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EMBALAGEM ATIVA DE BASE BIOLÓGICA: FILME COM EXTRATO DE ESTIGMA DE MILHO PARA CONSERVAÇÃO DE CARNE BOVINA REFRIGERADA

Santa Maria, RS
2022

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Tese apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia dos Alimentos (PPGCTA), da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Doutora em Ciência e Tecnologia dos Alimentos**.

Orientadora: Profª. Drª.: Claudia Severo da Rosa

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A persistência é o
caminho do êxito.

(Charlie Chaplin)

RESUMO

EMBALAGEM ATIVA DE BASE BIOLÓGICA: FILME COM EXTRATO DE ESTIGMA DE MILHO PARA CONSERVAÇÃO DE CARNE BOVINA REFRIGERADA

AUTORA: Caroline Pagnossim Boeira
ORIENTADORA: Claudia Severo da Rosa

Este trabalho teve por objetivo investigar a melhor condição de extração de compostos antioxidantes e antimicrobianos do estigma de milho e aplicá-la como ingrediente ativo em filmes de base biológica para conservação de carne bovina refrigerada. Para obtenção do extrato bioativo, um planejamento fatorial 2^2 foi proposto para investigar a influência do tempo e da relação sólido-solvente na extração. A extração assistida por ultrassom do estigma do milho reduziu o tempo de extração em 67% quando comparado ao método convencional. A condição ideal para a extração de compostos antioxidantes foi com o tempo de 5 minutos e a razão sólido-solvente de 0,05 g mL^{-1}. Utilizando a triagem por ESI-ToF-MS, foi possível identificar 27 compostos fitoquímicos. Além disso, os resultados demonstram que o extrato possui notável capacidade antimicrobiana frente aos 23 micro-organismos estudados. A condição ideal foi escolhida para ser incorporada como ingrediente ativo em filmes à base de gelatina e amido de milho para conservação de carne bovina refrigerada. Os filmes incorporados com o extrato de estigma de milho foram preparados com sucesso pelo método de *casting*. A espessura e umidade dos filmes não foram alteradas com a adição do extrato, porém foi observada, através da microscopia eletrônica de varredura, a formação de pequenos poros na superfície. Os resultados indicam ainda que o filme elaborado com 25% de extrato apresentou um caráter hidrofóbico, com baixa solubilidade em água e com fortes propriedades mecânicas e antioxidantes. O extrato de estigma de milho foi considerado eficaz contra a oxidação lipídica e o crescimento microbiológico em carne bovina. Os níveis de pH e os valores de TBARS diminuíram com o aumento da quantidade de extrato incorporada aos filmes, e o uso de 25% de extrato permitiu uma redução de 60% na oxidação lipídica. Da mesma forma para a contagem de micro-organismos mesófilos e psicrotróficos, o filme ativo contribuiu para a redução desses microrganismos após 3 dias de armazenamento. A utilização de extratos de resíduos vegetais como ingrediente ativo é uma forma inteligente e sustentável de valorizar esses subprodutos agrícolas. Assim, o estigma de milho é considerado uma alternativa promissora para incorporar compostos antioxidantes e antimicrobianos em embalagens de alimentos e melhorar a vida útil da carne bovina.

Palavras-chave: Extração assistida por ultrassom. Compostos antioxidantes e antimicrobianos. Filmes biodegradáveis. Conservação de produtos cárneos.

ABSTRACT

BIO-BASED ACTIVE PACKAGING: FILM WITH CORN STIGMA EXTRACT FOR REFRIGERATED BEEF PRESERVATION

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This work aimed to investigate the best condition for extracting antioxidant and antimicrobial compounds from corn stigma and apply it as an active ingredient in bio-based films for the conservation of refrigerated beef. To obtain the bioactive extract, a 2^2 factorial design was proposed to investigate the influence of time and solid-solvent ratio on extraction. Ultrasound-assisted extraction of corn stigma reduced extraction time by 67% when compared to the conventional method. The ideal condition for the extraction of antioxidant compounds was with a time of 5 minutes and a solid-solvent ratio of 0.05 g mL^{-1}. Using ESI-ToF-MS screening, it was possible to identify 27 phytochemical compounds. In addition, the results demonstrate that the extract has a remarkable antimicrobial capacity against the 23 microorganisms studied. The ideal condition was chosen to be incorporated as an active ingredient in films based on gelatin and corn starch for the conservation of refrigerated beef. The films incorporated with the corn stigma extract were successfully prepared by the casting method. The thickness and humidity of the films were not altered with the addition of the extract, but the formation of small pores on the surface was observed through scanning electron microscopy. The results also indicate that the film made with 25% extract showed a hydrophobic character, with low water solubility and strong mechanical and antioxidant properties. Corn stigma extract was found to be effective against lipid oxidation and microbiological growth in beef. The pH levels and TBARS values decreased with increasing amount of extract incorporated into the films, and the use of 25% extract allowed a 60% reduction in lipid oxidation. Likewise for the count of mesophilic and psychrotrophic microorganisms, the active film contributed to the reduction of these microorganisms after 3 days of storage. The use of extracts from plant residues as an active ingredient is an intelligent and sustainable way of valuing these agricultural by-products. Thus, corn stigma is considered a promising alternative to incorporate antioxidant and antimicrobial compounds into food packaging and improve the shelf life of beef.

Keywords: Ultrasound-assisted extraction. Antioxidant and antimicrobial compounds. Biodegradable films. Preservation of meat products.

LISTA DE ILUSTRAÇÕES

Artigo 1

Artigo 2

Artigo 3

LISTA DE TABELAS

Artigo 1

Artigo 2

Artigo 3

SUMÁRIO

1 INTRODUÇÃO

Os resíduos alimentares são produzidos em várias etapas do ciclo de vida dos alimentos, como na produção agrícola, no processamento industrial e na distribuição no mercado. Na produção agrícola, estima-se que pelo menos 40% da massa de matéria-prima inicial seja descartada como resíduo (GOMES-ARAÚJO, et al., 2021).

Dentre estes resíduos, destaca-se o estigma do milho, um subproduto agroindustrial da produção agrícola do milho (*Zea mays L*.) disponível em abundância e sem custo comercial. Pesquisas revelam que o estigma de milho é uma fonte renovável de compostos fenólicos e flavonoides, também é composto por proteínas, vitaminas, carboidratos, macronutrientes, óleos voláteis, esteroides, alcaloides e saponinas (WANG; ZHAO, 2019). Estudos farmacológicos demonstram ainda que os extratos de estigma de milho e seus componentes oferecem muitos benefícios à saúde humana (CHAIITTIANAN et al., 2017). No entanto, por se tratar de um subproduto pouco explorado é necessário novos estudos aprofundados sobre sua composição fitoquímica a fim de utilizá-lo como ingrediente funcional.

A valorização dos resíduos alimentares, através de diferentes técnicas de extração como alternativa potencial para obter ingredientes nutracêuticos e funcionais, vem sendo discutida por pesquisadores (VODNAR et al., 2017). Entretanto, alguns métodos de extração requerem o uso de grandes quantidades de solventes orgânicos, alto consumo de energia e longos tempos de extração. Com isso, a extração assistida por ultrassom (EAU) tem sido relatada como uma alternativa promissora para aumentar os rendimentos de extração de compostos bioativos de frutas e resíduos vegetais (KUMAR; SRIVASTAV; SHARANAGAT, 2021). O uso de EAU promove a extração exaustiva de ingredientes ativos de plantas com tempo relativamente curto e alta eficiência de extração quando comparado aos procedimentos convencionais (CHEMAT et al., 2017).

Os extratos vegetais mostraram-se consideravelmente promissores em uma variedade de aplicações na indústria alimentícia em virtude das propriedades antioxidantes e antimicrobianas que possuem (NEGI, 2012). Esses compostos podem ser incorporados na formulação do produto, revestidos em sua superfície ou incorporados ao material de embalagem a fim de inibir o crescimento de micro-organismos indesejáveis e reduzir reações química que comprometem o alimento, como, por exemplo, a oxidação lipídica em produtos cárneos (NIKMARAM, et al. 2018).

Associado a isso, a crescente preocupação ambiental com o uso de embalagens de base petroquímica impulsionou diversas pesquisas no desenvolvimento de polímeros de fontes

renováveis, biodegradáveis e ecológicas (GEYER, JAMBECK & LAW 2017). Essas embalagens permitem que compostos bioativos sejam adicionados intencionalmente para atuar mutuamente com o alimento embalado (BHARGAVA, 2020). Porém, é reconhecido que um dos maiores desafios enfrentados no desenvolvimento de embalagens biodegradáveis para alimentos são suas propriedades de engenharia insuficientes em comparação com materiais de embalagem de polímeros à base de petróleo (ZHANG E SABLANI, 2021).

Os polímeros naturais, como a gelatina e o amido de milho, são fontes potenciais para a formação de filmes biodegradáveis e comestíveis para embalagens de alimentos. Esses hidrocoloides se destacam por serem renováveis, abundantes e de baixo custo (ROSSETO, et al., 2019). A formação de *blends* com gelatina-amido de milho também vem ganhando espaço entre os pesquisadores, pois pode oferecer vantagens em termos de propriedades de barreira ao oxigênio e vapor d'água, comportamento mecânico e parâmetros ópticos (SILVA-WEISS, et al., 2014). É importante empregar tecnologias versáteis existentes e emergentes para superar as limitações no desenvolvimento de embalagens sustentáveis e aprimorar suas propriedades (ZHANG E SABLANI, 2021).

Diante ao exposto, a obtenção de compostos antioxidantes e antimicrobianos do estigma de milho, através da extração assistida por ultrassom, torna-se uma alternativa atrativa e inovadora frente aos métodos convencionais. Além disso, utilizar o extrato como ingrediente ativo na elaboração de embalagem biológica para conservação de carne bovina refrigerada valoriza esse subproduto da indústria agrícola.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Otimizar a extração de compostos antioxidantes e antimicrobianos de estigma de milho e avaliar o efeito da adição do extrato na produção de embalagem ativa de base biológica para conservação de carne bovina refrigerada.

2.1.1 Objetivos Específicos

- Otimizar a extração de compostos bioativos por extração convencional e assistida por ultrassom;
- Determinar a capacidade antioxidante dos extratos;
- Caracterizar os compostos fitoquímicos presentes no estigma de milho;
- Determinar a atividade antimicrobiana *in vitro* do estigma de milho;
- Investigar os efeitos citotóxicos do estigma de milho em células de fibroplastos embrionários (linhagem celular 3T3);
- Aplicar o extrato otimizado em filmes ativos de base biológica;
- Caracterizar os filmes obtidos quanto à espessura, à umidade, às propriedades ópticas, à permeabilidade ao vapor de água, à solubilidade em água, ao ângulo de contato, às propriedades mecânicas, microscopia eletrônica de varredura (MEV) e espectroscopia infravermelha de transformação de Fourier (FTIR).
- Determinar a atividade antioxidante *in vitro* dos filmes;
- Acompanhar a biodegradabilidade do filme depositado em solo;
- Aplicar os filmes elaborados como embalagem ativa para conservação de carne bovina refrigerada;
- Acompanhar a oxidação lipídica, o pH, o crescimento microbiológico e a atividade de água da carne bovina durante 7 dias de armazenamento refrigerado.

2 REVISÃO BIBLIOGRÁFICA

2.1 MILHO (*ZEA MAYS* L.)

O milho (*Zea mays* L.) vem sendo utilizado na América Latina desde os tempos mais remotos, como a principal e a mais tradicional fonte alimentar, ocupando hoje posição de destaque entre os cereais mais cultivados no mundo, precedido apenas pela cultura do trigo e do arroz (Figura 1). É uma espécie que pertence à família Gramineae/Poaceae e há mais de 8.000 anos está presente em diversas partes do mundo. A sua grande adaptabilidade, representada por variados genótipos, permite o seu cultivo em diferentes localidades. Pode ser encontrado em climas tropicais, subtropicais e temperados. Esta planta tem como finalidade de utilização a alimentação humana e animal, devido às suas elevadas qualidades nutricionais, contendo quase todos os aminoácidos conhecidos, com exceção da lisina e do triptofano (OLIVEIRA, et al., 2013).

Figura 1.Milho (*Zea mays* L.)

Fonte: Google Imagens
Disponível em: https://www.shutterstock.com/pt/search/zea+mays

A estrutura do milho é composta por raízes fasciculadas em que estão presentes raízes primárias e seminais, adventícias e de suporte. As folhas são longas e lanceoladas, com nervura central em forma de canaleta, bem vigorosa; as folhas são invaginantes e inserem-se por nós do colmo, apresentando pilosidades. O colmo suporta as folhas e partes florais, além de servir como órgão de reserva. Sendo o milho uma planta monoica, as flores masculinas se agrupam numa panícula no topo da planta, enquanto que as femininas são constituídas pelas espigas. O

florescimento ocorre aproximadamente de 50 a 100 dias após a semeadura e é afetado principalmente pela temperatura (LUÍS, 2014).

2.1.2 Estigma de milho (*Zea mays* L.)

O milho é uma planta monoica, ou seja, possui os órgãos masculinos e femininos na mesma planta em inflorescências diferentes. A inflorescência feminina, designada de espiga ou maçaroca, é constituída por um eixo, ao longo do qual se dispõe os alvéolos e onde se desenvolvem as espiguetas aos pares, sendo cada espigueta formada por duas flores, uma fértil e outra estéril. Cada flor tem um ovário com um único óvulo e a partir do ovário desenvolve-se o estilo-estigma. O conjunto do estilo-estigma irá constituir o estigma de milho (Figura 2). A função do cabelo de milho é capturar o pólen para polinização. Cada estigma pode ser polinizado para produzir um grão de milho. Pode ter 30 cm de comprimento ou mais, com um sabor levemente adocicado (CASTRO, 2009).

Figura 2. Estigma de milho (*Zea mays* L.)

Fonte: Agricultura e Abastecimento São Paulo
Disponível em: https://www.agricultura.sp.gov.br

O estigma do milho é um subproduto de baixo custo do cultivo do milho, rico em nutrientes e metabólitos secundários. Vem sendo consumido ao longo dos anos na medicina tradicional, em muitas partes do mundo, como um remédio terapêutico para várias doenças. Suas ações estão relacionadas ao tratamento de cistite, edema, cálculos renais, diuréticos, distúrbios da próstata e infecções urinárias, bem como enurese e obesidade (HASANUDIN et

al., 2012). Outros benéficos do estigma de milho incluem atividade anti-fadiga, atividade antidepressiva e caliurética (HU et al., 2010). Além disso, possui excelente capacidade antioxidante e demonstrou efeitos protetores na radiação e nefrotoxicidade (WANG; ZHAO, 2019)

É rico em compostos fenólicos, flavonoides, proteínas, vitaminas, carboidratos, sais de cálcio, potássio, magnésio e sódio, óleos voláteis, alcaloides e saponinas (NURRAIHANA, et al., 2018). Estudos anteriores sugerem que o estigma possui notáveis atividades bioativas, incluindo antioxidantes, antimicrobiana, hiperglicêmicos, antidepressivos, anti-fadiga e eficaz agente diurético (HASANUDIN et al., 2012). A composição centesimal do estigma de milho consiste em 9,65% de umidade, 3,91% de cinza, 0,29% gordura bruta, 17,6% de proteína bruta e 40% de fibra bruta (WANG, 2011).

2.2 RESÍDUOS VEGETAIS

O aumento da produção mundial de alimentos ocasiona um consequente aumento de resíduos gerados. Infelizmente, ainda existem poucas alternativas para a utilização da maior parte destes resíduos, alguns são usados como matéria-prima ou matéria-prima para bioprocessamento de valor agregado, mas muitos são simplesmente depositados em aterros (ZHANG; SABLANI, 2021). Com isso, surge a necessidade de estudos visando ao aproveitamento desses resíduos, uma vez que grande parte é rica em antioxidantes como o ácido ascórbico, tocoferóis, carotenoides e em compostos fenólicos (SAGAR, et al., 2018).

A composição dos resíduos do processamento de alimentos é extremamente variada e depende tanto da natureza da matéria-prima como da técnica de produção empregada. Exemplos típicos de materiais oriundos de resíduos vegetais são: bagaço de frutas, cascas, sementes, cascas de vegetais, biomassa de culturas ou frutas e vegetais inteiros de baixa qualidade (ZHANG; SABLANI, 2021).

Os produtos de origem vegetal contêm altos níveis de componentes biologicamente ativos e compostos nutricionais, que fornecem benefícios à saúde. Entre os componentes biologicamente ativos, destacam-se os compostos fenólicos e flavonoides, os quais têm despertado interesse devido a sua eficácia e segurança terapêutica, protegendo o corpo humano contra o estresse oxidativo, auxiliando na prevenção de doenças crônicas, como câncer e doenças cardiovasculares (SAHA; SADHUKHAN; SIL, 2016).

Os polifenóis derivados de resíduos de alimentos agrícolas e industriais são ambientalmente atraentes. Além disso, as perspectivas de agregação de valor ou valorização de

resíduos alimentares, que de outra forma não teriam valor econômico, tornariam essa opção econômica (BHARGAVA, et al., 2020).

2.3 TÉCNICAS DE EXTRAÇÃO DE COMPOSTOS BIOATIVOS

Metabólitos primários e secundários de vegetais podem ser extraídos por diferentes técnicas. Para compostos não voláteis, uma das técnicas mais conhecida é colocar a matéria-prima em contato com algum solvente (ex.: água, álcool ou óleo). Essa técnica é chamada de extração sólido-líquido, ou mais comumente maceração (NAVIGLIO, et al., 2019). Há vários anos, as chamadas técnicas de extração "modernas" têm sido desenvolvidas. O aprimoramento do desempenho vem do uso de tecnologia “assistida” (ou seja, ondas ultrassônicas, pressão, micro-ondas) que podem reduzir a duração do processo e induzir economia de energia e solvente por meio de vários mecanismos de intensificação (MEULLEMIESTRE, et al., 2016). Este tratamento físico também pode afetar o mecanismo de extração, possivelmente aumentando os rendimentos de extração e causando diferentes seletividades de extração em comparação com a maceração simples (LEFEBVRE; DESTANDAU; E LESELLIER, 2021).

Diversas metodologias e sistemas de solventes vêm sendo empregadas para a extração de compostos de interesse em amostras vegetais. Qualquer que seja o método de extração utilizado, a natureza química do solvente de extração é de primordial importância para favorecer a solubilidade do composto, ou seja, a recuperação da extração e a seletividade da extração (LEFEBVRE; DESTANDAU; E LESELLIER, 2021). A solubilidade desses compostos é diretamente afetada pela polaridade dos solventes utilizados, gerando dificuldades no desenvolvimento de procedimento de extração apropriado para todos os compostos fenólicos de interesse. Os solventes mais utilizados para a extração são metanol, etanol, acetona, água, acetato de etila, propanol, hexano, dimetilformaldeído e suas combinações (SOCACI et al. 2018).

Outros fatores, tais como tempo de extração, temperatura, pH, proporção sólido-líquido e tamanho das partículas afetam a disponibilidade dos compostos bioativos. Tempos prolongados de extração aumentam a chance de oxidação dos fenólicos, a menos que agentes redutores sejam adicionados ao sistema (ALARA; ABDURAHMAN; E UKAEGBU, 2021). Além disso, a razão amostra/solvente também influencia diretamente a recuperação de compostos fenólicos de plantas. Na extração sólido-líquido, os solutos migram do sólido (amostra vegetal) para o líquido (solvente de extração) dependendo das propriedades físico-químicas dos solutos e do solvente. Em virtude disso, o solvente de extração desempenha um

papel importante durante a extração e afete a seletividade (LEFEBVRE; DESTANDAU; ELESELLIER, 2021)

2.3.1 Extração Convencional

Muitos pesquisadores optam por métodos convencionais de extração devido ao seu baixo custo e facilidade de acesso. As técnicas convencionais de extração mais utilizadas para obtenção de compostos fenólicos são: maceração, decocção, percolação, infusão, digestão, extração exaustiva em série e extração soxhlet (LEFEBVRE; DESTANDAU; E LESELLIER, 2021).

A extração por maceração é um processo simples de imersão de uma amostra no solvente apropriado em sistema fechado, seguido de agitação constante ou esporádica, à temperatura ambiente ou pré-determinada (NAVIGLIO, et al., 2019). Após o período de extração, é aplicado um processo de separação entre a parte sólida e o solvente. Isso geralmente é alcançado por filtração, decantação ou clarificação (ALARA; ABDURAHMAN; E UKAEGBU, 2021). Apesar de ser uma técnica fácil, tem o demérito por ser demorada, exigir grandes volumes de solventes e elevado consumo energético. Além disso, o processo de extração convencional geralmente envolve uma etapa posterior de recuperação, que é a concentração do extrato por meio de um processo de evaporação, aumentando ainda mais o tempo do processo (REBOLLO-HERNANZ, et al., 2021).

A fim de encontrar a solução para os problemas acima citados os pesquisadores impulsionaram estudos visando o desenvolvimento de novos métodos extrativos. Tais métodos incluem extração de CO_2 supercrítico, extração assistida por micro-ondas, extração assistida por ultrassom, extração assistida por enzima, extração por fluido pressurizado, ou mesmo uma combinação dessas abordagens. De acordo com evidências cientificas, esses novos métodos de extração demonstram serem os mais apropriados em comparação com os métodos convencionais, pois oferecem inúmeras vantagens que incluem menor volume de solvente, maior rendimento, redução de resíduos tóxicos, melhor reprodutibilidade do processo e menor tempo de extração (LEFEBVRE; DESTANDAU; E LESELLIER, 2021).

2.3.2 Extração Assistida por Ultrassom

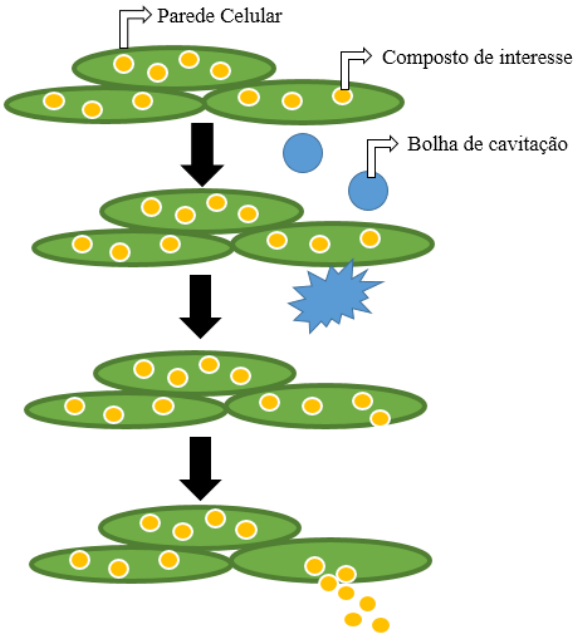
O uso de ultrassom apresenta-se como uma técnica promissora pois promove a extração exaustiva de princípios ativos de vegetais com gasto de energia relativamente pequeno,

economia de tempo e maior segurança no processo. Esse método é uma técnica de extração fácil que utiliza a influência mecânica induzida através da explosão de bolhas de tamanho micro para dar uma rápida desorganização do tecido que facilita a difusão de fitoquímicos da substância para o solvente. É um método simples e de baixo custo que pode ser usado tanto em pequena quanto em grande escala (CHEMAT, et al., 2017).

Nos últimos anos, inúmeros estudos propuseram o uso do ultrassom para obtenção de polifenóis de diferentes fontes (GÖRGÜÇ; BIRCAN E YILMAZ, 2019; DZAH, et al., 2020; HERRERA-POOL, et al., 2021). Algumas vantagens foram observadas, como, por exemplo, a redução do tempo de extração, o consumo reduzido de solvente e o uso de temperaturas mais brandas, evitando danos térmicos ao extrato e minimizando a perda de compostos ativos. Os efeitos do ultrassom no processo de extração dependem da frequência e capacidade do equipamento e do tempo empregado para extração (WANG; WELLER, 2006). O processo experimental geralmente requer o uso de ultrassom com faixa de frequência entre 20 e 2000 kHz para aumentar a permeabilidade da parede celular e produzir cavitação. De acordo com vários relatórios, o ultrassom garante uma extração mais rápida e melhor de polifenóis com quebra minimizada de compostos em relação a outras técnicas de extração (WEN, et al., 2018).

A cavitação é um fenômeno físico causado pela propagação de fortes ondas de ultrassom em líquidos, como pode ser observado na figura 3.

Figura 3. Colapso da bolha de cavitação



Fonte: A autora, adaptado de Chemat, Zill-E-Huma e Khan (2011).

As ondas geradas no processo propagam-se no meio líquido, originando variações de pressão, responsáveis pela criação e implosão de microbolhas de gás no centro de um líquido. Essa alta frequência forma ondas de choque no momento da implosão das bolhas e também produz um efeito vibratório na célula vegetal, capaz de levá-la a ruptura e, com isso, a liberação do seu conteúdo. Esses efeitos levam à redução do tamanho de partículas e ao aumento da taxa de reação através da transferência de massa da parede celular, sem causar as alterações da estrutura e função dos extratos (CHEMAT; ZILL-E-HUMA; KHAN, 2011).

Contudo, a cavitação não é o único mecanismo sobre a matriz, outros mecanismos independentes ou combinados como fragmentação, erosão, capilaridade, detexturização, sonoporação e tensão de cisalhamento também podem atuar. Esses efeitos podem quebrar a parede celular e aplicar com êxito nos processos de extração de componentes naturais do produto. (CRAVOTTO, et al., 2008).

2.4 EMBALAGENS DE ALIMENTOS

A embalagem de alimentos desempenha três funções básicas: contenção, preservação da qualidade e proteção de fatores ambientais, físicos e microbiológicos (BHARGAVA, et al., 2020). Os materiais mais comuns usados para embalagens de alimentos são: plástico, papel, vidro, alumínio, papelão e aço. Os plásticos à base de petróleo são comumente usados, pois oferecem várias vantagens em relação a outros materiais de embalagem em termos de baixo peso, estabilidade e robustez. No entanto, levam a sérios problemas ambientais, pois geram grandes volumes de resíduos não biodegradáveis (SUDERMAN; ISA; SARBON 2018).

Nos últimos tempos, em virtude das mudanças de preferências e expectativas do consumidor, a função da embalagem foi modificada para além de suas funções básicas. Agora, também está contribuindo para prolongar a vida de prateleira e atua como um indicador de qualidade dos produtos alimentícios embalados. O foco também está no desenvolvimento de sistemas de embalagens mais interativos, sendo classificados como embalagens ativas e inteligentes (GHAANI, et al., 2016).

Em embalagens ativas, materiais intencionalmente adicionados (absorventes ou emissores) interagem com o ambiente interno da embalagem para aumentar a vida útil do alimento. Já na embalagem inteligente os materiais adicionados monitoram o estado (tempo de armazenamento, temperatura, prazo de validade, etc.) dos produtos alimentícios embalados

(BHARGAVA, et al., 2020). Esse sistema de embalagem é denominado como *'Smart Packaging'* referem-se à embalagem com tecnologia de sensores incorporados a diferentes tipos de produtos. Estima-se que o mercado global de embalagens inteligentes atinja US$ 26,7 bilhões até 2024 (SCHAEFER; CHEUNG 2018).

Em outras palavras, a embalagem ativa é o componente que realiza alguma ação, enquanto a embalagem inteligente é o componente que detecta e compartilha a informação. A embalagem inteligente e a embalagem ativa podem trabalhar sinergicamente para produzir o que é definido como embalagem "inteligente", ou seja, um conceito de embalagem total que combina os benefícios advindos da tecnologia ativa e inteligente (GHAANI, et al., 2016).

2.4.1 Embalagem biodegradável

A produção de filmes para embalagens à base de materiais biológicos está crescendo e tem despertado cada vez mais atenção no meio científico. Esses materiais são ecologicamente corretos e desempenham um papel crucial na redução do impacto ambiental de resíduos plásticos (MATHEUS; MIYAHIRA; FAI 2020).

Os principais biopolímeros empregados no desenvolvimento de filmes biodegradáveis são as proteínas e os polissacarídeos. Os polissacarídeos estudados até agora incluem quitosana, carboximetilcelulose e amido. Proteínas comumente estudadas para o desenvolvimento de filmes biodegradáveis incluem proteína de soja, proteína do leite, como caseína e proteína de soro de leite, e gelatina (SUDERMAN; ISA; SARBON 2018).

Dentre todos os polímeros naturais, a utilização de amido e da gelatina justifica-se pelo baixo custo, fácil disponibilidade, renovabilidade e natureza não tóxica. Essas qualidades os tornam úteis como matéria-prima para a fabricação de filmes para embalagens (KUMAR; GHOSHAL E GOYAL 2020).

A gelatina possui excelentes propriedades filmogênicas, e por isso tem sido amplamente utilizada como biopolímero em estudos de filmes comestíveis e/ou biodegradáveis (SUDERMAN; ISA E SARBON 2018). Os filmes compostos de gelatina possuem boas propriedades mecânicas, mas são sensíveis à umidade e apresentam fracas propriedades de barreira ao vapor de água (NUR HANANI; ROOS; KERRY 2014).

Da mesma forma, o amido é um material muito atraente para novos sistemas de embalagem, pois tem a capacidade de formar uma matriz contínua e possui características termoplásticas, o que permite que seja facilmente processado. No entanto, os filmes formados são quebradiços e hidrofílicos, limitando seu processamento e aplicação (AZEVEDO, et al.,

2017). Para superar esses inconvenientes, o amido pode ser misturado com outros polímeros naturais. A mistura de polímeros é um processo bem conhecido usado para desenvolver novos materiais e otimizar as propriedades do polímero, resultando em materiais com melhores propriedades quando comparados aos elaborados com componentes puros (MENDES, et al., 2016)

Além disso, as propriedades funcionais dos filmes também podem ser melhoradas com a adição de plastificantes (polióis, como glicerol, sorbitol e polietilenoglicol) ou reforços que irão aumentar a sua extensibilidade, dispensabilidade, flexibilidade, elasticidade e propriedades mecânicas (SUDERMAN; ISA E SARBON 2018).

3 ARTIGOS CIENTÍFICOS INTEGRADOS

3.1 ARTIGO 1 - EXTRACTION OF ANTIOXIDANT AND ANTIMICROBIAL PHYTOCHEMICALS FROM CORN STIGMA: A PROMISING ALTERNATIVE TO VALORIZATION OF AGRICULTURAL RESIDUES

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FOOD TECHNOLOGY

Extraction of antioxidant and antimicrobial phytochemicals from corn stigma: a promising alternative to valorization of agricultural residues

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Extraction of antioxidant and antimicrobial phytochemicals from corn stigma: a promising alternative to valorization of agricultural residues

Extração de fitoquímicos antioxidantes e antimicrobianos do estigma do milho: uma alternativa promissora para valorização de resíduos agrícolas

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ABSTRACT

The discovery of new natural additives from agro-industrial waste is considered an important research topic. This study investigated the feasibility of ultrasound-assisted extraction (UAE) of antioxidant compounds from corn stigma (CS) and the effect of independent variables (time and solid-solvent ratio) and their interaction in the extraction of CS. Results indicated that the UAE method increases the antioxidant activity and reduces the extraction time by 67%. Optimized conditions for the simultaneous extraction of antioxidants and polyphenols from CS were obtained using 5 min and a solid-solvent ratio of 0.05 g mL^{-1}. The CS extract obtained by

UAE was characterized by ESI-ToF-MS and 27 phytochemicals were reported. The extract showed promising antifungal and antibacterial activities against 23 of the studied microorganisms. Therefore, the CS extract obtained by the UAE can be used as a source of bioactive and antimicrobial compounds for use as a functional ingredient in the food and pharmaceutical industry.

Key words: corn stigma, ultrasound-assisted extraction, antifungal activity, antibacterial activity.

RESUMO

A descoberta de novos aditivos naturais a partir de resíduos agroindustriais é considerada um importante tópico de pesquisa. Este estudo teve como objetivo investigar a viabilidade da extração assistida por ultrassom (EAU) de compostos antioxidantes do estigma do milho (EM) e o efeito de variáveis independentes (tempo e relação sólido-solvente) e sua interação na extração de EM. Os resultados indicaram que a EUA aumenta a atividade antioxidante e reduz o tempo de extração em 67%. Condições otimizadas para a extração simultânea de antioxidantes e polifenóis do EM foram obtidas com 5 min e uma relação sólido-solvente de 0,05 g mL^{-1}. O extrato de EM obtido pela EUA foi caracterizado por ESI-ToF-MS e 27 fitoquímicos foram encontrados. O extrato apresentou atividades antifúngicas e antibacterianas promissoras contra 23 dos micro-organismos estudados. Portanto, o extrato de EM obtido pela extração assistida por ultrassom pode ser utilizado como fonte de compostos bioativos e antimicrobianos para uso como ingrediente funcional na indústria alimentícia e farmacêutica.

Palavras-chaves: estigma do milho, extração assistida por ultrassom, atividade antifúngica, atividade antibacteriana.

INTRODUCTION

Food wastes are produced in several steps of the food life cycle, such as agricultural production, industrial processing, and market distribution. In the agricultural production, it is estimated that at least 40% of the initial feedstock mass is discarded as waste. Several studies proposed the valorization of food wastes, the extraction of value-added compounds from agricultural residues have been reported as a potential alternative to obtain nutraceuticals and functional ingredients (VODNAR et al., 2017).

Corn stigma (CS) has been used as a therapeutic medicine in many countries, such as Brazil, China, Turkey, United States, and France for the diseases treatment. The CS is a potential renewable source of phenolic compounds, and flavonoids, it is also composed by proteins, vitamins, carbohydrates, macronutrients, volatile oils, steroids, alkaloids and saponins. Pharmacological studies suggested that CS extracts and its bioactive components offer many benefits that can be used to ensure human health (CHAIITTIANAN et al., 2017). As it is an agro-industrial by-product available in abundance and without commercial cost, CS becomes a potential food additive, but for this detailed information about its phytochemical composition is needed to obtain an adequate functional ingredient.

Many extraction procedures also called “conventional extraction” have been proposed for the extraction of bioactive compounds from agricultural residues. These methods generally require the use of high amounts of organic solvents, high energy consumption, and long extraction times. Ultrasound-assisted extraction (UAE) has been reported as a potential alternative to increase the extraction yields of bioactive compounds from fruits and vegetable residues. The use of UAE promotes the exhaustive extraction of active ingredients from plants with relatively short time and high extraction efficiency when compared to conventional procedures (CHEMAT et al., 2017).

In this sense, the present research was proposed to investigate the effect of ultrasound as a feasible way for the extraction of bioactives compounds from CS. To ensure the applicability of CS extract in food processing, the antimicrobial action against 14 bacteria and 9 fungi was investigated. Additionally, the UAE extract was characterized by electrospray ionization with high resolution time-of-flight mass spectrometry (ESI-ToF-MS) and the compounds identified were directly related to the antioxidant activity demonstrated in vitro.

MATERIALS AND METHODS

The plant material used in this experiment was purchased from rural producers in Santa Maria (Brazil). The CS was dried in an oven at 45 °C (± 5) until constant weight and after crushed in a Willey knife mill (MA - 680; Marconi Ltda., Piracicaba, Brazil) using a 20 mesh sieve. The material was kept at a temperature of -18 ºC in an airtight container and protected from light until the analyzes were performed.

A factorial design 2^2 was proposed to investigate the effect of extraction time and the solid-solvent relationship on the dependent variables (ORAC and TPC). The CS was added to a solution of 70% ethanol at 60 °C, as proposed by JABBAR et al. (2014). The extractions were performed in different periods of time (X_1), with different solid-solvent ratios (X_2) (Table 1). The UAE experiments were performed using an ultrasonic probe (VC-750; Sonics & Materials, Inc., Newtown, CT, USA) operating at 20 kHz with 70% of US amplitude and 750 W of nominal power (SAEEDUDDIN et al., 2015). The shaking extraction (SE) procedures were carried out in an ultra-thermostatic bath (Marconi, model MA-184, São Paulo, Brazil) with constant agitation at 100 g the agitator (Marconi MA-039, São Paulo, Brazil). The CS extracts were then centrifuged at 202 g for 15 min (Centrifuga Coleman Model 90-1, São Paulo, Brazil) and the supernatants were collected as the extracts. Three repetitions were performed in each experiment. The extracts were stored at -18 °C for further analysis.

The determination of total phenolic compounds (TPC) was performed by the Folin-Ciocalteu method described by ROESLER et al. (2007). The content of total phenolic compounds was expressed in milligrams of gallic acid per gram of dry sample (mg GAE g^{-1}).

The oxygen radical absorbance capacity (ORAC) was analysed as proposed by DÁVALOS et al (2004). Results were expressed in μmol Trolox equivalents per gram of dry sample (μmol Trolox/g).

The extract obtained by UAE was submitted for the screening analysis by high-resolution electrospray ionization time-of-flight mass spectrometry (ESI-ToF-MS, model Xevo G2 Qtof, Waters Inc., Milford, USA), as proposed by BOEIRA et al. (2020).

For UAE extract, the antibacterial and antifungal activities were assayed using the broth microdilution method (NCCLS, 2017, 2018). A collection of twenty- three microorganisms was used, including five Gram-positive bactéria: *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Enterococcus fecalis* (ATCC 19433), *Enterococcus ssp*. (ATCC 6589), *Bacillus subtilis* (ATCC 19659), ten Gram-negative bacteria: *Salmonella enteric serovar* Typhimurium, (ATCC 14028), *Escherichia coli* (ATCC 25922), *Shigella sonnei* (ATCC 25931), *Enterobacter aerogenes* (ATCC 13048), *Salmonella enteritidis* (ATCC 13076), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeroginosa* (ATCC 27853), *Morganella morganii* (ATCC 25829), *Proteus mirabilis* (ATCC 25933), *Klebsiella pneumoniae* (clinical isolate) and nine yeasts: *Candida parapsilosis* (ATCC 22019), *Candida tropicalis* (ATCC 750), *Candida albicans* (ATCC 10231), *Candida glabrata* (ATCC 2001), *Candida dubliniensis* (ATCC MYA-577), *Candida krusei* (ATCC 6258), *Cryptococcus gatti* (ATCC 56990), *Cryptococcus neoformans* (ATCC 28952), *Saccharomyces cerevisiae* (ATCC 2601). Standard strains of the microorganisms were obtained from American Type Culture Collection (ATCC), and standard antibiotics chloramphenicol, ampicillin, fluconazole and nystatin were used in order to control the sensitivity of the microbial test. The minimal inhibitory concentration

(MIC) was determined on 96-well culture plates by a micro dilution method using a microorganism suspension at a density of 10^5 CFU mL^{-1} with Casein Soy Broth incubated for 24 h at 37 °C for bacteria and Sabouraud Broth incubated for 48 h at 25 °C for yeasts (NCCLS, 2017, 2018). The cultures that did not present growth were used to inoculate plates again (Casein Soy Broth and Sabouraud), in order to determine the minimal lethal concentration (MLC). Proper blanks were simultaneously assayed, and all samples were tested in triplicate.

The experiment was carried out in triplicate. The experimental data were submitted to analysis of variance (ANOVA) and the means were compared using the Tukey test through the statistical software STATISTICA® 10.0 (StatSoft, Inc., Tulsa, OK 74104, EUA), with a 95% significance level.

RESULTS AND DISCUSSION

The TPC and ORAC results for all conditions evaluated using ultrasound or shaking are shown in table 2. For shaking extraction (SE), the R^2 coefficients reached 0.841 for TPC and 0.854 for ORAC. Calculated R^2 values of the models for TPC and ORAC obtained by the UAE were 0.993 and 0.942, respectively.

For SE, it is observed that the highest yield of TPC (57.20 mg GAE g^{-1}) and ORAC (80.82 µmol Trolox g^{-1}) was obtained in the condition 15 min/0.05 g mL^{-1}. Some authors studied the extraction of bioactive compounds from CS, the use of long periods of extraction, exceeding 24 h of process combined with high temperatures, increase the possibility of loss of thermolabile compounds, resulting in the degradation of the phenolic compounds of interest (WONG-PAZ et al., 2017).

For UAE, using 5 min of extraction time and a solid-solvent ratio of 0.05 g mL^{-1}, the highest yields of TPC (65.31 mg GAE g^{-1}) and ORAC (124.44 µmol Trolox g^{-1}) were obtained. The ultrasound application increased the extraction rates with 67% less time when compared to SE. SILVA et al. (2020) when evaluating the use of ultrasound to extract bioactive compounds

from acerola residue, emphasized that the UAE allows extraction times up to 100 times shorter than necessary when using conventional methods. This is because the use of UAE increases mass transfer, reduces extraction time, and increases the solubilization of target compounds. This phenomenon can be explained by the action of cavitation bubbles in the liquid medium during ultrasound propagation. These effects provide a vibratory effect on plant cells, capable of breaking the cell wall and successfully applying to the processes of extracting components from natural products (CHEMAT et al., 2017)

The influence of extraction time and the solid-solvent ratio for TPC and ORAC, can be reported in table 2. Results demonstrated that both conventional extraction and ultrasound showed significant effects ($P<0.05$), indicating that the model is descriptive for the extraction. For SE, the interaction between the two variables (time and solid-solvent ratio) was significant ($P<0.05$) and negative. The isolated effect of time on TPC and ORAC was also significant ($P<0.05$) and positive, while the solid-solvent ratio had significant effect ($P<0.05$), but negative. It was observed that for TPC and ORAC showed similar behavior (significant, $P<0.05$).

Figure 1 shows the analysis of residues and represents a comparative analysis between the values of TPC and ORAC predicted by the model and those observed experimentally. The results showed that the responses are aligned, indicating the adequacy of the predicted data to the experimental values. Thus, the proposed model proved to be adequate to predict the extraction of bioactive compounds from CS.

The optimized conditions for the simultaneous extraction of antioxidants and polyphenols from CS was 15 minutes for SE and 5 minutes for UAE. The solid-solvent ratio was selected as 0.05 g mL^{-1} for both extraction methods. Considering the significant reduction of required extraction time by UAE, the optimized extract was evaluated for antimicrobial capacity, as well as for the chemical characterization of the phytochemicals present by ESI-ToF-MS.

The extract obtained by UAE, was submitted for the screening analysis by ESI-ToF-MS. The chemical structures were tentatively determined based on reference mass spectral data, and fragmentation pathways. An overview of all compounds identified in the extract is shown in table 3. Based on a multiple data processing approach, 50 ratio m/z in the CS extract were detected. Among them, 27 compounds belonging to different classes were identified, including 1 alkaloid, 1 carbohydrate, 1 amino acid, 3 phenolics, 3 flavonoids, 3 terpenes, 6 fatty acids, 6 organic acids, and 3 other compounds. As far as we know, most of these compounds (21 of them) were reported here in the CS extract for the first time. Only valine, disaccharide, tetracosanoic acid, 9-oxo-10E, 12Z octadecadienoic acid, linoleic acid, and palmitic acid had already been identified in CS.

The largest class of compounds reported in this study was fatty acids such as (linoleic acid, palmitic acid, DL-Cerebronic acid, and tetracosanoic acid). These compounds are produced by plants, which are responsible for the production of most biological lipids in the world. Organic acids, the second largest class observed in CS extract, are naturally present in vegetables, in minimal amounts or accumulating in certain species (MARTIN et al., 2006). In the present study, citric acid, trans-acotinic acid, D-xylonate, malic acid, 1-propanedioic acid-4-caffeoylquinic acid, and 2,4,5,6-tetrahydroxyhexanoic acid were found.

The antioxidant and bioactive activity shown by CS, in in vitro tests, may be related to the presence of phytochemicals in the class of phenolics, flavonoids, terpenes, and alkaloids. Previous studies have reported that the pharmacological effect of CS, such as antioxidants, anti-inflammatories, and diuretic activity, is attributed to the presence of these compounds (LIU et al., 2011). In the present study, 10 phytochemical compounds were reported.

The terpenoid compound Nardoaristolone A is described by MATOS et al. (2015), as an important precursor of secondary metabolites of flavonoids and isoflavonoids in plants. Its inhibitory effects on acute inflammation and chronic pain stand out, with significant activity in

the treatment of rheumatoid arthritis. Likewise, a study on the alkaloid Caesalpinin MH, demonstrated its anti-inflammatory actions as a selective modulator of glucocorticoids and having great potential for therapeutic use in inflammatory diseases (XIANG et al., 2018).

The screening by ESI-ToF-MS analysis, allowed the recovery and characterization of a large number of phytochemicals, many of which were not previously reported in the literature for this raw material. Previous studies confirm the bioactivity of corn stigma. ŽILIĆ et al. (2016) report the abundance of bioactive compounds, mainly hydroxycinnamic acid esters and luteolin derivatives. LI et al. (2019) found that CS tea reduced systolic blood pressure levels in hypertensive rats and inhibited the activity of the angiotensin-converting enzyme. In addition, HO et al. (2017) discovered a peptide with CS anti-inflammatory abilities. CS can be considered a safe ingredient for food application, according to data obtained by WANG et al. (2011). When performing subchronic toxicity tests, they reported that CS has no adverse effects and supports its safety for use in humans.

After the screening by ESI-ToF-MS, experiments regarding to antimicrobial activity of CS extract were performed. The results obtained in the screening to determine the antifungal and antibacterial activities are shown in table 4.

The MIC and MLC values of each of the tested fractions were compared with positive controls. The extract obtained by UAE exhibited significant inhibitory activity against all evaluated bacteria, with MIC values between 62.5 and 250 $\mu g.mL^{-1}$, with Gram-positive bacteria *Bacillus cereus*, *Bacillus subtilis* and *Enterococcus fecalis* (MIC of 62.5 $\mu g.mL^{-1}$) and the Gram-negative bacteria *Escherichia coli*, *Shigella sonnei*, *Salmonella* typhimurium and *Morganella morganii* (MIC of 62.5 $\mu g.mL^{-1}$) the most sensitive. The CS extract showed antifungal potential, inhibiting the growth of all evaluated fungi, with MIC values between 125 and 500 $\mu g.mL^{-1}$, with *Candida albicans*, *Candida krusei*, *Cryptococcus neoformans* and *Cryptococcus gatti* (MIC of 125 $\mu g.mL^{-1}$) the most sensitive.

The extract exhibited superior bactericidal potential compared to positive ampicillin control, for 7 of the 14 bacteria tested. Phytochemicals that present in MIC susceptibility tests between 100-1000 $\mu g.mL^{-1}$ in vitro, can be classified as antimicrobials (DOS REIS et al., 2019). Therefore, the extract obtained by ultrasound of corn stigma evaluated in this study can be considered a substance with antimicrobial potential (MIC $\leq$ 500 $\mu g.mL^{-1}$).

The antibacterial and antifungal effect observed in CS, is directly associated with its phytochemical composition. According to data obtained in the ESI-ToF-MS analysis, the presence of fatty acids was reported, among which linoleic acid, palmitic acid, DL-cerebronic acid and tetracosanoic acid stand out, which are described in the literature for having strong antimicrobial properties, acting against Gram-positive organisms. In addition, previous studies reveal that organic acids, the second largest class of compounds present in CS, also have action against different types of pathogens. These constituents of CS, act in reducing the internal pH of the microbial cell by ionization of acid molecules, not dissociated, and interrupting the transport of substrate by altering the permeability of the cell membrane or reduction of proton motive force (FENG et al., 2011).

CONCLUSION

The results of the study indicated that the ultrasound-assisted extraction (UAE) of corn stigma (CS) has the ability to increase the total phenolic compounds (TPC) and oxygen radical absorbance capacity (ORAC) values and reduce the extraction time by 67% when compared to the conventional method. The optimal condition for simultaneous extraction of antioxidants and polyphenols from CS by UAE was 5 minutes at a solid-solvent ratio of 0.05 g mL^{-1}. Using the screening by ESI-ToF-MS, it was possible identified 27 phytochemical compounds, of which 21 have not yet been described in the literature for CS. In addition, results also showed that CS has a more active and efficient bacteriostatic potential than the positive ampicillin control for 7

of the 14 bacteria studied. The results compiled in this study prove that corn stigma can be considered a source of bioactive metabolites with antioxidant and antimicrobial functions. This agricultural residue can be used in the food industry as a natural antioxidant ingredient, or it can be applied in bioactive packaging to extend the shelf life of food products.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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Table 1. Experimental runs using coded levels of time (min. X_1), and with different solid-solvent ratios (g mL^{-1} X_2) according to the factorial design 2^2.

	Coded variables levels	
Runs	X_1 (min)	X_2 (g mL^{-1})
1	5 (-1)	0.05 (-1)
2	15 (1)	0.05 (-1)
3	5 (-1)	0.1 (1)
4	15 (1)	0.1 (1)
5	10 (0)	0.075 (0)
6	10 (0)	0.075 (0)
7	10 (0)	0.075 (0)

Table 2. Values of TPC and ORAC after SE and UAE and the effect of the studied variables.

Analytical results				
	Shaking extraction (SE)		Ultrasound-assisted extraction (UAE)	
Runs	TPC (mg GAE g^{-1})	ORAC (µmol Trolox g^{-1})	TPC (mg GAE g^{-1})	ORAC (µmol Trolox g^{-1})
1	47.14±1.58	68.18±0.83	65.31±2.25	124.44±1.43
2	57.20±1.58	80.82±1.29	50.03±1.09	72.49±0.89
3	32.07±2.12	38.06±0.92	45.82±1.44	65.84±1.12
4	39.79±0.92	43.71 ±0.33	37.73±1.45	33.64 ±1.37
5	50.24±0.58	46.27±0.84	48.57±0.44	62.73±0.78
6	50.17±0.44	46.81±1.12	48.05±2.54	61.76±0.92
7	50.20±0.94	46.99±0.94	49.46±0.29	61.16±0.64

Effects						
	Shaking extraction (SE)			Ultrasound-assisted extraction (UAE)		
	TPC			TPC		
	Effect	Standard Error	*P*	Effect	Standard Error	*P*
Mean/Interaction	46.689	0.014	< 0.001*	49.287	0.269	< 0.001*
(X_1) Time	8.889	0.037	< 0.001*	-11.680	0.711	0.003*
(X_2) Solid-solvent ratios	-16.239	0.037	< 0.001*	-15.891	0.711	0.002*
X_1 by X_2	-1.1670	0.037	0.001*	3.597	0.711	0.036*
R^2 = 0.841				R^2 = 0.993		
Adj-R^2 = 0.682				Adj-R^2 = 0.986		
	ORAC			ORAC		
	Effect	Standard Error	*P*	Effect	Standard Error	*P*
Mean/Interaction	52.980	0.142	< 0.001*	68.865	0.299	< 0.001*
(X_1) Time	9.149	0.376	0.001*	-42.075	0.792	< 0.001*
(X_2) Solid-solvent ratios	-33.613	0.376	< 0.001*	-48.725	0.792	< 0.001*
X_1 by X_2	-3.494	0.376	0.011*	9.875	0.792	0.006*
R^2 = 0.854				R^2 = 0.942		
Adj-R^2 = 0.709				Adj-R^2 = 0.885		

Results are expressed as Mean±SD (n=3); TPC=Total phenolic content ORAC=Oxygen radical absorption capacity (*)There was significant effect considering 95% significance.

Table 3. Identification of the main chemical compounds of the extract of CS by ESI-ToF-MS.

No.	Experimental mass (m/z)	Theorical mass (m/z)	Error (ppm)	Possible molecular structure	Compounds	Classification
	Positive mode $[M+H]^+$					
1	629.1295	629.1280	2.4	$C_{33}H_{25}O_{13}$	Acremoxanthone D	Phenolic
2	589.4257	589.4248	1.5	$C_{39}H_{56}O_4$	Lupeol caffeate	Terpene
3	543.1350	543.1344	1.1	$C_{23}H_{27}O_{15}$	5,6-O-β-D-Diglucopyranosylangelicin	Flavonoid
4	531.2383	531.2401	3.4	$C_{32}H_{35}O_7$	Nardoaristolone A	Terpene
5	465.2125	465.2111	3.0	$C_{24}H_{33}O_9$	Caesalpinin MH	Alkaloid
6	381.0822	381.0801	5.5	$C_{17}H_{17}O_{10}$	Unknown phenolic glycoside	Phenolic
7	333.1855	333.1832	6.9	$C_{23}H_{25}O_2$	((3-(Benzyloxy)propoxy)methylene) dibenzene	Other compound
8	293.0661	293.0656	1.7	$C_{14}H_{13}O_7$	Planchol E	Phenolic
9	156.0421				Valine	Amino acid
	Negative mode $[M-H]^-$					
10	607.1630	607.1604	4.3	$C_{35}H_{27}O_{10}$	Australisine A	Terpene
11	575.1401	575.1394	1.2	$C_{27}H_{27}O_{14}$	Luteolin-8-C-6″deoxy-3″-hexoside-2″-O-ramnoside	Flavonoid
12	563.1401	563.1381	3.4	$C_{26}H_{28}O_{14}$	Schaftoside	Flavonoid
13	439.0877	439.0890	3.0	$C_{19}H_{20}O_{12}$	1-propanedioic acid-4-caffeoylquinic acid	Organic acid
14	411.0716	411.0741	6.1	$C_{21}H_{14}O_9$	Acetone adduct from polyozellic acid	Other compound
15	383.3525	383.3532	1.8	$C_{24}H_{48}O_3$	DL-Cerebronic acid	Fatty acid
16	367.3576	367.3545	7.8	$C_{24}H_{48}O_2$	Tetracosanoic acid	Fatty acid
17	341.1084	341.1076	2.3	$C_{12}H_{22}O_{11}$	Disaccharides	Carbohydrate
18	327.2899	327.2909	3.1	$C_{20}H_{39}O_3$	Ethyl 2-Acetylhexadecanoate	Other compound
19	311.2222	311.2237	4.9	$C_{18}H_{32}O_4$	(Z)-6-Hydroxy-4-oxo-octadec-11-enoic acid	Fatty acid
20	293.2117	293.2116	0.3	$C_{18}H_{30}O_3$	9-oxo-10E,12Z octadecadienoic acid	Fatty acid
21	279.2324	279.2344	7.2	$C_{18}H_2O_2$	Linoleic acid	Fatty acid
22	255.2324	255.2316	3.1	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid
23	195.0505	195.0506	0.5	$C_6H_{12}O_7$	2,4,5,6-tetrahydroxyhexanoic acid	Organic acid
24	191.0192	191.0185	3.7	$C_6H_8O_7$	Citric acid	Organic acid
25	173.0086	173.0100	8.0	$C_6H_5O_6$	Trans-Acotinic Acid	Organic acid
26	165.0399	165.0413	8.0	$C_5H_9O_6$	D-xylonate	Organic acid
27	133.0137	133.0146	6.8	$C_4H_6O_5$	Malic acid	Organic acid

Mode negative: 100 µL CS extract diluted in 1.5 mL of methanol with 20 µL of ammonium hydroxide (100 $mmolL^{-1}$) / Mode positive: 100 µL CS extract diluted in 1.5 mL of methanol with 20 µL of formic acid

Table 4. Minimum inhibitory and lethal concentrations for bacteria and fungi from CS extract obtained by UAE.

MIC 50: minimum inhibitory concentration capable of inhibiting 50% of the microorganism growth (μg mL-1);

Bacteria	Fractions (MIC 50/MLC μg mL^{-1})					
	Extract		Chloramphenicol		Ampicillin	
	MIC	MLC	MIC	MLC	MIC	MLC
Gram positive						
Bacillus cereus	62.5	500	3.12	12.5	200	200
Bacillus subtilis	62.5	>500	6.25	50	100	200
Staphylococcus aureus	125	500	1.56	6.25	200	200
Enterococcus fecalis	62.5	>500	3.12	12.5	1.56	12.5
Gram negative						
Pseudomonas aeruginosa	125	>500	3.12	12.5	50	200
Shigella flexneri	125	>500	1.56	3.12	12.5	200
Proteus mirabilis	250	>500	6.25	25	25	200
Escherichia coli	62.5	500	3.12	100	200	200
Shigella sonnei	62.5	500	1.56	3.12	25	200
Salmonella typhimurium	62.5	500	3.12	200	200	200
Salmonella enteritidis	125	>500	1.56	12.5	3.12	100
Klebsiella pneumoniae	125	500	6.25	200	100	200
Morganella morganii	62.5	500	6.25	50	200	200
Enterobacter aerogenes	125	>500	1.56	12.5	200	200
Fungal	Fractions (MIC 50/MLC μg mL^{-1})					
	Extract		Fluconazole		Nystatin	
	MIC	MLC	MIC	MLC	MIC	MLC
Candida albicans	125	500	25	100	50	100
Candida parapslosis	250	>500	1.56	25	1.56	100
Candida krusei	125	500	25	200	12.5	50
Candida tropicalis	500	500	50	200	100	200
Cryptococcus neoformans	125	500	3.12	12.5	25	100
Cryptococcus gatti	125	500	3.12	25	25	100
Candida dubliniensis	250	>500	3.12	12.5	50	100
Candida glabrata	500	>500	3.12	200	50	100
Saccharomyces cerevisiae	250	>500	1.56	25	1.56	3.12

MLC: minimum lethal concentration (μg mL-1); Chloramphenicol, Ampicillin, Fluconazole and Nystatin: positive controls.

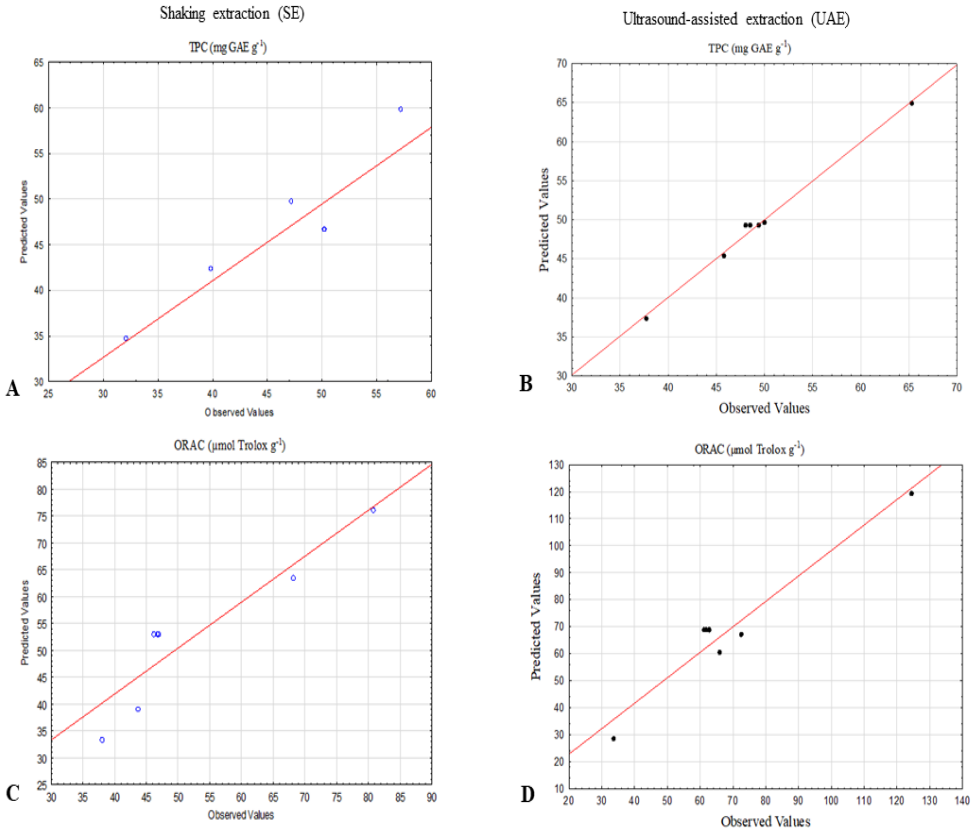

Figure 1. Values predicted versus observed experimentally in TPC and ORAC after SE and UAE. (A) TPC in SE and (B) UAE; (C) ORAC in SE and (D) UAE. TPC = Total phenolic content; ORAC = Oxygen radical absorption capacity; GAE = Gallic acid equivalent

3.2 ARTIGO 2 - EFFECT OF CORN STIGMA EXTRACT ON PHYSICAL AND ANTIOXIDANT PROPERTIES OF BIODEGRADABLE AND EDIBLE GELATIN AND CORN STARCH FILMS

Artigo em processo de revisão no periódico International Journal of Biological Macromolecules, ISSN: 0141-8130, Área de avaliação em Ciência de Alimentos A1.

Effect of corn stigma extract on physical and antioxidant properties of biodegradable and edible gelatin and corn starch films

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Highlights

- Gelatin/cornstarch films were prepared with the addition of corn stigma extract
- 25% extract improved mechanical property, contact angle and water vapor barrier
- SEM analysis revealed a homogeneous structure of the prepared films
- The complete biodegradability of the films occurred before the tenth day of the study
- Films with bioactive extract exhibited remarkable antioxidant activity.

Abstract

The development of bio-based food packaging with antioxidant properties is an important research topic and has gained prominence these days. In this study, bioactive films were developed based on gelatin (G) and corn starch (CS) incorporated with corn stigma extract at different concentrations (15% and 25%; w/v). In preliminary tests, the extract maintained cell viability above 90% indicating that it is safe for application as an active ingredient. Insertion of the extract did not influence the thickness of the films but caused a slight change in optical properties. Scanning electron microscopy (SEM) analysis revealed interactions between the extract's bioactive compounds with gelatin and corn starch compounds, which may have improved the mechanical properties (elongation at break, Young's modulus). The addition of 25% corn stigma extract increased the contact angle, giving the film a hydrophobic character. Furthermore, at this concentration, a 15% reduction in water vapor permeability was observed. The elaborated films showed complete biodegradability before the tenth day of the study. It can be inferred that the films with corn stigma extract have good antioxidant properties, indicating that they can be used as an ingredient for food packaging.

Keywords: Antioxidant packaging, Biodegradable packaging, Reuse of agricultural waste

1. Introduction

The use of plastic polymers by the packaging industry has increased exponentially over the past few decades[1]. As a result, the generation of waste from this material has grown worldwide. These residues are of particular concern, since studies reveal several adverse effects on human health and the environment[2]. According to Bala [3], packaging can contribute up to 45% of the environmental impacts of the food value chain. Because of this, hydrocolloids (proteins and polysaccharides) have been extensively investigated as material for bio-based packaging, as they can decrease the disposal of food residues packed in synthetic polymers such as plastics [4].

Natural polymers, such as gelatin (G) and corn starch (CS), are potential sources for forming biodegradable and edible films for packaging food. These hydrocolloids stand out for being renewable, abundant, and low cost [5]. The formation of blends with gelatin-corn starch (GCS) is also gaining ground among researchers, since this mixture may offer advantages in terms of oxygen and water vapor barrier properties, mechanical behavior, and optical parameters [6]. In addition, they also allow the incorporation of natural additives that can act not only as antioxidants but also as antimicrobial agents in the packaged product [7].

Agricultural residues are cheap sources of bioactive compounds that can be used in the formulation of innovative biopolymers [8]. In their composition, they have the presence of phytochemicals such as phenolic compounds, flavonoids, carotenoids, terpenoids, organic acids, fatty acids, among others, that act as antioxidants, reacting with a variety of free radicals, and can also inhibit the growth of different microorganisms [9]. Its action is essential to reduce or even eliminate some of the main causes of food deterioration, such as lipid oxidation, color changes, dehydration, microbiological growth, and unpleasant odors [10].

Corn stigma is a low-cost by-product of corn cultivation, rich in nutrients and secondary metabolites (flavonoids, polyphenols, and saponins). Studies reveal many promising applications with broad perspectives, including the discovery of their chemical constituents and pharmacological effects [11]. In addition, according to Jia et al [12], corn stigma has proven biological activities, such as anti-diabetics, lipid-lowering agents, antioxidants, anti-fatigue, anti-obesity, hepatoprotective and anticancer. However, as far as we know, information on the use of corn stigma extract as an active ingredient for making biodegradable packaging is still scarce. Thus, the objective of this work was to determine the structural, physical, and chemical properties of biodegradable films based on blends of gelatin and activated corn starch with different concentrations of corn stigma extract.

2. Materials and methods

2.1 Materials

Materials used for the production of the films were: gelatin type A (SM Empreendimentos Farmacêuticos LTDA, São Paulo, Brazil), native corn starch supplied by Neilar Indústria e Comércio de Alimentos LTDA (Santa Catarina, Brazil), and glycerol PA (Neon Comercial de Reagentes Analíticos, SP, Brazil).

2.2 Corn stigma extract production

The hydroethanolic extract of corn stigma was obtained using an ultrasound probe (VC-750; Sonics & Materials, Inc., Newtown, CT, USA) at 20 kHz operating at 70% of the amplitude and 750 W of nominal power, in a time of 5 minutes, temperature of 60 ºC and solid-solvent ratio of 0.05 g mL^{-1} [13]. Afterwards, the extract was centrifuged at 202 g for 15 min (Centrifuga Coleman Model 90-1, São Paulo, Brazil), filtered and concentrated by rotary

evaporator (Buchi R-210) using a temperature of 45 °C and the supernatant was collected for application in the film-forming solution.

2.7 In vitro cytotoxicity assays of corn stigma extract

2.7.1 Cell culture

The cell line 3T3 (murine Swiss albino fibroblasts) was grown in DMEM medium supplemented with 10% FBS (v/v) at 37 °C, 5% CO_2. The cells were routinely cultured in 75 cm^2 culture flasks and harvested using trypsin-EDTA when a confluence of about 80% was reached counted in an optical microscope to adjust cell density to $1x10^5$ cells mL^{-1} and then transferred to 96-well plates.

2.7.2 Cytotoxic assay

To assess the safety of the application of the use of the extract as an active ingredient in gelatin and corn starch films, it was performed to cytotoxic evaluated using MTT (2,5-diphenyl-3,-(4,5-dimethyl-2-thiazolyl) tetrazolium bromide) and NRU (Neutral Red Uptake) assays, following the procedures previously described by Nogueira, et al [14]. Cytotoxicity was expressed as a percentage of viability in relation to untreated control cells (the average optical density of untreated cells was adjusted to 100% viability) and in terms of IC50 (concentration that causes 50% death of the cell population), was calculated by adjusting the percentage cell survival curve in relation to the extract concentrations.

2.3 Preparation of films composed of gelatin and corn starch / bioactive extract of corn stigma

The film-forming solution was prepared as proposed by Boeira et al [15]. Corn stigma extract was added in gelatin-corn starch (GCS) blends in 15% and 25% proportions (w/v). The

plasticizer used was glycerol (10% in relation to the dry mass). 50 mL of the solution GCS was poured into polyester sheets and dried in an oven at 25 °C for 24 h. After drying, the films were detached and stored for 48 h in desiccators at 25 ± 3 °C with a relative humidity (RH) of 50 ± 3% before analysis. A film without corn stigma extract was prepared as a control.

2.4 Characterization and properties

2.4.1 Thickness and Water vapor permeability (WVP)

Five different points of the films were used to obtain the average thickness using a digital micrometer (Mitutoyo Co., Kawasaki, Japan). The results were used to obtain water vapor permeability values and mechanical properties. The WVP of the films was determined according to the American Society standard method E96/96M-16 for testing and material [16].

2.4.3 Water contact angle

The contact angle is measured using a Theta Lite optical tensiometer (Biolin Scientific, Finland) equipped with an electronic camera using the sessile fall method, as described by Jridi et al [18].

2.4.4 Mechanical properties

Tensile strength, elongation at break and Young's modulus were determined on a universal testing machine (Instron, model 3369, Instron Corp., Canton, MA, USA). The tensile strength (σ = force/ initial cross-section area), elongation at break (E) and Young's modulus (EM) values were obtained from the Instron Bluehill Universal Software (Norwood, MA, USA).

2.4.5 Color

The color of the samples was measured according to the method of Fan et al [18] based on the International Lighting Commission (CIE) system, using the parameters L*, a* and b* (CIELAB scale), with a Minolta CR-400 colorimeter (Minolta Sensing Inc. Konica , Japan) that was used with calibration mode at spectral reflectance, with illuminant D65 and observation angle of 10°. The color difference (ΔE) was obtained according to the equation below:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL = L of the gelatin film and corn starch –L of the gelatin film and corn starch with corn stigma extract. Δa* = a* of gelatin and corn starch film –a* of gelatin and corn starch film with corn stigma extract. Δb* = b* of gelatin and corn starch film –b* of gelatin and corn starch film with corn stigma extract

2.5 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM), model EVO LS15 (Carl Zeiss, Germany) was used to visualize the region of fracture surfaces and cross-sectional structures of the films. First, the samples were fixed on a bronze stump and covered with carbon tape before being sprayed and coated with gold and aspirated with Sputter Coater model Q150TE (Quorum, Italy) for 20 min. Visualizing analysis was done using magnification ranging from 1000 to 10.000x with an acceleration voltage of 5-10 kV.

2.6 Biodegradability of films on the ground

The qualitative test of biodegradability of the films was carried out as proposed by Jaramillo et al [19]. The vegetable compost (soil) was poured into a plastic tray (15 × 30 × 10 cm) up to a height of about 7 cm. Samples of the films (2 cm x 3 cm) were buried in the soil at a depth of approximately 2 cm. To simulate natural conditions, the plastic trays were kept at room temperature and water was sprayed twice a day to maintain the humidity of the medium. At different times (first, third, sixth and ninth day), the samples were carefully removed for photographic recording and visual assessment of biodegradation.

2.7 In vitro bioactive activity of films

Preliminary tests were carried out to define the ideal concentration of films as proposed by Zhang et al [20]. Conditioned films (400 mg) were dissolved in 10 mL of 70% hydroethanolic solution, under magnetic stirring (Biomixer model 78HW-1) at 40 ºC and afterwards and centrifuged at 8000 g for 10 min. The supernatant was used for the analysis of antioxidant activity (AA%). The AA% was estimated by the DPPH method (2,2-diphenyl-1-picryl-hydrazyl) was carried out according to Wu et al [21]. The results were expressed as percentage (%) of inhibition of DPPH radical oxidation.

2.8 Statistical analysis

The experiment was carried out in triplicate. The experimental data were submitted to analysis of variance (ANOVA) and the means were compared using the Tukey test through the statistical software STATISTICA® 10.0 (StatSoft, Inc., Tulsa, OK 74104, EUA), with a 95% significance level.

3. Results and discussion

3.1 In vitro cytotoxicity assays of corn stigma extract

Cellular cytotoxicity is characterized as the intrinsic ability of any material or compound to promote metabolic changes in cultured cells. This property can be measured by cell viability assays that compare the rates of surviving cells with those killed. Cytotoxicity assays are among the most common *in vitro* methods used to predict the potential toxicity of a substance in cell culture. *In vitro* systems are used primarily for screening purposes and to generate more comprehensive toxicological profiles [22,23]

There are a few reports in the literature regarding the mutagenic or cytotoxic activity of plant extracts. Thus, in order to evaluate the cytotoxicity potential of the corn stigma extract, two in vitro bioassays of the MTT and NRU type, which allow the evaluation of different cellular physiological mechanisms to study cell viability after exposure to toxic substances, were conducted and the data obtained are shown in Figure 1.

The result demonstrated low or no toxicity of corn stigma in the concentration range between 15.6 and 500 $\mu g.mL^{-1}$, showing cell viability in the range of 90.86 ± 1.01 to 97.07 ± 1.57% for the MTT assay and for the NRU test in the range of 92.01 ± 1.04 to 99.34 ± 1.85%. Therefore, corn stigma in the concentration range of 15.6 to 500 $\mu g.mL^{-1}$ is reasonably safe and used for other experiments.

In addition, the high level of safety of corn stigma from different cultivars can be concluded with supporting data from previous reports that showed no toxicity or any of the adverse effects of extracts conducted in in vitro and in vivo models. As reported by Chaiittianan et al [24], the corn stigma extract in concentrations in the range of 250-1000 $\mu g.mL^{-1}$ did not show toxicity. Wang et al [25], when assessing subchronic toxicity in male and female Wistar rats by dietary administration at concentrations of 0.5%, 2.0% and 8.0% (w/w) for 90 days, had no adverse effects affecting the safety of corn stigma. Similarly, Amani, Montazer and

Mahmoudirad [26] reported high cell viability (87%) for zinc oxide nanoparticles and corn stigma in human fibroblast cells.

3.2 Characterization and properties of gelatin and corn starch films with corn stigma extract

3.2.1 Thickness and Water vapor permeability (WVP)

One of the parameters that influence the properties of biofilms is thickness. The impact of incorporating corn stigma extract on the thickness of gelatin-based corn starch (GCS) films is shown in Table 1. The film thickness was in the range of 0.036 to 0.040 mm. One of the challenges when preparing biodegradable films is the control of thickness, especially in production processes such as *casting*. The high concentration of polymers makes the filmogenic solution very viscous and it does not flow under the force of its own weight, making it difficult to homogenize the material after drying [27]. In the present study, the addition of the extract slightly reduced the film thickness, however, there was no significant difference ($p>0.05$), suggesting that the process was homogeneous and efficient for the selected formulations. Xu et al [28] also observed thinner films when astaxanthin extracts were incorporated.

Water vapor permeability (WVP) is one of the determining factors in the quality of food packaging materials. The WVP of food packaging materials should be as low as possible to avoid and/or reduce water transfer between the food and the surrounding environment [29]. In the present study, the incorporation of corn stigma extract at 25% concentration in the films significantly ($p<0.05$) reduced the WVP values (0,348 ± 0.013 g mm/kPa h m^2). This can be attributed to the hydrogen bond interaction between the GCS network and the corn stigma extract, which can reduce the hydrophilic interaction between the hydrogen group and water, leading to a decrease in the film's affinity for water [29]. As shown in Table 1, the addition of 15% extract (0.373 ± 0.023 g mm/kPa h m^2) showed no significant difference compared to the

control film (0.413 ± 0.009 g mm/kPa h m^2) ($p<0.05$). In our study, all three films contained the same gelatin, corn starch and glycerol contents and, therefore, the reduction in WVP values in the films is due to the addition of the bioactive extract of corn stigma.

3.2.2 Water contact angle

Natural polymers such as gelatin and corn starch are known for their high sensitivity to water due to their hydrophilic nature [6]. Thus, the characterization of surface hydrophobicity by measuring the contact angle (CA) (θ), between water and the surface of the films, is one of the main properties of packaging materials. A low CA ($\theta < 65°$) indicates a hydrophilic surface and high CA ($\theta > 65°$) are characteristic of hydrophobic surfaces [30]. Figure 2 presents the results of the contact angle with water up to 25 s. The initial value of CA of the films made with corn stigma were higher than that of the control film, however all values indicated the hydrophobic character of the surface of the films, since the CA values were higher than 79° in all samples.

The increase in extract concentration caused a significant increase in CA values, from approximately 79° for the control film to 88° for films incorporated with 25% extract. Furthermore, after 25 s of water drop deposition, the CA values significantly decreased in the control treatment and in the film with 15% extract, compared to values at t = 0 s, while this decrease was significantly reduced after the addition of 25% of extract, indicating an increase in the resistance of the films to wettability. According to Boeira et al [15] these results are related to the phenolic compounds and flavonoids present in the chemical structure of the corn stigma extract. These compounds can form hydrogen bonds and covalent bonds and, thus, occupy the GCS functional group, decreasing the free hydrogen group, responsible for hydrophilic bonds with water.

3.2.3 Mechanical properties

The use of biodegradable films as packaging material depends on their mechanical properties, such as tensile strength (TS), elongation at break (EB) and Young's modulus (YM). The mechanical properties of the films are shown in Table 1. The GCS control film had a tensile strength value of 65.62 MPa. There was a gradual decrease in the TS values of the film with 15% corn stigma extract (65.39 MPa) and the film with 25% extract (60.82 MPa), differing significantly from the control treatment ($p>0.05$). According to Espino-Manzano, et al [31], the mechanical properties of films are affected by the interaction between the polymers that define their structure and the compounds used for their formation (water, hydrocolloids, plasticizers, antioxidant agents, among others). According to previous studies, the presence of these compounds generally lowers the TS of films [32]. The elongation at break (EB) for the films with 15% and 25% extract was 4.21% and 4.36%, respectively, while for the control film the value was statistically lower, this being 3.7% ($p>0.05$). This small increase in EB can be attributed to the increased mobility of the polymer chain caused by phenolic compounds from plant extracts, due to the formation of hydrogen bonds between the carboxylic groups of phenolic compounds and amine groups of the GCS. Furthermore, the GCS films showed a good Young's modulus (YM) ratio of 2,748.7 MPa. Corn stigma extract caused a notable reduction in YM (2,573.34 MPa) ($p>0.05$). Young's modulus is a parameter that is directly related to film stiffness. YM results were similar to those reported by Kaya et al [32], who observed a reduction in the values for chitosan films with the addition of fruit extract. Thus, the use of corn stigma extract can be applied in food packaging due to its good TS, EB and YM values.

3.2.4 Color

One of the important parameters for the development of food packaging is the surface color of the films, as it is directly related to consumer acceptance. According to Table 2, the

addition of corn stigma extract resulted in a slight decrease in L* values (lightness), indicating a trend towards dark ($p<0.05$). However, it can be observed that the L* values are expressively high, demonstrating that the elaborated films are bright (higher mean L* value) [33]. A reduction in a* values was observed especially for the film with 25% corn stigma extract, which exhibited the lowest value compared to the control film. The b* values increased as a function of increasing concentrations of corn stigma extract, demonstrating a tendency to yellow, which corroborates the difference in the intensity of the yellow color observed visually (Tabela 2.) These results indicated that the type and concentration of the extract play an important role in the color of films. Color changes due to incorporation of corn stigma extract can also be described using ΔE, an indicator of the degree of total color difference from the control film. The ΔE values obtained for the films with 15% and 25% extract were 3.18 and 3.98, respectively. Differences in color parameters for the elaborated films were expected, since the corn stigma extract has a yellow/orange color. Kadam et al [34] report that the addition of extracts to film formulations can result in color variation due to the presence of different bioactive phytochemicals.

3.3 Scanning Electron Microscopy (SEM)

The fracture surface and cross-section of the GCS films with and without incorporation of corn stigma extracts are shown in Figure 3. In general, the surface micrographs (Fig. 3 A, B, C) showed that the control film was homogeneous, smooth and had a continuous surface. However, it can be seen through the cross-section (Fig. 3 a, b, c) that the addition of the extract, in the proportions of 15% and 25%, caused a slight increase in the porosity of the matrix, which is associated with the distribution of the corn stigma extract in the polymer matrix [35]. These results are consistent with Rawdkuen et al [36] who observed rougher and more porous surface in smart gelatin films with the inclusion of anthocyanin extracts from different plants. Li et al

[37] also reported that the more antioxidant extracts are incorporated, the more heterogeneous and porous the surface-displayed for the film. The structural difference between the films may account for the lower WVP of the film with 25% corn stigma extract, albeit with lower mechanical properties [38]

3.4 Biodegradability of films on the ground

A qualitative study of the biodegradability of the films was carried out to observe the degradation of materials as a function of burial time in the plant compost. Figure 4 shows the evolution of the biodegradation of the elaborated films. After 3 days of testing, the films began to show changes in their integrity, suggesting the beginning of degradation. However, it can be observed that on the seventh day the films with 15% and 25% of corn stigma extract showed a lower degradation rate when compared to the control film (without additives), indicating that the biodegradation was delayed by the presence of extract. These results were expected since studies demonstrate that the use of antioxidant compounds in the formulation of biodegradable films affect the microbial activity present in the composting environment [39]. The complete biodegradability of the films occurred before the tenth day of the study. The short degradation time observed in this work proves that the residues of these elaborated films could be disposed of in urban gardens without industrial intervention, thus reducing expenses with the processing of packaging residues and preserving the environment.

3.5 In vitro bioactive activity of films

A promising technique to increase the shelf life of food products is the use of bioactive packaging. Table 1 shows the results obtained for the antioxidant activity of gelatin and corn starch-based films after the addition of corn stigma extract in the proportions 15% and 25%. The results suggested that the addition of 25% extract made the film possess the strongest

scavenging activity against the DPPH radical. The control film (without extract) has a small antioxidant capacity, attributed to the composition of gelatin, due to its content of amino acids that are known for the reducing action of the DPPH radical [38]. The application of the extract increased the antioxidant capacity by 60% when compared to the control treatment. This result is mainly related to the presence of phytochemicals with bioactive properties. According to the study by Boeira et al [13], corn stigma extract has 27 phytochemical compounds such as phenolic compounds, flavonoids, terpenoids, organic acids, fatty acids, among others, which confer these bioactive properties presented in the elaborated films. Therefore, the corn stigma extract added film has the potential to be applied to food packaging to extend shelf life.

4. Conclusion

In this study, gelatin and corn starch-based films incorporated with corn stigma extract were successfully prepared using the casting method. The thickness of the films was not changed with the addition of the extract. However, the films showed a homogeneous surface with formation of small pores by scanning electron microscopy after insertion of the extract. The results also indicate that the film made with 25% extract has greater resistance to water, giving the film a hydrophobic character and strong mechanical properties. Furthermore, a significant increase in antioxidant activity was observed with increasing extract concentration. Active films with corn stigma extract have great potential for use in food packaging, aiming at the reuse of this agricultural residue in potential value-added applications.

Conflict interest

The authors declare no conflict of interest.

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Legends:

Figure 1. Cytotoxic effect of the corn stigma extract against 3T3 fibroblast cells through the MTT and NRU assay. Values expressed as mean ± SD (n = 3), MTT = 2,5-diphenyl-3,-(4,5-dimethyl-2-thiazolyl) tetrazolium bromide; NRU = Neutral Red Uptake

Figure 2. Shape behavior at initial time (t = 0s) and contact angle kinetics of films as a function of time. GCS: blends films with gelatin-corn starch, CSE: corn stigma extract

Figure 3. Scanning Electron Microscopy (SEM) images of gelatin and corn starch (GCS) films prepared with different concentrations of corn stigma extract (CSE). A – C are images of the fracture surface of the films, and a – c are cross section images of the films.

Figure 4. Biodegradability of gelatin and corn starch (GCS) films added with corn stigma extract (CSE) in plant compost.

Figure 1.

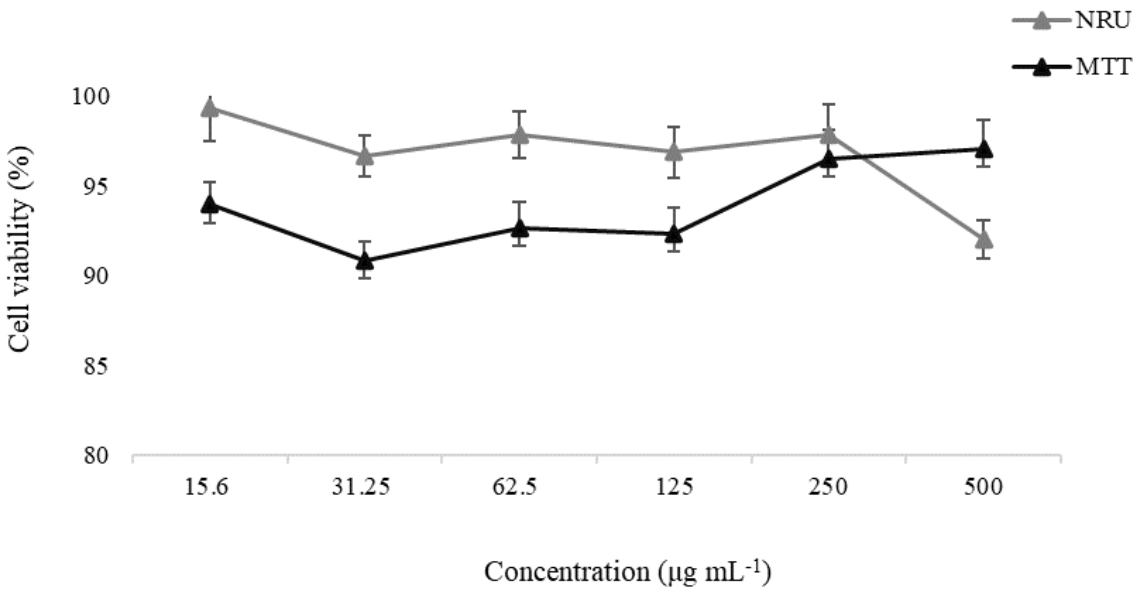

NRU
MTT
100
95
90
85
80
Cell viability (%)
15.6
31.25
62.5
125
250
500
Concentration (µg mL-1)

Fig 2.

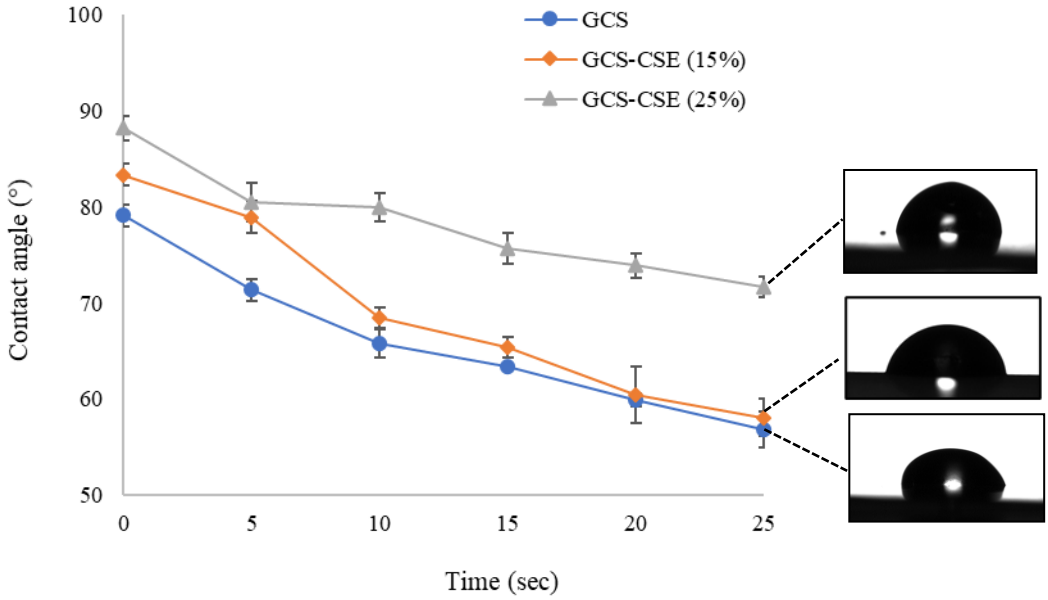
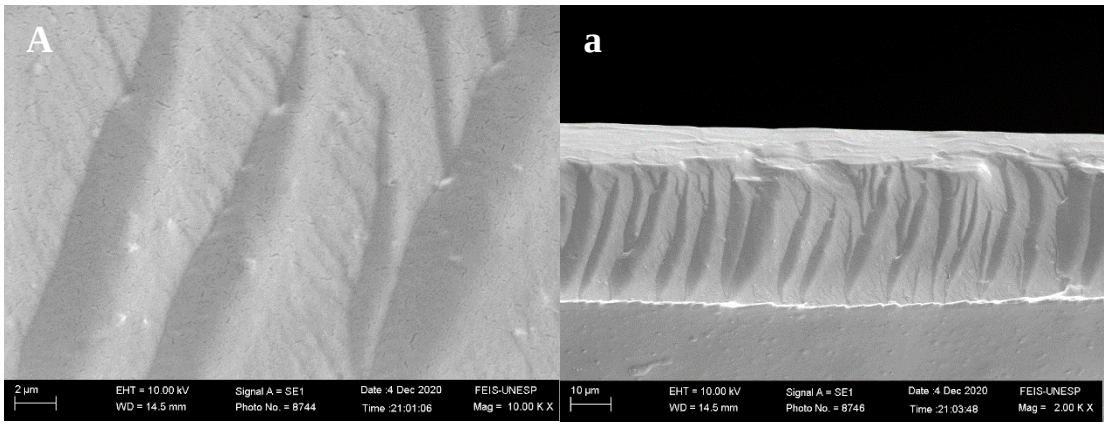
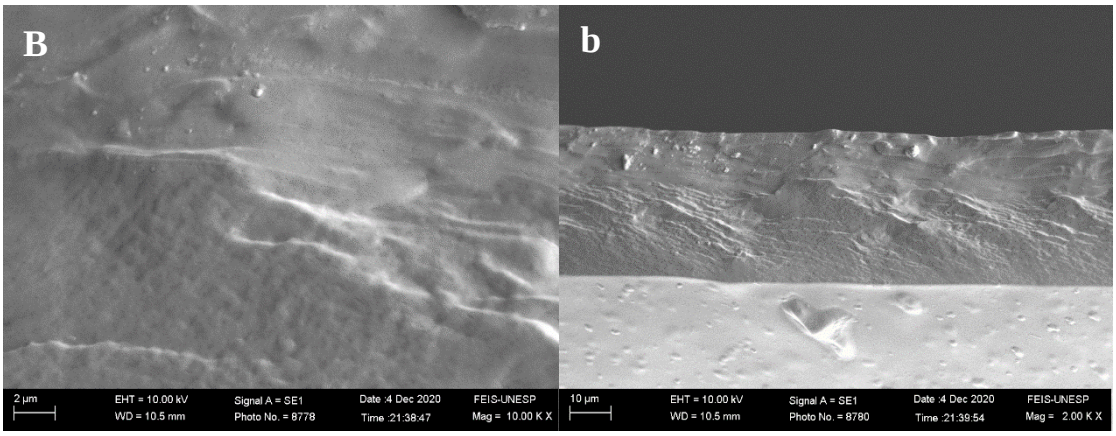

GCS
GCS-CSE (15%)
GCS-CSE (25%)
Contact angle (°)
100
90
80
70
60
50
0
5
10
15
20
25
Time (sec)

Fig. 3

GCS



GCS – CSE (15%)



GCS – CSE (25%)

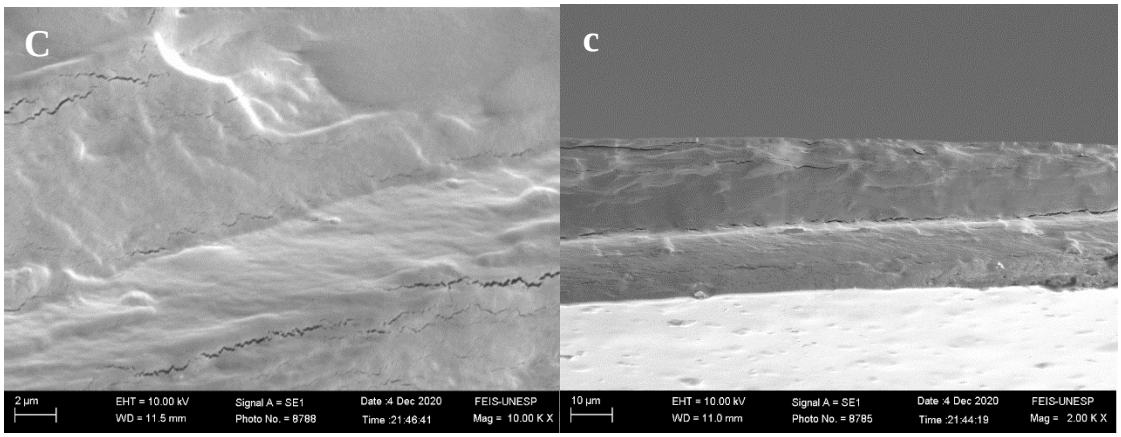


Fig. 4

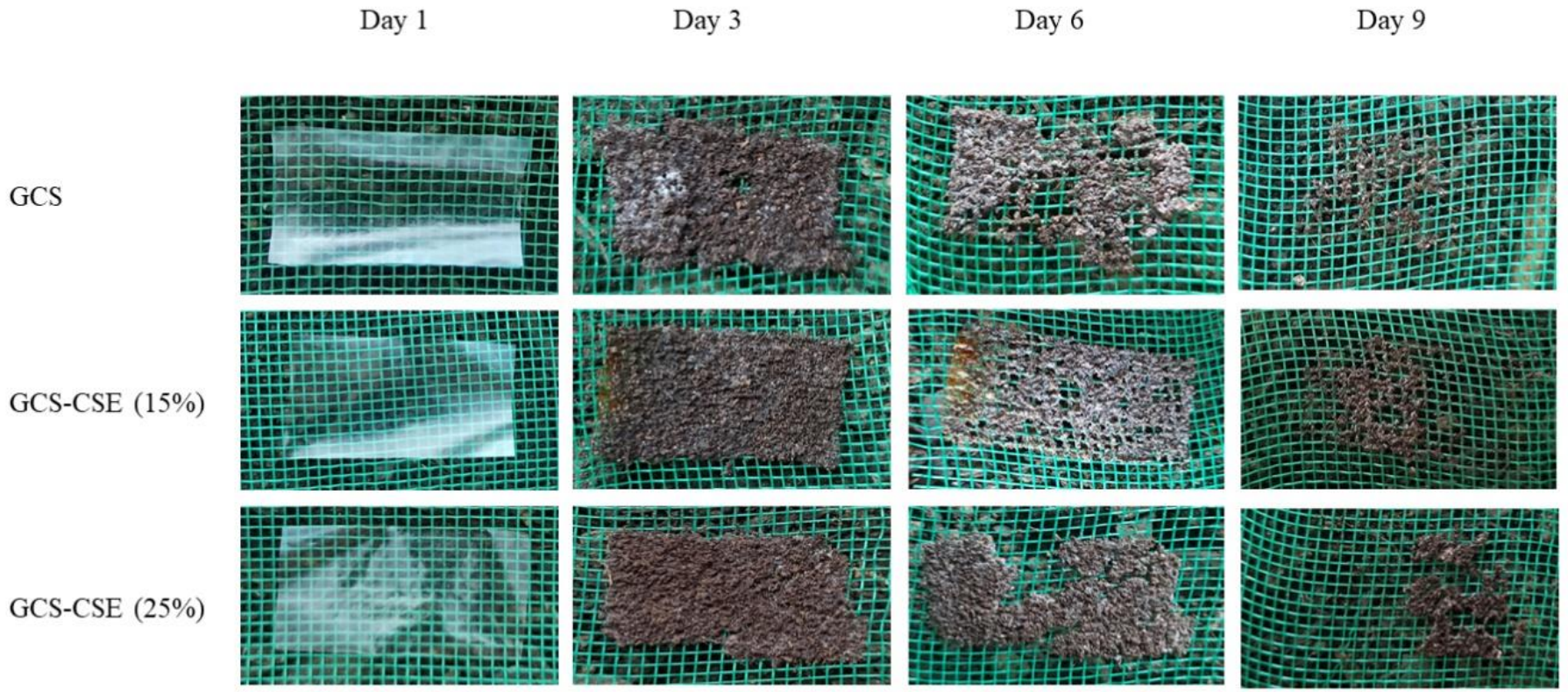
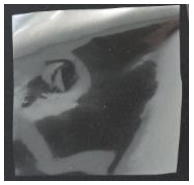


Table 1. Thickness, water vapor permeability, mechanical properties (tensile strength, elongation at break, Young's modulus) and antioxidant activity of gelatin and corn starch (GCS) films and those enriched with corn stigma extract (CSE).

Film Samples	Thickness (mm)	WVP (g mm/kPa h m^2)	Tensile strength (MPa)	Elongation at break (%)	Young's Modulus (MPa)	DPPH (%)
GCS	0.040 ± 0.003^{a}	0.413 ± 0.009^{a}	65.62 ± 7.948^{a}	3.70 ± 0.608^{b}	$2{,}748.78 \pm 63.341^{a}$	35.59 ± 0.856^{c}
GCS-CSE (15%)	0.036 ± 0.004^{a}	0.373 ± 0.023^{ab}	65.39 ± 7.285^{a}	4.21 ± 0.423^{a}	$2{,}675.65 \pm 41.872^{ab}$	89.06 ± 1.54^{b}
GCS-CSE (25%)	0.038 ± 0.002^{a}	0.348 ± 0.013^{b}	60.82 ± 5.279^{b}	4.36 ± 0.494^{a}	$2{,}573.34 \pm 43.754^{b}$	93.06 ± 0.345^{a}

Values represent a mean + standard deviation, n = 9. Different letters in the same column indicate significant differences ($p<0.05$). All films were previously stored at 25 °C and 50% RH for determination of mechanical properties

Table 2. Appearance and color parameters (L *, a *, b * and Δ E *) of gelatin and corn starch films (GCS) and those enriched with corn stigma extract (CSE).

Film Samples		Color values				
	Appearance	L*	a*	b*	ΔE	*Color
GC		90.51 ± 0.384[a]	1.81 ± 0.067[a]	-5.774 ± 0.282[a]	-	
GC-CS (15%)		90.02 ± 0.166[b]	0.912 ± 0.033[b]	-2.744 ± 0.107[b]	3.18 ± 0.011[b]	
GC-CS (25%)		89.80 ± 0.099[b]	0.704 ± 0.048[c]	-2.012 ± 0.203[c]	3.98 ± 0.029[a]	

Values represent a mean + standard deviation, n = 9. Different letters in the same column indicate significant differences ($p<0.05$). *Color was obtained using the following website: https://www.nixsensor.com/freecolor-converter/.

3.3 ARTIGO 3 - ANTIOXIDANT AND ANTIMICROBIAL EFFECT OF AN INNOVATIVE ACTIVE FILM CONTAINING CORN STIGMA RESIDUE EXTRACT FOR REFRIGERATED MEAT CONSERVATION

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ORIGINAL ARTICLE

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Antioxidant and antimicrobial effect of an innovative active film containing corn stigma residue extract for refrigerated meat conservation

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Antioxidant and antimicrobial effect of an innovative active film containing corn stigma residue extract for refrigerated meat conservation

Innovative active film for meat conservation

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Practical applications:

Through this study, was proved the antioxidant and antimicrobial action of corn stigma extract (CSE) incorporated in films to package refrigerated beef. The corn stigma extract demonstrated a strong antioxidant action, reducing lipid oxidation by 60% compared to the control, decreasing the count of mesophilic and psychrotrophic bacteria. The compiled results confirm that the active film containing CSE extract can be used as an alternative packaging.

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Abstract

A bioactive film based on gelatin and corn starch with corn stigma extract (CSE) was developed for application in beef. The physical and functional properties of the films were evaluated. It was found that the incorporation of CSE did not affect the thickness and moisture of the films, but reduced the solubility in water. The opacity of the active films was slightly higher than that of the control film. The incorporation of the CSE extract in the films considerably increased the bioactive and antioxidant properties. The application of CSE reduced lipid oxidation by 60% compared to the control (without additive). It is important to emphasize that the pH values remained low until the seventh day of shelf life. The active film also showed antimicrobial activity against mesophilic and psychrotrophic bacteria. The overall results emphasized the potential use of bioactive compounds from the CSE for the production of films intended for food packaging.

Keywords: Gelatin, Corn starch, Plasticizer, Film, Shelf life, Meat packaging

1. Introduction

The environmental concern with the use of petrochemical-based packaging has spurred new research focused on developing polymers from renewable, biodegradable, and ecological sources (Geyer, Jambeck & Law 2017). Besides that, severals studies have focused on developing biodegradable packaging with active functions. For this, compounds are intentionally added that can act mutually with the packaged food, avoiding the production of undesirable compounds and restricting the growth of pathogens (Bhargava, 2020). These compounds can be nutrients, dyes, essential oils, plant extracts, antioxidants, and antimicrobial compounds. These alternative additives can prolong the product's useful life and decrease the

risk of microbial growth, which is one of the biggest concerns, as they can result in foodborne diseases (Jafarzadeh et al., 2020). However, despite being a scientific innovation, the use of compounds with antioxidant and antimicrobial functions for the elaboration of bioactive films is a little explored area, so further research is necessary (Kalem et al., 2018).

Several materials can be used to produce biodegradable and edible films. Among the natural polymers capable of forming films, starch and gelatin are potential sources due to their low cost, easy availability, renewability and non-toxic nature (Kumar, Ghoshal & Goyal, 2021). Corn starch is a widely produced polysaccharide and gelatin differs from other hydrocolloids as a fully digestible protein, containing almost all essential amino acids in its composition (Fakhouri et al., 2015). These positive conditions make them a useful raw material for the manufacture of films for food packaging (Shah et al., 2016).

Meat and its derivatives are highly perishable products, which generate economic losses of up to 40% for processing (Domínguez et al., 2018). One of the biggest problems related to the quality of meat and its derivatives is lipid oxidation and microbiological growth. When these problems are associated, they can lead to the formation of unpleasant odors and flavors, as well as changes in the color of the product, loss of nutrients, and the formation of toxic compounds harmful to the health of the consumer (Mulla et al., 2017). As a result, the food industry needs to carry out strict hygiene processes and good manufacturing practices and inserting chemical additives to increase the shelf life of these products. However, consumers demand healthier foods, without synthetic additives, as these are well known for their harmful effects on human health (Lorenzo et al., 2018). In this way, interest arises in the discovery of new natural additives, with functions bioactive, which can be used directly in the product or as an additive in packaging, thus increasing the useful life of these foods (Domínguez et al., 2018).

Currently, according to McMillin (2017), there are many commercial packaging options for chilled and processed raw meat to enhance the desired properties for storage and display.

Among the main packaging options are air permeable (overwrap), vacuum and modified atmosphere (MAP) with low or high oxygen levels, and conventional packaging where the trays with the meat are wrapped with polyvinyl chloride (PVC) films or polyvinylidene chloride (PVDC). However, the use of active and biodegradable packaging for preserving and extending the useful life of meat and meat products is little explored, and more extensive research is needed to increase industrial application (Kalem et al., 2018).

Corn stigma (*Zea mays* L.), is an agricultural residue from maize production and is available in large quantities worldwide. It is widely used as a herbal medicine for urological diseases, such as cystitis, kidney diseases such as kidney stones, and nephritis, in addition to other similar diseases (Hasanudin, Hashim & Mustafa 2012; Wang & Zhao 2019). Chemical studies of corn stigma extract (CSE) reveal the presence of bioactive components, such as phenolics, polysaccharides, flavonoids, organic acids, fatty acids, and alkaloids, which promote positive effects on human health, thus enabling it to be used as a potential source of natural antioxidants (Tian et al., 2013; Guo et al., 2018).

Despite the potential use of plant extracts as active ingredients in films (Matta Fakhouri et al., 2019; Chen et al., 2020; Khedri et al., 2020), to date, no information has been found in the literature on the use of CSE as an ingredient in biodegradable films. Thus, the present study aimed to apply a new biodegradable film based on starch and gelatin with antioxidant and antimicrobial properties to delay changes associated with the cold storage of beef. The efficiency of the films was determined by TCP and AA% *in vitro*, as well as the ability to delay lipid oxidation, the growth of aerobic mesophilic and psychrotrophic microorganisms, and the physicochemical characteristics of the pH and water activity of beef during refrigerated storage.

2. Materials and methods

2.1 Materials

Materials used for the production of the films were: gelatin type A (SM Empreendimentos Farmacêuticos LTDA, São Paulo, Brazil), native corn starch supplied by Neilar Indústria e Comércio de Alimentos LTDA (Santa Catarina, Brazil), and glycerol PA (Neon Comercial de Reagentes Analíticos, SP, Brazil). The plant material used in this experiment was purchased from rural producers in Santa Maria, RS (Brazil). The corn stigma was dried in an oven at 45 °C (± 3) until constant weight and after crushed in a Willey knife mill (MA - 680; Marconi Ltda., Piracicaba, Brazil) using a 20 mesh screen. The material was kept at a temperature of -18 ºC in an airtight container and protected from light until the extract is produced. The meat used was the rump steak obtained from the "Nova Estancia" refrigerator in Santa Maria, RS, Brazil, and brought under refrigeration (4 ± 1 ºC) to the laboratory.

2.2 CSE extract production

Corn stigma was added to a 70% hydroethanolic solution as proposed by Jabbar et al. (2014) and the extraction was performed in an ultrasound probe (VC-750; Sonics & Materials, Inc., Newtown, CT, USA) at 20 kHz operating at 70% of the amplitude and 750 W of nominal power, for 5 minutes, at 60 ºC and using solid-solvent ratio of 0.05 g mL^{-1} (Saeeduddin et al., 2015). Afterward, the extract was centrifuged at 202 g for 15 min (Centrifuga Coleman Model 90-1, São Paulo, Brazil), filtered and concentrated by rotary evaporator (Buchi R-210) using a temperature of 45 °C and the supernatant was collected to perform the bioactive analyzes *in vitro* and subsequent application in the film-forming solution.

2.3 Elaboration of the films

The film-forming solution was prepared as proposed by Malherbi et al. (2019). The process flowchart can be seen in Figure 1. After all the steps, 50 mL of the film-forming solution for each treatment was poured onto polyester sheets (21 x 29 cm) and dried in an oven at 25 °C for 24 h. After drying, the films were detached and stored for 48 h in desiccators at 25 ± 3 °C, and 50 ± 3% RH, before applying the films to beef.

Figure 1

2.3.1 Elaboration of the film extract

Preliminary tests were carried out to define the ideal concentration of films as proposed by Zhang et al., (2019). Conditioned films (400 mg) were dissolved in 10 mL of 70% hydroethanolic solution, under magnetic stirring (Biomixer model 78HW-1) at 40 ºC for 10 minutes and then centrifuged at 8000 g for 10 min. The supernatant was used for the analysis of total phenolic content (TPC) and antioxidant activity (AA%).

2.4 Raw materials, processing and packaging

For storage, a quantity of 100 g of rump steaks was packed in polystyrene trays (7,7 x 13,0 x 2,2 cm), coated with two pieces of 150 cm^2 (10 × 15 cm) of the biodegradable film as shown in Figure 2, and then hermetically sealed with glue. The films used in this study included a gelatin and corn starch control film (without the addition of corn stigma extract), a film with 15% CSE and a film with 25% CSE, as proposed by Rasid et al. (2018). Afterward, the samples were stored under refrigeration at 4 °C, to simulate the most real use of meatpacking conditions. Quality attributes were determined on days 1, 3, 5 and 7.

Figure 2

2.5 Analyzes of bioactive activity in vitro

2.5.1 Total phenolic content (TPC)

TPC were estimated using the Folin-Ciocalteu method described by Roesler (2007). Four hundred microliters of samples previously diluted in a ratio of 1:10 (v v^{-1}) were added to 2 mL of 10% (v v^{-1}) of Folin-Ciocalteu reagent and 1.6 mL of 7.5% of sodium bicarbonate solution (w/v) in clean 15 mL centrifuge tubes. After 5 minutes of incubation at 50 °C, the absorbance of each reaction mixture was recorded at 765 nm spectrophotometrically (SP-220 Biospectro brand, São Paulo, Brazil). A standard curve was performed for using gallic acid as a positive control and the results expressed as milligram of gallic acid per gram of sample (mg GAE g^{-1}).

2.5.2 Total flavonoid content (TFC)

The content of total flavonoids was determined by the method proposed by Zhishen, Mengcheng & Janming (1999). To calculate, a calibration curve was performed using quercetin at concentrations of 25 to 200 mg.L^{-1}. The total flavonoid content was expressed in mg quercetin equivalent g^{-1} of sample (mg EQ g^{-1}).

2.5.3 Antioxidant activity by DPPH radical scavenging

The antioxidant activity (AA%) was estimated by the DPPH method (2,2-diphenyl-1-picryl-hydrazyl) was carried out according to Wu et al., (2013). The results were expressed as percentage (%) of inhibition of DPPH radical oxidation according to equation bellow:

$$AA\% = [(A_0 - A_s) \div A_0] \times 100$$

Where A_0 is control absorbance, A_S is the sample absorbance

For the calculation of the IC_{50}, the equation of the line obtained from the absorbance values (AA%) of the increasing concentrations of the samples was used, substituting the value of Y for 50, obtaining the value of X as the sample concentration with capacity to reduce 50% of DPPH. The IC_{50} value will be determined by the equation of the line plotted through the results containing the concentration values (mg mL^{-1}) used on the X axis and the percentages of protection found on the Y axis.

2.5.4 Oxygen radical absorption capacity (ORAC)

The oxygen radical absorption capacity (ORAC) was investigated as proposed by Dávalos, Gómez-Cordovés & Bartolomé (2004). In microplates, 25 μL aliquots of the extracts were mixed with 150 μL of the fluorescein solution (40 nM) and incubated at 37 °C for seven minutes before the addition of 25 μL of the AAPH solution (153 nM). All the reagents were prepared in phosphate buffer (75 mM, pH 7.1). The fluorescence intensity (excitation at 485 nm and emission at 525 nm) was monitored every minute for 120 minutes using a Sinergy Mx microplate reader (BioTeK, Winooski, USA). The standard curve was prepared with Trolox solution (0 to 96 mM) and the results were expressed in μmol equivalent of Trolox per gram of of dry sample (μmol Trolox g^{-1}).

2.6 Characterization and properties of films

2.6.1 Thickness, moisture and water solubility

Five different points of the films were used to obtain the average thickness using a digital micrometer (Mitutoyo Co., Kawasaki, Japan).

The moisture of the films was measured according to the method of Liu et al. (2016). The samples were cut to 4 × 4 cm and left in a desiccator (RH = 50% ± 3% to 25 ± 3% ºC) for 48 hours before analysis. Each film sample was then oven dried at 105 ºC for 24 h until it reached a constant weight (W_0). Weight before drying and weight loss after drying were measured and expressed as a percentage, based on the final weight of the film.

To determine the water solubility, the dry films were solubilized in 50 mL of water (25 °C), under agitation in an orbital shaker (MA-410, Marconi, Brazil) at 70 rpm. After immersion, the solutions were filtered and the undissolved samples were removed and dried again in an oven at 105 °C for 24 hours and weighed to determine the amount of dry matter not solubilized (W_1). Water solubility was calculated according to the equation below:

$$Water\ solubility\ (\%) = \frac{W_0 - W_1}{W_0} \times 100$$

The measurements were carried out in triplicate and the experiment was repeated three times.

2.6.2 Opacity

The opacity of the films was performed according to the procedure of Nur Amila Najwa, Mat Yusoff & Nur Hanani (2020). The films were cut into rectangular pieces (1×5 cm) to fit inside the spectrophotometer cuvette (BEL Photonics, model SF325NM - S05). The absorbance measurement of each film was recorded at 600 nm, and the opacity calculation was as follows:

$$Opacity = \frac{Abs600}{X}$$

where Abs600 is the absorbance value at 600 nm and X was the thickness of the film (mm).

2.6.3 Fourier transformation infrared spectroscopy (FTIR)

FTIR spectroscopy of the films was collected by a Nicolet Nexus 670 spectrometer (Nicolet Instrument Corporation, USA). The samples were ground with potassium bromide (KBr) and pressed to obtain pellets. All film samples were analyzed between 4000 and 400 cm^{-1} wavenumbers. at the spectra were collected at 4 cm^{-1} resolution and total 128 consecutive scans were recorded.

2.7 Analysis of rump steak during the the shelf life

2.7.1 Oxidative stability

Lipid stability was assessed by determining the values of substances reactive to thiobarbituric acid (TBARS) as proposed by Bruna et al. (2001). TBARS values were determined on storage days 1, 3, 5 and 7, and the results were expressed as “mg malonaldehyde per kg of sample” (mg MDA/kg).

2.7.2 pH and a

The pH was measured in a potentiometer (Marconi – MA-522), duly calibrated with solutions of pH 4.0 and pH 7.0. (AOAC, 2005). The determination of (a_w) was performed using an Aqualab digital device (Decagon, Pullman, WA, USA), at 25ºC.

2.7.3 Microbiological analysis

Microbiological analyzes were performed to check the stability of the rump steaks packed with the bioactive film. The counting analyzes of mesophilic and psychrotrophic aerobes were monitored using plate counting agar medium (PCA, Merck, Darmstadt,

Germany), using the counting technique in the plate, with incubation at 37 °C for 48 hours for mesophilic aerobes, and 7 °C for 7 days for psychotrophics (APHA, 2001).

2.8 Statistical analysis

The experiment was carried out in triplicate. The experimental data were submitted to analysis of variance (ANOVA) and the means were compared using the Tukey test through the statistical software STATISTICA® 10.0 (StatSoft, Inc., Tulsa, OK 74104, EUA), with a 95% significance level.

3. Results and discussion

3.1 Phytochemical contents in vitro of CSE

The *in vitro* phytochemical contents of the CSE was carried out by evaluating the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (Table 1).

Table 1

The results of TPC (66.13 mg GAE g^{-1} CSE) and TFC (7.69 mg QE g^{-1} CSE) obtained for CSE are in agreement with those obtained by Tian et al. (2013), and Žilić et al. (2016) for corn stigma. They reported a TPC content in the range from 36.74 to 101.60 mg GAE g^{-1}, and a TFC content within the range from 1.73 to 4.41 mg QE g^{-1}. Our results in terms of flavonoid content (7.69 mg QE g^{-1}) are higher than those reported by Tian et al. (2013). The observed difference can occur for several reasons, such as the extraction parameters used and the influence of genetic and environmental variability (Horincar et al., 2020).

The bioactive activity observed in this study may be related to the chemical characterization of the extract, as shown by studies by Wang et al. (2019). When extracting

bioactive components from the aqueous extract of the corn stigma and identified the presence of 76 compounds, including caffeic acid and ten of its derivatives, (E)-p-coumaric acid and two of its derivatives, ferulic acid and four of its derivatives and five flavones. In addition, another 12 metabolites are also described including: protocatechuic acid, vanillic aldehyde, vanillic acid, salicylic acid, caffeic acid, ferulic acid, luteolin and apigenin. It is worth mentioning that these phytochemicals exhibit various pharmacological activities, such as anticancer, antidiabetic, anti-obesity, anti-inflammatory, anti-edema, antimicrobials, antihepatotoxic, antioxidants, among other effects (Silva et al. 2018; Yin et al. 2019; Pernin et al. 2019). Likewise, El-Ghorab, El-Massry & Shibamoto (2007) investigated the volatile dichloromethane extract of Egyptian corn stigma by gas chromatography-mass spectrometry (GC-MS) and a total of 36 compounds were reported, the main constituents being werecis-α-terpinol (24.22%), citronellol (16.18%), 6,11-oxidoacor-4-ene (18.06%), trans-pinocamphone (5.86%), eugenol (4.37%), neo-iso-3-thujanol (2.59%) and cis-sabinene hydrate (2.28%). The extract also contained cinnamic derivatives, glucose, rhamnose and rich in minerals, including sodium (0.05%), potassium (15%), iron (0.0082%), zinc (0.016%) and chloride (0.25%) (Hasanudin, Hashim & Mustafa 2012).

The CSE inhibited the absorption of DPPH with a value of 91% (50 mg mL^{-1}) and had an IC_{50} of 1.93 mg mL^{-1}. These results indicate the presence of antioxidant molecules, since this technique consists of the elimination of the radical through the donation of hydrogen. These antioxidants donate hydrogen to free radicals, leading to non-toxic species and, therefore, inhibiting the propagation phase of lipid oxidation (Ordonez et al., 2006). The IC_{50} indicates the concentration of the sample needed to reduce the DPPH radical by 50%. The lower the IC_{50} value, the greater the antioxidant activity of the sample. The result is in accordance with the studies by Tian et al. (2013), in which the ethanolic extract of corn stigma inhibited DPPH activity with an IC_{50} of 1.84 mg mL^{-1}. The DPPH assay is convenient in its application,

however, it is limited because it uses a non-physiological radical. In contrast, the ORAC assay detects chemical change in a fluorescent molecule caused by a free radical attack. It is considered the most relevant method, as it uses peroxyl radicals that are a source of biologically relevant radical (Radošević et al., 2016). As far as we know, there are no data in the literature on the oxygen radical absorption capacity (ORAC) of corn stigma extracts obtained by ultrasound-assisted extraction. However, the result obtained in this study (123.84 μmol Trolox g^{-1}) is superior to that found by Saikaew et al. (2018) in waxy purple corn grains (31.67-48.33 μmol Trolox g^{-1}.). Thus, the CSE represents an important source of bioactive compounds and little data on their use as an active ingredient in the food industry are available, demonstrating the importance of new studies that value this agro-industrial residue.

3.2. Thickness, moisture and water solubility of the elaborated films

Results of thickness, moisture, and water solubility of gelatin and corn starch films incorporated with corn stigma extract (CSE) or not (Control) are shown in Table 2.

Table 2

The thickness of the films ranged from 0.036 mm to 0.040 mm, thus demonstrating that the incorporation of the CSE did not influence in this parameter ($p<0.05$). Likewise, there was no statistical difference between films for moisture. However, the addition of 15 and 25% CSE, in the gelatin and corn starch film, reduced the solubility by 57 and 65%, respectively.

These data are extremely important, since these polymers are highly hygroscopic, thus demonstrating that the extract enabled interactions, forming a less soluble network. A strong hydrogen bond can keep gelatin and corn starch together with the water molecule together to increase the diffusivity coefficient, resulting in an excellent water barrier property (Carvalho, Grosso & Sobral 2008). This behavior probably occurred due to the formation of a more

cohesive matrix, which made solubilization more difficult. Thus, as more CSE was incorporated into the films, the solubility rate decreased significantly ($p<0.05$). This stipulated that the addition of CSE helped to reduce the water absorption rate of the films. These results demonstrate an important advance for the application of films from natural sources such as food packaging, since most films based on proteins and carbohydrates have in their structure a strong interaction with water, which increases the solubility of the material. and makes it difficult to apply at an industrial level.

3.3 Film Opacity

The opacity of the film is an important optical property, especially if the films will be used as a food coating. As can be seen in Table 2, the film with the addition of corn stigma extract showed a slight increase in opacity compared to the control, differing statistically ($p <0.05$). According to Atar´es, Bonilla & Chiralt (2010), the addition of extracts affects the optical properties depending on the concentration used. These results are in line with those observed by Nur Hanani et al. (2018), according to which the addition of fruit peel extract increases the opacity of the gelatin/polyethylene bilayer films. This may be due to the light scattering effect, as supported by Nur Amila Najwa, Mat Yusoff & Nur Hanani (2020). However, it is important to note that the values for opacity of the elaborated films were low, indicating transparent films (Fig. 2), which is especially desirable for food packaging applications, as it affects the acceptance of the product by the consumer.

3.4 FTIR analysis

FTIR analysis is applied to elucidate the functional groups of composite films and obtain information about the molecular state of polymers by showing the interaction between their components (Hamdi et al., 2018). The FTIR pattern of gelatin and corn starch films made with

and without corn stigma extract (CSE) are shown in Fig.3. FTIR spectra indicate that all films showed similar major peaks (Wu et al., 2019).

Figure 3

The spectra of all films showed the main bands at 1640 cm^{-1} attributed to amide-I (C=O stretching/hydrogen bonding coupled with COO), and CSE (C=O stretching) and deformations of the aromatic ring due to the presence of flavonoids and amino acids; 1558 cm^{-1} (amide-II, due to the bending vibration of N-H groups and stretching vibrations of groups C-N and CC); and 1240 cm^{-1} (amide-III, representing the vibrations in the plane of groups C-N and N-H of bound amide and OH) (Hoque, Benjakul & Prodpran 2011). Peaks at 2920 cm^{-1} and 2850 cm^{-1} are attributed to the occurrence of proteins (Hamdi et al., 2018).

The characteristic peaks shifted to lower frequencies with increased extract concentration, which may be due to some interaction between functional groups of gelatin and corn starch and CSE (Balti et al., 2017). As expected, all characteristic peaks of gelatin and corn starch were observed in the FTIR spectra of the films with 15 and 25% CSE. The peak located around 1033 cm^{-1} may be related to the interactions that arise between the glycerol added as a plasticizer (OH group of the glycerol) and the structure of the film and COC (of the corn stigma extract - polysaccharides and nucleic acids) (Tongnuanchan, Benjakul & Prodpran 2012).

The elongation peak at 1640 cm^{-1} also suggests the presence of groups C=C due to deformations of the aromatic ring that can show the existence of flavonoids and amino acids: stretching vibration of C=O and C=C, flexion vibration asymmetric N-H, due to C=O elongation vibration of caffeic acid and its derivatives and due to the C=O elongation vibration of lipids and flavonoids (Oliveira et al., 2016). The band at 1462 cm^{-1} could be related to CH_3, CH_2, flavonoids and aromatic rings, where the vibrations would be the C-H flexion vibration and the aromatic stretching vibration (Chahardoli, Karimi & Fattahi 2018).

The characteristic absorption bands at 3425 cm^{-1} related to the OH balance are characteristic of phenolic compounds. In addition, a higher vibration intensity around 3500 cm^{-1} indicates N-H stretching vibration, which may be due to the overlapping of the O-H connection (Chahardoli, Karimi & Fattahi 2018). The bands assigned around 2850 and 2925 cm^{-1} are related to the stretching vibrations C-H ($–CH_3$ or $–CH_2–$) of the methyl and methoxy groups. The presence of alcoholic and phenolic hydroxyl groups can also be observed in the strong band in this region (Azadfar, et al., 2015).

From the FTIR, it is possible to verify that with the addition of the corn stigma extract (CSE), the phenolic and flavonoid compounds present in its structure can form hydrogen bonds and covalent bonds and, thus, occupy the functional group of gelatin and corn starch, subsequently decreasing the free hydrogen group that can form a hydrophilic bond with water (Siripatrawan & Harte 2010). As a result, the improved properties of water solubility (Tab. 2) and antioxidant activity (Fig. 4) were observed in the gelatin and corn starch films incorporated with CSE.

3.5 Total phenolic content (TPC) and antioxidant activity (AA%) of films

Phenolic compounds are important bioactive substances that function as reducing agents, hydrogenators and have antioxidant properties due to their high redox potential (Benbettaïeb et al., 2020). The TPC of the films and AA% was shown in Figure 4.

Figure 4

The total phenolic content of the film without additive (control film) was 4.33 mg $GAEg^{-1}$ film (Fig. 4.A.) Similarly, for antioxidant activity, which measured the ability of DPPH (2,2-diphenyl-1-picryl-hydrazyl) to eliminate free radicals, the control film showed 35% elimination (Fig. 4.B.). The control film exhibited a small phenolic content and intrinsic

antioxidant activity, which can be explained by the NH2 and OH groups of corn starch and gelatin that are involved in the elimination of radicals. Besides, the gelatine's bioactivity is related to its peptide fractions. These results are confirmed with the data obtained by the FTIR analysis (Fig. 3.) of this study, which suggests the presence of amino acids in the structure of the films, which are known to donate electrons and/or protons, thus showing the ability to scavenging free radicals (Benbettaïeb et al., 2020).

However, the TPC and AA% increased with the increasing concentration of CSE, differing statistically from the control ($p<0.05$). This is related to the chemical structure of the biomolecules of the CSE, due to the number and arrangement of hydroxyl groups, which are effective as hydrogen donors as well as the presence of electron donors. In addition, the high antioxidant activity of these biomolecules can be seen through the reaction with the stable free radical DPPH, leading to its reduced form (DPPH-H). An effective antioxidant can be seen when it reacts quickly leaving small amounts of unreduced DPPH (Souza et al., 2004).

When the CSE concentration was 25% in the films, the total phenolic content was up to 11.61 mg of GAE g^{-1} and the antioxidant activity was 93%. The same was observed by Wu et al. (2013), adding green tea extract also increased the bioactivity of the films. The results demonstrate that the incorporation of the CSE considerably increased the bioactive and antioxidant properties of the films.

3.6 The effect of adding CSE on films to protect the quality of chilled beef

3.6.1 Oxidative stability

One of the main changes that compromise the quality and acceptability of meat and meat products is lipid oxidation. The thiobarbituric acid reactive substance (TBARS) is often used to define the degree of lipid oxidation in these products. The TBARS values for the rump steaks samples packed with a bioactive film containing corn stigma extract were shown in Table 3.

Table 3

At the beginning of storage, the values of substances reactive to thiobarbituric acid (TBARS) for all samples was about 0.05 (mg MDA/kg) of meat. After 7 days, the values gradually increased. This increase can be seen in all samples, although it was smaller in the samples with active film compared to the control. On the third day of cold storage, TBARS values in the control sample were 41% higher (0.169 mg MDA/kg) compared to the group with 25% CSE (0.07 mg MDA/kg). This reduction in lipid oxidation was also observed by Moudache et al. (2017) in chilled pork packed with films containing 15% olive leaf extract.

It can be observed that the treatments with active extract of CSE (15% and 25%), the lipid oxidation during the shelf life was significantly ($p < 0.05$) lower compared to the control group. This demonstrates that the addition of CSE as an active ingredient in the film was sufficient to maintain the conservation of the rump steaks, being a promising way to extend the shelf life of this product for up to 7 days, which represents an important advantage in relation to conventional packaging (PVC and PVDC). On the seventh day of storage, TBARS values from treatment with 25% CSE were approximately 60% lower than the control.

Campo et al. (2006), reported a TBARS value of 2 mg malondialdehyde (MDA) per kg can be considered as a limit for the perception of oxidation flavor in beef, which is also emphasized in the studies by Zhan et al. (2019). As can be seen, none of the studied groups exceeded the acceptable limit described in the literature for lipid oxidation during the 7 days of storage. However, rump steaks packed with films containing 15% and 25% CSE showed the lowest values for substances reactive to thiobarbituric acid (TBARS), demonstrating the antioxidant effect of CSE on the lipid stability of meat.

This reducing effect on lipid oxidation demonstrated by the extract in meat samples may be associated with the presence of bioactive compounds in CSE. This is also proven in the *in vitro* tests of TPC and AA% (Fig. 4.). Chemical studies described in the literature revealed that

corn stigma has antioxidant properties due to the presence of volatile oils, steroids, alkaloids, flavonoids, organic acids and other phenolic compounds (Hasanudin, Hashim & Mustafa, 2012; Wang & Zhao, 2019). These compounds are known for their ability to reduce free radicals, delaying oxidation at different levels (Boeira, et al., 2020).

3.6.2 Physicochemical characterization of meat

One of the parameters related to the microbiological growth in meat and meat products is the water activity (a_w) since this represents the availability of water in the food. The a_w results for the rump steaks can be found in Table 3. During storage, there was no significant difference ($p<0.05$) between the observed values, which were from 0.97 to 0.98. However, it can be seen that there was a trend of decreasing values. Boeira et al. (2020) report that during storage, events of mass transfer in counterflow occur at the same time. This can be explained by the natural process of moisture exchange, which occurs from the inside of the meat. These results corroborate with Li et al. (2017), who observed a drop in water activity values at the end of storage in ground meat.

The pH results (Table 3) ranged from 5.54 to 5.86. These results are following the literature for beef (Caetano, et al., 2017). It is noticed that only on the first day of cold storage there was no significant difference between treatments ($p<0.05$). Afterward, the pH values of the meat increased progressively, however, the treatments with 15 and 25% of CSE maintained the lowest pH values. Afterward, the pH values of the meat increased progressively. According to Kaewprachu et al. (2015) microbiological growth in meat may be associated with the production of enzymatic actions by microorganisms, favoring the accumulation of alkaline compounds and consequently increasing the pH. However, the treatments with 15 and 25% of CSE maintained the lowest pH values. This is consistent with the lowest microbial count in samples packed with CSE. At the end of the seventh day of storage, the groups with active

CSE showed lower pH values, being 5.61 for films containing 25% CSE, and 5.72 for the treatment with 15% CSE. The control treatment (without the addition of extract), obtained values significantly ($p<0.05$) higher than the others, this being 5.86. Likewise, Azarifar et al. (2020) reported that the pH value of the meat sample wrapped with gelatin films incorporated with chitin nanofiber and essential oil of *Trachyspermum ammi* (6.12) was lower than that of the unpackaged sample (6.7) at 4 ° C after 12 days of store. Kuswandi & Nurfawaidi (2017), when evaluating the use of sensors in packaging for real-time monitoring of meat freshness, observed that for refrigerated meat, the onset of deterioration is detected on the seventh day. The results further demonstrate that normally deterioration of meat occurs at high pH (>6.0) than at normal pH (<5.8) of fresh meat. Thus, it is possible to conclude that biodegradable films containing antioxidant and/or antimicrobial compounds, control the increase in pH values in beef throughout its storage due to the decrease in microbial enzymatic activity by the bioactive activity of the films (Amjadi et al., 2020).

3.6.3 Microbiological analysis

To check the antimicrobial effect of the CSE on the films, counts of aerobic mesophilic and psychrotrophic microorganisms were performed on rump steaks (Figure 5). The initial counts of mesophiles (Fig. 5.A.) and psychrotrophic microorganisms (Fig. 5.B.) were 2.7 and 1.1 log UFC g^{-1}, respectively. There was no significant difference between treatments for the first day of cold storage ($p< 0.05$). From the third day on, the film-packed meat containing 25% CSE differed statistically ($p<0.05$) from the control group, thus demonstrating its ability to inhibit the multiplication of microorganisms. On the last day of storage, a reduction of 1.2 log UFC g^{-1} was observed in the growth of psychrotrophic in film-packed meat containing 25% CSE compared to the control. Similarly, Merlo et al. (2019) when applying pink pepper residue

extract in chitosan film to store salmon fillets, they observed effectiveness in maintaining the quality of the samples during cold storage, reducing microbiological count and lipid oxidation.

Figure 5

In chilled conditions, the bacteria that develop in the meat are predominantly psychrotrophic (Khan et al., 2016). This explains the higher final counts of psychrotrophic in meat samples compared to mesophiles. The maximum level of microbial count recommended by the International Commission for Microbiological Specification for Food (ICMSF) for raw meat in natura (ICMSF, 1986) is 7 log UFC g^{-1}, and when reaching this limit, the microbiological useful life must be considered closed. Behbahani, Noshad & Jooyandeh (2020), when studying the use of Shahri Balangu seed mucilage loaded with cumin essential oil as a bioactive edible coating on beef, observed that the control group (uncoated meat sample) on the third day was unsuitable for consumption, exceeding the maximum acceptable limits of microbiological growth. The results compiled in the present study and in the literature prove that beef in natura has a reduced useful life, as it is a product susceptible to contamination. For this reason, meats sold commercially are not exposed for more than 3 days to the consumer. Thus, it is possible to verify that the bioactive film developed has considerably increased the useful life of the rump steaks, since until the seventh day they have not exceeded the maximum limits of microbiological growth.

The reducing effect of the microbiological growth of CSE observed in rump steaks may be related to its phytochemical composition, according to the data obtained in this study in the *in vitro* analyzes of CSE phytochemicals, such as: phenolic compounds, flavonoids, alkaloids and organic acids, which are known to act as strong antimicrobial agents (Wang, et al., 2019; Arribas, et al., 2019; Boeira et al., 2020). The active film based on gelatin and corn starch incorporated with different concentrations of corn stigma extract could, therefore, suppress microbial growth in meat and, in turn, improve its refrigeration life.

4. Conclusion

In this study, films based on gelatin and corn starch incorporated with corn stigma extract were considered effective against lipid oxidation and microbiological growth in beef. The total phenolic content and antioxidant activity of films incorporated with CSE increased considerably. It was found that the incorporation of CSE did not affect the thickness and moisture of the films, but reduced the water solubility. The opacity of the active films was slightly higher than that of the control film. Possible interaction between the polymer matrix and the corn stigma extract was confirmed by Fourier transformation infrared spectroscopy (FTIR) analysis. The pH levels and TBARS values decreased with the increase in the amount of CSE incorporated in the films, and the use of 25% of CSE allowed a 60% reduction in lipid oxidation. For mesophilic and psychrotrophic microorganisms, the active film differed from the control film, contributing to the reduction of these microorganisms after 3 days of storage. The use of plant waste extracts as an active ingredient is an intelligent and sustainable way of valuing these agricultural by-products. Thus, CSE is considered a promising alternative for incorporating antioxidant and antimicrobial activity into food packaging and improving the shelf life of beef. Future studies should focus on the extension of the storage time of the meat, seeking in this way to elucidate all the interactions related to the application of the CSE in the packaging systems and to enhance its commercial use.

Conflict interest

The authors declare no conflict of interest.

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Table 1. Bioactive compounds (total phenolic content (TPC), total flavonoid content (TFC)) and antioxidant activities (2,2-diphenyl-1-picryl-hydrazyl (DPPH) and oxygen radical absorption capacity (ORAC)) for corn stigma extract (CSE).

Bioactive Compounds	
Total phenolic content (mg GAE g^{-1}).	66.13 ± 1.23
Total flavonoid content (mg QE g^{-1})	7.64 ± 0.33
Antioxidant Activity	
DPPH (%)	91.24 ± 0.20
IC_{50} (mg mL^{-1})	1.93 ± 1.06
ORAC (μmol Trolox g^{-1})	123.84 ± 1.10

Results are expressed as Mean ± SD (n=3). GAE = Gallic acid equivalent. QE: Quercetin equivalent.

Table 2. Thickness, water solubility, moisture and opacity of gelatin and corn starch films with and without corn stigma extract (CSE).

Film Samples	Thickness (mm)	Water Solubility (%)	Moisture (%)	Opacity
Control	0.040 ± 0.003^{a}	69.469 ± 1.966^{a}	7.578 ± 0.644^{a}	1.77 ± 0.10^{b}
15% CSE	0.036 ± 0.004^{a}	29.273 ± 1.183^{b}	7.735 ± 0.416^{a}	2.72 ± 0.13^{a}
25% CSE	0.038 ± 0.002^{a}	23.732 ± 0.904^{c}	7.973 ± 0.368^{a}	3.00 ± 0.14^{a}

Values are expressed as the mean ± SD (n=9). $^{a\text{-}c}$ Different letters in the same column indicate a significant difference ($p<0.05$). Control: without additive, CSE: corn stigma extract

Table 3. TBARS, aw and pH values in beef packed with active films during cold storage.

Parameters	Samples	Days of storage			
		1	3	5	7
TBARS	Control	0.051 ± 0.08^{aD}	0.169 ± 0.04^{aC}	0.392 ± 0.02^{aB}	0.482 ± 0.03^{aA}
	15% CSE	0.051 ± 0.02^{aB}	0.091 ± 0.01^{bB}	0.268 ± 0.03^{bA}	0.316 ± 0.02^{bA}
	25% CSE	0.049 ± 0.03^{aB}	0.079 ± 0.02^{bB}	0.233 ± 0.04^{bA}	0.287 ± 0.04^{bA}
a_w	Control	0.9838 ± 0.01^{aA}	0.9866 ± 0.00^{aA}	0.9850 ± 0.01^{aA}	0.9828 ± 0.00^{aA}
	15% CSE	0.9966 ± 0.00^{aA}	0.9965 ± 0.00^{aA}	0.9927 ± 0.00^{aAB}	0.9878 ± 0.01^{aB}
	25% CSE	0.9953 ± 0.01^{aA}	0.9916 ± 0.01^{aA}	0.9919 ± 0.00^{aA}	0.9885 ± 0.01^{aA}
pH	Control	5.63 ± 0.02^{aC}	5.69 ± 0.05^{aBC}	5.75 ± 0.04^{aB}	5.86 ± 0.04^{aA}
	15% CSE	5.62 ± 0.01^{aB}	5.6 ± 0.02^{abB}	5.67 ± 0.03^{aAB}	5.72 ± 0.04^{bA}
	25% CSE	5.63 ± 0.02^{aA}	5.54 ± 0.06^{bA}	5.57 ± 0.03^{bA}	5.61 ± 0.02^{cA}

Values are expressed as the mean ± SD (n=9). $^{a\text{-}c}$ Different letters in the same column indicate a significant difference ($p<0.05$). $^{A–D}$ Different letters in the same row presented indicate significant different ($p< 0.05$). Control: without additive, CSE: corn stigma extract

Figure legends

Figure 1. Simplified flowchart for the preparation of gelatin and corn starch films with the addition of corn stigma extract (CSE).

Figure 2. Films based on gelatin and corn starch incorporated with corn stigma extract (CSE) as packaging for beef. Control: without additives; CSE: corn stigma extract.

Figure 3. Fourier transformation infrared spectroscopy (FTIR) of gelatin and corn starch based films incorporated with corn stigma extract (CSE). Control: without additives; CSE: corn stigma extract.

Figure 4. Total phenolic content (A) and antioxidant activity (B) of corn stigma extract (CSE) in gelatin and corn starch films. Standard errors are represented by the error bars of the analyses performed in triplicate (n=9). $^{a\text{-}c}$ Different letters in the same column indicate a significant difference ($p<0.05$). Control: without additives; CSE: corn stigma extract

Figure 5. Counting of aerobic mesophilic (A) and psychrotrophic (b) microorganisms in rump steaks packed with active films. Standard errors are represented by the error bars of the analyses performed in triplicate (n=9). Control: without additives; CSE: corn stigma extract

Figure 1. Simplified flowchart for the preparation of gelatin and corn starch films with the addition of corn stigma extract (CSE).

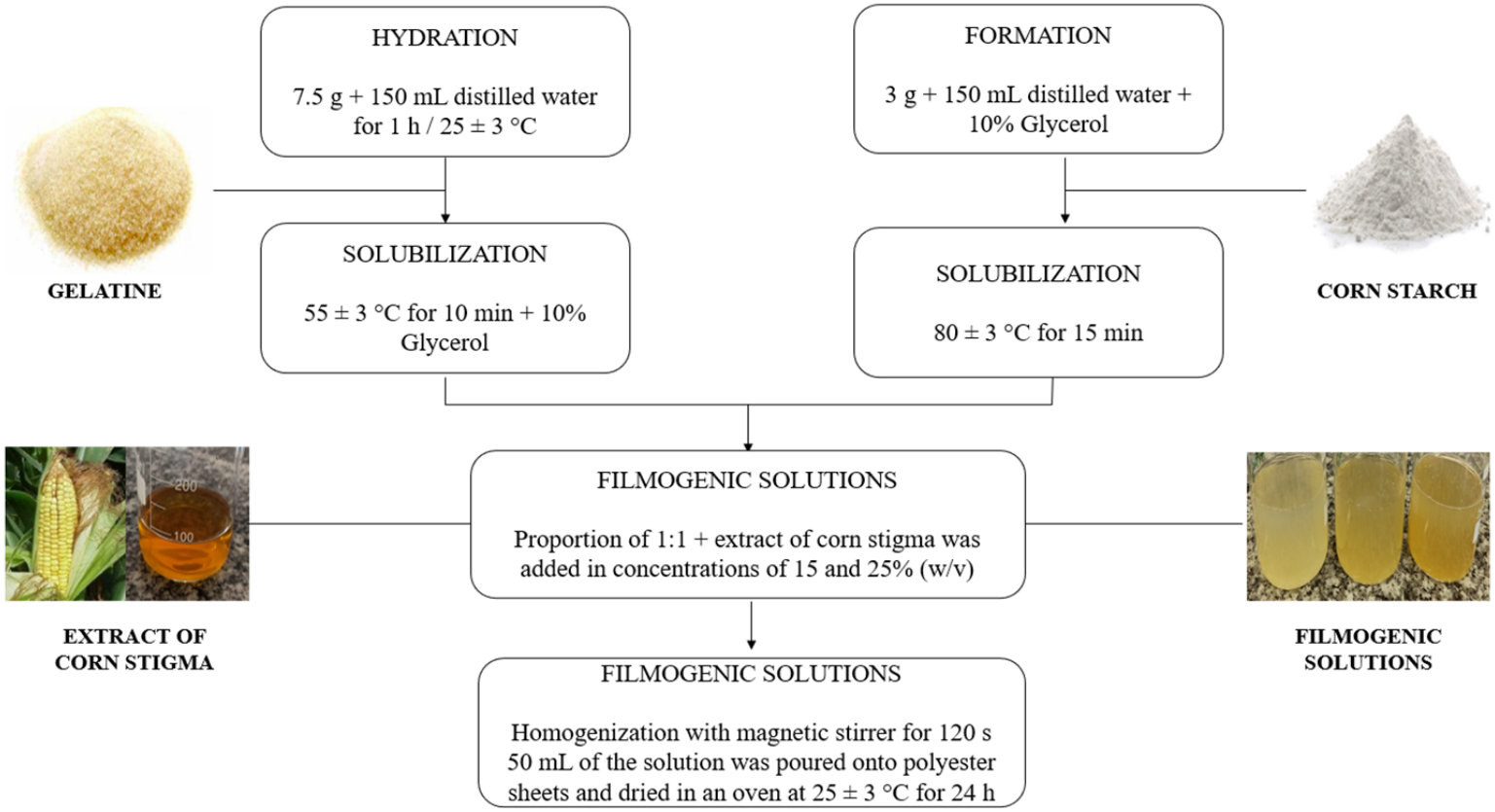
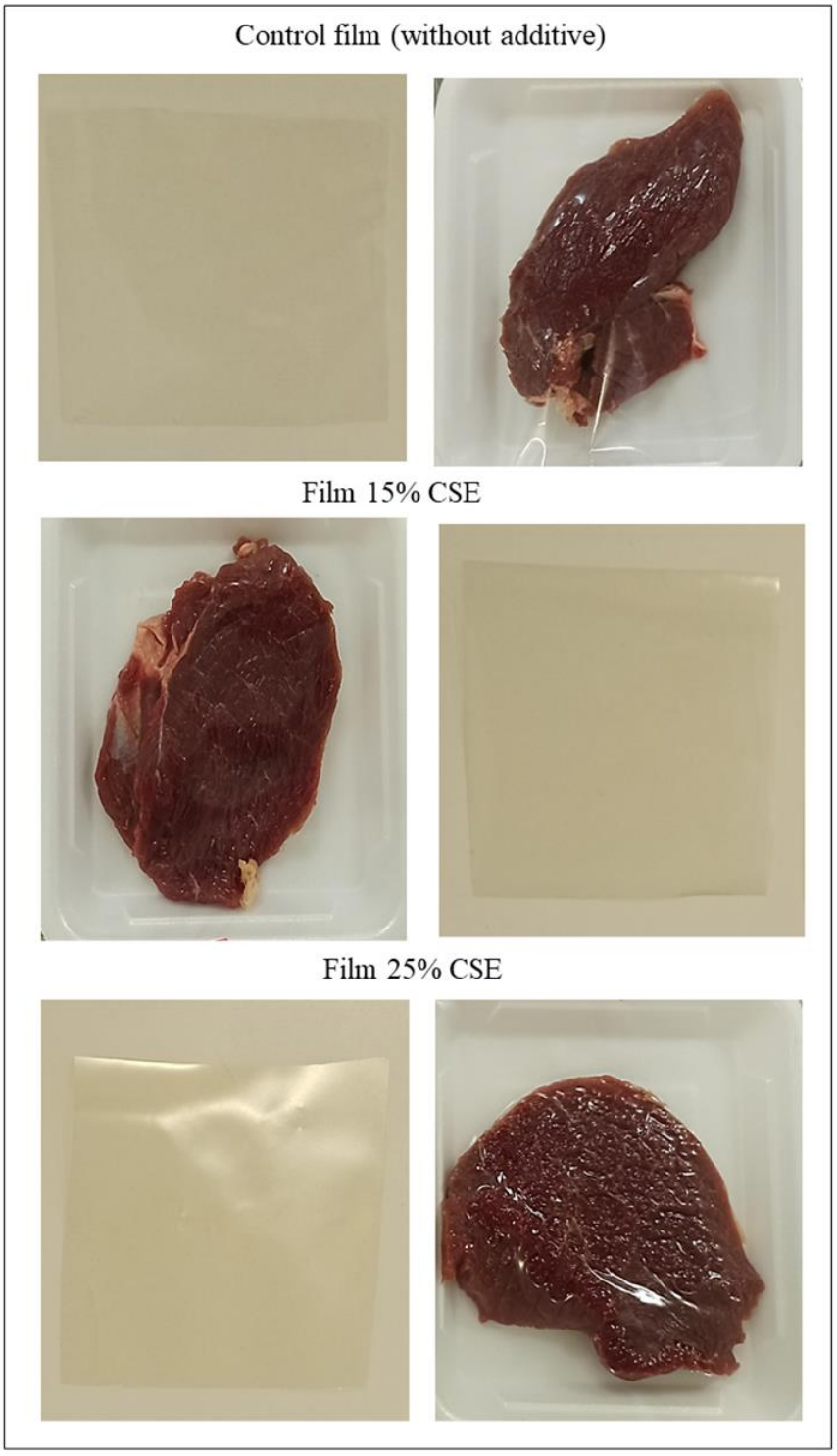
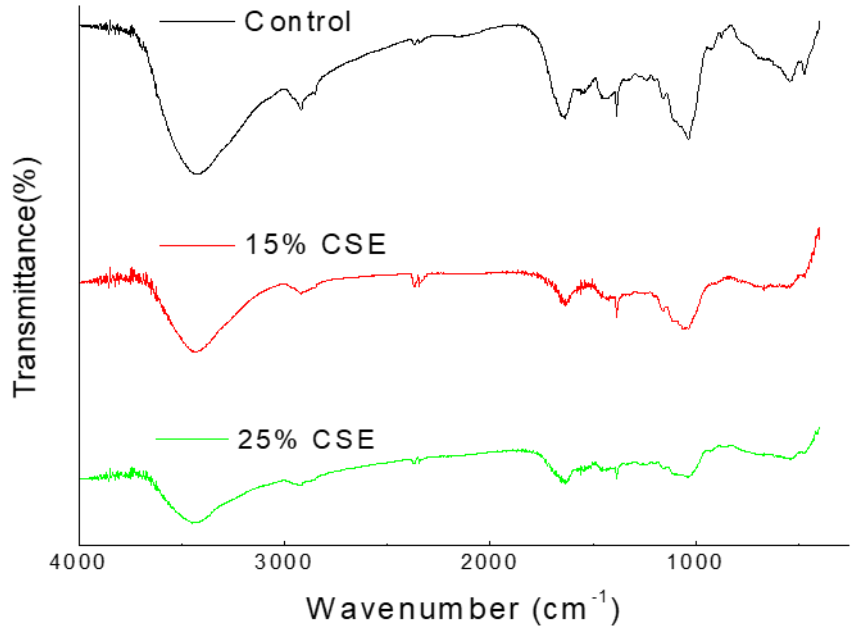


Figure 2. Films based on gelatin and corn starch incorporated with corn stigma extract (CSE) as packaging for beef.



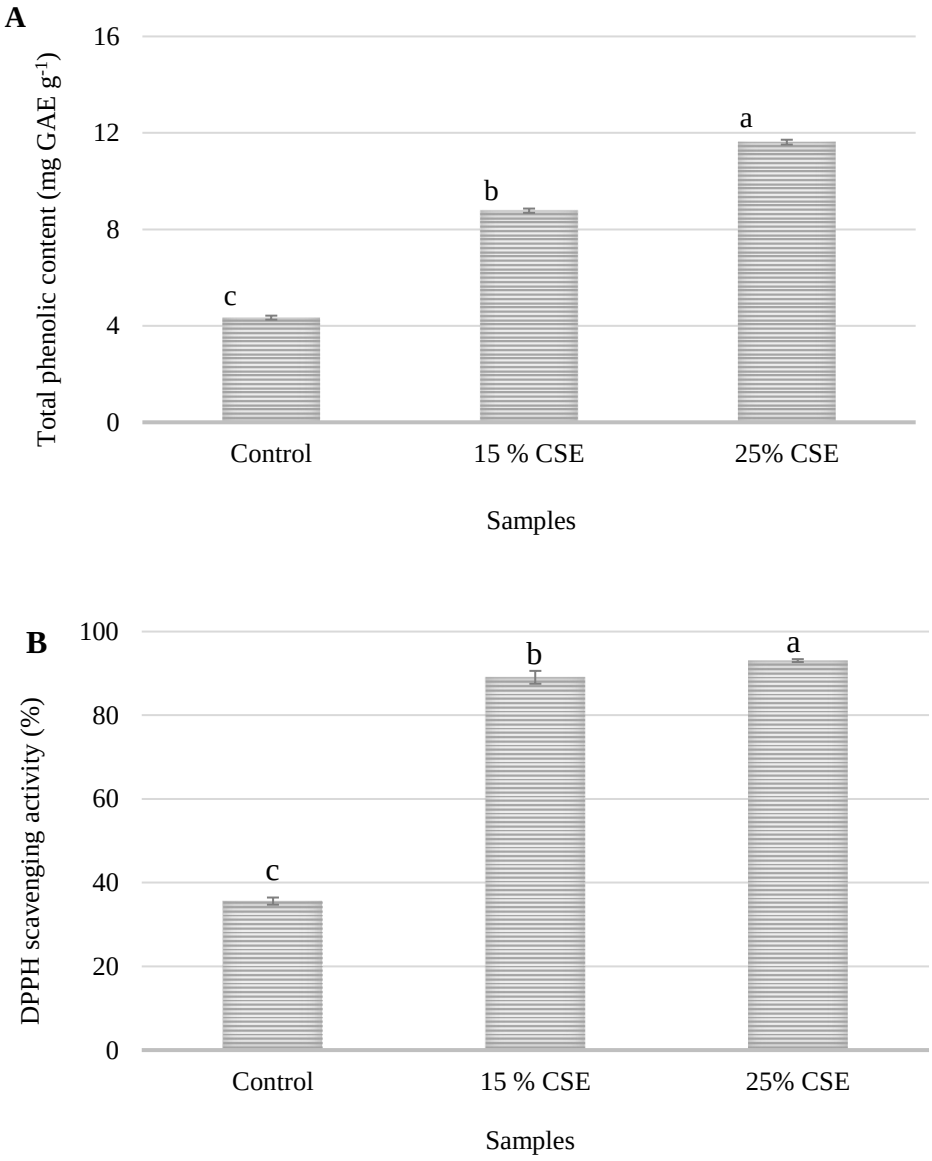
Control: without additives; CSE: corn stigma extract.

Figure 3. Fourier transformation infrared spectroscopy (FTIR) of gelatin and corn starch based films incorporated with corn stigma extract (CSE).



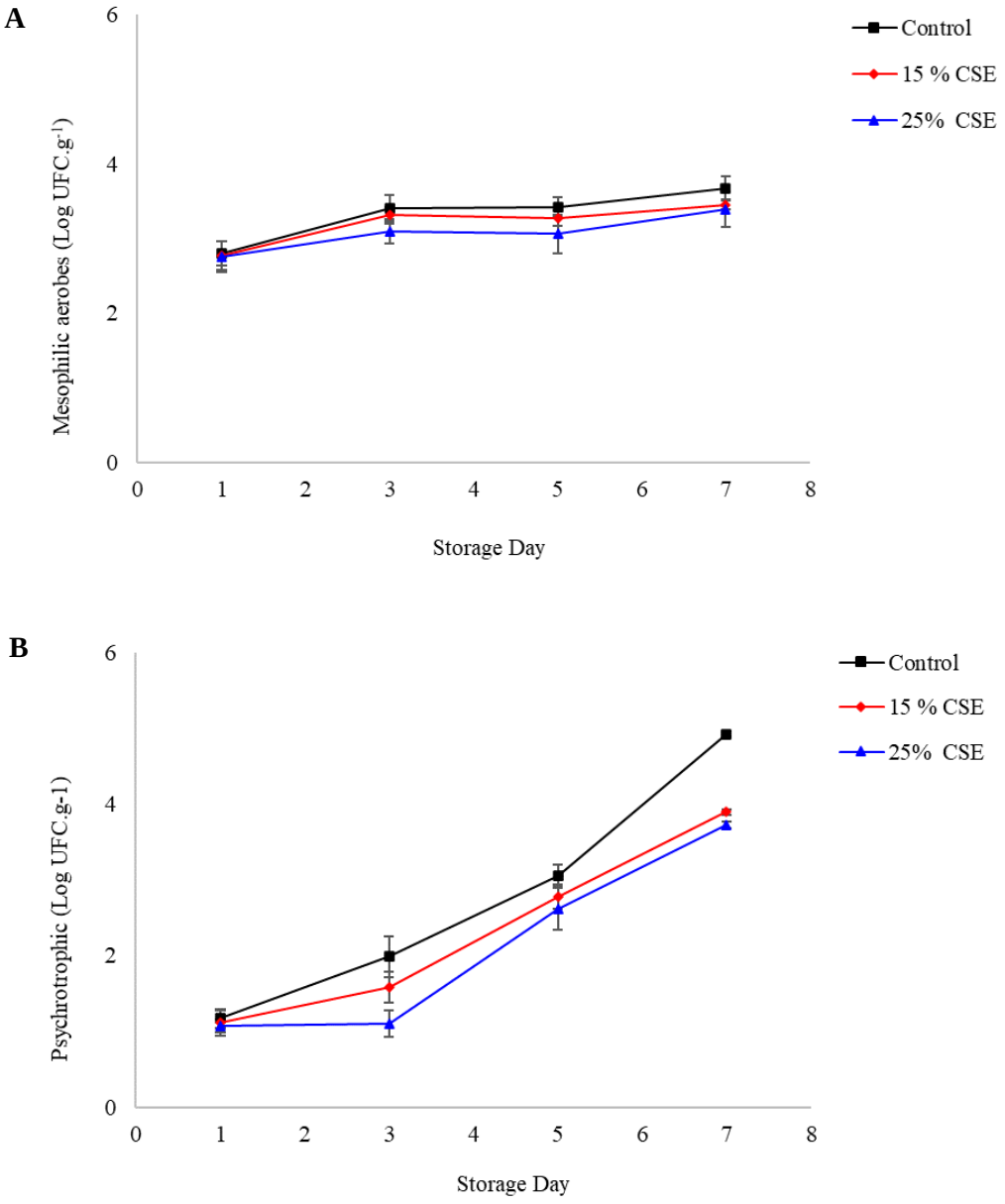
Control: without additives; CSE: corn stigma extract.

Figure 4. Total phenolic content (A) and antioxidant activity (B) of corn stigma extract (CSE) in gelatin and corn starch films.



Standard errors are represented by the error bars of the analyses performed in triplicate (n=9). [a-c] Different letters indicate a significant difference ($p<0.05$). Control: without additives; CSE: corn stigma extract

Figure 5. Counting of aerobic mesophilic (A) and psychrotrophic (b) microorganisms in rump steaks packed with active films.



Standard errors are represented by the error bars of the analyses performed in triplicate (n=9). Control: without additives; CSE: corn stigma extract

4 DISCUSSÃO GERAL

Os resíduos alimentares são produzidos em várias etapas do ciclo de vida dos alimentos, como na produção agrícola, no processamento industrial e na distribuição no mercado (GOMES-ARAÚJO, et al., 2021). A valorização dos resíduos alimentares através de diferentes técnicas de extração, como alternativa potencial para obter ingredientes nutracêuticos e funcionais, vem sendo discutida por pesquisadores. Dentre estes resíduos, destaca-se o estigma do milho, um subproduto agroindustrial da produção agrícola do milho (*Zea mays* L.) disponível em abundância e sem custo comercial. Pesquisas revelam que o estigma de milho é uma fonte renovável de compostos fenólicos e flavonoides, também é composto por proteínas, vitaminas, carboidratos, macronutrientes, óleos voláteis, esteroides, alcaloides e saponinas (WANG; ZHAO, 2019). Os extratos vegetais obtidos de resíduos alimentares mostraram-se promissores em uma variedade de aplicações na indústria alimentícia em virtude das propriedades antioxidantes e antimicrobianas que possuem (NEGI, 2012).

O primeiro artigo desta tese se propôs a investigar o efeito do ultrassom como uma via para a extração de compostos bioativos do estigma de milho bem como o efeito de variáveis independentes (tempo e relação sólido-solvente) e suas interações na extração. Os resultados indicaram que a extração assistida por ultrassom aumenta a atividade antioxidante e reduz o tempo de extração em 67% quando comparada à extração convencional. Condições otimizadas para a extração simultânea de antioxidantes e polifenóis do estigma de milho foram obtidas com 5 min e uma relação sólido-solvente de 0,05 g mL^{-1}. O extrato obtido foi caracterizado por ionização por eletrospray com espectrometria de massa de tempo de voo de alta resolução (ESI-ToF-MS) e 27 fitoquímicos foram encontrados. Os compostos identificados foram diretamente relacionados à atividade antioxidante demonstrada *in vitro*. O extrato apresentou atividades antifúngicas e antibacterianas promissoras contra 23 dos micro-organismos estudados. Os resultados compilados demonstram que o extrato de estigma de milho, obtido pela extração assistida por ultrassom, pode ser utilizado como fonte de compostos bioativos e antimicrobianos para uso como ingrediente funcional na indústria alimentícia.

A crescente preocupação ambiental com o uso de embalagens de base petroquímica impulsionou diversas pesquisas relacionadas ao desenvolvimento de embalagens de base biológica de fontes renováveis, biodegradáveis e ecológicas com propriedades antioxidantes (GEYER, JAMBECK & LAW 2017). Sendo assim, o segundo artigo dessa tese teve por objetivo desenvolver filmes bioativos à base de gelatina e amido de milho, incorporados com

extrato de estigma de milho em diferentes concentrações (15% e 25%; p/v) e determinar as propriedades estruturais, físicas dos filmes elaborados. Em testes preliminares de citotoxicidade, o extrato manteve a viabilidade celular acima de 90% indicando que é seguro para aplicação como ingrediente ativo. A inserção do extrato não influenciou na espessura dos filmes, mas causou pequenas alterações nas propriedades ópticas. A análise por microscopia eletrônica de varredura (MEV) revelou interações entre os compostos bioativos do extrato com a gelatina e os compostos de amido de milho, o que pode ter melhorado as propriedades mecânicas (alongamento à ruptura, módulo de Young). A adição de 25% de extrato de estigma de milho aumentou o ângulo de contato, conferindo ao filme um caráter hidrofóbico. Além disso, nesta concentração, observou-se uma redução de 15% na permeabilidade ao vapor de água. Os filmes elaborados apresentaram biodegradabilidade completa antes do décimo dia do estudo. Pode-se inferir que os filmes com extrato de estigma de milho possuem boas propriedades antioxidantes, indicando que podem ser utilizados como ingrediente para embalagens de alimentos.

Nesse sentido, o terceiro artigo dessa tese teve como objetivo aplicar os filmes desenvolvidos com propriedades antioxidantes e antimicrobianas como embalagem para retardar alterações associadas ao armazenamento refrigerado de carne bovina. A eficiência dos filmes foi determinada por conteúdo fenólico total e atividade antioxidante (AA%) in vitro, assim como a capacidade de retardar a oxidação lipídica, o crescimento de microrganismos aeróbios mesófilos e psicrotróficos, e as características físico-químicas como pH e atividade de água da carne bovina durante o armazenamento refrigerado. A incorporação do extrato de estigma de milho nos filmes aumentou consideravelmente as propriedades bioativas e antioxidantes. A aplicação do extrato reduziu a oxidação lipídica em 60% em relação ao controle (sem aditivo). É importante ressaltar que os valores de pH permaneceram baixos até o sétimo dia de vida de prateleira. O filme ativo também apresentou atividade antimicrobiana contra bactérias mesófilas e psicrotróficas. Os resultados gerais enfatizaram o uso potencial de compostos bioativos do extrato de estigma de milho para a produção de filmes destinados a embalagens de alimentos.

5 CONCLUSÃO GERAL

De maneira geral, os resultados do presente estudo demonstram que a extração assistida por ultrassom do estigma do milho foi promissora para aumentar os valores dos compostos

fenólicos totais e da capacidade antioxidante, além de reduzir o tempo de extração em 67% quando comparado ao método convencional. Além disso, possibilitou a recuperação de 27 compostos fitoquímicos pertencentes a diferentes classes, incluindo 1 alcaloide, 1 carboidrato, 1 aminoácido, 3 fenólicos, 3 flavonoides, 3 terpenos, 6 ácidos graxos, 6 ácidos orgânicos e 3 outros compostos. Os filmes à base de gelatina e amido de milho, incorporados com extrato de estigma de milho foram considerados eficazes contra a oxidação lipídica e o crescimento microbiológico em carne bovina. Os resultados compilados neste estudo comprovam que o estigma do milho pode ser considerado uma fonte de metabólitos bioativos com funções antioxidantes e antimicrobianas e pode ser aplicado como ingrediente funcional em embalagens de alimentos e melhorar a vida útil da carne bovina.

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