Universidade Estadual de Londrina

BEATRIZ MARJORIE MARIM

HIDROGÉIS BIOPOLIMÉRICOS SUPERABSORVENTES OBTIDOS POR EXTRUSÃO REATIVA

Londrina
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Tese apresentada ao Programa de Pós-graduação em Biotecnologia da Universidade Estadual de Londrina - UEL, como requisito parcial para a obtenção do título de Doutor.

Orientadora: Profa. Dra. Suzana Mali de Oliveira

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MARIM, Beatriz Marjorie. **Hidrogéis biopoliméricos obtidos por extrusão reativa para aplicação agrícola**. 2021. 111 f. Tese de doutorado (Qualificação de doutorado em Biotecnologia) – Universidade Estadual de Londrina, Londrina, 2021.

RESUMO

Hidrogéis são classificados como estruturas tridimensionais constituídas por polímeros reticulados que têm a capacidade de absorver e liberar, controladamente, grandes quantidades de água, nutrientes e substâncias iônicas, sem se dissolver. O presente trabalho teve como objetivo desenvolver hidrogéis à base de misturas biopoliméricas de amido de mandioca, celulose e gelatina para aplicações agrícolas como agentes de retenção de água. Inicialmente, o amido de mandioca foi modificado quimicamente via extrusão reativa utilizando-se o ácido cítrico (CA) e o ácido tartárico (TA) como agentes reticulantes. A celulose extraída da casca de aveia foi modificada via extrusão reativa, trimetafosfato de sódio (STMP) e ácido tartárico (TA) foram empregados como agentes reticulantes. Foram produzidos hidrogéis a partir das misturas de amido modificado, celulose modificada e gelatina. O processo de extrusão reativa foi eficaz na modificação química do amido e da celulose. Os resultados confirmaram a modificação química das cadeias poliméricas através do aparecimento de uma nova banda nos espectros de FTIR em 1730 cm-1 associada aos grupos carbonila das ligações ésteres formados para os amidos modificados com CA e TA em diferentes concentrações (2,5 a 20 g ácido/100 g amido). Foram obtidos amidos modificados com graus de substituição entre 0,023 a 0,365. A difração de raios-X indicou que a extrusão reativa resultou no desaparecimento de picos de difração do amido nativo, as amostras do amido reticulado apresentaram índices de cristalinidade variando de 8 a 11%. A análise morfológica mostrou que a estrutura granular do amido original foi perdida e substituída por uma estrutura mais áspera e irregular. A capacidade de retenção de água e a solubilidade dos amidos modificados obtidos por extrusão reativa foram superiores às do amido nativo e da amostra de controle (extrusadas sem os agentes de reticulação). No caso da celulose, o processo de extrusão reativa para reticulação com TA e STMP também foi eficaz, e os resultados confirmaram a modificação das cadeias poliméricas através do aparecimento de uma nova banda em 1735 cm-1, associada aos grupos carbonila das ligações ésteres que foram formadas nas amostras modificadas com TA. A modificação da celulose por extrusão reativa não alterou a morfologia e a cristalinidade das amostras. Os índices de cristalinidade (IC) variam de 42 a 33%, e as amostras modificadas apresentaram maior afinidade por solventes não polares em testes de molhabilidade, também confirmando a reticulação com TA e STMP. Quando foram produzidos pellets de hidrogel com a mistura de celulose, amido e gelatina, foi obtido um material poroso, com capacidade de retenção de água em diferentes pHs acima de 530%. Este estudo mostrou que a extrusão reativa foi eficiente tanto para a modificação do amido e da celulose, quanto na produção de hidrogéis no formato de pellets para serem empregados na agricultura como retentores de água, com as vantagens de simplicidade, baixa geração de efluentes, tempos de reação curtos e fácil adaptação para escala industrial.

Palavras-chave: celulose; amido; extrusão reativa; reticulação; ácidos orgânicos; trimetafosfato de sódio.

MARIM, Beatriz Marjorie. **Biopolymeric hydrogels obtained by reactive extrusion for agricultural application.** 2021. 111 p. Doctoral thesis (Doctorate in Biotechnology) – State University of Londrina, Londrina, 2021.

ABSTRACT

Hydrogels are classified as three-dimensional structures made of crosslinked polymers that are able to absorb and release, in a controlled, large amount of water, nutrients, and ionic substances, without dissolving. The present work aimed to develop hydrogel formulations based on biopolymeric mixtures of cassava starch, cellulose, and gelatin for agricultural applications as water retention agents. Initially, cassava starch was chemically modified via reactive extrusion using citric acid (CA) and tartaric acid (TA) as crosslinking agents. Cellulose extracted from oat hulls was modified via reactive extrusion, and sodium trimetaphosphate (STMP) and tartaric acid (TA) were used as crosslinking agents. Hydrogels were produced from mixtures of modified starch, modified cellulose and gelatin. The reactive extrusion process was effective in crosslinking starch and cellulose. Results confirmed that the chemical modification of polymer chains through the appearance of a new band in FTIR spectra at 1730 cm-1 associated with the carbonyl groups of the ester bonds formed for starches modified with CA and TA at different concentrations (2.5 to 20 g acid/100 g starch). Modified starches with degrees of substitution between 0.023 and 0.365 were obtained. The X-ray diffraction indicated that the reactive extrusion resulted in the disappearance of diffraction peaks of the native starch, the samples of the crosslinked starch had crystallinity indexes varying from 8 to 11%. Morphological analysis showed that the granular structure of the original starch was lost and replaced by a rougher and more irregular structure. The water holding capacity and solubility of modified starches obtained by reactive extrusion were superior to that of native starch and control sample (extruded without crosslinking agents). In the case of modified cellulose, the reactive extrusion process for crosslinking with TA and STMP was also effective, and the results confirmed the crosslinking of polymer chains through the appearance of a new band at 1735 cm-1, associated with the carbonyl groups of the ester bonds that were formed. The modification of cellulose by reactive extrusion did not change the morphology and crystallinity of the samples. Crystallinity indices (CI) ranged from 42 to 33%, and modified samples showed greater affinity for non-polar solvents in wettability tests, also confirming crosslinking with TA and STMP. When hydrogel pellets were produced with a mixture of cellulose, starch, and gelatin, a porous material was obtained, with water retention capacity at different pHs. This study showed that reactive extrusion was efficient both for the modification of starch and cellulose, and also in the production of hydrogels in the form of pellets to be used in agriculture as water retainers, with the advantages of simplicity, low effluent generation, low reaction times and easy adaptation to industrial scale.

Key words: cellulose; starch; reactive extrusion; crosslinking; organic acids; sodium trimetaphosphate.

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LISTA DE ABREVIATURAS E SIGLAS

CA	Ácido cítrico
DRX	Difração de Raios-X
FT-IR	Espectroscopia no Infravermelho com Transformada de Fourier
I_{002}	Intensidade máxima do pico correspondente ao plano (002)
I_{am}	Intensidade da parte amorfa
IC	Índice de Cristalinidade
K	Calor de sorção das multicamadas
m_0	Teor de água na monocamada
MEV	Microscopia Eletrônica de Varredura
r^2	Coeficiente de determinação
STMP	Trimetafosfato de sódio
TA	Ácido tartárico
TGA	Análise Termogravimétrica
$T_{máx}$	Temperatura de degradação na taxa de perda máxima
UR	Umidade Relativa
X_W	Umidade de equilíbrio

SUMÁRIO

1 INTRODUÇÃO

O desenvolvimento de novos produtos a partir de matérias-primas de fonte renovável e biodegradáveis tem sido foco de grande número de pesquisa nos últimos anos. Estes produtos podem ter aplicações em diversas áreas, como, agricultura, biomedicina, engenharia, em indústrias farmacêuticas e de alimentos, além de estudos recentes da utilização de hidrogéis para tratamento de águas residuais (FARHAT *et al.*, 2017; LEMOS *et al.*, 2021; LIPATOVA; YUSOVA, 2021; MAALOUL *et al.*, 2021).

Neste contexto, a utilização de polímeros naturais, nas últimas décadas tornou-se crescente, uma vez que a possibilidade de modificação desses polímeros através de reações químicas simples faz com que estes materiais se tornem versáteis e economicamente viáveis (ELGAIED-LAMOUCHI *et al.*, 2021; LI; CHEN, 2020). Entretanto, o uso exacerbado de polímeros sintéticos e seu consequente descarte promoveram o aumento da poluição, já que esses compósitos se decompõem lentamente no meio ambiente. Como forma de minimizar impactos ambientais, pesquisadores começaram a investir no estudo de biopolímeros e/ou polímeros biodegradáveis, que podem ser obtidos a partir de modificações com o uso mínimo de solventes químicos (KALENDOVA *et al.*, 2021; THOMBARE *et al.*, 2018; VARAPRASAD *et al.*, 2017).

Os hidrogéis são polímeros hidrofílicos reticulados física ou quimicamente, formando estruturas tridimensionais que podem absorver grandes quantidades de água ou soluções aquosas. Podem se apresentar em forma de pós, grãos ou fragmentos semelhantes a filmes plásticos flexíveis. Após a reticulação, os hidrogéis têm capacidade de absorver e liberar grandes quantidades de água e íons sem se dissolver. Quando hidratados, eles absorvem a água e adquirem uma consistência esponjosa ou gelatinosa, com peso de 10 a 1000 vezes maior que o inicial (CAGNIN *et al.*, 2021a; KALENDOVA *et al.*, 2021; SIMÕES *et al.*, 2019).

Atualmente, grande parte dos hidrogéis são produzidos a partir de polímeros sintéticos, o ácido acrílico e seus sais de sódio ou potássio (acrilatos) são frequentemente utilizados. A utilização desses materiais tem como consequência o acúmulo de resíduo sólido e seus produtos de degradação, que são considerados perigosos para o meio ambiente. Por esse motivo, há um esforço para substituir os hidrogéis sintéticos, de alto custo de produção, não renováveis, de difícil degradação e ambientalmente agressivos, por hidrogéis de origem natural e biodegradáveis. Além disso, estes materiais têm a capacidade de aumentar a aeração no solo e reduzir a perda de nutrientes por lixiviação. Neste sentido, o desenvolvimento de hidrogéis biodegradáveis é de extrema importância para o crescimento econômico sustentável, principalmente em países com intensa atividade agrícola (KALENDOVA *et al.*, 2021; WANG *et al.*, 2018; XIAO *et al.*, 2017).

Os hidrogéis produzidos a partir de biopolímeros como a celulose, o amido e a gelatina, podem apresentar resultados promissores, já que possuem vantagens atraentes como, baixo custo, segurança ambiental, são totalmente biodegradáveis, não tóxicos, além de possuírem excelentes propriedades hidrofílicas e capacidade de liberação controlada de água e/ou nutrientes, sendo assim são materiais apropriados para o uso no solo (ABDEL-RAOUF *et al.*, 2018; KOWALSKI *et al.*, 2019; LIU; LUAN; LI, 2019).

O amido é um homo polissacarídeo constituído por cadeias de amilose e amilopectina (FEKETE *et al.*, 2017; LEMOS *et al.*, 2021; YE *et al.*, 2019). Para aplicação do amido na formulação de hidrogel é importante o estudo da modificação do amido nativo, para a melhoria das propriedades mecânicas e de intumescimento do material, já que são características de menor desempenho quando comparadas aos materiais sintéticos já empregados.

A celulose é o biopolímero mais abundante na natureza, sua unidade estrutural básica é constituída de duas unidades de glicose (celobiose) (CAPANEMA *et al*.; GAN *et al*., 2017; MAALOUL *et al,* 2021; TAO; NONAKA, 2021). Possui grupos hidroxilas abundantes, podendo ser facilmente modificada e inserida em uma matriz polimérica reticulada para a produção de hidrogéis, apresentando excelentes caraterísticas físico-químicas, e a introdução de grupos funcionais como alquila, hidroxialquila e carbonila são os mais utilizados para esta finalidade. (MA; LI; BAO, 2015; REDDY; YANG, 2010). Embora a extração de celulose de resíduos agroindustriais requeira diversas etapas de purificação, sua elevada disponibilidade e baixo custo, fazem dela uma escolha promissora para a produção de materiais de maior valor agregado, como os hidrogéis (DEMITRI *et al.*, 2016; FEKETE *et al.*, 2017).

A gelatina é uma proteína obtida pela hidrólise parcial do colágeno, muito utilizada na indústria de alimentos como agente gelificante. É um polímero solúvel em água, e recentemente, há muitos estudos relatando a inserção da gelatina em matrizes de hidrogéis. Tem a capacidade de aumentar a elasticidade e estabilidade térmica dos hidrogéis produzidos. Esta proteína pode ser promissora para a obtenção de hidrogéis com o objetivo de liberação controlada de fertilizantes e nutrientes na agricultura (GÓMEZ-MASCARAQUE *et al.*, 2019; ULLAH *et al.*, 2015).

A inclusão de grupos funcionais nos biopolímeros pode ser realizada antes da reticulação do hidrogel, a fim de tornar o polímero mais adequado para o processamento (BIDUSKI *et al.*, 2018; REDDY; YANG, 2010). Os polissacarídeos, como o amido e a celulose podem ser modificados por diferentes métodos, nos métodos tradicionais são necessárias múltiplas etapas, que consomem tempo e energia, e também geram grande quantidade de resíduos químicos. Tecnologias inovadoras e menos poluentes, tanto para modificação de polímeros, quanto para produção de hidrogéis, têm sido investigadas, para produção com baixo custo, eficiência e menor impacto ambiental (CAI *et al.*, 2019; LIANG; WANG; CHEN, 2019; XIAO *et al.*, 2017).

A extrusão reativa, quando comparada a outros processos, apresenta diversas vantagens, se configurando como um processo eco-amigável, contínuo, e sem a geração de efluentes. Pode ser empregada para modificar polissacarídeos através da combinação de calor, pressão e atrito mecânico. O processamento via extrusão tem vários benefícios, sendo possível o emprego de matérias-primas com menor umidade, além da possibilidade de adicionar reagentes e aditivos como auxiliares de processamento e estabilizadores durante o processo, resultando em baixo custo, simplicidade e possibilidade de produção de materiais em larga escala (CAI *et al.*, 2019; LIU *et al.*, 2016; MALI; GROSSMAN, 1999; YE *et al.*, 2019a).

Os ácidos orgânicos policarboxílicos, como o ácido cítrico (CA) e o ácido tartárico (TA) podem ser utilizados como agentes reticulantes, configurando-se como uma opção viável tanto para modificação de polímeros como o amido e a celulose, como também para a obtenção dos hidrogéis (REDDY; YANG, 2010; SHEN *et al*., 2015; SIMÕES *et al.*, 2019; YE *et al*., 2019). Outro agente reticulante que pode ser usado é o trimetafosfato de sódio (STMF), seu uso é aprovado pela *Food and Drug Administration* (FDA), agência norte-americana que regula produção de alimentos e medicamentos.

Os hidrogéis têm extensas aplicações industriais, portanto as pesquisas sobre o desenvolvimento de novos métodos para sua obtenção estão aumentando significativamente. Por apresentarem propriedades como capacidade de intumescimento e não toxicidade, tornam-se um material adequado para diferentes aplicações, incluindo seu uso como absorventes de água para us o na agricultura (ASHRAFI; JOKAR; MOHAMMADI, 2018; PAN *et al.*, 2019).

Diante disso, o presente trabalho teve como proposta desenvolver hidrogéis a partir do emprego de biopolímeros, como amido, celulose e gelatina, modificados via extrusão reativa, para melhorar a taxa de intumescimento e propriedades de solubilidade dos materiais obtidos. A relevância científica do projeto está no uso do amido, celulose e gelatina, como precursores para síntese de materiais com interesse tecnológico a partir de reações que ainda são pouco estudadas. Ademais, os hidrogéis preparados apresentam potencial capacidade para aplicação na agricultura, tornando-se possíveis substitutos dos hidrogéis comerciais oriundos de precursores não renováveis

2 OBJETIVOS

2.1 GERAL

Desenvolver hidrogéis à base de misturas biopoliméricas de amido de mandioca, celulose e gelatina para aplicações agrícolas como agentes de retenção de água.

2.2 ESPECÍFICOS

- Modificar quimicamente o amido de mandioca via extrusão reativa através da esterificação e introdução de ligações cruzadas com os ácidos cítrico e tartárico visando aumentar a sua capacidade de retenção de água;
- Modificar quimicamente a celulose extraída da casca de soja via extrusão reativa através da esterificação e introdução de ligações cruzadas com trimetafosfato de sódio e ácido tartárico;
- Caracterizar os biopolímeros modificados em relação à sua morfologia, propriedades físico-químicas e capacidade de absorção de água;
- Desenvolver processo de extrusão para a produção de hidrogéis a partir das misturas de amido, celulose e gelatina;
- Caracterizar os hidrogéis produzidos por extrusão em relação à sua morfologia, densidade, propriedades físico-químicas e capacidade de absorção de água.

3 REVISÃO BIBLIOGRAFICA

3.1 HIDROGÉIS NA AGRICULTURA

A agricultura é uma atividade essencial por ser a principal fonte de alimentos para a nutrição humana e animal. Essa atividade depende de fatores climáticos para o fornecimento de água considerando-se que 90% do peso total das plantas é constituído de água (LOPES, 2017; MONTEIRO *et al.*, 2017; THOMBARE *et al.*, 2018).

A seca, o desmatamento, a desertificação do solo e a escassez de água para irrigação são os maiores problemas enfrentados pela agricultura. As mudanças climáticas ocorridas na última década agravaram ainda mais estas dificuldades, induzindo falhas ecológicas, agrícolas e até mesmo financeiras, em razão da escassez de chuvas. Para garantir a segurança alimentar é essencial a recuperação de solos degradados, além do uso de recursos naturais de forma correta e consciente, visando técnicas de economia de água e manejo agrícola adequado (BAI *et al*., 2010; HASIJA *et al*., 2018; NADA; BLUMENSTEIN, 2015; THOMBARE *et al*., 2018). De acordo com Monteiro *et al*. (2017), a atividade agrícola se destaca como a atividade socioeconômica que mais consome e desperdiça água em nível mundial. Portanto, consequências irreversíveis podem ocorrer caso não sejam adotadas tecnologias que minimizem o uso excessivo da água.

O Brasil tem destaque na produção agrícola mundial, porém nos últimos anos a condição climática teve efeitos negativo na safra, principalmente nas regiões norte e nordeste, onde o déficit hídrico é ainda mais severo, com o problema da seca, fator limitante para a produção agrícola. Os solos arenosos são caracterizados pela baixa capacidade de retenção de água e drenagem excessiva da chuva, levando a uma baixa utilização de água, por esse motivo, o manejo agrícola em solos arenosos enfrentam grandes dificuldades (LOPES, 2017; NADA; BLUMENSTEIN, 2015).

Neste sentido, a crescente demanda de alimentos e a escassez de recursos hídricos, aliados ao atual apelo mundial pelo crescimento sustentável, direcionam pesquisas para o desenvolvimento de novas alternativas mais sustentáveis para a agricultura, como o uso de hidrogéis biodegradáveis com capacidade de retenção de água, que podem proporcionar efeitos benéficos às plantas, auxiliando na melhoria da qualidade de solos áridos, além do aumento da capacidade de retenção de fertilizantes e água no solo, reduzindo a frequência de irrigação. Os hidrogéis vêm ganhando destaque na agricultura mundial, porém ainda são necessárias pesquisas para a produção de um material biodegradável e ambientalmente seguro para uso na agricultura, evitando prejuízos futuro no solo e ao meio ambiente (GUILHERME *et al.*, 2015; HASIJA *et al.*, 2018; MONTEIRO *et al.*, 2017).

Adicionalmente, a demanda por fertilizantes também está cada vez maior, e como grande parte dos fertilizantes utilizados não são devidamente fixados nas plantas, sendo perdidos por lixiviação, a utilização de hidrogéis como sistemas de liberação controlada pode favorecer a liberação dos nutrientes do solo, adaptando-se melhor ao ciclo de vida da planta, além minimizar os custos gerais de produção (RAAFAT; EID; EL-ARNAOUTY, 2012; SABADINI; MARTINS, 2015).

Portanto, a utilização de hidrogéis na agricultura para a otimização do uso de água e para a liberação controlada de fertilizantes é promissor. A capacidade de retenção de água pelos hidrogéis pode ser útil nos períodos de seca promovendo a liberação gradativa de água, fazendo com que o solo permaneça úmido. Grande parte dos fertilizantes utilizados não são fixados pela planta, o que leva a sua perda, principalmente por lixiviação. Com o uso do hidrogel como sistema de liberação controlada de fertilizantes, há aumento da presença dos nutrientes do solo, de acordo com a necessidade da planta. Há diversos benefícios dos sistemas de liberação controlada nos insumos agrícolas, tais como, diminuição dos custos, segurança no manuseio desses produtos, redução de contaminação ambiental e redução de riscos de toxicidade (BAI *et al.*, 2010; BIDUSKI *et al.*, 2018; XIE *et al.*, 2011).

Os hidrogéis podem ser definidos como materiais poliméricos funcionais constituídos por uma rede polimérica de estrutura tridimensional formada a partir da reticulação de polímeros hidrofílicos, com o objetivo de manter a estrutura, permitindo a absorção e retenção de grande quantidade de água sem se desintegrar (Figura 1). O hidrogel pode ser reticulado química ou fisicamente, a partir de polímeros sintéticos ou naturais. Os hidrogéis produzidos a partir de polímeros biodegradáveis tem se tornado materiais promissores por seu desempenho em diversas áreas como, agricultura, farmacêutica, biomédica, produtos de higiene, tratamento de efluentes e biossensores (GUTIÉRREZ; VALENCIA, 2021; KALENDOVA *et al.*, 2021; LIU; LUAN; LI, 2019; SALLEH *et al.*, 2019).

Figura 1 - Imagens de hidrogéis à base de amido na forma de pellets, pós e filmes secos e depois de colocados em contato com a água.

Fonte: Próprio autor

A estrutura tridimensional dos hidrogéis, geralmente não cristalina, apresenta grupos funcionais hidrofílicos ligados a cadeia principal do polímero, conferindo a capacidade de absorver água e inchar, sem se dissolver, sendo assim, um material estável na presença de água. A resistência à dissolução acontece em função das ligações cruzadas entre as cadeias da rede tridimensional. Os hidrogéis, quando secos apresentam-se quebradiços, quando em contato com água, tornam-se um gel elástico, e a sua forma original é preservada (AHMED, 2015; BIDUSKI *et al.*, 2018; SIMÕES *et al.*, 2019).

Segundo Ahmed (2015), os hidrogéis para aplicações agrícolas podem ser classificados de acordo com as seguintes características: a) origem/base (natural ou sintético); b) composição polimérica (hidrogéis homopoliméricos, hidrogéis copoliméricos, e hidrogéis poliméricos de interpenetração multipolimérica; c) tipo de ligações da rede polimérica (químicas ou físicas); d) aparência física (matriz, filme ou microesfera); e) carga elétrica da rede (não-iônico, iônico, anfotéricos). Portanto, de acordo com as características inerentes para cada tipo de hidrogel, podem ocorrer modificações quando há alteração das propriedades físicas (temperatura, campo elétrico, campo magnético, iluminação, pressão, som) e químicas (pH, força iônica, composição do solvente e espécie molecular).

As características desejadas dos hidrogéis são a alta capacidade de intumescimento, alta estabilidade durante o armazenamento, pH neutro, ausência de cheiro e cor, não tóxico, biodegradabilidade e baixo custo (THOMBARE *et al.*, 2018; ZHANG *et al.*, 2015). A alta capacidade de absorção de água dos hidrogéis garante as características de flexibilidade, elasticidade e permeabilidade ao material. Porém, estas características dependem dos grupos funcionais hidrofílicos ligados à cadeia do polímero. Desta forma, a escolha dos polímeros, sejam eles sintéticos ou naturais, são cruciais à obtenção de um material com todas as qualidades desejadas (GUILHERME *et al.*, 2015; SALLEH *et al.*, 2019; YE *et al.*, 2019).

3.2 POLÍMEROS BIODEGRADÁVEIS EMPREGADOS NA PRODUÇÃO DOS HIDROGÉIS

Os hidrogéis absorventes de umidade já são bastante estudados para aplicações agrícolas, tanto para retenção de água, quanto para sistemas de liberação controlada de fertilizantes. Porém, grande parte dos estudos descreve como base da cadeia polimérica polímeros sintéticos, especialmente poliacrilamida e poliacrilatos (CHANG *et al.*, 2021; GUO; CHU, 2005; HE *et al.*, 2017; KALALEH; TALLY; ATASSI, 2015; KARADAĞ *et al.*, 2000; OLAD *et al.*, 2018; SALIMI *et al.*, 2020; SCHMIDHALTER; GEESING; SCHMIDHALTER, 2004; SENNAKESAVAN *et al.*, 2020; TOMAR *et al.*, 2007; WEI *et al.*, 2016; ZAIN *et al.*, 2018). Esses polímeros apresentam excelentes qualidades, como baixo custo, fácil manuseio, além de ótimas propriedades químicas, físicas e mecânicas, tornando-se importantes no ponto de vista industrial. Entretanto, são polímeros não biodegradáveis, que demoram anos para degradação completa, colaborando assim, para o aumento de resíduo sólido, além de sua toxicidade. Portanto existe um problema ambiental com hidrogéis não biodegradáveis de origem sintética, essa questão é agravada quando esses polímeros são utilizados na agricultura, o acúmulo desses materiais no solo podem causar graves desequilíbrios ecológicos, afetando diretamente a fertilidade do solo (BAI *et al*., 2010; HE *et al*., 2017; SARUCHI *et al*., 2019; ZHANG *et al*., 2015).

Abdel-Raouf *et al.* (2018), He *et al.* (2017), Meng *et al*. (2017) e Motamedi *et al.* (2020) relatam o uso de misturas entre polímeros sintéticos derivados dos acrilatos e polímeros biodegradáveis para obtenção de hidrogéis para uso na agricultura, como forma de minimizar o impacto dos materiais não biodegradáveis. O uso de matérias-primas de fontes renováveis e biodegradáveis como substitutos dos materiais sintéticos vem chamando atenção de pesquisadores para a produção de hidrogéis biodegradáveis com aplicação na agricultura com as vantagens de serem atóxicos e apresentarem baixo custo, podem ser derivados de gomas vegetais (HASIJA *et al*., 2018; SARUCHI *et al*., 2019), amidos (KALENDOVA *et al.*, 2021; SUPARE; MAHANWAR, 2021), celulose e derivados (DEMITRI *et al*., 2008; 2013; RAAFAT; EID; EL-ARNAOUTY, 2012), hemicelulose (ZHANG *et al.*,2015), dentre outros. Esses polímeros reticulados podem ser utilizados no solo, reduzindo o consumo de água de irrigação, demonstrando melhora das propriedades físicas do solo, além de aumentar o cultivo em áreas áridas. A Figura 2 ilustra o mecanismo de funcionamento de hidrogéis na agricultura. (GUILHERME *et al*., 2015; DEMITRI *et al*., 2013).

Figura 2 - Mecanismo de funcionamento de hidrogéis na agricultura

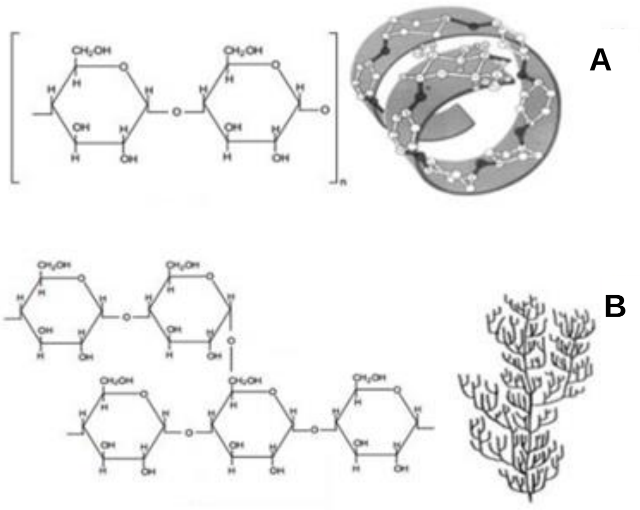
Fonte: Adaptado de Rosa, Bordado e Casquilho (1992)

Os hidrogéis elaborados a partir de polímeros naturais apresentam propriedades mecânicas inferiores aos polímeros sintéticos, isso faz com que essa matriz degrade precocemente, de tal forma que os nutrientes incorporados na matriz tridimensional não sejam liberados lentamente como esperado. Essas propriedades, dependem da densidade de ligações cruzadas, por isso, podem ser melhoradas através de estudos sobre os diferentes métodos de reticulação (física, química ou a combinação deles) para a incorporação de grupos funcionais na matriz polimérica, ou ainda, pela adição de outro polímero na matriz, visando a otimização da produção do material, podendo expandir sua faixa de aplicação (BIDUSKI *et al.*, 2018; CAPANEMA *et al.*, 2018; YE; CHANG; ZHANG, 2019).

3.2.1 Amidos na produção de hidrogéis

O amido é um polímero de interesse para a obtenção de hidrogéis, é o segundo carboidrato mais abundante na natureza, principal componente dos grãos de cereais e tubérculos. Apresenta características importantes, pois além de ser obtido de fontes renováveis, oferece vantagens como, baixo custo, facilidade de modificação química e eficiência na substituição de alguns polímeros sintéticos (GUILHERME *et al.*, 2015; LEMOS *et al.*, 2021; VON BORRIES-MEDRANO *et al.*, 2018). O amido é um homopolissacarídeo de glicose composto por cadeias de amilose (Figura 3a) e amilopectina (Figura 3b), frações lineares e ramificadas do amido, respectivamente. A proporção destas macromoléculas varia em função da origem botânica, das variedades da mesma espécie ou do estado fisiológico da planta (SABADINI; MARTINS, 2015).

Figura 3 - (A) Estrutura da amilose e sua conformação helicoidal, (B) estrutura da amilopectina e suas ramificações



Fonte: Adaptado de Biduski *et al*. (2018).

A amilose é composta por unidades de glicose unidas por ligações glicosídicas α-(1,4), dando origem a uma cadeia linear, enquanto a amilopectina é formada por unidades de glicose unidas em α-(1,4) e α-(1,6), originando uma estrutura ramificada. A amilose e a amilopectina, estão associadas paralelamente dentro do grânulo, formando ligações de hidrogênio, resultando em uma estrutura semicristalina. A cristalinidade é definida pelo grau de organização molecular dos grânulos de amido, sendo responsável pelo controle do comportamento do polímero em presença de água, conservação da estrutura, além de determinar a resistência aos ataques químicos e enzimáticos (SABADINI; MARTINS, 2015; YE *et al.*, 2019).

A amilose, aglomerada no centro do grânulo, participa das duplas hélices juntamente com a amilopectina. A estrutura em espiral tem capacidade de interagir com compostos apolares (lipídeo e iodo), esses compostos se encaixam na cavidade central da hélice. O amido é muito utilizado em indústria de alimentos, por contribuir em diversas propriedades de textura em alimentos, possuindo aplicações industriais como espessante, estabilizador, agente gelificante, entre outros (BIDUSKI *et al.*, 2018; GUILHERME *et al*., 2015; LEMOS *et al.*, 2021).

Para a obtenção de hidrogéis a partir do amido, são necessárias duas etapas, iniciando-se com a gelatinização do polímero. Quando o amido é aquecido em solução aquosa, ocorre o intumescimento dos grânulos até a sua ruptura, destruindo a organização estrutural, o que causa mudanças irreversíveis nas propriedades do polímero, esse fenômeno é definido como gelatinização, que é essencial para romper a estrutura cristalina do amido, possibilitando a interação entre as cadeias do polímero. A segunda etapa é a retrogradação do amido, com o resfriamento, o amido gelatinizado perde energia e as ligações de hidrogênio tornam-se mais fortes, as cadeias se organizam em um estado mais ordenado, ocasionando a formação de estrutura cristalina. O grau de retrogradação depende da quantidade de amilose liberada durante o processo de gelatinização, quando em grande quantidade no grânulo, tente a promover uma nova estrutura cristalina mais organizada e rígida, dando origem a um amido resistente. Enquanto uma matriz com baixa concentração de amilose tem tendência a formar uma estrutura menos coesa, com maior probabilidade em se desintegrar em contato com água (BIDUSKI *et al*., 2018; MINAKAWA; FARIA-TISCHER; MALI, 2019; MONROY; RIVERO; GARCÍA, 2018; VON BORRIES-MEDRANO *et al*., 2018;).

Amidos de diferentes origens podem ser considerados matrizes muito atrativas para a obtenção de hidrogéis biodegradáveis com maior valor agregado (ELGAIED-LAMOUCHI *et al*., 2021; LEMOS *et al*., 2021). Os agentes de reticulação são capazes de formar ligações intermoleculares de éter ou éster entre grupos hidroxila em moléculas de amido e, dependendo do nível de substituição, a rede de polímero é reforçada e resulta em mudanças na solubilidade, poder de expansão, viscosidade, digestibilidade, temperatura de gelatinização, entalpia, resistência à retrogradação e sinérese, coesividade, adesividade e translucidez dos materiais resultantes (SIMÕES *et al*., 2020; TUPA *et al*., 2020).

A reticulação para modificação química do amido por meio da reação com agentes bi ou polifuncionais é amplamente relatada na literatura (GHOSH DASTIDAR; NETRAVALI, 2012; LIU *et al.*, 2014b; OLSSON *et al.*, 2013a; OTACHE *et al.*, 2021; SHEN *et al.*, 2015; TUPA *et al.*, 2020; VOLKERT *et al.*, 2010; YE; CHANG; ZHANG, 2019). Essas mudanças estão diretamente relacionadas ao grau de substituição (GOLACHOWSKI *et al.*, 2020). A epicloridrina e o glutaraldeído têm sido amplamente usados como agente reticulante de polissacarídeos, no entanto, estes compostos apresentam toxicidade (LEMOS *et al.*, 2021; MANISH; AROCKIARAJAN; TAMADAPU, 2021; ULINIUC *et al.*, 2013; VON BORRIES-MEDRANO *et al.*, 2018; YE *et al.*, 2019). Em contraste, os ácidos policarboxílicos, como os ácidos cítrico e tartárico são reagentes de baixo custo e não tóxicos que podem ser usados com segurança para obter-se amidos reticulados (AČKAR *et al.*, 2015; GOLACHOWSKI *et al.*, 2020; MISKEEN *et al.*, 2021).

A interação entre os grupos carboxila dos ácidos orgânicos e os grupos hidroxila do amido pode melhorar sua estabilidade à água, reduzindo os grupos OH disponíveis do amido. Recentemente, vários autores relataram o uso de ácidos orgânicos como o ácido cítrico como agentes de reticulação para modificação do amido, enfatizando a segurança, eficiência e menor impacto ambiental de seu uso (AČKAR *et al.*, 2015; ALIMI; WORKNEH, 2018; FARHAT *et al.*, 2017; HONG *et al.*, 2020; KAPELKO-ZEBERSKA *et al.*, 2016; MISKEEN *et al.*, 2021; SHEN *et al.*, 2015; TUPA *et al.*, 2020; YE; CHANG; ZHANG, 2019; ZHOU *et al.*, 2016).

Xiao e colaboradores (2017), estudaram a obtenção de hidrogéis superabsorventes a partir do amido e concluíram que a reticulação desse polímero é influenciada por alguns fatores, tais como, fonte do amido, concentração e composição do reagente de reticulação. Quando incorporado grupos funcionas na estrutura do amido, há influência no poder de intumescimento do hidrogel produzido.

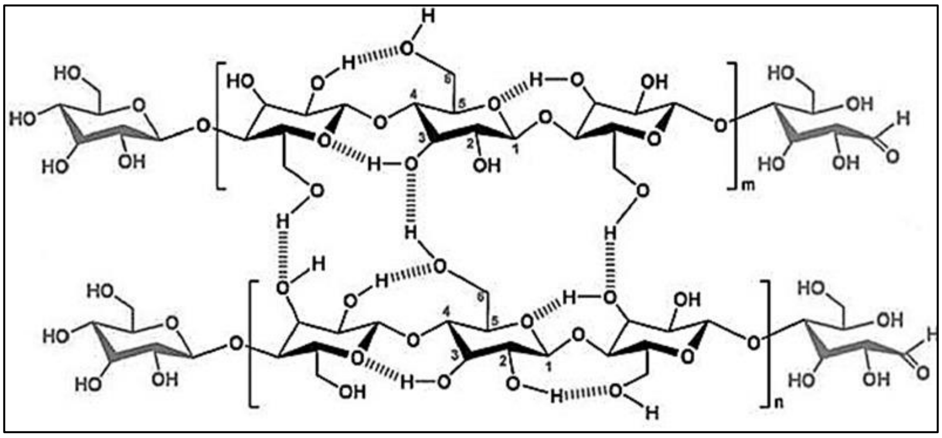
3.2.2 Celulose na produção de hidrogéis

A celulose é o polímero natural mais abundante no mundo, principal constituinte de tecidos vegetais superiores, é o material de construção de células fibrosas longas e tem como principal característica a alta resistência e rigidez. Pode ser encontrado também em algumas espécies de animais marinhos, algas, fungos, bactérias e pequenas quantidades em invertebrados (FEKETE *et al.*, 2017; GAZZOTTI *et al.*, 2017; MANTOVAN *et al.*, 2020).

A celulose $(C_6H_{10}O_5)_n$ é um homopolissacarídeo linear, constituído por unidades de glicose unidas por ligações β-(1,4)-glicosídicas, e sua unidade estrutural repetitiva é conhecida como celobiose, duas moléculas de glicose, o que confere uma estrutura única a celulose (Figura 4). Este polímero possui massa molar média de

100.000 g/Mol. O número de monômeros de glicose que compõe a cadeia da celulose determina seu grau de polimerização e varia de acordo com a origem botânica da celulose (COSCIA *et al.*, 2018; GAN *et al.*, 2017a; MARIM *et al.*, 2020)**.**

Figura 4 - Estrutura química da celulose



Fonte: adaptado de Trache *et al.* (2017)

Esse polímero possui uma extremidade redutora no carbono 1, e outra não redutora no carbono 4. As cadeias de celulose são próximas entre si, e a presença de hidroxilas em sua estrutura facilita as ligações de hidrogênio intra e intermoleculares. Essas ligações são permitidas devido a linearidade da celulose, e essas ligações impedem a solubilidade da celulose em água, dificultando a sua degradação (PADZIL *et al.*, 2015; SALLEH *et al.*, 2019).

Devido a essas ligações de hidrogênio, a celulose apresenta uma estrutura de elevada cristalinidade, com regiões com alto grau de ordenação, que são chamadas regiões cristalinas, e outras regiões com baixa organização ou desorganizadas, chamadas de regiões amorfas. O grau de cristalinidade é variável, e é determinado de acordo com a origem da celulose. A celulose extraída do algodão, por exemplo, possui cadeias extremamente ordenadas, chegando a uma cristalinidade de 70%, enquanto a celulose extraída de madeiras de árvores tem o índice de cristalinidade de aproximadamente 40%. A absorção de água só ocorre nas regiões amorfas, a cristalinidade da molécula influencia a solubilidade em água, dificulta a reatividade da química molécula e deixa a celulose menos susceptível ao ataque enzimático (GAZZOTTI *et al.*, 2017; MARIM *et al.*, 2020).

Há grande interesse no uso da celulose para produção de hidrogéis para retenção de umidade, no entanto, devido à sua insolubilidade em água, se faz necessária a introdução de substituintes na estrutura de celulose, melhorando assim sua solubilidade e as propriedades mecânicas (FEKETE *et al*., 2017; GIL- GIRALDO *et al.*, 2021; SALLEH *et al.*, 2019). Os hidrogéis de celulose podem ser empregados diferentes aplicações, tais como sistemas de liberação de medicamentos, separação de biomoléculas ou células, aditivos alimentares, e aplicações biomédicas (GAN *et al*., 2017).

Há vários relatos na literatura de hidrogéis de celulose e derivados em misturas com acrilatos e poliacrilamida para uso na agricultura (DONG *et al.*, 2013; SHAHZAMANI *et al.*, 2020), com excelente desempenho na retenção de água, porém é cada vez maior o interesse em substituir esses materiais por matrizes totalmente biodegradáveis (SHAHZAMANI *et al.,* 2020; ZHANG *et al*., 2017; ZHOU *et al*., 2013).

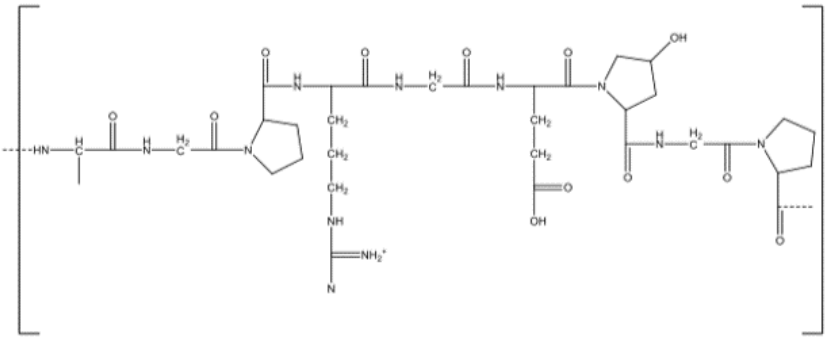
Li e Chen (2019) relataram diversos hidrogéis baseados em celulose de resíduos lignocelulósicos para aplicação na agricultura como forma de se controlar a umidade do solo e a liberação de nutrientes baseados em nitrogênio, fósforo e potássio, que são essenciais para o desenvolvimento das plantas. Comparando-se diferentes métodos de preparação, o estudo concluiu que apesar de mais rentáveis e com maiores capacidades de absorção de água, métodos químicos costumam ser mais nocivos ao meio ambiente do que os métodos físicos, que apesar de serem mais ambientalmente amigáveis, são mais caros e elaborados. O estudo apresentou combinações entre hidrogéis de celulose e diversos reticulantes que tiveram capacidade de absorver entre 35 e 2600 vezes o seu peso seco.

3.2.3 Gelatina na produção de hidrogéis

A gelatina é uma proteína derivada da desnaturação térmica, química ou física do colágeno encontrado em peles e ossos de animais. É um polímero solúvel em água, composto por uma mistura heterogênea de cadeias polipeptídicas, com a prevalência de resíduos de glicina (Gly), prolina (Pro), 4-hidroxiprolina (4Hyp) e glutamina (Glu). A estrutura típica da gelatina é composta pelos resíduos de aminoácidos -Ala-Gly-Pro-Arg-Gly-Glu-4HypGly-Pro- (Figura 5) (SABADINI; MARTINS, 2015; ULLAH *et al.*, 2015).

A indústria de alimentos utiliza a gelatina como agente gelificante ou modificador da textura, afim de melhorar a elasticidade, a consistência ou a estabilidade de produtos alimentícios, porém nos últimos anos, a gelatina tem sido empregada para diversas aplicações, tais como, na indústria farmacêutica, onde a função principal da gelatina é como um estabilizador, matriz para liberação controlada de substâncias químicas e na produção de tecidos orgânicos artificiais, entre outros (ULLAH *et al*., 2015; GÓMEZ-MASCARAQUE *et al*., 2019).

Figura 5 - Fragmento da estrutura da gelatina.



Fonte: Adaptado de Sabadini (2015)

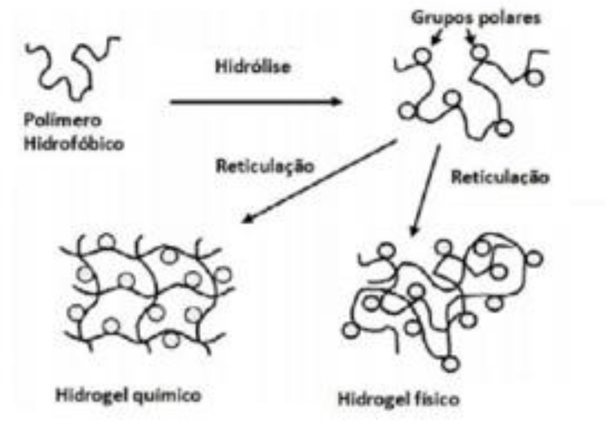
Por ser um polímero viscoso, biodegradável, não tóxico e de fonte renovável, tem a capacidade de produzir hidrogéis termicamente resistentes, sendo possível a substituição de alguns polímeros sintéticos (CHAUDHARY *et al.*, 2020; SABADINI; MARTINS, 2015; ULLAH *et al.*, 2015). Embora os hidrogéis à base de gelatina sejam relatados na literatura como adequados para produção de diferentes materiais para aplicação principalmente na área biomédica, os hidrogéis de gelatina pura são considerados mecanicamente instáveis quando em contato com elevadas proporções de água (> 80%), o que pode ser minimizado empregando-se a gelatina em misturas com outros biopolímeros (MANISH; AROCKIARAJAN; TAMADAPU, 2021). López-Velázquez *et al.* (2019) relataram a preparação de hidrogéis biodegradáveis de gelatina-quitosana-álcool polivinílico para potenciais aplicações agrícolas. Os hidrogéis apresentaram uma estrutura tridimensional densa, interligada e reticulada com capacidade de absorção de água correspondente à 12 vezes sua massa.

3.3 PROCESSOS DE PRODUÇÃO DE HIDROGÉIS

Para a obtenção do hidrogel, é primordial uma reação de reticulação, que é um método capaz de unir as cadeias poliméricas, que ocorre através da reação entre sítios reativos específicos presentes nas unidades estruturais do polímero e o reagente reticulante. Esse processo objetiva modificar as propriedades mecânicas, químicas e térmicas, rigidez estrutural, permeabilidade e capacidade de intumescimento do polímero (LIU; LUAN; LI, 2019; VARAPRASAD *et al.*, 2017; WANG *et al.*, 2018).

Os hidrogéis podem ser obtidos por métodos químicos ou físicos. Em hidrogéis produzidos com reticulação física, as cadeias poliméricas são estabilizadas por interações eletrostática, forças de Van der Waals e ligações de hidrogênio. Neste caso a reticulação pode ser destruída caso o material seja submetido a condições específicas. Já a reticulação química fornece uma estrutura estável, pois permite a obtenção de ligações permanentes na estrutura do polímero através de ligações covalentes, resultando em um material com maior resistência mecânica e maior grau de intumescimento. A Figura 6 compara os dois métodos de obtenção do hidrogel (ABDEL-RAOUF *et al.*, 2018; BIDUSKI *et al.*, 2018; DEMITRI *et al.*, 2016; VARAPRASAD *et al.*, 2017).

Figura 6 - Estrutura da matriz polimérica de hidrogéis reticulados por processo físico ou químico.

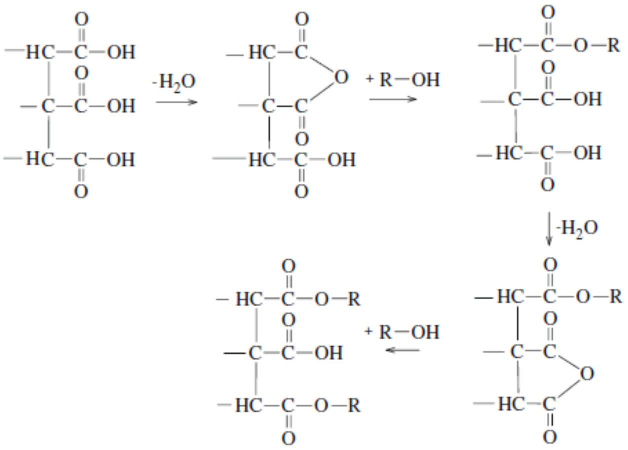


Fonte: Adaptado de Biduski (2018)

Dentre os principais agentes reticulantes para a obtenção de hidrogéis à base de biopolímeros podemos destacar a epicloridrina e o glutaraldeído, no entanto, estes compostos apresentam toxicidade relatada na literatura (LEMOS *et al.*, 2021; MANISH; AROCKIARAJAN; TAMADAPU, 2021; ULINIUC *et al.*, 2013). Por outro lado, alguns agentes polifuncionais menos tóxicos e catalogados como seguros pela *Food and Drug Administration* (FDA), têm sido relatados na literatura como reagentes em potencial para a modificação de amido e celulose com vistas à produção de hidrogéis. Pode-se destacar os ácidos orgânicos, em especial os ácidos policarboxílicos, como os ácidos cítrico, succínico e tartárico, considerados como alternativas viáveis, ecoamigáveis, de baixo custo e não tóxicas para obter-se biopolímeros reticulados (AČKAR *et al.*, 2015; GOLACHOWSKI *et al.*, 2020; MISKEEN *et al.*, 2021). O trimetafosfato de sódio (STMF) também tem sido relatado como agente reticulante de menor toxicidade quando comparado aos agentes reticulantes convencionais (SIMÕES *et al*., 2020; VON BORRIES-MEDRANO *et al.*, 2018; YE *et al.*, 2019).

A Figura 7 demonstra uma possível reação de reticulação da celulose com ácido cítrico (KUSUMAH; ASTARI; WIDYORINI, 2017; REDDY; YANG, 2010; VON BORRIES-MEDRANO *et al.*, 2018).

Figura 7 - Possível reação de reticulação entre celulose e ácido cítrico

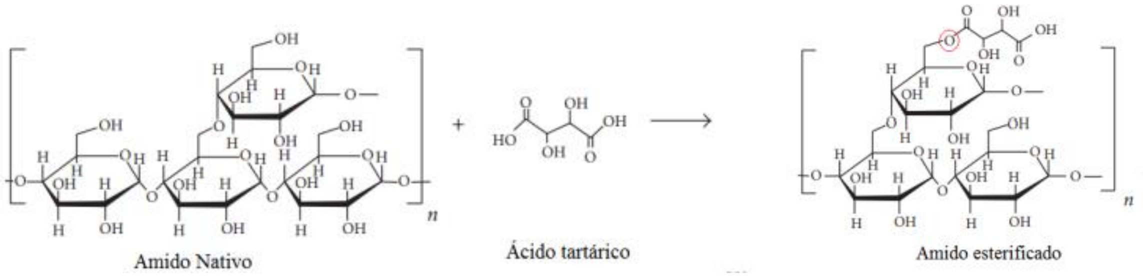


Fonte: Reddy e Yang (2010)

O ácido tartárico é um ácido orgânico dicarboxílico que faz parte da composição de frutas como banana e tamarindo. É um dos ácidos orgânicos mais importantes e tem sido amplamente aplicado na indústria a de alimentos, na preparação e preservação de alimentos (OLIVATO *et al.*, 2012; TUPA *et al.*, 2020). Olivato *et al*. (2012; 2013) concluíram que a adição de ácido tartárico contribuiu para a melhoria das propriedades mecânicas de filmes de amido/poli (adipato co-tereftalato de butileno) por promover ligações cruzadas entre as cadeias poliméricas.

Por ser um ácido dicarboxílico, o ácido tartárico pode promover a esterificação e/ou reticulação do amido e da celulose. A esterificação pode ocorrer por meio da substituição dos grupos hidroxila livres da molécula de amido ou celulose por grupos carboxílicos do ácido, estes novos grupos representam pontos reativos potenciais para as reações de reticulação (Figura 8) (MENDOZA, 2015; OLIVATO *et al.*, 2012, 2014; SPIRIDON *et al.*, 2013).

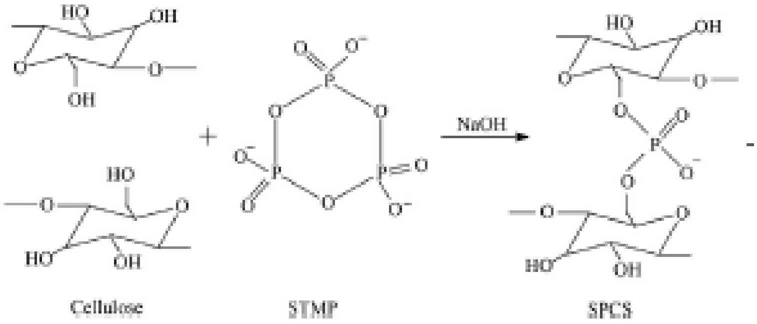
Figura 8 - Possível reação de reticulação entre amido e ácido tartárico



Fonte: Adaptado de Chin, Pang e Lim (2012)

O trimetafosfato de sódio (STMP) também vem sendo estudado como agente reticulante para a obtenção de hidrogéis absorventes de umidade, e a sua utilização tem como função reforçar as ligações de hidrogênio presentes no amido e na celulose. Esse intercruzamento reduz a ruptura da cadeia biopolimérica intumescida, produzindo assim uma rede absorvente mais estável. A Figura 9 apresenta o mecanismo de reticulação da celulose com STMP proposto na literatura (BIDUSKI *et al.*, 2018; CAGNIN *et al.*, 2021a; REDDY; YANG, 2010; SARUCHI *et al.*, 2019). Ma e colaboradores (2016) modificaram celulose com STMP, com o objetivo de introduzir grupos éster de fosfato na estrutura de celulose, resultando em um material com maior capacidade de absorção de água.

Figura 9 - Mecanismo da reticulação da celulose com STMP



Fonte: Adaptado de Ma *et al.*, (2016)

O método escolhido para a reticulação influencia algumas propriedades do material reticulado como, propriedades mecânicas, capacidade de absorção de água e grau de intumescimento. Portanto, é importante a escolha do melhor método visando as características necessárias para a aplicação do material. Existem diversos métodos para a síntese de hidrogel, entretanto, a síntese por extrusão reativa demonstra ser uma técnica rápida, simples e eficaz. Em geral é importante grande atenção na escolha do polímero, do agente de reticulação e do método de síntese para a obtenção do hidrogel desejado (FARHAT *et al.*, 2017; VON BORRIES-MEDRANO *et al.*, 2018; WANG *et al.*, 2018).

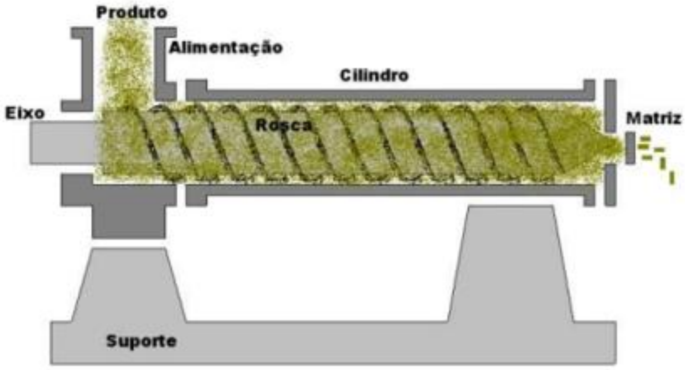
Os processos convencionais de reticulação do amido e da celulose têm sido realizados em reatores descontínuos ou de tanque agitado contínuo, com longos tempos de processamento, resultando em impactos ambientais dos efluentes gerados em todos os processos; assim, o uso de métodos físicos associados aos métodos químicos pode ser configurado como uma alternativa interessante para minimizar o uso excessivo de reagentes (CAI *et al.*, 2019; FARHAT *et al.*, 2017; MOAD, 2011; YE *et al.*, 2019).

3.3.1 Extrusão Reativa

A extrusão reativa é um processo muito explorado pela indústria de plásticos e alimentos. É um método utilizado para a mistura de polímeros, com a finalidade de obtenção de materiais termoplásticos, além da produção de modificações na estrutura do material processado. Este procedimento permite o processamento do material, sem a necessidade de solventes, por esse motivo é considerado um método de processamento ecoamigável para a reticulação de polímeros (CAI *et al.*, 2019; FARHAT *et al.*, 2017; VO *et al.*, 2017). A Figura 10 apresenta o esquema de uma extrusora monorosca.

As extrusoras oferecem a capacidade de controlar o tempo de residência do material, temperatura, além de ser capaz a adição de estabilizadores durante o processo. A extrusora monorosca é composta por apenas uma rosca sem fim dentro de um cilindro, possui a vantagem de fácil manuseio. A mistura dos polímeros ocorre através da ação combinada de calor, pressão e atrito mecânico, apresentando vantagens de alta produtividade, facilidade de manuseio e ausência de efluentes (WAHL *et al.*, 2013; MERCI *et al*., 2015; YE *et al*., 2019).

Figura 10 - Esquema simplificado de uma extrusora monorosca



Fonte: Merci *et al*. (2015)

A extrusão reativa é um método eficiente e versátil para modificar o amido e outros biopolímeros, sendo considerada uma solução tecnológica verde, uma vez que a extrusora é usada como um reator, onde reações químicas como esterificação, acetilação, hidroxipropilação e reticulação podem ser realizadas (CAI *et al.*, 2019; 2020; GUTIÉRREZ; VALENCIA, 2021). A extrusão reativa combina a energia termomecânica, catalisando a reação entre o biopolímero e os agentes reticulantes em um único processo sem o uso de outros reagentes. Além disso, a extrusão reativa é um processo contínuo que tem viabilidade comercial e é fácil de se adaptar a escalas industriais, oferecendo tempos de reação curtos (2–3 min) (FORMELA *et al*., 2018; HEEBTHONG; RUTTARATTANAMONGKOL, 2016; GIL-GIRALDO *et al*., 2021; MOAD, 2011; VOLKERT *et al*., 2010).

No caso do amido, quando é submetido ao processo de extrusão resulta em um produto gelatinizado. Esse processo já é utilizado em indústria de alimentos, para a obtenção de flocos de milho e outros alimentos. também utilizado para produção de raçoes animais (SIMÕES *et al.*, 2019; VON BORRIES-MEDRANO *et al.*, 2018)**.**

O uso de extrusão reativa para obter amido e celulose reticulados por meio de reação com ácidos orgânicos não foi totalmente explorado na literatura. Os grupos carboxílicos presentes nos ácidos orgânicos podem esterificar os grupos hidroxila dos biopolímeros, ao mesmo tempo reticular os polímeros com algumas vantagens, incluindo a segurança nutricional, não toxicidade e baixo custo. Recentemente, Farhat *et al.* (2017), Hong *et al.* (2020) e Ye *et al.* (2019) relataram o uso de extrusão reativa para obter citratos de amido de milho nativo, milho ceroso e amidos de arroz, respectivamente, e ressaltaram que esse processo pode ser considerado uma alternativa promissora para a obtenção de amidos reticulados. Hidrogéis de amido reticulados obtidos por reação com ácido tartárico por extrusão reativa não foram relatados na literatura. No trabalho de Ye e colaboradores (2019), foi estudada a reticulação do amido utilizando-se o ácido cítrico como agente de reticulação via extrusão reativa, esse estudo foi bem-sucedido, os hidrogéis desenvolvidos apresentaram excelente propriedades mecânicas, além da diminuição da dissolução do amido em água.

Bhandari *et al.* (2012) obtiveram carboximetilcelulose por extrusão reativa, enfatizando os tempos curtos de processamento. Zhang *et al.* (2014) relataram a modificação química da celulose por extrusão reativa, observando que a extrusão reativa pode destruir a regularidade das cadeias de celulose e da rede de ligações de hidrogênio de forma eficiente, favorecendo modificações químicas e a introdução de novos grupamentos químicos. Gil-Giraldo *et al.* (2021) relataram a introdução de ligações cruzadas na celulose extraída da casca de aveia via extrusão reativa com ácido cítrico. Tao e Nonaka (2021) relataram o uso de extrusão reativa para obtenção de celulose reticulada com ácidos orgânicos, e destacaram que este processo pode ser considerado uma alternativa promissora para obtenção de celulose reticulada.

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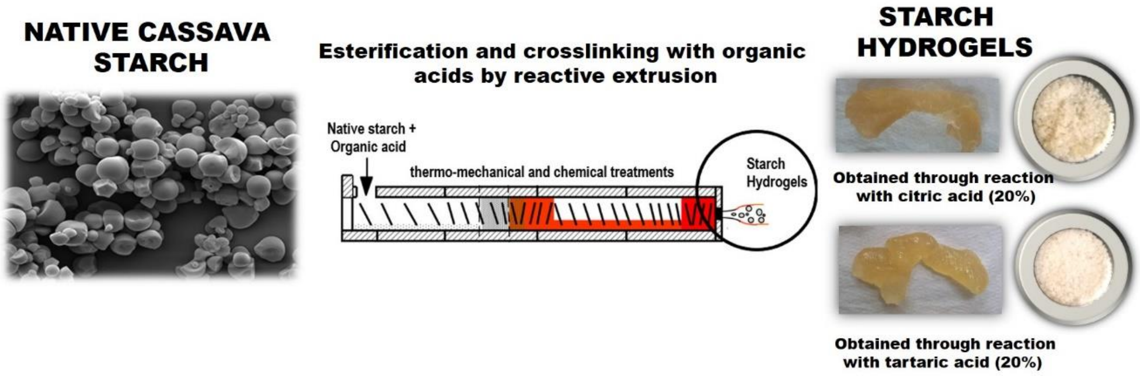
4 ARTIGO 1

Reactive extrusion-assisted process to obtain modified starch hydrogels through reaction with organic acids

Graphical Abstract



Abstract

A totally green process based on reactive extrusion was used for the production of cassava starch hydrogels by reactive extrusion through reaction with two organic esterifying agents, citric acid (CA) and tartaric acid (TA). CA and TA were used at different concentrations (0, 2.5, 5.0, 10.0, 15.0 and 20.0%). The degree of substitution (DS) of samples ranged from 0.023 to 0.365, which was confirmed by Fourier transform infrared spectroscopy (FT-IR) with a new band appearing at 1730 cm^{-1} associated with ester carbonyl groups. X-ray diffraction indicated that reactive extrusion resulted in the disappearance of diffraction peaks of native starch and samples with lower crystallinity indices ranging from 37% (native starch) to 8–11% in starch hydrogels. Morphology analysis showed that the original granular structure of starch was lost and replaced by a rougher and irregular structure. Water holding capacity and solubility of starch hydrogels obtained by reactive extrusion were both superior to those of native starch and the control sample (extruded without the crosslinking agents). Hydrogels obtained with the highest CA or TA concentrations (15.0 and 20.0%) resulted in the higher DS and swelling capacities. Reactive extrusion was effective in obtaining starch hydrogels by reaction with organic acids, resulting in an ecofriendly, simple and short reaction time process, which is easy to adapt to an industrial scale.

Keywords: Crosslinking; Citric acid; Tartaric acid; Green Chemistry.

1. INTRODUCTION

Hydrogels are materials formed by a porous three-dimensional network prepared from crosslinked natural or synthetic hydrophilic polymers that can hold large quantities of water or biological fluids in their structure without dissolving (ERDAGI, NGWABEBHOH, & YILDIZ, 2020; LEMOS, MARCELINO, CARDOSO, SOUZA, & DRUZIAN, 2021); generally, they can be prepared as membranes, films, scaffolds, injectable hydrogels, microgels, nanogels, or microspheres depending on the technique of polymerization and preparation.

Starch is a biodegradable, nontoxic, inexpensive, biopolymer available worldwide with readily free hydroxyl groups with potential for functionalization (GOLACHOWSKI, DROŻDŻ, GOLACHOWSKA, KAPELKO-ŻEBERSKA, & RASZEWSKI, 2020), which makes starch a versatile platform for chemical modifications, such as crosslinking reactions. Starches from different sources can be considered very attractive bases for the obtainment of biodegradable hydrogels with higher added value (ELGAIED-LAMOUCHI *et al*., 2021; LEMOS *et al.*, 2021), as a variety of physico-chemical changes can be made to their structural and nutritional properties, and they are suitable to be used in food, textile, papermaking, petrochemical, and pharmaceutical industries, depending on their different properties (LEMOS *et al.*, 2021; OTACHE, DURU, ACHUGASIM, & ABAYEH, 2020; TUPA, ALTUNA, HERRERA, & FORESTI, 2020).

The crosslinking for chemical modification of starch through reaction with bi- or polyfunctional agents is largely reported in the literature (DASTIDAR & NETRAVALI, 2012; LIU *et al*., 2014; OLSSON, HEDENQVIST, JOHANSSON, & JÄRNSTRÖM, 2013; OTACHE *et al*., 2021; SHEN, XU, KONG, & YANG, 2015; TUPA *et al*., 2020; VKERT, LEHMANN, GRECO, & NEJAD, 2010; YE *et al*., 2019). The crosslinking agents are capable of forming either ether or ester intermolecular linkages between hydroxyl groups on starch molecules, and depending on the level of substitution, the polymer network is reinforced and results in changes in solubility, swelling power, viscosity, digestibility, gelatinization temperature, enthalpy, resistance to retrogradation and syneresis, cohesiveness, adhesiveness, and translucency of the resulting materials (MEI, ZHOU, JIN, XU, & CHEN, 2015; SIMÕES *et al*., 2020; TUPA *et al*., 2020). These changes are directly correlated with the degree of substitution (GOLACHOWSKI *et al.*, 2020).

Starch hydrogels have several potential applications; however, in many cases, the high cost and the generation of toxic solvents are significant disadvantages. Epichlorohydrin and glutaraldehyde have been widely used to crosslink polysaccharide materials; however, they suffer from certain toxicity (LEMOS *et al*., 2021; MOAD, 2011; ULINIUC, HAMAIDE, POPA, & BĂCĂITĂ, 2013). In contrast, polycarboxylic acids, such as citric and tartaric acids, are inexpensive and nontoxic reagents that can be safely used to obtain crosslinked starches (AČKAR *et al*., 2015; GOLACHOWSKI *et al*., 2020; MISKEEN, HONG, CHOI, & KIM., 2021).

The interaction between the carboxyl groups of the organic acids and hydroxyl groups of starch can improve its water stability by reducing the available OH groups of starch. Recently, several authors reported the use of organic acids such as citric acid as crosslinking agents for starch modification, emphasizing the safety, efficiency, and lower environmental impact of their use (AČKAR *et al*., 2015; ALIMI & WORKNEH, 2018; FARHAT *et al*., 2017; HONG, CHUNG, LEE, & KIM, 2020; KAPELKO-ŻEBERSKA, ZIĘBA, PIETRZAK, & GRYSZKIN, 2016; MISKEEN *et al*., 2021; SHEN *et al*., 2015; SRIKAEO, HAO, & LERDLUKSAMEE, 2019; TUPA *et al*., 2020; YE *et al*., 2019; ZHOU, TONG, SU, & REN, 2016).

Conventional processes for starch modification have been performed in batch or continuous stirred tank reactors, with long processing times, and several reagents are usually required for gelatinization and acid neutralization, resulting in environmental impacts from the effluents generated in all processes; thus, the use of physical methods can be configured as an interesting alternative to minimize the excessive use of reagents (CAI *et al*., 2019; FARHAT *et al*., 2017; MOAD, 2011, YE *et al*., 2019).

Alternatively, the reactive extrusion process is an efficient and versatile method for modifying starch and is considered a green technological solution since the extruder is used as a reactor, where chemical reactions such as esterification, acetylation, hydroxypropylation, and crosslinking can be performed (CAI *et al*., 2019; CAI, TIAN, YU, SUN, & JIN, 2020; GUTIÉRREZ & VALENCIA, 2021). Reactive extrusion combines the thermomechanical energy necessary to break up the granular structure of starch, catalyzing the reaction between starch and organic acids in a single process without using other reagents. Additionally, reactive extrusion is a continuous process that has commercial viability, and it is easy to adapt to industrial scales, offering short reaction times (2–3 min) (FORMELA, ZEDLER, HEJNA, & TERCJAK,

2018; HEEBTHONG & RUTTARATTANAMONGKOL, 2016; MOAD, 2011; VKERT *et al*., 2010).

Organic acids have been reported as efficient crosslinking agents of starch, and the use of reactive extrusion to obtain crosslinked starches through reaction with organic acids has not been fully exploited in the literature. Recently, Farhat *et al*. (2017), Hong *et al*. (2020) and Ye *et al*. (2019) reported the use of reactive extrusion to obtain starch citrates from native corn, waxy corn, and rice starches, and they stressed that this process can be considered a promising alternative to obtain crosslinked starches. Crosslinked starch hydrogels obtained through reaction with tartaric acid by reactive extrusion have not been reported in the literature. Thus, this technology can be further explored for this purpose, considering that it is an ecofriendly and scalable alternative to industrial use.

Therefore, this study aimed to obtain cassava starch hydrogels by reactive extrusion using citric and tartaric acids as esterifying and crosslinking agents and to study their physicochemical and microstructural properties. This study contributes to the development of an environmentally friendly process for starch hydrogel obtainment.

2. MATERIAL AND METHODS

2.1. MATERIALS

Cassava starch (20% amylose and 80% amylopectin) was purchased from Pinduca Co. Ltd. (Araruna, Brazil). Citric acid (CA) and tartaric acid (TA) of analytical grade were purchased from Synthlab (Synthlab, Diadema, Brazil), similar to all other chemicals and solvents employed in this study.

2.2. METHODS

2.2.1. Reactive Extrusion Process

Citric acid (CA) and tartaric acid (TA) were employed at different concentrations (0, 2.5, 5.0, 10, 15, and 20% – g acid/100 g starch) as crosslinking agents. Crosslinked starch hydrogels were prepared by dissolving the different concentrations of CA or TA in distilled water. The acid solutions were mixed with the starch, resulting in a final moisture content of 32% in sealed bags, and were equilibrated for 1 h before extrusion. A control sample (S0) was extruded without any reagent except water, resulting in a final moisture content of 32%. Then, all the samples were extruded in a single screw extruder (AX Plastics, Diadema, Brazil) with a screw diameter of 1.6 cm and a screw length/diameter ratio (L/D) of 40, with four heating zones and a matrix of 0.8 cm in diameter. The temperature in all zones was 100 °C, and the screw speed was 60 rpm. The crosslinking starch extrudates were collected, placed in an oven, dried to constant weight at 45 °C, ground, and sieved through an 80-mesh sieve. The dry mixture was washed three times with absolute ethanol to remove the unreacted CA or TA. Finally, the washed starch hydrogel samples were air-dried at 45 °C. Starch hydrogel samples prepared by reaction with CA were labeled SC2.5, SC5, SC10, SC15, and SC20 throughout the study. Starch hydrogel samples prepared with TA were labeled ST2.5, ST5, ST10, ST15, and ST20 throughout the study.

2.2.2. Determination of the Degree of Substitution (DS)

The DS of each sample was determined in triplicate according to the method described by Vkert *et al*. (2010) by titration. All of the ester linkages were saponified by NaOH, and the amount of remaining excess NaOH was determined by titration with HCl.

2.2.3. Scanning Electron Microscopy (SEM)

SEM analyses were performed with an FEI Quanta 200 microscope (Oregon, USA). The samples were mounted for cross-section visualization on bronze stubs using double-sided tape. The surfaces were then coated with a thin gold layer (40–50 nm). All samples were examined using an accelerating vtage of 20 kV.

2.2.4. Fourier Transform-Infrared Spectroscopy (FT-IR)

The pulverized and dried samples were mixed with potassium bromide and compressed into tablets. The FT-IR analyses were carried out with a Shimadzu FT-IR-8300 (Kyoto, Japan), which has a spectral resolution of 4 cm^{-1} and a spectral range of 4000–500 cm^{-1}.

2.2.5. X-Ray Diffraction (XRD)

The crystallinity of each sample was investigated using XRD. The samples were finely powdered, and the analysis was performed using a Panalytical X'Pert PRO MPD diffractometer (Almelo, The Netherlands) with copper Kα radiation ($\lambda = 1.5418$ Å) under operational conditions of 40 kV and 30 mA. All assays were performed with a ramp rate of 1°/min. The relative crystallinity index (CI) was calculated from the ratio between the areas of the diffraction peaks and the area of the entire diffraction pattern after subtracting the amorphous background patterns using the method described by Cheetham & Tao (1998).

2.2.6. Differential Scanning Calorimetry (DSC)

DSC analyses were performed on a Shimadzu DSC 60 (Kyoto, Japan) calorimeter. Approximately 3.0 mg of each sample was placed in platinum containers and heated from 30 to 450 °C at a heating rate of 5 °C/min in a helium atmosphere.

2.2.7. Water Holding Capacity (WHC) and Solubility at 25 ºC

WHC was determined according to the methodology described by Butt, Ali, & Hasnain (2019), with minor modifications. Approximately 1.00 g of each sample was weighed, and 10 ml of water was added to a preweighed centrifuge tube (W_1). The samples were shaken for 30 min on a shaker at 25 °C and 200 rpm (Quimis Q 225M, Diadema, Brazil) and then centrifuged for 30 min at 2.200 rpm (Hettich Centrifuge, Universal Model 320R, Darmstadt, Germany). After centrifugation, the supernatant was decanted, and tubes with residue were weighed (W_2). The WHC was calculated as WHC (g/g) = (W_1 - W_2) - (initial mass of sample) /(initial mass of sample).

The solubility of the samples in water was calculated from the difference between the weight of the undissolved residue and the original weight of the sample.

2.2.8. Swelling Power at Different Times and Temperatures

The swelling at different times was determined according to the following procedure described by Yoshimura, Yoshimura, Seki, & Fujioka (2006). Dried samples (0.5 g) were left to swell within permeable nylon teabags (Japanese Industrial Standard, JIS K 7223) whose size was 200 and 100 mm in length and width. The tea bags were immersed in water at 25 °C for 0.5, 1, 24, and 48 h, and then each tea bag was removed from the water, and excess water was drained for 1 min. Then, the tea bags were weighed on an analytical scale. Later, the tea bags were placed in a shaking apparatus and heated at 10, 35, and 70 °C for 24 h. After incubation at those temperatures for 24 h, excess water was drained for 1 min, the weight of the teabag and samples was then measured (W_t), and the swelling was calculated according to the following equation: Swelling (g/g) = $((W_t – W_b) – W_p)/(W_p))$, where W_b is the weight of a blank tea bag after water treatment, and W_p is the weight of the dry sample.

2.2.9. Water Adsorption Isotherms

The samples (0.5 g) were dried for 7 days in a desiccator containing anhydrous calcium chloride, and then the samples were placed in desiccators containing different saturated salt solutions providing specific relative humidity (RH) (11, 33, 43, 60, 75, and 90%) and were held at 25 °C. The equilibrium moisture content of each sample was calculated after 7 days in a desiccator containing each RH, and it was determined in a ventilated oven (035 Marconi MA, Piracicaba, Brazil) by drying at 105 °C. The GAB (Guggenheim– Anderson-de Boer) model was used to fit the data from the sorption isotherms, and the monolayer values were calculated from the GAB isotherm model (Bizot, 1983) as follows: $M = (m_0CKa_w)/(1−Ka_w)\ (1−Ka_w+CKa_w)$, where M is the equilibrium moisture content at a given water activity (a_w), a_w is RH/100, m_0 is the monolayer value (g water/100 g solids), and C and K are GAB constants. All tests were conducted in triplicate.

2.2.10. Degree of Syneresis

The degree of syneresis of each sample was determined using the method described by Eliasson and Kim (1992). An aqueous suspension of starch (5 g/100 g) was heated in boiling water for 15 min, and then, the samples were stored for 4 weeks at 4 °C. The samples were adjusted to 20 °C, and the degree of syneresis (%) was determined from the vume of liberated water (%) after centrifugation at 5000 x g for 30 min at Days 1, 7, 14, 21, and 28.

2.2.11. Statistical Analysis

Analyses of variance (ANOVA) and Tukey's mean comparison test ($p \leq 0.05$) were performed with Statistica software version 7.0 (Statsoft, OK, USA).

3. RESULTS AND DISCUSSION

3.1. DEGREE OF SUBSTITUTION (DS)

According to Namazi & Dadkhah (2010), in modified starch samples, the DS can be defined as the number of hydroxyl groups substituted per glucopyranosyl unit. Considering that each glucopyranosyl unit presents three reactive hydroxyl groups, the maximum possible DS value is 3. DS values of starch samples are presented in Table 1. For both groups of samples modified with CA and TA, the increase in the acid concentration resulted in higher DS values, while comparing CA- and TA-modified samples, CA-modified samples had higher DS values (Table 1). Seidel *et al*. (2001) reported that the use of different polyfunctional carboxylic acids as esterifying and crosslinking agents results in different materials, which is related to their structures. Citric acid ($C_6H_8O_7$) is a tricarboxylic acid formed by two carboxylic groups spaced by three CH_2 groups with one OH group and one COOH group linked at the central carbon, while tartaric acid ($C_4H_6O_6$) is a dicarboxylic acid spaced by two CH_2 groups, each bearing one OH group. The difference in length of the spacer between CA and TA molecules and the kind and number of functional groups possibly resulted in a more efficient CA reaction with starch. Shen *et al*. (2015) reported that polycarboxylic acids with more than two carboxyl groups have better crosslinking effects than dicarboxylic acids. These authors stressed that citric acid (tricarboxylic) was more efficient as a modifying agent than succinic acid (dicarboxylic).

A higher DS value was obtained for the SC20 sample (DS = 0.365), and a lower DS value was obtained for the ST2.5 sample (DS = 0.023) (Table 1). These values were close to the values reported by Ye *et al*. (2019), who chemically modified rice starch by reactive extrusion with CA. They reported DS values ranging from 0.037 to 0.138 when the CA concentration ranged from 10 to 40% (g CA/100 g starch). Farhat *et al*. (2017) reported DS values of 0.52 to 0.99 in starch citrates obtained from corn starch by reactive extrusion with CA concentrations of 40 and 100% (g CA/100 g starch).

Table 1 - Degree of substitution (DS), water holding capacity (WHC), and solubility of native starch, control sample (S0) and starches hydrogels by reactive extrusion.

Samples	DS	WHC (g/g)	Solubility (%)
Native starch	0	1.84 ± 0.01^{g}	3.2 ± 0.05^{i}
S0	0	5.61 ± 0.05^{c,d}	12.14 ± 0.51^{h}
SC2.5	0.137	3.03 ± 2.01^{f}	28.69 ± 1.9^{c,d}
SC5	0.160	4.62± 1.12 ^{e}	28.15 ± 0.01^{c,e}
SC10	0.262	5.90 ± 2.13^{c}	26.55 ± 3.20^{d,e,f}
SC15	0.352	6.22 ± 2.07^{a,b}	24.30 ± 0.36^{f}
SC20	0.365	7.02 ± 3.03^{a}	21.30 ± 0.28^{g}
ST2.5	0.023	4.99 ± 0.07^{d,e}	29.68 ± 1.22^{c}
ST5	0.103	4.92 ± 0.05^{e}	32.70 ± 0.27^{b}
ST10	0.137	6.10 ± .03^{b,c}	39.73 ± 0.12^{a}
ST15	0.285	6.95 ± 0.04^{a}	25.35 ± 0.12^{e,f}
ST20	0.285	6.66 ± 0.09^{a,b}	24.65 ± 0.21^{f}

^{a}- Different letters in the same column indicate significant difference by Tukey test ($p \leq 0.05$).

The DS values of modified starch samples obtained in this study were slightly higher than those obtained for starch citrate prepared by high-temperature/long-term reactions in semidry conditions, as reported by other authors (MEI *et al*., 2015; SRIKAEO *et al*., 2019). Mei *et al*. (2015) reported that when the citric acid concentration was increased from 10 to 30%, the DS increased from 0.058 to 0.178 in cassava starch citrates obtained after 8 h of reaction at room temperature followed by drying at 50 °C for 24 h and at 130 °C for 5 h.

3.2. Scanning electron microscopy (SEM)

As observed in the SEM micrograph (Fig. 3), native cassava starch granules exhibited an elliptical or truncated elliptical shape, with a smooth surface without disruption. These results were consistent with those reported by other authors (MINAKAWA *et al.*, 2019; MONROY *et al*., 2018).

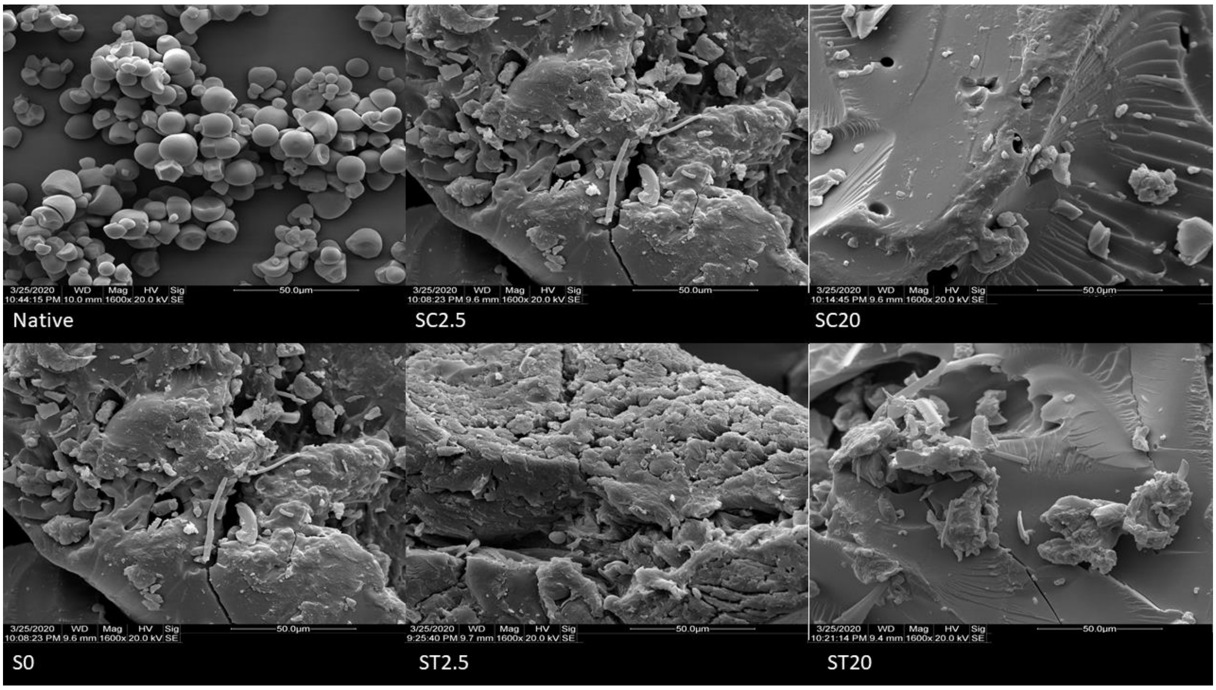


Fig. 1 - SEM images of native starch, the control sample (extruded without reagent) and starch hydrogels obtained by reactive extrusion through reaction with CA and TA.

After being subjected to extrusion, all extruded samples showed structural morphological differences in relation to native cassava starch. During reactive extrusion, the starch granules undergo changes in their structure, disintegrating totally or partially, depending on the input energy level, forming rougher structures with irregular and fragmented aggregates. SEM micrographs also demonstrated that the sample surfaces did not contain residual starch granules, which indicated that the granules were destroyed due to the thermal effects, shearing force, and chemical modification effects, which agreed with the XRD results. Other authors also reported the disintegration of the granular structure of starch subjected to reactive extrusion (CAI *et al*., 2019; FONSECA-FLORIDO *et al*., 2019; MURÚA-PAGOLA, BERISTAIN-GUEVARA & MARTÍNEZ-BUSTOS, 2009). In addition to the extrusion process, the hydrolytic action of CA and TA can also favor the breakdown of starch granules (SIMÕES *et al*., 2020). All samples presented several pores on their surfaces (Fig. 3); however, the SC20 and ST20 samples presented more compact structures, which can occur because the higher acid concentration possibly resulted in a lower viscosity of these samples during extrusion, yielding more homogeneous structures.

3.3. Fourier transform-infrared spectroscopy (FT-IR)

The FT-IR spectra of starch samples are shown in Fig. 2. It was possible to identify a new important band at 1730 cm^{-1} in all samples that indicates the stretching vibration of the carbonyl ester group, which provides clear evidence that the ester bonds were formed successfully. These results are consistent with those presented by other authors (FARHAT *et al*., 2017; MISKEEN *et al*., 2021; SIMÕES *et al*., 2020; TUPA *et al*., 2020; YE *et al*., 2019; ZHOU *et al*., 2016), who used CA and/or TA as esterifying and crosslinking agents for starch modification. It is important to highlight that all modified samples were washed with ethanol before analysis to prevent free citric or tartaric acids from remaining in the samples; thus, the band at 1730 cm^{-1} observed in all modified samples resulted from the formation of a covalent ester bond between the citric or tartaric acids and starch.

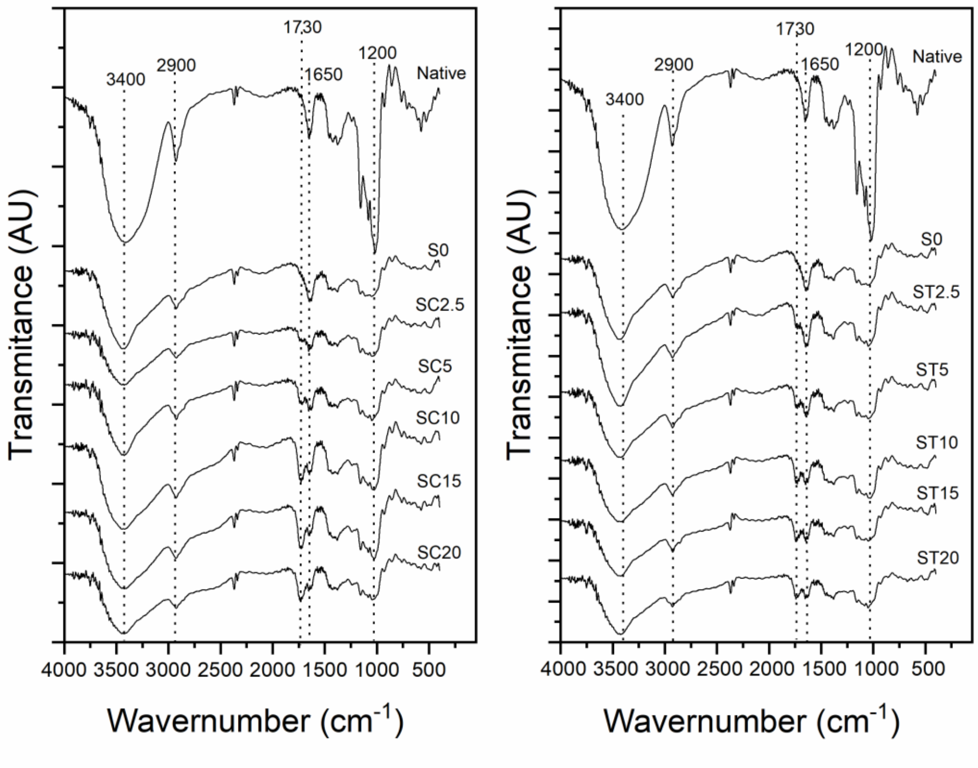


Fig. 2 - FT-IR spectra of native starch, the control sample (extruded without reagent) and starch hydrogels obtained by reactive extrusion through reaction with CA and TA.

Other bands are also observed in the FT-IR spectra of all samples: a broad band at 3400 cm^{-1} that is associated with O-H elongation and hydrogen bond vibration; a band at 2900 cm^{-1} that is associated with C-H asymmetric elongation and vibration; a band at 1650 cm^{-1} that is associated with H-O bending vibration; and a band at 1200 cm^{-1} that is attributed to the stretching vibration of C-O in C-OH groups (FARHAT *et al*., 2017; SIMÕES *et al*., 2020; YE *et al*., 2019; ZHOU *et al*., 2016).

3.4. X-RAY DIFFRACTION (XRD)

The X-ray diffraction patterns of native starch and starches hydrogels were investigated to provide additional information about changes in crystal structure during the reactive extrusion process. The diffractograms and relative crystallinity are shown in Fig. 3. Native cassava starch presented diffraction peaks at $2\theta = 15.03°$, 17.03°, 17.93° and 23.02° (Fig. 3), which are typical of an A-type crystallinity (MINAKAWA, FARIA-TISCHER, & MALI, 2019; MONROY, RIVERO, & GARCÍA, 2018; ZOBEL, 1988).

The crystallinity index (CI) of native starch was 37%, which is typical of a semicrystalline material, and a similar value (37.64%) was reported by Mei *et al*. (2015) for native cassava starch. The CI for all extruded samples (with and without reagent) decreased, and the CI values ranged from 8 to 11% (Fig. 2). During the reactive extrusion process, the starch granules were exposed to high temperatures and high shear forces, strongly affecting their crystalline structure, resulting in the disappearance of diffraction peaks and samples with low crystallinity indices (Fig. 2). These results agreed with other authors that used reactive extrusion to modify starches (CAI *et al*., 2019; YE *et al*., 2019). Cai *et al*. (2019) reported that rice starch crosslinked by reactive extrusion with sodium trimetaphosphate and propylene oxide had its crystalline structure almost completely disrupted, with CI values very close to those obtained in this study, ranging from 11 to 14%.

According to Schafranski, Ito, and Lacerda (2021), relative crystallinity affects the physico-chemical properties of starch, including its solubility in cold water. Some authors claim that the lower crystallinity of starch hydrogels can be important for their application as carriers of various compounds, such as in a drug and chemical element delivery system and in agricultural applications (BIDUSKI *et al.*, 2018; BORGES, SILVA, COELHO, & SIMÕES, 2015; MINAKAWA *et al*., 2019).

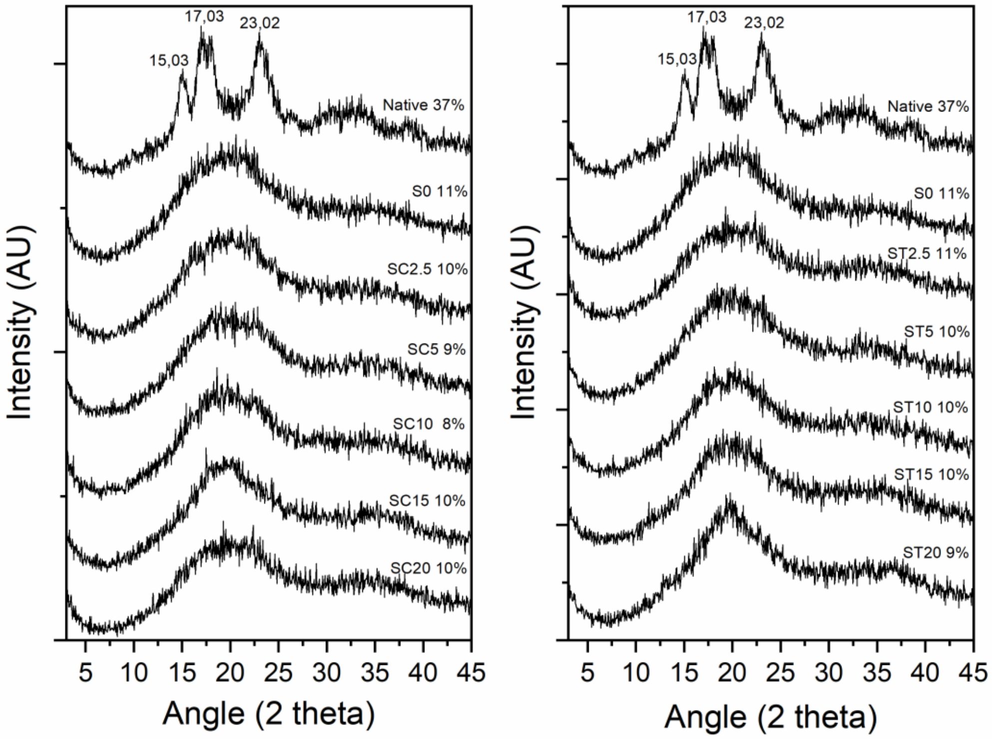


Fig. 3 - X-ray diffraction (XRD) patterns and relative crystallinity index (CI) of native starch, the control sample (extruded without reagent) and starch hydrogels obtained by reactive extrusion through reaction with CA and TA.

3.5. Differential scanning calorimetry (DSC)

Fig. 4 shows the DSC curves for native cassava starch and crosslinked starch hydrogels obtained by reactive extrusion through reaction with CA and TA. The DSC profile of native cassava starch shows two endothermic peaks, the first peak between 60–80 °C and the second at 310 °C, attributed to water loss and to the fusion of crystallites in the semicrystalline structure of starch, respectively (Fig. 4).

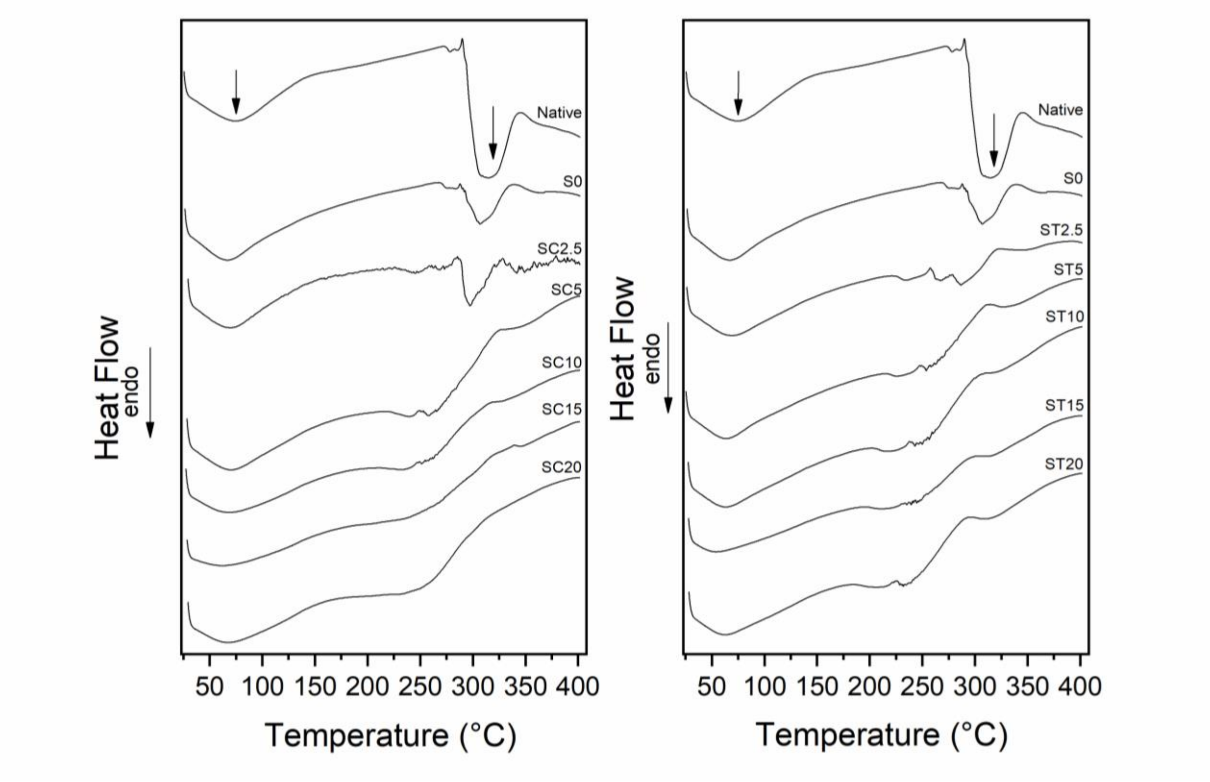


Fig. 4 - DSC thermograms of native starch, the control sample (extruded without reagent) and crosslinked starch hydrogels obtained by reactive extrusion through reaction with CA and TA.

For all extruded samples, small endothermic events were observed between 60 and 80 °C, which were also associated with water loss. Samples S0, SC2.5, and ST2.5 presented endothermic peaks between 310–343 °C, possibly associated with the fusion of the remaining crystalline structure; however, this peak was smoother in the samples modified with high concentrations of CA and TA, probably due to the effect of the modification, which led to the obtainment of amorphous structures with a polymer matrix reinforced through crosslinks between the starch chains (Fig. 2), consistent with the XRD analysis results.

3.6. WATER HOLDING CAPACITY (WHC) AND SOLUBILITY

The WHC and solubility of the samples are shown in Table 1. WHC is the capacity of the sample to absorb and hold water in its polymeric network (MEHFOOZ, ALI, AHSAN, ABDULLAH, & HASNAIN, 2021). Native cassava starch presented the lowest WHC (1.84 g/g) value. The S0 sample (extruded without reagent) and all starches hydrogels presented significant improvements in WHC values (Table 1).

WHC depends on the presence of hydrophilic groups that can absorb and bind water molecules, leading to more swollen granules. During reactive extrusion, the intramolecular bonds and the crystalline structure of starch granules were broken, increasing the retention of water at low temperature by the hydrophilic groups that were exposed, which was also reported by Ye *et al*. (2019). The sample with the higher WHC was prepared with 20% CA (SC20 sample, Table 1), which is an indicative that the reaction with CA and TA resulted in crosslinking of starch chains. According to Lemos *et al*. (2021), crosslinked starch chains are more reinforced than their native counterparts because of the covalent bonds formed, which improves their capacity to retain water. Native starches are almost insoluble in water at room temperature, as observed in this study; the solubility of cassava starch in water at 25 °C was 3.2% (Table 1), which agreed with Jivan, Yarmand, & Madadlou (2014), who reported that native starch solubility was negligible (~4%) in cold water at 25 °C.

After being subjected to extrusion, water starch granules are hydrated, swell and break down, resulting in starch destructurization and pregelatinization (MOAD *et al*., 2011; YE *et al*., 2018). This can be observed in this study; in the extruded samples (with or without reagents), the solubility significantly increased (Table 1) when compared to native starch. In hydrogel samples, the granular and semicrystalline structure of starch was destroyed, as observed by XRD and SEM, resulting in materials with lower crystallinity, which certainly contributed to the increases in the WHC and solubility of these samples. Heebthong & Ruttarattanamongkol (2016) reported that the thermomechanical energy from the extrusion process results in disruption of the starch molecular arrangement and that water can be transferred easily into its internal structure.

The control sample (S0, Table 1) extruded without reagent presented a solubility of 12.14%, while the solubility of crosslinked samples ranged from 21.30 to 39.73% (Table 1). The reaction with CA and TA increased the water *solubility* of starch samples, suggesting that CA- and TA-starches have large amounts of short amylose chains obtained by acid hydrolysis, which can dissociate and diffuse out of the hydrogel structure during swelling (HUNG, VIEN, & LAN PHI, 2016; KAPELKO-ŻEBERSKA *et al*., 2016; MEI *et al*., 2015).

3.7. SWELLING POWER AT DIFFERENT TIMES AND TEMPERATURES

The effects of time and temperature on the swelling of native cassava starch and starch hydrogels obtained by reactive extrusion through reaction with CA and TA can be observed in Fig. 5.

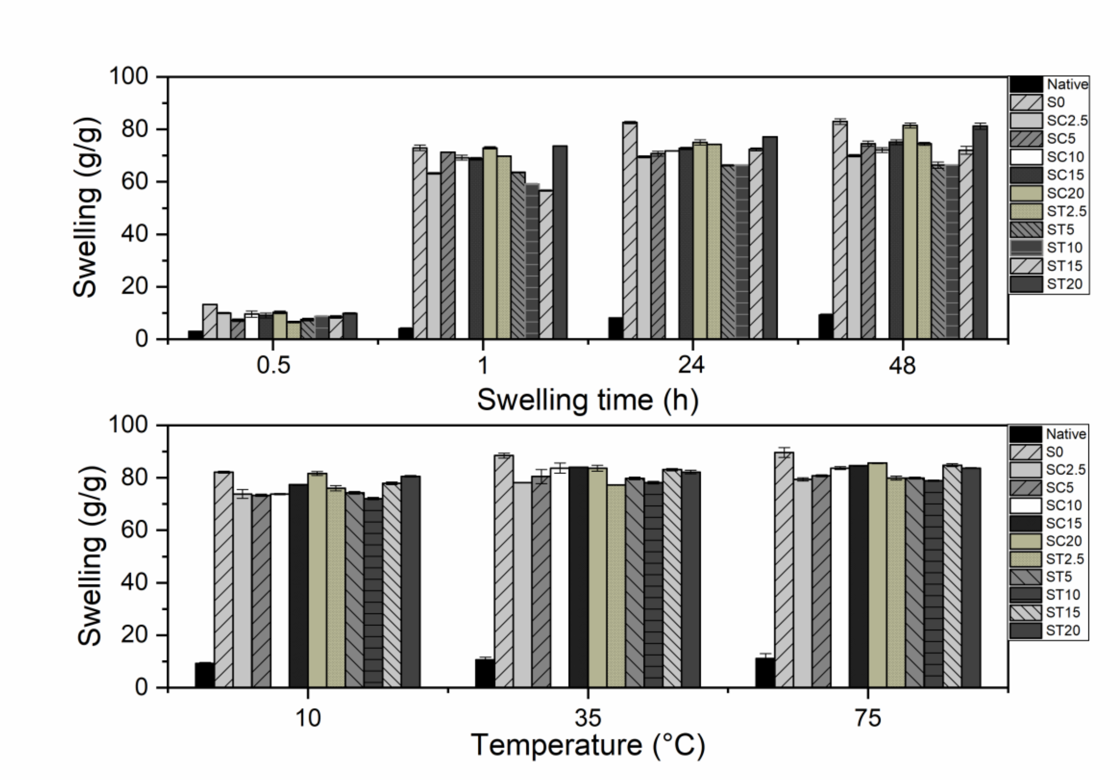


Fig. 5 - Effects of time and temperature on swelling of native starch, the control sample (extruded without reagent) and starch hydrogels obtained by reactive extrusion through reaction with CA and TA.

The swelling power was initially evaluated by time in the water, and then the time was fixed at 24 h, varying the temperature (Fig. 5). The swelling power of native starch was significantly lower than those of S0 and samples modified with CA and TA. The highest percentage of swelling observed was in sample S0 at 24 h with heating at 75 °C (88.66 g/g).

When studying only the time variation (Fig. 5), the swelling of samples subjected to extrusion with CA ranged from 3.01 (SC2.5, 1 min) to 75.02 (g/g) (SC20, 24 h), while in the samples modified with TA the values ranged from 2.86 (g/g) (ST2.5, 1 min) to 77.12 (g/g) (ST20, 24 h), suggesting that crosslinking was effective and resulted in samples that can swell and retain water at room temperature. When the temperature factor was evaluated, all the samples increased their swelling power when the temperature was above 60 °C. Hung *et al*. (2016) also described the increase in swelling of citric acid-crosslinked rice starch with increasing temperature.

During extrusion, the starch granules are subjected to high temperature and pressure, and the hydrogen bonds and the crystal structure were broken, resulting in disruption of their granular structure. The free hydroxyl groups of starch chains when in contact with water can form hydrogen bonds and swell (BUTT *et al*., 2019; DE GRAAF, BROEKROELOFS, & JANSSEN, 1998). Similar results have also been described by Monroy *et al*. (2018), who observed that ultrasound-modified starches lose their crystallinity and present increased swelling values.

The S0 sample presented the highest swelling value, ranging from 3.63 to 88.66 (g/g), values higher than those presented by the crosslinked samples with CA and AT. Crosslinked hydrogels with low CA and TA concentrations had the highest solubility and lowest swelling capacity (Table 1, Fig. 5). The high concentrations of CA and TA improved granule swelling due to better starch hydration, and samples SCA.20 and STA.20 also showed higher DS values (0.36 and 2.85, respectively). During reaction with 20% CA and TA, the starch acquires a three-dimensional matrix, which results in individual chains that repel each other due to steric hindrance, as starch derivatives try to exist in the lowest and most stable state. The repulsion between the chains can increase the capacity for expansion without dissolving, preserving the structure of the material (MURÚA-PAGOLA *et al*., 2009; TUPA *et al*., 2020).

3.8. MOISTURE SORPTION ISOTHERMS

The moisture sorption isotherms and parameters of the GAB model are shown in Fig. 6 and Table 2, respectively. An increase in the equilibrium moisture content was observed when there was an increase in the water activity (a_w) of the samples. The samples with a_w between 0.11 and 0.60 presented very close values of equilibrium moisture content, and it can be observed that with an increase in a_w from 0.75 and 0.90, the ST20 sample presented an equilibrium moisture content higher than that of the other samples, while native starch and S0 samples presented the lowest values (Fig. 6).

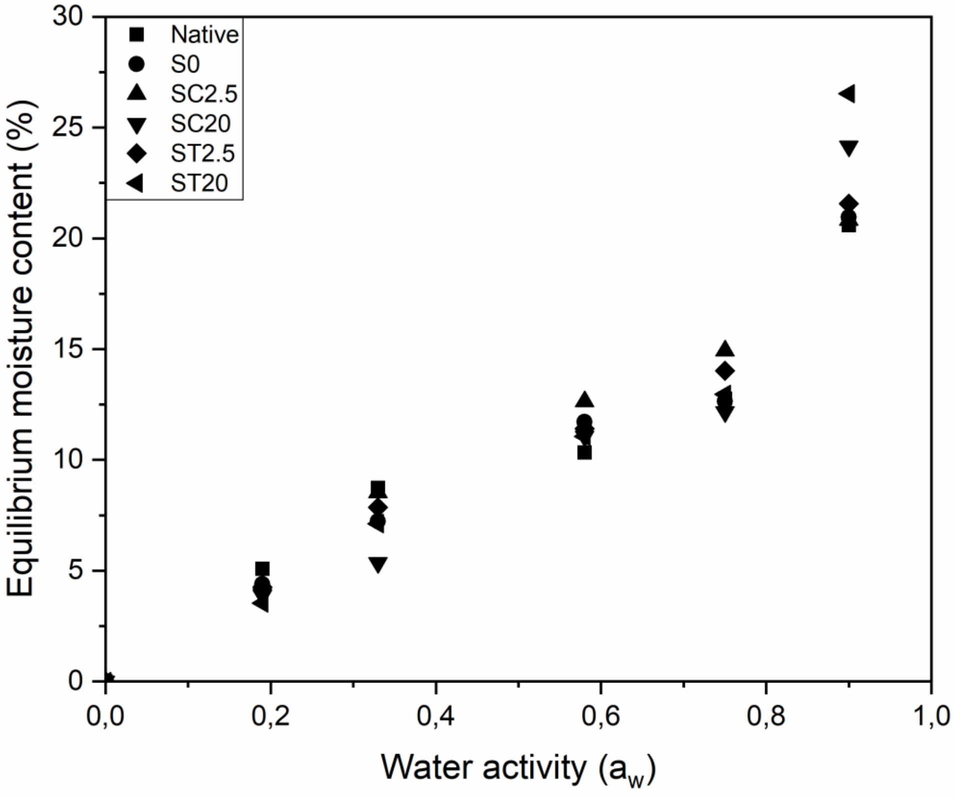


Fig. 6 - Moisture sorption isotherms of native starch, the control sample (extruded without reagent) and starch hydrogels obtained by reactive extrusion through reaction with CA and TA.

The correlation coefficients (R^2) calculated from the adjustment of the GAB model were satisfactory (R^2 = 0.99) (Table 2), indicating that the experimental data adequately fit the mathematical model used. The monolayer value (m_0) indicates the maximum amount of adsorbed water per dry weight of the sample that can be adsorbed in a single layer (GIL-GIRALDO, MANTOVAN, MARIM, KISHIMA, & MALI, 2021; MERCI, URBANO, GROSSMANN, TISCHER, & MALI., 2015). The sample of native cassava starch had the highest m_0 value (11.19 g water/100 g solids) among all samples, indicating a change in the adsorption properties of cassava starch when subjected to reactive extrusion, with or without the crosslinking agents.

Table 2 - GAB model parameters[a] of native starch, control sample (S0) and starches hydrogels obtained by reactive extrusion.

Samples	m_0	C	K	R^2
Native starch	5.47	58.01	0.81	0.99
S0	6.13	12.48	0.79	0.99
SC2.5	9.64	6.37	0.64	0.99
SC20	4.24	19.01	0.92	0.99
ST2.5	5.47	58.02	0.81	0.99
ST20	4.70	18.49	0.91	0.99

[a] $M = m_0CKa_w/(1 - Ka_w)(1 - Ka_w + CKa_w)$, where M is the equilibrium moisture content at a water activity (a_w), m_0 is the monolayer value (g water /100 g solids), and C and K are the GAB constants.

The extrusion process resulted in samples with lower adsorption capacity, which can possibly be attributed to the types of short amylose chains obtained, which can reassociate, forming less hygroscopic materials, and the hydrogels samples obtained with the highest CA or TA concentrations (SC20 and ST20) resulted in the lower m_0 values. In this case, the introduction of crosslinks possibly reduced the free hydroxyl groups that were able to interact with water. The understanding of the mechanisms of moisture adsorption of starch materials is complicated because of the complexity of their structure. Moisture sorption depends on their composition and structural properties, such as surface area, pore vume and crystallinity. As observed by SEM (Fig. 3), SCA20 and ST20 presented more compact structures than S0, SC2.5 and ST2.5; thus, the higher porosities and surface areas possibly affected the moisture adsorption in these samples.

.3.9. DEGREE OF SYNERESIS

Results of degree of syneresis are shown in Fig. 7. All starch hydrogels released water after 1 day, and the S0 sample showed a more stable pattern during storage than native and crosslinked starches, with values < 5%. The native cassava starch degree of syneresis increased from 5.5 to 22.0% at 1 and 28 days of storage at 4 °C, respectively (Fig. 7), and from Day 14, the release of water from the native starch was the highest among the samples. Although CA and TA acted as effective esterifying and crosslinking agents, they can also exert a hydrolytic action that can favor the breakdown of starch granules during extrusion (SIMÕES *et al*., 2020), and possibly, the higher release of water from CA and TA samples between days 1 and 7 can be attributed to the short amylose chains obtained by acid hydrolysis, which can be associated with retrograde degradation.

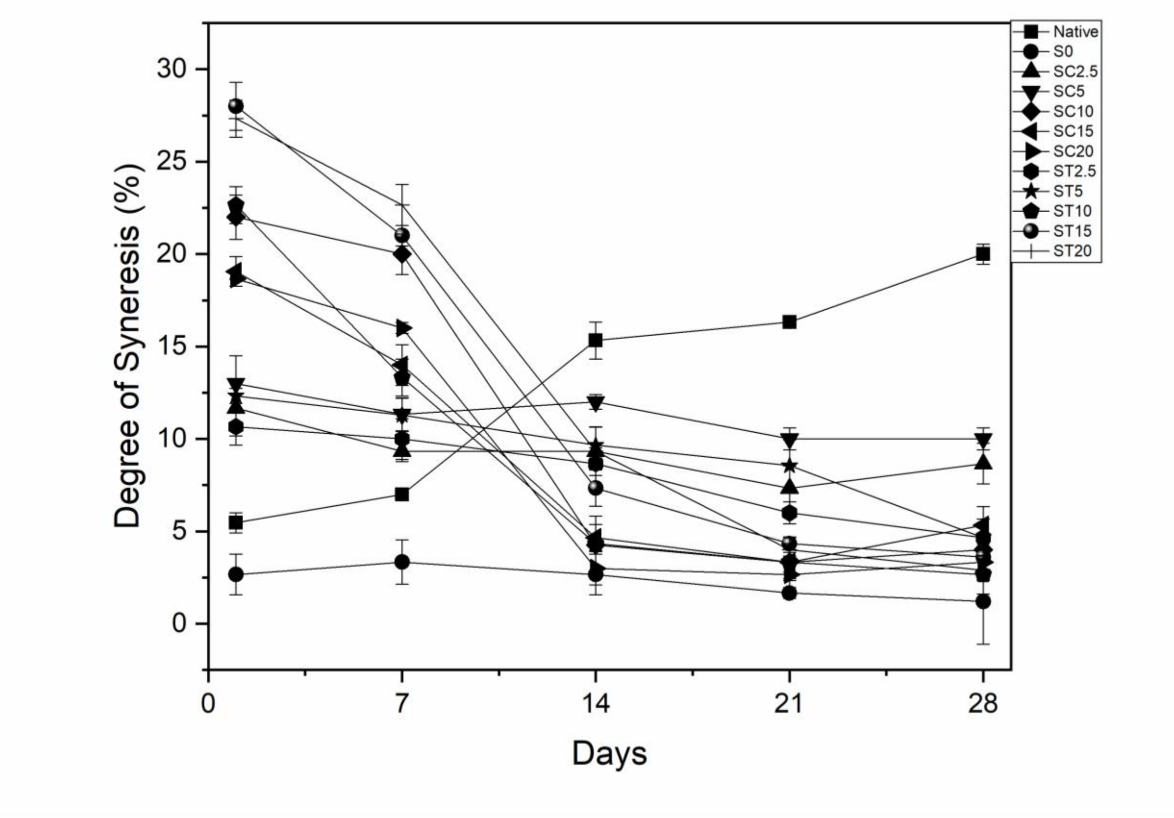


Fig. 7 - Degree of syneresis of native starch, the control sample (extruded without reagent) and starch hydrogels obtained by reactive extrusion through reaction with CA and TA.

On day 21, the percentage of water released from native starch hydrogel was 12%, and in modified samples, the percentage of water released varied between 1 and 8% (Fig. 7). The results showed that although starch hydrogels have a higher rate of water release after 1 day, they were more stable than native starch after 14 days of storage, and possibly the introduction of intermolecular linkages between hydroxyl groups on starch molecules reinforced the polymer network, resulting in more stable structures, as reported by other authors (LIU *et al.*, 2014; WŁODARCZYK-STASIAK *et al*., 2017). Ye, Hu, Zhang, Fang, Liu, & Luo (2016) also observed a decrease in the degree of syneresis of native and extruded rice starches after 14 days of storage, and they attributed this effect to the formation of gels with a rough structure after storage, by which part of the released water could be reabsorbed.

4. CONCLUSION

Cassava starch hydrogels were produced and esterified with citric acid and tartaric acids using reactive extrusion. Evidence of the occurrence of chemical modification was verified by FT-IR spectroscopy with the appearance of a new band at 1730 cm^{-1}, resulting in materials with different degrees of substitution, which were higher for hydrogels obtained by reaction with citric acid. XDR showed that the starch crystalline structure was broken during reactive extrusion, and morphology analysis showed that the original granular structure of starch was lost and replaced with a rougher and more irregular structure, which resulted in hydrogels with higher solubility and swelling capacity than native starch and the control sample (extruded without the modifying agents). Hydrogels samples obtained with the highest CA or TA concentrations (15 and 20%) resulted in the higher DS values, higher solubility values and swelling capacities.

Reactive extrusion was effective in obtaining starch hydrogels by reaction with organic acids, with the advantages of reduced processing times and low effluent generation when compared to conventional processes, with great possibilities to be adapted to an industrial scale, expanding the potential of starch hydrogels for several agricultural, food, and pharmaceutical applications.

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5. ARTIGO 2

Green modification of oat hull cellulose with sodium trimetaphosphate and tartaric acid by reactive extrusion

Abstract

The current study aimed to modify cellulose extracted from oat hull employing a simple and eco-friendly process based on reactive extrusion using sodium trimetaphosphate (STMP) and tartaric acid (TA) as esterifying and crosslinking agents. TA and STMP were used at different concentrations (0, 5, 12.5, and 20% for TA and 0.025, 0.05, 0.1% for STMP). Reactive extrusion did not change the morphology and crystallinity pattern of the modified samples. Crystallinity indexes (IC) ranged from 33 to 37% in modified samples, which presented higher affinity by the non-polar solvent in wettability tests, also confirming reaction with TA and STMP. This study proves that reactive extrusion can be an effective technology for the modification of cellulose, resulting in a material with new attractive characteristics, including.

Keywords: Lignocellulosic residue; Green Chemistry; Esterification; Crosslinking.

1. INTRODUCTION

The valorization of agroindustrial residues into new bio-based materials is according to the main premise of a circular economy, which encompasses the concept of industrial synergy that is encouraged by the reuse of residues within the industrial network, acting as a mechanism for a sustainable future. Additionally, the development of green technologies to produce new bio-based products with high added value can minimize the dependence on fossil-based resources and reduce the generation of solid wastes (DUQUE-ACEVEDO *et al.*, 2020; UBANDO, FELIX, CHEN, 2020; WANG, GANEWATTA, TANG, 2019).

The use of agroindustrial residues to obtain cellulose can be an interesting alternative to transform these materials, which generally contain about 20-50% cellulose, 20-30% hemicellulose and 20-30% lignin (DEBIAGI; FARIA-TISCHER; MALI, 2020; GIL GIRALDO *et al.*, 2021; MARIM *et al.*, 2020). Cellulose is a biopolymer that regardless its origin, its chemical structure is the same; D-glucose units linked by β-(1 → 4)-glycosidic bonds between the hydroxyl group of C4 and the C1 carbon atom. Cellulose has a linear polymeric chain with a large number of hydroxy groups, since each glucose unit presents three hydroxyl groups, one primary hydroxyl group on C-6 and two secondary hydroxyl groups on C-2 and C-3. Hydroxyl groups can participate in several chemical reactions, which makes cellulose a versatile platform for surface chemical modifications, including esterification and crosslinking (CAGNIN *et al.*, 2021a; CAGNIN *et al.*, 2021b; GIL GIRALDO *et al.*, 2021; HUBBE; GARDNER; SHEN, 2015; MA, LI; BAO, 2015; MAALOUL *et al.*, 2021; PANG *et al.*, 2019).

Cellulose is a renewable source, biodegradable and non-toxic material that has drawn much attention owing to its advantageous properties, such as its wide applicability in a variety of fields (GIL GIRALDO *et al.*, 2021; MARIM *et al.*, 2020), . However, some cellulose limitations can be related to dissolution of this biopolymer, cellulose is known hardly dissolved in water and most organic solvents (ZAINAL *et al.*, 2021), and the introduction of crosslinking through the reaction of esterification of hydroxyl groups provides a hydrophobic character to cellulose, making it more compatible with non-polar polymers and solvents (GIL-GIRALDO *et al.*, 2021).

Additionally, in recent years there is a great interest in the use of crosslinked cellulose in biodegradable hydrogels formulations to be employed in several applications, such as drug encapsulation, controlled release of bioactive compounds, water release for plants in agriculture. The use of cellulose hydrogels with incresased water retention have been widely reported, however few of them are be totally free of petro-based monomers, polymers or crosslinkers (CAGNIN *et al.*, 2021a; TAN *et al.*, 2021).

Sodium trimetaphosphate (STMP) is widely used as a crosslinking agent for starches and some gums because it is a solid of low toxicity with no reported adverse effects on humans. Crosslinking with STMP occurs by forming an intra- and/or intermolecular crosslinkage of one phosphate linked between hydroxyl groups in cellulose molecules in the amorphous region (CAGNIN; SIMÕES, 2020; LI *et al.*, 2009; MA *et al.*, 2016), and STMP is still little explored for the crosslinking of cellulose. Cagnin *et al*. (2021a; 2021b) reported the obtainment of starch-carboxymethyl cellulose blends crosslinked with STMP. Reshma, Reshmi, Shantikumar, & Deepthy (2020) reported the crosslinking of blends of sodium carboxymethyl cellulose and starch using a combination of STMP and aluminium sulphate as crosslinking agents.

The use of polycarboxylic acids is very studied to modify cellulose because they are crosslinking agents able to overcome toxicity and costs associated with other agents, such as glutaraldehyde and epichlorohydrin (OLIVATO *et al.*, 2012; SIMÕES *et al.*, 2020b; SUN *et al.*, 2018; TAO; NONAKA, 2021). Tartaric acid ($C_4H_6O_6$) is a dicarboxylic acid and its reaction with cellulose occurs due to the attachment of the carboxylic group via esterification with a cellulosic hydroxyl group, and also a further reaction via esterification with another cellulosic hydroxyl group can produce a crosslink between cellulose chains (SPIRIDON *et al.*, 2013; TAN *et al.*, 2021).

Conventionally, crosslinking reactions are performed in batches, demanding long reaction times and multiple steps (CAGNIN *et al*., 2021a; 2021 b), on the other hand, reactive extrusion is considered an eco-friendly technique that can be used for chemical modification of polymers since no solvent was involved during the process, being a continuous process with short reaction times (MOAD, 2011; FORMELA *et al.*, 2018; HEEBTHONG; RUTTARATTANAMONGKOL, 2016). Organic acids and STMP have been reported as efficient crosslinking agents of cellulose, and the use of these two crosslinking agents by reactive extruder has not yet been explored. Recently, (GIL GIRALDO *et al.*, 2021) and (TAO; NONAKA, 2021), reported the use of reactive extrusion to obtain cellulose crosslinked with organic acids, and they stressed that this process can be considered a promising alternative to obtain modified cellulose.

Therefore, this study aimed to modify cellulose extracted from oat hull employing a simple and eco-friendly process based on reactive extrusion using sodium trimetaphosphate (STMP) and tartaric acid (TA) as esterifying and crosslinking agents.

2. MATERIAL AND METHODS

2.1. MATERIALS

The oat hull sample used in this study was supplied by a local oat processing industry (SL Cereais e Alimentos, Paraná - Brazil). Cellulose was extracted from oat hulls using peracetic acid as bleaching agent by the methodology described by Marim et al. (2021). The cellulose and hemicellulose contents of the raw and bleached oat hulls were determined by the Van Soest method (1965) and the lignin content was determined according to a standard method of the Technical Association of Pulp and Paper Industry (1999).

Sodium trimetaphosphate (STMP) was purchased from Sigma Aldrich

(Co., St. Louis, MO, USA) and tartaric acid (TA) from Synthlab (Diadema, Brazil).

2.2. METHODS

2.2.1. Crosslinking of cellulose by reactive extrusion

Cellulose was crosslinked by reactive extrusion using TA and STMP. Different proportions of TA and STMP (TA: 5, 12.5, 20 % - g acid/100 g cellulose, and STMP: 0.025, 0.05 and 0.1 % - g STMP/100 g cellulose) were employed to obtain the modified cellulose; these proportions were based on literature data (CAGNIN *et al*., 2021a). The samples were prepared by dissolving different proportions of TA or STMP in distilled water, and the obtained solutions were mixed with cellulose, resulting in samples with a final moisture content of 32 % (g/g), which remained in sealed plastic bags at room temperature for 1h before extrusion. A control sample was extruded without any reagent other than water, resulting in final moisture content of 32% (g/g). Then, all samples were extruded in a single screw extruder (AX Plastics, Diadema, Brazil) with a screw diameter of 1.6 cm and a screw length/screw diameter ratio (L / D) of 40, with four zones of heating and a matrix of 0.8 cm in diameter. The temperature in all zones was 100°C and the screw speed was 60 rpm. The cellulose extrudates were collected, placed in an oven, dried to constant weight at 45 °C, ground, and sieved in an 80-mesh sieve. The dry mixture was washed three times with absolute ethanol to remove unreacted TA or STMP. Finally, the washed cellulose samples were air-dried at 45 °C. Cellulose samples modified with TA were labeled as TA5, TA12.5, and TA20, respectively, throughout the study. These cellulose samples modified with STMP were labeled as STMP0.025, STMP0.05, STMP0.1 throughout the study.

2.2.2. Degree of substitution (DS) of modified samples

The DS of samples modified with TA was determined in triplicate according to the method described by Volkert *et al*. (2010) by titration. All of the ester linkages were saponified by NaOH, and the amount of remaining excess NaOH was determined by titration with HCl. For the samples modified with STMP, the phosphorus content (%) was analyzed in triplicate by colorimetry in 600 nm (spectrophotometer Varian - Cary 50 Conc, São Paulo, Brazil), and the degree of substitution (DS) was calculated as follows: $DS = 162*P/ (3100 - 102*P)$, where: P is phosphorus content (%); 162 is the molecular weight of the monomeric unit of cellulose, 3100 is the atomic weight of phosphorus multiplied by 100; and 102 is the group phosphate weight.

2.2.3. Scanning electron microscopy (SEM)

SEM analyses were performed with an FEI Quanta 200 microscope (Oregon, USA). The foam pieces were mounted for cross-section visualization on bronze stubs using double-sided tape. The surfaces were then coated with a thin gold layer (40–50 nm). All samples were examined using an accelerating voltage of 20 kV.

2.2.4. X-ray diffraction (XRD)

The samples were powdered (particles 0.149 mm), and the analysis was performed using a Panalytical X'Pert PRO MPD diffractometer (Almelo, The Netherlands) with copper Kα radiation (λ = 1.5418 Ǻ) under the operational conditions of 40 kV and 30 mA. All of the assays were performed with a ramp rate of 1° / min. The relative crystallinity index (CI) was calculated from the ratio between the areas of the diffraction peaks and the area of the entire diffraction pattern after subtracting the amorphous background patterns, using the method described by (CHEETHAM; TAO, 1998).

2.2.5. Fourier Transform-Infrared Spectroscopy (FT-IR)

The pulverized and dried samples were then mixed with potassium bromide and compressed into tablets. The FT-IR analyses were carried out with a Shimadzu FT-IR -8300 (Kyoto, Japan), which has a spectral resolution of 4 cm-1 and a spectral range of 4000–500 cm-1.

2.2.6. Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC) and solubility.

WAC and OAC of samples were determined according to the methodology described by Lu *et al* (2011), with minor modifications. Approximately 1 g of each sample (M0) and 30g of water (or soybean oil) (M1) were added to a previously weighed centrifuge tube. The samples were kept in a water bath for 30 min and then centrifuged for 30 min at 9000 rpm (Hettich Centrifuge, Universal model 320R, Darmstadt, Germany). After centrifugation, the supernatant was weighed (M2) and the water (or soybean oil) absorbed by the samples was estimated as the difference between M1 and M2. WAC was calculated as: WAC (g/g) = (M1 - M2)/M0 and OAC was calculated as: OAC (g/g) = (M1 - M2)/M0.

The solubility of the samples in water was calculated from the difference between the weight of the undissolved residue and the original weight of the sample.

2.2.7. Thermal properties

A differential scanning calorimeter (DSCShimadzu DSC 60, Japan) was used for thermal analysis. The scanning temperature ranged from 30 to 450˚C at a heating rate of 5 °C/min in a helium atmosphere. Thermogravimetric Analysis (TGA) of the samples were be performed using the Shimadzu TGA-50 (Japan) equipment. The scans were be performed at room temperature up to 600 °C with a heating rate of 20 °C/min under a nitrogen flow of 20 mL/min.

2.2.8. Wettability

Wettability was determined using the method described by (NAMAZI; DADKHAH, 2010). Samples were mixed in a system containing two immiscible solvents with different densities, water (d: 1.000 g cm^{-3}) and dichloromethane (d: 1.335 g cm^{-3}), and water and chloroform (d: 1.490 g cm^{-3}), thus, allowing to observe the affinity of the samples for each substance.

2.2.9. Water adsorption isotherms

The samples (0.5 g) were dried for 7 days in a desiccator containing anhydrous calcium chloride, and then the samples were placed at 25 °C for 7 days in desiccators containing different saturated salt solutions providing specific water activities (aw) for the samples (0.11, 0.33, 0.43, 0.60, 0.75, and 0.90). Samples were dried at 105 °C in a ventilated oven (035 Marconi MA, Piracicaba, Brazil) to determine their equilibrium moisture content at each aw condition. The GAB model (Guggenheim– Anderson-de Boer) was used to fit the sorption isotherm data, and the monolayer values were calculated from the GAB isothermal model (Bizot, 1983) as follows: $M = (m_0CK_{aw}) / (1 - K_{aw}) (1 - K_{aw} + CK_{aw})$, where M is the equilibrium moisture (g water / 100 g solids), a_w is the water activity, m_0 is the monolayer value (g water / 100 g solids), and C and K are GAB constants.

2.2.10. Statistical analysis

Analyses of variance (ANOVA) and Tukey's mean comparison test ($p \leq 0.05$) were performed with Statistica software version 7.0 (Statsoft, OK, USA)

3 RESULTS AND DISCUSSION

The non-cellulosic fraction present in the oat hull was removed by the bleaching with peracetic acid, resulting in a cellulose rich material. Raw oat hull had 26% cellulose, 30% hemicellulose, and 21% lignin, and the bleached material presented 78% cellulose, 8% hemicellulose, and 3% lignin. The process yields 30%, each 100 g of raw oat hull resulted in 30 g of this bleached material.

3.1. DEGREE OF SUBSTITUTION (DS)

DS values of cellulose-modified samples are presented in Table 1. For TA modified samples, the greater the amount of TA resulted in increase of DS; the highest DS value was obtained for the TA20 (DS = 2.96), and the lowest DS value was obtained for the TA5 sample (DS = 1.58) (Table 1).

For samples modified with STMP, the amount of the reagent did not affect significantly the DS values, for all samples the DS value 0.01 (Table 1). According to Cagnin *et al*. (2021a), DS can be consider a good indicative of the occurrence of crosslinking of the phosphate groups grafted onto the cellulose chain (CAGNIN *et al*., 2021a). Dong and Vasanthan (2010) and Maoni *et al.* (2010) also reported that the quantity of incorporated phosphorus content can be directly used to indicate the degree of crosslinking in modified starches. Cagnin *et al*. (2021a) obtained samples with DS values of 0.05 for hydrogels obtained by reactive extrusion from cassava starch and carboxymethylcellulose crosslinked with STMP; however, they used a higher content of STMP (4 g/100 g).

Table 1 - Degree of substitution (DS) of cellulose modified by reactive extrusion with TA and STMP.

Samples	DS
Cellulose	-
Control	-
TA5	1.58 ± 0.06
TA12.5	1.72 ± 0.12
TA20	2.96 ± 0.09
STMP0.025	0.01 ± 0.001
STMP0.05	0.01 ± 0.001
STMP0.1	0.01 ± 0.001

3.2 SCANNING ELECTRON MICROSCOPY (SEM)

As observed in the SEM micrograph (Fig. 1), raw oat hull exhibited a typical aspect of a lignocellulosic material, with a compact and uniform structure. These results were consistent with those reported by other authors (DEBIAGI; FARIA-TISCHER; MALI, 2020; GIL-GIRALDO *et al.*, 2021; MANTOVAN *et al.*, 2021; MARIM *et al.*, 2020). The materials changed from a compact surface structure in raw oat hull to cellulosic fibers that appeared as individualized bundles, confirming that lignin and hemicellulose removal was effective using peracetic acid, agreeing with the results of composition of cellulose after bleaching. Several authors reported that peracetic acid is an efficient bleaching agent for the extraction of cellulose from lignocellulosic residues, as it has low environmental impact when compared to that of the chlorinated reagents employed in conventional processes (MANTOVAN *et al.*, 2021; MARIM *et al.*, 2020).

For samples of cellulose modified with TA and STMP, individual fibers similar to unmodified cellulose were observed in the SEM micrographs. Gil-Giraldo *et al*. (2021) also observed that the extrusion of cellulose with citric acid (40 g/100 g) did not affect the morphology of cellulosic fibers, suggesting that the modification occurred mainly on the fibers surfaces.

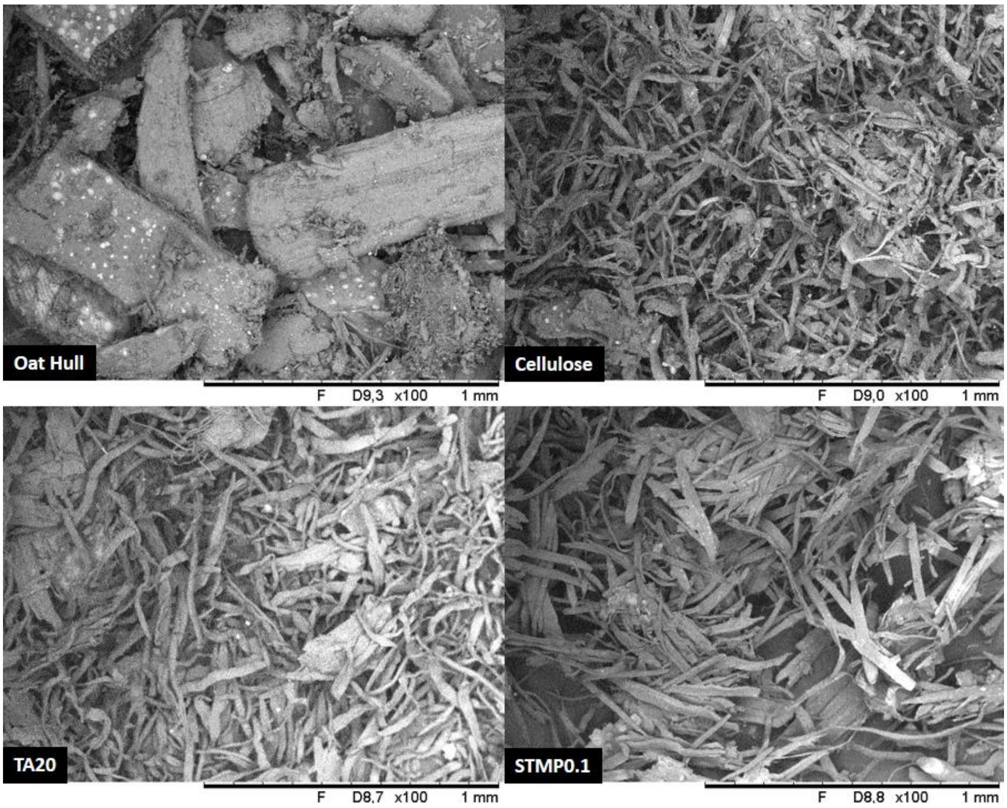

Fig. 1 - SEM images of cellulose, control sample (extruded without reagent) and modified cellulose obtained by reactive extrusion through reaction with TA and STMP.

3.3. X-RAY DIFFRACTION (XRD) AND RELATIVE CRYSTALLINITY

The X-ray diffraction patterns of cellulose and modified cellulose were investigated to provide additional information about changes in crystal structure during the reactive extrusion process. The diffractograms and relative crystallinity are shown in Fig.2. Debiagi *et al*. (2020) reported that individual fibrillary units from cellulose are formed by long periods of ordered regions (crystallites) interrupted by completely disordered regions. In all samples (Fig. 2), there were three typical peaks of cellulose type I located at approximately $2\theta = 16°$, 22°and 34.5°. The amorphous valley and crystalline peak are located between the angles $18° \leq 2\theta \leq 19°$ and $22° \leq 2\theta \leq 23°$, respectively (MANTOVAN *et al.*, 2021; TAN *et al.*, 2021). The cellulose reaction with TA and STMP by reactive extrusion did not affect the inherent crystalline structure of cellulose, and possibly this occurred because both crosslinking with TA and STMP have occurred in the amorphous fractions of the cellulose fibrils.

Gil-Giraldo *et al*. (2021) reported that reactive extrusion of cellulose in presence of citric acid resulted in any variation in the polymorph type of cellulose compared to the original raw material, which demonstrated that this process does not change the crystal organization of cellulose. Ramirez, Fortunati, Kenny, Torre (2017) reported that the initial crystalline structure of cellulose remained unaffected after reaction with citric acid.

Crystallinity index (CI) values observed for modified cellulose samples ranged from 33 to 37%, lower values than the values observed for unmodified cellulose (42%). For both groups of samples, the higher concentration of the crosslinking agent resulted in lower CI values (Fig. 2). Reactive extrusion is a physical method that combines thermo-mechanical and chemical treatments, which can affect the proportion of crystalline and amorphous fraction in cellulose samples, and possibly the higher concentrations of the crosslinking agents resulted in lower amounts of free hydroxyls, which are responsible to the formation of hydrogen bonding in the crystalline structure of cellulose. Ma *et al.* (2016) reported that STMP-modified cellulose had lower CI after the reaction between cellulose hydroxyl groups and STMP because it was dependent on hydrogen bond interactions of the free hydroxyl groups.

Dong and Vasanthan (2020) studied the crosslinking of corn starch with STMP, and they reported that STMP-crosslinking had no pronounced effect on the XRD patters, indicating that the phosphate crosslinks must have occurred in the amorphous regions of the starch granules. They also reported that crosslinking resulted in the loss of crystallinity, suggesting that the reduction of crystallinity after crosslinking indicated the action of crosslinking disrupted the crystallinity of starch.

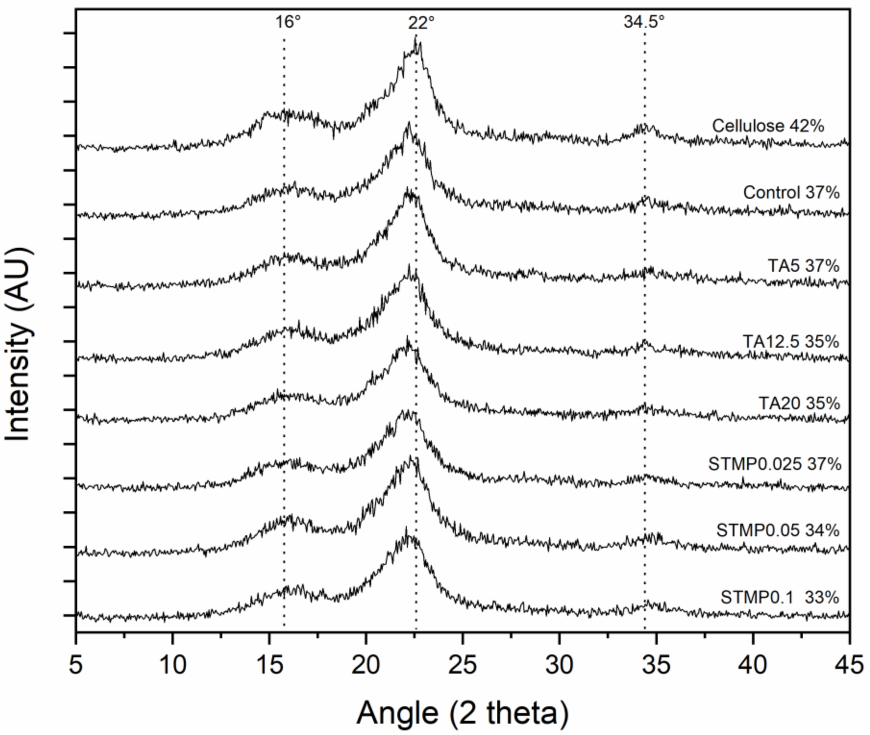

Fig 2 - X-ray diffraction (XRD) patterns and relative crystallinity index (CI) of cellulose, control sample (extruded without reagent) and modified cellulose obtained by reactive extrusion through reaction with STMP and TA

3.4. FOURIER TRANSFORM-INFRARED SPECTROSCOPY (FT-IR)

FT-IR spectra of all samples are presented in Fig. 3, and they were used to identify functional groups of cellulose and modified cellulose samples. Bands around 3400 cm^{-1} were attributed to stretching of OH groups from cellulose, and this band was observed in all samples, and also the bands observed at 2900 cm^{-1} corresponding to the CH stretching vibration of alkyl groups. The band at 1640 cm^{-1} that appeared in all spectra corresponded to the bending vibration of the hydroxyl groups of the water absorbed in the structure of cellulose (MANTOVAN *et al.*, 2020; MARIM, B.M. *et al.*, 2020). The characteristic bands of cellulose were also distinguished at 1017 cm^{-1} (pyranose ring vibration C – O – C) and 895 cm^{-1} (flexion of the β-glycosidic bond) (MAALOUL *et al.*, 2021).

The specific bands of the phosphate groups were not noticeable in the spectrum of samples modified with STMP, probably due to the low concentration of

STMP used. Some authors have also reported that they did not detect these phosphate bands in STMP crosslinked polymers, and they attributed this to the low concentration of phosphate groups (CAGNIN *et al.*, 2021a; SECHI; MARQUES, 2017). In addition, characteristic bands of elongation P=O found at 1214 cm^{-1} and band PO bond at 889 cm^{-1} can be covered by other bands due to the similarity of the wavenumbers.

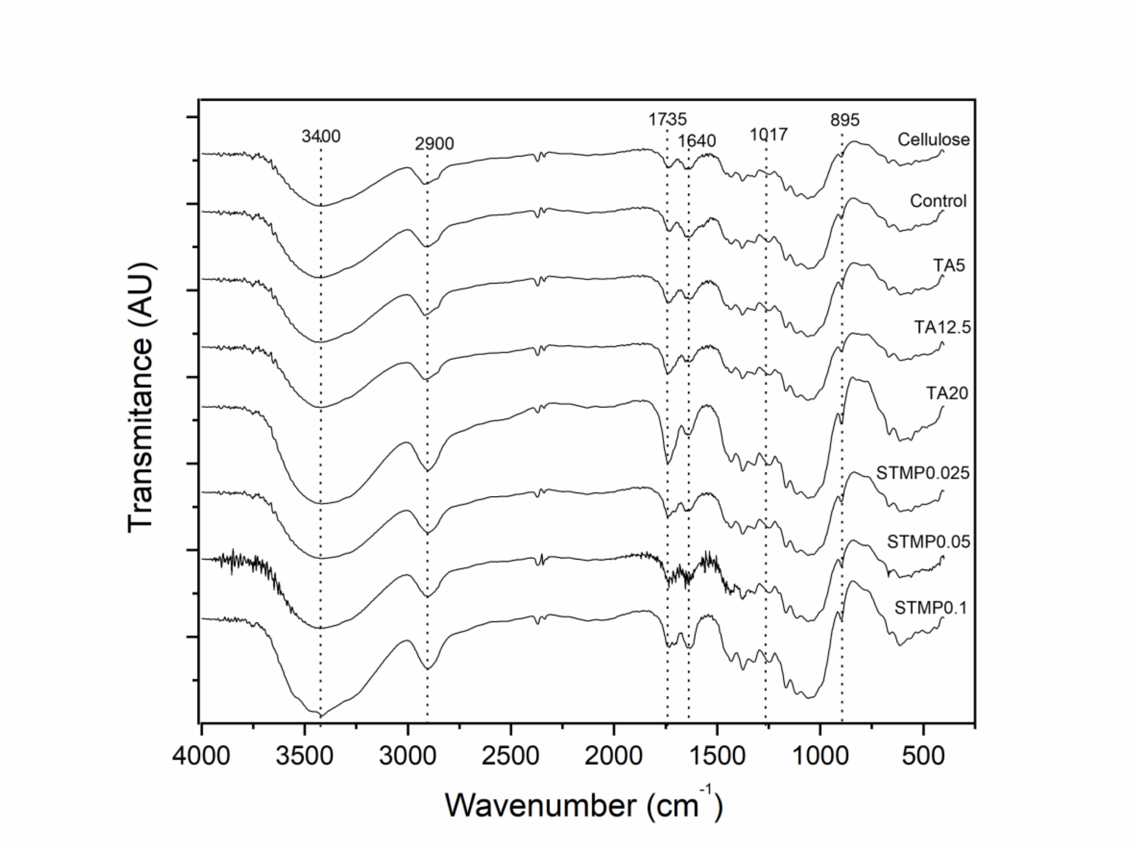

Fig. 3 - FT-IR spectra of cellulose, control sample (extruded without reagent) and modified cellulose obtained by reactive extrusion through reaction with TA and STMP.

In all spectra the band it was observed a band at 1735 cm^{-1}, since the cellulose was extracted from the oat hull, there is a trace of hemicellulose, originating from the lignocellulosic residue, this band corresponds to the acetyl or uronic groups of the hemicelluloses (MARIM *et al.*, 2020). However, in the samples crosslinked with TA this band at 1735 cm^{-1} appeared with higher intensity, which can be assigned to the stretching vibration of ester carbonyl groups (TAN *et al.*, 2021), which is an indicative that the TA molecules were successfully grafted and crosslinked onto the cellulose chains. According to Tan *et al*. (2021), TA is grafted onto the cellulose chain through the ester linkage, and additionally carboxyl and hydroxyl groups in TA molecule undergo this esterification reaction to form a crosslinking network, and the stretching vibration of ester carbonyl groups indicated that TA molecules were successfully grafted onto the cellulose chains and crosslinked with the side chains constituting other tartaric acids.

3.5. WATER ABSORPTION CAPACITY (WAC), OIL ABSORPTION CAPACITY (OAC) AND SOLUBILITY.

WAC, OAC, and solubility are presented in Table 1. Cellulose presented WHC value of 19.24 g/g. The control sample (extruded without reagent) presented the lowest WAC value (9.50 g/g), while all cellulose samples crosslinked with TA and STMP showed significant improvements in WAC values, which ranged from 35.01 to 37.20 g/g, which is evidence of the crosslinking of both group of samples. The level or type of crosslinking agent did not affect significantly WAC values. According to Tan *et al*. (2021), TA play a role as an esterifying agent for cellulose, but also as crosslinker agent, which results in increased stability of biomolecular network and water absorption capacity. Cagnin *et al*. (2021a) reported that reaction with STMP results in reinforcement of the biopolymer network structure, which allows the water molecules to be adsorbed.

OAC significantly decreased in all samples modified by reactive extrusion when compared to unmodified cellulose, and the level or type of crosslinking agent did not affect significantly OAC values (Table 2). These results is an indicative that the modified cellulose had its hydrophilic character altered by the decrease of free hydroxyl groups on the cellulose surface by reaction with TA and STMP.

The solubility of cellulose in water at 25° C was 9.32% (Table 1), which agreed with Mantovan *et al.* (2021). In TA modified samples, the solubility increased significantly (Table 1) when compared to cellulose, and higher TA levels resulted in higher solubility values. However, in the samples extruded with STMP with a concentration of 0.1%, the solubility did not show any significant difference with the cellulose extracted from the oat hull. Seker and Hanna (2006) also observed a decrease in the water solubility promoted by crosslinking of starch of STMP by reactive extrusion.

Table 2 – Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC) and solubility of cellulose, control sample (extruded without reagent) and modified cellulose obtained by reactive extrusion through reaction with TA and STMP.

Samples	WAC	OAC	Solubility
Cellulose	19.24 ± 0.31^{b}	10.02 ± 0.01^{a}	9.32 ± 0.02^{e}
Control	9.50 ± 0.32^{c}	6.30 ± 0.36^{c}	12.81 ± 0.22^{d}
TA5	35.01 ± 0.11^{a}	7.26 ± 0.31^{bc}	16.92 ± 0.11^{c}
TA12.5	37.20 ± 0.66^{a}	8.60 ± 0.37^{ab}	20.78 ± 0.67^{b}
TA20	34.83 ± 0.01^{a}	8.71 ± 0.61^{ab}	25.19 ± 0.34^{a}
STMP0.025	35.07 ± 0.16^{a}	7.52 ± 0.13^{bc}	17.08 ± 0.23^{c}
STMP0.05	36.05 ± 0.36^{a}	7.43 ± 0.13^{bc}	14.17 ± 0.89^{d}
STMP0.1	35.74 ± 2.12^{a}	7.88 ± 0.27^{bc}	8.65 ± 0.55^{e}

Data are the means of replicate determinations ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$) between means (Tukey test).

3.6. THERMAL PROPERTIES

Fig. 4 shows the DSC curves for cellulose and modified cellulose obtained by reactive extrusion through reaction with TA and STMP. In all samples, an endothermic peak between 50°C and 125°C was observed, which was attributed to water evaporation, which was also observed by other authors (DEMITRI *et al.*, 2008; MARIM *et al.*, 2020).

For all samples, the presence of exothermic peaks around 350 and 360°C was observed (Fig. 4), mainly attributed to exothermic reactions due to cellulose depolymerization. Other authors (CAPANEMA *et al.*, 2018; GIL-GIRALDO *et al.*, 2021) have also reported the same peaks in cellulose-rich materials extracted from lignocellulosic residues.

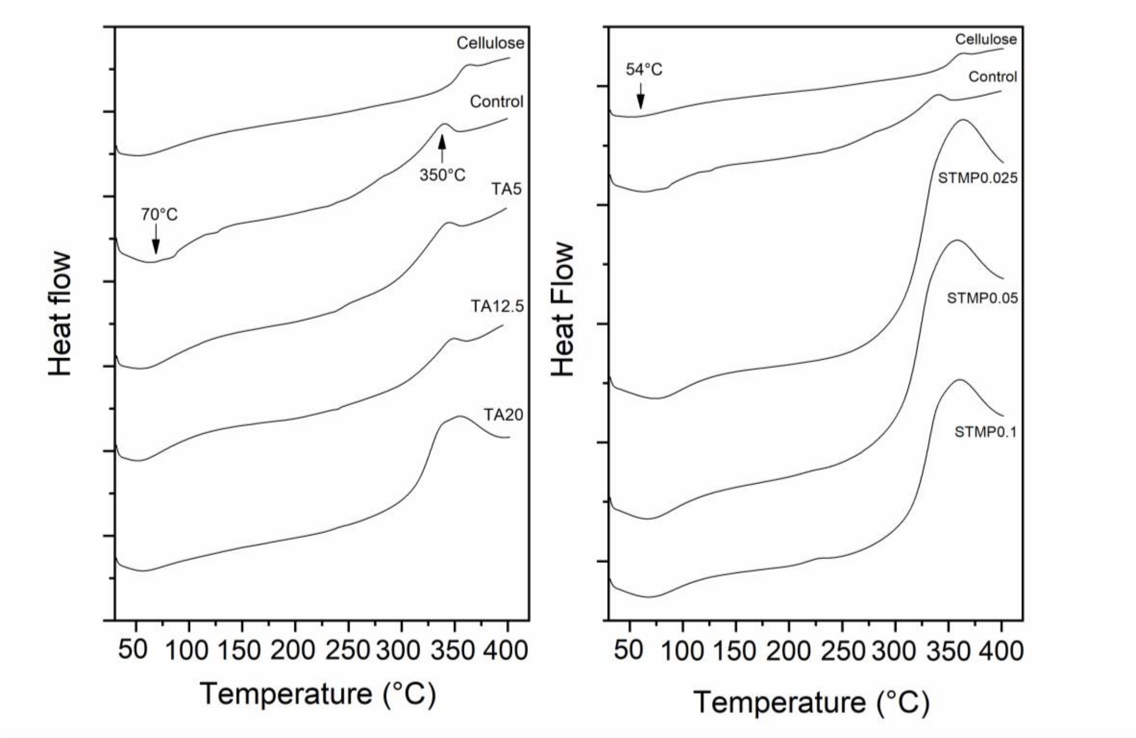

Fig. 4 - DSC thermograms of cellulose, the control sample (extruded without reagent) and modified cellulose obtained by reactive extrusion through reaction with TA and STMP.

TGA and DTGA curves are shown in Fig. 5. All samples show a first stage of degradation at 50-100°C, which is associated with loss of water or low molecular weight compounds (GIL-GIRALDO *et al.*, 2021; MIRANDA *et al.*, 2013). It was possible to observe that the control samples, cellulose and the samples modified with TA, the main weight loss occurred between 344 and 357 °C (Figure 5), which may be associated with polymerization reactions, dehydration and decomposition of glycosidic units, these reactions break down the cellulose. According to Oliveira *et al*. (2017), the depolymerization of hemicellulose occurs between 180 and 350°C, randomly cleaving the glycosidic bond of cellulose between 275 and 350°C and the degradation of lignin between 250 and 500°C.

In the samples where the cellulose was modified by STMP, it can be observed that the sample STMP0.1 has the main weight loss at 320 °C, because the materials containing phosphorus when heated release a polymeric form of phosphoric acid, this acid accelerates the degradation of the material (ALHARBI *et al.*, 2018).

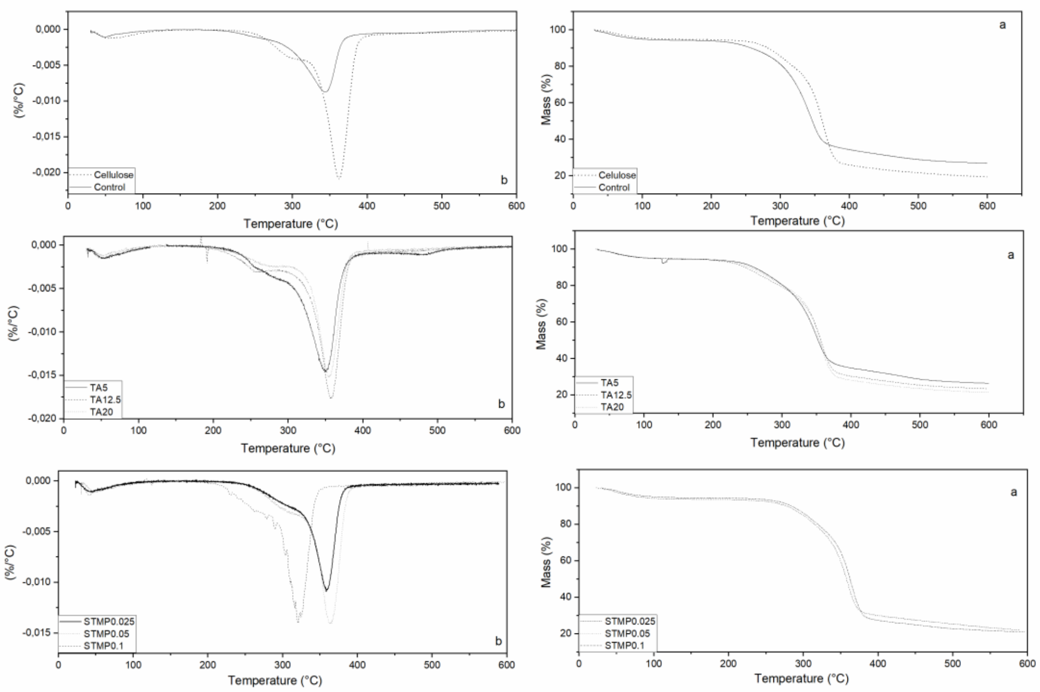

Fig 5 - TGA/DTGA curves of cellulose, control sample (extruded without reagent) and modified cellulose obtained by reactive extrusion through reaction with TA and STMP.

3.7. WETTABILITY

Wettability is the tendency of one fluid to spread on or adhere to a solid surface in the presence of other immiscible fluids. The wettability property is directly influenced by the polar and non-polar groups present on the surface of the molecule (AHMED, 2019; HUBBE; GARDNER; SHEN, 2015). Fig. 6 show the cellulose wettability results with water/chloroform. Cellulose and control (extruded in presence of water) samples showed an affinity for water (polar solvent), while all the crosslinked cellulose with STMP and TA showed affinity for chloroform (non-polar solvent). Unmodified cellulose has a hydrophilic character; thus, it has an affinity for water, unlike crosslinked samples. This difference evidences the hygroscopic alteration suffered by cellulose after chemical modification with TA and STMP.

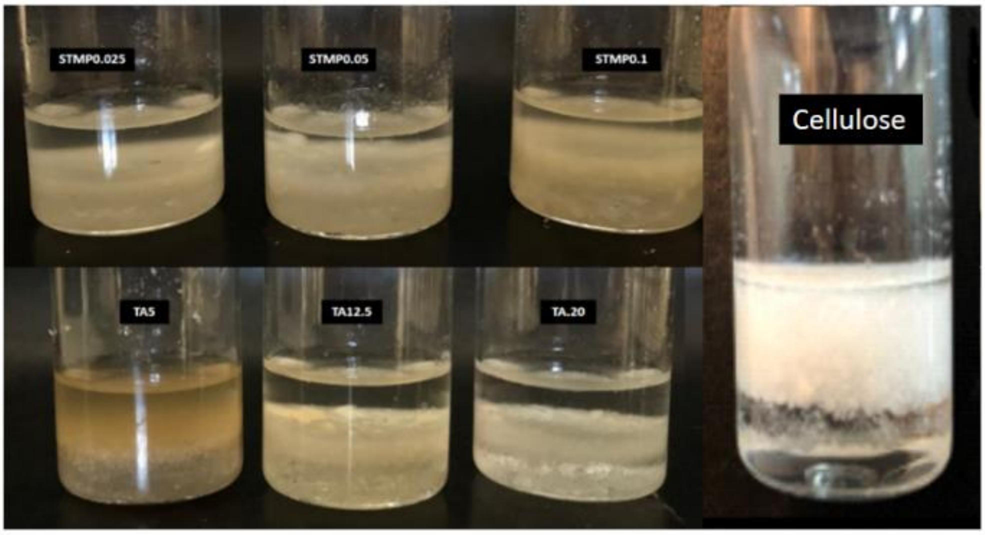

Fig. 6 - Dispersion of cellulose and modified cellulose in a water/chloroform system.

3.8. MOISTURE SORPTION ISOTHERMS

The moisture sorption isotherms and parameters of the GAB model are shown in Fig. 7 and Table 3, respectively. All samples presented the typical type II (sigmoidal) isotherm reported in the literature for other cellulosic materials content than the other samples, mainly in a_w of 0.75 and 0.90 (MANTOVAN *et al.*, 2021; MARIM *et al.*, 2020). The monolayer value (m_0) indicates the maximum amount of water that can be adsorbed on the primary layer of the material, by dry matter mass, it is a measure of the number of water sorption sites in the sample (CUNHA *et al.*, 2014; MERCI *et al.*, 2015; VERCELHEZE *et al.*, 2019).

According to the parameters presented in Table 3, cellulose had the highest monolayer value (37.10/100g) while the sample modified with 0.1% STMP (STMP0.1) had the lowest one (2.42/100g). These results indicated that there was some alteration in the water sorption sites of the cellulose samples by reaction with TA and STMP, resulting in samples with lower hygroscopicity. Possibly, the reaction of some free hydroxyls of cellulose surface with the crosslinking agents resulted in a lower capacity of interaction with water, generally free hydroxyl groups interact to water in cellulose samples.

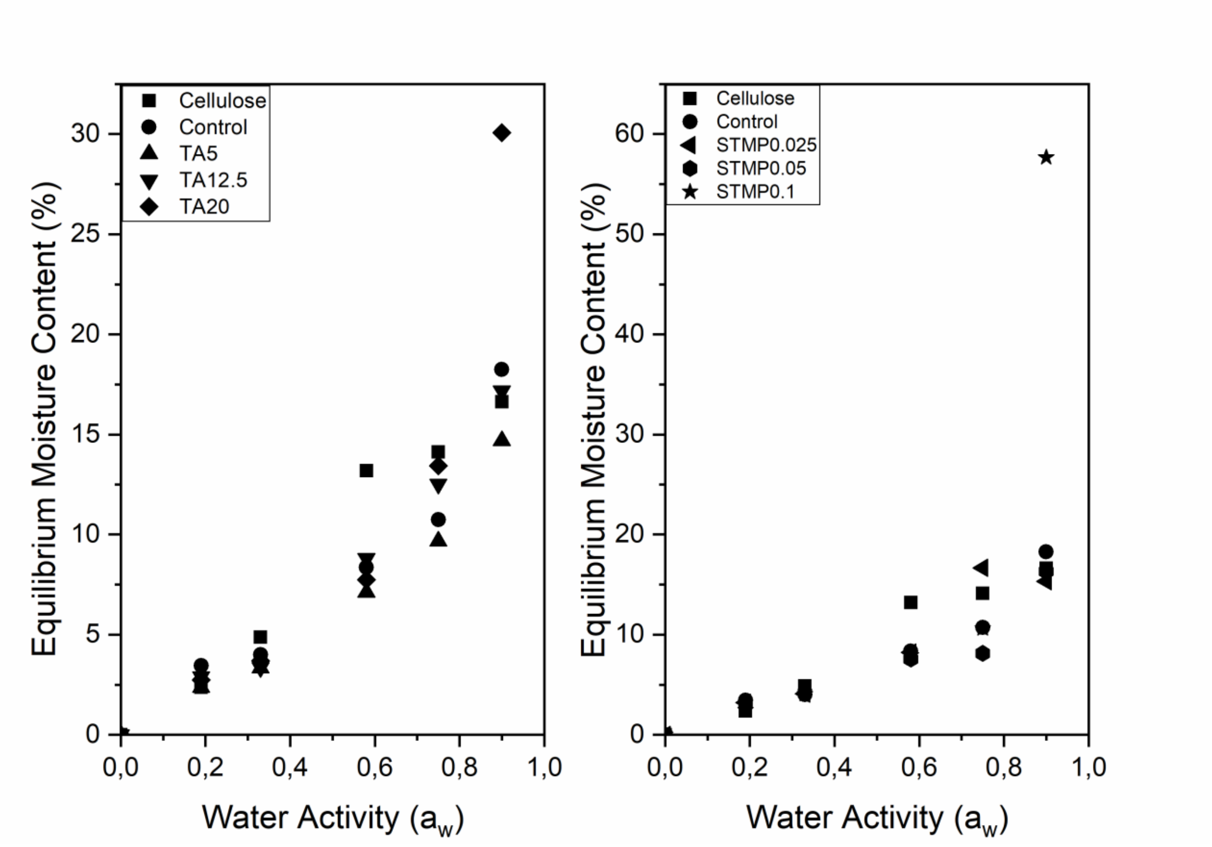

Fig. 7 - Moisture sorption isotherms of cellulose, control sample (extruded without reagent) and modified cellulose obtained by reactive extrusion through reaction with TA and STMP.

Table 3 - GAB model parameters of cellulose, control sample and cellulose samples modified by reactive extrusion.

Samples	m_0	C	K	R^2
Cellulose	37.10	2.16	0.22	0.97
Control	10.51	1.87	0.63	0.99
TA5	5.06	3.44	0.77	0.99
TA12.5	8.26	2.36	0.568	0.99
TA20	3.93	4.69	0.97	0.99
STMP0.025	16.63	0.44	0.19	0.99
STMP0.05	3.14	23.81	0.89	0.99
STMP0.1	2.42	27.34	1.06	0.99

4. CONCLUSION

Reactive extrusion proved to be an effective process for cellulose modification using TA and STMP, with the advantages of low effluent generation, being considered a promising environmentally safe alternative to this polymer modification. Reactive extrusion allowed the modification of cellulose without cellulose dissolution, maintaining the original morphology of the cellulosic fibers.

Modified samples showed greater thermal stability than unmodified cellulose, and all modified samples presented higher affinity for non- polar solvents and increased oil absorption capacity, while unmodified cellulose presented affinity for water.

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6 ARTIGO 3

Biodegradabe hydrogels based on starch, cellulose and gelatin obtained by reactive extrusion

Abstract

Biopolymeric hydrogels are materials with the advantage of having a variety of applications, such as agricultural practice materials, drug delivery systems, biosensors, and food packaging. This work investigated the production and characterization of biodegradable hydrogels using a mixture of starch, cellulose and gelatin as a polymeric matrix to be used for water retention in agriculture. Before being incorporated to the hydrogels formulations, cassava starch and cellulose were modified by reactive extrusion through reaction with citric acid and sodium trimetaphosfate (STMP) employed as crosslinking agents, respectively. The hydrogels were also produced by reactive extrusion as porous pellets, which were characterized according to their porosity, thermal properties, degree of swelling at different times and pHs, and water adsorption capacities. After 48 h soaked in pH 7, the hydrogel sample formulated with both CA-modified starch and STMP-modified cellulose, and gelatin, presented the highest values of porosity (>45%) and open pores (>5%), and the higher degree of swelling (607%), which makes these materials potential candidates for application as water retainers in agricultural systems, with important advantages, including their biodegradability and low toxicity. It is worth mentioning that the reactive extrusion process used is a continuous process, with low effluent generation and scalable for large-scale production.

Keywords: agriculture; biodegradability; swelling; porosity.

1. INTRODUCTION

Hydrogels have a three-dimensional structure with hydrophilic functional groups attached to the polymer main chain, giving the ability to absorb water and swell, without dissolving, thus being a stable material in the presence of water. Resistance to dissolution occurs as a function of crosslinks between the chains of the three-dimensional polymeric network. When dry, hydrogels are brittle, when in contact with water, they become an elastic gel, and their original shape is preserved (KHAN *et* *al.*, 2020; SIMÕES *et al.*, 2018).

There are several applications for these materials, such as agriculture, pharmaceuticals, biomedical, hygiene products, effluent treatment and biosensors, and they are being widely applied in agriculture to increase the retention capacity of fertilizers and water in the soil, reducing the frequency of irrigation. In addition, these hydrogels have been used as seed coatings and root dips to aid germination and improve their habitability (CAMPOS *et al.*, 2014; CHEN *et al.*, 2018; ZHANG *et al.*, 2017).

Polyacrylamide or polyacrylic acid are largely reported in literature as synthetic polymers that result in high performance hydrogels (DENG *et al.*, 2020; KHAN *et al.*, 2020; LI; CHEN, 2020; LV *et al.*, 2020; WANG; GUO, 2020), however these polymers are non-biodegradable synthetic polymers obtained from non-renewable sources (PADZIL *et al.*, 2020). Recently, several hydrophilic biopolymers are being extensively studied for the production of hydrogels, and hydrogels consisting of a mixture of polymers are highlighted because there is greater interaction between chains of different structures, improving the characteristics of the material, thus the application of the hydrogel is expanded (CUI *et al.*, 2022; GOPINATH *et al.*, 2022; LIU *et al.*, 2022; LUPINA *et al.*, 2022).

Starch, gelatin and cellulose are considered interesting raw materials for the obtainment hydrogels, they are materials with numerous possibilities of chemical and physical modifications described in the literature, and also with several advantages, including its non-toxicity, biodegradability, biocompatibility, low cost, and wide availability. Additionally, they are listed as generally recognized as safe (GRAS) and included in the Food and Drug Administration Inactive Ingredient Guide (BAO *et al.*, 2019; RODRÍGUEZ-CASTELLANOS *et al.*, 2015). According to Gopinath *at al.* (2022), chemically modified cellulose and starch derivatives have been emerged as potential raw materials for obtainment of new hydrogels formulations.

Generally, the most employed crosslinking agents for biopolymeric hydrogels based on starch, cellulose and gelatin are epichlorohydrin and glutaraldehyde, however they are toxic compounds and are not environmentally friendly (KHAN *et al*., 2022). Recently, an increased interest was observed in the use of low toxicity crosslinking agents such as citric acid or sodium trimetaphosphate to obtain new hydrogels formulations, especially for natural polymer-based hydrogels (GIL-GIRALDO *et al*., 2021; ZHU *et al*., 2021)

Reactive extrusion can be used for hydrogels production and recent research reports the effectiveness of this method. Cagnin *et al.* (2021a; 2021b) reported the production of hydrogels based on carboxymetilcellulose and starch by reactive extrusion using sodium trimetaphosfate (STMP) as a crosslink agent. Simões *et al*. (2020) reported the use of reactive extrusion for the obtainment of hydrogels based on starch and xanthan gum using citric acid as a crosslinker.

This study aimed the production and characterization of biodegradable hydrogels using a mixture of starch, cellulose and gelatin as a polymeric matrix to be used for water retention in agriculture. Before being incorporated to the hydrogels formulations, cassava starch and cellulose were modified by reactive extrusion though reaction with citric acid and sodium trimetaphosfate (STMP), respectively, which were employed as crosslinking agents. The hydrogels were also produced by reactive extrusion as porous pellets, and they were characterized according to their porosity, thermal properties, degree of swelling at different times and pHs, and water adsorption capacities.

2. MATERIAL AND METHODS

2.1. MATERIALS

The hydrogel formulations were prepared with: 1) cassava starch (20% amylose and 80% amylopectin) purchased from Pinduca Co. Ltd. (Araruna, Brazil); 2) cellulose extracted from oat hulls using peracetic acid as a bleaching agent by the methodology described by Marim et al. (2021); and 3) gelatin (150 to 220 g gel strength, Biotec, São Paulo, Brazil).

Glycerol (Synth) were employed as plasticizer and, citric acid (CA) (Synthlab, Diadema, Brazil) and sodium trimetaphosphate (STMP) (Sigma Aldrich, St. Louis, USA) were employed to obtain modified starch and cellulose, respectively.

2.2. METHODS

2.2.1. Modification of cellulose and starch by reactive extrusion

CA (20% - g acid/100 g starch) and STMP (0.1% - g STMP/100 g cellulose) were employed to obtain the modified cassava starch and cellulose from oat hull, respectively, based on method described by Gil-Giraldo *et al.* (2021). The samples were prepared by dissolving different proportions of CA or STMP in distilled water, and the obtained solutions were mixed with starch and cellulose, respectively, resulting in samples with a final moisture content of 32 % (g/g), which remained in sealed plastic bags at room temperature for 1h before extrusion. Starch and cellulose were extruded in a single screw extruder (AX Plastics, Diadema, Brazil) with a screw diameter of 1.6 cm and a screw length/screw diameter ratio (L / D) of 40, with four zones of heating and a matrix of 0.8 cm in diameter. The temperature in all zones was 100°C and the screw speed was 60 rpm. The starch and cellulose extrudates were collected, placed in an oven, dried to constant weight at 45 °C, ground, and sieved in an 80-mesh sieve. Samples were washed three times with absolute ethanol to remove unreacted CA or STMP. Finally, the washed samples were air-dried at 45 °C.

Degree of substitution (DS) of modified starch was calculated according to Volkert *et al*. (2010) by titration. For modified cellulose, the phosphorus content (%) was analyzed in triplicate by colorimetry in 600 nm (spectrophotometer Varian - Cary 50 Conc, São Paulo, Brazil) and DS was calculated as follows: $DS = 162*P/ (3100 - 102*P)$, where: P is phosphorus content (%); 162 is the molecular weight of the monomeric unit of cellulose, 3100 is the atomic weight of phosphorus multiplied by 100; and 102 is the group phosphate weight.

2.2.2. Hydrogels production by reactive extrusion

Four formulations (Table 1) were employed to obtain the biopolymeric hydrogels by reactive extrusion using different proportions of native and modified starch, cellulose and modified cellulose, and these proportions were determined after preliminary tests (data not presented), the formulations that were used were those that resulted in intact and easily processable pellets during the extrusion process. Gelatin and glycerol concentrations were fixed.

The formulations were prepared by manually mixing the components with 35% glycerol. The final mixtures were processed in a laboratory single-screw extruder (model EL-25, BGM, São Paulo, Brazil) with a screw diameter (D) of 25 mm and a with a screw diameter (D) of 25 mm, a screw length of 26D, using a die with six 2 mm diameter holes. Barrel temperature profile was 90/120/130/115 ºC, from the feed zone to the die zone, and a screw speed of 35 rpm was employed. Subsequently, the samples were pelleted dried in a circulation oven at 60 ºC for 3 h and used for characterization.

Table 1 - Formulations used in the preparation of 100 g of hydrogels by reactive extrusion.

Components (g/100 g)	**Formulations**			
	S-C-G	**S-MC-G**	**S.MS-C-G**	**S.MS-MC-G**
Native starch (S)	51.0	51.0	30.6	30.6
Modified starch (MS)			20.6	20.6
Unmodified cellulose (C)	7.0	-	7.0	-
Modified cellulose (MC)	-	7.0	-	7.0
Gelatin (G)	7.0	7.0	7.0	7.0
Glycerol	35.0	35.0	35.0	35.0

2.2.3. Porosity

Porosity and pore size were determined by X-ray computed tomography (X-ray CT) in SkyScan-Bruker equipment (model 1172 MCT, Kontich, Belgium). The conditions were 50 kV, 80 μA, exposure time of 300 ms and spatial resolution of 5 mm.

2.2.4. Fourier Transform-Infrared Spectroscopy (FT-IR)

Approximately 1 mg of modified starch, modified cellulose and hydrogels were pulverized, dried and mixed with potassium bromide (KBr) and compressed into tablets in a mass ratio of approximately 1:100 (sample to KBr). FT-IR spectroscopy for the samples was performed with a Shimadzu FT-IR -8300 spectrometer (Kyoto, Japan), the scanning range was 4000 to 500 cm^{-1} with spectral resolution of 4 cm^{-1}.

2.2.5. Thermal properties

Thermogravimetric Analysis (TGA) of the samples were be performed using the Shimadzu TGA-50 (Japan) equipment. The scans were be performed at room temperature up to 600 °C with a heating rate of 20 °C/min under a nitrogen flow of 20 mL/min.

2.2.6. Water adsorption isotherms

The samples (0.5 g) were dried for 7 days in a desiccator containing anhydrous calcium chloride, and then the samples were placed at 25 °C for 7 days in desiccators containing different saturated salt solutions providing specific water activities (a_w) for the samples (0.11, 0.33, 0.43, 0.60, 0.75, and 0.90). Samples were dried at 105 °C in a ventilated oven (035 Marconi MA, Piracicaba, Brazil) to determine their equilibrium moisture content at each a_w condition. The GAB model (Guggenheim– Anderson-de Boer) was used to fit the sorption isotherm data, and the monolayer values were calculated from the GAB isothermal model (Bizot, 1983) as follows: $M = (m_0CK_{aw}) / (1 - K_{aw}) (1 - K_{aw} + CK_{aw})$, where M is the equilibrium moisture (g water / 100 g solids), a_w is the water activity, m_0 is the monolayer value (g water / 100 g solids), and C and K are GAB constants.

2.2.7. Degree of swelling at different times and different pHs

Degree of swelling was determined by gravimetric method using previously weighed cylindrical devices (3.0 x 3.5 cm) containing at one end a screen with a nominal opening of 138 x 75 mm, as described by (PEREIRA; LONNI; MALI, 2022). About 1g of each sample was weighed and placed inside the devices and then immersed in 10 mL of a different pH solution (4.0, 7.0 and 9.0) at 25 ^oC. At predetermined times (30min, 24h and 48h), the devices containing the hydrogel pellets were removed from the solutions, the excess was removed with filter paper and then weighed. From the value obtained, the degree of swelling (g/g) of each sample was calculated in relation to its initial dry weight. Analyzes were performed in triplicate.

2.2.8. Statistical analysis

Analyses of variance (ANOVA) and Tukey's mean comparison test ($p \leq 0.05$) were performed with Statistica software version 7.0 (Statsoft, OK, USA).

3. RESULTS AND DISCUSSION

3.1. Modification of starch and cellulose

Cassava starch was modified by reactive extrusion, and degree of substitution (DS) was 0.365 for citric acid-modified starch. Citric acid ($C_6H_8O_7$) is a tricarboxylic acid that acts as an esterifying agent for starch, but also as crosslinker agent, which results in increased stability of biopolymeric matrix (MISKEEN *et al.*, 2021; SIMÕES *et al.*, 2020). Ye *et al*. (2019) modified rice starch by reactive extrusion with CA and observed DS between 0.037 and 0.138 when the CA levels ranged from 10 to 40% (g CA/100 g starch).

Regarding to the STMP-modified cellulose sample, the DS was 0.01. DS can be consider a good indicative of the occurrence of crosslinking of the phosphate groups grafted onto the polysaccharides chain (CAGNIN *et al.*, 2021a; CAGNIN *et al.*, 2021b; DONG; VASANTHAN, 2020). The value observed in this study was lower when compared to DS reported by Cagnin *et al*. (2021a), who reported DS values of 0.05 for hydrogels obtained by reactive extrusion from cassava starch and carboxymethylcellulose crosslinked with STMP (4 g/100 g).

Crosslinking agents as CA and STMP are capable of forming ester intermolecular linkages between specific groups on starch and cellulose molecules, and depending on the level of substitution, the polymer network is reinforced and results in changes in solubility and degree of swelling power (CAGNIN *et al.,* 2021a; SIMÕES *et al*., 2020; TUPA *et al*., 2020), and according to Golachowski *et al*. (2020), these changes are directly correlated with the degree of substitution.

3.2 Porosity

Table 2 presents the results of radial expansion and porosity of hydrogels pellets produced by reactive extrusion.

Table 2. Porosity, open pores, and closed pores of samples.

Samples	Porosity (%)	Open Pores (%)	Closed Pores (%)
S-C-G	34.99 ± 0.03^{a,b}	34.33 ± 1.28^{b}	0.82 ± 0.31^{a}
S-MC-G	37.14 ± 0.14^{a,b}	36.42 ± 1.52^{b}	1.12 ± 0.02^{a,b}
S.MS-C-G	28.52 ± 0.09^{b}	30.49 ± 1.09^{b}	5.25 ± 1.99^{b,c}
S.MS-MC-G	45.34 ± 0.10^{a}	42.51 ± 1.19^{a}	5.53 ± 1.05^{c}

Data are the means of replicate determinations ± standard deviation. Different letters in the same column indicate significant differences (p≤ 0.05) between means (Tukey test).

S.MS-C-G sample had the lowest porosity when compared to the other samples (28.52%, Table 2), and the sample S.MS-MC-G prepared with modified starch and cellulose had the higher porosity values (45.34%, Table 2) and open pores (42.51%). Possibly, the modification of starch and cellulose resulted in a more expandable polymeric matrix because of the introduction of new linkages between polymeric chains, resulting in a more stable melted material, which expanded at the exit of the extruder without losing the porous structure.

During reactive extrusion, the high temperatures induce rapid evaporation of water with the exit of water from the melted material resulting in the formation of pores. Evaporation depends on the resistance of the material to the pressure exerted on the water vapor and also depends on the variables of the reactive extruder (temperature, screw speed), these variables tend to increase the porosity of the hydrogel (WŁODARCZYK-STASIAK; JAMROZ, 2009). However, in this work the same conditions were standardized for all samples, thus the differences in the results must be attributed to the components of the formulations. (CAGNIN *et al*., 2020) reported lower porosity values (below 11%) for carboxymetilcellulose and starch hydrogels obtained by reactive extrusion using sodium trimetaphosfate (STMP) as crosslink agent.

Considering the intended application for the hydrogels as a polymeric matrix to be used for water retention in agriculture, higher porosity and open pores are desirable. These open pores have communication channels with the outside, improving the swelling properties of the hydrogel. Closed pores are interesting for the mechanical strength and elasticity of the material.

3.3 Fourier Transform-Infrared Spectroscopy (FT-IR)

As seen in Figure 1a, a new important band at 1730 cm^{-1} appeared in modified starch (MS) sample, it was attributed to the stretching vibration of the carbonyl ester group, indicating that the ester bonds were formed successfully. These results agreed with those presented by other authors (FARHAT *et al.*, 2017; SIMÕES *et al.*, 2020; YE *et al.*, 2019; ZHOU *et al.*, 2016), who used CA as esterifying and crosslinking agent for starch modification.

FT-IR spectra of hydrogel samples are shown in Fig. 1b. It was possible to identify an important band at 1730 cm^{-1}, in the samples that were prepared with modified starch (S.MS-C-G and S.MS-MC-G), attributed to the stretching vibration of the carbonyl ester group, which provides evidence of ester bonds formed in the CA-modified starch used to prepare the hydrogels

Other bands are observed in the FT-IR spectra (Fig. 1b) of all hydrogels samples: a broad band at 3400 cm^{-1} attributed to stretching of OH stretching in hydrogen bonds; a band at 2900 cm^{-1} that was associated CH group stretching vibration (CH_2 groups)(FARHAT *et al.*, 2017; MARIM *et al.*, 2020). The characteristic e bands of cellulose were also distinguished at 1017 cm$^{−1}$ (C–O–C pyranose ring vibration) and 895 cm$^{−1}$ (β-glycosidic bond bending) (MAALOUL *et al.*, 2021).

Specific bands of the phosphate groups at 1214 cm^{-1} (P=O elongation), 1129 cm^{-1} (symmetric and asymmetric stretching of the PO_2 group), and 889 cm^{-1} (PO bond) (Fig.1b) appeared in all hydrogels samples without any difference in intensity or shift, including the sample prepared with STMP-modified cellulose (S.MS-MC-G**).** This occurred probably due to the low concentration of STMP used, and additionally these bands may be covered by other bands between 1200 and 800 cm^{-1}, which is characteristic of starch and cellulose (Mantovan et al., 2021; Pozo et al., 2018). Some authors reported that they did not detect these phosphate bands in crosslinked STMP starch and cellulose, and they attributed this to the low concentration of phosphate groups (CAGNIN *et al.*, 2021a; DA SILVA MIRANDA SECHI; MARQUES, 2017).

KHAN *et al.*, (2020) reported that pure gelatin presents characteristics bands at 3430 cm$^{−1}$ due to amide –N–H elongation and at 1670 cm$^{−1}$ due to amine groups. It can be seen that in all hydrogels samples (Fig. 1b) da band at 1650 cm$^{−1}$, which can be an indicative of the formation of hydrogen bonds between OH groups of starch and cellulose with amine groups of gelatin (LU *et al.*, 2017). Lu *et al*. (2017) also reported that free carboxylic groups of gelatin can be esterified with hydroxyl groups of other polymers, such as cellulose and starch.

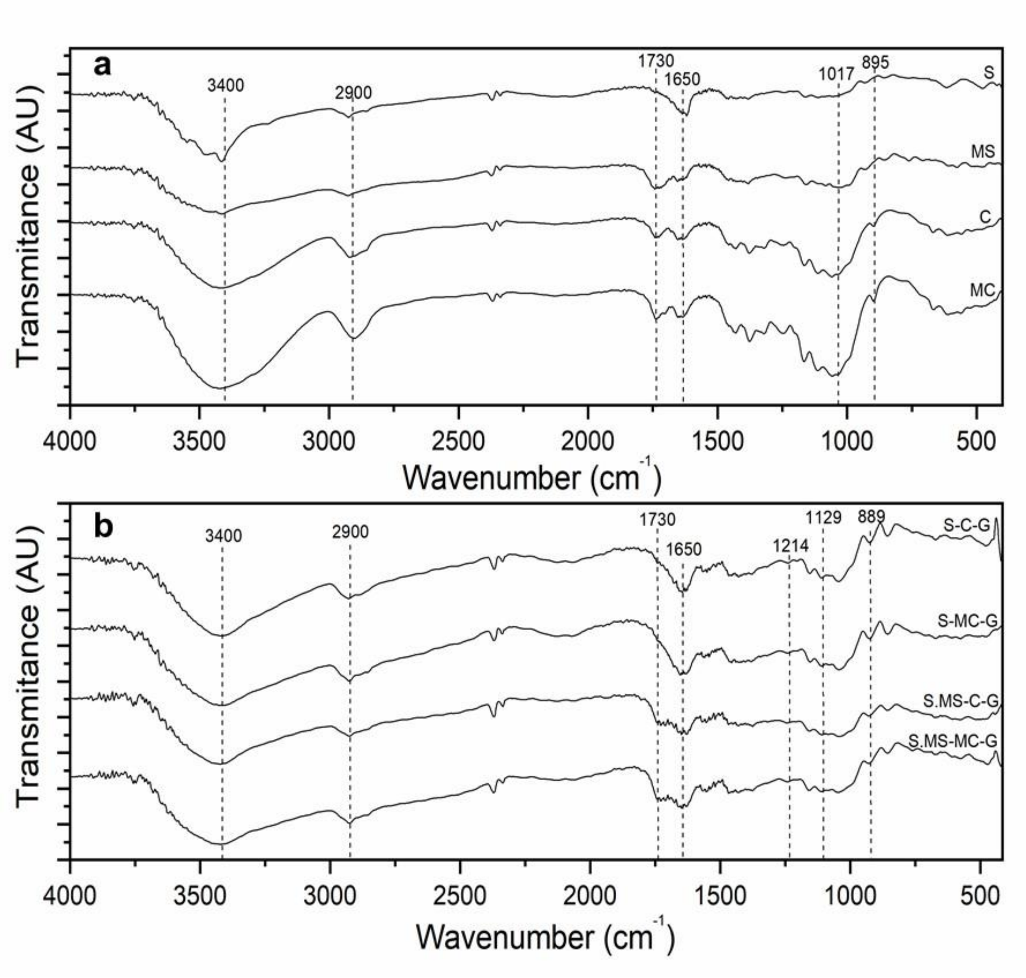

Fig. 1 - FT-IR spectra of samples.

S (native starch); MS(modified starch); C (cellulose); MC(modified cellulose); S-C-G (hydrogel formulated with native starch, cellulose and gelatin); S-MC-G (hydrogel formulated with native starch, modified cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, modified cellulose, and gelatin)

3.4 Thermal properties

TGA and DTGA curves are shown in Fig. 2. For all hydrogel samples, the main weight loss occurred between 320 to 330°C, (Figure 3), which can be associated with the degradation of starch and cellulose (ALHARBI *et al.*, 2018; RODRÍGUEZ-CASTELLANOS *et al.*, 2015), and other peaks were observed at 160°C. These results are interesting results, indicating that the obtained hydrogels can support up to a temperature of 300 °C without being significantly degraded. Rodríguez-

Castellanos et al (2015) produced starch - gelatin hydrogels reinforced with cellulose by reactive extrusion, and they described that the weight loss observed at 160 - 170 °C may be associated with the glycerol content in the polymer matrix, while the peaks at 266 °C were associated to the degradation of long chains of gelatin. The peaks observed at 490 °C indicate denaturation of the gelatin.

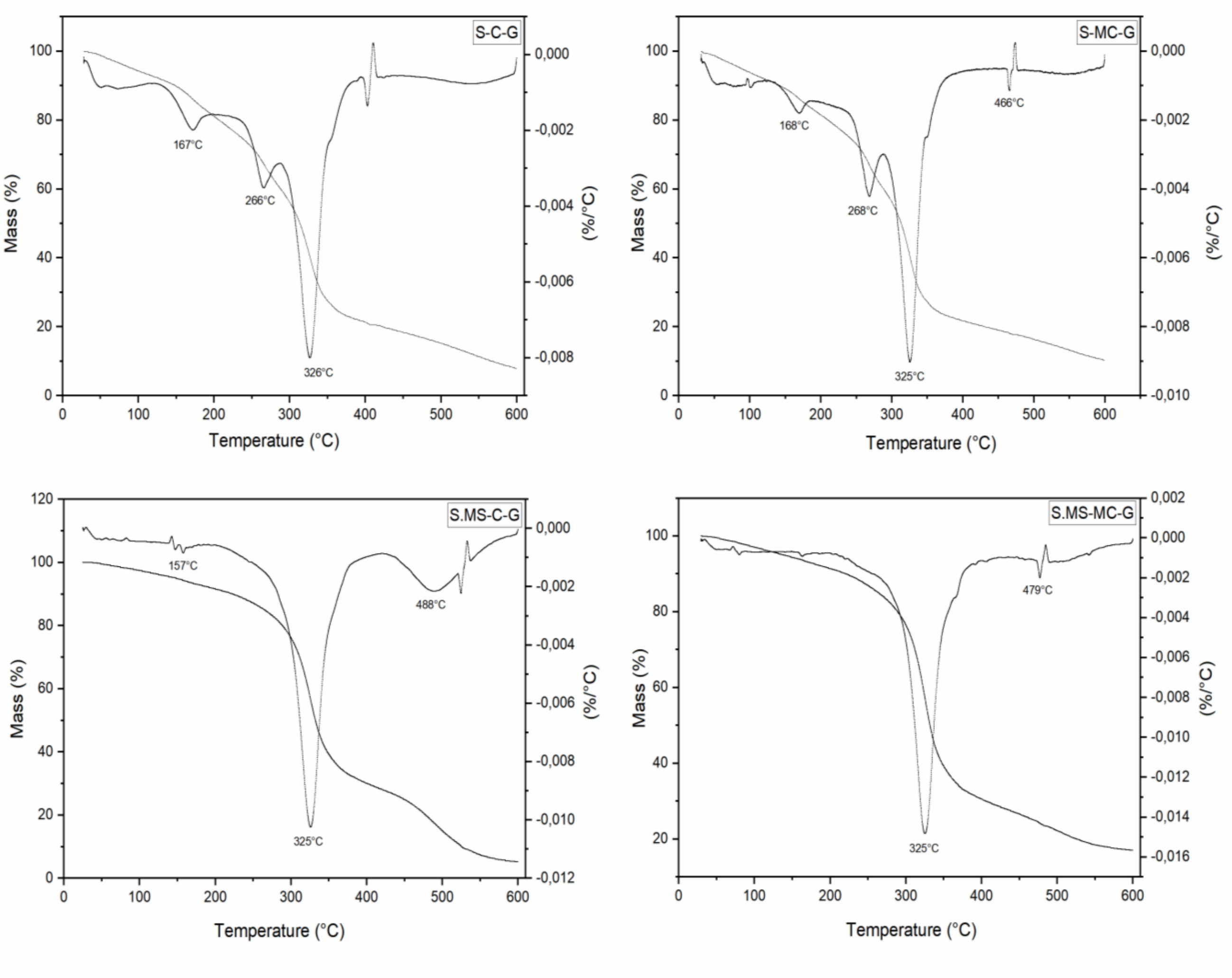

Fig 2 - TGA/DTGA curves of hydrogels obtained by reactive extrusion.

S-C-G (hydrogel formulated with native starch, cellulose and gelatin); S-MC-G (hydrogel formulated with native starch, modified cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, modified cellulose, and gelatin)

3.5. Moisture sorption isotherms

The moisture sorption isotherms and parameters of the GAB model are shown in Fig. 3 and Table 3, respectively. All samples had a similar shape characteristic of hydrophilic materials and most starch and cellulose based materials (KOHLER *et al.*, 2006; MANTOVAN *et al.*, 2021; MÜLLER; LAURINDO; YAMASHITA, 2009; SILVA *et al.*, 2013), which means that hydrogels produced by reactive extrusion have a high affinity for water when exposed to high activity of water (a_w). The GAB model was efficient to describe the moisture sorption isotherms of the hydrogel samples ($R^2 = 0.99$).

The sorption isotherms of all samples showed similar patterns, with an increase in water activity (a_w) when there was observed an increase in equilibrium moisture contents.

The monolayer value (m_0) indicates the maximum amount of water adsorbed in a single layer per gram of dry material that can be related to the sorption sites (GIL GIRALDO *et al.*, 2021; MARIM *et al.*, 2020). According to the parameters presented in Table 1, the sample produced with native starch and unmodified cellulose had the highest m_0 (53.25/100g) while samples prepared with modified starch had the lowest values (S.MS-C-G and S.MS-MC-G). These results indicate that the addition of modified starch in the samples decreased their capacity of adsorb water when stored in environments with higher relative humidities. Possibly, starch modification with CA resulted in a reinforced polymeric matrix, when free hydroxyl groups of starch were esterified, which resulted in a lower ability to interact with water.

The parameter k is related to the heat of sorption of the multilayer region and, generally, the values of this parameter are less than 1, and the values of k ranged from 0.46 (S/C/G) and 3.59 (S/SCA/C/G), respectively (Table 3). The parameter C is related to the sorption heat of the first layer, 0.58 to 0.88 (S/SCA/C/G and S/SCA/CSTMP/G).

Table 3 - GAB model parameters of hydrogels crosslinked by reactive extrusion.

Samples	m_0	C	K	R^2
S-C-G	53.25	0.46	0.58	0.99
S-MC-G	39.92	0.62	0.59	0.99
S.MS-C-G	5.86	3.59	0.88	0.99
S.MS-MC-G	6.51	2.41	0.88	0.99

S-C-G (hydrogel formulated with native starch, cellulose and gelatin); S-MC-G (hydrogel formulated with native starch, modified cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, modified cellulose, and gelatin)

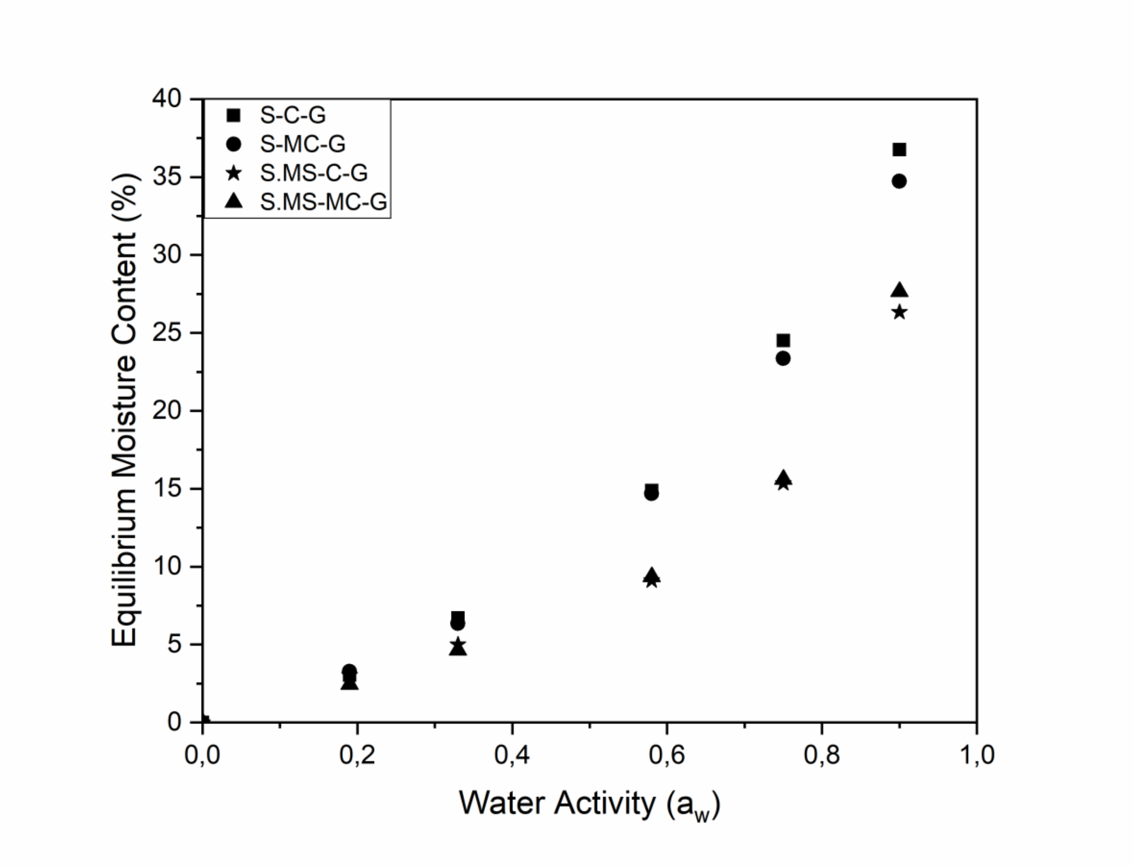

Fig. 3 - Moisture sorption isotherms curves of hydrogels obtained by reactive extrusion.

S-C-G (hydrogel formulated with native starch, cellulose and gelatin); S-MC-G (hydrogel formulated with native starch, modified cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, modified cellulose, and gelatin)

3.6. Degree of swelling at different times and pHs

The effects of time and pH on the degree of swelling of hydrogels obtained by reactive extrusion can be seen in Figs. 3 and 4 and the effect of pH after 48 h of contact with water can be seen on Table 4.

A first visual analysis was performed to determine how long the granules could be immersed in distilled water without losing their structure. This criterion was established with a focus on the possible future application of hydrogel pellets, for which structural integrity is critical. However, all samples could remain submerged in water for more than 48h without dissolving or lose their shape. In Fig. 4 it was possible to observe the intact and swollen pellets after 48 immersed in water.

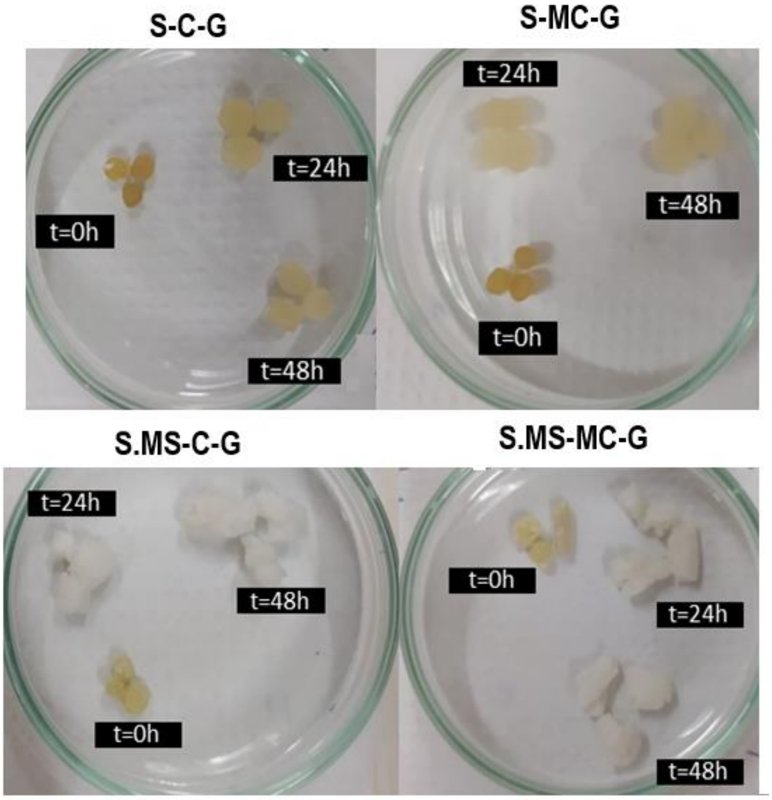

Fig. 4 - Appearance of hydrogels pellets after being immersed in water for 0, 24 and 48 h.

S-C-G (hydrogel formulated with native starch, cellulose and gelatin); S-MC-G (hydrogel formulated with native starch, modified cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, modified cellulose, and gelatin)

In Fig. 5 it can be observed that the swelling of hydrogels pellet increased rapidly after 30 min of contact with water, but after 24 h the swelling degree stabilizes. Chemical and physical forces induce the liquid in a polymeric matrix, being responsible for the swelling of hydrogels. In the beginning there is rapid sorption of water, as there is a lot of free space between the chains, then the absorption rate decreases, as the molecules cannot bind at the crosslinking points, which act as barriers, bringing the material to equilibrium. The time required for the hydrogel to equilibrate depends on the crosslink density, extent of porosity and chemical composition, which determine characteristics such as hydrophilicity, charge and intermolecular interactions (CHAUDHARY *et al.*, 2020; GHAREKHANI *et al.*, 2017; HASIJA *et al.*, 2018).

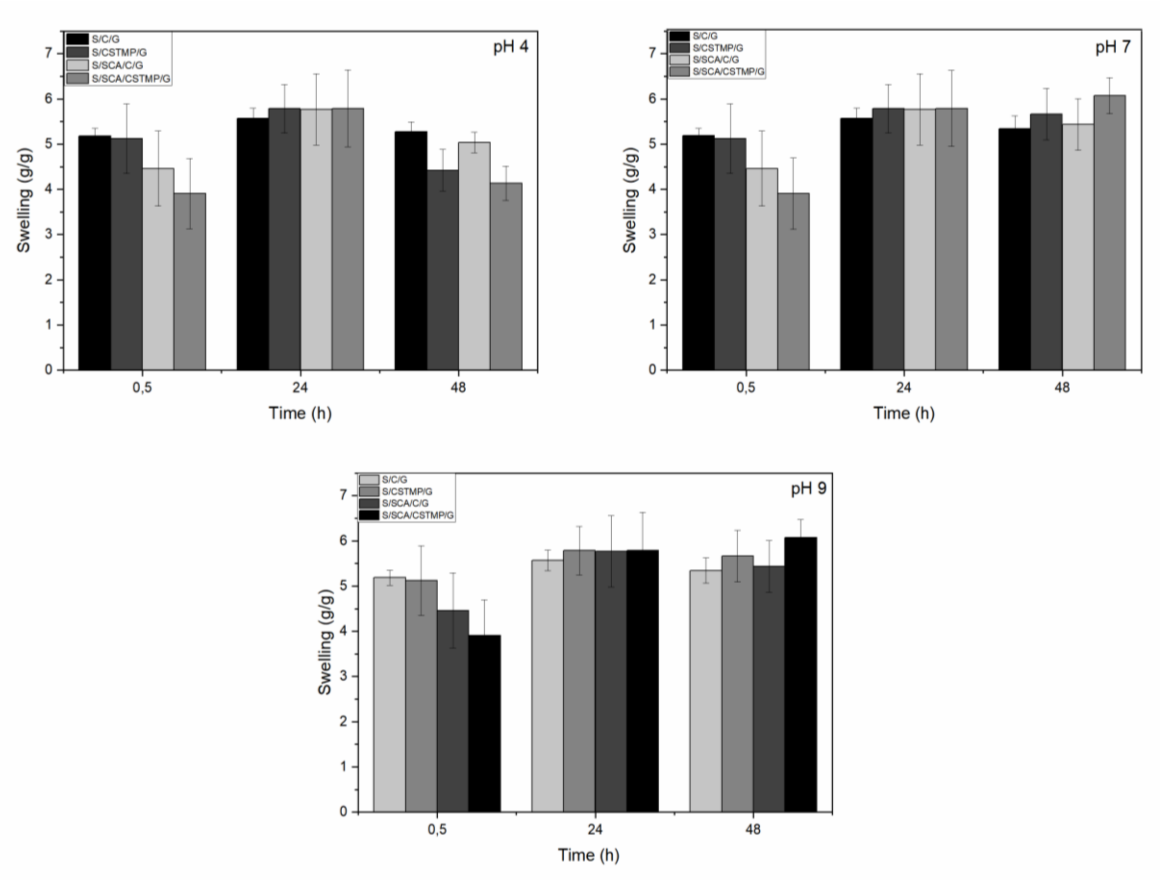

Fig. 5 - Effects of time and pH on swelling of hydrogels obtained by reactive extrusion.

S-C-G (hydrogel formulated with native starch, cellulose and gelatin); S-MC-G (hydrogel formulated with native starch, modified cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, cellulose, and gelatin); S.MS-C-G (hydrogel formulated with native starch, modified starch, modified cellulose, and gelatin)

In Table 4 it was observed that there was no significant difference between the degree of swelling of the different hydrogels samples at pH 7 after 48 h immersed in water, except for sample S.MS-MC-G that presented the higher significantly value (6.07 g/g). This sample, which was formulated with both CA-modified starch and STMP-modified cellulose, also presented the highest values of porosity and open pores, which favored water absorption in this sample. Modification of starch and cellulose resulted in the introduction of new linkages between polymeric chains. When samples were subjected to pH 4 and pH 9, small variations were observed in the different formulations, however it was difficult to related these variation to hydrogels formulations.

Table 4 - Degree of swelling of hydrogel samples for 48 h at different pH values

Samples	Degree of swelling (g/g)		
	pH 4	**pH 7**	**pH9**
S-C-G	5.27 ± 0.03^{aA}	5.34 ± 0.05^{aA}	4.21 ± 0.25^{a,bB}
S-MC-G	5.04 ± 0.14^{b,aA}	5.63 ± 0.22^{aA}	3.22 ± 0.05^{bB}
S.MS-C-G	4.42 ± 0.09^{b,c,B}	5.43 ± 0.99^{aA}	4.65 ± 0.07^{a,bB}
S.MS-MC-G	4.13 ± 0.10^{c,bB}	6.07 ± 0.11^{bA}	3.74 ± 0.53^{bA}

Data are the means of replicate determinations ± standard deviation. Different small letters in the same column indicate significant differences (p≤ 0.05) between means, and different capital letters in the same line indicate significant differences (p≤ 0.05) between means (Tukey test).

Comparing the effect of pH on degree of swelling of hydrogels samples, it can be observed that the highest values were observed for all samples at pH 7, with values ranging from 5.37 to 6.07 g/g (537 – 607% of swelling), however in pH 9 degree of swelling decreased for all samples (Table 4). According to Khan *et al.* (2022), when the water absorption content can exceed more than 95 percent of the total weight or volume of the hydrogel, these hydrogels can be referred to as superabsorbent hydrogels, thus, the hydrogels obtained in this study can be considered as superabsorbent hydrogels, which in the most important property in a material that will be used for water retention on agricultural systems.

4. CONCLUSION

Reactive extrusion process was efficient to produce hydrogels based on citric acid-modified starch, STMP-modified cellulose and gelatin. After 48 h soaked in pH 7, they obtained higher values of degree of swelling (up to 530%), and the hydrogel sample formulated with both CA-modified starch and STMP-modified cellulose presented the highest values of porosity (>45%) and open pores (>5%), and the higher degree of swelling (607%), which makes these materials potential candidates for application as water retainers in agricultural systems, with important advantages, including their biodegradability, low toxicity and low environmental impact resulting from their production process. Additionally, it is worth mentioning that the reactive extrusion process used is a continuous process, with low effluent generation and scalable for large-scale production.

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7 CONSIDERAÇÕES FINAIS

Hidrogéis de amido de mandioca foram produzidos e esterificados com ácido cítrico e ácido tartárico por extrusão reativa. O processo de extrusão resultou em materiais com diferentes graus de substituição, que foram maiores para os hidrogéis obtidos por reticulação com ácido cítrico. A estrutura cristalina do amido foi rompida durante a extrusão reativa, e a análise da morfologia mostrou que a estrutura granular original do amido foi perdida e substituída por uma estrutura mais áspera e irregular, o que resultou em hidrogéis reticulados com maior solubilidade e capacidade de inchamento do que o amido nativo e a amostra de controle (extrudada sem os agentes de reticulação). As amostras de hidrogéis obtidas com as concentrações mais altas de ácido cítrico ou tartárico (15 e 20%) resultaram em valores mais altos de DS, valores de solubilidade e capacidades de intumescimento mais altos.

A celulose foi extraída da casca da aveia e modificada com ácido tartárico e trimetafosfato de sódio por extrusão reativa apresentou pequeno decréscimo na cristalinidade, no entanto, o padrão cristalino das amostras não mudou. A morfologia da superfície das amostras não foi afetada pela modificação com ácido tartárico e trimetafosfato de sódio. As amostras modificadas mostraram maior estabilidade térmica do que a celulose não modificada. Todas as amostras modificadas apresentaram maior afinidade para solventes apolares, enquanto a celulose não modificada apresentou afinidade para água.

Foram produzidos hidrogéis no formato de pellets com a mistura de celulose, amido e gelatina, que após 48 h em contato com a água em pH 7, obtiveram elevados valores de grau de intumescimento (até 530%). A amostra de hidrogel formulada com amido nativo, amido modificado com ácido cítrico 20%, celulose modificada com STMP 0,1% e gelatina apresentou os maiores valores de porosidade (>45%) e poros abertos (>5%), e o maior grau de inchamento (607%), o que torna esses materiais candidatos potenciais para aplicação como retentores de água em sistemas agrícolas, com vantagens importantes, incluindo sua biodegradabilidade, baixa toxicidade e baixo impacto ambiental resultante de seu processo de produção.

A extrusão reativa foi eficiente tanto para a modificação do amido e da celulose, quanto na produção de hidrogéis no formato de pellets para serem empregados na agricultura como retentores de água, com as vantagens de simplicidade, baixa geração de efluentes, tempos de reação curtos e fácil adaptação para escala industrial.