^{d}$ | 27.7±1.4 $^{c}$ | 30.9±1.2 $^{b}$ | 32.8±0.6 $^{b}$ | 36.3±0.3$^{a}$ |
| Phosphomolybdenum (mg AAE $g^{-1}$) | 43.9±0.1 | 171.8±0.1$^{e}$ | 174.9±0.1$^{d}$ | 175.7±0.1$^{c}$ | 176.4±0.1$^{b}$ | 177.4±0.1$^{a}$ |
| β-carotene/linoleicacid ($\%AA_{\beta-carotene}$) | 90.3±0.6 | 24.5±2.1 $^{e}$ | 31.2±1.6 $^{d}$ | 37.6±1.2 $^{c}$ | 41.3±1.8 $^{b}$ | 44.1±0.6$^{a}$ |

Means ± standard deviation of three repetitions followed by the same lowercase letters (comparison between samples at the same temperature) in the same row do not differ statistically by Tukey's test at 5% ($p<0.05$).

The content of total phenolic compounds in an oil is an indication of its efficiency as an antioxidant agent, considering that these phenolic compounds are responsible for inhibiting lipid peroxidation and stabilizing the free radicals present in the medium (Kumar et al., 2020). The pracaxi oil exhibited a TPC value of 56.7±1.3 mg GAE 100 $g^{-1}$ oil. This value is much higher than those foundby Teixeira et al. (2020), who extracted pracaxi oil using supercritical $CO_2$ at different pressures. The oil in this study was extracted by cold pressing. This difference in TPC contents probably occurs due to the processing conditions of refining and the condition of the vegetable oil extractions (Kumar et al., 2020). Phenolic acids are the main bioactive compounds present in seed oils, along with flavonoids, which contribute to reducing the speed of oxidative processes in extracted oils (Teixeira et al., 2020).

The determination of the antioxidant activity by the DPPH radical method consists of the change in the purple coloration of the DPPH solution in the presence of antioxidants. The IC50 indicates thevoil amountvrequired to reduce the initial concentration of the DPPH radical by 50%, therefore, the lower the IC50 value, the greater the antioxidant activity. The pracaxi oil showed an IC50 value of 19.8±2.4 mg $mL^{-1}$. The antioxidant activity of the pracaxi oil found in this study was higher than those reported in the literature for different

oils, such as essential oil from basil grown in Beni Suef Province(55.2 mg $mL^{-1}$) (Ahmed et al., 2019), lavender essential oil (21.6 mg $mL^{-1}$) (Blažeković et al., 2018) and pracaxi oil extracted using supercritical $CO_2$ (Teixeira et al., 2020).

The phosphomolybdenum assay has been widely used for the determination of the antioxidant activity of both plant extracts and oils. Pracaxi oil, for example, reached a value of 43.9±0.1 mg ascorbic acid $g^{-1}$ of oil. According to Hifney et al. (2016), contents higher than 9.0 mg ascorbic acid $g^{-1}$ are considered to indicate high antioxidant activity; therefore, pracaxi oil exhibited excellent antioxidant activity.

The β-carotene bleaching test consists of the discoloration of the orange color of β-carotene in the absence of an antioxidant (Amiri, 2012). By presenting a protection of 90.3 ± 0.6 %, pracaxi oil prevented the bleaching of β-carotene, as shown in Table 3, thus the oil proved to be an excellent antioxidant. The antioxidant activity by bleaching β-carotene in this study was higher than that observed by Amiri (2012) with the oils of *T. kotschyanus* (69.2 ± 0.5 %), *T. eriocalyx* (57.6 ± 0.6 %) and *T. Daenensis subs plancifolius* (88.4 ± 0.8 %). It was also superior to *Plantago lanceolata* extracts where ethyl acetate, methanol, n-hexane and water were used for extraction and the antioxidant activities were 71 ± 4, 75 ± 1, 78 ± 1 and 77 ± 1 %, respectively. These results indicate that pracaxi oil is an excellent antioxidant.

Regarding the bionanocomposites, there was a significant increase in the antioxidant action of pectin films with the addition of pracaxi oil (Table 3). The increase in antioxidant action was evidenced by all the methodologies employed (DPPH, β-carotene/linolenic acid and phosphomolybdenum). An increase in the content of total phenolic compounds (TPC) was also evidenced. The highest increase in $\%AA_{DPPH}$ occurred in the film with 0.4% wt. pracaxi oil added into the nanoemulsion, and when compared to the control film with 0% oil, an increase of 56% in DPPH free radical scavenging was observed. These results corroborate those obtained by Nisar et al. (2018), who reported an increase in DPPH with the addition of clove oil to pectin films.

The pectin films without the presence of pracaxi oil nanoemulsion also showed antioxidant activity. This activity can be explained by the presence of hydroxyl groups in the pectin chain and the xylitol; these hydroxyl groups can act as proton donors and as antioxidants, as evidenced by the DPPH, β-carotene/linolenic acid and phosphomolybdenum results for pure pectin (Wathoni et al., 2019). The antioxidant action of the pectin films can also be explained by the high content of total phenolic compounds (TPCs) found.

According to Table 3, increasing the concentration of nanoemulsion present in the film (0 – 0.4% wt. pracaxi oil) increased the content of total phenolic compounds, as well as in the

antioxidant action of the films. The highest antioxidant activity was observed for pectin films containing 0.4% wt. pracaxi oil in the nanomembrane. These results indicate synergy between the nanoemulsion of pracaxi oil and pectin.

The antioxidant activity for DPPH of the Bionanocomposites with 0.4 % wt. pracaxi oil (36.3 ± 0.3 %) is higher than those found with mucilage films of *dicotoma Cordia* fruit with *Salvia Mirzaiani* essential oil nanoemulsion (approximately 26 %) (Hasheminya & Dehghannya, 2021), soybean polysaccharide films with incorporated cinnamon essential oil nanoemulsions (10.8 ± 0.17 %) (Ghani et al., 2018), and active coatings of chitosan and *ferulago angulata* oil nanoemulsion (30.17 ± 0.44 %) (S. Shokri et al., 2020). For the antioxidant activity by the β-carotene/linoleic acid system, the results found here (44.1 ± 0.6) are similar to those found in chitosan films with 0.5 % *Mentha Pulegiums* essential oil (47.42 ± 4.18 %) and Whey Protein Isolate (WPI) films with 0.5% *Mentha Pulegiums* oil (40.23 ± 3.57 %) (Z. Shokri & Kamkar, 2018).

### 3.6. Application of bionanocomposites in butter

An unpleasant taste, color and nutrient loss are the results of secondary oxidation of lipids that occur in unsaturated fatty acids. Secondary oxidation results in the formation of byproducts that correspond to volatile and nonvolatile products comprising aldehydes, ketones, alcohols, epoxy compounds, and organic acids. Secondary oxidation directly impacts the shelf life of various foods. It can be analyzed using thiobarbituric acid reactive substances (TBARS) (Asdagh & Pirsa, 2020; Carpena et al., 2021; Nor Adilah et al., 2020).

To evaluate the effect of pectin bionanocomposites and pracaxi oil nanoemulsions on the secondary oxidation of butter, TBARS analysis was performed for 60 days. Figure 6 indicates the results obtained for butter samples wrapped with the bionanocomposites films and a control treatment, where the samples were wrapped with PVC film, after 0, 30 and 60 days of storage.

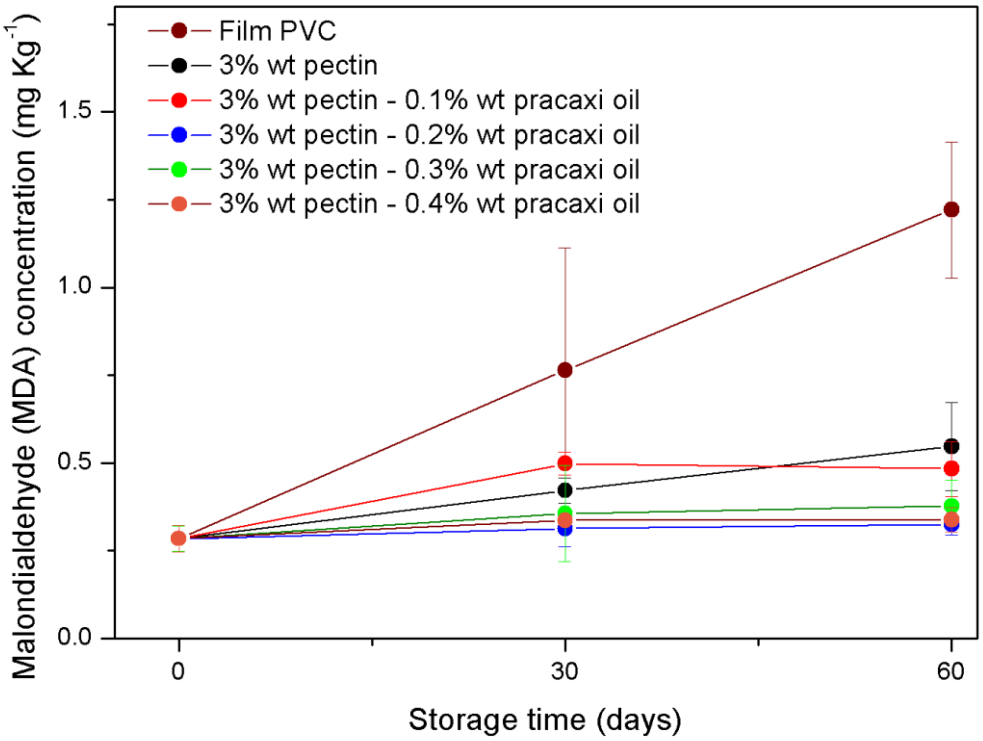



**Figure 6.**Oxidative properties - Malondialdehyde (MDA) content of butter packed in control film and pectin films with pracaxi oil for 60 days.

In the 30-day storage period, the butter coated in the PVC film exhibited a statistically higher value (0.764±0.245 mg of MDA $kg^{-1}$ of butter) than the bionanocomposites when compared to the initial butter values. The butter coated with the pure pectin film showed no significant difference in MDA values after 30 days of storage compared to the initial MDA concentration. This result indicates that the presence of hydroxyl and carboxyl groups in the pectin and xylitol is sufficient for the film to present antioxidant action sufficient to protect the butter samples from secondary oxidation. However, after 60 days of storage, it was possible to observe a significant difference between the MDA values for the butter samples coated with the pectin film in relation to the initial value obtained. This result indicates that after 60 days, the pure pectin films are not sufficient to prevent the secondary oxidation of butter samples.

However, even after 60 days, all butter samples coated with the bionanocomposites did not present malondialdehyde (MDA) values with significant differences ($p<0.05$) in relation to the butter samples at the beginning of the experiment. These results indicate that the bionanocomposites were efficient in reducing the secondary oxidation of butter samples

and thus enabled the increased storage time of this food. However, between the concentrations of pracaxi oil on the bionanocomposites, there was no significant difference in the MDA values after 60 days of storage.

The antioxidant activity of the bionanocomposites occurs due to the migration of phenolic compounds to butter. These compounds act in stabilizing the free radicals that are generated during the oxidation process of unsaturated fatty acids present in butter. The reduction in the concentration of free radicals directly affects the concentration of the primary compounds of oxidation, hydroperoxides and consequently limits the production of side products within the butter, thus preventing unpleasant taste and increasing the shelf life of butter (Asdagh & Pirsa, 2020; Moura et al., 2018; Nor Adilah et al., 2020).

## 4. CONCLUSION

Nanoemulsions of pracaxi oil were synthesized by ultrasonication and used in the production of pectin-based bionanocomposites. The presence of polar groups in the Tween 80 molecule caused the affinity for water molecules on the surface of the bionanocomposites to increase with the incorporation of the nanoemulsion, which was confirmed through contact angle and thermodynamic properties ($q_{st}$ and$S_d$). However, the incorporation of the nanoemulsion, a physical barrier, increased the barrier properties, as confirmed by the water vapor permeability and activation energy. An increase in antioxidant activity was also confirmed through DPPH, phosphomolybdenum and β-carotene protection analysis, as well as an increase in TPC contents. The presence of antioxidants, especially the presence of phenolic compounds, in the bionanocomposites was determined to enable greater protection against butter oxidation during the 60 days of storage. Therefore, the use of this packaging for butter presents an interesting potential application because it can protect against the formation of compounds that cause unpleasant taste, thus enabling longer shelf life for this product and greater food safety.

## ACKNOWLEDGMENTS

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**REFERENCES**

Ahmed, A. F., Attia, F. A. K., Liu, Z., Li, C., Wei, J., & Kang, W. (2019). Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (Ocimum basilicum L.) plants. *Food Science and Human Wellness*, *8*(3), 299–305. https://doi.org/10.1016/j.fshw.2019.07.004

Alexandre, E. M. C., Lourenço, R. V., Bittante, A. M. Q. B., Moraes, I. C. F., & Sobral, P. J. do A. (2016). Gelatin-based films reinforced with montmorillonite and activated with nanoemulsion of ginger essential oil for food packaging applications. *Food Packaging and Shelf Life*, *10*, 87–96. https://doi.org/10.1016/j.fpsl.2016.10.004

Almasi, H., Azizi, S., & Amjadi, S. (2020). Development and characterization of pectin films activated by nanoemulsion and Pickering emulsion stabilized marjoram (Origanum majorana L.) essential oil. *Food Hydrocolloids*, *99*(June 2019), 105338. https://doi.org/10.1016/j.foodhyd.2019.105338

Amiri, H. (2012). Essential oils composition and antioxidant properties of three thymus species. *Evidence-Based Complementary and Alternative Medicine*, *2012*. https://doi.org/10.1155/2012/728065

Arranz, S., Cert, R., Pérez-Jiménez, J., Cert, A., & Saura-Calixto, F. (2008). Comparison between free radical scavenging capacity and oxidative stability of nut oils. *Food Chemistry*, *110*(4), 985–990. https://doi.org/10.1016/j.foodchem.2008.03.021

Asdagh, A., & Pirsa, S. (2020). Bacterial and oxidative control of local butter with smart/active film based on pectin/nanoclay/Carum copticum essential oils/β-carotene. *International Journal of Biological Macromolecules*, *165*, 156–168. https://doi.org/10.1016/j.ijbiomac.2020.09.192

ASTM. (2009). *ASTM D882-09, Standard Test Method for Tensile Properties of Thin Plastic Sheeting*.

ASTM. (2012). *ASTM E104-02, Standard Practice for Maintaining Constant Relative Humidity by Means of Aqueous Solutions1*.

Bellinazo, P. L., Vitola, H. R. S., Cruxen, C. E. dos S., Braun, C. L. K., Hackbart, H. C. dos S., da Silva, W. P., & Fiorentini, Â. M. (2019). Probiotic butter: Viability of Lactobacillus casei strains and bixin antioxidant effect (Bixa orellana L.). *Journal of Food Processing and Preservation*, *43*(9). https://doi.org/10.1111/jfpp.14088

Bertuzzi, M. A., Castro Vidaurre, E. F., Armada, M., & Gottifredi, J. C. (2007). Water vapor permeability of edible starch based films. *Journal of Food Engineering*, *80*(3), 972–978. https://doi.org/10.1016/j.jfoodeng.2006.07.016

Blažeković, B., Yang, W., Wang, Y., Li, C., Kindl, M., Pepeljnjak, S., & Vladimir-Knežević, S. (2018). Chemical composition, antimicrobial and antioxidant activities of essential oils of Lavandula × intermedia 'Budrovka' and L. angustifolia cultivated in Croatia. *Industrial Crops and Products*, *123*, 173–182. https://doi.org/10.1016/j.indcrop.2018.06.041

Buransompob, A., Tang, J., Mao, R., & Swanson, B. G. (2003). Rancidity of walnuts and almonds affected by short time heat treatments for insect control. *Journal of Food Processing and Preservation*, *27*(6), 445–464. https://doi.org/10.1111/j.1745-4549.2003.tb00529.x

Carpena, M., Nuñez-Estevez, B., Soria-Lopez, A., Garcia-Oliveira, P., & Prieto, M. A.

(2021). Essential oils and their application on active packaging systems: A review. In *Resources* (Vol. 10, Issue 1, pp. 1–20). MDPI AG. https://doi.org/10.3390/resources10010007

Chinma, C. E., Ariahu, C. C., & Alakali, J. S. (2015). Effect of temperature and relative humidity on the water vapour permeability and mechanical properties of cassava starch and soy protein concentrate based edible films. *Journal of Food Science and Technology*, *52*(4), 2380–2386. https://doi.org/10.1007/s13197-013-1227-0

Ciannamea, E. M., Castillo, L. A., Barbosa, S. E., & De Angelis, M. G. (2018). Barrier properties and mechanical strength of bio-renewable, heat-sealable films based on gelatin, glycerol and soybean oil for sustainable food packaging. *Reactive and Functional Polymers*, *125*, 29–36. https://doi.org/10.1016/j.reactfunctpolym.2018.02.001

Dhavamani, S., Poorna Chandra Rao, Y., & Lokesh, B. R. (2014). Total antioxidant activity of selected vegetable oils and their influence on total antioxidant values in vivo: A photochemiluminescence based analysis. *Food Chemistry*, *164*, 551–555. https://doi.org/10.1016/j.foodchem.2014.05.064

do Lago, R. C., de Oliveira, A. L. M., de Amorim dos Santos, A., Zitha, E. Z. M., Nunes Carvalho, E. E., Tonoli, G. H. D., & de Barros Vilas Boas, E. V. (2021). Addition of wheat straw nanofibrils to improve the mechanical and barrier properties of cassava starch–based bionanocomposites. *Industrial Crops and Products*, *170*, 113816. https://doi.org/10.1016/j.indcrop.2021.113816

Espitia, P. J. P., Du, W. X., Avena-Bustillos, R. de J., Soares, N. de F. F., & McHugh, T. H. (2014). Edible films from pectin: Physical-mechanical and antimicrobial properties - A review. In *Food Hydrocolloids* (Vol. 35, pp. 287–296). https://doi.org/10.1016/j.foodhyd.2013.06.005

Esquerdo, V. M., Monte, M. L., & Pinto, L. A. de A. (2019). Microstructures containing nanocapsules of unsaturated fatty acids with biopolymers: Characterization and thermodynamic properties. *Journal of Food Engineering*, *248*, 28–35. https://doi.org/10.1016/j.jfoodeng.2018.12.015

Galus, S. (2018). Functional properties of soy protein isolate edible films as affected by rapeseed oil concentration. *Food Hydrocolloids*, *85*, 233–241. https://doi.org/10.1016/j.foodhyd.2018.07.026

Galus, S., & Kadzińska, J. (2016). Whey protein edible films modified with almond and walnut oils. *Food Hydrocolloids*, *52*, 78–86. https://doi.org/10.1016/j.foodhyd.2015.06.013

Ghani, S., Barzegar, H., Noshad, M., & Hojjati, M. (2018). The preparation, characterization and in vitro application evaluation of soluble soybean polysaccharide films incorporated with cinnamon essential oil nanoemulsions. *International Journal of Biological Macromolecules*, *112*, 197–202. https://doi.org/10.1016/j.ijbiomac.2018.01.145

Gómez-Estaca, J., López-de-Dicastillo, C., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2014). Advances in antioxidant active food packaging. In *Trends in Food Science and Technology* (Vol. 35, Issue 1, pp. 42–51). https://doi.org/10.1016/j.tifs.2013.10.008

Grunert, K. G. (2005). Food quality and safety: Consumer perception and demand. *European Review of Agricultural Economics*, *32*(3), 369–391. https://doi.org/10.1093/eurrag/jbi011

Gutiérrez-Jara, C., Bilbao-Sainz, C., McHugh, T., Chiou, B. sen, Williams, T., & Villalobos-Carvajal, R. (2020). Physical, mechanical and transport properties of emulsified films

based on alginate with soybean oil: Effects of soybean oil concentration, number of passes and degree of surface crosslinking. *Food Hydrocolloids*, *109*. https://doi.org/10.1016/j.foodhyd.2020.106133

Harikrishnan, A. R., Das, S. K., Agnihotri, P. K., & Dhar, P. (2017). Particle and surfactant interactions effected polar and dispersive components of interfacial energy in nanocolloids. *Journal of Applied Physics*, *122*(5). https://doi.org/10.1063/1.4997123

Hasheminya, S. M., & Dehghannya, J. (2021). Development and characterization of novel edible films based on Cordia dichotoma gum incorporated with Salvia mirzayanii essential oil nanoemulsion. *Carbohydrate Polymers*, *257*. https://doi.org/10.1016/j.carbpol.2020.117606

Hifney, A. F., Fawzy, M. A., Abdel-Gawad, K. M., & Gomaa, M. (2016). Industrial optimization of fucoidan extraction from Sargassum sp. and its potential antioxidant and emulsifying activities. *Food Hydrocolloids*, *54*, 77–88. https://doi.org/10.1016/j.foodhyd.2015.09.022

Jridi, M., Sellimi, S., Lassoued, K. Ben, Beltaief, S., Souissi, N., Mora, L., Toldra, F., Elfeki, A., Nasri, M., & Nasri, R. (2017). Wound healing activity of cuttlefish gelatin gels and films enriched by henna (Lawsonia inermis) extract. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *512*, 71–79. https://doi.org/10.1016/j.colsurfa.2016.10.014

Kanmani, P., & Rhim, J. W. (2014). Properties and characterization of bionanocomposite films prepared with various biopolymers and ZnO nanoparticles. *Carbohydrate Polymers*, *106*(1), 190–199. https://doi.org/10.1016/j.carbpol.2014.02.007

Karkhanis, S. S., Stark, N. M., Sabo, R. C., & Matuana, L. M. (2021). Potential of extrusion-blown poly(lactic acid)/cellulose nanocrystals nanocomposite films for improving the shelf-life of a dry food product. *Food Packaging and Shelf Life*, *29*, 100689. https://doi.org/10.1016/j.fpsl.2021.100689

Kuai, L., Liu, F., Chiou, B. Sen, Avena-Bustillos, R. J., McHugh, T. H., & Zhong, F. (2021). Controlled release of antioxidants from active food packaging: A review. In *Food Hydrocolloids* (Vol. 120). Elsevier B.V. https://doi.org/10.1016/j.foodhyd.2021.106992

Kumar, S. S., Manasa, V., Tumaney, A. W., B. K., B., Chaudhari, S. R., & Giridhar, P. (2020). Chemical composition, nutraceuticals characterization, NMR confirmation of squalene and antioxidant activities of: Basella rubra L. seed oil. *RSC Advances*, *10*(53), 31863–31873. https://doi.org/10.1039/d0ra06048h

Lara, B. R. B., Dias, M. V., Guimarães Junior, M., de Andrade, P. S., de Souza Nascimento, B., Ferreira, L. F., & Yoshida, M. I. (2020). Water sorption thermodynamic behavior of whey protein isolate/ polyvinyl alcohol blends for food packaging. *Food Hydrocolloids*, *103*. https://doi.org/10.1016/j.foodhyd.2020.105710

Li, Y., & Liu, S. (2012). Reducing lipid peroxidation for improving colour stability of beef and lamb: On-farm considerations. In *Journal of the Science of Food and Agriculture* (Vol. 92, Issue 4, pp. 719–729). John Wiley and Sons Ltd. https://doi.org/10.1002/jsfa.4715

Lopes-Lutz, D., Alviano, D. S., Alviano, C. S., & Kolodziejczyk, P. P. (2008). Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. *Phytochemistry*, *69*(8), 1732–1738. https://doi.org/10.1016/j.phytochem.2008.02.014

Ma, Y., Cao, X., Feng, X., Ma, Y., & Zou, H. (2007). Fabrication of super-hydrophobic film from PMMA with intrinsic water contact angle below 90°. *Polymer*, *48*(26), 7455–7460. https://doi.org/10.1016/j.polymer.2007.10.038

Martins, J. T., Cerqueira, M. A., & Vicente, A. A. (2012). Influence of α-tocopherol on physicochemical properties of chitosan-based films. *Food Hydrocolloids*, *27*(1), 220–227. https://doi.org/10.1016/j.foodhyd.2011.06.011

Merino, F. J. Z., Oliveira, V. B., Paula, C. S., Cansian, F. C., Souza, A. M., Zuchetto, M., Hirota, B. C. K., Duarte, A. F. S., Kulik, J. D., Miguel, M. D., & Miguel, O. G. (2015). Análise fitoquímica, potencial antioxidante e toxicidade do extrato bruto etanólico e das frações da espécie Senecio westermanii Dusén frente à Artemia salina. *Revista Brasileira de Plantas Medicinais*, *17*(4), 1031–1040. https://doi.org/10.1590/1983-084X/14_137

Moura, L. E., de Souza, C. O., de Oliveira, E. A. S., Lemos, P. V. F., & Druzian, J. I. (2018). Bioactive efficacy of low-density polyethylene films with natural additives. *Journal of Applied Polymer Science*, *135*(34). https://doi.org/10.1002/app.46461

Nisar, T., Wang, Z.-C., Yang, X., Tian, Y., Iqbal, M., & Guo, Y. (2018). Characterization of citrus pectin films integrated with clove bud essential oil: Physical, thermal, barrier, antioxidant and antibacterial properties. *International Journal of Biological Macromolecules*, *106*, 670–680. https://doi.org/10.1016/j.ijbiomac.2017.08.068

Noori, S., Zeynali, F., & Almasi, H. (2018). Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (Zingiber officinale) essential oil and its effect on safety and quality attributes of chicken breast fillets. *Food Control*. https://doi.org/10.1016/j.foodcont.2017.08.015

Nor Adilah, A., Noranizan, M. A., Jamilah, B., & Nur Hanani, Z. A. (2020). Development of polyethylene films coated with gelatin and mango peel extract and the effect on the quality of margarine. *Food Packaging and Shelf Life*, *26*. https://doi.org/10.1016/j.fpsl.2020.100577

Norcino, L. B., Mendes, J. F., Natarelli, C. V. L., Manrich, A., Oliveira, J. E., & Mattoso, L. H. C. (2020). Pectin films loaded with copaiba oil nanoemulsions for potential use as bio-based active packaging. *Food Hydrocolloids*, *106*. https://doi.org/10.1016/j.foodhyd.2020.105862

Owens, D. K., & Wendt, R. C. (1969). Estimation of the surface free energy of polymers. *Journal of Applied Polymer Science*, *13*(8). https://doi.org/10.1002/app.1969.070130815

Ozturk, S., & Cakmakci, S. (2006). The effect of antioxidants on butter in relation to storage temperature and duration. *European Journal of Lipid Science and Technology*, *108*(11), 951–959. https://doi.org/10.1002/ejlt.200600089

Rhim, J. W., Park, H. M., & Ha, C. S. (2013). Bio-nanocomposites for food packaging applications. In *Progress in Polymer Science* (Vol. 38, Issues 10–11, pp. 1629–1652). Elsevier Ltd. https://doi.org/10.1016/j.progpolymsci.2013.05.008

Rodsamran, P., & Sothornvit, R. (2019). Lime peel pectin integrated with coconut water and lime peel extract as a new bioactive film sachet to retard soybean oil oxidation. *Food Hydrocolloids*, *97*. https://doi.org/10.1016/j.foodhyd.2019.105173

Rufino, M. do S. M., Alves, R. E., Morais, S. M. de, Sampaio, C. de G., Pérez-Jiménez, J., & Saura-Calixto, F. D. C. D. da A. A. T. em F. pela C. do R. L. D. (2007). Metodologia Científica: Determinação da Atividade Antioxidante Total em Frutas pela Captura do Radical Livre DPPH. *Embrapa, (Comunicad*.

Shivangi, S., Dorairaj, D., Negi, P. S., & Shetty, N. P. (2021). Development and characterisation of a pectin-based edible film that contains mulberry leaf extract and its bio-active components. *Food Hydrocolloids, 121*. https://doi.org/10.1016/j.foodhyd.2021.107046

Shokri, S., Parastouei, K., Taghdir, M., & Abbaszadeh, S. (2020). Application an edible active coating based on chitosan- Ferulago angulata essential oil nanoemulsion to shelf life extension of Rainbow trout fillets stored at 4 °C. *International Journal of Biological Macromolecules, 153*, 846–854. https://doi.org/10.1016/j.ijbiomac.2020.03.080

Shokri, Z., & Kamkar, A. (2018). Study the antioxidant activities of edible chitosan and whey protein isolate films containing Mentha Pulegiums essential oil. *Carpathian Journal of Food Science and Technology*, *10*, 150–156.

Siripatrawan, U., & Noipha, S. (2012). Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. *Food Hydrocolloids*, *27*(1), 102–108. https://doi.org/10.1016/j.foodhyd.2011.08.011

Spatafora Salazar, A. S., Sáenz Cavazos, P. A., Mújica Paz, H., & Valdez Fragoso, A. (2019). External factors and nanoparticles effect on water vapor permeability of pectin-based films. In *Journal of Food Engineering* (Vol. 245, pp. 73–79). Elsevier Ltd. https://doi.org/10.1016/j.jfoodeng.2018.09.002

Teixeira, G. L., Maciel, L. G., Mazzutti, S., Gonçalves, C. B., Ferreira, S. R. S., & Block, J. M. (2020). Composition, thermal behavior and antioxidant activity of pracaxi (Pentaclethra macroloba) seed oil obtained by supercritical CO2. *Biocatalysis and Agricultural Biotechnology*, *24*. https://doi.org/10.1016/j.bcab.2020.101521

Tian, Y., Zhao, Y., Zhang, X., Zhang, Y., Zhu, L., & Shi, Q. (2020). Sodium alginate coating pretreatment improved storage stability of heat pump dried scallop adductors: from thermal and thermodynamic points of view. *Journal of Thermal Analysis and Calorimetry*. https://doi.org/10.1007/s10973-020-10087-1

Wathoni, N., Yuan Shan, C., Yi Shan, W., Rostinawati, T., Indradi, R. B., Pratiwi, R., & Muchtaridi, M. (2019). Characterization and antioxidant activity of pectin from Indonesian mangosteen (Garcinia mangostana L.) rind. *Heliyon*, *5*(8). https://doi.org/10.1016/j.heliyon.2019.e02299

Zhou, X., Zong, X., Wang, S., Yin, C., Gao, X., Xiong, G., Xu, X., Qi, J., & Mei, L. (2021). Emulsified blend film based on konjac glucomannan/carrageenan/ camellia oil: Physical, structural, and water barrier properties. *Carbohydrate Polymers*, *251*. https://doi.org/10.1016/j.carbpol.2020.117100

**SUPPLEMENTARY MATERIAL**

**Table S1.** Water activity values of saturated salt solutions at different temperatures.

| Salt | $a_w$ (5°C) | $a_w$ (35°C) | $a_w$ (45°C) | Reference |
|---|---|---|---|---|
| LiCl | 0.129 | 0.113 | 0.112 | (Bertuzzi et al., 2007) |
| $MgCl_2$ | 0.335 | 0.321 | 0.311 | |
| NaCl | 0.757 | 0.749 | 0.745 | |
| KCl | 0.868 | 0.830 | 0.817 | |
| $K_2SO_4$ | 0.985 | 0.967 | 0.961 | (Agustinelli et al., 2014; Kitic et al., 1986) |

**Table S2.** Reference values of the surface energy components of liquids.

| Liquid | $\gamma_L$ (mN m$^{-1}$) | $\gamma_L^d$(mN m$^{-1}$) | $\gamma_L^p$(mN m$^{-1}$) |
|---|---|---|---|
| Water (WA) | 72.8 | 21.8 | 51.0 |
| Glycerol (GLY) | 63.4 | 37.0 | 26.4 |
| 1-bromonaphthalene (BNL) | 44.6 | 44.6 | 0.0 |
| Diiodomethane (DIM) | 50.8 | 48.5 | 2.3 |

$\gamma_L$: global surface energy of the liquid; $\gamma_L^d$: dispersive component surface energy of the liquid; $\gamma_L^p$: polar component surface energy of the liquid.

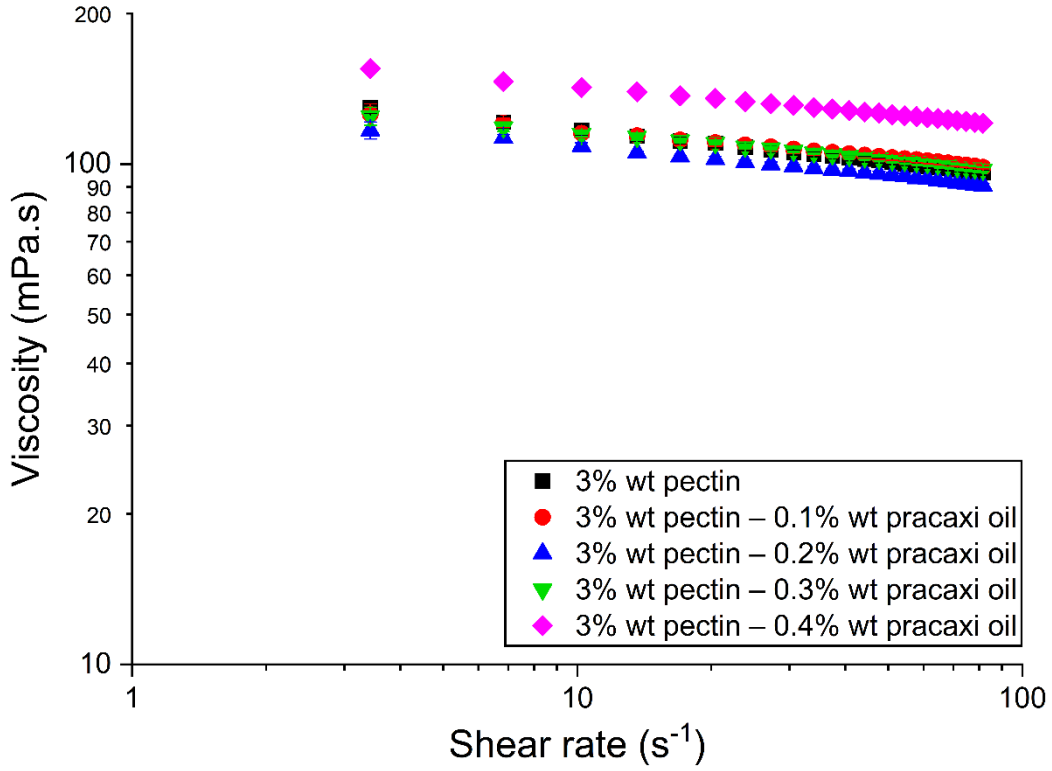


**Figure S1.** Viscosity of the pectin and nanobiocomposites solutions.

**REFERENCES**

Agustinelli, S. P., Salvadori, V. O., & Yeannes, M. I. (2014). Determination of the moisture sorption behavior of osmotically dehydrated mackerel fillets by means of binary and ternary solutions. *Food Science and Technology International*, *20*(5), 353–363. https://doi.org/10.1177/1082013213488610

Bertuzzi, M. A., Castro Vidaurre, E. F., Armada, M., & Gottifredi, J. C. (2007). Water vapor permeability of edible starch based films. *Journal of Food Engineering*, *80*(3), 972–978. https://doi.org/10.1016/j.jfoodeng.2006.07.016

Kitic, D., Jardim, D. C. P., Favetto, G. J., Resnik, S. L., & Chirife, J. (1986). Theoretical Prediction of the Water Activity of Standard Saturated Salt Solutions at Various Temperatures. *Journal of Food Science*, *51*(4), 1037–1041. https://doi.org/10.1111/j.1365-2621.1986.tb11227.x

**ARTIGO 2- Bionanocomposites of cellulose acetate and a hybrid of nanoclay and clove essential oil as active packaging for cooked ham.**

Artigo submetido ao conselho editorial do periódico "Food Packaging and Shelf Life" e formatado conforme normas do referido periódico.

# Bionanocomposites of cellulose acetate and a hybrid of nanoclay and clove essential oil as active packaging for cooked ham.


## ABSTRACT

This paper describes a new formulation composed of cellulose acetate and nano clay hybrids impregnated with clove essential oil and its application in the preservation of sliced cooked ham. The morphology of the bionanocomposites and their properties were evaluated by scanning electron microscopy (SEM), water vapor permeability, colorimetry, mechanical tests, antioxidant and antimicrobial activity. Also, its application in cooked ham samples. The morphological analysis indicated a good dispersion of the nano clay in the films and the presence of the nano clay impregnated with clove oil reduced the water vapor permeability of the bionanocomposites. There was a significant increase in antioxidant and antimicrobial activity. And a slight reduction in bacterial count, lipid oxidation and pH of the cooked ham samples packaged with bionanocomposites. This work demonstrated that bionanocomposites of cellulose acetate and nano clove oil impregnated nano clay hybrids can act as active packaging for slices of cooked ham.


**Keywords**: biopolymer, cloisite, shelf-life, meat, antimicrobial, antioxidant

## 1. INTRODUCTION

The emergence of new demands for healthy, safe, natural, and ecological products with high nutritional value has driven the search for alternatives to replace synthetic polymers in food packaging. Thus, the number of studies aimed at the development of packaging based on natural polymers has been growing in recent years. These materials can function through excellent transport of bioactive compounds, thereby ensuring preservation and food safety (Ait Ouahioune et al., 2022; Farhan & Hani, 2020)

Cellulose is one of the most abundant naturally occurring biopolymers. It has many applications and can undergo different chemical modifications. Among these modifications, esterification occurs through the acetylation of cellulose, forming cellulose acetate (CA), which is a biodegradable thermoplastic polymer. CA has excellent advantages for application in food packaging due to its outstanding optical transparency and high toughness. In addition, CA can be used for the production of active packaging when antioxidant and antimicrobial compounds are incorporated into a polymer matrix of CA (Arrieta et al., 2020; Harini & Sukumar, 2019).

Several nanomaterials, especially montmorillonite (MTT), have been widely used in the development of food packaging. MTT is a clayey material belonging to the phyllosilicate family that exhibits excellent interaction with various biomolecules and readily diffuses in polymer layers. The use of MTT as a nanocarrier for bioactive compounds such as essential oils has been of interest in recent years because these compounds have high antioxidant and antimicrobial activity (Asdagh&Pirsa, 2020; de Oliveira et al., 2022; Giannakas et al., 2021). In addition, the use of MTT may improve the mechanical and barrier properties of packaging (Alves-Silva et al., 2022).

Several studies successfully used nanominerals loaded with essential oils to extend the shelf life of foods: chitosan/MTT films incorporated with essential oils of rosemary and ginger were effective against oxidation in fresh beef and chicken (Pires et al., 2018); cassava starch/sodium bentonite clay and cinnamon essential oil films were produced and tested on meatballs and found to be suitable in terms of antimicrobial activity (Iamareerat et al., 2018); and protein/MTT-based films loaded with clove essential oil demonstrated antimicrobial and antioxidant activity and efficient application for bluefin tuna samples (Echeverría et al., 2018).

The main objective of the present study was to prepare a cellulose acetate bio-nanocomposite with nanoclay modified with clove essential oil and to evaluate its effectiveness with ham samples. The optical, structural, antioxidant, and antimicrobial

properties of the bio-nanocomposites were investigated. The optical properties, microbiological properties, oxidation, and pH of ham when packaged with the bio-nanocomposite for 28 days of storage were also evaluated.

## 2. MATERIALS AND METHODS

### 2.1. Materials

CA in the form of flakes (density: 1.33 g/cm³, with a particle size of 15 mm (90%) and <0.5 mm (0.5%) with a degree of substitution of 2.5) was supplied by Rhodia Acetow. Organically modified commercial grade montmorillonite nanoclay (Cloisite 20A), organically modified with methyl tallow bis (2-hydroxyethyl) quaternary ammonium salt, was supplied by Southern Clay Products, Inc. (Texas, USA). Acetone (Sciavicco, Sabará/MG, Brazil), clove essential oil (Ferquímica, Vargem Grande Paulista/SP, Brazil), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Steinheim, Germany), Folin-Ciocalteu reagent(Êxodo Científica, Hortolândia/SP, Brazil), epoxidized soybean oil (GotalubeAditivos, Itaberaba/SP, Brazil),bacteriological peptone (Becton Dickison and Company, United States), tryptic soy agar (TSA), plate count agar (PCA), yeast extract (Merck, Germany), MRS broth (Scharlb SL, Spain), and MRS agar (Neogem, United States) were also used.

### 2.2. Characterization and quantification of clove essential oil

The clove essential oil was characterized by gas chromatography–mass spectrometry (GC/EMShimadzu, model QP 5050A). A DB5 fused silica capillary column with dimensions of 30 mx 0.25 mm and a film thickness of 0.25 μm was used. The carrier gas (He) had a continuous flow of 1.18 mL $min^{-1}$. The oven temperature was initially 60 °C, then was increased by 3 °C $min^{-1}$ to 240 °C, by 10 °C$min^{-1}$ to 300 °C, andheld constant for 7 minutes. The temperatures of the injector, detector, and ion source were 220 °C, 240 °C, and 200 °C, respectively. The essential oil sample was diluted in hexane, and 1.0 μL of the dilution was injected. The partition rate of the injected volume was 1:100, and the column pressure was 71.0 kPa. The mass spectrometer conditions for the qualitative analysis of the constituents were as follows: detector scan 1,000; scanning intervals of 0.50 fragments, and fragments detected in the range of 45 to 500 Da. The Van den Dool and Kratz equation for the homologous series of n-alkanes (nC8-nC18) was used to calculate the retention index.

The identification of the constituents was performed by comparing the spectra obtained for each component with the database present in the library of the equipment and compared with the Kovats retention indices (Adams, 2007). Quantitative analysis of the

essential oil constituents was performed using a Shimadzu CG-17A gas chromatograph equipped with flame ionization detector (FID), and the operating conditions were identical to those used to identify the constituents.

### 2.3. Preparation of bionanocomposites

A mixture was prepared as described by Torin et al. (2018) with minor modifications. Cloisite 20A was dissolved in acetone, and clove essential oil was added in a 1:2 ratio. The mixture was sonicated under high-end ultrasound for 30 minutes. CA bionanocomposites were prepared using solution *casting* according to the method proposed byRodríguez et al.(2014), Cherifi et al. (2022), and Torin et al.(2018) with some modifications and with the amounts described in Table 1. CA was dissolved in the mixture with the hybrid in the percentage by weight (5% m/v). The varying concentrations of modified nanoclay used were 0, 7.5, 15, 22.5, and 30.0% (m/m relative to CA). The mixture was stirred for 2 h on a magnetic stirrer until the CA was completely dissolved. Then, epoxidized soybean oil (5% w/w of the dry base) was added as a plasticizer and stirred vigorously for 30 minutes at room temperature. Then, 40 mL of the mixture was added to a glass petri dish (diameter 15 cm). The plates were covered and dried in an oven with forced air circulation at 30 °C for 24 h. Finally, the films were removed from the plate and stored in polyethylene bags under ambient conditions (approximately 25 °C and 53% relative humidity) for 3 days before characterization.

Table 1. Description of the fractional weight of the components used to prepare the bionanocomposites.

| Bionanocomposites | Components | | | |
|---|---|---|---|---|
| | Cellulose acetate (% wt) | Clay (% wt) | Clove oil (% wt) | Soybean oil (% wt) |
| Cellulose acetate | 95.0 | - | - | 5.0 |
| Cellulose acetate - 7.5% hybrid | 87.5 | 2.5 | 5.0 | 5.0 |
| Cellulose acetate – 15.0% hybrid | 80.0 | 5.0 | 10.0 | 5.0 |
| Cellulose acetate - 22.5% hybrid | 72.5 | 7.5 | 15.0 | 5.0 |

| | | | | |
|---|---|---|---|---|
| Cellulose acetate – 30.0% hybrid | 65.0 | 10.0 | 20.0 | 5.0 |

**2.4. Mechanical properties**

The bionanocomposites were subjected to uniaxial tensile testing in a universal testing machine DL3000 (EMIC, São Paulo-SP, Brazil). The tests were performed according to ASTM D822-09 (ASTM, 2009) and other studies (Abral et al., 2019; Saricaoglu et al., 2018). The samples were at least 10 mm wide and 100 mm long. The specimens were subjected to thickness determination (measured using a Mitutoyo IP65 digital micrometer at three random positions) per treatment and were stretched to 25 mm $min^{-1}$ using an initial clamp-to-clamp distance of 50 mm and a load cell of 50 kgf. The elastic modulus was determined from the linear slope of the stress versus strain curves. The tensile strength was calculated by dividing the maximum force by the initial cross-sectional area. The elongation at break (ε) was calculated by Equation 1, where d is the final displacement and $d_0$ is the initial clamp-to-clamp distance:

$$\varepsilon(\%) = \frac{d-d_0}{d_0} x\ 100 \quad (1)$$

The elastic modulus was determined from the linear slope of the stress versus strain curves.

**2.5. Water vapor permeability**

The water vapor permeability (WVP) was measured according to the methodology described in ASTM E96 (ASTM, 2009). 40-mL glass vials with 14-mm diameter lids were used, with 3/4 of their volume containing dry silica. The bionanocomposite samples were cut so that their areas corresponded to the opening of the lid and were placed between the lids and the ampoules. The vials containing the sample and silica were placed in a desiccator maintained at 11 °C, with the relative humidity controlled with saturated NaCl solution (75%). The flasks were weighed every 24 h for a period of seven days.

The WVP was calculated from the slope (G) of a linear regression of the weight gain over time, as shown in Equation 2.

$$WVP = \frac{Gx}{A\Delta p} \quad (2)$$

where x is the film thickness, A is the area of the exposed film, and Δp is the partial pressure of water vapor across the film.

### 2.6. Optical properties

The determination of the color and transparency of the films was determined as proposed Gomide et al. (2021). The color parameters *L**, *a**, and *b** were measured with the aid of an HPSPRO colorimeter (Coralis, São Paulo, Brazil) with a D65 light source, observation angle of 10°, and specular component included (SCI).

The transparency was measured using a UV–VIS spectrophotometer (Nova Instruments) at 600 nm. Specimens measuring 3 X 1 cm were used, which were fixed so that the light beam could pass through unobstructed.

The transparency (*T*) was calculated according to Equation (3):

$$T = \frac{Log\ \%T}{e} \quad (3)$$

where *e* is the film thickness (mm).

### 2.7. Antioxidant activity of bio-nanocomposites

#### 2.7.1. Preparation of cellulose acetate bionanocomposite extract

The extract was prepared according to Farhan & Hani (2020) with some modifications and used for the determination of the total phenolic compounds (TPC) and the antioxidant activities by the DPPH, β-carotene/linolenic acid, and phosphomolybdenum methods. In a container, 25 mg of the bionanocompositewas cut into small pieces, and 5 mL of methanol/water (80:20 v/v) was added. The mixture was subjected to ultrasound for 30 minutes at room temperature to obtain the extract.

#### 2.7.2. DPPH method

The antioxidant activity was determined by the DPPH method as described by Farhan & Hani (2020), with some modifications. 0.1 mL of each film extract was mixed with 3.9 mL of DPPH (0.06 mM) in methanol solution. After 60 minutes of reaction in the dark, the remaining DPPH was determined by absorbance at 515 nm using a UV–VIS spectrophotometer (Nova Instruments). The absorbance of a control was also obtained so that the absorbance of the DPPH solution before reacting with the film extract sample could be determined. The antioxidant activity was calculated according to Equation 4.

$$\%AA = 100 \times \left[1 - \left(\frac{A_{sample}}{A_{control}}\right)\right] \quad (4)$$

### 2.7.3. Phosphomolybdenum method

The determination of the antioxidant molybdenum (VI) reduction activity was performed as proposed by Merino et al. (2015) and Hifney et al.(2016) Briefly, with some modifications, a 0.1 mL aliquot of the bio-nanocomposite extract was added to 3.0 mL of reagent (4 mM ammonium molybdate, 28 mM sodium phosphate, 0.6 M sulfuric acid). The tubes were closed and placed in a water bath at 95 °C for 90 minutes. After heating, readings were obtained with a UV–VIS spectrophotometer (Nova Instruments) at 695 nm. The standard curve at different concentrations of ascorbic acid was used, and the results were expressed as mg of ascorbic acid $g^{-1}$ of film.

### 2.7.4. Total phenolic compounds

The determination of TPC was performed by the Folin-Ciocalteu method, as reported by Nisar et al. (2018). Then, 0.5 mL of the bionanocomposite extracts was added to 2.5 mL of Folin-Ciocalteu reagent (10%) and 2.0 mL of sodium carbonate solution ($Na_2CO_3$). After homogenization and 2 hours of resting, readings were obtained with a UV–VIS spectrophotometer (Nova Instruments) at 720 nm. The standard curve with different concentrations of gallic acid equivalent (GAE) was used, and the results were expressed as mg GAE 100 $g^{-1}$ of film.

### 2.8 Inoculum activation, standardization and maintenance

Enteropathogenic *Escherichia coli* (EPEC) strains INCQS 00181 (CDC O55), *Listeria monocytogenes* ATCC 19117 and *Lactobacillus sakei* ATCC 15521 from the Food Microbiology laboratory of the Food Science Department of the Federal University of Lavras were used. The stock cultures were stored in freezing medium (glycerol, 15 mL; bacterial peptone, 0.5 g; yeast extract, 0.3 g; NaCl, 0.5 g and distilled water, 100 mL, pH 7.0). The inocula were reactivated by inoculating 100 µL aliquots of the stock cultures into tubes containing 10 mL of tryptone soy broth (TSB) for EPEC, TSB plus 0.6% yeast extract (TSB+YE) for L. monocytogenes, and Man Rogosa & Sharp broth (MRS) for L. sakei. The tubes containing the EPEC and L. monocytogenes inocula were incubated at 37°C and L. sakei at 30°C for 24 hours. Standardization of the inocula was performed using the 0.5 degree of the Mac Farlande scale, approximately $10^8$ CFU $mL^{-1}$.

### 2.8.1 Antimicrobial activity by contact

Antibacterial tests were performed to evaluate the antibacterial effects of the CA bio-nanocomposites with nanoclay modified with clove essential oil. Direct contact on agar was performed as proposed by Lammi et al. (2019), with some modifications of bacterial strains (*Lactobacillus sakei, Listeria monocytogenes,* and *Escherichia coli*). The tests were performed on young precultures of the bacteria in broth. Then, 100 µl aliquots of suspensions at $10^8$ CFU/ml were inoculated on the surface of plates containing MRS agar for *Lactobacillus sakei* bacteria, tryptic soy soy agar (TSA) + YE for *Listeria monocytogenes* bacteria, and TSA for *Escherichia coli* bacteria. Then, rectangles of each bio-nanocomposite with dimensions of 15 mm x 10 mm were placed on the plates. The analyses were performed with three replicates. The plates were incubated at 37 °C for 24 h for *Escherichia coli* and *Listeria monocytogenes* and at 30 °C for 48 h for *Lactobacillus sakei*. The occurrence of an antibacterial effect was evidenced by the appearance of a clear region below where the films were placed. And evaluated as total inhibition, partial inhibition and no growth inhibition.

**2.9 Preparation, packaging, and storage of ham samples**

The bionanocomposite with 30% modified nanoclay was investigated in terms of its storage efficiency for ham and evaluated through microbiological (mesophiles, psychrotrophs, and lactic acid bacteria (LAB) and physicochemical (color, pH, and lipid oxidation) analyses, where lipid oxidation was determined by the formation of thiobarbituric acid-reactive substances (TBARS). As a control, the CA film was used without the presence of the nanoclay hybrid.The ham used was purchased from the local market, cut into rectangular slices of approximately 15 g and into separate batches for microbiological and physicochemical determinations, and packaged separately, one for each sampling time and in three replicates. The samples were stored in a refrigerator at 11 °C (temperature abuse) and 30% relative humidity. The microbiological and physicochemical quantifications were performed at 0, 7, 14, 21 and 28 days of storage.

**2.9.1. Microbiological analyses of ham**

The microbiological analyses of the ham samples were performed as proposed by Pateiro et al. (2019) with minor modifications. Ten grams of ham was homogenized with 90 mL of sterile 0.1% w/v peptone water in a Stomacher (Metroterm, Brazil; 490 strokes/min) for 3 minutes at room temperature. Serial decimal dilutions were prepared for each sample in 0.1% peptone solutions, and 10 µL of the samples in appropriate dilutions, in duplicate, were pipetted for total count and selective agar plates.

Mesophile and psychrotrophic counts were obtained on plate count agar (PCA) (with incubation at 37 °C for 24 h and 11 °C for 5 days, respectively. LAB counts on MRS agar after incubation at 30 °C for 48 hours were obtained.

### 2.9.2 Physicochemical analyses

**Instrumental color**

The instrumental color of the ham samples was evaluated using a CM700 spectrophotometer colorimeter (Konica Minolta Sensing Inc., Japan) according to the recommendations of Ramos & Gomide (2017). The readings were obtained with a D65 illuminant, an observer angle of 10°, and specular component excluded (SCE mode). At two different points of the sample, the CIELAB color indices were recorded: luminosity (L*), red index (a*), yellow index (b*), saturation (C*), and hue angle (h, degrees).

**Evaluation of pH**

The pH was determined according to the methodology described byLima et al. (2017). Five grams of the sample was weighed and homogenized (Ultraturrax, IKA T10, United States) in 50 mL of distilled water for 1 minutes, and the pH was measured using a digital pH meter (Tecnopon mPA-201, Piracicaba, São Paulo, Brazil).

**Thiobarbituricacid-reactive substances**

Lipid oxidation was assessed by the determination of TBARS as described byPikul et al. (1989) with some adaptations. Fifteen milliliters of 3.86% perchloric acid and 1 mL of the antioxidant hydroxybutyl toluene (HBT) 0.15% (w/v) were added to 5 g of ground ham sample. The mixture was homogenized (Ultraturrax, IKA T10, United States) for 60 s and filtered. A 2 mL aliquot of the filtrate was added to 2 mL of 0.02 M thiobarbituric acid (TBA). The tubes were then boiled for 30 minutes in a water bath and then cooled for 15 minutes in an ice bath. The absorbance of the mixture was measured in a spectrophotometer (Nova Instruments) at 532 nm. A blank (0.02 M TBA and 3.86% perchloric acid in the proportion of 1:1) was used. The concentration of malondialdehyde (MDA) was determined from an analytical curve prepared with 1,1,3,3-tetraethoxypropane (TEP), and the results were expressed in milligrams of MDA per kilogram of sample (mg MDA/kg).

### 2.10 Statistical analysis

The results were subjected to analysis of variance (ANOVA), and the means were

compared by Tukey's test at a confidence level of 95% ($p < 0.05$) using the statistical software SISVAR 5.4.

## 3 RESULTS AND DISCUSSION

### 3.1 Chemical composition of clove oil

Table 2 shows the results of the chromatographic analysis of clove essential oil, which revealed the presence of four chemical compounds, with eugenol being the major compound, with a peak area of 83.28%, followed by eugenol acetate (10.25%), caryophyllene (6.09%), and α-caryophyllene (0.38%).

Table 2 . Chemical compounds present in clove essential oil

| Retention time (min) | Peak area (%) | Compound |
|---|---|---|
| 23.205 | 83.28 | Eugenol |
| 26.001 | 6.09 | Caryophyllene |
| 27.474 | 0.38 | α-Caryophyllene |
| 29.846 | 10.25 | Eugenol acetate |

These results are similar to those reported by Vinicius de Oliveira Brisola Maciel et al. (2020) with clove essential oil, which was found to be eugenol (80.15%) and caryophyllene (14.03%). However, Radünz et al. (2019) found a slightly different chemical composition for clove essential oil, with eugenol (50.06%), caryophyllene (39.63%), and α-caryophyllene (4.31%). These differences in composition can be attributed to factors such as the type of plant (species), geographical location, soil type, harvest time, genetic factors, plant age, and the methods used in extraction (Varghese et al., 2020).

### 3.2 Scanning electron microscopy

To investigate the effect of nanoclay and clove essential oil hybrids on CA bio-nanocomposites, scanning electron microscopy (SEM) images of the fracture surfaces of the films were captured. Figure 1 shows a uniform and smooth cryogenic fracture surface. With the addition of the modified nanoclay (Figure 1(b) - 1(e), the fracture surfaces remained smooth; however, the fracture surface was observed to increase in roughness, possibly due to the dispersion of the intercalated/exfoliated clay in the biopolymer matrix (Torin et al., 2018). The efficiency of the distribution and dispersion of nanoclay in biopolymer matrices has already been reported in the literature (Salarbashi et al. (2018)). In these studies, contents of up to 1% w/w nanoclay in a soybean polysaccharide matrix were found to exhibit good

dispersion and distribution. However, the SEM results indicated that higher concentrations led to agglomeration of the nanoclay in the biopolymer matrix. For the bio-nanocomposites of CA and nanoclay impregnated with clove oil, a good dispersion was observed at concentrations of up to 10% w/w nanoclay (30% w/w hybrid). This result can be attributed to the polarity of CA and nanoclay impregnated with clove oil, as well as the strong electrostatic interactions of CA, Cloisite, and clove oil.

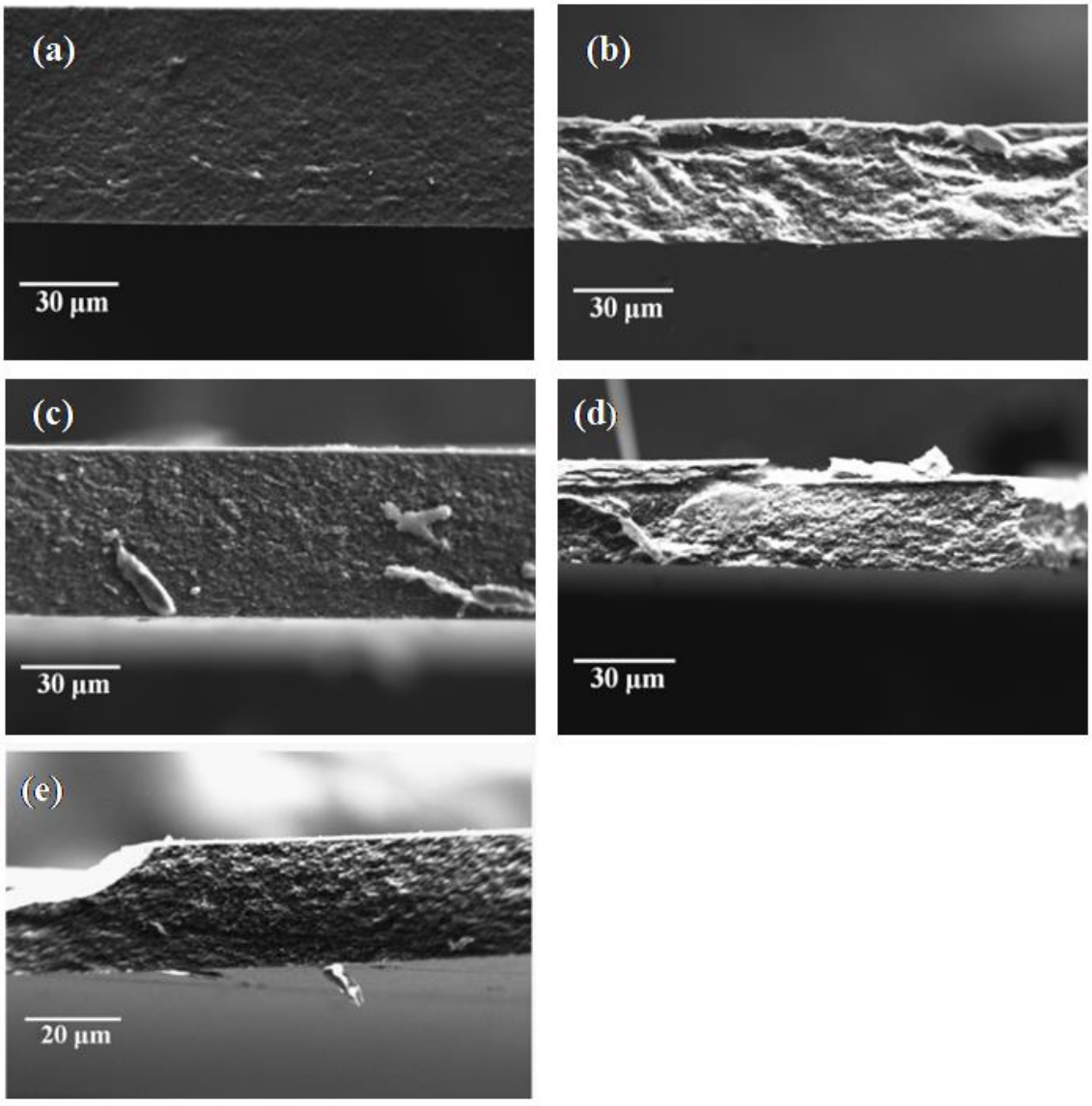


Figure 1. Cross-sectional SEM images of (a) cellulose acetate; (b) cellulose acetate - 7.5% hybrid; (c) cellulose acetate - 15.0% hybrid; (d) cellulose acetate - 22.5% hybrid cellulose acetate - 7.5% hybrid and (e) cellulose acetate - 30.0% hybrid.

### 3.2 Mechanical properties

The effects of incorporating the nanoclay and clove essential oil into CA films on the

elastic modulus, tensile strength and elongation at break were investigated, and the results are shown in Table 3. The introduction of the mixture did not cause significant changes ($p < 0.05$) in the elasticity module of the films. However, for tensile strength, there was a significant reduction ($p < 0.05$), decreasing from 59.92 MPa in the CA film to 35.52 MPa when 30% mixture was added. According to da Silva Scudeler et al. (2020), this reduction in tensile strength occurs due to the heterogeneous structure of the film, as this results in discontinuities in the structure of the network; as a result, points more susceptible to breakage confer greater fragility, thus reducing the tensile strength.

A significant reduction in elongation at break was also observed, varying from 16.74% in the CA film to 8.05% when 30% mixture was added. This reduction in elongation possibly occurs as a result of the crosslinking effect; interactions between CA and the nanoclay can cause a compact bubble-shaped structure, resulting in a decrease in the elongation values (Marand et al., 2021).

Table 3. Mean values and standard deviation for mechanical properties in cellulose acetate bionanocomposites incorporated with different concentrations of the hybrid of nano clay and clove essential oil.

| Bionanocompósitos | Modulus of Elasticity (MPa) | Tensile strength (MPa) | Elongation at rupture (%) |
|---|---|---|---|
| Cellulose acetate | 1355.45±426.75$^{a}$ | 59.92±14.19$^{a}$ | 16.74±9.99$^{a}$ |
| Cellulose acetate - 7.5% hybrid | 1580.89±371.31$^{a}$ | 50.05±8.57$^{ab}$ | 8.16±5.45$^{ab}$ |
| Cellulose acetate – 15.0% hybrid | 1480.70±330.71$^{a}$ | 41.85±7.42$^{ab}$ | 8.09±4.34$^{ab}$ |
| Cellulose acetate - 22.5% hybrid | 1348.02±234.46$^{a}$ | 40.87±6.19$^{bc}$ | 13.24±8.70$^{ab}$ |
| Cellulose acetate – 30.0% hybrid | 1276.35±150.29$^{a}$ | 35.52±5.22$^{c}$ | 8.05±3.26$^{b}$ |

Means ± standard deviation of ten repetitions followed by the same lower case letters (comparison between samples at the same temperature) in the same column do not differ statistically by Tukeya's Test 5% ($p<0.05$).

### 3.3. Optical properties

The CAbio-nanocomposites modified with nanoclay presented smooth, homogeneous, and translucent surfaces (Figure 2). The optical characteristics of packaging for application in

food are one of the most important factors for customer selection in terms of product choice. According to the data presented in Table 4, the incorporation of modified nanoclay caused significant changes ($p<0.05$) in the color and transparency characteristics of the bio-nanocomposites. The control films were lighter, presenting higher luminosity (L) values. The addition of nanoclay impregnated with essential oil caused the brightness values to reduce from 91.90 to 80. A decrease in transparency was also observed, changing from 18.23 to 13.66. The redness index (a*) and the yellowness index (b*) increased with the presence of nanoclay and essential oil, increasing from -0.65 to 4.05 and from -0.77 to 16.11, respectively.

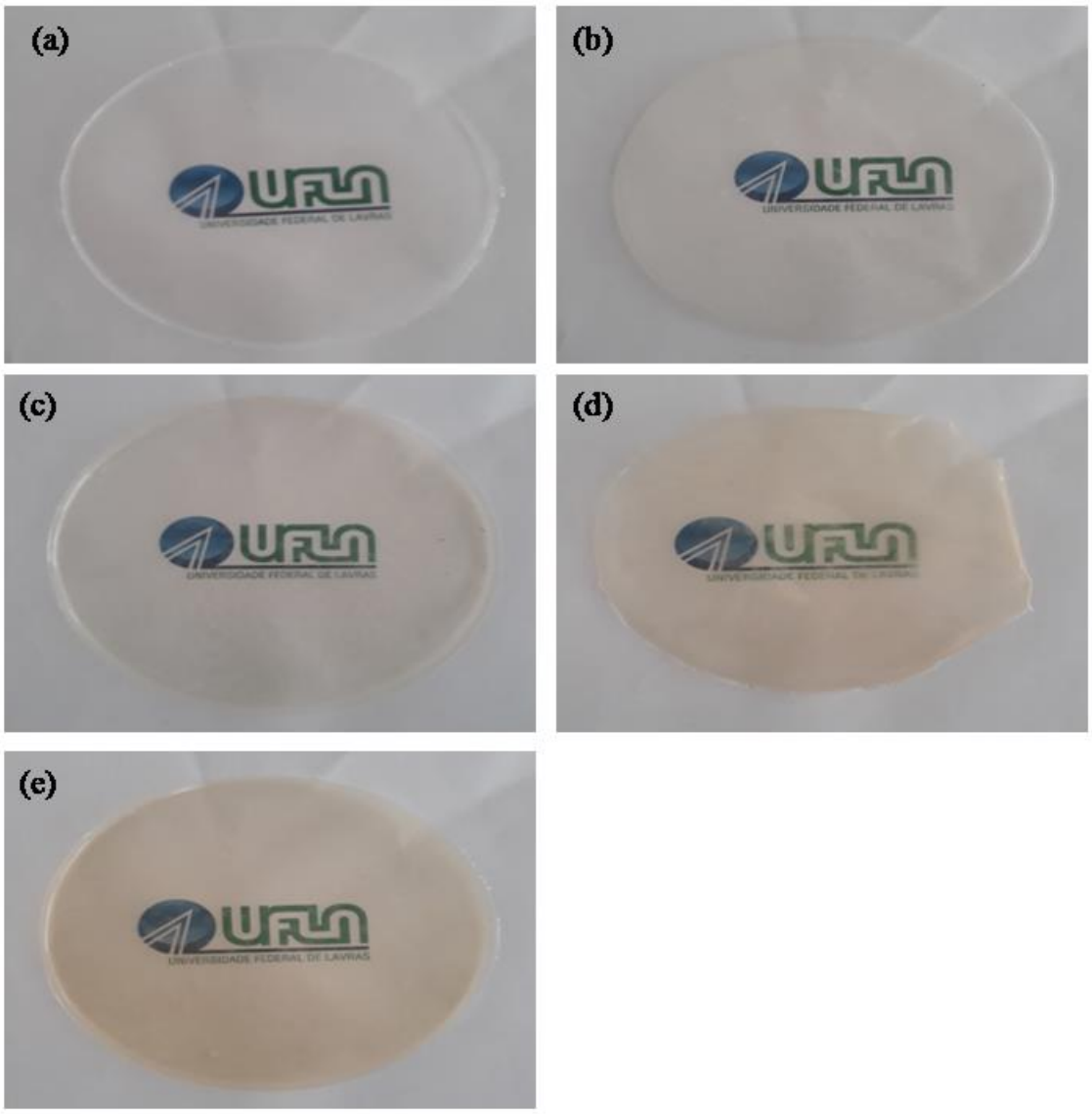


Figure 2 . Images of the bionanocomposites (a) cellulose acetate; (b) cellulose acetate - 7.5% hybrid; (c) cellulose acetate -15.0% hybrid; (d) cellulose acetate- 22.5% hybrid cellulose acetate - 7.5% hybrid and (e) cellulose acetate - 30.0% hybrid

The variation in the parameters of color and transparency can be attributed to the natural yellow color of the pigments present in clove essential oil (Nisar et al., 2018) as well as the intrinsic colors of the nanoclay (Micó-Vicent et al., 2020). Similar results for color and transparency parameters were reported by Nisar et al. (2018) and Asdagh & Pirsa (2020). These results for the optical properties of bionanocomposites do not necessarily negatively affect customer selection, as Matta Fakhouri et al. (2019), in studies with starch films and cranberry powder, obtained darker films, which were well accepted by the panelists in a sensory analysis.

Table 4. Color and transparency in cellulose acetate bionanocomposites incorporated with different concentrations of modified nano clay.

| Color | % w/w of hybrid | | | | |
|---|---|---|---|---|---|
| Parameters | 0 | 7.5 | 15.0 | 22.5 | 30.0 |
| L | 91.9±1.3$^{a}$ | 91.3±1.3$^{a}$ | 85.1±0.84$^{b}$ | 83.1±1.9$^{c}$ | 80.0±1.5$^{d}$ |
| a* | -0.6±0.2$^{e}$ | -0.04±0.26$^{d}$ | 1.9±0.4$^{c}$ | 2.6±0.3$^{b}$ | 4.0±0.7$^{a}$ |
| b* | -0.8±0.2$^{e}$ | 3.8±0.3$^{d}$ | 10.7±1,1$^{c}$ | 12.0±1.1$^{b}$ | 16.1±1.0$^{a}$ |
| Transparency | 18.2±1.1$^{a}$ | 18.6±0.5$^{a}$ | 15.2±1.4$^{b}$ | 12.1±0.8$^{a}$ | 13.7±0.5$^{ab}$ |

Means ± standard deviation of ten repetitions followed by the same lower case letters (comparison between samples at the same temperature) in the same row do not differ statistically by Tukeya's Test 5% ($p<0.05$).

### 3.3. Water vapor permeability

The presence of moisture in foods can cause a number of undesirable changes, such as the proliferation of microorganisms, in addition to changes in the physical-chemical parameters. Thus, knowing the water vapor barrier properties of films for application in food becomes fundamental and essential (Marand et al., 2021). The results of this study (Figure 3) showed that the incorporation of nanoclay modified with clove essential oil resulted in a significant reduction ($p<0.05$) in water vapor permeability. The CA films without nanoclay presented a permeability of $1.923x10^{-12}$ $gm^{-1}$ $s^{-1}$ $Pa^{-1}$, while the bionanocomposite with 30% nanoclay presented a WVP of 0.544 $x10^{-12}gm^{-1}s^{-1}Pa^{-1}$, a reduction of approximately 72%.

This reduction in WVP values may possibly be associated with the hydrophobic nature of essential oils. Thereby, the nanoclay impregnated with clove oil acts as a barrier to the diffusion of water molecules in the CA matrix (Iamareerat et al., 2018; Lee et al., 2018).

The results of this study are in agreement with those reported by Marand et al. (2021), who observed a reduction in WVP in active films based on Plantago seed gum,

nanoclay, and fennel essential oil. Additionally, Lee et al., (2018) observed a reduction in WVP values with the addition of halloysite and clove essential oil. However, in both studies, the absolute values of WVP were higher than those in this study, but the experimental conditions were also different.

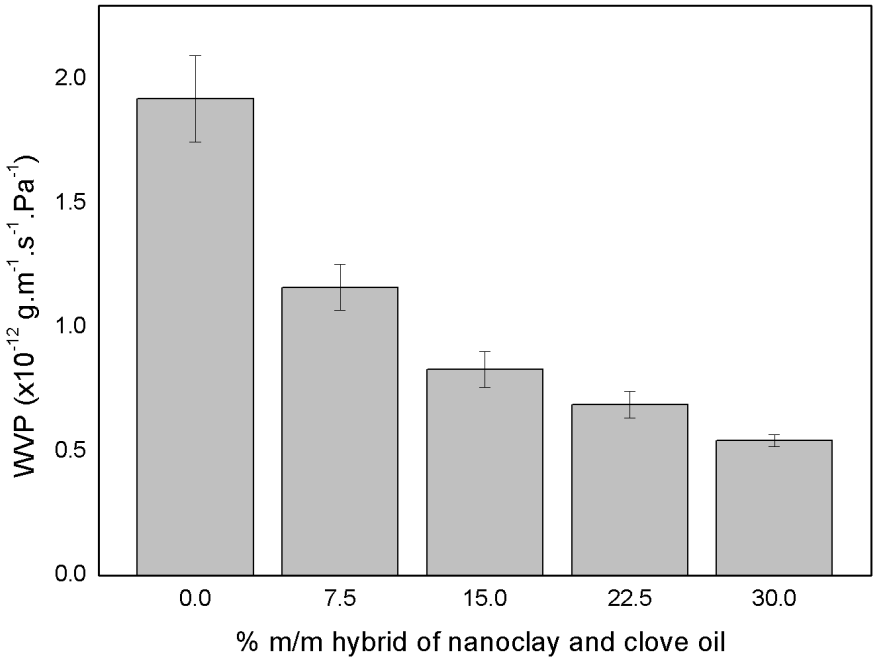


Figure 3. Results of water vapor permeability (WVP) determinations on cellulose acetate bionanocomposites incorporated with nano clove oil modified nano clay.

### 3.4. Antimicrobial activity of cellulose acetate films

Antimicrobial activity is a fundamental property for the determination of active packaging systems. Thus, *Escherichia coli* (gram-negative) and *Listeria monocytogenes* and *Lactobacillus sakei* (gram-positive), as pathogenic (Gracia-Vallés et al., 2022) and spoilage (Veselá et al., 2022) bacteria in cooked ham samples, were chosen for testing. Table 5 shows the antimicrobial activity of the bionanocomposites against the bacteria according to testing by the contact method. The CA films without nanoclay impregnated with clove oil did not show antimicrobial activity, as no inhibition or reduction of growth was observed for the three bacterial strains tested. This result is in agreement with that reported by El Fawal et al. (2019). The addition of nanoclay impregnated with clove essential oil inhibited or reduced growth for all bacterial strains investigated. At a concentration of 7.5% w/w, only a reduction in the bacterial growth of all 3 bacterial strains was observed. *Listeria monocytogenes* was the most

sensitive bacterium because at concentrations higher than 15% modified nanoclay, there was no bacterial growth in the contact area. The CA bionanocomposite and 30% of the hybrid showed total inhibition in the contact area for all three bacterial strains tested.

The antimicrobial activity of the bio-nanocomposites with the highest percentage of nanoclay and clove essential oil is possibly due to the MTTs being clays organically modified with a quaternary ammonium salt; the presence of ammonium groups in the salt are able to react with the proteins and lipids of the cell wall and cause the death of microorganisms (M. Ramos et al., 2020). Clove essential oil also contains eugenol (Table 1), which is a phenolic monoterpenoid compound with a hydroxyl group that is highly reactive and forms hydrogen bonds with the active sites of the bacterial target enzymes, inactivating it. As a result, there is a disturbance or rupture in the cell membrane (A. C. Guimarães et al., 2019). These compounds can interact with the lipid bilayer fraction and cause an increase in permeability, causing cell leakage, leading to cell death and resulting in antimicrobial activity (Abou Baker et al., 2020; Ozogul et al., 2020).

Table 5. Antimicrobial effect of cellulose acetate bionicomposites with nano clove essential oil modified nanoclay against *Lactobacillus sakei*, *Listeria monocytogenes* and *Escherichia coli* by contact technique.

| | Bionanocomposites (% w/w hybrid) | | | | |
|---|---|---|---|---|---|
| Bacteria | 0 | 7.5 | 15.0 | 22.5 | 30.0 |
| *Escherichia coli* | - | + | + | + | ++ |
| *Listeria monocytogenes* | - | + | ++ | ++ | ++ |
| *Lactobacillus sakei*. | - | + | + | + | ++ |

The - sign indicates that there was no inhibition; + indicates that there was a reduction in bacterial growth, and ++ total inhibition.

### 3.5. Antioxidant activity and total phenolic compounds of cellulose acetate films

The antioxidant activity of the bio-nanocomposites was determined in terms of TPC and by two types of methods: DPPH and phosphomolybdenum. The results are described in Table 6. The addition of the nanoclay hybrid and clove oil to the CA films resulted in a significant increase in TPC, varying from 1.64±0.10 mg GAE in the CA film to 103.77±0.25 mg GAE in the 30% w/w hybrid bionanocomposite.

Phenolic compounds are often present in essential oils, with eugenol being the major phenolic compound present in clove essential oil (Mande &Sekar, 2021). The presence of the

aromatic ring in their structure allows phenolics to eliminate reactive oxygen species in oxidative processes through the transfer of hydrogen atoms, as they has the ability to stabilize due to the presence of resonance in their structure (Alfikri et al., 2020; Mande & Sekar, 2021) These properties allow the neutralization of free radicals and the decomposition of peroxides. Thus, when applied in food systems, phenolics can restrict the effects of degradation associated with the oxidation of lipids and fats present in ham and thus increase the shelf life of these foods (Alves-Silva et al., 2022).

The increase in TPC was accompanied by a significant increase in antioxidant activity, as measured by the DPPH and phosphomolybdenum methods. The absence of antioxidant activity is observed for the CA film due to the lack of functional groups with antioxidant capacity available in the CA chain (Tedeschi et al., 2018).

The DPPH radical is purple in color, and the presence of antioxidants in the sample causes the transfer of a hydrogen atom and an observed change in color from purple to yellow (Nisar et al. 2018; Rambabu et al., 2019). Thus, the nanoclay modified with clove oil caused the antioxidant activity of DPPH to increase from 2.79% to 88.70% when 30% w/w nanoclay and clove oil were added.

The presence of nanoclay at a percentage of 30% caused an increase of approximately 889-fold in the antioxidant activity according to phosphomolybdenum testing. This method is based on the estimation of the antioxidant activity of polyphenols and other nonvolatile antioxidant compounds by reducing molybdenum (VI) phosphate to molybdenum phosphate (V), generating a green complex (Ait Ouahioune et al., 2022).

The antioxidant activity found in this study is higher than that reported in the literature for pectin films with nanoemulsion of pracaxi oil (Candido et al., 2022), CA films incorporated with the essential oils of rosemary and aloe vera (El Fawal et al., 2019), and films of chitosan, MTT, and α-tocopherol nanoparticles (Yan et al., 2019)

Table 6. Total phenolic compounds and antioxidant activity by DPPH, β-carotene/linolenic acid and phosphomolybdenum in cellulose acetate bionanocomposites incorporated with nano clove essential oil modified nano clay.

| Bionanocomposites | DPPH (AA%) | Phosphomolybdenum (mg AAEq/g) | TPC (mg GAEq/g) |
|---|---|---|---|
| Cellulose acetate | 2.79±0.34 [d] | 0.99±0.20 [e] | 1.64±0.10 [e] |

| | | | |
|---|---|---|---|
| Cellulose acetate - 7.5% hybrid | 62.72±2.16 [c] | 205.19±3.59 [d] | 35.95±0.58 [d] |
| Cellulose acetate – 15.0% hybrid | 81.79±0.75 [b] | 438.08±7.77 [c] | 79.55±0.83 [c] |
| Cellulose acetate - 22.5% hybrid | 85.87±0.63 [a] | 554.9 9±11.30 [b] | 94.16±0.74 [b] |
| Cellulose acetate – 30.0% hybrid | 88.70±0.83 [a] | 889.98±25.27 [a] | 103.77±0.25 [a] |

Means ± standard deviation of three repetitions followed by the same lower case letters (comparison between samples at the same temperature) in the same column do not differ statistically by Tukeya's Test 5% ($p<0.05$).

### 3.6. Ham microbiology

The microbiological evaluation of the ham samples consisted of counting total LAB, mesophiles, psychrotrophs during the 28 days of storage. From the 7th day, a significant difference between the control film and the film with 30% modified nanoclay was observed for the mesophiles, psychrotrophs, and LAB (Figure 4) in the ham samples. This difference is probably due to the migration of clove oil through the ham since the antimicrobial power is correlated with the presence of phenolic compounds (Pateiro et al., 2019). Thus, it can be inferred that the bio-nanocomposites exert bacteriostatic action by reducing the growth of bacterial strains present in ham samples for up to 21 days of storage (Priyadarshi et al., 2021). However, from the 21st day onward, no static difference in microbial growth was observed in the ham samples packed with the control film and the bio-nanocomposites. These results are an indication that the processes of diffusion and sorption of clove oil from the bio-nanocomposites to the ham matrix reached thermodynamic equilibrium and that the growth rate of the bacteria reestablished.

Other studies in the literature indicate the application of active packaging for samples of ham. Films of synthetic polymers, such as polyethylene and polyethylene terephthalate containing green tea plant extract, were observed to exert bacteriostatic action from 14 days to 21 days in which the experiment was conducted. However, in films with the same polymer but using another active compound (1% mixture of green tea extract and oregano essential oil), the microbiological evaluation revealed that the film was not efficient in increasing the shelf life of the ham (Pateiro et al., 2019). In addition, when testing chitosan films and thymine essential oil applied during 28 days of ham storage, no significant differences between the active packaging and the control were detected (Quesada et al., 2016). Nanoclay reduces the mobility of active compounds such as eugenol (electrostatic interaction), and thus,

CA bionanocomposites with nanoclay hybrid and clove oil exhibit superior bacteriostatic action.

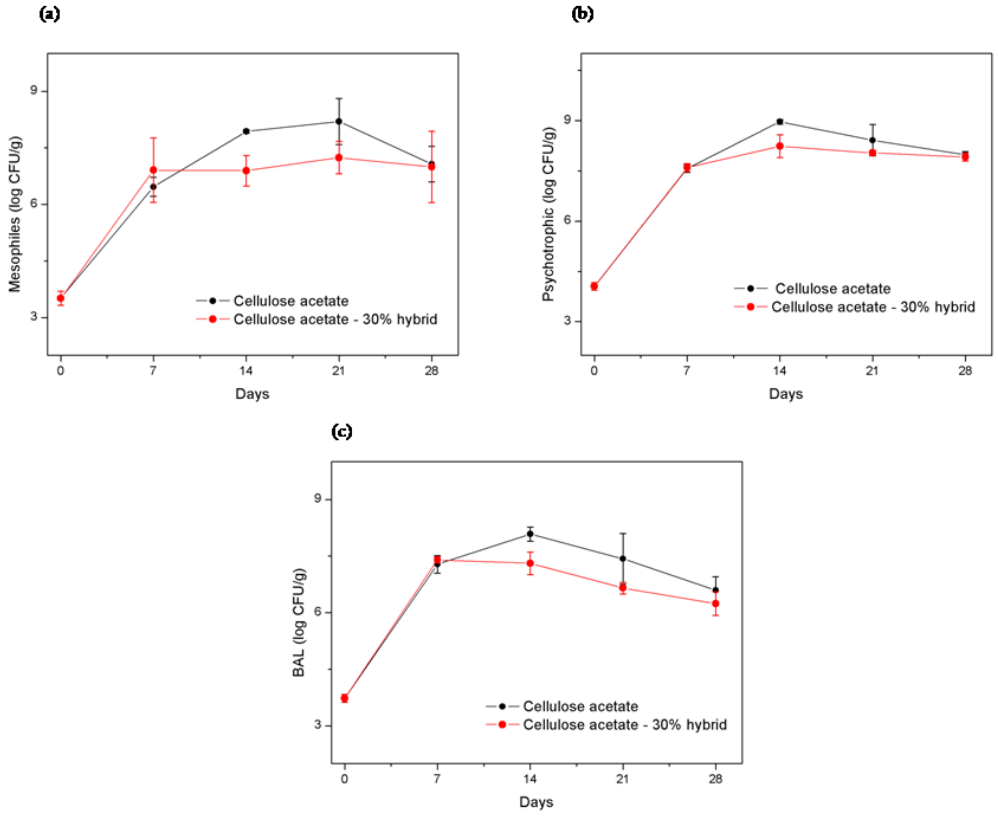


Figure 4. Results of microbiological determinations: (a) mesophiles, (b) psychotrophs and (c) lactic acid bacteria BAL during the storage time of the ham samples.

### 3.7. Physicochemical properties of ham samples

#### 3.7.1. TBARS

Lipid oxidation is one of the main causes of deterioration of meat products that can affect the organoleptic properties of meat samples (Pattarasiriroj et al., 2020). To evaluate the efficiency of the bionanocomposite containing 30% w/w nanoclay impregnated with clove oil in inhibiting the oxidation of ham, the secondary oxidation of lipids, which is associated with the presence of off-flavors, was evaluated using TBARS (Yan et al., 2019). As shown in Figure 5a, up to 14 days of storage, no significant differences ($p<0.05$) were observed between the CA film and the bio-nanocomposite containing 30% w/w nanoclay impregnated with clove oil. However, samples stored for a period of time longer than 14 days showed significant differences in MDA concentration. Figure 4a shows that the ham samples packed with CA film showed a constant concentration of TBARS (0.09 mg/kg) in the first 7 days of storage. There was a progressive increase in the concentration of these substances until the

conclusion of the experiment after 28 days of storage (0.2 mg/kg). The ham samples packed with CA bionanocomposite containing 30% w/w nanoclay impregnated with clove oil showed no significant variation in MDA concentration (0.09 mg/kg) throughout the experiment. The protective action of the bio-nanocomposite against oxidation of the ham is the result of the presence and migration of antioxidant substances (Table 3) present in clove oil that act as free radical scavengers (Candido et al., 2022). The change in the optical properties (Table 2) of the bionanocomposites in relation to the CA films are also important factors in the reduction of the lipid oxidation of the ham samples. This occurs because more translucent packages reduce the transmission of visible and ultraviolet light, reducing the degradation and oxidation of food products (Alves-Silva et al., 2022; Pattarasiriroj et al., 2020).

### 3.7.2. pH

The pH analysis of ham samples during storage is an important parameter for the evaluation of the quality of the samples. An increase in the pH of the ham indicates the growth of the population of mesophilic and psychotropic, which leads to the accumulation of ammonia and other substances of a basic nature. The pH variation of the ham samples packed for a period of 28 days in CA films and bio-nanocomposites containing 30% w/w nanoclay impregnated with clove oil are shown in Figure 5b. The ham samples packed with bio-nanocomposite films exhibited an approximately constant pH value of 6.6 during the 28 days of storage. In turn, the ham samples packed with CA film showed an increase in pH from 6.6 at the beginning of the experiment to 8.1 after 28 days of storage at 11 °C. This higher pH value in the ham samples packed with CA films is possibly related to the increase in the population of mesophilic and especially the psychotropic ones (Figure 3). Reduction in the bacterial population in ham samples packed with bio-nanocompositeshas been reported in the literature (Amor et al., 2021) and may be associated with the migration of active compounds from the bio-nanocomposite to the food matrix.

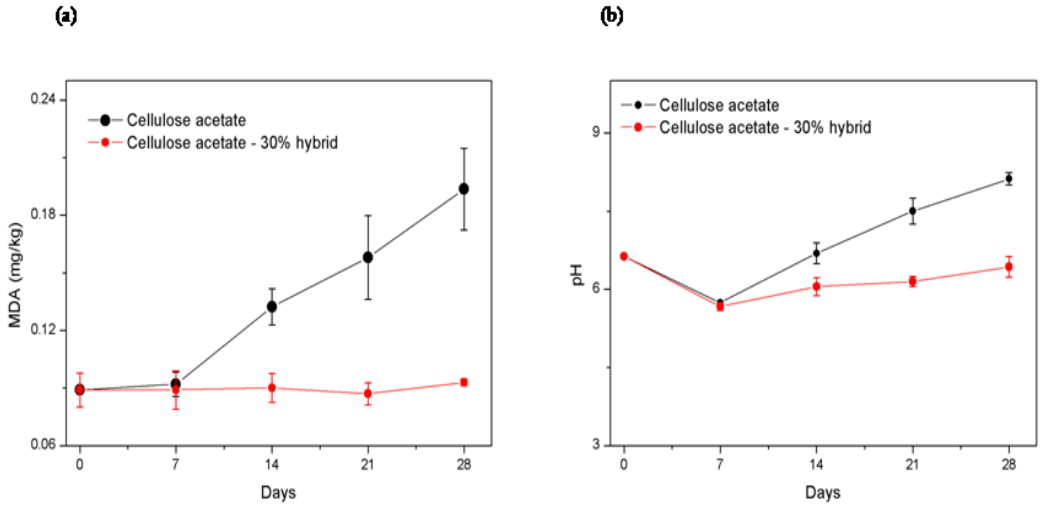


Figure 5. Results of TBARS (a) and pH (b) analyses during the storage time of the ham samples.

### 3.7.3. Optical properties of ham

The color of food products is one of the parameters that most influences consumer acceptability because it is an accessible reference of quality (Pateiro et al., 2019). Table 7 shows the results of the instrumental color changes of the ham subjected to treatment with the active packaging and the control during the 28 days of storage. The results show that there were no significant differences ($p<0.05$) at the same storage time in the parameters ofL*, a*, b*, and C*. However, the h parameter (hue angle) showed significant differences ($p<0.05$) after 21 days of storage. The samples of ham subjected to CA film exhibited higher values of h, which indicates less red coloration (A. S. Guimarães et al., 2022; Quesada et al., 2016). Thus, the presence of nanoclay modified with clove essential oil in the CA biopolymer may have increased the preservation of the red hue (because it exhibited lower h values) during ham storage.

Table 7. Color coordinates L* (brightness), a* (red-green axis), b* (blue-yellow axis), C* (chroma), h (hue) of cooked ham submitted with the control film and the film with 30% nano clay modified with clove essential oil during 28 days of storage at 11 °C.

| Color parameter | Days of storage | Cellulose acetate | Cellulose acetate - 30.0% m/m hybrid |
|---|---|---|---|
| L* | 0 | 68.60±0.04[a] | 68.60±0.04[a] |
| | 7 | 65.68±0.87[a] | 63.53±4.10[a] |
| | 14 | 66.74±1.34[a] | 67.70±4.33[a] |
| | 21 | 59.59±2.98[a] | 66.99±0.64[a] |
| | 28 | 59.13±0.78[a] | 63.16±1.82[a] |
| a* | 0 | 10.10±0.29[a] | 10.10±0.29[a] |
| | 7 | 9.92±0.95[a] | 10.89±1.25[a] |
| | 14 | 9.51±1.11[a] | 9.28±0.42[a] |
| | 21 | 5.99±0.93[a] | 7.92±0.79[a] |
| | 28 | 6.78±1.64[a] | 9.48±1.00[a] |
| b* | 0 | 7.82±0.29[a] | 7.82±0.29[a] |
| | 7 | 7.22±0.36[a] | 8.28±0.18[a] |
| | 14 | 8.29±0.81[a] | 8.56±1.51[a] |
| | 21 | 10.75±0.29[a] | 9.98±1.51[a] |
| | 28 | 11.94±1.59[a] | 9.40±1.20[a] |
| C* | 0 | 12.78±0.41[a] | 12.78±0.41[a] |
| | 7 | 12.28±0.91[a] | 13.70±0.96[a] |
| | 14 | 12.65±0.46[a] | 12.64±1.32[a] |
| | 21 | 12.32±0.71[a] | 12.80±0.68[a] |
| | 28 | 13.75±2.09[a] | 13.49±0.16[a] |
| h | 0 | 44.34±0,41[a] | 44.34±0.41[a] |
| | 7 | 41.87±3.18[a] | 44.00±5.54[a] |
| | 14 | 50.69±9.25[a] | 52.69±7.06[a] |
| | 21 | 104.06±12,42[a] | 73.38±17.90[a] |
| | 28 | 102.92±15.41[b] | 57.79±13.82[a] |

Means ± standard deviation of three repetitions followed by the same lower case letters (comparison between samples at the same temperature) in the same row do not differ statistically by Tukey's Test at 5% ($p<0.05$).

## 4. CONCLUSION

The incorporation of the nanoclay and clove essential oil mixture resulted in important changes in the properties of the bionanocomposites, with a decrease in mechanical properties and an increase in antimicrobial activity against the bacteria *Escherichia coli*, *Listeria monocytogenes*, and *Lactobacillus sakei.* An increase in antioxidant activity was also confirmed by the DPPH and phosphomolybdenum methods, as well as an increase in TPC levels.

The active packaging system developed was suitable for the preservation of cooked ham, as slight reductions in the counts of mesophiles, psychrotrophs, and LAB were observed. The ham was also protected from oxidation, as measured by TBARS, during storage. pH analysis also showed that the application of the active packaging maintained the pH value of the ham during the storage time. The reddish hue in the ham samples packaged with the active bio-nanocomposite was also preserved.

## REFERENCES

Abou Baker, D. H., Al-Moghazy, M., & ElSayed, A. A. A. (2020). Antimicrobialactivity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens. *Bioorganic Chemistry*, *95*, 103559. https://doi.org/10.1016/j.bioorg.2019.103559

Adams, R. P. (2007). *IDENTIFICATION OF ESSENTIAL OIL COMPONENTS BY GAS CHROMATOGRAPHY/ MASS SPECTROMETRY, 4th Ed*.

Ait Ouahioune, L., Wrona, M., Becerril, R., Salafranca, J., Nerín, C., & Djenane, D. (2022). Ceratonia siliqua L. kibbles, seeds and leaves as a source of volatile bioactive compounds for antioxidant food biopackaging applications. *Food Packaging and Shelf Life*, *31*, 100764. https://doi.org/10.1016/j.fpsl.2021.100764

Alfikri, F. N., Pujiarti, R., Wibisono, M. G., & Hardiyanto, E. B. (2020). Yield, Quality, and Antioxidant Activity of Clove ( *Syzygium aromaticum* L.) Bud Oil at the Different Phenological Stages in Young and Mature Trees. *Scientifica*, *2020*, 1–8. https://doi.org/10.1155/2020/9701701

Alves-Silva, G. F., Santos, L. G., Martins, V. G., & Cortez-Vega, W. R. (2022). Cassava starch films incorporated with clove essential oil and nanoclay as a strategy to increase the shelf life of strawberries. *International Journal of Food Science & Technology*. https://doi.org/10.1111/ijfs.16014

Amor, G., Sabbah, M., Caputo, L., Idbella, M., De Feo, V., Porta, R., Fechtali, T., & Mauriello, G. (2021). Basil Essential Oil: Composition, Antimicrobial Properties, and Microencapsulation to Produce Active Chitosan Films for Food Packaging. *Foods*, *10*(1), 121. https://doi.org/10.3390/foods10010121

Arrieta, M. P., Garrido, L., Faba, S., Guarda, A., Galotto, M. J., & López de Dicastillo, C. (2020). Cucumis metuliferus Fruit Extract Loaded Acetate Cellulose Coatings for

Antioxidant Active Packaging. *Polymers*, *12*(6), 1248. https://doi.org/10.3390/polym12061248

Asdagh, A., & Pirsa, S. (2020). Bacterial and oxidative control of local butter with smart/active film based on pectin/nanoclay/Carum copticum essential oils/β-carotene. *International Journal of Biological Macromolecules*, *165*, 156–168. https://doi.org/10.1016/j.ijbiomac.2020.09.192

ASTM. (2009). *ASTM D882-09, Standard Test Method for Tensile Properties of Thin Plastic Sheeting*.

Candido, G. S., Natarelli, C. V. L., Carvalho, E. E. N., & Oliveira, J. E. (2022). Bionanocomposites of pectin and pracaxi oil nanoemulsion as active packaging for butter. *Food Packaging and Shelf Life*, *32*, 100862. https://doi.org/10.1016/j.fpsl.2022.100862

da Silva Scudeler, C. G., de Lima Costa, T., Cortez-Vega, W. R., Prentice, C., & Fonseca, G. G. (2020). Development and characterization of Nile tilapia (Oreochromis niloticus) protein isolate-based biopolymer films incorporated with essential oils and nanoclay. *Food Packaging and Shelf Life*, *25*. https://doi.org/10.1016/j.fpsl.2020.100542

de Oliveira, L. H., Trigueiro, P., Souza, J. S. N., de Carvalho, M. S., Osajima, J. A., da Silva-Filho, E. C., & Fonseca, M. G. (2022). Montmorillonite with essential oils as antimicrobial agents, packaging, repellents, and insecticides: an overview. *Colloids and Surfaces B: Biointerfaces*, *209*, 112186. https://doi.org/10.1016/j.colsurfb.2021.112186

Echeverría, I., López-Caballero, M. E., Gómez-Guillén, M. C., Mauri, A. N., & Montero, M. P. (2018). Active nanocomposite films based on soy proteins-montmorillonite- clove essential oil for the preservation of refrigerated bluefin tuna (Thunnus thynnus) fillets. *International Journal of Food Microbiology*, *266*, 142–149. https://doi.org/10.1016/j.ijfoodmicro.2017.10.003

El Fawal, G. F., Omer, A. M., & Tamer, T. M. (2019). Evaluation of antimicrobial and antioxidant activities for cellulose acetate films incorporated with Rosemary and Aloe Vera essential oils. *Journal of Food Science and Technology*, *56*(3), 1510–1518. https://doi.org/10.1007/s13197-019-03642-8

Farhan, A., & Hani, N. M. (2020). Active edible films based on semi-refined κ-carrageenan: Antioxidant and color properties and application in chicken breast packaging. *Food Packaging and Shelf Life*, *24*. https://doi.org/10.1016/j.fpsl.2020.100476

Giannakas, A. E., Salmas, C. E., Karydis-Messinis, A., Moschovas, D., Kollia, E., Tsigkou, V., Proestos, C., Avgeropoulos, A., & Zafeiropoulos, N. E. (2021). Nanoclay and Polystyrene Type Efficiency on the Development of Polystyrene/Montmorillonite/Oregano Oil Antioxidant Active Packaging Nanocomposite Films. *Applied Sciences*, *11*(20), 9364. https://doi.org/10.3390/app11209364

Gomide, R. A. C., Oliveira, A. C. S., Luvizaro, L. B., Yoshida, M. I., Oliveira, C. R., & Borges, S. V. (2021). Biopolymeric films based on whey protein isolate/lignin microparticles for waste recovery. *Journal of Food Process Engineering*, *44*(1). https://doi.org/10.1111/jfpe.13596

Gracia-Vallés, N., Ruiz-Torrubia, F., Mitchell, S. G., Nerín, C., & Silva, F. (2022). Developing ethyl lauroyl arginate antimicrobial films to combat Listeria monocytogenes in cured ham. *Food Control*, *141*, 109164. https://doi.org/10.1016/j.foodcont.2022.109164

Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M. C. C., Endringer, D. C., Fronza, M., & Scherer, R. (2019). Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. *Molecules*, *24*(13), 2471. https://doi.org/10.3390/molecules24132471

Guimarães, A. S., Guimarães, J. S., Rodrigues, L. M., Fontes, P. R., Ramos, A. de L. S., & Ramos, E. M. (2022). Assessment of Japanese radish derivatives as nitrite substitute on the physicochemical properties, sensorial profile, and consumer acceptability of restructured cooked hams. *Meat Science*, *192*, 108897. https://doi.org/10.1016/j.meatsci.2022.108897

Harini, K., & Sukumar, M. (2019). Development of cellulose-based migratory and nonmigratory active packaging films. *Carbohydrate Polymers*, *204*, 202–213. https://doi.org/10.1016/j.carbpol.2018.10.018

Hifney, A. F., Fawzy, M. A., Abdel-Gawad, K. M., & Gomaa, M. (2016). Industrial optimization of fucoidan extraction from Sargassum sp. and its potential antioxidant and emulsifying activities. *Food Hydrocolloids*, *54*, 77–88. https://doi.org/10.1016/j.foodhyd.2015.09.022

Iamareerat, B., Singh, M., Sadiq, M. B., & Anal, A. K. (2018). Reinforced cassava starch based edible film incorporated with essential oil and sodium bentonite nanoclay as food packaging material. *Journal of Food Science and Technology*, *55*(5), 1953–1959. https://doi.org/10.1007/s13197-018-3100-7

Lammi, S., Gastaldi, E., Gaubiac, F., & Angellier-Coussy, H. (2019). How olive pomace can be valorized as fillers to tune the biodegradation of PHBV based composites. *Polymer Degradation and Stability*, *166*, 325–333. https://doi.org/10.1016/j.polymdegradstab.2019.06.010

Lee, M. H., Kim, S. Y., & Park, H. J. (2018). Effect of halloysite nanoclay on the physical, mechanical, and antioxidant properties of chitosan films incorporated with clove essential oil. *Food Hydrocolloids*, *84*, 58–67. https://doi.org/10.1016/j.foodhyd.2018.05.048

Lima, Í. A., Gomes, H. B., Ramos, A. de L. S., Piccoli, R. H., & Ramos, E. M. (2017). Caracterização física, química e microbiológica de presunto cru desossado adicionado de lactulose. *Brazilian Journal of Food Technology*, *20*(0). https://doi.org/10.1590/1981-6723.2816

Mande, P., & Sekar, N. (2021). Comparative study of chemical composition, antibacterial and antioxidant activity of essential oils isolated from the seeds of Amomum subulatum by using microwave extraction and hydro-distillation methods. *Journal of the Indian Chemical Society*, *98*(11), 100201. https://doi.org/https://doi.org/10.1016/j.jics.2021.100201

Marand, S. A., Alizadeh Khaledabad, M., & Almasi, H. (2021). Optimization and Characterization of Plantago major Seed Gum/Nanoclay/Foeniculum vulgare Essential Oil Active Nanocomposite Films and Their Application in Preservation of Local Butter. *Food and Bioprocess Technology*, *14*(12), 2302–2322. https://doi.org/10.1007/s11947-021-02724-w

Matta Fakhouri, F., Nogueira, G. F., de Oliveira, R. A., & Velasco, J. I. (2019). Bioactive Edible Films Based on Arrowroot Starch Incorporated with Cranberry Powder: Microstructure, Thermal Properties, Ascorbic Acid Content and Sensory Analysis. *Polymers*, *11*(10), 1650. https://doi.org/10.3390/polym11101650

Merino, F. J. Z., Oliveira, V. B., Paula, C. S., Cansian, F. C., Souza, A. M., Zuchetto, M., Hirota, B. C. K., Duarte, A. F. S., Kulik, J. D., Miguel, M. D., & Miguel, O. G. (2015). Análise fitoquímica, potencial antioxidante e toxicidade do extrato bruto etanólico e das frações da espécie Senecio westermanii Dusén frente à Artemia salina. *Revista Brasileira de Plantas Medicinais*, *17*(4), 1031–1040. https://doi.org/10.1590/1983-084X/14_137

Micó-Vicent, B., Viqueira, V., Ramos, M., Luzi, F., Dominici, F., Torre, L., Jiménez, A., Puglia, D., & Garrigós, M. C. (2020). Effect of Lemon Waste Natural Dye and Essential Oil Loaded into Laminar Nanoclays on Thermomechanical and Color Properties of Polyester Based Bionanocomposites. *Polymers*, *12*(7), 1451. https://doi.org/10.3390/polym12071451

Nisar, T., Wang, Z.-C., Yang, X., Tian, Y., Iqbal, M., & Guo, Y. (2018). Characterization of citrus pectin films integrated with clove bud essential oil: Physical, thermal, barrier, antioxidant and antibacterial properties. *International Journal of Biological Macromolecules*, *106*, 670–680. https://doi.org/10.1016/J.IJBIOMAC.2017.08.068

Ozogul, Y., Kuley Boğa, E., Akyol, I., Durmus, M., Ucar, Y., Regenstein, J. M., & Köşker, A. R. (2020). Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens. *Food Bioscience*, *36*, 100635. https://doi.org/10.1016/j.fbio.2020.100635

Pateiro, M., Domínguez, R., Bermúdez, R., Munekata, P. E. S., Zhang, W., Gagaoua, M., & Lorenzo, J. M. (2019). Antioxidant active packaging systems to extend the shelf life of sliced cooked ham. *Current Research in Food Science*, *1*, 24–30. https://doi.org/https://doi.org/10.1016/j.crfs.2019.10.002

Pattarasiriroj, K., Kaewprachu, P., & Rawdkuen, S. (2020). Properties of rice flour-gelatine-nanoclay film with catechin-lysozyme and its use for pork belly wrapping. *Food Hydrocolloids*, *107*, 105951. https://doi.org/10.1016/j.foodhyd.2020.105951

Pikul, J., Leszczynski, D. E., Kummerow, F. A., & Moore, H. E. (1989). *Food Chem* (Vol. 37). https://pubs.acs.org/sharingguidelines

Pires, J. R. A., de Souza, V. G. L., & Fernando, A. L. (2018). Chitosan/montmorillonite bionanocomposites incorporated with rosemary and ginger essential oil as packaging for fresh poultry meat. *Food Packaging and Shelf Life*, *17*, 142–149. https://doi.org/10.1016/j.fpsl.2018.06.011

Priyadarshi, R., Kim, H.-J., & Rhim, J.-W. (2021). Effect of sulfur nanoparticles on properties of alginate-based films for active food packaging applications. *Food Hydrocolloids*, *110*, 106155. https://doi.org/10.1016/j.foodhyd.2020.106155

Quesada, J., Sendra, E., Navarro, C., & Sayas-Barberá, E. (2016). Antimicrobial Active Packaging including Chitosan Films with Thymus vulgaris L. Essential Oil for Ready-to-Eat Meat. *Foods*, *5*(4), 57. https://doi.org/10.3390/foods5030057

Radünz, M., da Trindade, M. L. M., Camargo, T. M., Radünz, A. L., Borges, C. D., Gandra, E. A., & Helbig, E. (2019). Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (Syzygium aromaticum, L.) essential oil. *Food Chemistry*, *276*, 180–186. https://doi.org/10.1016/j.foodchem.2018.09.173

Ramos, E. M., & Gomide, L. A. M. (2017). *Avaliação da qualidade de carnes: fundamentos e metodologias* (UFV (org.); 2°).

Ramos, M., Fortunati, E., Beltrán, A., Peltzer, M., Cristofaro, F., Visai, L., Valente, A. J. M., Jiménez, A., Kenny, J. M., & Garrigós, M. C. (2020). Controlled Release,

Disintegration, Antioxidant, and Antimicrobial Properties of Poly (Lactic Acid)/Thymol/Nanoclay Composites. *Polymers*, *12*(9), 1878. https://doi.org/10.3390/polym12091878

Rodríguez, F. J., Torres, A., Peñaloza, Á., Sepúlveda, H., Galotto, M. J., Guarda, A., & Bruna, J. (2014). Development of an antimicrobial material based on a nanocomposite cellulose acetate film for active food packaging. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *31*(3), 342–353. https://doi.org/10.1080/19440049.2013.876105

Tedeschi, G., Guzman-Puyol, S., Paul, U. C., Barthel, M. J., Goldoni, L., Caputo, G., Ceseracciu, L., Athanassiou, A., & Heredia-Guerrero, J. A. (2018). Thermoplastic cellulose acetate oleate films with high barrier properties and ductile behaviour. *Chemical Engineering Journal*, *348*, 840–849. https://doi.org/10.1016/j.cej.2018.05.031

Torin, R. F. da S., Camani, P. H., da Silva, L. N., Sato, J. A. P., Ferreira, F. F., & Rosa, D. dos S. (2018). Effect of clay and clay/essential oil in packaging films. *Polymer Composites*, *39*(11), 4034–4040. https://doi.org/10.1002/pc.24457

Varghese, S. A., Siengchin, S., & Parameswaranpillai, J. (2020). Essential oils as antimicrobial agents in biopolymer-based food packaging - A comprehensive review. *Food Bioscience*, *38*. https://doi.org/10.1016/j.fbio.2020.100785

Veselá, H., Dorotíková, K., Dušková, M., Furmančíková, P., Šedo, O., & Kameník, J. (2022). The Pork Meat or the Environment of the Production Facility? The Effect of Individual Technological Steps on the Bacterial Contamination in Cooked Hams. *Microorganisms*, *10*(6), 1106. https://doi.org/10.3390/microorganisms10061106

Vinicius de Oliveira Brisola Maciel, M., da Rosa Almeida, A., Machado, M. H., Elias, W. C., Gonçalves da Rosa, C., Teixeira, G. L., Noronha, C. M., Bertoldi, F. C., Nunes, M. R., Dutra de Armas, R., & Manique Barreto, P. L. (2020). Green synthesis, characteristics and antimicrobial activity of silver nanoparticles mediated by essential oils as reducing agents. *Biocatalysis and Agricultural Biotechnology*, *28*, 101746. https://doi.org/10.1016/j.bcab.2020.101746

Yan, W., Chen, W., Muhammad, U., Zhang, J., Zhuang, H., & Zhou, G. (2019). Preparation of α-tocopherol-chitosan nanoparticles/chitosan/montmorillonite film and the antioxidant efficiency on sliced dry-cured ham. *Food Control*, *104*, 132–138. https://doi.org/10.1016/j.foodcont.2019.04.026