



KATIANY MANSUR TAVARES

**FILMES DE AMIDO E CARBOXIMETILCELULOSE ATIVOS
PELA ADIÇÃO DE ANTIMICROBIANOS À BASE DE
PEPTÍDEOS**

**LAVRAS-MG
2020**

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Engenharia de Biomateriais, área de concentração em Produtos e Nanoprodutos Alimentícios, para a obtenção do título de Doutor.

Prof. Dr. José Manoel Marconcini
Orientador

Prof. Dr. Juliano Elvis de Oliveira
Prof. Dra. Adriana de Campos Pastre
Coorientadores

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2020**

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KATIANY MANSUR TAVARES

**FILMES DE AMIDO E CARBOXIMETILCELULOSE ATIVOS PELA ADIÇÃO DE
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**STARCH AND CARBOXYMETHYLCELLULOSE FILMS ACTIVE BY THE
ADDITION OF PEPTIDE BASED ANTIMICROBIALS**

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Dr. Lourival Marin Mendes UFLA

Dr. João de Deus Souza Carneiro UFLA

Dra. Adriana de Campos Pastre CTC

Dra. Roberta Hilsdorf Piccoli UFLA

Prof. Dr. José Manoel Marconcini
Orientador

Prof. Dr. Juliano Elvis de Oliveira
Profa. Dra. Adriana de Campos Pastre
Coorientadores

**LAVRAS-MG
2020**

“A educação é a arma mais poderosa que você pode usar para mudar o mundo.”

(Nelson Mandela)

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RESUMO GERAL

Filmes de amido apresentam grande potencial para serem empregados em embalagens, apesar de estarem sujeitos à ação da água e de microrganismos, que dificultam esta utilização. Sendo assim, a adição de carboximetilcelulose (CMC) e de peptídeo BP100 visa superar estas limitações, ampliando as propriedades mecânicas e a vida de útil dos alimentos, respectivamente. O objetivo deste trabalho foi avaliar as propriedades mecânicas, de barreira ao vapor de água e de atividade antimicrobiana de filmes de amido com CMC ativos pela adição de BP100. Avaliações preliminares de filmes de amidos de milho e de mandioca mostraram que a adição de 50 % (m/m) de CMC aumentou as propriedades mecânicas e reduziu a permeabilidade ao vapor de água dos filmes, independente da origem botânica do amido. <https://doi.org/10.1016/j.carbpol.2019.115055>. Em seguida, diferentes concentrações de CMC foram adicionadas em filmes de amido de milho e constatou-se que teores de 20 e 40% (m/m) foram responsáveis pelo aumento das propriedades mecânicas e de barreira ao vapor de água dos filmes. <https://doi.org/10.1016/j.carbpol.2020.116521>. Em um terceiro momento, BP100 foi adicionado aos filmes de amido de milho contendo 20 e 40% (m/m) de CMC e verificou-se que houve aumento nas propriedades mecânicas e de barreira a vapor de água, além da inclusão de propriedades antimicrobianas a bactérias gram-negativas e gram-positivas.

Palavras-chave: Polímeros naturais. Embalagens ativas. Atividade antimicrobiana. Propriedades mecânicas. Hidrofobicidade.

GENERAL ABSTRACT

Starch films have great potential to be used in packaging, despite being subjected to the action of water and microorganisms, which hinder this use. Thus, the addition of carboxymethylcellulose (CMC) and peptide BP100 aims to overcome these limitations, expanding the mechanical properties and shelf life of food, respectively. The objective of this work was to evaluate the mechanical, water vapor barrier and antimicrobial properties of starch films with active CMC by the addition of BP100. Preliminary evaluations of corn or cassava starch films showed that the addition of 50 wt. % CMC increased the mechanical properties and reduced the water vapor permeation of the films, regardless of the botanical origin of the starch. <https://doi.org/10.1016/j.carbpol.2019.115055>. Then, different concentrations of CMC were added in corn starch films and it was found that contents of up to 40 wt. % were responsible for the increase of mechanical and water vapor barrier properties of the films. <https://doi.org/10.1016/j.carbpol.2020.116521>. In a third moment, BP100 was added to the films of corn starch with 20 and 40 wt.% of CMC and it was verified that there was an increase in the mechanical and water vapor barrier properties, besides the appearance of antimicrobial properties to gram-negative and gram-positive bacteria.

Keywords: Natural polymers. Active packaging. Antimicrobial activity. Mechanical properties. Hydrophobicity.

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PRIMEIRA PARTE

1 INTRODUÇÃO

O grande volume de resíduos poliméricos gerados nos últimos anos e seu acúmulo no meio ambiente tem se tornado uma preocupação ambiental em todo o mundo (KARAN *et al.*, 2019; GEYER; JAMBECK; LAW, 2017). O desenvolvimento de novos materiais baseados em recursos renováveis tem sido uma alternativa, pois estes apresentam vantagens como sustentabilidade, disponibilidade, baixo custo, não toxicidade e - ampla gama de aplicações (SESSINI *et al.*, 2019; KAKAEI; SHAHBAZI, 2016; DAI *et al.*, 2015; GONZÁLEZ; IGARZABAL, 2015; BENHAMOU *et al.*, 2014; RHIM *et al.*, 2013).

Neste contexto, o amido tem sido considerado um material promissor para a produção de embalagens devido à sua origem natural, abundância, viabilidade econômica, comportamento termoplástico e biodegradabilidade (DUFRESNE; CASTAO, 2017; JIMÉNEZ *et al.*, 2012; CAO *et al.*, 2008). De maneira geral, os filmes de amido apresentam boas propriedades de barreira ao oxigênio, dióxido de carbono (CO₂) e lipídeos, sendo suas características influenciadas pela fonte botânica, tipo de plastificante e forma de processamento (MA *et al.*, 2017). Porém, a resistência mecânica e barreira ao vapor de água dos filmes de amido são inferiores quando comparados aos materiais convencionais, sendo estes, fatores limitantes para sua aplicação (MIRI *et al.*, 2015).

Visando superar essas deficiências, outros polímeros como a quitosana (HARI *et al.*, 2018; MENDES *et al.*, 2016), a pectina (PREZOTTI *et al.*, 2014; MOREIRA *et al.*, 2012a), a celulose e a carboximetilcelulose (CMC) (CAMPOS *et al.*, 2017; LIMA *et al.*, 2017; NAWAB *et al.*, 2017; MOREIRA *et al.*, 2012b; MA; CHANG; YU, 2008) têm sido empregados para a formação de filmes de amido. A adição de CMC em filmes de amido aumenta a resistência à tração e a barreira contra o oxigênio e lipídios (MA *et al.*, 2017; TONGDEESOONTORN *et al.*, 2011; GHANBARZADEH; ALMASI; ENTEZAMI, 2010), além de reduzir a permeabilidade ao vapor de água (TAVARES *et al.*, 2020; TAVARES *et al.*, 2019), originando filmes transparentes que é uma característica desejada pelo consumidor.

Além das questões ambientais, os problemas causados pela contaminação de alimentos por microrganismos deteriorantes e patogênicos têm despertado o interesse na adição de agentes antimicrobianos em embalagens, tornando-as ativas e agregando assim, uma função adicional (DAIRI *et al.*, 2019). As embalagens ativas possuem constituintes que interagem com o alimento ou com o meio (atmosfera, microrganismos, etc.) e desempenham - papel

crucial na extensão da vida útil e na manutenção da qualidade do produto, bem como na segurança dos consumidores MOHSENABADI *et al.*, 2018; MEIRA *et al.*, 2017).

Por outro lado, os consumidores estão cada vez mais conscientes dos riscos à saúde humana representados pelo uso de conservantes químicos em alimentos. Assim, a tendência de “rótulo limpo” está se tornando cada vez mais comum e a busca pelo consumo de alimentos sem conservantes tornou-se uma tendência (GRANT & PARVEEN, 2017). Assim, o uso de peptídeos antimicrobianos em embalagens apresenta vantagens, pois são considerados seguros, uma vez que podem ser facilmente degradados por enzimas proteolíticas do trato gastrointestinal (TKACZEWSKA, 2020; SILVA *et al.*, 2018).

Os peptídeos antimicrobianos (AMPs) têm despertado o interesse de pesquisadores das mais diversas áreas como medicina, controle de doenças, terapêutica e agricultura, devido ao amplo espectro de ação e pela dificuldade das bactérias em desenvolver resistência contra eles (GONZÁLEZ *et al.*, 2017; YI; CHOWDHURY; HUANG, 2014; BUNDÓ *et al.*, 2014). Podem ser encontrados em diversos organismos e constituem um componente principal de seu sistema imune inato, produzidos em resposta à ação de bactérias, fungos ou vírus (VALE; AGUIAR; GOMES, 2014; MANGONI *et al.*, 2008).

Os AMPs têm sido utilizados para retardar a deterioração de alimentos, incorporados em matrizes poliméricas, visando ao desenvolvimento de embalagens ativas devido à sua notável eficiência inibitória contra microorganismos (ZHONG *et al.*, 2020; CORRALES-URENA *et al.*, 2020; HE; FEI; LI, 2020; AHMAD *et al.*, 2017; MEIRA *et al.*, 2017; BROGDEN *et al.*, 2011; SETTANNI; CORSETTI, 2008). O BP100 é um peptídeo antimicrobiano sintético curto (KKLFKKILKYL-NH₂), obtido a partir de uma combinação de cecropina A e melitina, altamente ativo e seletivo contra bactérias gram-negativas, que apresenta baixa citotoxicidade e hemólise ((WADHWANI *et al.*, 2014; BADOSA *et al.*, 2007).

2 OBJETIVO GERAL

O objetivo deste trabalho é avaliar os filmes de amido/CMC ativos pela adição de peptídeos antimicrobianos.

2.1 Objetivos específicos

Avaliar a interação da CMC com filmes de amido de milho e de mandioca e sua correlação com o conteúdo de amilose e amilopectina.

Estabelecer a quantidade de CMC necessária para a produção de filmes poliméricos de amido com propriedades mecânicas satisfatórias para aplicação em embalagens de alimentos.

Avaliar as propriedades mecânicas, interação com a água e atividade antimicrobiana de filmes de amido/CMC ativos com BP100.

3 REFERENCIAL TEÓRICO

3.1 Embalagens para alimentos

De acordo com a ANVISA (Agência Nacional de Vigilância Sanitária) a embalagem para alimentos é o material que está em contato direto com alimentos, destinado a contê-los desde a sua fabricação até a entrega ao consumidor, com a finalidade de protegê-los de agentes externos, de alterações e de possíveis contaminações (ANVISA, 2018).

Uma embalagem pode apresentar diversas funções, no entanto, quando utilizada para alimentos, ela os protege preservando suas características através das propriedades de barreira aos fatores ambientais como luz, umidade, oxigênio e microrganismos. Assim, a embalagem auxilia para que não ocorra alterações indesejáveis como, por exemplo, oxidação e contaminação por patógenos durante toda a vida útil do produto (SARANTÓPOULUS *et al.*, 2002).

As embalagens de alimentos são as que mais utilizam polímeros termoplásticos, devido, principalmente às suas características de flexibilidade, leveza, baixo custo, variedade entre outras (GALLEGO-SCHMID; MENDOZA; AZAPAGIC, 2019; SOUZA *et al.*, 2012). No entanto, a maioria dos polímeros utilizados é de origem petroquímica (fonte não renovável), tornando-se um material que atualmente não há substitutos competitivos no mercado para esta aplicação (LAGARON; LOPEZ-RUBIO, 2011; GONZÁLEZ; IGARZABAL, 2013).

As embalagens plásticas produzidas com os polímeros convencionais de fonte petroquímica degradam-se muito lentamente no ambiente, pois são bastante resistentes às radiações, ao calor, ao ar, à água e ao ataque imediato de microrganismos. Isso gera problemas ambientais, visto que a degradação desses materiais leva centenas de anos (ARVANITOYANNIS; BILLIARERIS, 1999).

O aumento do seu consumo resulta inevitavelmente em problemas socioeconômicos, como a escassez e o aumento do preço do petróleo, e ambientais como a geração e acúmulo de resíduos sólidos. Isso ocorre porque além de ocuparem muito espaço, permanecem por longo tempo, pois possuem uma decomposição lenta, gerando uma grande quantidade de resíduos. Além disso, quando não descartados corretamente, contribuem para a poluição de águas e ameaçam a segurança de animais (BRAVO-REBOLLEDO *et al.*, 2013; CARSON *et al.*, 2013; BRITO *et al.*, 2011).

Várias alternativas têm sido investigadas para minimizar o impacto ambiental dos polímeros convencionais, incluindo a utilização de polímeros biodegradáveis. Em comparação com os polímeros sintéticos, os polímeros naturais como materiais de embalagens de alimentos apresentam vantagens como a biodegradabilidade e obtenção a partir de recursos renováveis (QUINTERO *et al.*, 2012). Para reduzir a quantidade de resíduos, o estudo de embalagens e revestimentos para a indústria de alimentos a partir de recursos naturais tornou-se um tema de grande interesse (ASSIS; ZAVAREZE, 2012; THARANATHAN, 2003).

3.2 Polímeros naturais

Os polímeros naturais podem ser classificados em três categorias: hidrocoloides (tais como proteínas e polissacarídeos), lipídios (ácidos graxos, acilglicerol e ceras) e compósitos (DASHIPOUR *et al.*, 2015). Atualmente, os materiais originados de fontes renováveis estão sendo bastante utilizados no desenvolvimento de embalagens como resultado da preocupação ambiental e do esgotamento dos recursos fósseis, como pode ser demonstrado pelo número exponencialmente crescente de patentes e publicações sobre esses materiais (CALEGARI; FREITAS, 2016; SILVA *et al.*, 2009).

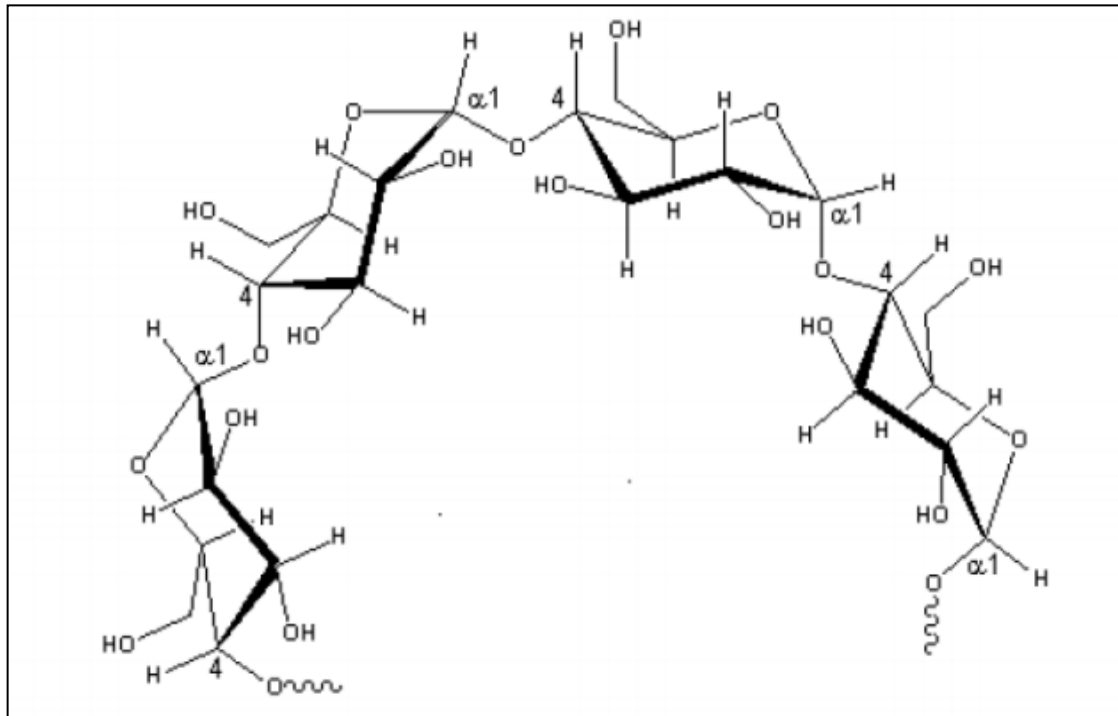
Dentre os polímeros naturais, o amido tem sido considerado um material promissor para a produção de embalagens devido à sua abundância, viabilidade econômica e comportamento termoplástico (DUFRESNE; CASTAO, 2017; JIMÉNEZ *et al.*, 2012; CAO *et al.*, 2008).

3.2.1 Amido

O amido é um polímero natural formado por unidades de glicose ligadas entre si e pode ser representado pela fórmula geral $(C_6H_{10}O_5)_n + xH_2O$ (FRANCO *et al.*, 2002). É formado por dois tipos de polímeros de glicose: a amilose e a amilopectina com estruturas e funcionalidades diferentes.

A proporção de amilose / amilopectina varia de 15:85 a 35:65 em diferentes tipos de amido, dependendo da origem botânica. A amilose é um polímero linear com unidades de glicose ligadas em α -1,4 (FIGURA 1) (VILAPLANA; HASJIM; GILBERT, 2012). A amilopectina é um polímero altamente ramificado com unidades de glicose com ligação α -1,4 e α -1,6 e possui um tamanho muito maior que a amilose (LI *et al.*, 2011).

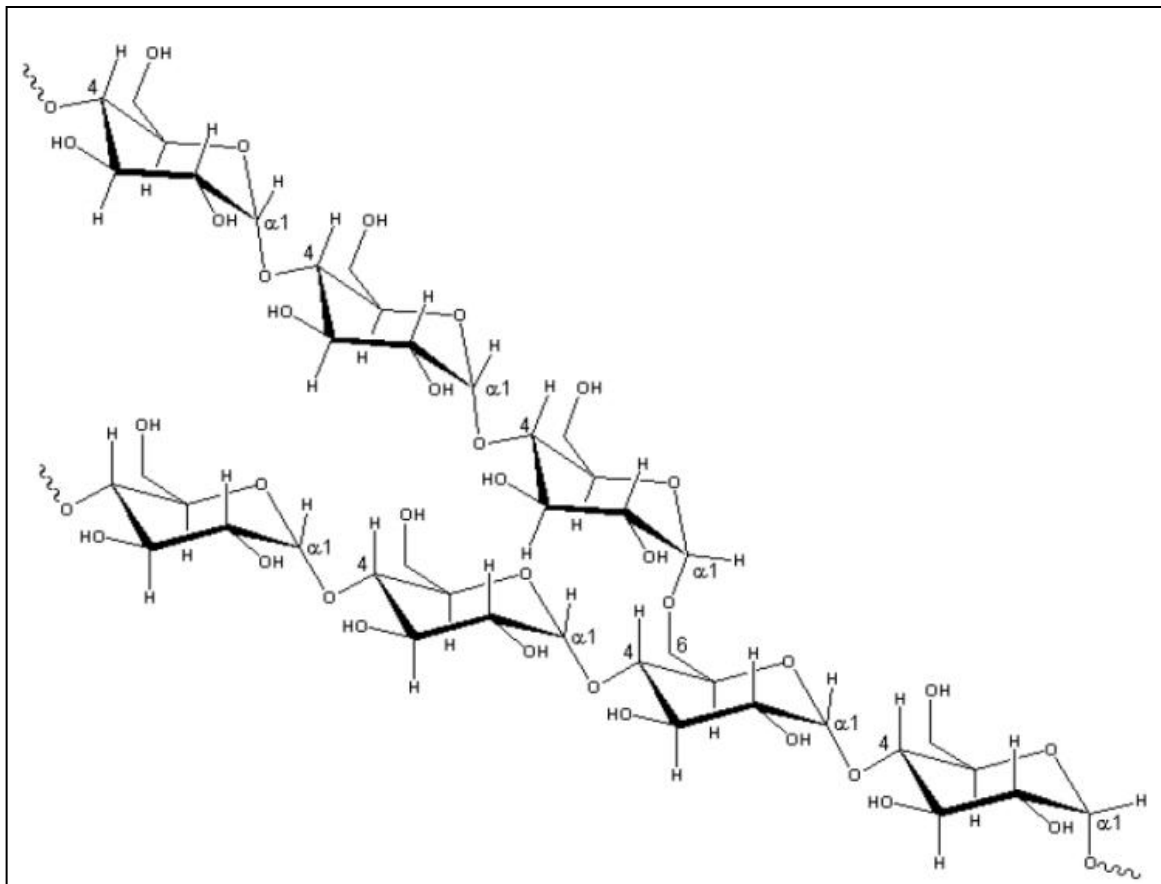
Figura 1- Estrutura da amilose [polímero linear composto por D-glicoses unidas em α -(1-4)].



Fonte: Adaptado de Lajolo & Menezes (2006).

A amilose é relatada como um excelente material formador de filmes, com cadeias lineares e uma rede estabilizada por ligações de hidrogênio (MENZEL *et al.*, 2015; ROMERO-BASTIDA *et al.*, 2015). Em contraste, as cadeias poliméricas de amilopectina, devido à sua ramificação e comprimento mais curto, podem ser altamente enroscadas em conjunto, levando à formação de ligações de hidrogênio entre cadeias como mostrado na Figura 2 (RINDLAV-WESTLING *et al.*, 1998).

Figura 2 - Estrutura da amilopectina [polímero ramificado composto por D-glicoses unidas em α -(1-4) e α -(1-6)].



Fonte: Adaptado de Lajolo & Menezes (2006).

É um polissacarídeo de reserva de energia dos vegetais e está presente nos plastídios de vegetais superiores. Está disponível em abundância na natureza e pode ser obtido de diferentes fontes vegetais como cereais, raízes e tubérculos, assim como de frutas e legumes. No entanto, sua extração comercial restringe-se aos cereais como o milho, o trigo e o arroz, e de tubérculos como mandioca e batata (CHIVRAC; POLLET; AVÉROUS, 2009).

O milho é reconhecido mundialmente como a maior fonte comercial de amido (aproximadamente 65%), seguido por batata-doce (13%) e mandioca (11%) (MERCADOS GLOBAIS DE AMIDO, 2018; GARCIA *et al.*, 2009; SOUZA; DITCHFIELD; TADINI, 2010).

A mandioca destaca-se com alto rendimento de amido, sendo o Brasil o quarto maior produtor dessa raiz (LUCHESE; SPADA; TESSARO, 2017). O amido de milho tem uma

média de 28% de amilose, enquanto a mandioca tem aproximadamente 18% em massa (MENDES *et al.*, 2016).

As propriedades dos grânulos de amido são determinadas principalmente pelo arranjo das moléculas lineares e ramificadas distribuídas em seu interior. A cristalinidade dos grânulos de amido é atribuída, principalmente, à amilopectina e a parte amorfa à amilose que, embora seja linear, apresenta uma conformação helicoidal, que dificulta sua associação regular com outras cadeias (BULÉON *et al.*, 1998).

A absorção de água pelos grânulos é permitida pela parte amorfa, enquanto a parte cristalina apresenta maior resistência à entrada de água e mantém a estrutura dos grânulos (DENARDIN; SILVA, 2009).

Quando o amido é aquecido na presença de água, ocorre uma transição ordem-desordem irreversível denominada de gelatinização. Neste processo, os grânulos absorvem água, gelatinizam e incham até atingir a capacidade máxima de absorção de água, ocasionando a perda da cristalinidade e a lixiviação de amilose (JENKINS; DONALD, 1998).

3.2.2 Propriedades de filmes à base de amido

Os filmes à base de amido vêm sendo estudados desde 1950 (COFFIN, 1950 citado por FISHMAN; FRIEDMAN; HUANG, 1994). A utilização desta macromolécula na produção de filmes baseia-se nas propriedades químicas, físicas e funcionais da amilose para formar géis e na sua capacidade para formar filmes. As moléculas de amilose em solução, devido à sua linearidade, tendem a se orientar paralelamente, aproximando-se o suficiente para que se formem ligações de hidrogênio entre hidroxilas de polímeros adjacentes. Como resultado, a afinidade do polímero por água é reduzida, favorecendo a formação de pastas opacas e filmes resistentes (WURZBURG, 1986).

São produzidos, em sua maioria utilizando a técnica “casting” que consiste na solubilização do polímero em um solvente com posterior evaporação deste solvente. O amido gelatinizado apresenta capacidade de formar filmes, devido ao rompimento de sua estrutura cristalina, permitindo a formação de uma matriz polimérica homogênea e amorfa (GENNADIOS; WELLER, 1990).

As características dos filmes de amido variam muito de acordo com a origem botânica, o teor de plastificante, do tipo e condições de processamento (BASIAK; LENART; DEBEAUFORT, 2017; JOSHI *et al.*, 2013; JIMÉNEZ *et al.*, 2012). Diversos estudos relataram as boas propriedades formadoras de filmes de amido provenientes de diferentes

fontes naturais como o milho (FABRA *et al.*, 2018; MENDES *et al.*, 2016; LÓPEZ *et al.*, 2011), (BONILLA *et al.*, 2013), a mandioca (PINEROS-HERMANDEZ *et al.*, 2017), o arroz (DIAS *et al.*, 2010), a batata (SESSINI *et al.*, 2016), o inhame (MUKURUMBIRA *et al.*, 2017), entre outros.

As diferenças nas propriedades mecânicas e na capacidade de barreira dos filmes de amido podem ser explicadas pelo fenômeno da retrogradação, no qual as moléculas de amido recristalizam durante a gelificação e o envelhecimento (MARIOTTI *et al.*, 2006). Os filmes de amido apresentam boas propriedades de barreira ao oxigênio, dióxido de carbono (CO₂) e lipídeos (MA *et al.*, 2017). Porém, suas propriedades mecânicas e de resistência à água são inferiores às dos filmes sintéticos (MIRI *et al.*, 2015).

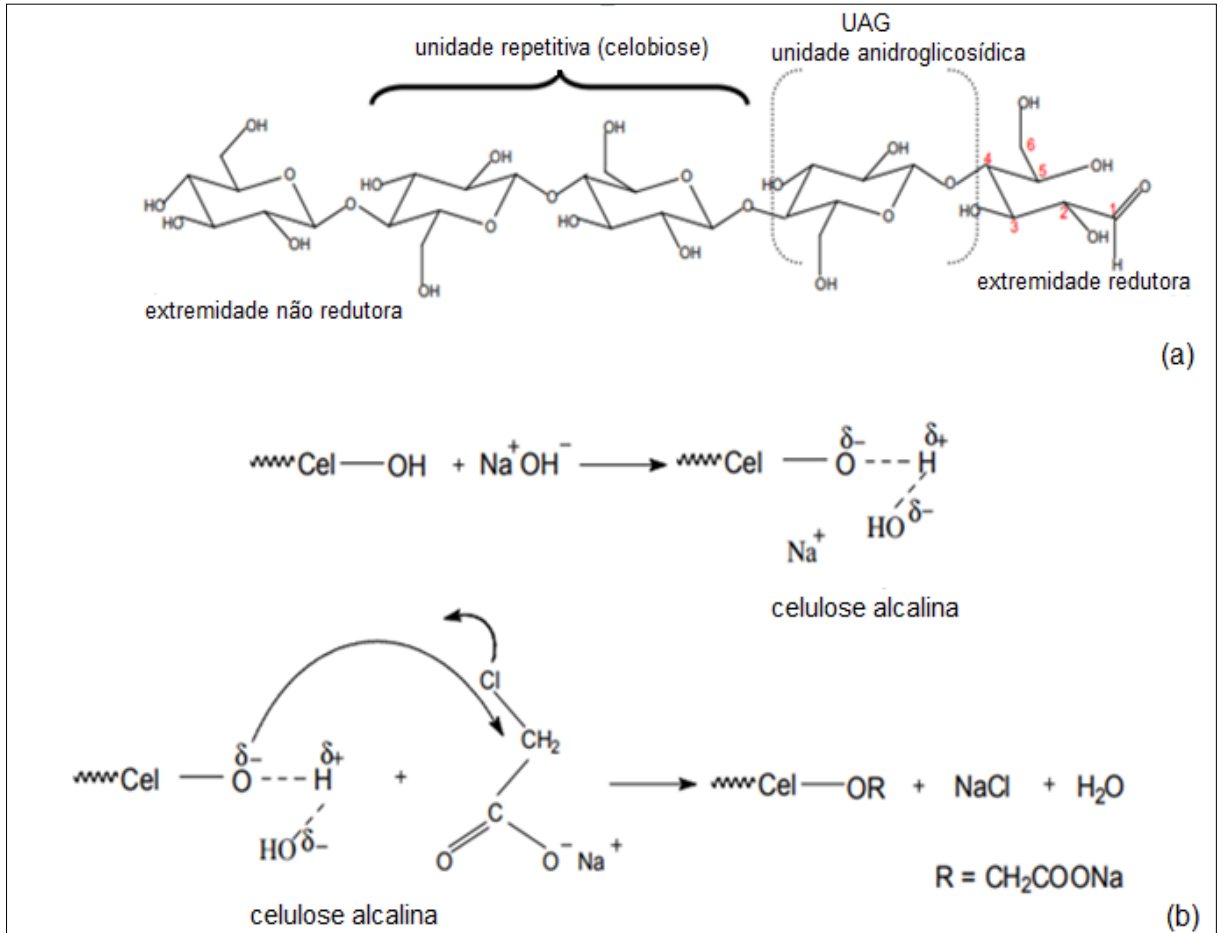
Para aumentar suas propriedades mecânicas, os filmes de amido são comumente associados a outros materiais como o glicerol (SELIGRA *et al.*, 2016; CORRADINI *et al.*, 2007) e outros polímeros oriundos de fontes naturais como a carboximetilcelulose (CMC) (CAMPOS *et al.*, 2017; LIMA *et al.*, 2017; NAWAB *et al.*, 2017; MOREIRA *et al.*, 2012a; MA; CHANG; YU, 2008).

3.2.3 Carboximetilcelulose (CMC)

A carboximetilcelulose (CMC) é um polímero aniônico derivado de celulose, comercializada na forma de um sal de sódio, que pode ser produzida à pressão atmosférica (KLEMM *et al.*, 1998). É amplamente utilizada nas indústrias farmacêutica, alimentícia, agrícola, como também na construção civil, na produção de tintas, detergentes, adesivos e cosméticos, e devido as suas propriedades físico-químicas, é também empregada como agente espessante, doador de viscosidade, estabilizante de fluidos, emulsificante, engomante e agente adesivo, de suspensão e tixotrópico. Foi sintetizada por Jensen em 1918 na Alemanha e, em 1920, produzida comercialmente (HEINZE; KOSCHELLA, 2005).

A produção da CMC ocorre com a mercerização da celulose com o monocloroacetato. Nesse processo ocorre uma reação de substituição para a produção da carboximetilcelulose, onde a celulose interage com o monocloroacetato de sódio (CRUZ, 2011). A utilização de hidróxido de sódio, em concentrações adequadas, ativa as hidroxilas da celulose para a substituição nucleofílica pelos grupos do agente eterificante, o ácido monocloacético (CERRUTTI; FROLLINI, 2009). A Figura 3 mostra a representação esquemática da reação da celulose que leva a CMC.

Figura 3 - Parte da estrutura da celulose (a); Representação esquemática da reação de formação da CMC (b).



Fonte: Cerrutti e Frollini (2009).

A adição de CMC em filmes de amido propicia um aumento na resistência à tração e melhora suas propriedades de barreira ao oxigênio e lipídios (MA *et al.*, 2017; TONGDEESOONTORN *et al.*, 2011). Porém, os filmes de amido termoplástico com CMC ainda apresentam caráter bastante hidrofílico, resultando em alta sensibilidade à água e baixa capacidade de barreira ao vapor de água, o que pode comprometer sua aplicação como embalagem de alimentos.

3.3 Embalagens ativas

Vários estudos estão sendo realizados na busca por embalagens de alimentos com alto desempenho mecânico, térmicos, barreira aos gases e ao vapor de água e com atividade antimicrobiana com o intuito de preservar a qualidade, integridade e estender o prazo de validade dos alimentos (DAIRI *et al.*, 2019).

As embalagens ativas são um grupo de embalagens desenvolvidas para proteger e interagir com o produto, como forma de preservar a qualidade e segurança deste durante o armazenamento (SARANTÓPOULOS; MORAES, 2009). Elas também podem ser definidas como embalagens em que elementos adicionais são deliberadamente incluídos no material estrutural da embalagem ou no espaço livre desta, como na forma de sachês, para melhorar seu desempenho (GONTARD; GUILBERT; CUQ, 1993; SARANTÓPOULOS; MORAES, 2009).

Existem vários mecanismos de atuação das embalagens ativas, dentre eles: absorção de oxigênio, absorção de etileno, absorção de odores, absorção de umidade, liberação de compostos antioxidantes e antimicrobianos (GONTARD; GUILBERT; CUQ, 1993). Dentre as vantagens do uso de embalagens ativas, destacam-se: maior durabilidade dos produtos embalados e a redução da necessidade de utilização de conservantes químicos (GONTARD; GUILBERT; CUQ, 1993; JONGJAREONRAK *et al.*, 2008).

3.3.1 Peptídeos Antimicrobianos (AMPs)

Os peptídeos antimicrobianos (AMPs) são, em grande maioria, moléculas anfipáticas e catiônicas (DEMPSEY *et al.*, 2010, BROGDEN, 2005). Possuem de 10 a 80 resíduos de aminoácidos sendo, frequentemente, 50% resíduos hidrofóbicos e 50% resíduos hidrofílicos carregados positivamente em pH fisiológico. A região catiônica da molécula é responsável por sua atração por membranas aniônicas de bactérias e, por fim, a conformação e composição dos AMPs permitem a formação de estruturas que causam a morte do micro-organismo (FERRE *et al.*, 2009; BROGDEN, 2005).

A composição de aminoácidos e a estrutura secundária de um AMP são importantes para definir as suas características hidrofílicas e hidrofóbicas, promovendo maiores ou menores interações com os diferentes tipos de membranas. A sua composição é capaz de definir estruturas em α -hélice, dobrada (em grampo), randômica e outras, dependendo do meio em que a molécula se encontra, e ainda determinam domínios que irão interagir com

superfícies ou se inserir no interior hidrofóbico da membrana dos micro-organismos (ZHANG; ROZEK; HANCOCK, 2001; JENSSEN; HAMILL; HANCOCK, 2006; GIANGASPERO; SANDRI; TOSSI, 2003; EPAND *et al.*, 2010; NGUYEN *et al.*, 2010). (ZHANG *et al.*, 2001; JENSSEN *et al.*, 2006; GIANGASPERO *et al.*, 2001; EPAND *et al.* 2010; NGUYEN *et al.* 2010).

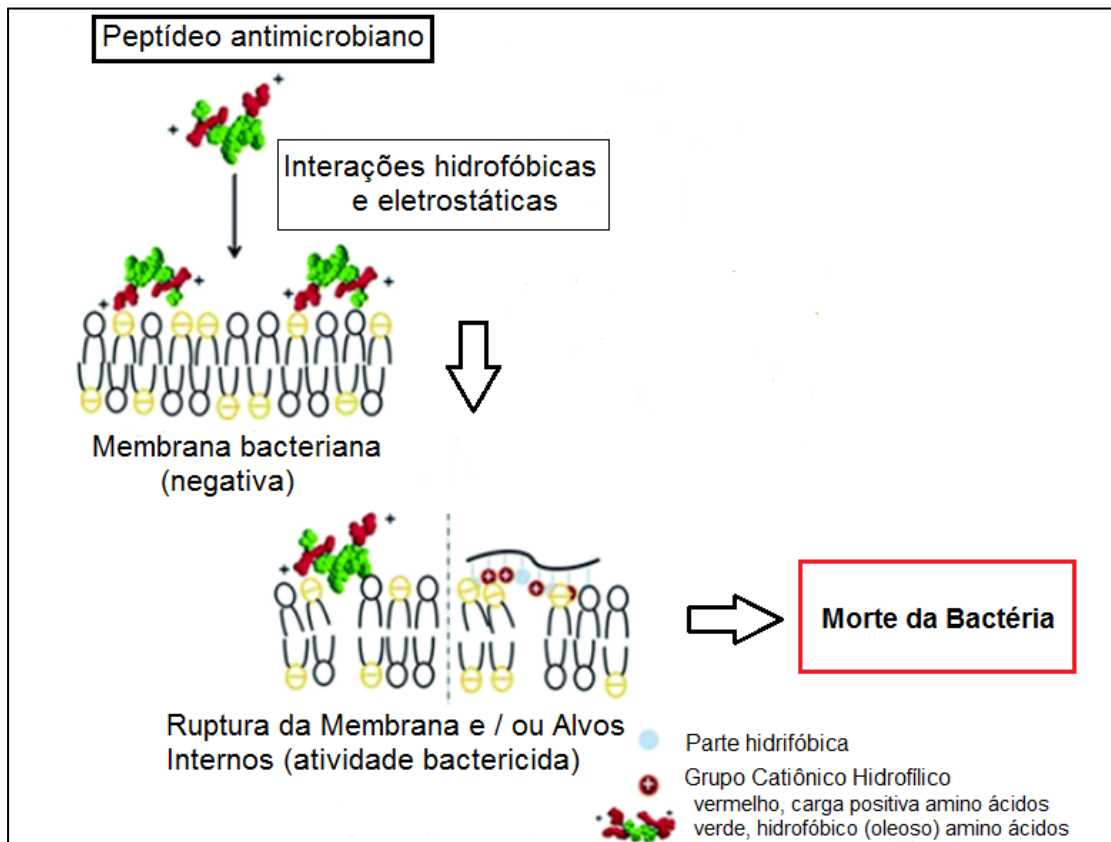
Independentemente do tipo de distúrbio que o peptídeo exerce sobre a membrana, a interação inicial do AMP é eletrostática, uma vez que estas moléculas são policatiônicas e as membranas das bactérias são carregadas negativamente (HUANG, 2000). Apesar de bactérias Gram-positivas e Gram-negativas serem muito mais complexas que modelos de membranas, estes micro-organismos apresentam - carga superficial negativa oriunda da presença de fosfolípídeos ácidos, grupos fosfato dos lipopolissacarídeos (bactérias Gram-negativas) e do ácido teicóico (bactérias Gram-positivas) (BROGDEN, 2005).

Dentre os vários AMPs existentes, dois foram amplamente estudados, a cecropina A e a melitina. Merrifield e colaboradores (1995) sintetizaram híbridos desses peptídeos com o intuito de aumentar sua atividade antimicrobiana e diminuir os efeitos prejudiciais nas membranas do hospedeiro dados pelo alto índice terapêutico (TI) que é a razão entre concentrações tóxicas e antimicrobianas (BOMAN *et al.*, 1989; ANDREU *et al.*, 1992; SATO *et al.*, 2006).

Dos híbridos obtidos a partir de uma combinação de cecropina A e melitina, BP100 se destaca por ser um peptídeo antimicrobiano sintético curto (KKLFFKKILKYL-NH₂), altamente ativo e seletivo contra bactérias gram-negativas, além de apresentar baixa citotoxicidade e hemólise (WADHWANI *et al.*, 2014; BADOSA *et al.*, 2007). Além disso, outra vantagem é a ausência de resíduos de aminoácidos propensos à oxidação, como triptofano e metionina (Chan & White, 2000). Seu mecanismo de ação é dependente da neutralização da carga superficial microbiana (MANZINI *et al.*, 2014). A concentração inibitória mínima (MIC) para várias bactérias gram-negativas está entre 2,5 e 5µM (TORCATO *et al.*, 2013; ALVES *et al.*, 2010; BADOSA *et al.*, 2007).

Os peptídeos antimicrobianos têm sido utilizados para retardar a deterioração de alimentos com resultados promissores (AHMAD *et al.*, 2017; MEIRA *et al.*, 2017; BROGDEN *et al.*, 2011). A Figura 4 mostra a representação esquemática do mecanismo de ação dos peptídeos antimicrobianos contra as bactérias.

Figura 4 – Representação esquemática do mecanismo de ação dos peptídeos antimicrobianos.



Fonte: Adaptado de Yin et al. (2017).

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SEGUNDA PARTE – ARTIGOS**ARTIGO 1 - Corn and cassava starch with carboxymethyl cellulose films and its mechanical and hydrophobic properties**

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Katiany Mansur Tavares^{a,*}; Adriana de Campos^b; Milene Corso Mitsuyuki^b, Bruno Ribeiro Luchesi^c, José Manoel Marconcini^b

^a Postgraduate Program in Biomaterials Engineering (PPGBiomat), Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil,

^b National Laboratory of Agribusiness Nanotechnology (LNNA), Embrapa Instrumentação, São Carlos, São Paulo, Brazil.

^c Postgraduate Program in Materials Science and Engineering (PPGCEM), Federal University of São Carlos (UFSCar), São Carlos, São Paulo, Brazil.

*Corresponding author: mansurtavares@yahoo.com.br

Abstract

The amylose and amylopectin content of starch influences its functional characteristics and its interaction with other materials. Thus, it is important to study the mechanical strength and hydrophilicity of starch films of different botanical origins to elucidate the influence of their constituents in the interaction with carboxymethyl cellulose (CMC). The objective of this work was to evaluate the mechanical and physico-chemical properties of corn and cassava starch films and their blends with CMC by casting. The addition of CMC improved the tensile, increasing the strength (206% for its blends with corn starch and 51% for its blends with cassava starch), the rupture stress (89% and 74%, respectively), the rupture strain (381% and 57%, respectively), the elastic modulus (20% and 18%, respectively) and the water vapor permeability (48 and 40%, respectively). The corn starch film was more hydrophobic due to its higher amylose content, which contributed to the interaction between starch and glycerol OH groups and CMC COOH groups. This interaction was evidenced by FTIR and contact angle analysis, turning corn starch/CMC films into less hydrophilic material and reducing its water vapor permeability rate, which made this material promising for several applications, including food packaging.

Keywords: Starch; Botanic source; Carboxymethylcellulose; Natural sources; Polymer packages.

1 INTRODUCTION

The environmental pollution caused by large incorrect disposal of non-biodegradable plastics has aroused interest in the development of polymer packaging using biodegradable materials (Dai et al, 2015; Benhamou et al, 2014; Kakaei & Shahbazi, 2016; Rhim, Park & Ha, 2013). Among natural-sourced and biodegradable materials, starch is one of the most promising due to its good film forming properties, to its abundance and consequent low cost

and its ease to be processed in conventional equipments (Cao, Chang & Hunealt, 2008; Dufresne & Castao, 2017, Jiménez et al, 2012).

Starch can be obtained from different botanical sources and consists mainly of linear and branched chains of glucose molecules, designed by amylose and amylopectin, respectively. Its botanical source influences the physicochemical properties of starch-based films as well as the interaction of those constituents with other materials for polymer blends production (Joshi et al, 2013).

Corn is recognized worldwide as the largest commercial source of starch (approximately 65%), followed by sweet potato (13%) and cassava (11%) (Global Markets for Starch Products, 2018; Garcia et al, 2009; Souza, Ditchfield & Tadini, 2010). Cassava stands out with a high yield of starch, being Brazil the fourth largest producer of this root (Luchese, Spada & Tessaro, 2017). Corn starch has an average of 28% amylose, while cassava has approximately 18% by mass (Mendes et al, 2016).

Cassava starch as a matrix for polymeric films offers advantages such as availability, low cost, high amylopectin content and high viscosity, which leads to good film forming properties (Ma et al, 2017). Studies have reported that a higher amount of amylose strengthens starch films mechanically and improves its processing conditions (Basiak, Lenart & Debeaufurt, 2017; Forssell, et al, 2002).

In general, starch films have good barrier properties to oxygen, carbon dioxide (CO₂) and lipids, thus protecting foods against lipid oxidation that compromises their sensory characteristics (Ma et al, 2017). However, the mechanical properties and moisture resistance of these materials are inferior when compared to conventional polymeric films and therefore are limiting factors for their industrial application (Miri et al, 2015).

In order to increase starch films tensile and water vapor barrier properties, natural polymers such as chitosan (Hari, Francis & Nair, 2018; Mendes et al, 2016) and cellulose

(Nawab et al, 2017) have been used for blending with carboxymethyl cellulose (CMC) (Campos et al, 2017; Li et al., 2008; Pongsawatmanit et al., 2018), which have innovative properties (Ghanbarzadeh, Almasi & Entezami, 2010; Sionkowska, 2011).

Other materials from cellulose such as cellulose nanofibers (CNF) and cellulose nanocrystals (CNC) have been widely used as mechanical reinforcement in polymeric materials for packaging (Claro et al., 2019; Li et al., 2019; Claro et al., 2018; Tarrés et al., 2018, Campos et al., 2017.; Wei & McDonald, 2016; Oun & Rhim, 2015). Cellulose nanofibers are used to reinforce starch to take advantage of the chemical similarity between the starch and the cellulose, which results in good interfacial bonding (Saetun et al., 2015). However, when small fillers are used, the major problem is the difficulty of making them disperse in a thermoplastic starch matrix (Aouada et al., 2011). In addition, cellulose nanocrystals from different sources have different characteristics due to hydrophobicity, depending on the aspect ratio of the remaining lignin in their production process (Azeredo et al., 2017).

Fazeli, Keley and Biazar (2018) used CNF as a reinforcing material in corn starch films and reported that 0.4% of CNF increased the tensile strength and the modulus of elasticity of the starch film related to 80% and 170%, respectively. According to the authors, above 0.4% of CNF the material begins decrease the tensile strength.

Carboxymethylcellulose was used in this work due to good solubility in water and in starch solution, which allows evaluating the chemical interaction (hydrogen bonding) between its constituents and the constituents of the starch.

CMC is used as food additive in order to change its texture and to act as a binder or a fluid absorbent in food packaging (Young et al., 2018). CMC with rice starches of different amylose content were studied by Li et al. (2008) who observed crosslinks between waxy rice starch and CMC, which were due to ester bonds formed between hydroxyl groups in amylopectin branches of starch and carboxyl acid groups of CMC.

Mustapha et al (2019) evaluated the potential of a cassava starch biopolymer containing CMC and turmeric oil as kraft paper coating for food packaging and reported that the addition of 10% of CMC as a reinforcing material in the cassava starch package contributed to increased resistance of 12.9 MPa to 15.3 MPa.

Pongsawatmanit et al. (2018) investigated the effect of CMC on the properties of wheat flour-tapioca starch blend films and observed that its use increased the viscosity and consistence without changing the gelatinization process. The authors observed that the use of CMC with starch acted as oil barrier after frying products.

The effect of extrusion on starch/CMC blends were analyzed by Veronese et al. (2018) that observed a different behavior in the films properties when CMC was added after starch extrusion and when it was added before the extrusion, which led to stable extruded gels. They also observed that increases in CMC concentration generate increases in viscosity. The authors have not observed changes on thermal properties once no new covalent linkages were observed.

Here, CMC was added to our formulations of corn and cassava starch, which have different content of amylose and amylopectin, to improve water vapor permeability and hydrophobicity.

In this sense, some studies have evaluated the influence of amylose and amylopectin contents in starch from different botanical sources films while other ones have evaluated the influence of different ratios of CMC addition in starch films. However, the influence of different botanical source with CMC has not been reported yet. Thus, the objective of this work was to evaluate the influence of the botanical source of corn (*Zea mays L.*) and cassava starches (*Manihot esculenta L.*) in the mechanical and physicochemical properties of CMC films produced by casting, more specifically on its water vapor permeability and hydrophobicity.

2 METHODOLOGY

Cassava starch (18% amylose and 82% amylopectin) from Amazon Amido Pará and corn starch (28% amylose and 72% amylopectin) from Unilever Food Solutions were used. Carboxymethyl cellulose was purchased from Synth and glycerol, from Proquimica (São Paulo, Brazil)

2.1 Samples Prepare

Neat starch films were obtained by solvent-cast of aqueous mixtures comprising 75wt % starch (cassava or corn), 30wt % glycerol and 97wt % of deionized water. Each mixture was solubilized at 90 °C for 1 hour in a glycerin bath with mechanic stirring. Afterwards, each mixture was spread on a flat and uniform plate. The film-forming process was conducted in an air circulating oven at 50 °C for 17 h.

Neat carboxymethyl cellulose (CMC) 1wt % was solubilized in deionized water at 40 °C for 1h with magnetic stirring. Afterwards, the mixture was spread on a flat and PTFE uniform plate. The film-forming process was conducted in an air circulating oven at 50 °C for 17 h.

CMC/starch (50/50 wt %) film from cassava or corn starch were obtained from the previous solutions described at the same stirring and film-forming conditions.

2.2 Characterizations

Water vapor permeability rate (WVPR)

Water vapor permeability rate was determined by gravimetry according the standard method of ASTM E96-00. The specimens were cut and placed in acrylic capsules containing silica, oven dried at 100°C for 24 hours and sealed with silicone. The capsules were conditioned in desiccators containing a saturated solution of sodium chloride, providing 75% of relative humidity. The permeability of the film was calculated by linear regression between the weight gain (g) and the time (h), in order to find the angular coefficient values that

determine the amount of water acquired by time ($t \rightarrow \infty$). The water vapor permeability rate (WVPR) of the film was calculated by Equation 1, as follows.

$$WVPR = \frac{tg_{\infty}}{A} \quad (1)$$

With WVPR expressed in $\text{g H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{day}$ and the area A expressed in m^2 .

Water vapor permeability (WVP) was calculated by Equation 2.

$$WVP = \frac{100 WVPR t}{p RH h} \quad (2)$$

With t being the film thickness (mm), p the pure water vapor pressure at 20°C (mmHg), RH the relative humidity at 25°C and h the time in hours. WVP is expressed in $\text{g H}_2\text{O} \cdot \text{mm} \cdot \text{m}^{-2} \cdot \text{day}^{-1} \cdot \text{mmHg}^{-1}$.

Contact angle tests

Samples were submitted to a contact angle analysis in KSV Cam101 equipment. The absorption of a water drop by the blend films was analyzed for 1min and the angles between this drop and the surface of the blend films were calculated by the software Cam2008.

Residual humidity content of the films

Residual humidity content of the films was measured in a thermal scale (model ID50, Marte) with 1 g of the film heated at 105°C .

Mechanical Tests

Samples were tested using a smooth mechanical testing machine (Stable Micro Systems TA.XT Plus Texturometer), with an initial gap of 20 mm and rate of $0.1 \text{ mm} \cdot \text{s}^{-1}$. The analysis was carried out under ASTM D882 standard method (2013). Significant differences among tensile strength, elongation at break and elastic modulus values were determined at 5% significance level by analysis of Variance (ANOVA) and Games-Howell comparative

analyses to indicate cases with heterogeneity of variance between treatments. Statistical analyses were performed with R software, version 3.5.1 (2018).

Fourier transform infrared spectroscopy

Spectroscopic analyses were performed on a Perkin Elmer FT-IR analyzer Vertex 70 (Bruker) using a resolution of 4 cm^{-1} and 32 accumulation scans per measurement.

X-Ray Diffraction

XRD patterns were measured in a Lab X-XRD 6000 diffractometer (Shimadzu) employing $\text{CuK}\alpha$ radiation ($\lambda = 1.5406\text{ \AA}$) at 30 kV and 30 mA.

Crystallinity index was determined using Magic Plot Student software by deconvolution analysis with Gaussian function.

Scanning Electron Microscopy (SEM)

The morphology of films was analyzed by scanning electron microscopy (JEOL microscope, model JSM 6510) at 2kV. Films fractured surfaces were obtained by submerging samples in liquid nitrogen, fracturing with tweezers and conditioning the fractured samples in a desiccator with controlled temperature and relative humidity. Samples were mounted with the fractured surfaces facing up onto aluminum specimen stubs using double-sided adhesive carbon tape. Specimens were sputter-coated with a thin layer of gold.

Thermogravimetric analysis – TGA

The thermal profile of the samples (TGA and DTGA curves) was obtained in a TGA - Shimadzu, model 60H. Samples with mass between 5 and 10 mg were heated from $25\text{ }^{\circ}\text{C}$ to $600\text{ }^{\circ}\text{C}$ using a rate of $10^{\circ}\text{C}\cdot\text{min}^{-1}$. The measurements were performed under dynamic atmospheres of nitrogen and synthetic air, with a flow rate of $60\text{ mL}\cdot\text{min}^{-1}$. One sample was analyzed for each study material.

Statistical analysis

In order to compare the materials for the properties of maximum stress, rupture stress, rupture deformation and elastic modulus, Welch's Variance Analysis (1951) and Games-Howell's (1976) multiple comparison test were used. This methodology was adopted due to the heterogeneity of the material variances in the studied properties. For the WP, moisture and contact angle were used Analysis of Variance, assuming homogeneity of variance and test of multiple comparisons of Duncan. The Bartlett test was applied to verify the condition of homogeneity of variance. The level of significance was 5% and the software used in the analysis was R³ version 3.5.1 (2018).

3 RESULTS

The WVP, contact angle and humidity films values for neat cassava and corn starches, CMC and its blends are shown in Figure 1.

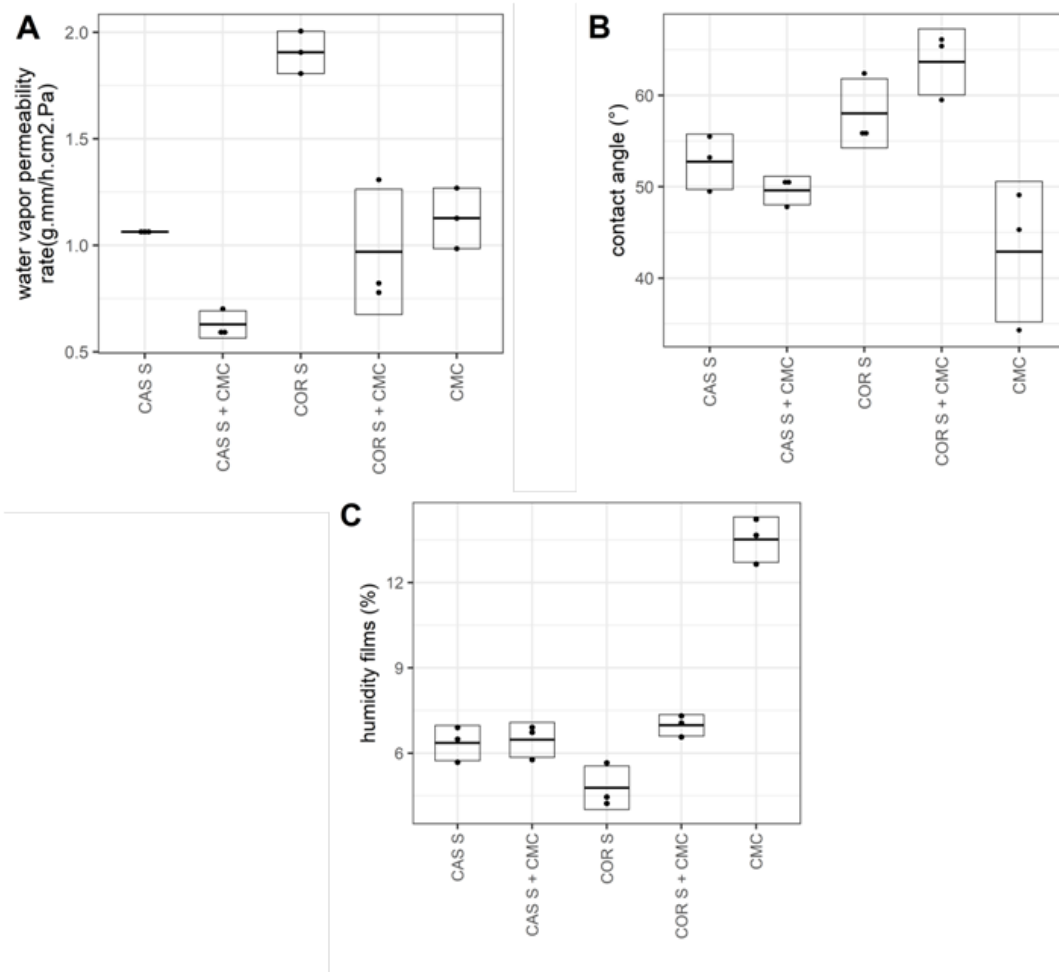


Figure 1 Dot plot with the data of water vapor permeability (A), contact angle (B) and humidity films (C). The box represents the mean values; mean-dp; mean +dp. Samples with the same letter did not present significant differences between their averages, by the Games-Howell (A) or Duncan (B, C) test.

The addition of CMC has decreased significantly the affinity of the corn starch film with water. The amylose and amylopectin present in the starches can influence the interaction between different source starches and CMC. Cassava starch has higher amylopectin content, while corn starch has more amylose. The amount of amylose significantly affects the physico-chemical and functional properties of the starch (Alcázar-alay & Meireles, 2015; Basiak et al, 2017).

It is considered that amylose from corn starch network has a great intermolecular force with glycerol molecules (Luchese, Spada & Tessaro, 2017), which was related to the increased difficulty for water to enter within the molecule of amylose in corn starch films.

The higher water vapor permeability of corn starch films compared to cassava starch ones can be explained by the higher amylose and lower amylopectin contents of the former. Thus, the numerous branching present in amylopectin reduces water vapor permeability by introducing a tortuous path to vapor molecules inside the corn starch matrix (Basiak et al, 2017; Luchese, Spada & Tessaro, 2017).

The blending of CMC and cassava starch has considerably reduced the water vapor permeability of the films due to chemical interactions present as hydrogen bonding between starch and CMC molecules. Those bonding have restricted starch chains mobility and have stabilized its hydrophilic matrix by enhancing the water molecules path in the matrix and reducing its diffusion through the starch/CMC matrix (Kaushik, Singh & Verma, 2010; Ma et al, 2017; Pelissari et al, 2017).

Corn starch films and starch/CMC blends showed the highest contact angle values, indicating a relative hydrophobicity. Cassava starch films and cassava starch/CMC blends showed higher affinity for water than corn starch ones, in agreement with the results of Luchese, Spada & Tessaro (2017).

The final content of water in a polymeric film, especially those from natural and biodegradable sources, is of paramount importance in determining its application, since several processing technique are related mainly to the water absorbed in the polymer and the hydrogen bonding present, as well as their water content interactions (Sessini et al, 2018). CMC has higher intramolecular water content, therefore its addition increased the final water content of the corn starch film and did not affect the residual moisture of the cassava starch film, which may be related to the higher content of amylopectin present in the cassava starch

which is characterized as being the most branched molecule and makes the path more tortuous for water entry in the film (Alcázar-alay & Meireles, 2015; Basiak et al, 2017).

The value of WVP at different relative humidity (RH) gradients is useful to evaluate how well films promote or inhibit the water vapor exchange between the product and the environment. In other words, it is possible to identify whether the films are potentially applicable as food packaging or as films for surface coating since WVP decreased with CMC addition. The RH conditions used for film conditioning modify its crystallinity, as well as its mechanical and barrier properties (Muller et al, 2009).

The mass gain of the films was calculated by means of the water vapor permeability test and the results are presented in Figure 2.

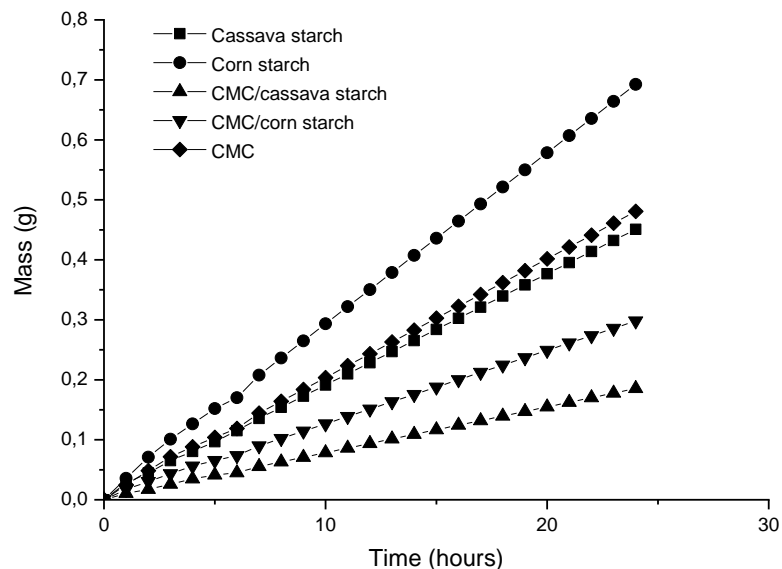


Figure 1 Curves of mass gain as a function of time for the samples of neat cassava and neat corn starches, neat CMC and its blends.

The WVP values for the cassava starch, corn starch, CMC and its blends are shown in Figure 1(A). It was observed that the presence of CMC in both starch films has significantly decreased the WVP. In this case, it is suggested that the chemical interaction by hydrogen

bonds between starch and CMC molecules has restricted the mobility of the former, increasing the films water vapor barrier property such that the water molecules had to follow an increased or tortuous path to diffuse through the matrix of starch / CMC (Kristo & Biliaderis, 2007).

Starch films are hydrophilic due to the chemical composition of starch (amylose and amylopectin), where its hydroxyl groups interact with water molecules by hydrogen bonding (Kuciel & Liber-knec, 2009). Besides that, the addition of a plasticizer increases the material affinity for moisture (Mathew & Dufresne, 2002; Pelissari et al, 2017), once this molecule is also hydrophobic.

The barrier properties of polymeric systems can be altered by the formation of composites and nanocomposites (Almasi et al, 2010; Reis et al, 2017; Silva et al, 2009). The water barrier efficiency depends on the polymeric matrix characteristics, which can alter the diffusion of the penetrating molecule (Reis et al, 2017; Tongdeesoontorn et al, 2011). An increase in barrier properties may also be observed in cases where the polymer matrix relaxation temperature is changed due to the chemical interaction with the added phase (Ma, et al, 2008, Muller; Laurindo & Yamashita, 2009).

Although the water uptake of the CMC films was higher than that of neat cassava starch ones, the blending of CMC and cassava starch reduced the water absorption of the films. It is due to the intermolecular interaction that occurs between starch hydroxyl groups and CMC carboxyl groups during the synthesis process of the films, giving rise to a dense network structure and reducing the migration capacity of the water molecules (Li et al, 2008; Ma et al, 2017).

The hydroxyl and carboxyl groups of the CMC can form strong hydrogen and ester bonds, respectively, with starch hydroxyl groups, thereby improving the interactions between these molecules and the cohesion of the matrix of the polymer blend and decreasing the

absorption capacity of water (Li et al, 2008; Ma et al, 2008; Ghanbarzadeh et al, 2010; Tongdeesoontorn et al, 2011).

Samples submitted to tensile analysis are presented in Figure 3 and in Table 1.

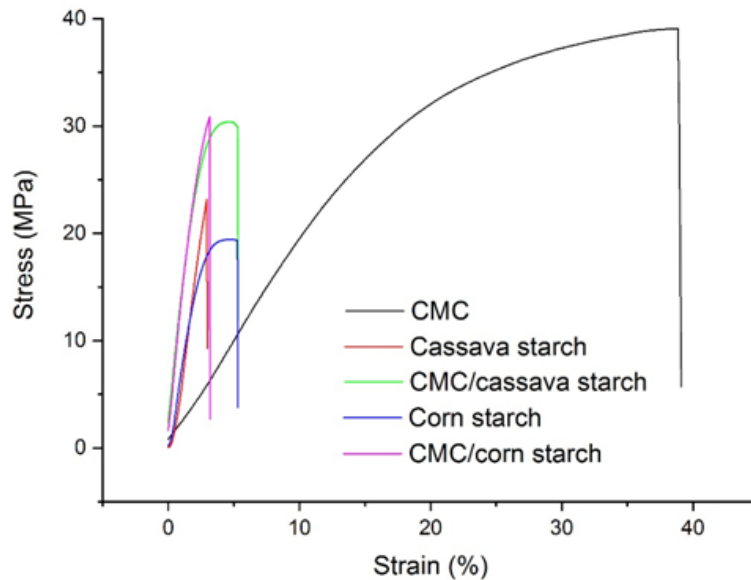


Figure 2 Stress-strain curves for CMC, cassava and corn starches and their blends films.

The curves represent an average of five samples tested for each composition.

Table 1 Tensile strength, stress and strain at rupture and elastic modulus of CMC, cassava and corn starches and their blends films. According to the ANOVA statistical analysis, different letters represent samples with significant differences.

Film sample	Tensile Strength (MPa)	Stress at Rupture (MPa)	Strain at Rupture (%)	Elastic Modulus (MPa)
CMC	38.9 ± 4.8 ^a	44.4 ± 5.0 ^a	43.8 ± 7.8 ^a	2.0 ± 0.1 ^a
Cassava starch	25.3 ± 4.1 ^b	24.8 ± 4.3 ^{b,c}	2.7 ± 0.2 ^b	11.8 ± 1.4 ^b
CMC/cassava	30.4 ± 1.4 ^b	31.2 ± 1.7 ^b	5.1 ± 0.3 ^b	11.7 ± 1.0 ^b
Corn starch	17.3 ± 3.0 ^c	18.2 ± 2.6 ^c	1.8 ± 0.4 ^b	10.8 ± 1.3 ^b
CMC/corn	32.1 ± 3.3 ^{a,b}	31.7 ± 3.6 ^b	17.1 ± 1.2 ^c	12.5 ± 0.6 ^b

CMC addition in starch films increased significantly its maximum stress, regardless the botanical source of the starch, approximating the mechanical properties of the blend films

to those of the neat CMC ones. This increase can be attributed to the chemical similarity of the materials and to the interaction of the starch hydroxyl groups with the CMC carboxyl groups (Li et al., 2008; Ma et al, 2008). The existence of this interaction was previously reported by Avérous et al (2001) and, according to those authors, occurs mainly during the drying of the films, replacing the original hydrogen bonding between starch molecules, making the molecular structure of the starch/CMC blends more compact and therefore more resistant to tensile stress (Almasi et al, 2010; Ma et al, 2017; Tongdeesoontorn et al, 2011).

Although the amylose and amylopectin contents of corn starch may influence the mechanical properties of its films with CMC, however, in this study, the botanical source of starch did not influence in the stress at rupture. Wang et al (2017) reported that higher amylose concentrations in starch may increase the mechanical strength of the starch-gelatin composite films. Therefore, it is expected that the different amylose contents for corn and cassava starch may alter the mechanical properties of its blend films, as showed by Luchese, Spada & Tessaro (2017).

The interactions between the starch, glycerol and CMC molecules present in the films can be evidenced by FTIR spectroscopy.

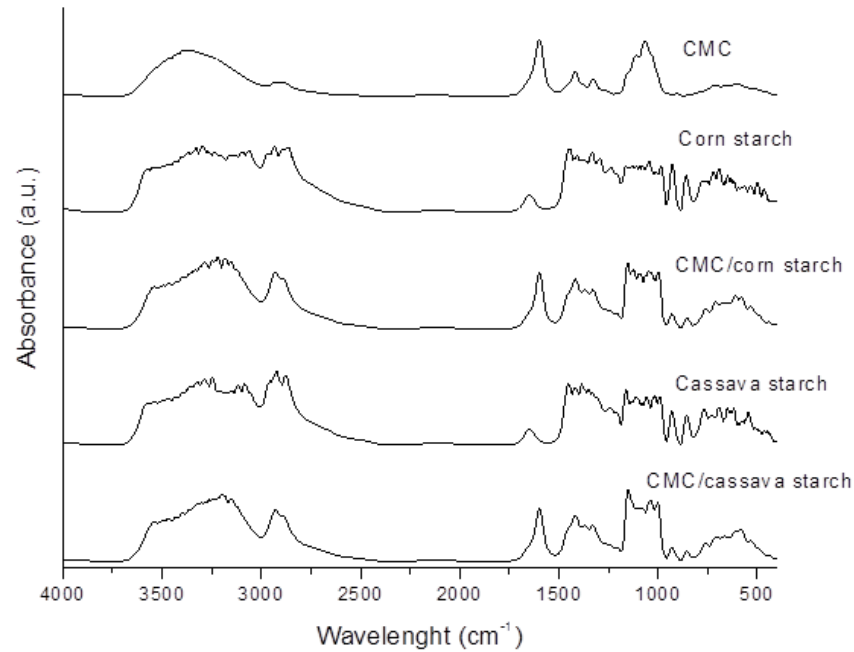


Figure 3 FTIR of CMC, cassava starch, cassava starch/CMC, corn starch and corn starch/CMC films.

Spectra of both starch films showed absorption bands at 920 cm^{-1} , 1022 cm^{-1} and 1148 cm^{-1} , which are characteristic of C-O stretching, 1423 cm^{-1} of glycerol, 1648 cm^{-1} of bound water, 2914 cm^{-1} of CH stretching and 3277 cm^{-1} of simple OH groups binding (Ma et al, 2017; Mendes et al, 2016).

A wide absorption band at 3268 cm^{-1} is evident in all spectra, characterizing an OH group elongation frequency due to interaction with CMC and also water residues (Tongdeesontorn et al, 2011). Spectra of CMC presents a band in the 1597 cm^{-1} related to C=O; however, the spectra of the blends presented higher intensity for this band with the addition of CMC in both starch films, evidencing the occurrence of intermolecular interaction between starch hydroxyl groups and CMC carboxyl groups, forming a more compact and rigid structure (Almasi et al, 2010; Li et al, 2008).

CMC films also presented a band in the region of 1648 cm^{-1} , indicative of bound water in the matrix structure, and showed a displacement to 1597 cm^{-1} for CMC blending with both starches, indicating a possible interaction between the components. Some CMC bands were suppressed by the starch bands because they present clusters in the same spectral region (Ma et al, 2017; Tongdeesoontorn et al, 2011). At 1411 cm^{-1} and 1365 cm^{-1} , there are bands attributed to the folding of the CH_2 and C-OH groups, respectively. Stretching of the C-O-C group can be observed at 1144 cm^{-1} region. The bands occurring in the 1000 cm^{-1} region are related to the crystalline structure of the starch, so the bands observed suggest that the film has an amorphous structure (Tongdeesoontorn et al, 2011).

X-Ray diffractograms and crystallinity index of the neat and blend films are present in the Figure 5 and Table 2, respectively.

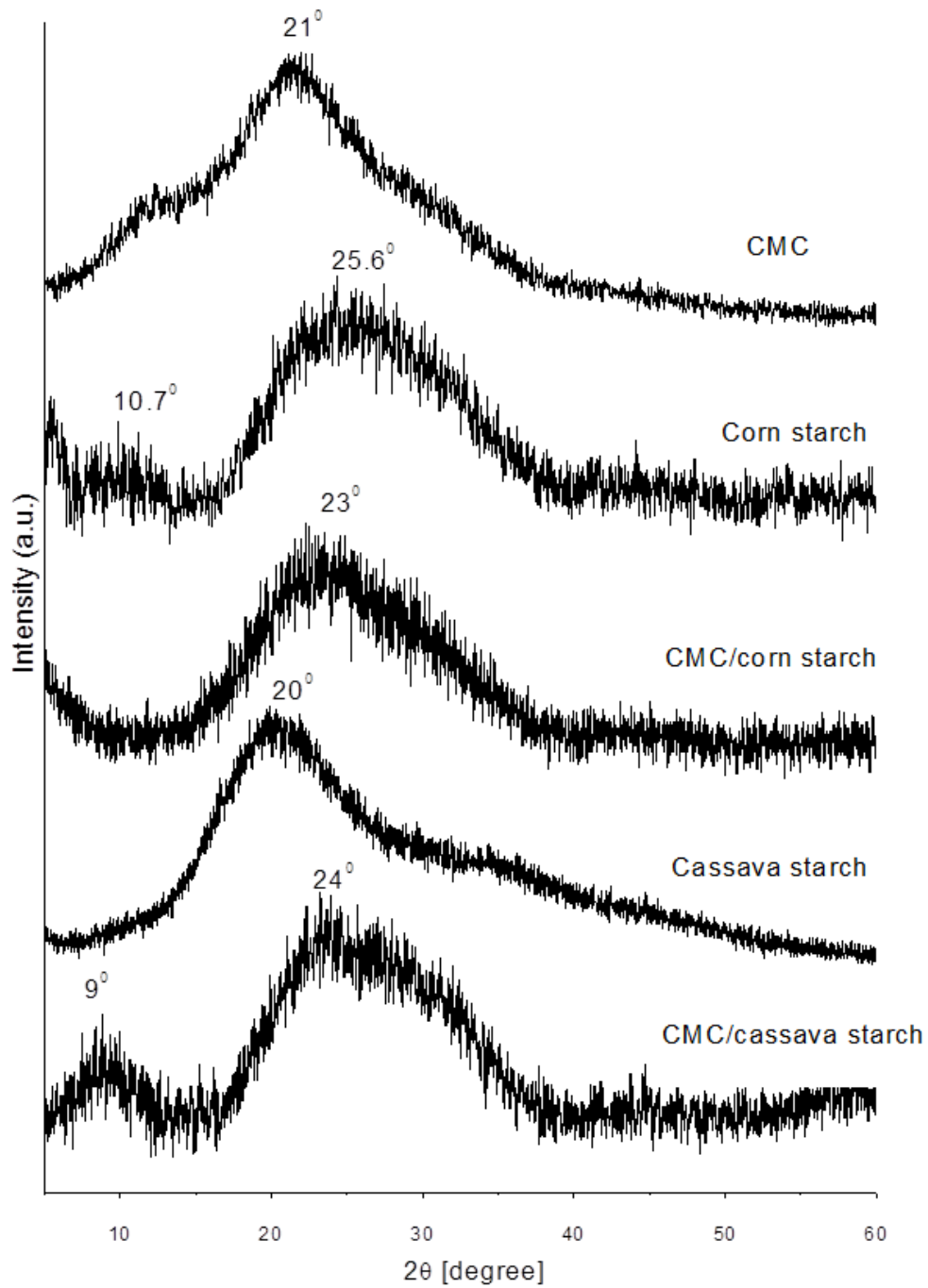


Figure 4 X-Ray diffractograms of: CMC film, cassava starch film, corn starch film, cassava starch/CMC blend film and corn starch/CMC blend film.

Amylose associated with large branches of amylopectin molecules comprises the amorphous region of starch, while amylopectin molecules with short branches comprise the

crystalline region. Therefore, a higher amylopectin content of amylopectin in starch granules results in higher crystallinity (Cheetham & Tão, 1998). Thus, cassava starch films tend to have higher crystallinity than corn starch ones.

Table 2 Crystallinity index of the neat and blend films.

Samples	Cristallinity (%)
CMC	51 ± 2.5
Corn starch	33 ± 2.8
CMC/Corn starch	24 ± 1.4
Cassava starch	35 ± 1.4
CMC/Cassava starch	30 ± 2.1

CMC diffraction pattern displays a broad peak at 51° associated with the low crystallinity of CMC structure that has been previously observed (Chai & Isa, 2013; Hazirah et al, 2016; Shang, Shao, & Chen, 2008). Cassava and corn starch films presented a broad peak at 26° and 20°, respectively, which are related to amorphous peaks attributed to no significant residual A-type crystallinity (Campos et al, 2017).

Cassava starch/CMC and corn starch/CMC blends presented peaks at 24° and 23°, respectively, which represent starch and CMC with no significant residual crystalline structure. The slight shift of the blends peaks is due to starch and CMC interactions. Hazirah et al (2016) also observed a slight shift to 23° related to gelatin and CMC interactions. Normally, the starch crystals are type V and this occurs due to the complexation that occurs between glycerol and amylose (Campos et al, 2013; Magalhães & Andrade, 2009).

Starch granules have between 15% and 45% of crystalline fraction whose composition varies with the starch origin. For cereal starches, such as corn one, the chains arrange themselves in a highly condensed monoclinic structure, comprising type A. For

tuberculous starches, such as cassava one, the structure is hexagonal and is called type B. A third type, C or V, is a mixture of the previously described ones (Waterschoot et al., 2015). According to Campos et al (2017), this crystallization depends on the degree of hydration of the starch and can be classified as V_A type which is characteristic of starch less hydrated than V_H .

The cryogenic and surface fracture morphologies of the films were visualized by scanning electron microscope and the results are presented in Figures 6 and 7, respectively.

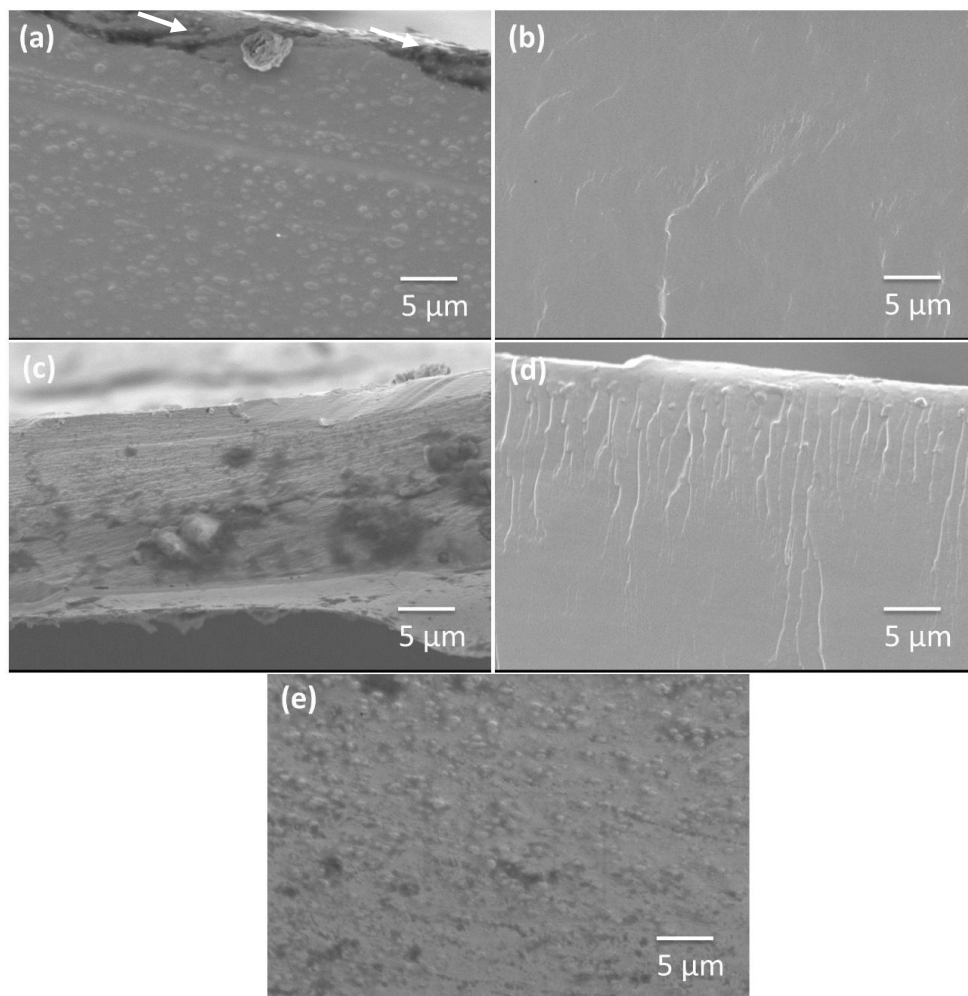


Figure 5 SEM of cryogenic fracture of films: (a) CMC, (b) cassava starch, (c) cassava starch/CMC blend, (d) corn starch and (e) corn starch/CMC blend.

Cross-section of starch films showed that no starch granules remained after the gelatinization process. The blends presented a dense and compact structure and the micro-

cracks observed in the fractures of the neat films decreased after the addition of CMC, which shows a good interaction among their constituents, making it possible to obtain a compact film (Kibar et al, 2017; Kibar et al., 2013). No interruption of the starch/CMC interface was observed, which shows that there was good interfacial adhesion between its constituents. The similarity in the chemical structure contributed to a good interaction between corn and cassava starch with CMC as shown as the structural integrity of the film. This is probably a consequence of hydrogen bonding between hydroxyl groups (Pelissari et al, 2017). In Figure 6e, it is possible to observe some bubbles and irregularities that suggest the presence of CMC particles in the film. Similar results were reported by Salleh, Muhamad & Khairuddin (2009) for starch and chitosan films obtained by casting. Figure 7 shows the surface area of corn and cassava starches, CMC and its blends.

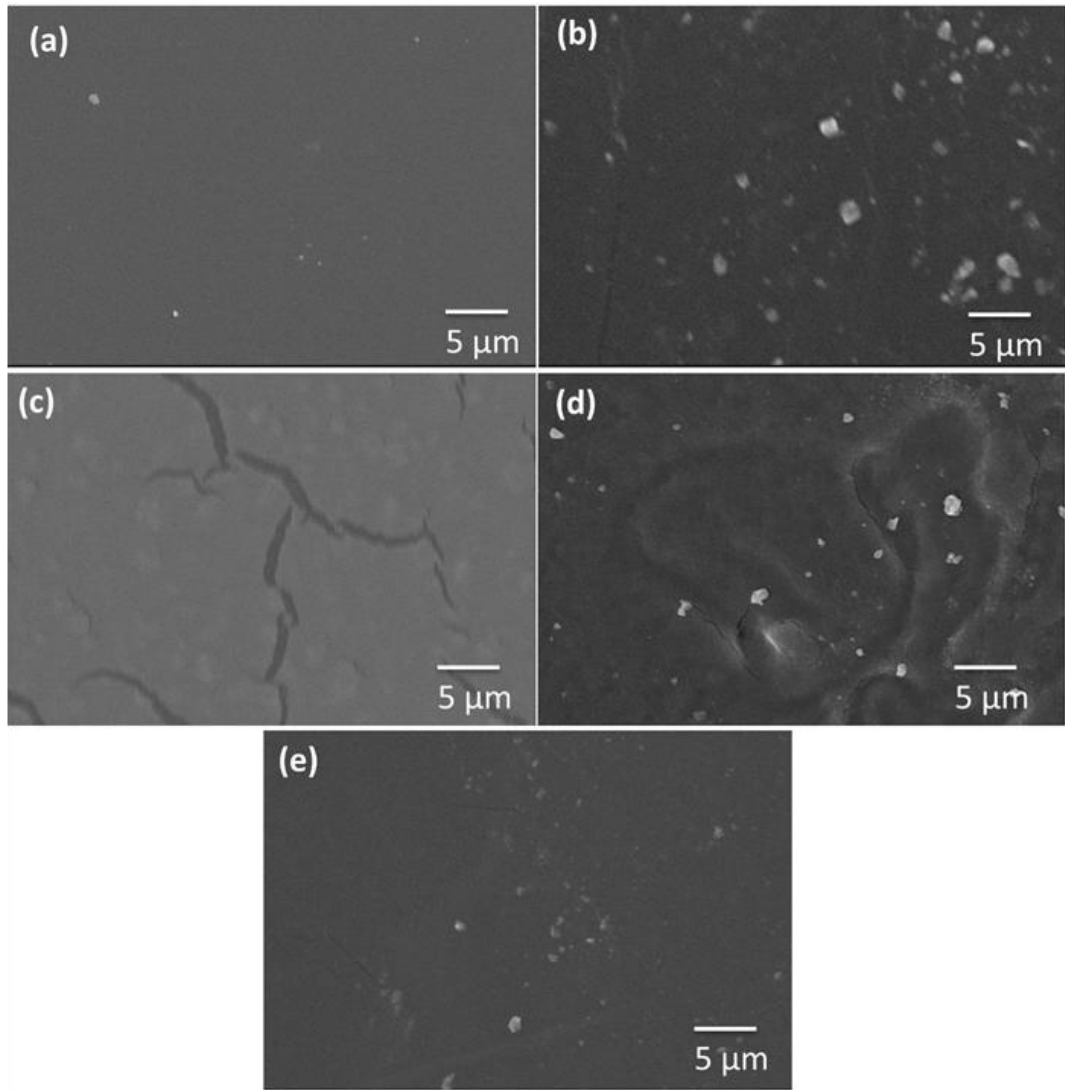


Figure 6 SEM of films surface: (a) CMC, (b) cassava starch, (c) cassava starch/CMC blend, (d) corn starch and (e) corn starch/CMC blend.

In general the films showed bubbles, irregularities and cracks in the surfaces. It is observed the presence of bubbles on the surface of the neat starch films, being corn starch films the ones with the higher density and size of bubbles. The increase in the size of these bubbles caused the films rupture. Higher density and size of the bubbles observed in corn starch films can be explained by their higher amylose content that retrogrades faster than amylopectin because it has a strong tendency to interact by hydrogen bonds with other

adjacent molecules, forming crystalline structures of double helices with the reduction of temperature and with the passage of time (Denardin; Silva, 2008).

After the addition of CMC in starch matrix, the films presented a smoother surface with few smaller bubbles, which suggest good intermolecular interaction (hydrogen bonds) between groups of CMC and starch, according observed by FTIR and reported by Suriyatem et al. (2018). Thus, the presence of higher amylopectin content favors the production of less dense and heterogeneous films, therefore, more transparent, since higher amylose content may lead to thicker and opalescent films (Basiak et al, 2017).

The thermal stability of the films was analyzed by TGA and the results are shown in Figure 8.

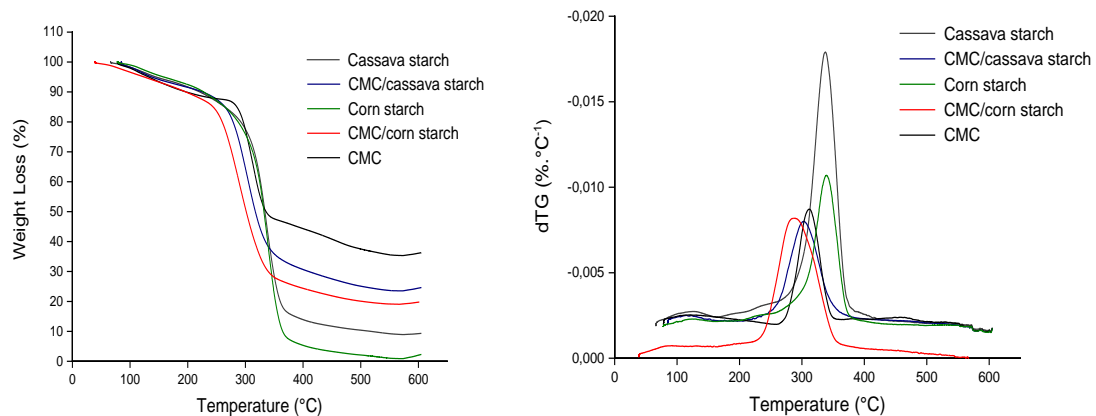


Figure 7 TGA and DTGA thermograms of cassava and corn starch films and their blends with CMC.

Thermogravimetric analysis was used to evaluate the thermal stability of cassava and corn starches films, CMC films and their blends. In addition, the derivative of TGA curves was used to determine the thermal decomposition temperature of the samples, which occurred in three main steps.

The first degradation temperature of all samples occurred at approximately 95°C and referred to water loss. The second stage of degradation showed a maximum degradation rate at 190°C- 225°C and was associated to glycerol and structural bound water loss, which represented 6% of weight decrease (Mu et al., 2012). The third stage referred to the degradation of the constituents of starch and of CMC and occurred in the range of 250°C to 350°C, in agreement with other results reported previously (Suriyatem; Auras; Rachtanapun, 2018; Jaramillo et al., 2016).

A weight loss of approximately 10% for all samples occurred in the first stage and corresponded to water evaporation. The botanical origin of the starch does not change the thermal stability of the neat films. However, the stability of the starch films was changed after the addition of CMC, since the peaks associated with the degradation of the starch-rich phases were reduced, as shown in Table 2.

Table 3 Initial film degradation temperatures and percentage of residues.

Samples	Tonset (°C)	% Residual	T peak (dTG)°C
Cassava starch	291	9	313
CMC/Cassava starch	267	24	277
Corn starch	295	2	315
CMC/Corn starch	257	19	289
CMC	269	37	286

The interaction between starch and CMC resulted in films with greater sensitivity to heat. According to Ghanbarzadeh et al. (2010), the addition of CMC in starch films can act as a lubricating agent and decrease the intermolecular interaction between starch chains, which in turn decreases the degree of crystallinity, as shown in Table 1.

CMC addition reduced the blends thermal stability showing Tonset and Tpeak decrease temperature when compared to neat starch films.

On the other hand, the addition of CMC increased the final residue content of the films. After the addition of CMC, the final weight loss of corn starch films decreased from 91% to 76% and, for cassava starch films, from 98% to 81 %. Similar results were reported by Suriyatem et al. (2018), who studied rice starch films with CMC and determined that the weight loss of blends with CMC tends to decrease when the CMC content increases. According to these authors, the higher thermal stability of the blend films was related to the lower carbon content when compared to carbon content of rice starch.

The results found in the present study are of great relevance and show that starch is a promising material to replace the conventional materials used for the production of food packaging such as poly-(lactic acid) (PLA). As an additional advantage, starch has smaller production costs and can be used a precursor of PLA, once one of the ways to obtain PLA is through the fermentation of starch to produce lactic acid (Mckeen, 2014). PLA has excellent mechanical properties, rigidity and resistance comparable to conventional petroleum-based synthetic polymers such as polyethylene terephthalate (PET) and polystyrene (PS), making it a promising material. However, it has some shortcomings as a higher cost of production compared to non-biodegradable counterparts derived from oil. Another limiting factor is the fact that it is intrinsically fragile, despite its tunable tactic, microstructure and mechanical properties (Koh, Zhang & He, 2018). Therefore, an exclusively PLA-based material with a good balance of toughness and stiffness is still difficult to obtain (Farah, Anderson & Langer, 2016).

In order to turn PLA into a more attractive substitute for conventional petroleum-based polymers, one alternative is to mix it with a lower-cost polymer. Starch has been this alternative due to its low cost, complete biodegradability and its renewable character, making PLA / starch blends objects of several studies and patents for the production of food

packaging (Lu, Xiao & Xu, 2009; Schmidt et al. al, 2014, Koh, Zhang & He, 2018; Alexy et al., 2019).

The main challenge to be overcome by PLA and starch blends is the low interfacial adhesion between their components caused by the difference between the starch hydrophilic and PLA hydrophobic characters. This difficulty leads to additional steps to solve the problem, which makes PLA/starch blends more costly and time-consuming when compared to TPS/CMC blends (Arroyo et al., 2010; Park et al., 2000; Huneault et al., 2007; Kovács & Tabi, 2011; Kim et al., 1998; Chapleau, Huneault, Li, 2007; Ferrarezi et al., 2013).

Thermoplastic starch (TPS, obtained by adding low water contents to the starch) is considered more flexible because of the ability of the plasticizer present therein to decrease the amount of hydrogen bonds between the polymer chains of the starch. (Stepito, 2003). Mixtures of TPS with other biodegradable polymers such as CMC are effective ways of improving their mechanical and water vapor barrier properties, making it more competitive against the conventional materials used for food packaging (Brandelero et al., 2011; Tawakkal et al., 2014). In addition to the advantages cited, (lower cost of starch to conventional polymers and their total biodegradability), TPS can also be produced through conventional thermoplastic processing equipment (injection, extrusion or compression molding at high temperature and shear, for example), which makes it a promising material for the production of sustainable packaging (Averous et al., 2009; Prachayawarakorn et al., 2010; Ochoa-Yepes et al., 2019).

Thus, the results of this work showed that the addition of CMC increased the mechanical and water vapor barrier properties of starch films, both corn starch and cassava starch ones. However, there are still some problems to be overcome, such as the hydrophilicity of the material. To overcome this challenge, additional studies of coating these

films with more hydrophobic materials are being carried out, mainly using the layer-by-layer technique by immersion and/or spraying.

4 CONCLUSION

The addition of CMC increased the maximum tensile stress of both starches films without affecting its elastic modulus. In contrast, there is a small but notable reduction in the thermal stability of the films, independent of the botanical origin of starch. Intermolecular bonding between starch and CMC was observed, which reduced the affinity of starch films with water and considerably reduced the water vapor permeability rate (48% and 40% for corn and cassava starch films, respectively), thus improving its barrier properties. The higher amylose content of the corn starch favored the production of a more hydrophobic film when compared to the cassava starch one, since this higher content contributes to a greater interaction of starch and glycerol molecules, providing stronger intermolecular bonding.

5 ACKNOWLEDGEMENTS

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ARTIGO 2 - Effect of carboxymethyl cellulose concentration on mechanical and water vapor barrier properties of corn starch films

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Katiany Mansur Tavares^{a,*}; Adriana de Campos^b; Bruno Ribeiro Luchesi^c, Ana Angélica Resende^a; Juliano Elvis de Oliveira^d; José Manoel Marconcini^b

^a Program in Biomaterials Engineering (PPGBioma), Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil,

^b National Laboratory of Agribusiness Nanotechnology (LNNA), Embrapa Instrumentação, São Carlos, São Paulo, Brazil.

^c Postgraduate Program in Materials Science and Engineering (PPGCEM), Federal University of São Carlos (UFSCar), São Carlos, São Paulo, Brazil.

^d Department of Engineering, Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil.

Corresponding authors: *mansurtavares@yahoo.com.br; jose.marconcini@embrapa.br

Abstract

The main objective of this study was to evaluate the effect of the addition of different concentrations of CMC (0, 20, 40, 60, 80 and 100%) on the mechanical and water vapor barrier properties in corn starch films produced by casting. The addition of CMC 40% was sufficient to significantly increase its mechanical properties (tensile strength, elongation at break and elastic modulus), and water vapor barrier of the starch films, thus improving its functionality as a packaging material for food. CMC incorporation led to a small reduction in the thermal stability of the films. CMC in low content dispersed well in the starch matrix, ensuring interaction between its constituents that formed a network structure, thus improving mechanical properties and making diffusion of water difficult.

Keywords: polymer blends, biodegradable film, packaging, tensile strength, thermal stability, water vapor permeability.

1 INTRODUCTION

The demand for polymeric plastic packaging materials has increased in recent years due to its properties, such as malleability, versatility, lightness and low cost, which confer numerous advantages to the polymers in this type of application. Environmental and economic concerns associated with the accumulation of non-degradable waste have led to a global interest in replacing non-biodegradable petroleum-based polymers with biodegradable ones, derived from renewable sources (Sessini et al., 2019; Tawakkal et al., 2014).

The use of agricultural products in industrial applications can be considered as a way to reduce environmental pollution and to consolidate the use of these products for other purposes (Sessini et al., 2019; Wjotowicz et al., 2009). In this context, starch is an ideal and sustainable alternative to petroleum-based plastics, mainly due to its abundance, renewability, biodegradability, non-toxicity and low cost (Muthuraj, Misra & Mohanty, 2018). These

properties come from its different sources such as cereals, roots and tubers (Chivrac, Pollet & Avérous, 2009). However, its commercial scale extraction is still restricted to cereals (corn, wheat and rice) and tubers (cassava and potato) (Tabasum et al., 2019).

Corn starch is typically composed of 72% amylopectin and 28% amylose. Amylose is a linear polymer with α -1,4 linked glucose units, while amylopectin is a polymeric structure highly branched with α -1,6 bonds between glucose units, in addition as the previously mentioned α -1,4 bonds. Amylopectin has a much larger size than amylose ($M_w = 10^7 \text{ g mol}^{-1}$ and $M_w = 10^5 \text{ g mol}^{-1}$, respectively) (Vilaplana et al., 2012; Li et al., 2011a).

As a packaging material, starch main deficiencies are low mechanical properties and high permeability to water vapor, which makes its use unfeasible on a large scale (Khan et al., 2017; Miri et al., 2015; Zhang, Rempel & Liu, 2008). The formation of a polymeric blend using the starch together with another natural polymer has been an alternative to overcome those deficiencies and to achieve an increase in the properties that could justify the application of starch as a package material (Hari, Francis & Nair, 2018; Nawab et al, 2017, Sionkowska, 2011, Ghanbarzadeh, Almasi, Entezami, 2010).

In general, starch films have good barrier properties to oxygen, carbon dioxide (CO₂) and lipids (Ma et al, 2017). However, they show lower mechanical properties, specially its tensile strength, and higher water vapor permeability when compared to conventional polymeric films and therefore are limiting factors for their industrial application (Miri et al, 2015).

In order to increase starch films tensile and water vapor barrier properties, natural polymers such as cellulose and carboxymethyl cellulose (CMC) (Li et al, 2008; Campos et al, 2017; Nawab et al, 2017; Pongsawatmanit et al, 2018) have been used for blending with starch (Ghanbarzadeh, Almasi & Entezami, 2010; Sionkowska, 2011).

Carboxymethylcellulose (CMC) is a cellulose derivative, often used as a reinforcing material in biodegradable blends with starch due to their chemical compatibility, which results in a good interaction between starch and CMC and leads to an increase in mechanical and moisture resistances (Suriyatem et al., 2019; Ma et al., 2017; Kibar et al., 2013; Tongdeesoontorn et al., 2011; Almasi, Ghanbarzadeh & Entezamil, 2010; Ghanbarzadeh, Almasi & Entezami, 2010; Ma, Chang & Yu, 2008).

Several studies have reported the effects of CMC on starch films from different sources such as rice (Suriyatem et al., 2019), cassava (Tongdeesoontorn et al., 2011; Ma et al. 2017), pea (Ma et al., 2008) and maize (Ghanbarzadeh, Almasi & Entezami, 2010; Kibar et al., 2013). However, those studies presented more constituents in its blends than just starch and CMC. The starch film proposed in this work has low starch concentration, which makes the material cheap without decreasing its mechanical and functional properties for various applications including food packaging.

Thus, the objective of this work is to evaluate the effect of different concentrations of CMC on the polymer matrix of corn starch, aiming improvements in mechanical and water vapor barrier properties of the films.

2 METHODOLOGY

Corn starch (28wt % amylose and 72wt % amylopectin) by Corn Products Brazil (Amidex 3001) was used. Carboxymethyl cellulose was purchased from Synth and glycerol from Produquimica (São Paulo, Brazil)

2.1 Samples Preparation

Neat starch films were obtained by solvent-cast of aqueous mixtures comprising

75wt% starch and 30wt% glycerol (dry basis) and 97wt% of deionized water. The mixture was solubilized at 90°C for 1 hour in a glycerin bath under mechanical stirring. After that, 100mL of each polymeric solution was verted on non-stick 14 x 14 cm acrylic plates lined with PET substrates to enhance the non-stick effect. The PET substrate was only used as a non-stick material to improve the non-stick character of PTFE plates. The film-forming process was conducted in an air circulating oven at 50 °C for 17 h.

Neat carboxymethyl cellulose (CMC) 1wt % was solubilized in deionized water at 40 °C for 1h under mechanical stirring. Afterwards, 100mL of each polymeric solution was verted on non-stick 14 x 14 cm acrylic plates lined with PET substrates to enhance the non-stick effect. The PET substrate was only used as a non-stick material to improve the non-stick character of PTFE plates. The film-forming process was conducted in an air circulating oven at 50 °C for 17 h.

CMC (0, 20, 40, 60, 80 and 100wt %) and corn starch blends were obtained from the previous solutions previously described at the same procedures.

2.2 Characterizations

Zeta potential

The presence of surface charges in the solutions constituents were evaluated by zeta potential analysis using a Malverne 3000 Zetasizer NanoZS (Malvern Instruments, UK) equipment. Aliquots were prepared by the addition of 1 mL of the polymeric solutions, kept at 25°C. Three measurements were done for each solution.

Mechanical Tests

Samples were tested using a smooth mechanical testing machine (Stable Micro Systems TA.XT Plus Texturometer), with an initial gap of 20 mm and rate of 0.1 mm.s⁻¹. The

analysis was carried out under ASTM D882 standard method (2013). Significant differences of tensile strength, elongation at break and elastic modulus values were determined at 5% significance level by analysis of Variance (ANOVA) using Past software (Hammer et al., 2001).

Fourier transform infrared spectroscopy

Spectroscopic analyses were performed on a Perkin Elmer FTIR analyzer Vertex 70 (Bruker) using the range between 4000 cm^{-1} and 400 cm^{-1} , resolution of 4 cm^{-1} and 32 accumulation scans per measurement. Bands intensities are related to the content of starch and CMC in the samples, as expected by the Law of Lambert-Beer (Smith, 1979).

X-Ray Diffraction

The diffractograms were recorded on a Lab X-XRD 6000 Shimadzu diffractometer operating at 30 kV, 30 mA and $\text{CuK}\alpha$ radiation ($\lambda = 1540 \text{ \AA}$). The samples were scanned in 2θ range varying from 4 to 40° and with scan speed of $0.5^\circ \text{ min}^{-1}$. Crystallinity index (CI) of neat and blend films were determined by the Lorentzian deconvolution method using the software Magic Plot Student 2.5.1. The relation between the areas under the amorphous and crystalline peaks (I_{AM} and I_C , respectively) was used to calculate CI, as expressed in equation 1 (Asthana & Kiefer, 1982; Park et al., 2010).

$$CI = \left(\frac{I_{AM}}{I_{AM} + I_C} \right) \times 100\% \quad (1)$$

Water vapor permeability rate (WVP)

Water vapor permeability rate was determined gravimetrically according to ASTM E96-00 standard method. The specimens were cut and placed in acrylic capsules containing silica, oven dried at 100 $^\circ\text{C}$ for 24 hours, and sealed with silicone. The capsules were conditioned in desiccators containing a saturated solution of sodium chloride, providing 75%

of relative humidity. The permeability of the film was calculated by linear regression between the weight gain (g) and the time (h), in order to find the angular coefficient values that determine the amount of water acquired by time (tg^∞). The water vapor permeability rate (WVPR) of the film was calculated by Equation 2, as follows.

$$WVPR = \frac{tg^\infty}{A} \quad (2)$$

WVPR expressed in g H₂O. m⁻².h and the area **A** expressed in m².

Water vapor permeability (WVP) was calculated by Equation 3.

$$WVP = \frac{100WVPRt}{pRHh} \quad (3)$$

Which t being the film thickness (mm), p the pure water vapor pressure at 20 ° C (mmHg), RH the relative humidity at 25 ° C and h the time in hours. WVP is expressed in g H₂O.mm.m⁻².h⁻¹.mmHg⁻¹. WVP results were statistically analyzed by Scott-Knot ANOVA tests in SISVAR software (Ferreira, 2010).

Surface wettability to water

The surface wettability to water was measured using a contact angle meter (KSV Instruments), calculating the angle with the equipment software (Cam2008). Three values were taken, at $t = \text{zero}$, $t = 60\text{s}$ and $t = 120\text{s}$. Significant differences among the values were determined at 5% significance level by analysis of Variance (ANOVA) using Past software (Hammer et al., 2001).

Scanning Electron Microscopy (SEM)

The morphology of films was analyzed by scanning electron microscopy (JEOL microscope, model JSM 6510) at 5 kV. Films fractured surfaces were obtained by submerging samples in liquid nitrogen, fracturing with tweezers and conditioning the fractured samples in a desiccator with controlled temperature and relative humidity. Samples were mounted with

the fractured surfaces facing up onto aluminum specimen stubs using double-sided adhesive carbon tape. Specimens were sputter-coated with a thin layer of gold.

Termogravimetry analysis – TGA

The thermal profile of the samples (TG and DTG curves) was obtained in a Q500 equipment (TA Instruments, USA), previously calibrated with a zinc standard. Samples with mass between 8 and 10 mg were heated from 25 °C to 600 °C using a heating rate of 10 °C.min⁻¹. The measurements were performed under dynamic atmospheres of nitrogen and synthetic air, with a flow rate of 60 mL.min⁻¹. One sample was analyzed for each study material.

Dynamical-mechanical thermal analysis – DMTA

The dynamical-mechanical analysis were performed in a DMA Q800 equipment (TA Instruments, USA) with samples of 30 mm in length, 5 mm in width and 0.06 mm in thickness. The measurements were made in temperatures between -80 °C and 600 °C, heating rate of 2 °C min⁻¹, constant frequency of 1 Hz and strain amplitude of 10 µm. One sample was analyzed for each study material.

3 RESULTS AND DISCUSSION

Figure 1 presents the samples images showing its transparence kept with CMC content.



Figure 1 Samples image of polymeric films of starch, CMC and its blends.

The zeta potential provides an indirect measure of surface charge density and is an indicator of system stability. The zeta potential of starch, CMC and its blends solutions were measured and are presented in Table 1. The more negative zeta potential for CMC solutions (indicative of higher surface charges) were expected, once there was the presence of carboxylic groups in sodium carboxymethylcellulose ($-\text{CH}_2\text{COO}^- \text{Na}^+$) that are responsible for higher charge density and solubility in aqueous CMC media (Duro et al., 1998; Wang & Somasundaran, 2005). The addition of CMC to starch solutions increases their zeta potential values, confirming that CMC modifies the electrical profile of the solution, causing attraction and electrostatic repulsion between CMC and starch molecules (Cerrutti & Frollini, 2009). Starch solution charges increased as the CMC concentration in the solution increased, which is associated with the presence of CMC COO^- groups. Similar results were reported by Cerrutti & Frollini (2009), who evaluated the CMC zeta potential for application as a stabilizing agent of aqueous alumina suspensions. The authors concluded that, after the addition of CMC, the zeta potential increased once CMC charges prevented the aggregation of alumina particles.

The addition of the lowest concentration of CMC (20%) was able to significantly increase the tensile strength and elongation at break. Samples with 20% and 40% of CMC did not differ statistically for tensile strength, while 20%, 40%, and 60% of CMC did not differ for elongation at break. At concentrations above 60%, the addition of CMC induced a very significant reduction (approximately 63%) in the elongation at break of the starch films. CMC has been previously reported as a booster in starch films, mainly for increasing mechanical strength (Mikus et al., 2014). The increase of the films stiffness, as evidenced by the increase of the elastic modulus (Mali et al., 2005), occurred due to the higher energy required to deform the angles and the distances of the bonds between atoms of the polymer chain, energy arose from the good interaction between starch and CMC constituents, mainly between starch

OH and CMC COOH (Li et al., 2008, Mikus et al., 2014, Mendes et al., 2016; Ma et al., 2017).

This interaction has been reported in other studies that evaluated the properties of starch films blended with carboxymethyl cellulose and, according to the authors, this type of interaction occurs mainly during the drying of the films in which there is the substitution of the hydrogen bonds between starch OH groups by hydrogen bonds between those same OH groups and CMC hydroxyl groups. This substitution form stronger bonds between the chains, which makes the blend structure more compact, reducing the free volume available for chains mobility and demanding more energy to break the blend chains apart during the traction effort (Ma, Chang & Yu, 2008; Almasi, Ghanbarzadeh & Entezamil, 2010, Tongdeestoon et al., 2011; Suriyatem et al., 2019), increasing both tensile strength and elastic modulus values.

CMC films showed elastic modulus 14.5 times higher than the pure starch ones. Even the CMC film exhibiting a larger elastic modulus, the addition of up to 40wt % CMC in starch films has not caused significant differences in the stiffness of the material. Above 40wt % CMC, the increase in tensile strength and elongation at the rupture of the films can be associated with the phase inversion in which CMC becomes the matrix over starch. This fact explains the abrupt increase in tensile strength and elastic modulus values above 40wt % CMC added (Fig. 2, Table 1). The integrity of a film used as packaging is directly related to its ability to withstand mechanical stresses during its application, handling and transportation. In other words, the films must withstand some resistance to rupture and flexibility, being able to deform without causing their rupture. Thus, S60:CMC40 blend proved to be more suitable for this application as packaging, since the addition of CMC increased tensile strength and elongation at break without altering its modulus of elasticity.

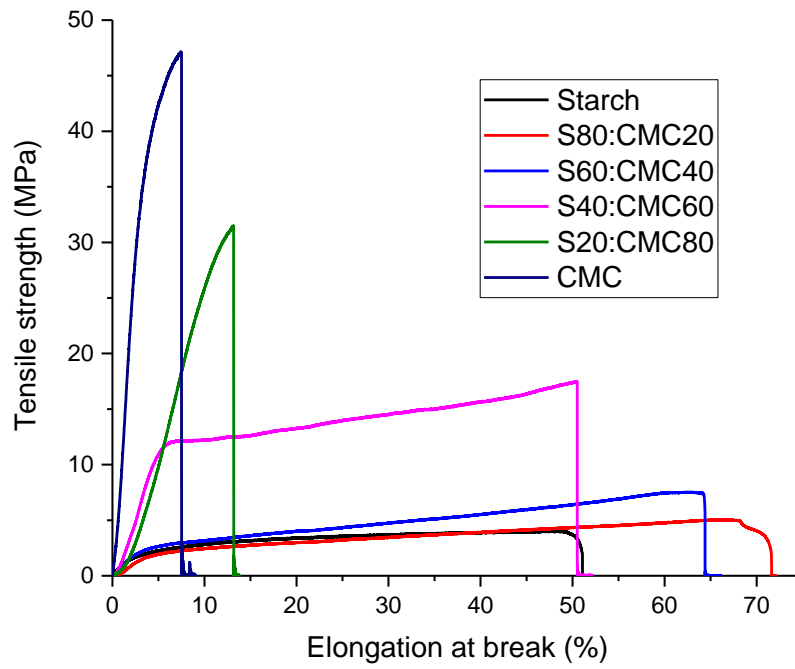


Figure 2 Tensile strength-elongation at break for starch, CMC and its blends.

The FTIR spectra of the films and their blends are shown in Figure 3 and the relative absorbances of two bands (OH and C=O) were calculated and are shown in Table 1.

Table 1. Zeta potential e mechanical properties and relative intensities of absorbance between OH and C=O bands of starch films and starch and CMC blends for corn starch, CMC and its blends.

Samples	Zeta potential (mV)	Tensile Strength (MPa)	Elongation at Break (%)	Elastic Modulus (MPa)	Absorbance relative (A_R)
Corn Starch	-7.61	3.8±0.2 ^a	35.1±8.5 ^a	47.3±12.5 ^{a,b}	0.80
S80:CMC20	-35.4	4.7±0.5 ^b	64.8±6.8 ^b	40.3±11.5 ^a	0.80
S60:CMC40	-43.8	5.5±0.8 ^b	60.8±4.3 ^b	63.1±7.2 ^b	0.81
S40:CMC60	-61.9	17.0±0.7 ^c	56.8±4.1 ^b	295.6±39.8 ^c	0.90
S20:CMC80	-64.3	32.6±2.1 ^d	21.2±4.3 ^c	250.6±2.3 ^c	1.09
CMC	-65.8	50.2±6.9 ^e	7.6±2.2 ^d	684.3±49.1 ^d	1.39

* Equal letters (superscript) in the same column do not differ from each other according to ANOVA at 5% significance.

* A_R = OH relative absorbance/C=O relative absorbance.

CMC has been previously reported as a booster in starch films, mainly for increasing mechanical strength (Mikus et al., 2014). The increase of the films stiffness, as evidenced by the increase of the elastic modulus (Mali et al., 2005), occurred due to the higher energy required to deform the angles and the distances of the bonds between atoms of the polymer chain, energy arose from the good interaction between starch and CMC constituents, mainly between starch OH and CMC COOH (Li et al., 2008, Mikus et al., 2014, Mendes et al., 2016; Ma et al., 2017).

This interaction has been reported in other studies that evaluated the properties of starch films blended with carboxymethyl cellulose and, according to the authors, this type of interaction occurs mainly during the drying of the films in which the substitution of the hydrogen bonds between the OH groups of the starch chains by hydrogen bonds between the OH groups of the starch and the hydroxyl groups of the CMC chains occurs, thus making the blend structure more compact and requiring more tensile strain during the traction effort (Ma, Chang & Yu, 2008; Almasi, Ghanbarzadeh & Entezamil, 2010, Tongdeestoon et al., 2011; Suriyatem et al., 2019).

CMC films showed elastic modulus 14.5 times higher than the pure starch ones. Even the CMC film exhibiting a larger elastic modulus, the addition of up to 40wt % CMC in starch films has not caused significant differences in the stiffness of the material. Above 40wt % CMC, the increase in tensile strength and elongation at the rupture of the films can be associated with their reduction in flexibility (7 times less), which influences their application. The integrity of a film used as packaging is directly related to its ability to withstand mechanical stresses during its application, handling and transportation. In other words, the films must withstand some resistance to rupture and flexibility, being able to deform without causing their rupture. Thus, S60:CMC40 blend proved to be more suitable for this application

as packaging, since the addition of CMC increased tensile strength and elongation at break without altering its modulus of elasticity.

The FTIR spectra of the films and their blends are shown in Figure 2 and the relative absorbances of two bands (OH and C=O) were calculated and are shown in Table 1. In general, when the same bands are observed in the samples, their relative intensities differ. Based on this law, and in agreement with that observed by Gedeon and Ngyuen (1985), an understanding of the limitations of the use of FTIR bands intensity for quantitative analysis, the data should be placed as a function of the percentage of the composition. Then, the mean values of relative absorbance between two bands (OH) and (C = O) were calculated and plotted against the content of starch and CMC in films to overcome problems of thickness variation, as also reported by Ferreira et al. (2018). The ratio of the relative intensities (Table 1) showed that there was a slight increase for samples with concentrations greater than 40% of CMC, which is related to the higher value observed for CMC, suggesting good interaction with the constituents of starch.

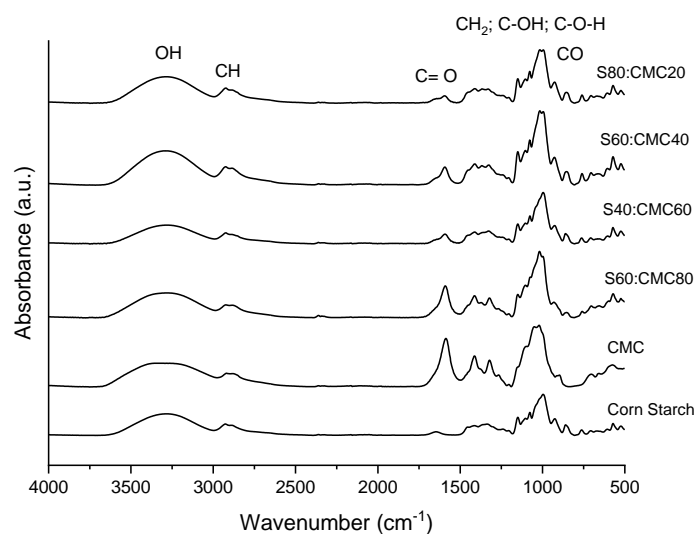


Figure 3 FTIR of CMC, starch and starch/CMC blend films.

The interaction between the constituents of starch and CMC was investigated by FTIR spectroscopy and the main bands appear in two regions (3600 cm^{-1} to 2800 cm^{-1} and 1700 cm^{-1} to 700 cm^{-1}), as also reported in the literature, with absorptions in 917 cm^{-1} , 1024 cm^{-1} and 1140 cm^{-1} (characteristic of the CO stretching), 1425 cm^{-1} for glycerol, 1588 cm^{-1} for C=O and COOH deprotonation and 3299 cm^{-1} for binding of simple OH groups (MA et al., 2017; MENDES et al, 2016).

A wide range of absorption at 3299 cm^{-1} , characterizing an OH group elongation frequency and residual moisture, is evident in all spectra, being more intense in the blends with 40wt % CMC (Ma et al., 2017; Tongdeesoontorn et al., 2011). The determination of the relative absorbance between two binding bands present in the starch molecule (OH and C=O) at 3299 cm^{-1} showed that the S20:CMC80 blend has the highest value (1.09) among the formulations, as shown in Table 1, due to the greater concentration of CMC in the blend, suggesting better interaction between groups of starch (OH) and CMC (COOH). This stretching in the OH group in starch occurs due to the formation of a hydrogen bond between them and the CMC carboxyl (COOH) groups, which makes the film more compact (Almasi, Ghanbarzadeh & Entezamil, 2010; Li et al, 2008).

CMC films also showed bands at 1415 cm^{-1} and 1331 cm^{-1} , which are attributed to folding by plane flexion of CH_2 groups and to COH bond flexion, respectively. At 1147 cm^{-1} , the asymmetric stretching of the COC group occurs (Ma et al., 2017; Tongdeesoontorn et al, 2011). These bands were intensified in the blends due to the interaction between their constituents, which may explain the increase in mechanical properties by the addition of CMC.

Deprotonation of the CMC carboxyl groups can also occur and is observed by stretching of carbonyl (-CO) and of protonated carboxylic acid (-COOH) groups in bands

occurring at 1588 cm^{-1} (due to asymmetric $-\text{COO}-$ bending) and in 1412 cm^{-1} (due to symmetrical $-\text{COO}-$ stretching) (Gonzaga et al., 2018).

Other bands at 995 cm^{-1} and 1144 cm^{-1} (CO stretching) and 2930 cm^{-1} (CH asymmetric stretching) are present in all spectra, but with displacements due to the interactions between the constituents of the blends (Ma et al, 2017; Mendes et al., 2016, Rachtanapun et al., 2012). Some bands of CMC were suppressed by starch bands because they had clusters in the same spectral region (Ma et al, 2017; Tongdeesoonorn et al, 2011).

The bands occurring in the 1000 cm^{-1} region, attributed to the hydrogen bonding of C6 hydroxyl group of starch structure, are related to the crystalline structure of the starch and, according to studies by Van Soest et al. (1995). The authors evaluated the influence of water content on the crystalline structure of starch and suggested that the film had an amorphous structure due to the high amount of amylopectin present in corn starch (about 75wt %), which made the carbon 6 in the crystalline structure became practically inaccessible to the hydroxyl.

According to the authors, changes and displacements of the band attributed to C-OH groups can be attributed to variations in the molecular environment of the primary hydroxyl groups of amylose, resulting from changes in intramolecular hydrogen bonding. In addition, it is possible to note that the intensity of this band in the starch film increases with the addition of CMC as shown in the diffractograms (Figure 4) and Table 2.

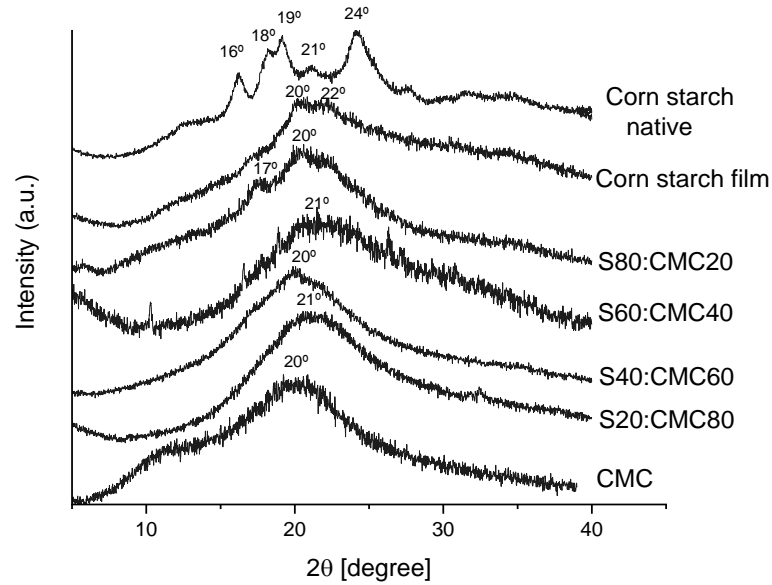


Figure 4 X-Ray diffractograms of: Corn starch native, Corn starch film, Starch/CMC blend film and CMC film.

Figure 4 shows XRD patterns of native corn starch, plasticized corn starch, corn starch/CMC and CMC films used as samples. Main diffraction peaks of native corn starch were at 2θ values of 16° , 18° , 19° , 21° and 24° , which indicated the type A crystalline structure, characteristic of cereal starches (Ramirez et al, 2010; Guimarães et al., 2010). Starch granules have between 15% and 45% of crystallinity, depending on its origin. In a previous study, the authors obtained cassava starch films with a crystalline fraction of 36%, while corn starch films had 33%. The peak of pure starch film showed that its gelatinization occurred successfully and that its structure is predominantly amorphous in shape. According to Campos et al. (2017) and Van Soest et al. (1996), crystallization depends on the degree of hydration of the starch and can be classified as V_A or V_H type.

The low crystalline index of starch films is attributed to the interaction of its chains with the plasticizer and/or the CMC, which reduces the number of hydrogen bonds between

the starch chains and prevent their approximation to form the crystalline arrangement. The acetyl groups in the starch increase the hydrogen bonds between starch and water, thus promoting the melting of the granular starch (Niranhana-Prabhu & Prashantha, 2018).

CMC diffraction patterns exhibit characteristic peaks at 10° and at 15° - 25° , showing its semi-crystalline structure, and crystallinity index of 51%, as also reported in other studies due (Hazirah et al, 2016; Chai & Isa, 2013; Shang, Shao & Chen, 2008; Ikhuoria et al., 2017; Parid et al., 2018; Kimani et al., 2016). Ikhuoria et al. (2017) obtained CMC with high crystallinity index (57%). The authors showed that the crystallinity of CMC can be related to the synthesis method applied in obtaining the cellulose prior to CMC synthesis. Crystallinity in CMC from bleached fibers compared to cellulose from neat fibers tends to be higher, since lignin and hemicellulose is known to contribute to its amorphousity. Parid et al (2018) extracted bleached fibers from oil palm empty fruit bunch, with crystallinity index of 88.6% due to withdrawal of lignin and hemicellulose. According to the authors, the cellulose molecules treated with an alkaline solution during the carboxymethylation process cause swelling in the cellulose particles that exert pressure on the crystalline part in the molecules and distort them favorably. The dissociation and distortion of the crystalline part caused by the swelling of cellulose molecules further reduce crystallinity to 45.0% for CMC. Li et al. (2011b) also studied the effect of oxidation on the degree of crystallinity of CMC. Based on this, the crystallinity index reported by the authors was reduced (CI = 80%, 70%, 64%, and 61%, respectively) almost proportionally to the oxidation level of the initial CMC (aldehyde content = 0%, 45%, 68%, and 81%, respectively). The authors considered that the loss of crystallinity results from the opening of the glucopyranose rings, therefore the higher the level of oxidation, the lower the degree of crystallinity.

The crystalline indexes and water vapor permeability of the films was evaluated and the results are presented in Table 2.

Table 2 Crystallinity index and water vapor permeability (WVP) of starch films and their blends with CMC.

Samples	Cristallinity index (%)	WVP (g H ₂ O.mm.m ⁻² .h ⁻¹ .mmHg ⁻¹)*
Corn Starch	33	2.65±0.43 ^d
S80:CMC20	16	1.57±0.28 ^b
S60:CMC40	19	1.14±0.56 ^a
S40:CMC60	25	0.94±0.40 ^a
S20:CMC80	33	0.85±0.17 ^a
CMC	51	2.28±0.28 ^c

*Mean + standard deviation. Samples with the same letter in the column did not present significant differences among the means by the Scott-Knott test ($p < 0.05$).

The addition of CMC in the starch films reduces the crystallinity index. This reduction in crystallinity may be related to the interaction between the starch OH and the CMC COOH groups, which restricts the mobility of starch chains and difficult the recrystallization. Suriyatem et al. (2019) studied rice starch films with CMC and reported similar results. According to the mentioned study, the reduction of crystallinity is related to the limitation on the formation of amylose-glycerol complexes after the introduction of CMC, suggesting that the regularity of starch films can be interrupted by the intermolecular bonds between starch and CMC groups. Increasing the amount of CMC in the starch/CMC blends causes increases in the crystallinity index because of the higher CMC crystallinity when compared to the corn starch film, as seen in Table 2.

The high water vapor permeability (WVP) of starch films has been considered as a limiting factor for their application as packaging material. This parameter is useful for evaluating how well the films promote or inhibit the exchange of water vapor between the product and the environment and how vulnerable are the effects of moisture on its mechanical

properties. Moreover, it is possible to evaluate whether the films are potentially applicable as food packaging or as films for coating surfaces (Muller et al, 2009).

The presence of CMC in the blends significantly decreased the WVP. The addition of 20% CMC reduced WVP by 40% and that the concentration of 40% reduced WVP by 56%. Above 40wt % of CMC, the reduction was not significant. This is because, in concentrations of up to 40% of CMC, the number of groups (COOH) was sufficient to interact with the groups (OH) of the starch. This interaction decreases the number of OH available in starch. With low charge content, the CMC probably dispersed well in the polymeric starch matrix, interacting with its constituents and forming a compact network that acts as a block against water vapor. However, an excess of CMC can induce an agglomeration between its molecules, which decreases the effective content of CMC in the blend in order to reduce its efficiency against water vapor permeation. This result indicates that the formation of CMC polymeric blends with starch improves water resistance to some extent, as CMC must also be considered to be a hydrophilic material (Ma et al., 2008).

This behavior was previously reported by Ghanbarzadeh et al. (2010) and, according to these authors, low CMC contents are better dispersed in starch matrix and allow the occurrence of hydrogen bonds between starch and CMC chains. The interaction between starch and CMC groups restricts the mobility of the starch chains that leads to a longer and more tortuous path for water vapor molecules through the structure of starch/CMC films, reducing their diffusion and, consequently, the permeability to water vapor (Kristo & Biliaderis, 2007).

According to Li et al. (2008), during the heating and drying processes, CMC carboxyl groups react with starch hydroxyl groups to form an ester bond, which leads to the formation of a more structured matrix and to the consequent reduction in the number of OH available, preventing the diffusion of water vapor molecules.

This reduction of water vapor permeability of starch films results in better functional properties, considering the hydrophilic characteristics of the matrix. The decrease of the WVP by the incorporation of another biopolymer was previously reported in other studies with starch blends for packaging applications (Arvanitoyannis & Biliaderis, 1999; Ma et al., 2008; Fama et al., 2009; Ma et al., 2017).

Contact angle results (Table 3) show the same trend in surface wettability as the one observed for the WVP analysis, except for the CMC value.

Table 3 Contact angles for corn starch, CMC and their blends films.

Samples	Contact Angle (°)		
	0s	60s	120s
Corn starch	68.21 ± 4.45 ^a	62.38 ± 3.99 ^a	61.47 ± 3.28 ^a
S80:CMC20	64.08 ± 2.06 ^{a,b}	61.11 ± 4.54 ^{a,c}	58.58 ± 5.53 ^{a,b}
S60:CMC40	56.47 ± 6.26 ^b	54.91 ± 4.30 ^{a,c}	54.34 ± 2.87 ^b
S40:CMC60	66.54 ± 3.51 ^{a,c}	57.40 ± 2.66 ^{a,c}	52.97 ± .67 ^{b,c}
S20:CMC80	66.72 ± 3.89 ^{a,c}	56.73 ± 4.02 ^{a,c}	52.31 ± 3.65 ^{b,c}
CMC	62.02 ± 6.37 ^{a,b}	53.95 ± 4.76 ^{a,c}	53.14 ± 4.30 ^{b,c}

*Mean + standard deviation. Samples with the same letter in the column did not present significant differences among the means by the Scott-Knott test ($p < 0.05$).

There was little difference between CMC and all the blends contact angle values, with a slight decrease tendency, which shows that CMC reduced the surface hydrophobicity of starch films. Surface CMC carboxyl groups that had not interacted with starch OH groups by hydrogen bonds were free to interact with water molecules, which increase the contact area between the CMC film surface and the water drop on it. The great interaction seen by CMC carboxyl groups and water molecules at the surface increased the adsorption step of permeability and, consequently, the values of WVP for CMC films, as seen previously.

The films cryogenic fracture morphologies are presented in Figure 5.

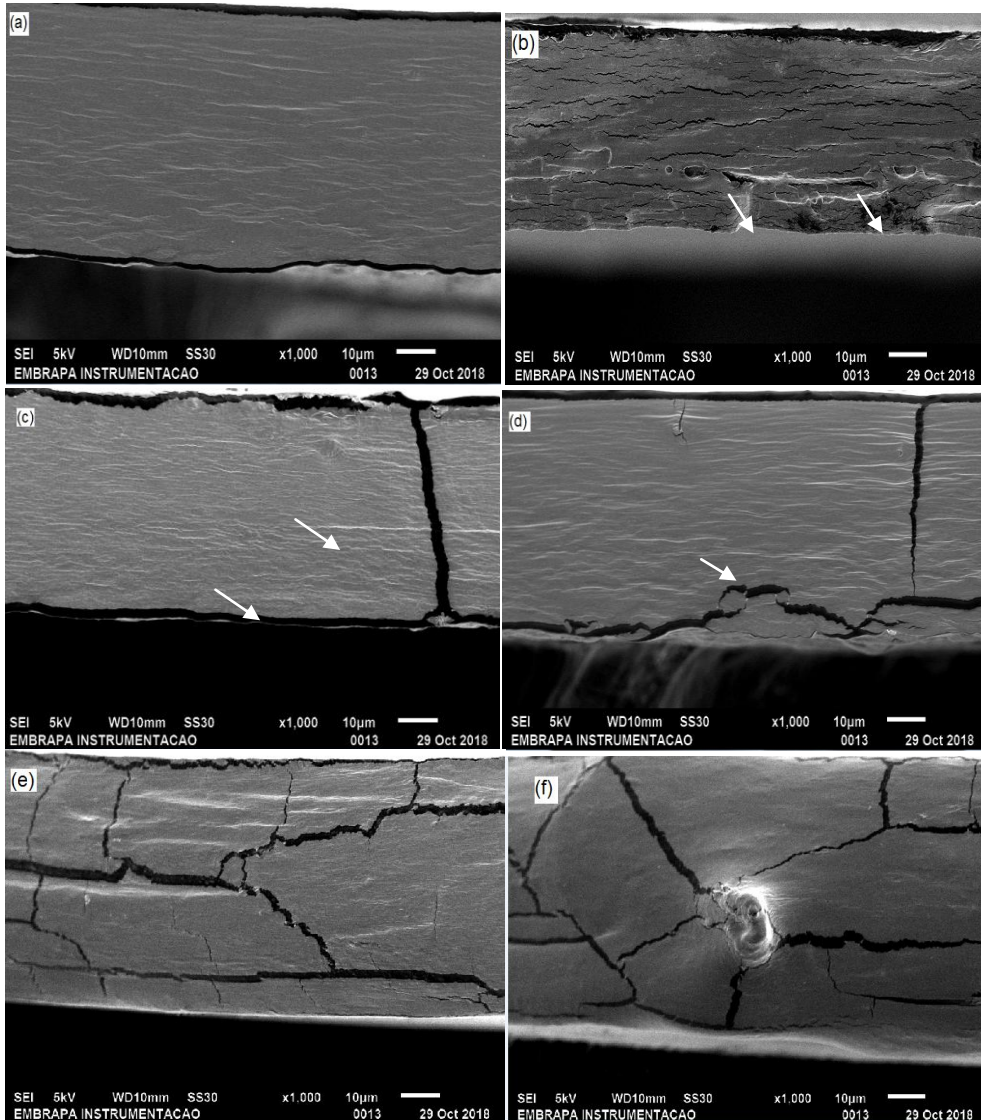


Figure 5 SEM of cryogenic fracture of films: (a) CMC, (b) Corn Starch, (c) S80:CMC20, (d) S60:CMC40, (e) S40:CMC60 and (f) S20:CMC80.

The cross section of the films showed an absence of starch granules, indicating that gelatinization was successful. The blends presented a dense and compact structure and the micro-cracks observed in the fractures of pure starch films decreased, which was highlighting the good interaction between their constituents and the possibility of making a compact film (Ma et al, 2017). The blends presented a homogeneous and compact structure, showing no

interruption of the interface of starch films when added up to 40wt % of CMC, which relates to the good interfacial adhesion among its constituents.

The similarity in the chemical structure contributed to the good interaction between starch and CMC, as demonstrated by the structural integrity of the film. This is a consequence of the hydrogen bonding between its constituents groups (Pelissari et al, 2017). Similar results were reported by Salleh, Muhamad & Khairuddin (2009) for starch and chitosan films obtained by casting.

In Figures 5e and 5f, related to starch films with 60wt % and 80wt % of CMC, respectively, it is possible to observe the presence of cracks inside the films, suggesting that the interaction is no longer effective as once observed for the blends with 20wt % and 40wt % of CMC, related to the excess of CMC in the film. Figure 6 shows the surface area of starch, CMC and their blends films.

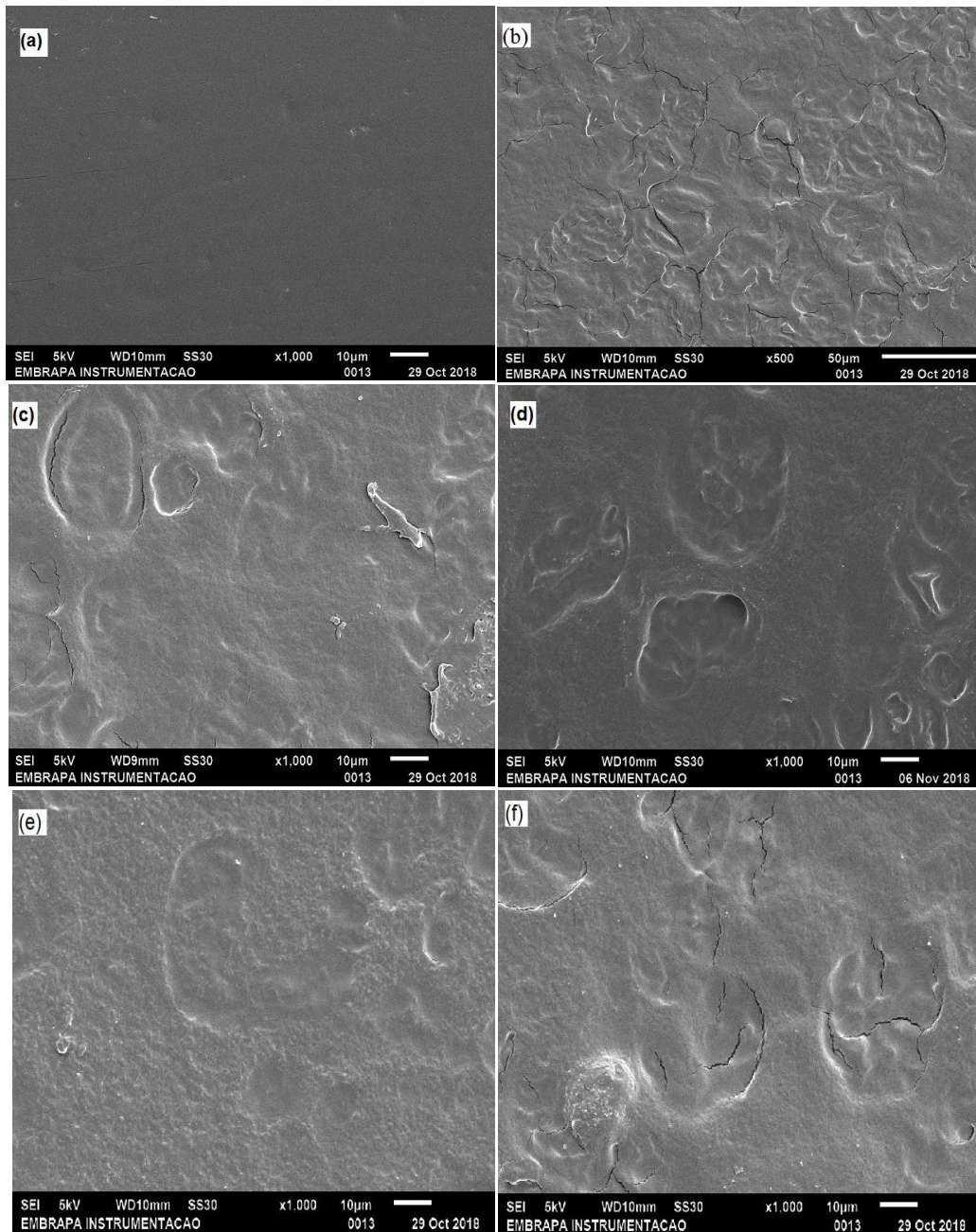


Figure 6 SEM of films surface: (a) CMC, (b) Corn Starch, (c) S80:CMC20 blend, (d) S60:CMC40 blend, (e) S40:CMC60 blend and (f) S20:CMC80 blend.

Pure starch films showed cracks and high density of bubbles on the surface, whose increase in size caused the rupture of the film during the mechanical tests. The high bubble density observed in the pure starch film may be related to its higher amylose content, which is

recrystallized faster than amylopectin and has a stronger tendency to interact with adjacent molecules via hydrogen bonds, forming crystalline structures of double helices (Denardin & Silva, 2008).

After the addition of CMC in the starch matrix, the films presented a smoother surface with fewer amounts of bubbles, suggesting good intermolecular interaction between CMC and starch groups, as evidenced by FTIR and reported by Suriyatem et al. (2019). This interaction is also responsible for the increase in the mechanical and water vapor barrier properties of the films. However, excess CMC can cause cracking on the surface of the film, as shown in Figure 6f and also in Figure 5e and 5f.

The thermal stability of the films was analyzed by TG and the results are shown in Figure 7.

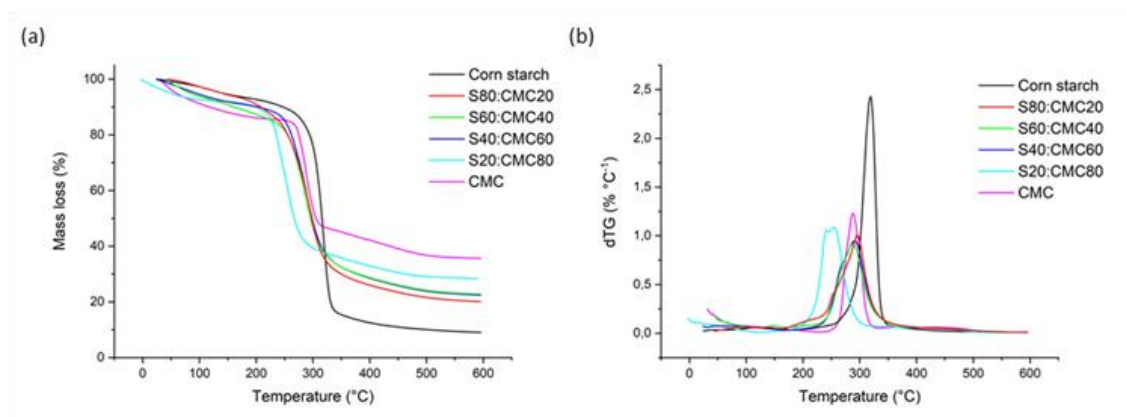


Figure 7 (a) TGA and (b) DTGA thermograms of starch films and their blends with CMC.

Thermogravimetry was used to evaluate the thermal stability of CMC and starch films and their blends. In addition, the derivative of TGA curves was used to determine the thermal decomposition temperature of the material, which occurred in three main steps.

The first degradation temperature of the films occurred at approximately 95°C and refers to the loss of water; the second step of the thermal degradation of the films is related to

the volatilization of glycerol and occurs between 145°C and 160°C and the third stage is due to the degradation of the constituents of starch and CMC and occurred in the range of 250°C to 350°C and is in agreement with other results reported previously (Suriyatem, Auras & Rachtanapun, 2019; Jaramillo et al., 2016).

The degradation temperatures of the films were determined and the results are shown in Table 4.

Table 4. Initial film degradation temperatures and percentage of residues.

Samples	T _{onset} (°C)	% Residual	T _{peak} (dTG)°C
Corn Starch	294	9	318
S80:CMC20	234	20	295
S60:CMC40	253	23	288
S40:CMC60	252	28	291
S20:CMC80	255	28	271
			283
CMC	259	36	288

Approximately 5% of mass loss of films occurred in the first stage and is related to the evaporation of water and glycerol. The stability of the starch films was altered after the addition of CMC, since the peaks associated with degradation of the starch-rich phases were reduced from 294 °C to 234 °C and 255 °C for the lowest and highest CMC film content, respectively. According to Ghanbarzadeh et al. (2010) the lower level of CMC can act as a lubricating agent and decrease the intermolecular interaction and the association in the matrix of the starch film, which in turn decreases the degree of crystallinity, as shown in Table 2. This change in the peak position indicates that higher levels of CMC favor the formation of large crystalline domains and reduce the mobility of amylopectin (Mondragón, Arroyo & Romero - Garcia, 2008).

The blends presented lower thermal stability than starch film and residual mass was 20% for S80:CMC20 blend and 28% for S40:CMC60 and S20:CMC80 blend. It is not worthy that the S40: CMC60 film exhibited similar thermal stability to S20: CMC80.

The addition of CMC reduced the thermal stability of the films because both the T_{onset} and T_{peak} was reduced, showing that there was loss of mass for the blends at a temperature lower than the view for the pure starch film. This fact may be related to the lower thermal stability of CMC as also reported by Ma et al. (2008) and Suriyatem et al. (2019). However, the mass loss rate (given by the dTG value in Table 4) was lower for the blends when compared to the neat films, which is related to the more compact structure of the blends, as a result of starch OH and CMC COOH hydrogen bonding. The chains in the blend films are not so exposed as in the neat ones, fact that turns difficult their degradation and consequent mass loss. The peaks of the dTG for the thermal degradation of the S20:CMC80 blend shows secondary reactions occurring in two steps, suggesting the presence of thermo degradation of two materials at different temperatures due to the excess of CMC in the blend.

A Figure 8 illustrates the dynamic mechanical test results for the films of neat starch and CMC films containing 20 – 80 wt% starch. The loss modulus may be related to energy dissipation of viscoelastic response of the polymer as well their blends in a wide range of temperatures, by the relative slippage of their chains, which is evident in the glass transition temperature of the samples, a peak in the curves. The loss modulus was sensitive to the molecular motions and its peak related to the glass transition temperature (Ma et al., 2018)

Starch film presented biphasic structure due to the partial miscibility between starch and glycerol, as observed by Campos et al. (2017). The two decays in the temperature of loss and storage modulus (Fig. 8) are observed; the first transition temperature decays was at -54°C , related to α -relaxation of glycerol-rich phase. The second transition beginning decays at

47 °C, correspondent to α -relaxation of starch-rich phase, which was regarded as the glass transition temperature of starch materials. Although, CMC present monophasic structure, showing decay in the temperature centered at approximately 4 °C.

The temperature of loss and storage modulus (Fig. 8a) for Starch/CMC was higher than that neat TPS, which was related to stiffness increase due to starch and CMC interactions. Both starch-phase could form intermolecular interactions with CMC, which was observed by both upper transition and lower transition shifted to higher temperature. However, the shift was more pronounced in the upper transition, as also observed by Ma et al. (2008).

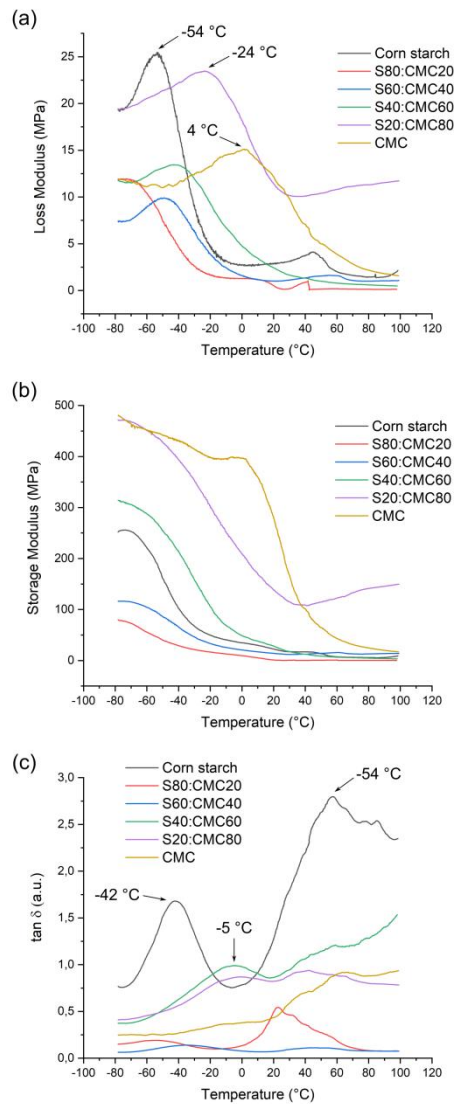


Figure 8 Behavior of (a) loss modulus, (b) logarithm storage modulus and (c) $\tan \delta$ upon increasing temperature of glycerol plasticized starch film and its respective blends with different CMC contents.

The interaction between the CMC chains is more intense than the interaction between the starch chains due to the presence of highly polar groups in the former (COH), which induce a greater number of hydrogen bonds between the chains and hinders their relative slippages. Leading to higher glass transition temperatures. For corn starch, the interaction between its OH groups is less intense due to its lower polarity. Therefore, the higher the

starch content and the lower the CMC content, the interaction between the chains becomes weaker, facilitating relative movement and reducing the transition temperature.

The storage modulus, related to the ability of the chains to recover a strain imposed on them, decreases with temperature as the free volume between the chains increases and the interaction between them is reduced to allow relative sliding. The increasing of CMC in the blend showed the drop of this module with temperature increase. The intermolecular interaction of starch and CMC reduced the free volume and brought adjacent starch chains closer, raising the T_g of the blends, as also observed at damping modulus (tan delta) (Fig. 8). The CMC chains interacted with the starch molecules via hydrogen bonds, which approached these molecules, reducing the free volume between them and, consequently, increasing the tan delta peak temperature, as also observed by Ma, Chang & Yu (2008).

4 CONCLUSIONS

The addition of 40% CMC in the starch matrix is sufficient to increase the tensile strength, the elongation at break and the barrier property of the films. The flexibility of the films is not altered for the formulations with up to 40% CMC. In contrast, there is a small but notable reduction in the thermal stability of the films. The increase of the mechanical properties and reduction of the water vapor permeability of the blends are evidenced by the FTIR spectrum and by the morphological analysis that show the good interaction of starch and CMC constituents, leading to the formation of a transparent, compact and without phase separation films. In general, blended corn starch films with up to 40% CMC are promising materials for packaging application.

5 ACKNOWLEDGEMENTS

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ARTIGO 3 - Antimicrobial activity, mechanical and structural properties of active starch / CMC films by the addition of BP100 antimicrobial peptides

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Atividade antimicrobiana, propriedades mecânicas e estruturais de filmes ativos de amido / CMC pela adição de peptídeos antimicrobianos BP100.

Katiany Mansur Tavares^{a,*}; Adriana de Campos^b; Bruno Ribeiro Luchesi^c, Marcelo Porto Bemquerer^d; Juliano Elvis de Oliveira^a José Manoel Marconcini^b

^a Programa de Pós-Graduação em Engenharia de Biomateriais (PPGBiomat), Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brasil,

^b Laboratório Nacional de Nanotecnologia Aplicada ao Agronegócio (LNNA), Embrapa Instrumentação, São Carlos, São Paulo, Brasil.

^c Programa de Pós-Graduação em Ciência e Engenharia de Materiais (PPGCEM), Universidade Federal de São Carlos, São Carlos, São Paulo, Brasil.

^d Embrapa Recursos Genéticos e Biotecnologia, Brasília, Distrito Federal, Brasil.

Corresponding authors: *mansurtavares@yahoo.com.br; & jose.marconcini@embrapa.br

Resumo

O amido de milho e a CMC foram utilizados para o desenvolvimento de novas embalagens antimicrobianas com o peptídeo BP100. As concentrações de CMC foram estabelecidas em estudo anterior e a quantidade de BP100 foi baseada na concentração mínima inibitória (MIC). Os filmes foram avaliados quanto à cor e transparência, interação com a água, propriedades mecânicas (tração), estruturais (FTIR e DRX), térmicas (TG), microscopia eletrônica de varredura (MEV) e atividade antimicrobiana. O peptídeo não modificou a cor e a transparência dos filmes. A boa interação entre o amido, CMC e a presença de grupos hidrofóbicos no BP100, evidenciadas pelos espectros de FTIR, favoreceu um incremento na resistência à tração e no módulo de elasticidade e reduziu a deformação na ruptura e, além disso, aumentou a hidrofobicidade dos filmes. A cristalinidade do material foi reduzida após a inserção de CMC e do peptídeo e as micrografias mostraram que BP100 provocou irregularidades na superfície. Um aumento na temperatura de degradação térmica dos filmes foi observado após a adição do peptídeo. *S. aureus* foi a cepa mais resistente ao BP100, sendo necessária uma maior concentração para inibir seu crescimento, e *Salmonella* foi a mais sensível, necessitando um baixo teor do peptídeo para garantir a atividade antimicrobiana do filme. Assim, os filmes propostos apresentaram potencial para serem utilizados como material antimicrobiano inibindo o crescimento de *Salmonella*, *E. coli* e *S. aureus*.

Keywords: filmes poliméricos, amido, peptídeos antimicrobianos, embalagem ativa, atividade antibacteriana.

1 INTRODUÇÃO

Os filmes poliméricos à base de amido têm sido estudados para a produção de embalagens visando minimizar os impactos ambientais causados pelo grande volume de produção e descarte dos materiais convencionais, pois além de sua origem natural, são abundantes, de baixo custo e biodegradáveis (Meira et al., 2017; Dufresne; Castao, 2017; Jiménez et al., 2012). No entanto, estes apresentam baixa resistência mecânica e sensibilidade ao vapor de água (Miri et al., 2015), sendo comumente misturados à outros polímeros como a carboximetilcelulose (CMC) para a produção de embalagens visando aumentar a resistência à tração, hidrofobicidade, barreira ao vapor de água, ao oxigênio e lipídeos (Tavares et al., 2020; Tavares et al., 2019; Ma et al., 2017; Tongdeesoontorn et al., 2011; Ghanbarzadeh; Almasi; Entezami, 2010).

Além disso, a deterioração de alimentos e os surtos de origem alimentar impulsionam a demanda por novas tecnologias para controlar o crescimento de microrganismos patogênicos principalmente na superfície dos alimentos (Huang et al., 2019; Meira et al., 2017; Arfat et al., 2014). Assim, embalagens ativas têm sido desenvolvidas para oferecer mais segurança e qualidade aos produtos alimentícios. Neste tipo de embalagem, o produto e o ambiente interagem para prolongar o tempo de armazenamento, melhorar a segurança alimentar ou as propriedades sensoriais, mantendo a qualidade do alimento (Zhong et al., 2020; Santos et al., 2018; Soares et al., 2009; Vermeiren et al., 1999).

Embalagens ativas contendo peptídeo antimicrobiano (AMP) surgiram como uma abordagem promissora para retardar a deterioração e aumentar a segurança alimentar e a vida útil dos produtos alimentícios (Al-Tayyar, Youssef, Al-hindi, 2020; He, Fei, Li, 2020; Huang et al., 2019; Sung et al., 2013; Han, 2003). Dessa forma, a

utilização de peptídeos antimicrobianos de ocorrência natural ou análogos sintéticos tem sido considerada promissora para superar esse desafio contra infecções bacterianas (Corrales-Urena et al., 2020; Santos et al., 2018; Meira et al., 2017; Manzini et al. 2014; Sobczak et al., 2013).

O uso de AMPs exibe uma série de propriedades vantajosas tais quais a eficácia contra ampla gama de bactérias - incluindo cepas resistentes a antibióticos - baixa toxicidade para células de mamíferos, reduzido tamanho molecular e alta estabilidade química (Corrales-Urena et al., 2020; He, Fei, Li, 2020; Santos et al., 2018; Meira et al., 2017; Sobczak et al., 2013; Yoshinari et al., 2010; Huang, 2009). Peptídeos antimicrobianos têm sido utilizados na preservação de alimentos há muitos anos (Zhong et al., 2020; He, Fei, Li, 2020; Meira et al., 2017, Ahmad et al., 2017; Settanni & Corsetti, 2008; Cutter, Willett & Siragusa, 2001).

Estes peptídeos podem ser incorporados em matrizes poliméricas para desenvolver embalagens ativas antimicrobianas devido à sua notável eficiência inibitória contra micro-organismos, mesmo em baixas concentrações (Corrales-Urena et al., 2020; Yin et al., 2017). Muitos AMPs apresentam alta estabilidade térmica e sua incorporação em resinas poliméricas é possível mesmo através de extrusão ou outros métodos de processamento dependentes de calor (Corrales-Urena et al., 2020; Phambu et al., 2017; Zehetmeyer et al., 2015; Zang et al., 2015; Bhatia & Bharti, 2014; Steven & Hotchkiss, 2008).

O BP100 é um peptídeo antimicrobiano sintético curto (KKLFFKKILKYL-NH₂), obtido a partir da combinação de cecropina A e melitina, sendo altamente ativo e seletivo contra bactérias gram-negativas, além disso apresenta baixas citotoxicidade e hemólise (Wadhvani et al., 2014; Badosa et al., 2013; Badosa et al., 2007). Outra

vantagem é a ausência de resíduos de aminoácidos propensos à oxidação, como triptofano e metionina (Chan & White, 2000). Seu mecanismo de ação é dependente da neutralização da carga superficial microbiana e a concentração inibitória mínima (MIC) para várias bactérias gram-negativas está entre 2,5 e 5 μ M (Manzini et al., 2014; Torcato et al., 2013; Alves et al., 2010; Badosa et al., 2007). O BP100 tem aplicação potencial contra *Escherichia coli* (Alves et al., 2010) e outras bactérias Gram-negativas (Badosa et al., 2007), porém não apresentou atividade significativa contra *S. aureus*, bactérias Gram-positivas testadas (Alves et al., 2010).

Apesar do grande potencial dos peptídeos em diversas áreas biotecnológicas, como, por exemplo, embalagens de alimentos e curativos para a pele, a atividade antimicrobiana do BP100 em matrizes poliméricas ainda não foi avaliada. Outros peptídeos antimicrobianos têm sido incorporados em filmes poliméricos com ação antimicrobiana satisfatória para uso em embalagens de alimentos (Woraprayote et al., 2018; Meira et al., 2017, Zehetmeyer et al., 2015; Zhang et al., 2015; Espitia et al., 2013; Steven & Hotchkiss, 2008). Assim, o objetivo deste trabalho foi desenvolver filmes de amido/CMC ativo incorporando o peptídeo BP100 e avaliar sua atividade antimicrobiana, propriedades mecânicas, funcionais e de resistência ao vapor de água.

2 METODOLOGIA

Foi utilizado amido de milho (28% de amilose e 72% de amilopectina) fornecido pela Corn Products Brazil (Amidex 3001). A carboximetilcelulose foi adquirida da Synth e glicerol da Produquímica (São Paulo, Brasil).

2.1 Síntese dos peptídeos em fase sólida

BP100 (H-KKLFKKILKYL-NH₂) foi sintetizado pelo método em fase sólida (Chan & White, 2000; Albericio, 2004), com a utilização da resina “Rink Amide” (Peptides International, Louisville, KY, Estados Unidos). As reações de desproteção do grupamento Fmoc foram conduzidas com uma solução de 4-metilpiperidina em *N,N*-dimetilformamida (DMF) a 20% durante 20-30 minutos (em duas etapas de 10-15 minutos). Os acoplamentos para formação das ligações peptídicas foram conduzidos com 1,3-diisopropilcarbodiimida (DIC) e acetato de etil-2-ciano-2-(hidroxiimino) (Oxyma®, Subirós-Funosa et al., 2009) e *N,N'*-diisopropiletilamina (DIEA) em DMF durante 60 a 120 minutos. As etapas de desproteção e acoplamento foram monitoradas pela reação de ninhidrina (Chan & White, 2000; Friedman, 2004). Após cada etapa de desproteção e acoplamento, a resina foi lavada três vezes com metanol e DMF, de modo alternado. Completada a síntese, a reação de desproteção final e de desligamento entre o peptídeo e a resina foi conduzida em solução de ácido trifluoroacético na presença de nucleófilos (*e.g.* triisopropilsilano, 1,2-etanoditiol, tioanisol) como sequestradores de carbocátions durante 120 minutos à temperatura ambiente. Após precipitação do material bruto com éter diisopropílico e quatro a seis lavagens com o mesmo solvente, o peptídeo foi recolhido por filtração em funil de placa porosa, extraído com água e liofilizado. Os peptídeos foram purificados por cromatografia em fase reversa e foram caracterizados por espectrometria de massa (MALDI-TOF) e por cromatografia em fase reversa analítica.

2.2 Microrganismos, manutenção e padronização dos inóculos

Foram utilizadas as cepas *Escherichia coli* INCQS, *Salmonella enterica* Typhimurium INCQS e *Staphylococcus aureus* INCQS obtidas da coleção de culturas da Fundação Oswaldo Cruz (Rio de Janeiro, RJ, Brasil).

As culturas foram mantidas e reativadas em caldo triptona de soja (TSB) a 37°C por 24h. Após reativação, as culturas foram padronizadas em aproximadamente 10^8 UFC/mL utilizando-se o grau de 0,5 da escala de McFaddin.

2.3 Determinação da Concentrações Mínimas Inibitórias (CMI) de BP100

A CMI de BP100 sobre *E.coli*, *S.Typhimurium* e *S. aureus* foi determinada utilizando-se a técnica de macrodiluição em caldo (CLSI, 2019) com adaptações. Soluções contendo BP100 em diferentes concentrações foram elaboradas em TSB adicionados em microtubos. As concentrações utilizadas foram de $3,26 \times 10^8$ µg/mL de *S. aureus*, $4,17 \times 10^8$ µg/mL de *S.Typhimurium* e $5,12 \times 10^8$ µg/mL. Alíquotas de 22 µL das culturas padronizadas foram inoculadas e os tubos incubados a 37°C por 24h. A absorbância (DO 625 nm) das culturas foram medidas após o período de 18 a 24h de incubação. A CMI foi definida como a menor concentração de BP100 capaz de inibir completamente o crescimento das bactérias

2.4 Obtenção dos filmes antimicrobianos

2.4.1 Preparo do filme

Os filmes de amido puro foram obtidos por *casting* com misturas aquosas compreendendo 75% (m/v) de amido e 30% (m/m) de glicerol e 97% de água deionizada. A mistura foi solubilizada a 90 ° C por 1 h em banho de glicerina sob

agitação mecânica. Depois disso, 100 mL de cada solução polimérica foram vertidos em placas de acrílico antiaderente de 14 x 14 cm revestidas com substratos de PET (Polietileno Tereftalato) para melhorar o efeito antiaderente. O substrato PET foi usado apenas como material antiaderente para melhorar o caráter antiaderente das placas de PTFE (Politetrafluoretileno). O processo de formação de filme foi conduzido em estufa com circulação de ar a 50 ° C por 17 h.

A carboximetilcelulose pura (CMC) 1% em peso foi solubilizada em água deionizada a 40 ° C por 1 h sob agitação mecânica. Posteriormente, 100 mL de cada solução polimérica foram vertidos em placas de acrílico antiaderente de 14 x 14 cm revestidas com substratos de PET para melhorar o efeito antiaderente. O substrato PET foi usado apenas como material antiaderente para melhorar o caráter antiaderente das placas de PTFE. O processo de formação do filme foi conduzido em estufa com circulação de ar a 50 ° C por 17 h.

As misturas de CMC (0, 20, 40 e 100% em peso) e amido foram obtidas por meio de misturas das soluções anteriores.

2.5 Incorporação de BP100 nos filmes

Foram adicionados 5mg, 10mg e 15mg de BP100 por 100 mL de solução polimérica. O peptídeo foi adicionado diretamente nas soluções poliméricas puras e nas blendas, depois de preparadas, no momento em que seriam vertidas nas placas e levadas para a etapa de *casting*, resultando em um filme ativo de 14 cm x 14 cm com espessura variando de 0,072 a 0,109 μm .

2.6 Atividade Antimicrobiana dos Filmes

A atividade antimicrobiana dos filmes contendo BP100 foi avaliada empregando-se a técnica de disco-difusão (CLSI, 2019) com adaptações. Em placas de Petri contendo ágar tripton de soja (TSA) foram espalhados 100 µL das culturas padronizadas, seguida da disposição dos discos dos filmes (aproximadamente 5 mm de diâmetro) contendo 5mg, 10mg e 15mg de BP100 por 100 mL de solução polimérica. As placas foram incubadas a 37°C por 48 horas. Após incubação, os diâmetros dos halos de inibição formados foram mensurados com auxílio de um paquímetro. O experimento foi realizado em triplicata com três repetições.

2.7 Caracterizações

Cor da superfície dos filmes

Os parâmetros de cores dos filmes foram medidos com um colorímetro (Minolta, CR-400, Osaka, Japão). As medições foram realizadas na escala CIELAB, na qual cada medição é expressa em L * (indicando os valores de luminosidade, escuro para claro variando de 0 a 100), a * (positivo na direção vermelha e negativa na direção verde), e b * (positivo na direção amarela e negativo na direção azul), auxiliando na quantificação das variações de coloração apresentado pelas amostras. Os valores padrão referem-se à placa branca de calibração (L = 94,23, a = -0,55 e b = 9,68). Os resultados foram expressos como a média de cinco medidas realizadas aleatoriamente em diferentes áreas de cada filme.

Transparência

As amostras de filme foram cortadas em retângulos e colocadas diretamente em uma célula de teste de espectrofotômetro (Shimadzu UV-1800, Kyoto, Japão), tomando a cubeta em branco como referência. A transmitância da luz foi determinada no comprimento de onda de 600 nm e o valor da transparência foi calculado a partir da seguinte equação (Han & Floros, 1997).

$Transparência = -\frac{\log T_{600}}{X}$ em que T_{600} é a transmitância a 600 nm e X é a espessura do filme (milímetros).

Ângulo de contato

A molhabilidade da superfície dos filmes foi medida usando um medidor de ângulo de contato (KSV Instruments), calculando o ângulo com o software do equipamento (Cam2008). Foram tomados três valores, em $t = \text{zero}$, $t = 60\text{s}$ e $t = 120\text{s}$.

Absorção de umidade

A absorção de umidade foi medida de acordo com o método de Angles e Dufrense (2000). Filmes com 20 mm x 20 mm de diâmetro foram acondicionados a 0% de RH (sílica gel) por 24 h. Após pesagem, foram acondicionados em dessecador contendo solução saturada de NaBr entre 20 ° C e 25 ° C para garantir umidade relativa de 55%. As amostras foram pesadas em intervalos desejados até que o estado de equilíbrio fosse alcançado. A absorção de umidade das amostras foi calculada da seguinte forma:

$$\text{Absorção de umidade (\%)} = Mf - \frac{Mi}{Mf} \times 100 \quad (1)$$

Onde M_f e M_i são o peso da amostra após 24h a 55% UR e o peso inicial da amostra, respectivamente. Todas as medições foram realizadas em triplicata.

Taxa de permeabilidade ao vapor de água (WVP)

A taxa de permeabilidade ao vapor de água foi determinada gravimetricamente de acordo com o método padrão ASTM E96-00. As amostras foram cortadas e colocadas em cápsulas de acrílico contendo sílica, secas em estufa a 100 ° C por 24 h e seladas com silicone. As cápsulas foram acondicionadas em dessecador contendo uma solução saturada de cloreto de sódio, proporcionando 75% de umidade relativa. A permeabilidade do filme foi calculada por regressão linear entre o ganho de peso (g) e o tempo (h), a fim de encontrar os valores do coeficiente angular que determinam a quantidade de água adquirida pelo tempo ($tg \infty$). A taxa de permeabilidade ao vapor de água (WVPR) do filme foi calculada pela Eq. (1):

$$TPVA = \frac{tg \infty}{A} \quad (1)$$

Onde, TPVA expresso como g H₂O. m⁻².dia e A, área em m².

A permeabilidade ao vapor de água (PVA) foi calculada por meio da equação 2.

$$PVA = \frac{100 TPVA . e}{p UR} \quad (2)$$

Onde, e consiste na espessura do filme (mm), p é a pressão de vapor de água pura na temperatura de 20 °C, expressa como mmHg e UR a umidade relativa a 25 °C. A PVA é expressa como gH₂O.mm .m⁻².dia⁻¹ .mmHg⁻¹.

Os resultados do WVP foram analisados estatisticamente pelos testes Scott-Knot ANOVA no software SISVAR (Ferreira, 2010).

Ensaaios Mecânicos

As amostras foram testadas usando uma máquina de teste mecânico lisos (Texturômetro Stable Micro Systems TA.XT Plus), com uma abertura inicial de 20 mm e taxa de $0,1 \text{ mm.s}^{-1}$. A análise foi realizada de acordo com o método padrão ASTM D88 (2013). Diferenças significativas dos valores de resistência à tração, alongamento na ruptura e módulo de elasticidade foram determinadas no nível de significância de 5% por análise de Variância (ANOVA) usando o software Past (Hammer, Harper & Ryan, 2001).

Microscopia Eletrônica de Varredura (MEV)

A morfologia dos filmes foi analisada por microscopia eletrônica de varredura (microscópio JEOL, modelo JSM 6510) a 5 kV. As superfícies fraturadas dos filmes foram obtidas submergindo as amostras em nitrogênio líquido, fraturando com pinça e condicionando as amostras fraturadas em um dessecador com temperatura e umidade relativa controlada. As amostras foram montadas com as superfícies fraturadas voltadas para cima em *stubs* (espécime de alumínio) usando fita adesiva dupla face de carbono. Os espécimes foram revestidos com uma camada fina de ouro.

Espectroscopia no infravermelho por transformada de Fourier (FTIR)

As análises espectroscópicas foram realizadas em um analisador Perkin Elmer FTIR Vertex 70 (Bruker), usando a faixa entre 4000 cm^{-1} e 400 cm^{-1} , resolução de 4 cm^{-1} e 32 varreduras por medida. As intensidades das bandas estão relacionadas ao

conteúdo de amido e CMC nas amostras, conforme esperado pela Lei de Lambert-Beer (Smith, 1979).

Difração de Raios X

Os difratogramas foram registrados em um difratômetro Lab X-XRD 6000 Shimadzu operando a 30 kV, 30 mA e radiação CuK_{α} ($\lambda = 1540 \text{ \AA}$). As amostras foram digitalizadas na faixa 2θ variando de 4 a 40° e com velocidade de varredura de $0,5^{\circ} \text{ min}^{-1}$. O índice de cristalinidade (CI) de filmes puros e misturados foi determinado pelo método de deconvolução de Lorentzian usando o software Magic Plot Student 2.5.1. A relação entre as áreas sob os picos amorfo e cristalino (I_{AM} e I_C , respectivamente) foi usada para calcular o IC, conforme expresso na Eq. (4) (Asthana & Kiefer, 1982; Park et al., 2010).

$$CI = \frac{I_c - I_{am}}{I_c} \times 100 \quad (1)$$

Análise de Termogravimetria – TGA

O perfil térmico das amostras (curvas TG e DTG) foi obtido em um equipamento Q500 (TA Instruments, EUA), previamente calibrado com um padrão de zinco. Amostras com massa entre 8 e 10 mg foram aquecidas de 25°C a 600°C usando uma taxa de aquecimento de $10^{\circ}\text{C}\cdot\text{min}^{-1}$. As medições foram realizadas em atmosferas dinâmicas de nitrogênio e ar sintético, com vazão de $60 \text{ mL}\cdot\text{min}^{-1}$. Uma amostra foi analisada para cada material de estudo.

Análise estatística

Os resultados foram submetidos à análise de variância (ANOVA) e as médias foram comparadas pelo teste de Scott-kott, no nível de 95% de significância, utilizando o software SISVAR (versão 5.3).

3 RESULTADOS E DISCUSSÃO

Atividade antimicrobiana

A atividade antimicrobiana dos filmes contendo BP100 sobre *E. coli*, *S. Typhimurium* e *S. aureus* é mostrada na Tabela 1 e ilustradas nas Fig 1a-c. Os resultados mostraram que os filmes adicionados do BP100 (filmes de amido, CMC e suas blendas) apresentaram ação inibitória sobre as bactérias estudadas, sendo considerados materiais de embalagem ativa.

Tabela 1 Diâmetro dos halos de inibição dos filmes de amido ativos sobre *Escherichia coli*, *Salmonella typhimurium* e *Staphylococcus aureus*.

Filmes	Halo de Inibição (mm)		
	<i>E. coli</i>	<i>S. Typhimurium</i>	<i>S. aureus</i>
Amido_Pt_5	+	6,60 ±0,13 ^{a1}	N
Amido_Pt_10	6,53±0,13 ^{a1}	7,04±0,08 ^{a3}	-
Amido_Pt_15	-	-	+
A:CMC20_Pt_5	+	6,83±0,06 ^{a2}	N
A:CMC20_Pt_10	6,73±0,15 ^{a2}	7,40±0,13 ^{a5}	-
A:CMC20_Pt_15	-	-	+
A:CMC20_Pt_50	-	-	7,47±0,13 ^{a1}
A:CMC40_Pt_5	+	6,96±0,03 ^{a3}	N
A:CMC40_Pt_10	6,79±0,08 ^{a3}	7,21±0,04 ^{a4}	-
A:CMC_Pt_15	-	-	+
A:CMC40_Pt_50	-	-	7,64±0,10 ^{a2}
CMC_Pt_5	+	7,68±0,23 ^{a6}	N
CMC_Pt_10	7,81±0,06 ^{a4}	8,17±0,18 ^{a7}	-
CMC_Pt_15	-	-	+

*+ = a área de inibição foi restrita ao perímetro do filme.

*N= não houve inibição; - = não houve teste.

*Os tratamentos seguidos pela mesma letra na mesma coluna não são significativamente diferentes ($P > 0,05$) pelo teste de Scott-Knott.

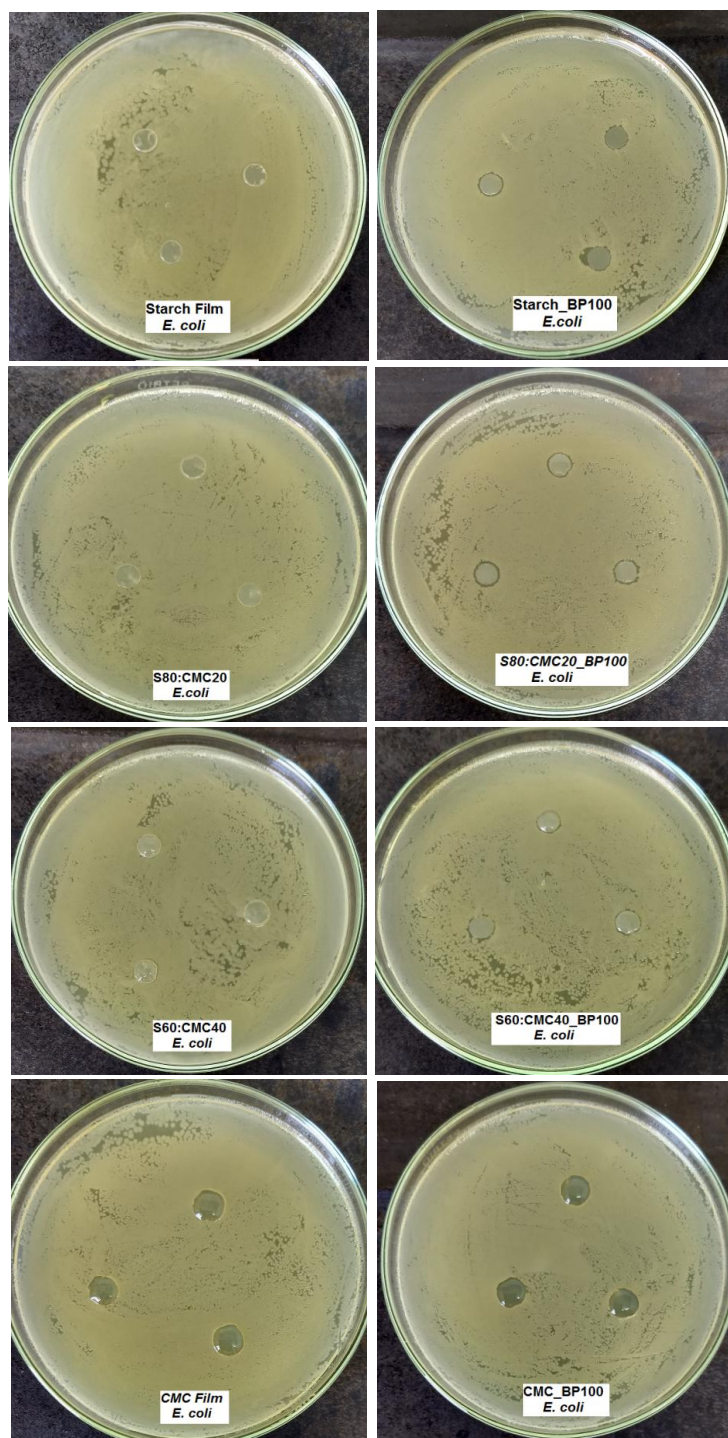


Figura 1 (a). Halos de inibição dos filmes de amido, CMC e suas belandas, ativos pela adição de peptídeo antimicrobiano (BP100_10%) e das amostras controle contra *E. coli*.

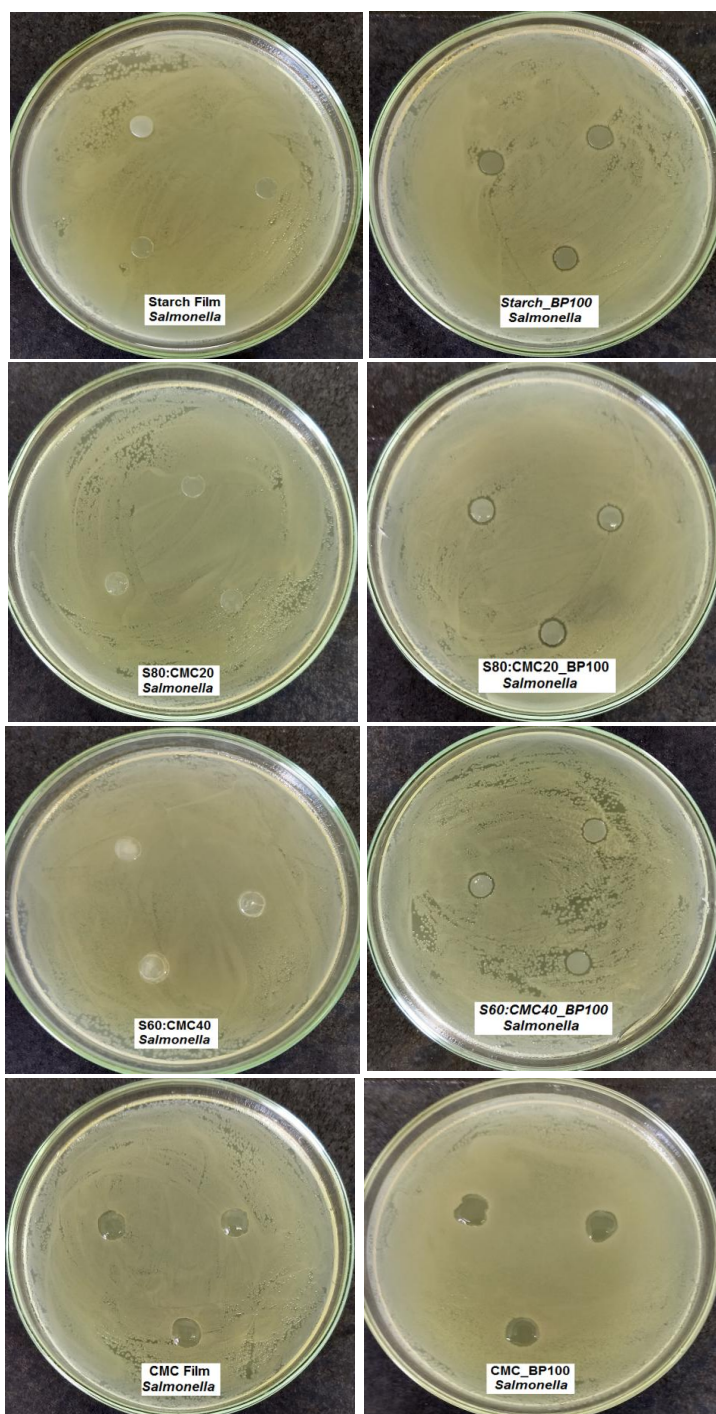


Figura 1 (b). Halos de inibição dos filmes de amido, CMC e suas blendas, ativos pela adição de peptídeo antimicrobiano (BP100_10%) e das amostras controle contra Salmonella.

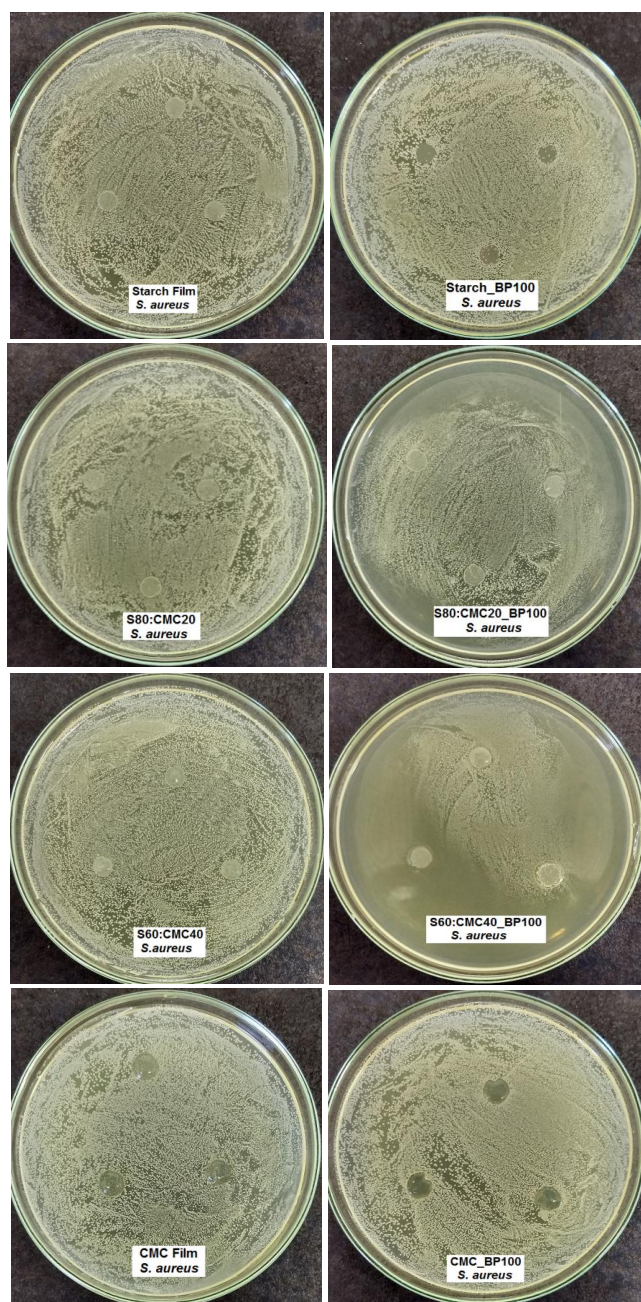


Figura 1 (c). Halos de inibição dos filmes de amido, CMC e suas blendas, ativos pela adição de peptídeo antimicrobiano (BP100_10%) e das amostras controle contra *S. aureus*.

Dentre as bactérias estudadas *S. Typhimurium* foi a mais sensível ao filme ativo, sendo observado o maior halo de inibição ($6,60 \pm 0,13$) na menor concentração testada (5mg/100mL). Já para *E. coli* essa concentração de BP100 (5mg/100mL solução polimérica) promoveu apenas a inibição por contato. Entretanto, ao se dobrar a concentração de BP100 (10mg) observou-se o aumento do diâmetro do halo, sendo a menor atividade antimicrobiana observada para o filme de amido puro e o maior halo formado para o filme de CMC. Para as blendas, o halo de inibição foi maior para o filme com maior teor de CMC.

A eficácia dos peptídeos antimicrobianos contra *E. coli* tem sido relatada em outros estudos, como exemplo, por Zhang et al. (2015) que avaliaram filmes de amido de milho contendo ϵ -Poly-L-lysina e observaram que além da atividade antimicrobiana, a boa interação entre peptídeo-polímero resultou em aumento na resistência à tração e alongamento na ruptura.

O comportamento antimicrobiano do peptídeo no filme foi semelhante tanto para *Salmonella* quanto para *E. coli*, com a menor inibição para o filme de amido puro e a maior para filme de CMC, sendo os maiores halos observados nos filmes de CMC puro, mesmo na menor concentração de BP100 testada. Nas blendas, o tamanho do halo de inibição aumentou com o incremento no teor do peptídeo e de CMC.

Staphylococcus aureus foi a cepa mais resistente ao BP100 não havendo inibição na menor concentração testada (Tabela 1) e quando esta foi aumentada para 10mg, o halo de inibição observado foi restrito ao perímetro do filme, ou seja houve inibição do crescimento bacteriano apenas por contato (dados não mostrados). Assim, para esta cepa foi testada uma concentração maior do peptídeo (50mg) e houve o crescimento do halo de inibição, ou seja, BP100 foi capaz de inibir o crescimento de *S. aureus*, sendo o

maior halo de inibição observado para o filme contendo a maior quantidade de CMC. Resultados semelhantes foram relatados por Yasir et al., (2019) que estudaram um híbrido de BP100 (Mel4) que mostrou menor interação com as membranas celulares e a morte de *S. aureus* quando comparada ao seu precursor (melimina). Segundo os autores, é possível que o mecanismo de ação de Mel4 contra *S. aureus* envolva a liberação de autolisinas seguida pela morte celular, além de uma menor ruptura da membrana do micro-organismo, o que está relacionada à neutralização da carga superficial microbiana (Manzini et al., 2014).

O potencial de outros peptídeos antimicrobianos como a pediocina em embalagens de alimentos foi relatado, incorporados em filmes de amido de milho (Meira et al.2017), celulose (Espitia et al., 2013; Soares et al., 2009) e filmes de poli (ácido láctico) (Woraprayote et al., 2013) com inibição efetiva contra bactérias como *Listeria*, entre outras. Portanto, o peptídeo BP 100 mostrou-se eficiente antimicrobiano contra as bactérias estudadas gram negativas.

Para todas as cepas estudadas, as melhores atividades antimicrobianas foram observadas para filmes de CMC ou blendas com maior concentração deste polímero. Segundo Torcato et al (2013), na presença de vesículas aniônicas, o BP100 mostra transição da forma não estruturada para a forma helicoidal, revelando mudança na conformação após a inserção da membrana. A conformação helicoidal anfipática maximiza as interações eletrostáticas e hidrofóbicas com a membrana, pois a face positiva promove a ligação aos grupos aniônicos da cabeça, enquanto a face apolar favorece o contato com a parte hidrofóbica da membrana e permite a inserção da molécula no núcleo hidrofóbico da bicamada lipídica (Zelezetsky & Tossi; Seelig, 2004).

Assim, o caráter aniônico da CMC parece ter favorecido o mecanismo de ação de BP100 que exerce sua ação visando a superfície bacteriana aniônica e rompendo a integridade da membrana. Um mecanismo de ação dependente da ligação à superfície bacteriana aniônica, seguido pela inserção do peptídeo na membrana e permeabilização também foi observada por Manzini et al., 2014; Torcato et al., 2013; Alves et al., 2010; Ferre et al., 2009.

Quando comparados ao estudo de Meira et al. (2017) que utilizaram a nisina e a pediocina em filmes de amido, nossos resultados demonstraram que os filmes propostos apresentam potencial para ser utilizados como embalagens ativas, pois pequenas quantidades de BP100 são necessárias para inibir o crescimento de *Salmonella* e *E coli* na superfície de alimentos embalados.

Além disso, estes resultados demonstraram que os materiais propostos possuem potencial para utilização na área farmacológica, pois têm sido amplamente utilizados como materiais de curativos de feridas da pele (Sadeghi et al., 2020; Maver et al., 2019; Antosik et al., 2019; Rakhshaei & Namazi, 2017) que tem como principal causa de infecções a contaminação por *S. aureus*, entre outros (Silva et al., 2020; Yu et al., 2015). Segundo Sun et al., (2018), os hidrogéis poliméricos antibacterianos preparados a partir de conjugados de AMP-polímero são candidatos promissores para o fechamento e tratamento de feridas devido às inúmeras vantagens como boa biocompatibilidade e biodegradabilidade, alta absorção de água, reticulação rápida, forte adesão, propriedade antibacteriana de amplo espectro e baixa suscetibilidade de desenvolvimento de resistência.

As características de cor e a interação com a água dos filmes de amido, CMC e suas blends com e sem adição do peptídeo antimicrobiano (Pt) estão apresentadas na Tabela 2.

Tabela 2. Resultados das análises de cor, transparência, ângulo de contato, absorção de água e permeabilidade ao vapor de água (WVP) de filmes de amido, CMC e suas blendas ativos pela adição de BP100 e amostras controle.

Amostras	Cor			Transparência	Ângulo de	Absorção de	PVA
	L	A	B		Contato (°)		Água (%)
Amido	95.6 ± 4.5 ^a	-0.130 ± 0.02 ^c	2.5 ± 0.05 ^a	3.06 ± 0.09 ^a	47.8 ± 1.4 ^b	6.88 ± 0.01 ^b	1.90 ± 0.14 ^c
A:CMC20%	97.0 ± 0.5 ^a	-0.084 ± 0.01 ^b	2.9 ± 0.1 ^c	3.09 ± 0.05 ^a	54.2 ± 3.2 ^c	5.62 ± 0.005 ^a	1.34 ± 0.35 ^b
A:CMC40%	96.8 ± 0.3 ^a	-0.060 ± 0.03 ^a	2.8 ± 0.15 ^{b,c}	3.14 ± 0.07 ^a	65.3 ± 1.1 ^d	5.45 ± 0.001 ^a	1.20 ± 0.32 ^b
CMC	96.5 ± 0.5 ^a	-0.196 ± 0.03 ^d	3.3 ± 0.11 ^d	3.30 ± 0.01 ^b	42.3 ± 5.1 ^a	6.57 ± 0.002 ^b	1.63 ± 0.20 ^c
Amido_Pt	96.8 ± 0.2 ^a	-0.090 ± 0.01 ^b	2.4 ± 0.1 ^a	3.07 ± 0.08 ^a	57.8 ± 1.2 ^c	4.81 ± 0.01 ^a	0.96 ± 0.23 ^a
A:CMC20%_Pt	96.9 ± 0.2 ^a	-0.100 ± 0.02 ^b	2.7 ± 0.06 ^b	3.17 ± 0.03 ^a	79.6 ± 0.8 ^e	5.34 ± 0.003 ^a	0.60 ± 0.03 ^a
A:CMC40%_Pt	96.1 ± 1.6 ^a	-0.062 ± 0.02 ^a	2.9 ± 0.07 ^c	3.13 ± 0.02 ^a	69.3 ± 1.8 ^d	5.40 ± 0.005 ^a	0.61 ± 0.02 ^a
CMC_Pt	96.7 ± 0.8 ^a	-0.042 ± 0.03 ^a	2.9 ± 0.02 ^c	3.27 ± 0.02 ^b	67.9 ± 3.5 ^d	5.78 ± 0.006 ^a	1.38 ± 0.06 ^b

*Os tratamentos seguidos pela mesma letra (coluna) não são significativamente diferentes ($P > 0,05$) pelo teste de Scott-Knott.

Para o parâmetro L^* , os filmes controle, suas blendas e as amostras contendo o peptídeo antimicrobiano (Pt) não diferiram estatisticamente. Para a variável a^* observa-se uma redução nos valores dos filmes puros de amido e CMC após a adição do peptídeo, no entanto, para as blendas não houve diferença significativa. Esse comportamento está, possivelmente, relacionado às interações químicas (ligação de hidrogênio) que ocorrem entre os constituintes do amido e da CMC (OH e COOH, respectivamente), que pode restringir a interação com o peptídeo.

Assim, nota-se que os filmes são transparentes com tendência levemente amarelada. Para a CMC e para a blenda A:CMC_20, a adição de BP100 reduziu os valores de b^* , tornando os filmes menos amarelados. Para as demais amostras não foram observadas diferenças significativas entre a coloração. Meira et al. (2017) e Basch et al. (2013) relataram redução dos valores de L^* e aumento nos valores de b^* após a adição de nisina em filmes de amido de tapioca, o que pode ser explicado devido à coloração amarelada do peptídeo em pó, tornando portanto os filmes mais amarelados.

O BP100 apresenta-se na forma de pó de cor branca e não modificou a cor dos filmes. Além disso, a boa interação entre os grupos hidroxila e carboxila dos polímeros da matriz parece ter restringido a influência do peptídeo na alteração da cor, luminosidade e aparência dos filmes, sendo estas propriedades relevantes para o uso como embalagens de alimentos. Entretanto, a incorporação de pediocina, outro peptídeo antimicrobiano () na formulação de filmes nanocompósitos com ZnO produziu filmes levemente amarelados, ocorrendo diminuição significativa da luminosidade dos filmes (Espitia, Soares, et al., 2013), o que não foi observado neste estudo.

Além da cor, a transparência do filme é uma propriedade relevante, pois afeta diretamente a aparência do produto embalado. A Figura 2 apresenta as imagens dos filmes mostrando sua transparência mantida com CMC e BP100.

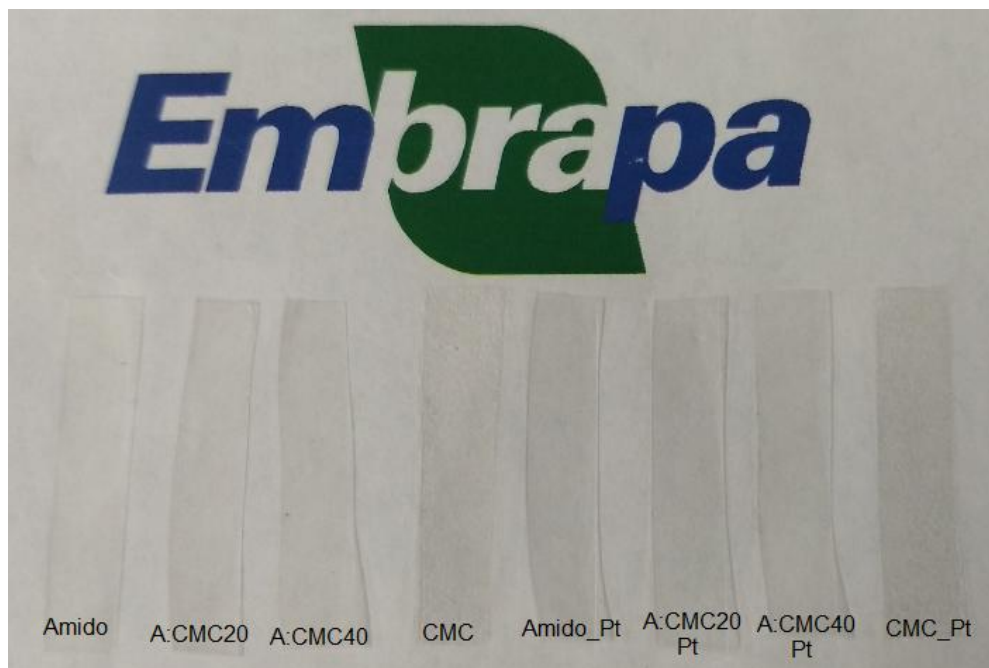


Figura 2. Amostras de filmes de amido, CMC e suas blendas puras e com o peptídeo (Pt) para efeito de comparação.

Pode-se observar que os filmes de amido e suas blendas com CMC apresentaram transparência semelhante (Tabela 2 e Fig. 2), independente da adição do peptídeo antimicrobiano, sendo estas condições desejáveis pelos consumidores, permitindo a plena visualização do produto embalado. Os filmes de CMC, independentemente da adição de BP100, mostraram-se mais transparentes que os demais filmes analisados.

Os valores de transparência reportados nesse estudo variaram de 3,06 (amido puro) a 3,30 (CMC pura) e foram superiores aos relatados por Meira et al. (2017), que variaram de 1,75 a 2,68. No entanto, os autores também relataram que a adição de peptídeos antimicrobianos (nisina e pediocina) não afetou a transparência dos filmes de amido obtidos por casting. Quando comparados aos valores de transparência de filmes comerciais utilizados como embalagens, os resultados desse trabalho foram superiores aos relatados para o polipropileno orientado (1,67) e inferiores ao do polietileno de baixa densidade (4,26) (Guerrero et al., 2011).

Na figura 3 é mostrado os resultados dos ensaios de UV-visível, na faixa de 200 a 900 nm. Independente do comprimento de onda analisado, todas as amostras mostraram o mesmo comportamento.

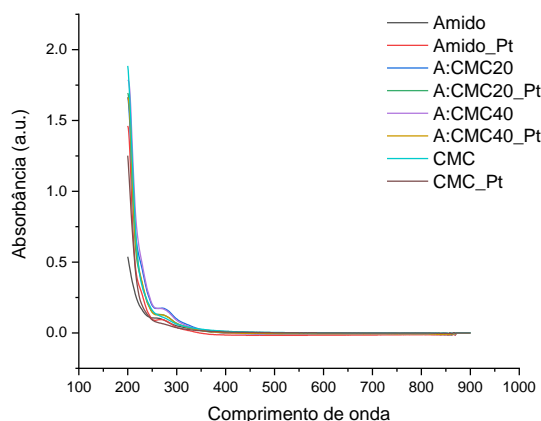


Figura 3. Espectroscopia UV-Vis de filmes de amido e CMC puros e suas blendas com BP100.

Nos comprimentos de onda de 200 nm até 400 nm (faixa ultravioleta), houve uma queda expressiva na absorção, indicando que os filmes absorvem raios de maior frequência de vibração. Na faixa do visível (400 - 700 nm), bem como na faixa do infravermelho próximo (700 - 900 nm), não houve absorção de nenhum comprimento de onda, indicando que os filmes são transparentes nos comprimentos de onda na faixa do visível e para radiações infravermelhas.

A adição de CMC e do peptídeo antimicrobiano em filmes de amido aumentou de forma significativa os valores do ângulo de contato (Tabela 2), o que sinaliza um aumento no caráter hidrofóbico dos filmes de amido. A adição do peptídeo não causou diferenças significativas para o filme de A:CMC40. O amido possui grupos hidroxila em sua estrutura, assim como o CMC possui grupos carboxila, conforme demonstrado pelos resultados da FTIR (Fig. 6). Esses dois grupos são hidrofílicos e interagem facilmente com a água, o que explica a hidrofobicidade do amido puro e dos filmes de CMC puro, apresentando os menores valores de

ângulo de contato entre todas as amostras. Quando as misturas foram obtidas, os grupos hidroxila e carboxila interagiram entre si por ligações de hidrogênio, levando à blindagem mútua. Assim, o número de grupos disponíveis para interagir com a água foi reduzido e, em geral, os filmes de amido e mistura de carboximetilcelulose se mostraram mais hidrofóbicos do que seus filmes puros.

Quando o peptídeo foi adicionado, um aumento em todos os valores dos ângulos de contato foi notado. BP100 é um peptídeo formado por lisina, leucina, fenilalanina, isoleucina e tirosina, aminoácidos com resíduos catiônicos e hidrofóbicos (Manzini et al., 2014; Wadhvani et al., 2014; Carretero et al., 2018). As unidades catiônicas do peptídeo interagiram com grupos hidroxila de amido e carboxila CMC de filmes puros e suas misturas, aumentando sua repulsão à água. Além disso, as unidades hidrofóbicas repeliram a molécula de água. Portanto, houve efeito aditivo entre esses dois fatores, levando a valores de ângulo de contato mais elevados para as blendas do que aqueles observados para matrizes puras com o peptídeo.

A sensibilidade à umidade de filmes de amido é um desafio fundamental para sua aplicação em substituição as embalagens convencionais. A adição de CMC e BP100 reduziu a absorção de água dos filmes de amido (Tabela 2), independentemente da concentração de CMC, o que pode ser explicado pela boa interação entre os constituintes de ambos os polímeros e o peptídeo. O efeito complementar da adição de BP100 nas blendas não foi significativo.

Em estudo realizado por Meira et al. (2017), a adição de pediocina (peptídeo antimicrobiano) reduziu a umidade dos filmes de amido de milho ao diminuir suas capacidades de absorção de água. Já em estudo anterior realizado por Basch et al. (2013), a adição de nisina e natamicina em filmes comestíveis de amido/HPMC (hidroxipropil-metilcelulose) não causou mudanças na absorção de água dos filmes.

Filmes de amido e CMC puro apresentaram maiores valores de permeabilidade ao vapor de água (Tabela 2), uma vez que as blendas apresentaram menores valores com a adição de CMC aos filmes de amido. Resultados semelhantes foram observados pelos autores em estudo anterior (Tavares et al., 2020). Essa redução ocorre devido às ligações de hidrogênio que ocorrem entre grupos hidroxila do amido e grupos carboxila da CMC, ligações estas que reduzem a quantidade de OH livres que interagem com moléculas de água, tornam a estrutura mais compacta e o caminho livre das moléculas de água mais tortuoso dentro dos filmes, reduzindo assim a taxa de difusão (Ma et al., 2017; Li et al., 2008).

Entre as blendas A:CMC20 e A:CMC40, a redução da taxa de permeabilidade ao vapor de água não foi significativa, o que já havia sido relatado em estudo anterior (Tavares et al., 2020). Isso ocorre porque um excesso de CMC é irregularmente disperso na rede polimérica, induzindo a aglomeração de suas moléculas e prejudicando a formação de uma rede mais compacta, o que, por consequência, reduz o efeito de barreira à água. Outros autores reportaram resultados semelhantes em que um baixo teor de CMC se dispersou bem na matriz polimérica de amido, interagindo com seus constituintes e formando uma espécie de bloqueio contra o vapor de água (Ghanbarzadeh, Almasi & Entezami, 2010; Ma et al., 2008).

A adição de peptídeos antimicrobianos reduziu ainda mais a permeabilidade ao vapor de água dos filmes de amido, de CMC e de suas blendas (Tabela 2). No entanto, não houve diferenças significativas entre o filme de amido com BP100 e suas blendas com CMC. Quando os resultados são comparados com o filme de amido puro, a adição de BP100 reduziu pela metade a taxa de permeabilidade ao vapor de água; para as blendas A:CMC20 e A:CMC40, a redução foi de 55% e 49%, respectivamente; para o filme de CMC, a redução da PVA foi de 15%.

A adição de agentes antimicrobianos pode interferir na estrutura da rede polimérica formada entre biopolímeros através de ligações de hidrogênio entre o peptídeo e o polímero, o

que substitui as interações polímero-polímero e inibe a formação de ligações de hidrogênio entre o polímero e a água nas regiões amorfas da matriz (Flores et al., 2007).

Resultados semelhantes foram descritos por Meira et al. (2017) que também relataram uma menor taxa de PVA em filmes de amido de tapioca com pediocina quando comparado aos filmes controle e em amostras contendo pediocina e halloysite como material de reforço mecânico. Por outro lado, Basch et al. (2013) relataram um aumento significativo na PVA em filmes de amido/HPMC devido à adição de nisina e atribuíram esse resultado à maior ruptura da matriz dos biopolímeros causada pela presença do antimicrobiano.

Dessa maneira, o peptídeo avaliado em nosso estudo (BP100) apresenta ser compatível com o amido, resultando em uma distribuição homogênea ao longo da matriz polimérica dos filmes, como mostrado pelos resultados de microscopia de varredura dos filmes (Fig. 5).

As morfologias (superfície e fratura) dos filmes de amido e suas blendas puras e com adição de BP100 estão apresentadas nas Figuras 4 e 5, respectivamente.

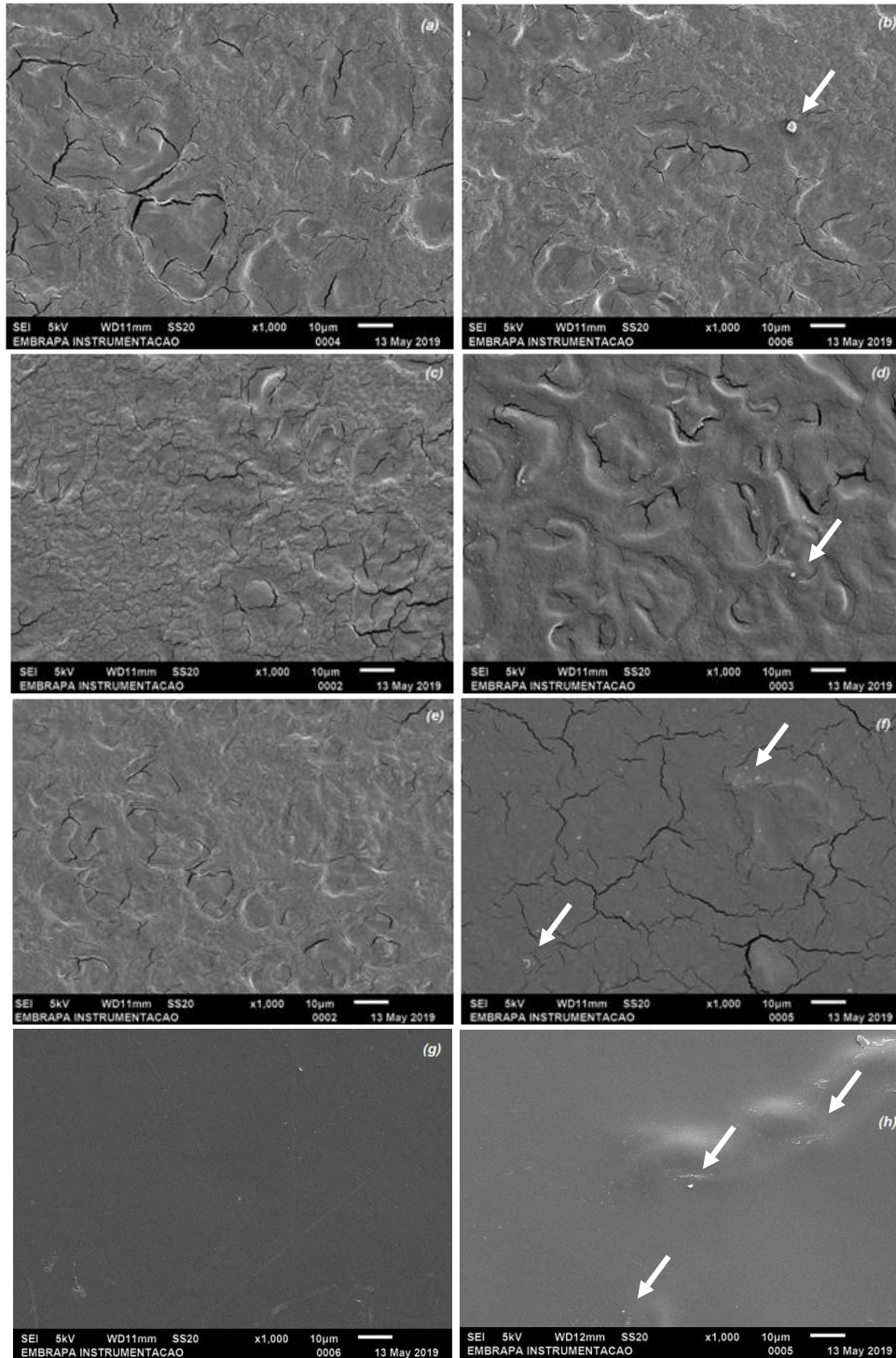


Figura 4. Micrografias de superfície dos filmes de Amido (a), Amido_Pt (b), A:CMC20 (c), A:CMC20_Pt (d), A:CMC40 (e), A:CMC40_Pt (f), CMC (g) e CMC_Pt(h).

As micrografias de superfície dos filmes mostraram a formação de bolhas e de microfissuras, sendo que a maior densidade e tamanho das bolhas foram observados para os filmes de amido e suas blendas. A adição de CMC tornou a superfície dos filmes de amido mais lisa e reduziu o tamanho das bolhas, o que pode estar relacionado à boa interação entre os grupos hidroxilas do amido e carboxilas da CMC (Tavares et al., 2020; Suriyatem, Auras, Rachtanapun, 2019).

Com a inclusão do peptídeo, foi observado aumento no tamanho das bolhas e das fissuras, sugerindo a presença de partículas de BP100 na superfície dos filmes, como também observado por Meira et al., (2017) em seus filmes de amido de milho e de tapioca com nisina e por Ollé Resa et al. (2014) em filmes de amido de tapioca também com nisina. Ambos relataram a presença de cristais de peptídeos em filmes de celulose contendo pediocina (Espitia et al., 2013).

Para avaliar o efeito da adição de CMC e BP100 na estrutura dos filmes, foi realizada a microscopia na fratura criogênica dos filmes (Figura 5).

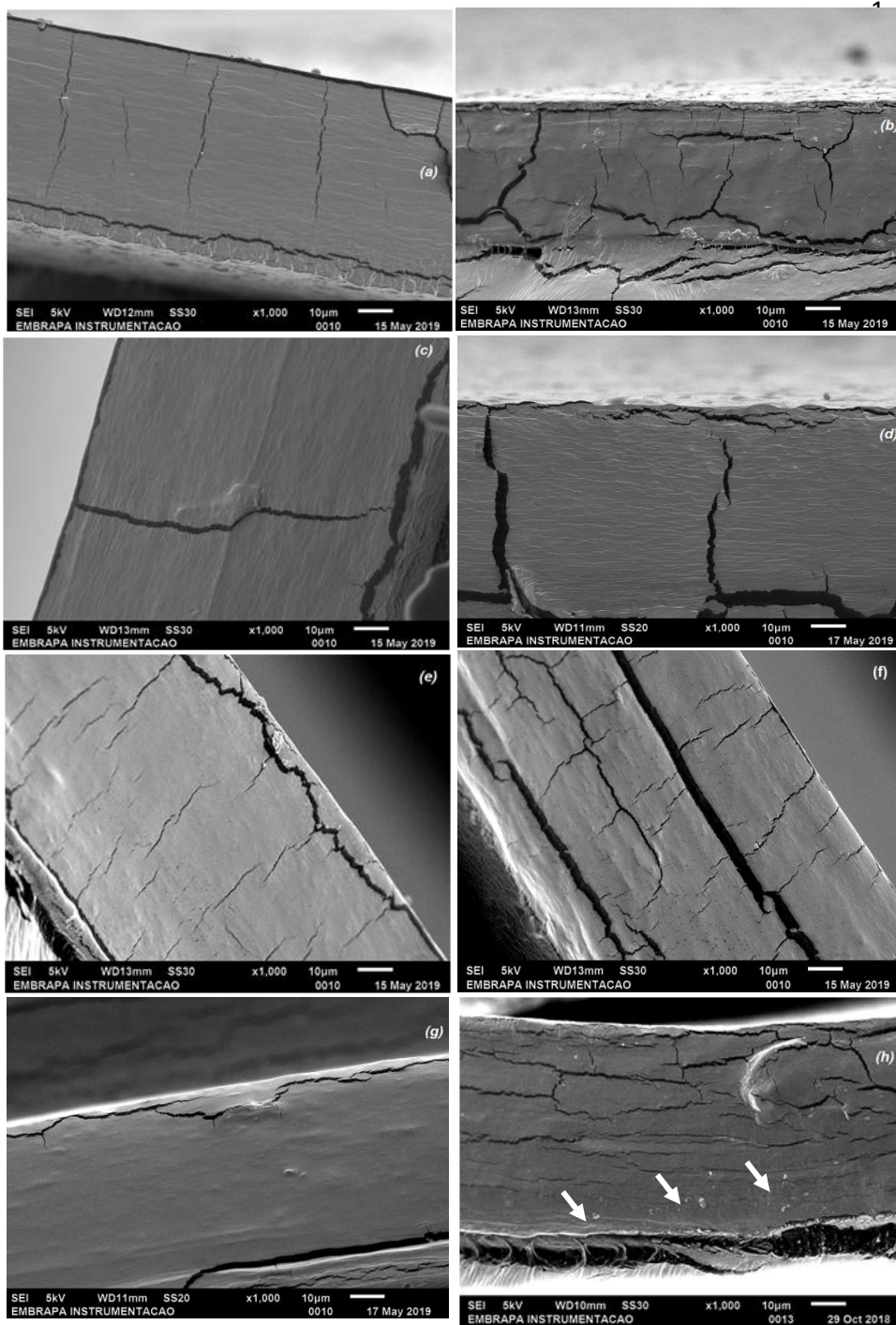


Figura 5. Micrografias de fratura dos filmes de Amido (a), Amido_Pt (b), A:CMC20 (c), A:CMC20_Pt (d), A:CMC40 (e), A:CMC40_Pt (f), CMC(g) e CMC_Pt (h).

É possível observar que os filmes das blendas de amido e CMC apresentaram estrutura densa e compacta, resultado da boa interação entre os constituintes dos polímeros utilizados (Tavares et al., 2020; Ma et al., 2017). A adição do peptídeo aumentou o número de trincas e microfissuras no interior da rede polimérica. Na estrutura do filme de CMC_Pt foi possível observar a presença de microbolhas no interior da rede polimérica.

A ocorrência de bolhas na superfície e no interior de filmes de amido de milho pode estar relacionada ao seu alto teor de amilose, propiciando maior retrogradação e forte tendência de interagir com outras moléculas por meio de ligações de hidrogênio, o que leva à formação de estruturas cristalinas durante a redução da temperatura e o armazenamento dos filmes (Denardim & Silva, 2008; Tavares et al., 2019).

Do ponto de vista prático, é desejável manter ou aumentar as propriedades de barreira do material de embalagens a fim de limitar a deterioração dos alimentos. Assim, é importante notar que a adição de BP100 melhorou significativamente a barreira da água dos filmes. Os resultados de PVA variaram de 1,90 a 0,60 g H₂O.mm.m⁻².h⁻¹.mm.Hg⁻¹ para Starch e blendas de amido/CMC_Pt, respectivamente. Estes resultados mostraram uma redução de 68% na taxa de permeabilidade ao vapor de água do filme de amido após a adição de 20% CMC e 10% de BP100.

As propriedades mecânicas dos filmes ativos (blendas de amido/CMC/BP100) e controles (filmes sem BP100) estão descritos na Tabela 3.

Tabela 3 Resultados dos ensaios mecânicos dos filmes de amido, CMC e suas blendas com o peptídeo (Pt). Letras iguais significam valores estatisticamente iguais.

Amostras	Resistência à Tração (MPa)	Deformação na Ruptura (%)	Módulo Elástico (MPa)
Amido	2.16 0.05 ^a	74.60±2.75 ^d	15.85 ± 3.46 ^a
A:CMC20	5.64±0.11 ^c	54.95±1.96 ^c	106.00 ± 4.84 ^c
A:CMC40	13.48±0.25 ^d	38.61±3.82 ^b	379.46 ± 34.42 ^d
CMC	50.44±2.65 ^f	5.30±0.31 ^a	1687.84 ± 103.89 ^f
Amido__Pt	3.18±0.05 ^b	76.08 ±6.75 ^d	51.63 ± 2.07 ^b
A:CMC20_Pt	13.99±0.31 ^d	5.35 ± 0.11 ^a	520.31 ± 36.43 ^e
A:CMC40_Pt	15.95±0.83 ^e	5.67 ±0.35 ^a	505.40 ± 46.92 ^e
CMC_Pt	55.21±3.86 ^f	5.27 ± 0.75 ^a	1808.72 ± 67.15 ^f

Conforme mostrado em trabalho anterior dos autores (Tavares et al., 2020), a adição de CMC ao amido aumentou o módulo de elasticidade dos filmes devido à alta energia necessária para deformar o ângulo e a distância de ligação entre os átomos em as cadeias da matriz, especialmente as ligações de hidrogênio entre os grupos OH do amido e COOH da CMC (Ma et al., 2017; Mendes et al., 2016; Mikus et al., 2014; Li et al., 2008). A mesma tendência foi observada no presente trabalho, com a resistência à tração e o módulo de elasticidade dos filmes aumentados com a incorporação do CMC, enquanto a deformação na ruptura foi reduzida.

Quando BP100 foi adicionado aos filmes, houve um aumento na resistência à tração e módulo de elasticidade dos filmes. Estatisticamente, não houve diferenças nos valores de deformação na ruptura e módulo elásticos entre as blendas com o BP100, indicando que as cadeias da matriz interagem de forma semelhante com o peptídeo, conforme observado nos resultados do FTIR. Basiak et al. (2015) relataram que a adição de soro de leite em filmes de amido de trigo aumentou a flexibilidade e o alongamento na ruptura. Outros autores

reportaram que a adição de pediocina aumentou o alongamento na ruptura dos filmes de metilcelulose, indicando que o peptídeo apresentou um efeito plastificante na matriz polimérica (Espitia et al., 2013).

No presente trabalho, o peptídeo atuou como uma ponte entre as cadeias de amido e CMC, conectando-se por pontes de hidrogênio a grupos polares em cada cadeia. Isso restringiu ainda mais o movimento dessas cadeias, levando ao aumento da rigidez e da resistência à tração e à redução da deformação. O espectro FTIR dos filmes é mostrado na Figura 6.

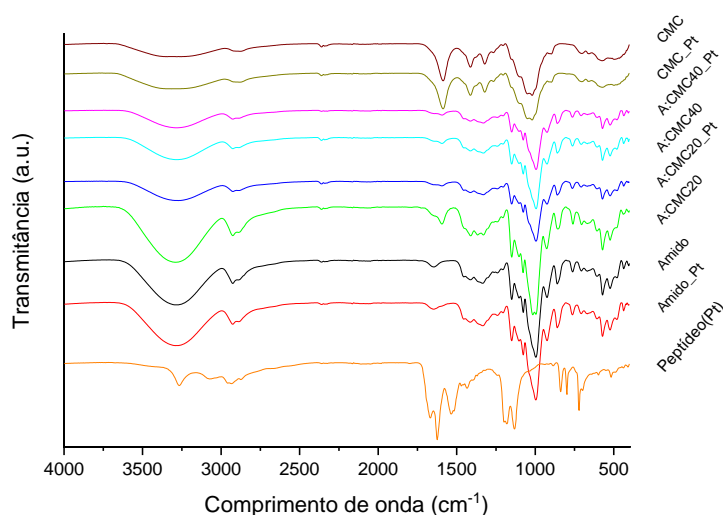


Figura 6. FTIR de filmes de amido, CMC e suas blendas puras e com peptídeo antimicrobiano (Pt).

A interação entre os constituintes das blendas foi investigada em duas intensidades de bandas principais (3600 cm^{-1} a 2800 cm^{-1} e 1700 cm^{-1} a 700 cm^{-1}), bem como em 917 cm^{-1} , 1024 cm^{-1} e 1140 cm^{-1} (característica do alongamento da ligação CO), 1425 cm^{-1} (glicerol), 1588 cm^{-1} (desprotonação dos grupos COOH) e 3299 cm^{-1} (estiramento OH e C=O) (Ma et al., 2017; Mendes et al, 2016).

Uma ampla variação em 3299 cm^{-1} indicou que houve um alongamento dos grupos OH das cadeias de amido (Ma et al., 2017; Tongdeesoontorn et al., 2011). Esse alongamento ocorreu devido à formação de ligações de hidrogênio entre esses grupos hidroxila do amido e carboxila da CMC (Almasi, Ghanbarzadeh & Entezamil, 2010; Li et al, 2008).

Os filmes de CMC também mostraram bandas em 1415 cm^{-1} , 1321 cm^{-1} e 1147 cm^{-1} , atribuídas ao dobramento dos grupos CH_2 , à flexão dos grupos COH e ao estiramento da ligação COH, respectivamente (Ma et al. , 2017; Tongdeesoontorn et al, 2011). Além dessas, bandas em 1588 cm^{-1} e 1412 cm^{-1} mostraram a desprotonação dos grupos carboxila CMC por dobramento assimétrico e alongamento simétrico de COO^- , respectivamente (Gonzaga et al., 2018). Algumas bandas CMC foram suprimidas pelas bandas de amido (Ma et al, 2017; Tongdeesoontorn et al, 2011).

O espectro do peptídeo BP100 também é mostrado na Figura 6, onde é possível ver bandas características em 1650 cm^{-1} , banda de estiramento de amida (NH_2 e $\text{C}=\text{O}$), presente nos aminoácidos que compõem o peptídeo, 1192 cm^{-1} (tirosina), 840 cm^{-1} e 798 cm^{-1} (anel aromático de tirosina e fenilalanina), 767 cm^{-1} (lisina) e 721 cm^{-1} (tirosina) (Pearson & Slifkn, 1972; Wolpert & Hellwig, 2006). Ao comparar os espectros das amostras sem adição do peptídeo e aquelas com BP100, pode-se concluir que a presença do peptídeo não alterou significativamente os espectros das amostras de amido de milho puro e CMC. Porém, o peptídeo interagiu com as blendas, uma vez que as bandas próximas a 3300 cm^{-1} , relativas aos grupos hidroxila, 1650 cm^{-1} , relacionadas ao NH_2 dos aminoácidos, e 1590 cm^{-1} , relativas à carbonila CMC, tiveram suas intensidades reduzidas.

Os difratogramas de DRX dos filmes de amido estão mostrados na Figura 7.

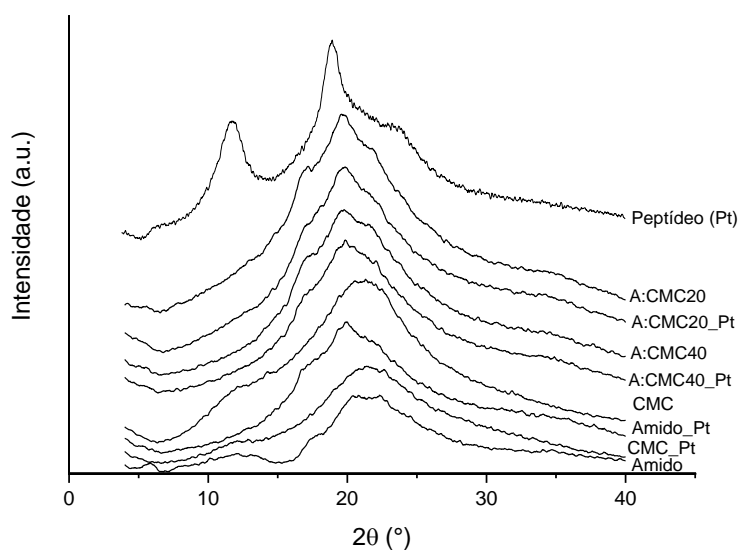


Figura 7. Difractogramas de raios-X de filmes de amido, CMC e de suas blendas puras e com a adição de peptídeo antimicrobiano (Pt).

Tabela 4. Índice de cristalinidade para os filmes de amido, CMC e PB100.

Amostras	% C	
	sem peptídeo	c/ peptídeo
Amido	39.2	35.3
A:CMC20	35.4	18.1
A:CMC40	40.0	14.8
CMC	49.9	44.0
Peptídeo (Pt)	11.9	

O índice de cristalinidade do filme de amido de milho puro foi de 39%, valor ligeiramente superior ao encontrados em estudos anteriores (33%) (Tavares et al., 2019), o que está relacionado ao processo de retrogradação do amido e tempo de armazenamento dos filmes. O CMC apresentou índice de cristalinidade de 49%, valor próximo a outros estudos semelhantes (Parid et al., 2018; Ikhuori et al., 2017; Hazirah et al., 2016; Kimani et al., 2016; Chai & Isa, 2013; Shang, Shao, Chen, 2008).

A incorporação do CMC reduziu a cristalinidade dos filmes de amido (Tavares et al., 2020), devido à interação entre os grupos OH do amido e COOH do CMC que restringe a mobilidade de suas cadeias e dificulta a cristalização. Suriyatem et al. (2019) estudaram a incorporação de CMC no amido de arroz e obtiveram resultado semelhante. Segundo o autor, o CMC interrompe o arranjo regular das cadeias de amido pela formação de ligações de hidrogênio entre os grupos hidroxila e carboxila, reduzindo a cristalinidade das blendas.

O difractograma do BP100 é mostrado na figura 7. É possível observar um pico de difração a $11,9^\circ$, relacionado ao empacotamento ortorrômbico de leucina, isoleucina e fenilalanina, e outro a $19,1^\circ$, relacionado ao empacotamento monoclinico de lisina e tirosina (Marsh, 1967; Gurskaya, 1968). Tais picos não são encontrados nas curvas dos filmes de amido e CMC e suas blendas incorporadas ao peptídeo. Isso reforça o fato de que as moléculas de BP100 estão ligadas às cadeias da matriz por meio de ligações de hidrogênio, agindo como uma ponte entre essas cadeias. Portanto, o peptídeo não está livre para se organizar em um arranjo ordenado e os picos que foram vistos anteriormente desaparecem. Devido ao papel desempenhado pelo BP100, a cristalinidade de todos os filmes é reduzida quando este peptídeo é incorporado, uma vez que as cadeias da matriz (amido, CMC ou ambos) têm sua mobilidade reduzida e, portanto, não são capazes de se organizarem ordenadamente, reduzindo o valor de cristalinidade, conforme tabela 3. Não houve diferenças significativas no índice de cristalinidade das blendas, indicando que a composição não altera o arranjo das cadeias.

A termogravimetria foi utilizada para avaliar a estabilidade térmica dos filmes, bem como a derivada das curvas TGA, utilizadas para determinar a temperatura de decomposição térmica do material, que ocorreu em três etapas principais: (1) perda de massa em aproximadamente 95°C devido à evaporação da água; (2) volatilização do glicerol entre 145°C e 160°C ; e (3) degradação dos constituintes do amido e CMC e ocorreu na faixa de 250°C

°C a 350 °C e está de acordo com outros resultados relatados anteriormente (Suriyatem, Auras, Rachtanapun, 2019; Jaramillo et al., 2016)

A estabilidade térmica dos filmes de amido foi reduzida com a incorporação de CMC, uma vez que os picos de dTG relacionados à fase rica em amido foram reduzidos de 291 °C para 252 °C e 242 °C para as blendas com 20 e 40% de CMC, respectivamente, que possui menor estabilidade térmica quando comparada ao amido, resultado semelhante obtidos por Ma et al. (2008) e Suriyatem et al. (2019) (Figura 8 e Tabela 4).

Pequenos aumentos na temperatura de degradação são vistos com a incorporação do BP100 para todos os filmes. O aumento na temperatura de início de degradação foi de 2,75% para o filme de amido puro, 1,85% para CMC, 3,17 e 4,13% para as blendas 20 e 40% respectivamente. Isso ocorre porque o peptídeo funciona como uma ponte entre as cadeias de amido, CMC ou ambos da matriz, reduzindo a mobilidade e dificultando a cisão destas cadeias, aumentando a energia térmica. Perdas secundárias de massa foram vistas para todas as formulações, exceto para o amido puro, indicando que houve um excesso de CMC nas blendas.

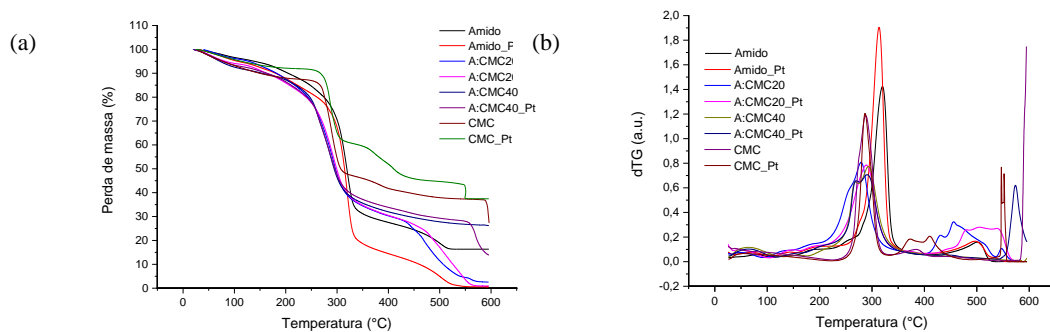


Figura 8. (a) Termogramas TGA e (b) DTGA de filmes de amido e suas misturas com CMC e BP100.

Tabela 5. Valores de TGA.

Amostra	T _{onset} (°C)	T _{peak} (°C)	Resíduo (%)
Amido	291	320	16.3
Amido_Pt	299/469	313/497	0.6
A:CMC20	252/431	279/456	2.6
A:CMC20_Pt	260/472	289/505	1.0
A:CMC40	242	268/293	26.3
A:CMC40_Pt	252/559	270/291/574	13.8
CMC	269/590	288/594	27.3
CMC_Pt	274/360/550	287/373/552	37.5

A adição de CMC aos filmes de amido reduziu a estabilidade térmica dos filmes, conforme mostrado nas temperaturas T_{onset} (início de degradação), que partiram de 291 °C no filme de amido puro e atingiram 252 °C e 242 °C nos filmes com adição de 20% e 40% de CMC, respectivamente. Além disso, T_{peak} (temperatura média de degradação) nos primeiros estágios de degradação foi de 320 °C para o filme de amido, 279 °C para A:CMC20 e 268 °C para A:CMC40, o que se deve à menor estabilidade da CMC em relação ao amido e à interação entre os constituintes do amido e da CMC com o glicerol - cuja temperatura de degradação está em torno de 160 °C.

Por outro lado, a adição de CMC aumenta o conteúdo residual dos filmes de amido, como resultado do maior teor de resíduo da degradação da CMC. Resultados semelhantes foram relatados por Suryatem et al. (2018) e por Ahmad et al (2014). Na curva do TGA da CMC (Fig. 8), observa-se uma perda de massa inicial em torno de 100 °C que corresponde à perda de água adsorvida pelo material. Em seguida, ocorre uma de perda de massa mais significativa referente à quebra das cadeias poliméricas nas ligações entre carbonos (Shehap,

2008). Esse resultado está de acordo com o relatado na literatura, no qual a temperatura de início da degradação (T_{onset}) do material ocorre a aproximadamente 289 °C. O material apresenta uma massa residual de 27%, que não sofreu termo degradação até a temperatura de 600 °C, e que aumentou para 37% após a adição do peptídeo.

O processo de degradação térmica da CMC ocorre em três etapas de perdas de massa. Durante a pirólise, compostos voláteis são gerados, consumidos e liberados. Os materiais voláteis produzidos por reações intermediárias no exterior e interior do CMC seriam pirolisados acima 100 °C durante a execução do TGA. Portanto, a primeira etapa do processo térmico degradação pode ser atribuída à evaporação da água da CMC. A segunda etapa da degradação térmica ocorre em um curto intervalo de temperatura, entre 250 e 375 °C. Esta etapa pode estar associada à quebra de ligações associada aos grupos funcionais e quebra da cadeia polimérica em moléculas menores. A terceira etapa está associada à degradação térmica de produtos desidratados do carbono como alcanos, alcenos, dienos e ciclização aromática de resíduo de carvão vegetal (Ahmad; Wahab, Al Omar, 2014).

A adição do peptídeo ao filme de amido formou ligações e compostos que resultaram em maior resistência mecânica (Tabela 2) e menor estabilidade térmica (Tabela 4). Tendência oposta foi observada para o filme de CMC.

A adição do peptídeo reduziu o conteúdo do resíduo dos filmes, propiciando outros estágios de degradação em maiores temperaturas, devido às novas ligações de hidrogênio existentes entre carbono e nitrogênio, presentes na molécula do peptídeo, além de carbono e oxigênio já existentes nos filmes de amido e CMC. Seus estágios de degradação térmica ocorrem com reações primárias de desidratação de formação de CO_2 , seguida da formação de H₂CO, CO e HCN em temperaturas acima de 400°C (Moldoveanu, 2010).

As reações de pirólise dos aminoácidos são compostas por reações complexas que envolvem descarboxilação, desaminação, desidratação e reações de condensação. Os produtos

da decomposição térmica são principalmente compostos inorgânicos simples como CO₂, H₂O, NH₃ e CO, com uma variedade de compostos orgânicos voláteis como aminas, nitrilos, amidas e hidrocarbonetos e alguns compostos orgânicos menos voláteis, além da formação de dipeptídeos cíclicos como a 2,5 Diketopiperazinas (DPK) (Puerto et al., 2018; Basiuk, 1998; Giuseppe & Galletti, 1992).

A literatura relata curvas de TGA dos peptídeos com diferentes fases de degradação, pois a maioria dos aminoácidos apresenta perda parcial de massa em 600 °C, ao passo que os aminoácidos alifáticos volatilizam completamente a temperaturas abaixo de 400°C (Lien & Nawar, 1974; Rodante, 1992).

4 CONCLUSÕES

A adição de CMC e do BP100 tornaram os filmes de amido de milho menos hidrofílicos devido à boa interação entre seus constituintes, além de unidades hidrofóbicas presentes no peptídeo, evidenciadas pelo FTIR. Esta interação reduziu a cristalinidade e aumentou a resistência à tração e o módulo de elasticidade e reduziu a deformação na ruptura dos filmes. A adição de BP100 induziu um pequeno incremento na temperatura de degradação térmica dos filmes. As micrografias mostraram que CMC tornou os filmes de amido mais lisos, compactos e com uma menor densidade de bolhas. Já o peptídeo mostrou efeito contrário. *Salmonella* foi a cepa mais sensível, enquanto *S. aureus* foi mais resistente, sendo necessária uma maior concentração de BP100 para inibir o crescimento da bactéria. O conteúdo de CMC favoreceu a atividade antimicrobiana, devido ao seu caráter aniônico, capaz de provocar mudanças conformacionais na estrutura do BP100, favorecendo interações eletrostáticas e hidrofóbicas. Os filmes propostos mostraram-se promissores para a utilização como embalagens ativas antimicrobianas.

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CONSIDERAÇÕES FINAIS

Espera-se que esta pesquisa contribua tanto no ramo da engenharia de biomateriais, quanto na área de embalagens de alimentos, proporcionando conhecimento científico a ambas as áreas, além de superar barreiras e limitações da etapa de conservação de alimentos.

Aumentar a resistência à tração dos filmes de amido, além da inclusão de uma nova funcionalidade aos filmes ativos pela adição dos peptídeos antimicrobianos BP100, visando assim, aumentar a vida de prateleira de alimentos.

Trabalhos futuros visam o estudo da atividade dos filmes ativos em produtos alimentícios para avaliar seu efeito na vida de prateleira, bem como sua atividade in vivo como material para curativos da pele. Outros estudos visam, ainda, a modificação superficial dos filmes de amido/CMC para torná-lo menos hidrofílicos.