Universidade
Estadual de Londrina

JANAINA MANTOVAN

APROVEITAMENTO DO BAGAÇO DE LARANJA PARA EXTRAÇÃO DE MATERIAIS RICOS EM CELULOSE EMPREGANDO-SE DIFERENTES MÉTODOS FÍSICOS E QUÍMICOS

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Biotecnologia

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Biotecnologia da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Doutora em Biotecnologia.

Orientador: Profª. Drª. Suzana Mali de Oliveira.

Londrina
2021

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

Mantovan, Janaina .
APROVEITAMENTO DO BAGAÇO DE LARANJA PARA EXTRAÇÃO DE MATERIAIS RICOS EM CELULOSE EMPREGANDO-SE DIFERENTES MÉTODOS FÍSICOS E QUÍMICOS / Janaina Mantovan. - Londrina, 2021.
108 f. : il.

Orientador: Suzana Mali de Oliveira.
Tese (Doutorado em Biotecnologia) - Universidade Estadual de Londrina, Centro de Ciências Exatas, Programa de Pós-Graduação em Biotecnologia, 2021.
Inclui bibliografia.

1. tratamentos de etapa única - Tese. 2. tratamento alcalino - Tese. 3. autoclavagem; ultrassonicação; extrusão; - Tese. 4. nanocelulose - Tese. I. Mali de Oliveira, Suzana . II. Universidade Estadual de Londrina. Centro de Ciências Exatas. Programa de Pós-Graduação em Biotecnologia. III. Título.

CDU 66

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AGRADECIMENTOS

Agradeço a Deus, o centro de minha vida, pela força e direcionamento em minha caminhada.

Á minha família, em especial minha mãe, Norma Mantovan, e avó, Elvira Bianchi, que já partiu, por serem duas mulheres incríveis que sempre me apoiaram nos momentos alegres e difíceis. Também às minhas irmãs, Luciana Mantovan e Carolina Mantovan, por torcerem muito pelo meu sucesso.

Á minha orientadora, Suzana Mali, que me acolheu em seu grupo de pesquisa com muito carinho, sempre me ajudando e motivando através de sua grande dedicação à pesquisa e ao ensino, mesmo diante das dificuldades.

Aos meus amigos, por proporcionarem grandes momentos de leveza em meio às dificuldades. Em especial, Talita Caretta, Gabrielly T. Bersanettie G. Alejandra G. Giraldo, por serem grandes companheiras que compartilharam essa mesma caminhada comigo.

Ao meu grupo de pesquisa, em especial à G. Alejandra G. Giraldo, Beatriz M. Marime João Otávio F. Kishima, pela amizade, pelas diversas colaborações na pesquisa e pela parceria na bancada de trabalho.

Aos técnicos e colaboradores do departamento de Biotecnologia, em especial à Sandra e ao Nelson, que são pessoas sempre prontas para ajudar.

À Universidade Estadual de Londrina (UEL) porto da minha formação acadêmica, desde a graduação até o doutorado, e muito mais que isso, um lugar que proporcionou uma trajetória e momentos maravilhosos.

A todos os professores do Departamento de Bioquímica e Biotecnologia da UEL, pelo suporte e pela dedicação ao ensino e à pesquisa.

Á CAPES por ceder o apoio financeiro que permitiu aminha formação.

Mantovan, Janaina. **Aproveitamento do bagaço de laranja para extração de materiais rico sem celulose empregando-se diferentes métodos físicos e químicos.** 2021. 108 f. Tese (Doutorado em Biotecnologia) –Universidade Estadual de Londrina, Londrina, 2021.

RESUMO

O bagaço de laranja é um resíduo lignocelulósico abundante no Brasil e pouco explorado como matéria-prima para a obtenção de celulose e nanocelulose. O presente estudo teve como objetivos empregar diferentes combinações de tratamentos químicos (hidróxido de sódio, ácido peracético, ácido sulfúrico e peróxido alcalino) e físicos (explosão a vapor em autoclave, ultrassonicação e extrusão), realizados preferencialmente em processos de etapa única, para a extração de celulose do bagaço de laranja e, ainda, caracterizar os materiais obtidos de acordo com sua composição, morfologia, cristalinidade, estabilidade térmica, capacidade de absorção de água e óleo, solubilidade, capacidade de intumescimento e isotermas de sorção. Nanocelulose também foi produzida e caracterizada quanto à sua composição, morfologia e cristalinidade. O teor de celulose dos materiais obtidos pelas diferentes combinações de processos variou de 12,1 a 85,4%, com rendimentos de 60,5 a 100% em relação ao conteúdo inicial de celulose no bagaço de laranja. Os tratamentos em etapa única resultaram em materiais com maiores teores e rendimentos de celulose, reduzindo os tempos de reação e a quantidade de reagentes químicos empregados quando comparados aos processos convencionais, geralmente realizados em várias etapas. O tratamento em etapa única combinando tratamento alcalino e explosão a vapor em autoclave foi o mais eficiente na obtenção de um material com maior teor de celulose, maior cristalinidade e estabilidade térmica. Os tratamentos químicos combinados à ultrassonicação foram os menos eficazes na obtenção de materiais ricos em celulose. O processo de extrusão foi considerado efetivo para a obtenção de matérias ricos em celulose. A nanocelulose obtida apresentou 60% de cristalinidade e 100% de celulose, com rendimento de 4,4% (g/100g de bagaço). As propriedades funcionais dos materiais foram afetadas principalmente por sua cristalinidade e morfologia da superfície. Os materiais à base de celulose obtidos neste estudo podem ser usados como fonte de fibras em produtos alimentícios, como substratos em processos de fermentação, e também podem ser usados para obter nanocelulose conforme realizado neste estudo.

Palavras-chave: tratamentos de etapa única; tratamento alcalino; autoclavagem; ultrassonicação; extrusão; nanocelulose.

Mantovan, Janaina. **Utilization of Orange bagasse to extract cellulose-rich materials employing diferente physical and chemical methods.** 2021. 108 f. Thesis (PhD in Biotechnology) - State University of Londrina, Londrina, 2021.

ABSTRACT

Orange bagasse is na abundant lignocellulosic residue in Brazil, and few studies have explored this raw material for celulose and nanocellulose obtainment. The present study aimed to employ different combinations of chemical (sodium hydroxide, peracetic acid, sulfuric acid and alcaline peroxide) and physical (autoclaving, extrusion, and ultrasonication) treatments, performed preferably in one-step processes, for celulose extraction from Orange bagasse and also to characterize the obtained materials according to their composition, morphology, crystallinity, thermal stability, water and oil absorption capacity, solubility, and swelling capacity. Nanocellulose was also produced and characterized according to its composition, morphology, and crystallinity. The celulose content of materials obtained from Orange bagasse by the different combinations of processes ranged from12.1 to 85.4%, with a celulose yield of 60.5 to 100% in relation to the initial celulose content in the Orange bagasse. The one-step treatments resultedin materials with higher celulose contents and yields, reducing reaction times and the number of chemical reagents used When compared to conventional processes, usually carried out in several stages. The one-step treatment combining alcaline treatment and steam explosionin na autoclave was the most efficient in obtaining a material with a higher celulose content, greater crystallinity and termal stability. Chemical treatments combined with ultrasound were the less effective in obtaining materials with higher celulose contents. The extrusion process was considered effective in obtaining materials rich in cellulose. The nanocellulose obtained had 60% crystallinity with ayield of 4.4%(g/100g of bagasse). Crystallinity and surface morphology of samples affected their functional properties. Cellulose-based materials obtained in this study can be used as fibers source in food products, as substrates in fermentation processes, and it can also be used to obtain nanocellulose as performed in this study.

Keywords: one-step treatments; alcaline treatments; autoclaving; ultrasonication; extrusion; nanocellulose.

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SUMÁRIO

1. INTRODUÇÃO

Em um cenário global de maior preocupação com impactos ambientais, cresce a busca por materiais biodegradáveis e provenientes de fontes renováveis. A celulose é o biopolímero mais abundante do planeta, principal componente da parede celular vegetal, que se alinha à demanda por produtos sustentáveis, podendo ser empregada em diversos setores da indústria, como papel, alimentos, construção, saúde, entre outros (GANGULY *et al*., 2020; LAVOINE *et al*., 2012).

A proporção entre celulose, hemicelulose e lignina na parede celular vegetal pode variar de acordo com as fontes botânicas, dentre as quais estão diversos resíduos (subprodutos) agroindustriais lignocelulósicos, como palha de arroz e trigo, sabugo de milho, bagaço de cana-de-açúcar, que podem então ser utilizados para extração da celulose, o que garante agregação de valor aos subprodutos e diminuição dos impactos ambientais do seu descarte inadequado (CYPRIANO *et al*., 2018; GARCÍA *et al*., 2016).

A extração do suco de laranja é um exemplo de processamento industrial que resulta em resíduo lignocelulósico. A polpa cítrica ou o bagaço de laranja é o resíduo sólido que resta depois que as frutas frescas são espremidas para obtenção do suco e remoção posterior do óleo essencial. As laranjas são as frutas mais cultivadas em todo o mundo (ZEMA *et al*., 2018), e o Brasil é o maior produtor mundial de suco de laranja (*Citrus* × *sinensis L*.), responsável por mais de três quartos das exportações mundiais. Na safra 2018/2019, a produção brasileira de suco de laranja foi de 1,23 milhão de toneladas resultando em grandes quantidades de subprodutos (USDA, 2019).

O bagaço fresco é composto pelas cascas, sementes e polpas, equivalentes a cerca de 40-50% do peso de cada fruto, com aproximadamente 82% de umidade. Após secagem, apresenta cerca de 7 a 9% de umidade, 2 a 3% de cinzas, 4 a 8% de proteínas, 0,6 a 4% de lipídios, 80 a 87 de carboidratos, 42 a 64% de fibras totais, das quais 22 a 46% de fibras insolúveis e 15 a 25% de fibras solúveis (CRIZEL *et al*., 2013; MACAGNAN *et al*., 2015). De acordo com de la Torre *et al*. (2017), o bagaço de laranja apresenta cerca de 18,6% de celulose, 14,3% de hemicelulose e 6,5% de lignina.

O bagaço de laranja é um resíduo lignocelulósico que apresenta alto valor agregado, além do limoneno, outros compostos que podem ser extraídos desse resíduo incluem hesperidina, pectina e açúcares solúveis (CYPRIANO *et al*., 2018). Pode ainda gerar biocombustíveis, como biogás e bioetanol (BOUKROUFA *et al*., 2015; LOHRASBI *et al*., 2010) e biomateriais adsorventes para poluentes (TOVAR *et al*., 2019). No entanto, existem poucos relatos na literatura que descrevem a extração de celulose e nanocelulose do bagaço de laranja (BICU; MUSTATA, 2011; 2013; CYPRIANO *et al*., 2018; HIDENO *et al*., 2014; MARIÑO *et al*., 2015; 2018; TSUKAMOTO *et al*., 2013).

Considerando a heterogeneidade dos materiais lignocelulósicos, é um desafio generalizar tratamentos para a extração de celulose a partir desses materiais e na última década houve um interesse crescente no uso de métodos menos poluentes, empregando-se processos totalmente livres de cloro (*totally chlorine free* -TCF). A combinação de processos físicos e químicos também é uma importante estratégia para reduzir o consumo de energia e os custos gerais para produção de celulose comercialmente competitiva (AHMED-HARAS *et al*., 2020; MIRI *et al*., 2015).

Os materiais ricos em celulose são a base para a obtenção de vários produtos de interesse industrial, como hidrogéis (XIE *et al*., 2020), oleogéis (JIANG *et al*. 2018), membranas (WENG *et al*., 2020), celulose regenerada (PHINICHKA *et al*., 2018) e nanocelulose (MARIÑO *et al*. 2018). Também podem ser aplicados como fonte de fibras insolúveis na dieta e como substrato nos processos fermentativos, uma vez que os materiais obtidos são mais acessíveis à hidrólise enzimática (ZHANG *et al*., 2020).

Assim, a proposta deste estudo inclui a obtenção de diferentes materiais à base de celulose a partir do bagaço de laranja empregando-se tratamentos menos poluentes e mais rápidos por meio da combinação de métodos químicos e físicos e avaliar como os diferentes tratamentos afetam as características e propriedades funcionais desses materiais, a fim de disponibilizá-los para diferentes potenciais aplicações.

2. OBJETIVOS

2.1 Geral

Empregar diferentes combinações de tratamentos químicos (hidróxido de sódio, ácido peracético, ácido sulfúrico e peróxido alcalino) e físicos (explosão a vapor em autoclave, extrusão e ultrassonicação), realizados preferencialmente em processos de etapa única, para a obtenção de materiais ricos em celulose a partir do bagaço de laranja.

2.2 Específicos

Determinar a composição química dos materiais obtidos (celulose, hemicelulose e lignina).

Caracterizar os materiais obtidos por meio das análises de: microscopia eletrônica de varredura (MEV); calorimetria diferencial de varredura (DSC); análise termogravimétrica (TGA); difração de raios-X (DRX), espectroscopia no infravermelho com transformada de Fourier (FT-IR).

Avaliar as propriedades funcionais dos materiais obtidos por meio dos testes de isotermas de sorção, capacidade de absorção de água (CAA), capacidade de absorção de óleo (CAO), solubilidade em água e capacidade de intumescimento.

Produzir nanocelulose a partir do bagaço da laranja tratado e caracterizá-la por meio de microscopia de força atômica (MFA) e difração de raios-X (DRX).

3. CAPÍTULO I - REVISÃO DA LITERATURA

3.1 Resíduos agroindustriais

O processamento de produtos agrícolas gera grande quantidade de resíduos lignocelulósicos, o tipo de biomassa mais abundante, como cascas, bagaços, palhas, espigas, entre outros (Tabela 1), que muitas vezes são comercializados com baixo valor econômico. Os materiais lignocelulósicos envolvem uma mistura de três componentes principais: celulose, hemiceluloses e lignina, que constituem a parede celular vegetal, sendo a celulose o biopolímero mais abundante do planeta (GANGULY *et al*., 2020; LAVOINE *et al*., 2012).

Tabela 1 - Teor de celulose, hemiceluloses e lignina em % (base seca) de matérias-primas lignocelulósicas.

Categoria	Biomassa celulósica	Celulose	Hemicelulose	Lignina
Matéria-prima (fonte primária)	Algodão	87,5	17,1	0,0
	Fibras de linho	75,9	20,7	3,4
	Eucalipto	52,7	15,4	31,9
	Grama	48,7	38,4	12,9
	Pinho	48,1	23,5	28,4
	Capim-elefante	31,5	34,3	34,2
Resíduos não processados (fonte secundária)	Haste de algodão	66,2	18,4	15,4
	Cascas de girassol	56,5	28,0	15,5
	Palha de arroz	52,3	32,8	14,9
	Palha de cevada	48,6	29,7	21,7
	Cobre de coco	52,2	28,4	19,4
	Palha de milho	47,4	30,3	22,3
	Caules de tabaco	44.6	30.2	25.2
	Espiga de milho	48.1	37.2	14.7
	Palha de trigo	44.5	33.2	22.3
	Formas de linho	39,9	26,8	24,2
	Palha de legume	29,2	35,5	35,3
	Casca de madeira	25,2	30,3	44,5
	Cascas de azeitona	25,0	24,6	50,4
Resíduos processados (fonte terciária)	Jornal	45,6	31,3	23,1
	Bagaço da cana-de-açúcar	47,4	29,1	23,5
	Resíduo de chá	33,3	23,2	43,5

Fonte: adaptado de García *et al*. (2016).

Os resíduos agroindustriais têm ganhado destaque como importantes matérias-primas renováveis para obtenção de produtos industriais a custos mais baixos (BATISTA-MENESES *et al*., 2020). A extração de celulose representa uma das principais atividades de interesse, além da extração de hemiceluloses e lignina. Os resíduos podem ser empregados em processos fermentativos para produção de enzimas, polissacarídeos e biocombustíveis, e ainda como componentes de alimentos, fonte de moléculas de interesse econômico, como pectina e hesperidina, entre outros (CRIZEL *et al*., 2013; CYPRIANO *et al*., 2018; GONÇALVES *et al*., 2015; ZHANG *et al*., 2020).

A utilização da biomassa lignocelulósica proveniente de resíduos pode aumentar seu valor econômico e solucionar problemas ambientais, correspondendo ao conceito de biorrefinaria (GANGULY *et al*., 2020), que para Cherubini (2010), "engloba uma ampla gama de tecnologias capazes de separar recursos de biomassa (como madeira, gramíneas e milho) em seus blocos de construção (como carboidratos, proteínas e triglicerídeos) que podem ser convertidos em produtos de valor agregado, biocombustíveis e produtos químicos. Uma biorrefinaria é uma instalação (ou rede de instalações) que integra processos e equipamentos de conversão de biomassa para produzir biocombustíveis para transporte, energia e produtos químicos".

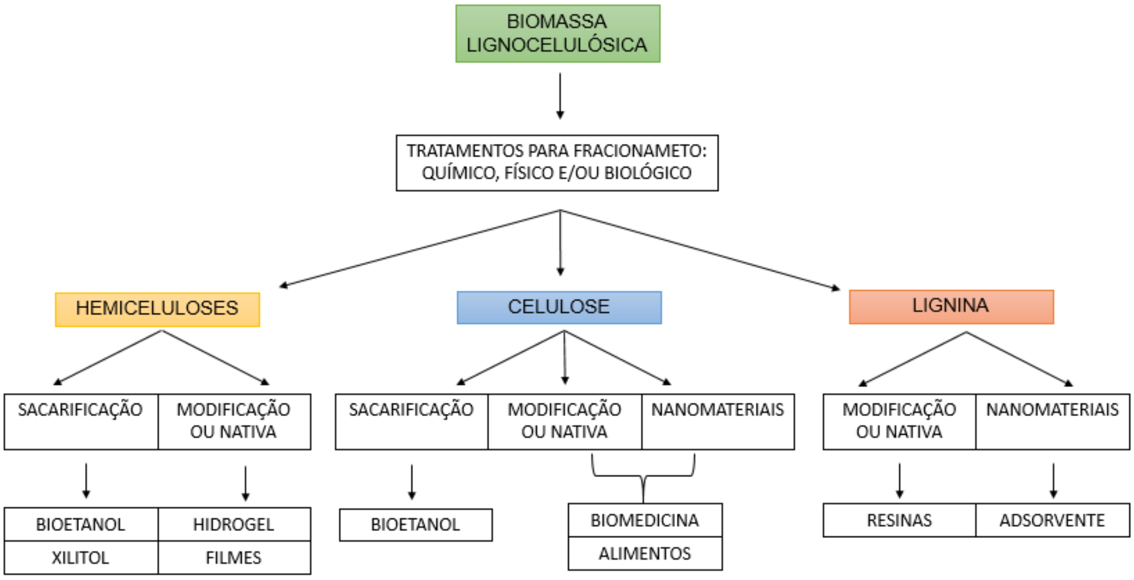
Segundo a Agência Internacional de Energia (AIE) "os conceitos integrados de biorrefinaria convertem uma variedade de matérias-primas, incluindo resíduos, em um portfólio de produtos com maior eficiência na cadeia energética, economia e efeitos ambientais, em comparação com processos autônomos que geralmente produzem apenas um ou dois produtos (IAE, 2017).

De acordo com a matéria-prima a ser processada, as biorrefinarias podem ser classificadas em: 1ª geração (1G), que usam alimentos diretos, como açúcar e óleo, 2ª geração (2G), usam fontes não alimentares, como resíduos agroindustriais e 3ª geração (3G), utilizam biomassa de algas. As biorrefinarias 2G e 3G ainda não estão tão estabelecidas quanto 1G (PARADA; OSSEWEIJER; DUQUE, 2017).

As biorrefinarias lignocelulósicas exigem maior número de tecnologias de processamento, que podem envolver mais de uma etapa de tratamento da biomassa (físico, químico ou biológico), para separar as frações dos componentes (celulose, hemicelulose e lignina) (BILAL *et al*., 2017). Em compensação, podem gerar maior variedade de produtos em comparação com as refinarias de petróleo, já que os materiais lignocelulósicos possuem uma composição química diversa (BILAL *et al*., 2017; NASCIMENTO; REZENDE, 2018; SHARMA; SAINI, 2020).

Além da utilização bem estabelecida da celulose na indústria de papel, os três componentes dos materiais lignocelulósicos podem gerar outros importantes bioprodutos, a Figura 1 mostra alguns exemplos de produtos ou áreas de aplicação. Para isso, celulose, hemiceluloses e lignina podem ser usadas diretamente em sua forma nativa ou podem ser submetidas a processos de transformação, como sacarificação, derivatização e produção de nanomateriais, entre outros (BATISTA-MENESES *et al*., 2020; DU *et al*., 2017; GRISHKEWICH *et al*., 2017; NAIDU; HLANGOTHI; JOHN, 2018; NISHIGUCHI AND TAGUCHI, 2020; PANG *et al*., 2017a; PENG; SHE, 2014; PUTRO *et al*., 2017).

Figura 1 - Biomassa lignocelulósica e exemplos de suas aplicações.



Fonte: Próprio autor.

A celulose é um componente com grande destaque, portanto, é importante explorar diversas fontes, que podem ser classificadas em (Tabela 1): (I) primárias, onde a fibra é produzida como produto principal para as indústrias de têxteis, papel, madeira e bioetanol, (II) secundárias, correspondem aos subprodutos não processados provenientes da indústria alimentar ou resíduos de atividades agrícolas/florestais (cascas, palhas e folhas) e (III) terciária, resíduos do uso, transformação e conversão de biomassa celulósica (polpa, bagaço e resíduos alimentares). Como mostra a Tabela 1, diferentes materiais lignocelulósicos de fontes secundárias e terciárias estão sendo empregados para obtenção de celulose. A proporção entre celulose, hemicelulose e lignina varia de acordo com a fonte (BATISTA-MENESES *et al*., 2020; GARCÍA *et al*., 2016).

A biomassa lignocelulósica pode ser dividida ainda em biomassa lignocelulósica de madeira (dura e macia) e não-madeireira (resíduos agrícolas, plantas nativas e fibras vegetais não-madeireiras, como bagaço de cana-de-açúcar, mudas e fibra de algodão). Essas podem diferir em suas composições químicas e propriedades físicas, e, portanto, cada uma apresenta vantagens e desvantagens específicas. No quesito ambiental, a biomassa não-madeireira é mais vantajosa (TYE *et al*., 2016). A biomassa de madeira apresenta maiores teores de lignina, que podem dificultar o acesso à celulose, tornado o processo de extração mais prejudicial ao ambiente (GARCÍA *et al*., 2016).

O Brasil apresenta uma ampla variedade e capacidade de produção agrícola (CONAB, 2017), evidenciando um grande potencial econômico aos resíduos lignocelulósicos por meio da extração de celulose.

3.2 Bagaço da laranja

As culturas de citrinos, que incluem laranjas, toranjas, limões, limas, tangerinas e mandarim, são muito populares e cultivadas em diversas regiões. Entre esses, a laranja é o fruto mais popular, correspondendo a aproximadamente 70% da produção e consumo total de citros. O consumo do fruto é de até 50 milhões de toneladas por ano, o que resulta na geração de grande quantidade de resíduos, cerca de 45 a 60% do peso total do fruto, como cascas, polpas e sementes (de la TORRE *et al*., 2017; SATARI *et al*., 2018).

O Brasil é responsável por 60% da produção mundial de suco de laranja. São produzidas mais de 18 milhões de toneladas de laranja anualmente, ou cerca de 30% da safra mundial, indicando assim a ampla disponibilidade deste produto (USDA 2019; MINISTÉRIO DA AGRICULTURA, 2019). Estima-se que, da produção comercial, 80% seja destinada às indústrias processadoras de suco e 20% destinada ao mercado *in natura* (CONAB, 2014).

A extração do suco de laranja é um exemplo de processamento industrial que resulta em resíduo lignocelulósico ou bagaço de laranja. O bagaço é composto de 60-65% (m/m) de cascas, 30-35% (m/m) de tecido interno e o restante de sementes. Apresenta na sua composição cerca de 7 a 9% de umidade, 2 a 3% de cinzas, 4 a 8% de proteínas, 0,6 a 4% de lipídios, 80 a 87% de carboidratos, 42 a 64% de fibras totais, das quais 22 a 46% são fibras insolúveis e 15 a 25% são fibras solúveis (MACAGNAN *et al*., 2015; CRIZEL *et al*., 2013). Na Tabela 2 pode-se observar a composição em base seca do bagaço de laranja.

Se descartado inadequadamente em aterros, em condição anaeróbica, ocorre uma decomposição descontrolada ocasionando diversos problemas, isso porque as cascas da laranja apresentam alta demanda química de oxigênio (1085 mg de O_2.g^{-1}), pH baixo (3-4) e alto conteúdo orgânico (cerca de 95% m/m dos sólidos totais). Esse processo gera metano, gás potencialmente explosivo em contato com o oxigênio atmosférico e também um poderoso gás causador do feito estufa (NEGRO *et al*., 2016).

Tabela 2 - Análise de composição sólida dos resíduos de laranja.

Componentes	***Base seca (% m/m)***
Extratos aquosos	38,0
Cinzas	3,7
Pectina	18,6
Lignina	6,5
Hemiceluloses	14,3
Celulose	18,6
Sólidos totais	19,5

Fonte: adaptado de de la Torre *et al*. (2017).

Assim, é necessário explorar os bioprodutos que podem ser extraídos ou gerados a partir do bagaço da laranja. Os resíduos de citros podem ser fonte de óleos, açúcares, ácidos, carboidratos insolúveis, enzimas, flavonóides, óleos essenciais, pigmentos e vitamina C (BOUKROUFA *et al*., 2015; SATARI *et al*., 2018; ZHANG *et al*., 2020).

Os óleos essenciais podem ser considerados como um dos subprodutos mais importantes da cadeia produtiva do suco de laranja. São produtos voláteis que se originam do metabolismo secundário de plantas aromáticas. A obtenção do óleo essencial ocorre simultaneamente ao processamento de suco. O D-limoneno, um monoterpeno monocíclico, é o componente mais abundante entre os óleos essenciais e apresenta muitas aplicações em diversos setores (saúde, cosméticos e produtos químicos em geral) (NEGRO *et al*., 2016).

As fibras presentes no bagaço podem ser incorporadas diretamente na formulação de alimentos, contribuindo com sua qualidade nutricional (CRIZEL *et al*., 2013). Podem também ser fonte de polifenóis, antioxidantes naturais, que podem ser usados em alimentos para evitar a rancidez e oxidação, substituindo o uso de antioxidantes químicos. Além de apresentar outras aplicações na área da saúde (BOUKROUFA *et al*., 2015; ZHANG *et al*., 2020).

As pectinas são constituintes da parede vegetal, tratam-se de heteropolissacarídeos formados por unidades de ácido D-galacturônico unidas por ligações α-1,4 com cadeias laterais de açúcares neutros tais como ramnose, arabinose, xilose e galactose. Podem ser usadas nas indústrias de alimentos como agente gelificante, espessante, texturizante, emulsionante e estabilizador para modificar propriedades de produtos alimentares. De acordo com o método empregado, pode-se obter altos rendimentos de pectina do bagaço da laranja (WUSIGALE *et al*., 2020; YANG *et al*., 2016).

Os resíduos da laranja podem atuar como adsorventes de baixo custo, uma aplicação muito explorada, principalmente, para remoção de metais pesados de soluções aquosas. Um adsorvente feito com microfibras de pectina e celulose também foi capaz de remover azul de metileno, um corante orgânico proveniente das indústrias de têxteis e papel, que é liberado para o meio ambiente (LESSA *et al*., 2017).

O bagaço ainda pode ser usado para obter açúcares fermentescíveis, principalmente glicose, frutose e sacarose. Para isso, é necessário estabelecer cuidadosamente as variáveis no processo de sacarificação, como pH, temperatura e agitação (de la TORRE *et al*., 2017). Os açúcares podem ser usados para produção de bioetanol (CHOI *et al*., 2015).

Conforme a composição mostrada na Tabela 2, o bagaço da laranja apresenta cerca de 18,6% de celulose, portanto, é possível utilizar esse resíduo como fonte do polímero. Em comparação com outras fontes (Tabela 1), o teor de celulose no bagaço da laranja não é tão alto, no entanto, se torna vantajoso sua utilização devido à alta disponibilidade no Brasil. Outra vantagem é o baixo teor de lignina (6,5%) (Tabela 2) em comparação a outros resíduos (Tabela 1).

Trata-se de uma fonte de celulose classificada como terciária (GARCÍA *et al.*, 2016), não-madeireira (TYE *et al.*, 2016), que, portanto, se encaixa no conceito de biorrefinarias de 2ª geração. Existem poucos trabalhos na literatura objetivando a extração de celulose deste resíduo (BICU; MUSTATA, 2011; 2013; CYPRIANO *et al.*, 2018; HIDENO *et al.*, 2014; MARIÑO *et al.*, 2015; 2018; TSUKAMOTO *et al.*, 2013).

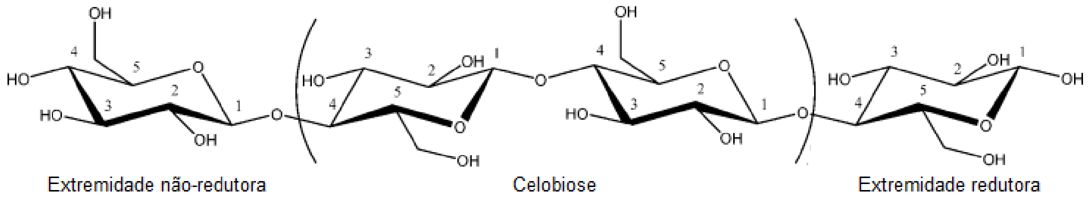
3.3 Celulose

3.3.1 Estrutura e organização da celulose de origem vegetal

A celulose é um homopolímero linear formado por unidades de D-glicopiranose unidas por ligações glicosídicas β-1,4 (Figura 2), configuração que indica que o grupo OH encontra-se do lado oposto da cadeia do anel hemiacetal (C_1 – O – C_4). Essa ligação implica em uma rotação de 180° entre duas moléculas de glicose, resultando assim em unidades que se repetem ao longo da cadeia, denominadas celobiose. Trata-se de uma substância fibrosa, resistente e insolúvel em água.

Cada cadeia de celulose apresenta uma assimetria química direcional em relação ao terminal do seu eixo molecular, ou seja, uma extremidade é redutora e a outra é a extremidade não-redutora nominal, que possui um grupo hidroxila pendente (HABIBI; LUCIA; HOJAS, 2010).

Figura 2 - Estrutura da celulose formada por unidades de D-glicopiranose a partir de ligações glicosídicas β-1,4, destacando a unidade básica, celobiose.



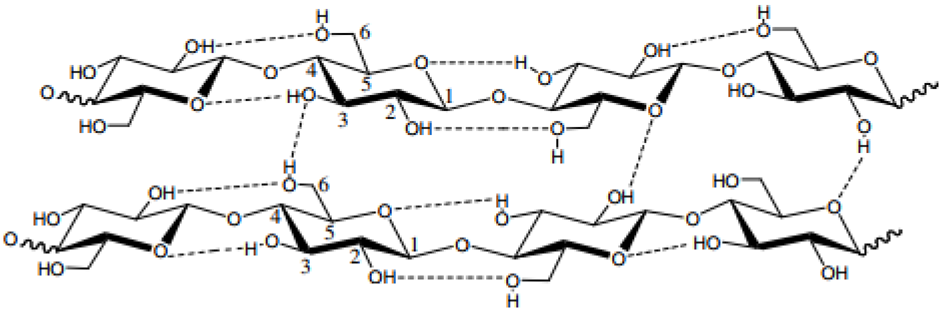
Fonte: adaptado de HABIBI *et al.*, 2010.

O grau de polimerização (DP) da celulose pode variar entre 1000 e 30.000, de acordo com a fonte, gerando cadeias com comprimentos de 500 a 15.000

nm (IOELOVICH, 2008). A massa molar é calculada multiplicando-se DP por 162, valor que corresponde à massa molar da glicose (162 g.mol^{-1}), porém, obtém-se um valor aproximado, pois a fibra de celulose consiste em uma mistura de macromoléculas de vários tamanhos.

Ocorrem ligações de hidrogênio intramoleculares, responsáveis pela rigidez das cadeias, e ligações intermoleculares, responsáveis pela formação da estrutura supramolecular fibrilada (Figura 3) (FENGEL; WEGENER, 1989). As ligações intramoleculares se formam entre a hidroxila O(3′)-H e o oxigênio do anel O(5) da próxima unidade e entre a hidroxila do O(2)-H e hidroxila O(6′) do próximo resíduo.

Figura 3 - Ligações de hidrogênio intra e intermolecular da celulose.



Fonte: EL SEOUD *et al*., 2013.

Como consequência dessas ligações, são formadas regiões cristalinas, ou seja, ordenadas, e regiões não ordenadas, denominadas amorfas, que são mais susceptíveis a ataques químicos. Para compostos cristalinos, ao longo dos eixos cristalográficos (eixos principais das estruturas cristalinas) existem unidades que se repetem, tratam-se de arranjos geométricos, denominados células unitárias, que possuem dimensões bem definidas. A proporção entre as regiões cristalinas e amorfas determina o grau de cristalinidade e as características dimensionais dos domínios cristalinos, e, consequentemente, determina as aplicações do polímero, sendo variável de acordo com a fonte da celulose (SILVA; D'ALMEIDA, 2009).

Estudos com ressonância magnética (RMN), infravermelho e difração mostraram a existência de polimorfos da celulose, denominados I, II, III_I, III_{II}, IV_I e IV_{II}, que podem ser interconvertidos de acordo com tratamentos específicos (O'SULLIVAN, 1996). A celulose II pode ser preparada por duas rotas distintas: mercerização (tratamento alcalino) e regeneração (solubilização e posterior recristalização). As celuloses $\mathrm{III_I}$ e $\mathrm{III_{II}}$ podem ser formadas a partir de celuloses I e II, respectivamente, por tratamento com amônia líquida e a reação é reversível. As celuloses $\mathrm{IV_I}$ e $\mathrm{IV_{II}}$ podem ser obtidas por aquecimento de celuloses $\mathrm{III_I}$ e $\mathrm{III_{II}}$, respectivamente (HAYASHI *et al*., 1975).

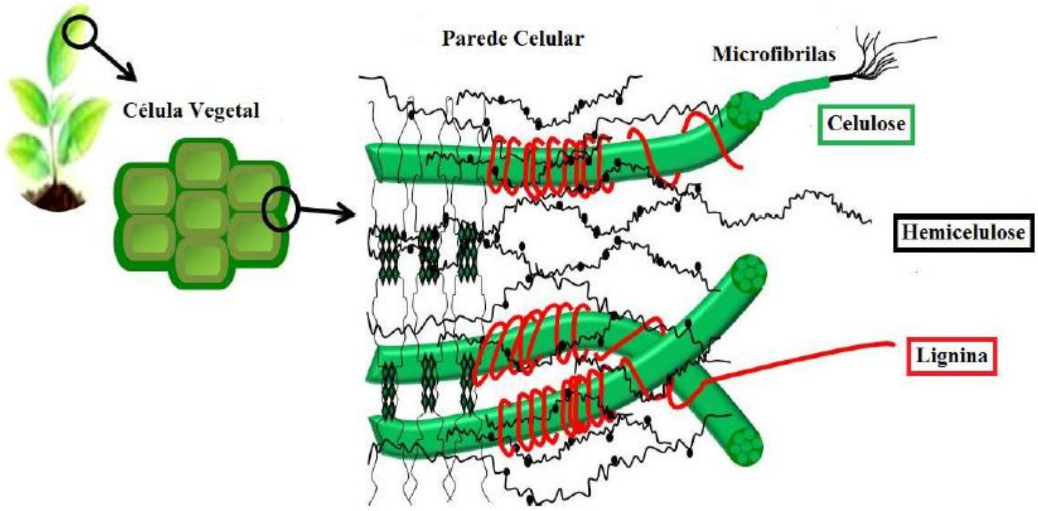
A celulose I é forma nativa, cuja célula unitária pode assumir duas formas, denominadas celulose Iα (triclínicas) e Iβ (monoclínicas), sendo que as quantidades relativas de celuloses Iα e Iβ variam de acordo com a origem da celulose, a forma Iβ é dominante em plantas superiores (ATALLA; VANDERHART, 1984).

Conforme exposto, a capacidade de formar fortes ligações de hidrogênio conferem à celulose importantes propriedades, em particular (I) a estrutura microfibrilada em escala múltipla, (II) organização hierárquica (regiões cristalinas e amorfas) e (III) natureza altamente coesiva (com uma temperatura de transição vítrea superior à sua temperatura de degradação) (LAVOINE *et al*., 2012).

Existem diversas classificações para celulose de acordo com a fonte de obtenção. A principal diz respeito à origem na natureza; a celulose pode ser vegetal (GARCÍA *et al*., 2016) ou bacteriana (KAWEE; LAM; SUKYAI, 2018), também é possível obter celulose a partir de algas (EL ACHABY *et al*., 2018).

Na parede vegetal, a celulose tem função de sustentação e encontra-se associada com hemicelulose e lignina. Esses três componentes estão ligados fisicamente e quimicamente (UMMARTYOTIN; MANUSPIYAB, 2015) (Figura 4). A hemicelulose está associada com a celulose através de ligações de hidrogênio e à lignina através de ligações covalentes conhecidas como complexos de lignina-carboidrato, dos quais compreendem ligações glicosídicas fenil, ésteres e éteres benzil (FARTHAT *et al*., 2017).

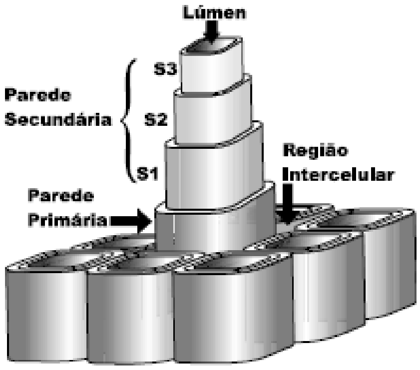
Figura 4 - Associação entre celulose, hemicelulose e lignina na parede celular vegetal.



Fonte: IOELOVICH, 2008.

Juntos formam diferentes camadas da parede celular (Figura 5), que são denominadas de acordo com a ordem de deposição durante o desenvolvimento vegetal: primária (P), secundária (S) e terciária (T). As camadas primária e terciária são mais finas. A parede secundária (S) apresenta maior espessura (3-5 μm) e é composta por três camadas (S1, S2 e S3), sendo que S2 é a mais espessa. Nas diversas camadas, as fibrilas encontram-se orientadas em diferentes posições em relação ao eixo longitudinal da célula (SERAD; SANDERS, 1979).

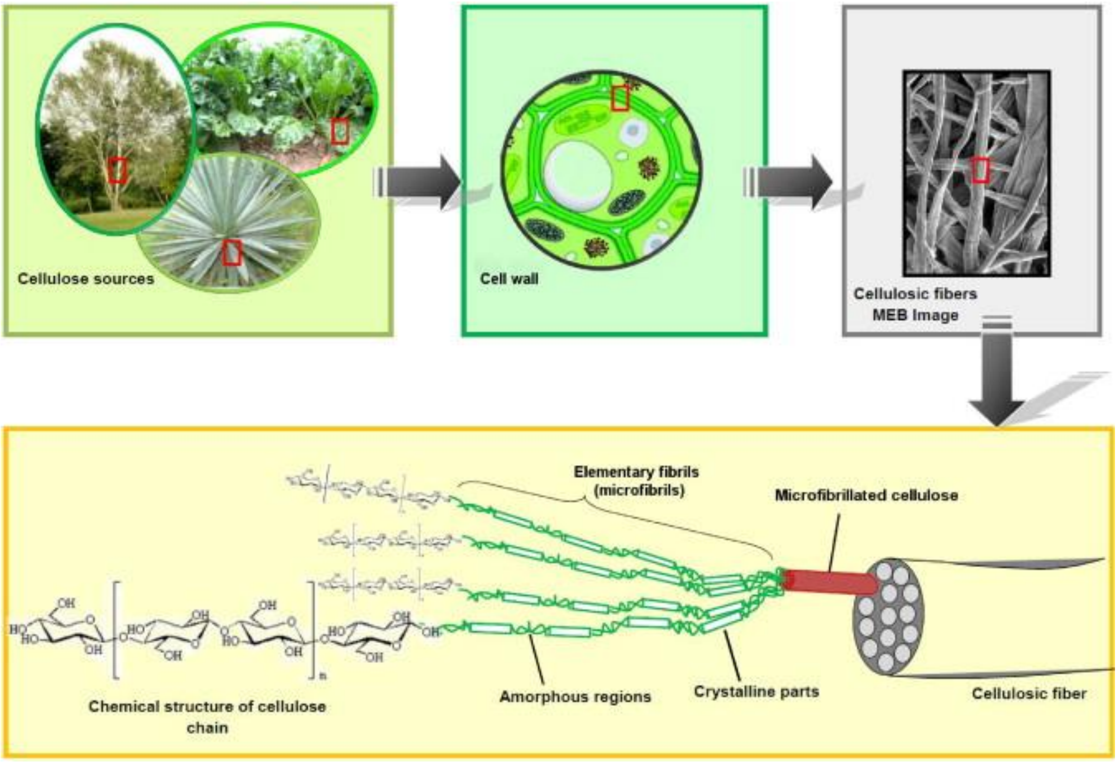
Figura 5 - Estrutura morfológica da célula vegetal.



Fonte: SERAD; SANDERS, 1979.

As moléculas de celulose não ocorrem isoladas, mas sim formam estruturas organizadas de forma hierárquica (Figura 6) no momento da biossíntese na parede vegetal. A maior estrutura formada, a fibra celulósica, é composta por unidades chamadas de microfibrilas (20-50 nm de diâmetro cada), que são formadas pela união de estruturas denominadas nanofibrilas ou fibrilas elementares (5 nm de diâmetro cada), que por sua vez são formadas por aproximadamente 36 moléculas de celulose individuais. As microfibrilas apresentam regiões cristalinas alternadas por amorfas (HABIBI; LUCIA; HOJAS, 2010).

Figura 6 - Organização hierárquica das fibrilas de celulose provenientes da parede celular vegetal.



Fonte: LAVOINE *et al*., 2012.

3.3.2 Aplicações da celulose

A celulose e seus derivados podem ser transformados em diversos produtos, como produtos de papel, têxteis, compósitos poliméricos, farmacêuticos, alimentos, bebidas, biocombustíveis, baterias, revestimentos, entre outros, devido às suas conhecidas vantagens, incluindo a fonte renovável (HOKKANEN *et al*., 2016), sustentabilidade (DAI *et al*., 2019), biodegradabilidade (WANG *et al*., 2016), disponibilidade generalizada (LIU *et al*., 2019), baixa toxicidade (XU *et al*., 2018) e alta flexibilidade (DUTTA *et al*., 2017).

A celulose pode ser usada para produzir diversos derivados, como acetato de celulose, metilcelulose, etilcelulose, hidroxietilcelulose, hidroxipropilcelulose, carboximetilcelulose de sódio, entre outros. Os derivados não demonstraram toxicidade em humanos ou animais, garantindo assim, diversas aplicações, inclusive para biomedicina (HABIBI *et al*., 2010). A celulose derivada também foi empregada com sucesso para remoção de metais pesados de soluções aquosas (JIAO *et al*., 2017; JILAL *et al*., 2018).

Em estudo recente, Oliveira *et al*. (2017) utilizaram fibras de celulose de arroz e casca de aveia para produzir hidrogéis. A maior capacidade de absorção de água foi apresentada pelo hidrogel da celulose de arroz a 25 °C, mostrando que a fonte da celulose pode interferir nas suas propriedades e aplicações. Os hidrogéis de celulose podem ser aplicados em diversos setores da indústria, como saúde, agricultura e alimentação.

Celulose e seus derivados têm sido empregados com sucesso para substituir os polímeros comumente usados em impressão 3D; destaca-se o uso dos derivados éteres por suas funções versáteis, incluindo boa solubilidade, alta estabilidade química, segurança fisiológica e boa biodegradabilidade. No entanto, matérias-primas celulósicas para impressão 3D geralmente precisam receber adição de outros materiais para melhorar a qualidade do produto (LI *et al*., 2018; YANG *et al*., 2020).

O setor de combustíveis celulósicos também apresenta grande potencial, várias instalações pioneiras estão produzindo etanol a partir de resíduos lignocelulósicos agrícolas com capacidade ≥ 10 milhões de galões por ano. No entanto, ainda existem desafios técnicos para tornar o preço mais competitivo, em especial quando a celulose é extraída de resíduos lignocelulósicos (LYND *et al*., 2017).

A celulose extraída de resíduos da agroindústria pode ser usada ainda para obtenção da nanocelulose. O termo nanocelulose refere-se à materiais celulósicos, oriundos de diferentes fontes, que apresentam dimensões na escala nanométrica. Estes materiais podem ser produzidos por diferentes métodos a partir de diversas fontes lignocelulósicas. São relatados dois tipos de nanoceluloses obtidos de fontes vegetais na literatura: nanocristais e nanofibrilas.

Para obtenção dos nanocristais de celulose, as regiões amorfas das nanofibrilas são removidas, resultando em um composto altamente cristalino, com um comprimento tipicamente inferior a 500 nm e diâmetros entre 5-100 nm. São constituídos por 100 % de celulose e possuem alta cristalinidade (54 a 88 %). Obtidos principalmente a partir da hidrólise ácida das fibras de celulose (CURVELLHO *et al*., 2019; DEBIAGI *et al*., 2020a; 2020b).

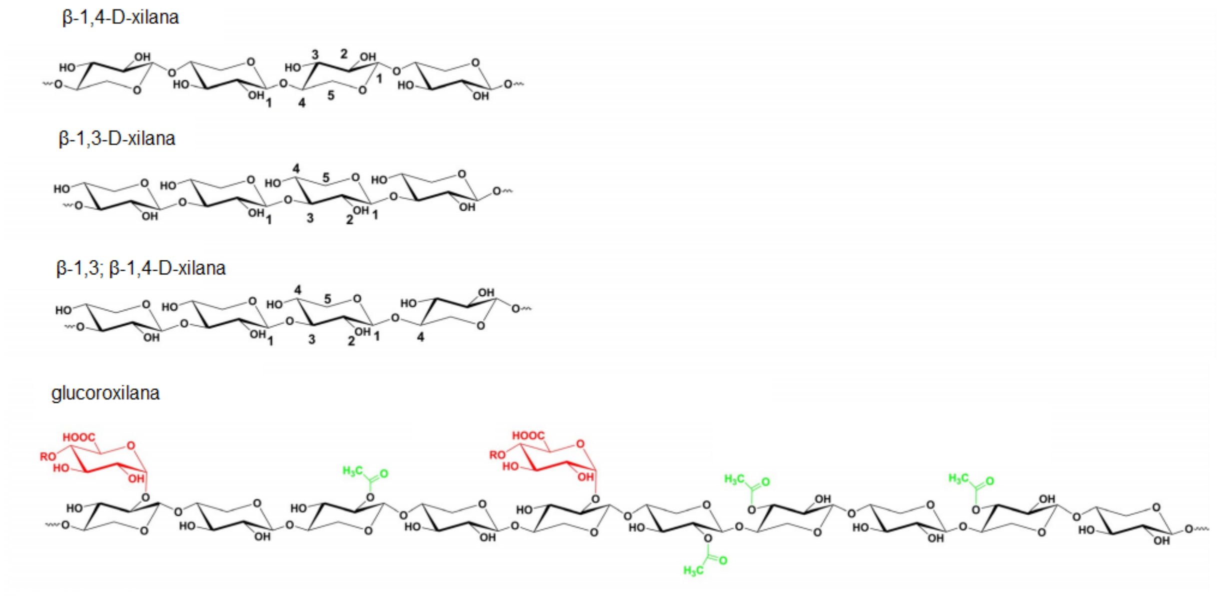
As nanofibrilas ou celulose nanofibrilada correspondem às cadeias de celulose contendo regiões cristalinas alternadas com regiões amorfas, com diâmetros na escala nanométrica (5 - 100 nm) e comprimentos de até vários micrômetros. Apresentam flexibilidade estrutural devido à sua elevada razão de aspecto (razão L/D), que é definida pela relação entre o comprimento (L) da fibra e o seu diâmetro (D). A razão de aspecto para a celulose nanofibrilada varia de 100 a 150, enquanto que os nanocristais apresentam razões de aspecto entre 10 e 100 (ABDUL-KHALIL; BHAT; YUSRA, 2012).

As nanoceluloses podem ser empregadas no setor biomédico, como agentes antimicrobianos, para encapsulamento e veículo para biomoléculas, biomarcadores, imobilização de proteínas e enzimas, adsorventes, entre outros (CURVELLHO, *et al*., 2019; NISHIGUCHI; TAGUCHI, 2020). Possuem grande potencial como supercapacitores devido à grande área de superfície que facilita o armazenamento de carga em baterias de íons de lítio e células solares. Existem pesquisas ampliando o uso para setores como petróleo e gás, cuidados pessoais, aditivos alimentares e embalagens, entre outras aplicações (DU *et al*., 2017; GRISHKEWICH *et al*., 2017; PUTRO *et al*., 2017).

3.4 Hemiceluloses

As hemiceluloses podem ser definidas como uma classe heterogênea de polímeros de estruturas lineares e altamente ramificados, como xilanos, mananos, xiloglucanos e galactanos. Os xilanos (Figura 7) são os mais abundantes, sendo os principais componentes de hemiceluloses de paredes celulares secundárias. São formados por diferentes grupos funcionais, que incluem pentoses (D-xilose e L-arabinose), hexoses (D-manose, D-galactose e D-glicose), ácidos hexurônicos (ácido 4-O-metil-D-glucurônico, ácido D-glucurônico e ácido D-galacturônico) e grupos acetila, bem como pequenas quantidades de ramnose e fucose (NAIDU; HLANGOTHI; JOHN, 2018).

Figura 7 - Alguns tipos de xilanos constituintes de hemiceluloses.



β-1,4-D-xilana

β-1,3-D-xilana

β-1,3; β-1,4-D-xilana

glucoroxilana

Fonte: modificado de ZHOU *et al*., 2016.

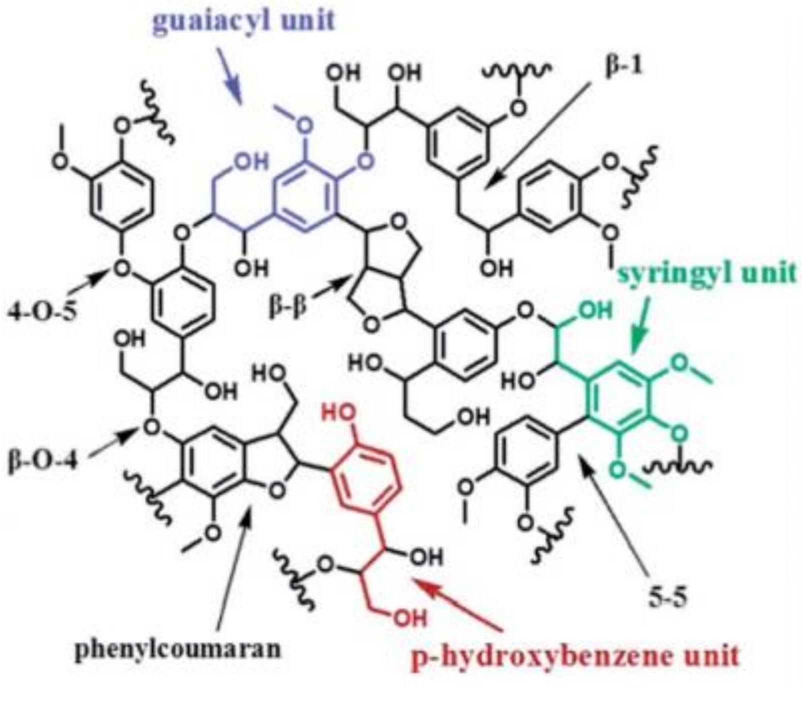
As hemiceluloses atuam como um revestimento entre as fibrilas de celulose, contribuindo com a rigidez do tecido vegetal. A abundância e as estruturas das hemiceluloses variam de acordo com as fontes de biomassa. O grau de polimerização (DP) médio das hemiceluloses é de 80 – 200 (PENG; SHE, 2014).

A fração de hemiceluloses pode ser usada para conversão em biocombustíveis, sendo indispensável para o sucesso econômico do etanol a partir dos materiais lignocelulósicos (GÍRIO *et al*., 2010). As hemiceluloses podem ser utilizadas também para obtenção de diversos produtos de interesse econômico, como xilitol (usado na indústria alimentícia, como adoçante e conservante), ácido lático, xilo-oligossacarídeos, furfural, filmes, hidrogel, veículo, biossurfactante, aditivo de papel, agente floculante, entre outros. Podem ser usadas na forma nativa ou modificada (NAIDU; HLANGOTHI; JOHN, 2018; PENG; SHE, 2014). No estudo de Farhat *et al* (2017), as hemiceluloses foram reticuladas com zircónio para desenvolver um gel resistente à água para revestimentos ou aplicações adesivas. Podem também ser empregadas como matéria-prima para impressão 3D (YANG *et al*., 2020).

3.5 Lignina

A lignina é o segundo biopolímero mais abundante na Terra, constitui um material heterogêneo de origem natural com uma estrutura muito complexa, altamente ramificado, de alta massa molar e amorfo. É formado por diferentes unidades denominadas p-hidroxifenila (H), guaiacila (G) e siringila (S). Cada unidade é sintetizada por precursores de álcoois aromáticos (monolignóis): p-coumarila, coniferila e sinapila, respectivamente (Figura 8). As propriedades físico-químicas da lignina variam de acordo com a matéria-prima utilizada e o método de extração. Encontra-se presente na parede celular secundária das plantas, atuando com um adesivo, que garante a integridade estrutural. Devido ao seu caráter hidrofóbico, desempenha também um papel importante no transporte de água da planta (PINKERT *et al*., 2011; ZHU *et al*., 2014).

Figura 8 - Representação esquemática da molécula da lignina.



Fonte: adaptado de ZHU *et al*., 2014**.**

A lignina pode ser convertida em combustíveis e produtos químicos valiosos (YUNPU *et al*., 2016). Para as aplicações, pode ser utilizada em sua forma nativa ou quimicamente modificada. Derivados de lignina como sulfonato, fenólico, organossolv, kraft e sulfonato de sódio apresentam boas propriedades mecânicas, químicas e físicas (NASEEM *et al*., 2016; YEO *et al*., 2017). A estrutura fenólica da lignina a torna aplicável à produção de produtos químicos e materiais de valor agregado, tais como resinas de lignina-fenol-formaldeído (PANG *et al*., 2017b).

Atua como aglutinante natural, inclusive em baterias de lítio recarregáveis (DOMÍNGUEZ-ROBLES *et al*., 2017). Apresenta, propriedades adesivas. Pode ser utilizada como agente dispersante, floculante, espessante, auxiliar para revestimentos, tintas, impressão 3D, entre outros (NASEEM *et al*., 2016; YANG *et al*., 2020). A lignina pode ainda ser usada para produção de nanopartículas, que possuem muitas aplicações potenciais, como adsorção de íons de metais pesados em águas residuais e estabilização de emulsões de Pickering (PANG *et al*., 2017b).

Dois tipos de lignina podem ser gerados, de acordo com o método de obtenção: (I) lignina contendo enxofre, proveniente de processos comerciais de polpação química e (II) lignina livre de enxofre, proveniente de processos de produção de etanol celulósico. A lignina natural é incolor ou amarelo pálido, mas no tratamento com ácido ou álcali, sua cor muda para marrom ou castanho escuro (NASEEM *et al*., 2016).

3.6 Processos de extração da celulose e nanocelulose de resíduos lignocelulósicos

A utilização dos materiais lignocelulósicos, geralmente, exige o fracionamento, total ou parcial, de seus componentes. Isso é feito por meio de tratamentos específicos, que devem estar ajustados ao produto de interesse a ser obtido e à fonte do material lignocelulósico (BATISTA-MENESES *et al*., 2020). A recalcitrância dos materiais lignocelulósicos indica o quão fácil é tratar, fracionar e modificar a biomassa, e está diretamente relacionada ao grau de cristalinidade, polimerização da celulose, área de superfície acessível, proteção da celulose pela lignina, revestimento por hemiceluloses, força da fibra e sua porosidade e à espessura da matéria-prima. A biomassa lenhosa, que contém maior teor de lignina, apresenta maior recalcitrância (GARCÍA *et al*., 2016).

O bagaço de laranja apresenta menor teor de lignina do que outros materiais lignocelulósicos, como bagaço de cana (20%) (KATAKOJWALA; MOHAN, 2020), casca de aveia (26%) (OLIVEIRA *et al*., 2019), palha de milho (20%) (XU *et al*., 2018) e casca de arroz (20%) (HAFEMANN *et al*., 2020), o que pode ser considerada uma vantagem. Outros componentes, além da estrutura lignocelulósica, como a pectina, podem interferir no processo de extração (RAJINIPRIYA *et al*., 2018).

Os processos tradicionais para deslignificação empregados na indústria são os processos Kraft, Soda e Sulfito. O processo Kraft envolve um tratamento com solução aquosa de hidróxido de sódio e sulfeto de sódio em altas temperaturas. No processo Sulfito o material é submetido a uma solução aquosa de sulfito ou bissulfito em altas temperaturas. O processo Soda utiliza solução aquosa de hidróxido de sódio em altas temperaturas. No entanto, muitos estudos têm investigado o emprego de matérias-primas e processos mais favoráveis ambientalmente, que envolvem quantidades reduzidas de reagentes e que sejam livres de cloro. Além disso, é importante buscar processos economicamente viáveis e em etapas reduzidas, capazes de gerar menos efluentes (HAFEMANN *et al*., 2020). Os tratamentos podem ser físicos (moagem, aquecimento, resfriamento, vapor, pressão, descompressão e micro-ondas), biológicos (microrganismos e enzimas), químicos (ácidos, álcalis, oxidantes e solventes orgânicos) ou combinação desses (BATISTA-MENESES *et al*., 2020; BILAL *et al*., 2018; CHENG *et al*., 2018; DAGNINO *et al*., 2017).

Os processos empregados devem atender alguns requisitos, tais como favorecer o acesso aos carboidratos ou habilitar estes carboidratos para a hidrólise enzimática, evitar a degradação ou perda de carboidrato, evitar a formação de subprodutos inibitórios para a subsequente hidrólise e fermentação, e, ainda, serem economicamente viáveis (SUN; CHENG, 2002).

Dentre os tratamentos químicos empregados, podemos ressaltar o uso de reagentes alcalinos. Os tratamentos alcalinos são amplamente empregados aos resíduos lignocelulósicos como parte de um processo sequencial que envolve outros agentes de branqueamento, são eficientes na remoção de lignina, hemicelulose e pectina, com a vantagem de baixo custo e por serem ecologicamente amigáveis (AHUJA *et al*., 2018; BICU; MUSTAFA 2013; MORIANA *et al*. 2016).

O tratamento alcalino pode romper as ligações de hidrogênio na estrutura das fibras, aumentando assim a rugosidade da superfície. Este tratamento remove hemicelulose e lignina, podendo também despolimerizar a celulose, expondo as suas regiões cristalinas. O tratamento reduz o diâmetro da fibra. A extração alcalina libera as hemiceluloses através da separação total ou parcial das ligações éster entre lignina e hemicelulose nas paredes celulares, resultando na dissolução das hemiceluloses. Os íons hidróxido causam o intumescimento da celulose e ruptura das ligações de hidrogênio intermoleculares entre celulose e hemiceluloses (AKIL *et al*., 2011; BICU; MUSTATA, 2013; FARHAT *et al*., 2017).

O hidróxido de sódio (NaOH) é o reagente alcalino mais empregado, e o seu uso está relacionado ao aumento significativo da porosidade da biomassa e do conteúdo de celulose, no entanto, elevadas concentrações de NaOH podem levar a produção de compostos alcalinos nos efluentes de lavagem que são difíceis de tratar, portanto, a combinação do tratamento alcalino com outros processos pode ser interessante, uma vez que pode se empregar menores concentrações do reagente sem perdas de rendimento (YAN *et al*., 2020).

O uso do peróxido de hidrogênio alcalino também tem sido relatado nos últimos anos por alguns autores (CARDOSO *et al*. 2016a; 2016 b; MALIK *et al*., 2020; YAN *et al*., 2020). No tratamento com peróxido de hidrogênio alcalino, forma-se o íon hidroperóxido (HOO-). Este ânion é um forte nucleófilo que, preferencialmente, ataca grupos etilênicos e carbonila presentes na lignina (SUN *et al*., 2000). O uso deste reagente apresenta baixo custo e provoca a oxidação da lignina e consequentemente a descoloração da mesma de forma eficiente. Além disso, outras reações acontecem nesse processo, como a ruptura dos anéis aromáticos e hidrólise da lignina, ou até mesmo a conversão dos grupos fenólicos da lignina em aldeídos, quinonas e carbonila (CARDOSO *et al*., 2016a).

Os perácidos têm sido considerados como potenciais substitutos para os reagentes clorados empregados para a remoção da lignina de resíduos agroindustriais. Os perácidos são geralmente preparados pela mistura de peróxido de hidrogênio (soluções de 50 a 70 %) e o ácido correspondente. Além disso, o branqueamento com este tipo de reagente têm apresentado bons resultados em termos de resistência, indicando baixos índices de degradação da celulose. Durante o branqueamento, os perácidos são consumidos em reações de oxidação da lignina e em decomposições espontâneas e catalisadas por metais de transição. A decomposição espontânea dos peracéticos envolve a formação de oxigênio (BRASILEIRO *et al*., 2001).

Um dos perácidos mais estudados é o ácido peracético (PA) que, desde o final da década de 40 vem sendo investigado como reagente de branqueamento e deslignificação. Pode ser preparado pela oxidação do ácido acético por peróxido de hidrogênio, esta reação é um equilíbrio, em que estão presentes ácido acético, peróxido de hidrogênio e água. O ácido peracético, em meio ácido, é considerado um agente deslignificante altamente seletivo, devido à sua capacidade de oxidar estruturas ricas em elétrons, como os anéis aromáticos da lignina, formando quinonas e intermediários hidroxilados ainda mais reativos do que as estruturas iniciais (ABDEL-HALIM; AL-DEYAB, 2011; LIANG; WANG, 2015).

O ácido peracético se mostrou eficiente na remoção da lignina em resíduos lignocelulósicos, tais como a casca de arroz (NASCIMENTO *et al*., 2016), ou a casca de aveia (PASCHOAL *et al*., 2015), sendo que a fonte e o tipo de tratamento influenciaram nas características da celulose obtida

Dentre os métodos físicos, os tratamentos hidrotérmicos mostram eficiência no rompimento do complexo celulose-hemicelulose-lignina, como é o caso da explosão a vapor realizada em autoclave, que é capaz de reforçar a abertura de fibras lignocelulósicas, promover solubilização dos açúcares hemicelulósicos e solubilização parcial e redistribuição de polímeros de lignina (OLIVA *et al*., 2017). Os tratamentos de explosão a vapor realizados em autoclave são capazes de gerar um material rico em celulose que pode ser usado após purificação adicional. O pré-tratamento a vapor modifica a estrutura da parede celular da planta, gerando um material marrom a partir do qual a hemicelulose parcialmente hidrolisada é facilmente recuperada por lavagem com água, deixando uma fração insolúvel em água composta de celulose, hemicelulose residual e uma lignina quimicamente modificada (DEBIAGI *et al*., 2020).

No estudo de Debiagi *et al*. (2020), o farelo de trigo e a cascas de aveia tratadas com explosão a vapor em autoclave em meio ácido (H_2SO_4) apresentaram maior liberação total de açúcares e menores teores de lignina e hemicelulose na fração sólida residual. No estudo de Feng *et al*. (2018), o tratamento contínuo de explosão a vapor eliminou a parede primária e os materiais de ligação relacionados à lamela intermediária, afrouxou a estrutura da parede celular da fibra e promoveu nanofibrilação sequencial das fibras de celulose.

A ultrassonicação é uma tecnologia emergente para o tratamento de resíduos lignocelulósicos que se baseia nos efeitos cavitacionais especiais causados por ondas acústicas de ultrassom na faixa de frequência de 10 kHz a 20 MHz, ocorrem assim um transporte de massa devido à mistura turbulenta e transmissão acústica, danos superficiais em interfaces líquido-sólido devido às ondas de choque e micro jatos, colisões interpartículas em alta velocidade e fragmentação mecânica de grandes partículas sólidas aumentando a área superficial (BHUTTO *et al*., 2017).

No estudo de Abdullah *et al*. (2016), foram utilizados métodos químicos e físicos para extração da celulose. O tratamento ultrassônico com peróxido de hidrogênio produziu 49% de celulose com 68,7% de cristalinidade, contra 64% de celulose obtida com tratamento em autoclave. O peróxido de hidrogênio se mostrou eficaz na separação entre celulose, hemicelulose e lignina, com a vantagem de baixo custo (SU *et al*., 2015). A combinação do tratamento ultrassônico com alcalino foi considerado eficaz para deslignificação. Junto ao tratamento alcalino, o ultrassom gera radicais livres que aumentam a transferência de massa dentro da solução (MOHAPATRA *et al*., 2017).

A extrusão é uma tecnologia que foi usada inicialmente para conformação de metais, mas logo se expandiu para muitas outras aplicações em diversos campos, como cerâmica, borracha, processamento de alimentos, química, polímeros, compósitos e indústrias de energia. A extrusão é um processo termo-mecânico que se baseia na ação de um ou dois parafusos que giram em um cilindro sob elevada pressão, equipado com controle de temperatura. Esta ação produz altas forças de cisalhamento entre a matéria-prima, a rosca e o cilindro que levam ao aumento local da temperatura e da pressão ao longo da extrusora. Essa tecnologia tem sido aplicada com sucesso para promover a modificação de biomassa lignocelulósica; diversos estudos com resíduos de cereais e grãos, como soja e aveia, foram desenvolvidos a fim de obter-se materiais ricos em celulose e fibras (DEBIAGI *et al*., 2018; DUQUE *et al*., 2017; GARCIA-AMEZQUITA *et al*., 2019). No entanto, o tratamento de resíduos de frutas ainda é pouco explorado, principalmente o bagaço da laranja (GARCIA-AMEZQUITA *et al*., 2019; HUANG *et al*., 2019).

Já para a obtenção de nanocelulose, a primeira etapa consiste da extração da celulose do complexo celulose-hemicelulose-lignina, isto é, o desmembramento deste complexo por meio de técnicas de deslignificação e pré-tratamentos, sem a destruição das fibras celulósicas. Desta forma é possvível separar seletivamente a lignina e as hemiceluloses, removendo-as da fibra por métodos químicos, térmicos, físicos, biológicos, ou por combinações destes. O emprego dos métodos depende da qualidade de separação que se deseja obter.

A obtenção de nanocelulose, geralmente, requer processos em múltiplas etapas que envolvem a hidrólise controlada de regiões amorfas; a transição do resíduo ao nanomaterial envolve mudanças referentes à morfologia e à cristalinidade (DEBIAGI *et al*., 2020; MELIKOĞLU *et al*. 2019). As etapas iniciais são importantes na redução da fração não celulósica, permitindo um ataque ácido eficaz para a obtenção de nanofibras. Hideno *et al*. (2014) relataram que a eliminação de compostos da camada em torno das microfibrilas de celulose é crucial para a preparação de nanofibras a partir de cascas de laranja. Cypriano *et al*. (2018) produziram nanocelulose com 60% de cristalinidade a partir do resíduo do suco de laranja empregando hidrólise enzimática. Mariño *et al*. (2018) empregaram várias etapas para obter nanocelulose a partir do bagaço de laranja industrial: extração ácida de pectina, extração alcalina de celulose, clorito de sódio seguido de branqueamento com peróxido alcalino e preparação de nanocristais de celulose por diferentes métodos alcalinos ou ácidos. O índice de cristalinidade variou de 55 a 77%, e as nanofibras de celulose apresentaram diâmetros médios de 20,5 ± 7,0 nm.

As características da celulose e nanocelulose obtidas podem variar de acordo com o tipo de método de extração e fonte vegetal. É importante compreender e caracterizar como o material é modificado após o tratamento, a fim de avaliar o custo-benefício dos métodos empregados e estabelecer as possíveis aplicações (MORIANA *et al.*, 2016; OLIVEIRA *et al*., 2019). Materiais celulósicos com altos valores de capacidade de absorção de água e de intumescimento podem ser incorporados em produtos alimentícios como fontes de fibra dietética, como produtos assados, geleias ou sucos de frutas, carnes, molhos e laticínios. Fibras com maior capacidade de adsorção de óleo podem auxiliar no processamento de alimentos gordurosos (LUNDBERG *et al*., 2014). A fibra dietética para rações de animais domésticos também é uma possibilidade (BRAMBILLASCA *et al*., 2013). Materiais amorfos podem ser usados em processos de fermentação, pois permitem melhor acesso enzimático. Materiais que apresentam maior grau de purificação são importantes fontes de celulose de baixo custo e podem ser utilizados em etapas posteriores para obtenção de nanocelulose (MARIÑO *et al*., 2018).

O bagaço de laranja, que possui grande potencial econômico no Brasil, ainda é uma matéria-prima pouco explorada para a extração de celulose e nanocelulose, por isso foi objeto de estudo deste trabalho, onde foram empregadas várias combinações de processos químicos e físicos, realizados preferencialmente em processos de etapa única, para a extração de celulose do bagaço de laranja.

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4. CAPÍTULO II – ARTIGO I: VALORIZATION OF ORANGE BAGASSE THROUGH ONE-STEP PHYSICAL AND CHEMICAL COMBINED PROCESSES TO OBTAIN A CELLULOSE-RICH MATERIAL

Artigo publicado na revista "Journal of the Science of Food and Agriculture".
Referências e citações conforme a revista.

Valorization of orange bagasse through one-step physical and chemical combined processes to obtain a cellulose-rich material

Abstract

Background

Orange bagasse (OB) is an agroindustrial residue of great economic importance that has been little explored for the extraction of cellulose. The present study aimed to investigate different combinations of chemical (sodium hydroxide, peracetic acid and alkaline peroxide) and physical (autoclaving and ultrasonication) treatments performed in one-step processes for cellulose extraction from OB and to characterize the obtained materials according to their composition, morphology, crystallinity and thermal stability.

Results

The processing yields ranged from 14 to 82 %, with a recovery of 72 to 100 % of the original cellulose. Treatments promoted morphological changes in the fiber structure, resulting in materials with higher porosity, indicating partial removal of the noncellulosic fractions. The use of combined chemical treatments with autoclaving was more efficient at obtaining high cellulose contents. Samples with higher cellulose content were processed by autoclaving with NaOH, resulting in 71.1 % cellulose, 0 % hemicellulose and 19.0 % lignin, with a crystallinity index of 42 %.

Conclusion

The one-step treatments were able to result in materials with higher cellulose contents and yields, reducing reaction times and quantity of chemical reagents employed in the overall processes when compared to multistep conventional processes.

Keywords: orange bagasse; ultrasound; autoclaving; lignocellulosic residues.

4.1 Introduction

Oranges are the most widely cultivated fruit worldwide,[1] and Brazil is the largest world producer of orange (*Citrus* × *sinensis* L.) juice, accounting for over three-quarters of global orange juice exports. In the 2018/2019 harvest, the Brazilian production of orange juice is forecast to be 1.23 million tons,[2] resulting in large quantities of byproducts. Citrus pulp or orange bagasse is the solid residue that remains after fresh fruits are squeezed for their juice, which suffers from posterior essential oil removal that is obtained through biomass pressing.[3]

Orange bagasse (OB) is composed of peel (60 to 65 %), albedo, spongy white inner part (30 to 35 %) and seeds (0 to 10 %). These tissues are rich in structural fibers along with pectin and fermentable soluble sugars [3]. The exact composition varies according to the type and yield of the orange crop, but it usually consists of ashes (2 – 3 %), proteins (4 to 8 %), lipids (0.6 to 4 %) and carbohydrates (80 to 87 %), of which 22 to 46 % are insoluble fibers and 15 to 25 % are soluble fibers. The insoluble fiber content comprises cellulose, hemicellulose and lignin.[4-5]

According to Rezzadori *et al*.,[6] residues from orange juice production generally have no economic value, and the most common utilization of orange bagasse is for animal feed, accounting for up to 70 - 80 % of production; however, the bitter taste reduces its palatability.[1] Other uses have also been reported in the literature, including the extraction of bioactive compounds such as carotenoids, polyphenols and flavonoids,[7-8] energy production,[9-10] ethanol[11] and fermentation substrate for lactic acid production.[12]

There are few studies in the literature exploring the use of orange juice industry waste for cellulose extraction. Bicu and Mustata[13-14] successfully extracted cellulose from orange peel through sequential chemical treatments, including alkaline and sulfite pulping and bleaching steps with hypoclorites and hydrogen peroxide. Mariño *et al*.[15] reported the use of an alkaline treatment combined with bleaching with sodium chlorite to obtain a cellulose-rich material employed for the production of nanocellulose from industrial orange bagasse. Mariño *et al*.[16] reported the use of alkaline and acid treatments, followed by bleaching with sodium hypochlorite to obtain cellulose for use in nanocellulose production. Cypriano *et al*.[3] also reported the use of multistep sequential physical-chemical treatments with sodium hydroxide and sodium chlorite to obtain nanocellulose by enzymatic hydrolysis.

The extraction of cellulose from lignocellulosic residues requires the rupture of the complex structure of lignocellulosic biomass, which is hierarchically organized into fibrils until forming the cellulosic fiber, surrounded by hemicellulose and lignin. Physical, chemical or combined routes are employed to separate the different fractions. In conventional processes, higher amounts of reagents are required, including several steps of acid and alkaline pretreatments and bleaching, generally using chlorites and hypochlorites. Chlorine reagents oxidizes lignin, consequently leading to its degradation and solubilization, but resulting in long processes and higher amounts of toxic effluents.[17-18]

Considering the heterogeneity of the lignocellulosic materials, it is challenging to generalize treatments for cellulose extraction from these materials, and in the past decade, there has been an increasing interest in the use of less polluting methods, employing totally chlorine-free (TCF) processes with nonchlorine chemicals. Non-chlorine reagents have been used successfully in the delignification of wood lignocellulose and agro-industrial wastes.[19-20-21] Peroxide and peroxy acids, and the combination of physical and chemical processes as a strategy to reduce the energy consumption and overall processing costs in producing commercially competitive cellulose.[17-22-23]

In this study, the objectives were (1) to investigate different combinations of chemical (sodium hydroxide (NaOH), peracetic acid and alkaline peroxide) and physical (autoclaving and ultrasonication) treatments performed in one-step processes for cellulose extraction from orange bagasse and (2) to characterize the obtained materials according to their composition, morphology, crystallinity and thermal stability. Less-polluting processes have been proposed as alternatives for the delignification of orange bagasse instead of chlorine bleaching agents, as well as the combination of chemical and physical methods that can reduce the reaction times and the quantity of chemical reagents employed in the overall processes. The cellulose-rich materials are the basis for obtaining various products of industrial interest, such as cellulose-rich oleogels,[24] membranes,[25] regenerated cellulose, [26] hydrogels[27] and nanocellulose[15-16] and also can be applied as a source of dietary insoluble fibers in functional foods and as a substrate in fermentation processes, since the obtained materials will be more accessible to enzymatic hydrolysis.

4.2 Materials and methods

4.2.1. Materials

Orange bagasse (OB) was obtained from a Brazilian orange-juice processing industry (Integrada Cooperativa - Paraná, Brazil) and dried at 50 °C in an air-circulating oven (Marconi MA 415, Piracicaba, Brazil) until constant moisture content was achieved and milled to yield particles < 0.30 mm (IKA-A 11 Basic Mill, Germany). Sodium hydroxide PA (NaOH beads, Synth, Brazil), glacial acetic acid PA-ACS (99 %, Synth, Brazil), and hydrogen peroxide PA (H_2O_2 35 %, Synth, Brazil) were employed in the experiments.

4.2.2 Cellulose extraction

OB was submitted to three treatment groups as described in Table 1: (1) chemical treatment (CH) with sodium hydroxide, peracetic acid (50 % acetic acid, 38 % hydrogen peroxide 30 volumes and 12 % distilled water v/v) or peroxide alkaline (H_2O_2 16 % v/v in NaOH 5 % w/v); (2) chemical treatment combined with autoclaving (AC) and (3) chemical treatment combined with ultrasonication (US). The AC treatment was performed in a vertical autoclave (Prismatec, model CS, São Paulo, Brazil). The reaction mixture was subjected to the autoclaving process for 30 min at 121 °C and 1.0 bar. In US treatment, the bagasse was immersed in reagent, and the reaction mixture was ultrasonicated at a frequency of 20 kHz and 50 % amplitude using a Fisher Scientific Sonicator model 505 (Pittsburgh, PA - USA) coupled with a probe with a tip diameter of 1.27 cm (Fisher Scientific model FB 4219, Pittsburgh, PA – USA) for 1 h at the initial ambient temperature and varied to 60 °C.

Table 1 - Processing conditions to obtain cellulose from OB.

Sample	Chemical treatment	Physical treatment
	Chemical reagents (1 h, 60 °C; agitation)	
CH_{SH}	Sodium hidroxide - NaOH	-
CH_{PA}	Peracetic acid	
CH_{PX}	Peroxide alcaline	
CH_{CT}	Water	
Chemical treatment combined with high-pressure steam in autoclave (HP) – One-step process		
AC_{SH}	Sodium hidroxide - NaOH	Autoclave 30 min, 121 °C, 1.0 bar
AC_{PA}	Peracetic acid	
AC_{PX}	Peroxide alcaline	
AC_{CT}	Water	
Chemical treatment combined with ultrasonication (US) – One-step process		
US_{SH}	Sodium hidroxide - NaOH	Ultrasound 50 % power output, 1 h, 25-60 °C
US_{PA}	Peracetic acid	
US_{Px}	Peroxide alcaline	
US_{CT}	Water	

For each treatment group, a control test was performed using water instead of chemical reagents. In all chemical treatments, the same solid (OB): liquor ratio (1:15 w/v) was employed. All treatments were conducted in triplicate.

After each treatment, the residual solid fractions were washed with distilled water close to neutrality (pH 5.5-6.5), dried until constant weight at 50 ºC for 12 to 24 h in a ventilated oven (035 Marconi MA - São Paulo, SP, Brazil), milled (IKA-A 11 Basic Mill, Germany) and sieved to yield particles from 100 to 180 µm. The process yields of the cellulosic-rich material obtained from each treatment were given on a dry weight basis related to the starting OB.

4.2.3 Cellulose, hemicellulose and lignin contents

The cellulose and hemicellulose contents of the raw OB and OB subjected to the different treatments were determined by the Van Soest method[28] and the lignin content was determined according to a standard method of the Technical Association of Pulp and Paper Industry.[29] The cellulose yields were calculated from the dry weight of cellulose related to the starting cellulose content of OB.

4.2.4 Pectin content

The pectin content was determined in the raw OB according to Kratchanova *et al*. method[30] with some modifications: the dry OB sample (20 g) was mixed with 800 mL of extraction solvent (HCl 0.05 M) and then heated to 80 °C in continuous stirring for 1 h. The solid fraction (fibers) was separated by filtration, and pectin was precipitated from the liquid fraction using an equal volume of 96 % ethanol and kept for two hours in a refrigerator. The coagulated pectin was filtrated and washed using 70 % acidic ethanol (0.5 % HCl), followed by 70 % ethanol and then 96 % ethanol. Samples were dried at 50 ºC for 12 - 24 h in a ventilated oven (035 Marconi MA - São Paulo, SP, Brazil). Pectin content was calculated from the dry weight of pectin related to the starting OB sample.

4.2.5 Scanning electron microscopy (SEM)

The morphology of the raw OB and OB subjected to the different treatments was observed by scanning electron microscopy (SEM). The SEM analyses were performed with an FEI Quanta 200 microscope (Oregon, USA). The dried samples were mounted for 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 30 kV.

4.2.6 X-ray diffraction (XRD)

The analysis was performed using a PANalytical X'Pert PRO MPD diffractometer (Almelo, The Netherlands) under the operation conditions of 40 kV and 30 mA. The relative crystallinity index (CI) was calculated using the Segal *et al*.[31] method as follows: CI (%): $([I_{002}-I_{am}]/I_{002})$*100, where I_{002} is the intensity of the 002 peak (at approximately 2θ = 20- 22°) and I_{am} is the intensity corresponding to the peak at 2θ = 18°.

4.2.7 Fourier transform-infrared spectroscopy (FTIR)

FTIR analysis was carried out in a Shimadzu FT-IR - 8300 instrument (Japan), with a spectral resolution of 4 cm^{-1} and a spectral range of 4000 - 500 cm^{-1}. Dried samples were mixed with potassium bromide and compressed into tablets.

4.2.8 Differential scanning calorimetry (DSC)

The samples were placed in platinum containers and heated from 30 to 450 °C at a heating rate of 5 °C/min in a helium atmosphere. Analyses were performed on a Shimadzu DSC 60 (Japan) calorimeter.

4.2.9 Statistical analysis

The data were analyzed using R software (R Foundation for Statistical Computing, Vienna, Austria), and Tukey's test was employed to evaluate differences between means ($p \leq 0.05$).

4.3 Results and discussion

4.3.1 Cellulose, hemicellulose, lignin and pectin contents of raw OB

The raw OB employed in this study presented 12.4 ± 0.6 % cellulose, 7.5 ± 0.1 % hemicellulose, 8.9 ± 0.5 % lignin and 12.3 ± 0.7 % pectin; and the insoluble dietary fiber content (calculated from the sum of cellulose, hemicellulose and lignin contents) was 28.8 %. De la Torre *et al*.[32] found contents of approximately 18, 14, 6 and 18 % for cellulose, hemicellulose, lignin and pectin, respectively, while Cypriano *et al*.[3] reported values of 20, 6, 4 and 18 %, respectively, for orange bagasse. Thus, the composition of the OB varies according to the source of the product or the type of processing. These variables interfere with the bagasse response to the extraction treatment.[16]

Considering the great economic importance of the orange juice industry in Brazil and the great availability of its main residue, OB can be considered an interesting source of cellulose that has not been fully exploited for the purpose of obtaining cellulose and its derivatives. Although its cellulose content is lower than that of other residues that have been largely reported as a cellulose source, such as sugarcane bagasse, which has cellulose contents of approximately 40 % and 18 % lignin,[33] or wheat straw, with 30 % cellulose and 15 % lignin,[34] its lower lignin content when compared to these residues can be considered an advantage.

The higher pectin content is particular to OB; pectin is a major component of the primary cell walls of dicotyledonous plants;[35] however, lignocellulosic residues are mainly composed of secondary cell walls, which only contain minor amounts of pectin.[36] In this study, the focus was the extraction of cellulose from the OB and the analysis of the resulting cellulose-rich material, but the integrated removal of the pectin should also be considered. In this work, the suggested application of the released pectin and hemicellulose is their use as substrates in fermentation processes.

4.3.2 Cellulose, hemicellulose, lignin and pectin contents of OB subjected to different treatments

The extraction of cellulose from a lignocellulosic matrix in a reasonable content without large yield losses is a technical challenge; generally, obtaining high concentrations of cellulose results in low processing yields. Cellulose content increased significantly for all employed treatments, including the controls (Table 2). The AC_{SH} sample (combination of chemical treatment with NaOH with autoclaving), CH_{SH} (chemical treatment with NaOH), AC_{PA} (combination of chemical treatment with peracetic acid with autoclaving) and AC_{PX} (combination of chemical treatment with alkaline peroxide with autoclaving) showed higher cellulose contents after processing, with contents of 71.1, 54.7, 56.1 and 52.5 %, respectively (Table 2).

Among all the processes, the samples subjected to autoclaving (AC) combined with chemical treatments had the high cellulose contents (Table 2), and the sample obtained in the presence of NaOH (AC_{SH}) had higher cellulose concentrations. Autoclaving pretreatment performed in an autoclave is considered an effective method for deconstructing the three-dimensional structure of lignocellulose, resulting in a cellulose-rich material that can be used after further purification.[37-39] The steam pretreatment modifies the plant cell wall structure, and partially hydrolyzed hemicellulose is easily recovered by water washing, leaving a water-insoluble fraction composed of cellulose, residual hemicellulose and a chemically modified lignin.[40] The use of an alkaline medium results in a cleaving of the ester bonds between lignin and hemicellulose, the swelling of cellulose and the disruption of intermolecular hydrogen bonds between cellulose and hemicellulose, releasing noncellulosic fractions and leaving the cellulose as the solid residue.[41-42]

Table 2 - Cellulose, hemicellulose, lignin contents, process yield, cellulose yield and crystallinity index (CI) of raw OB and OB subjected to different treatments.

Treatment	Cellulose content (%)	Hemicellulose content (%)	Lignin content (%)	Process yield (g cellulosic-rich material/ 100 g OB)	Cellulose yield (g cellulose/ 100 g original cellulose)	CI (%)
Raw OB	12.4 ± 0.4^{h}	7.5 ± 0.1^{bc}	8.9 ± 0.5^{d}	-	-	18
CH$_{SH}$	54.7 ± 1.0^{b}	0.0 ± 0.0^{d}	9.1 ± 0.1^{d}	25	100	26
CH$_{PA}$	12.1 ± 0.8^{h}	8.3 ± 1.8^{b}	10.9 ± 1.6^{d}	75	85	17
CH$_{PX}$	29.5 ± 0.4^{d}	8.4 ± 2.2^{b}	9.2 ± 0.8^{d}	50	100	21
CH$_{CT}$	20.0 ± 1.2^{f}	8.1 ± 0.1^{b}	10.4 ± 0.1^{d}	82	100	18
AC$_{SH}$	71.1 ± 1.0^{a}	0.0 ± 0.0^{d}	19.0 ± 0.5^{b}	14	82	42
AC$_{PA}$	56.1 ± 1.5^{b}	0.0 ± 0.0^{d}	22.3 ± 1.0^{a}	16	72	39
AC$_{PX}$	52.5 ± 1.0^{b}	0.0 ± 0.0^{d}	13.0 ± 0.1^{d}	24	100	30
AC$_{CT}$	22.7 ± 0.1^{e}	7.7 ± 0.4^{bc}	10.1 ± 0.3^{d}	80	100	20
US$_{SH}$	33.7 ± 1.0^{c}	7.8 ± 0.1^{bc}	10.0 ± 0.1^{d}	36	97	21
US$_{PA}$	32.5 ± 0.5^{c}	6.2 ± 0.3^{c}	5.7 ± 0.1^{e}	57	100	19
US$_{Px}$	22.8 ± 0.7^{e}	10.5 ± 0.3^{a}	9.2 ± 0.2^{d}	57	100	18
US$_{CT}$	15.5 ± 0.5^{g}	8.5 ± 0.2^{b}	10.4 ± 0.1^{d}	80	100	21

$^{a-h}$- Different letters in the same column indicate significant differences ($p \leq 0.05$) between means (Tukey's test).

There was also complete hemicellulose removal in all OB samples processed by the combination of all chemical treatments with autoclaving (Table 2). Hemicelluloses were easily degraded, but total lignin removal was not possible in any treatment employed in this work. Cellulose and lignin are more resistant to degradation than hemicelluloses.[43]

Comparing the chemical reagents employed in this study (Table 2), the use of NaOH resulted in higher cellulose contents than the use of peracetic acid or alkaline peroxide. According to Ahuja *et al.*,[41] the use of an alkaline treatment with NaOH is effective in lignin and hemicellulose removal, with some advantages such as low cost and eco-friendliness. Bicu and Mustafa[14] reported that NaOH was effective in solubilizing and dissolving lignin but also in the despectination of orange juice wastes by breaking the ester linkages between carboxyl groups of pectin and hydroxyl groups of cellulose and hemicellulose.

Peracetic acid is an efficient bleaching agent that can substitute for hypochlorite in bleaching processes; however, in this work, the use of PA was more efficient when performed in combination with autoclaving (Table 2). Peracetic acid is considered a highly selective delignifying agent due to its capacity to oxidize electron-rich structures, such as aromatic rings of lignin, and it can also react with acid groups linked to xylan hemicelluloses.[44-45]

The use of ultrasound was less efficient in removing hemicellulose from OB compared to the other processes employed in this study, and the lowest contents of cellulose were found in the US group (Table 2). However, interesting results were observed for lignin removal, and the US_{PA} sample had the lowest lignin concentration (5.7 %).

It can be seen in Table 2 that all employed processes resulted in samples with higher insoluble dietary fiber contents (sum of cellulose, hemicellulose and lignin), which indicated that other components, such as pectin, lipids, proteins and ashes, were removed from the OB. The samples with higher cellulose contents, AC_{SH}, CH_{SH}, AC_{PA} and AC_{PX}, presented 90.1, 63.8, 78.4 and 65.5 % insoluble dietary fibers, respectively, while the raw OB presented 28.8 %.

Considering the yields of each process, higher cellulose content lowers the yields (Table 2), which agreed with Bicu and Mustafa,[14] who reported that maximum cellulose contents result in minimum yields. Cellulose yields ranged from 72 to 100 % (Table 2), and they were calculated from the recovery of cellulose in relation to the original cellulose. The obtained results indicated that there was little loss of the original cellulose during the employed processes and in the washing steps. The samples submitted to AC in combination with NaOH and PA (AC_{SH} and AC_{PA}) presented lower processes and cellulose yields, and both samples had higher cellulose contents. Bicu and Mustafa[13] employed a multistep protocol to extract cellulose from orange peel employing sulfite treatments followed by bleaching steps with hypochlorite and hydrogen peroxide and obtained materials with 75 to 81 % cellulose and 13 to 18 % hemicelluloses, with processing yields of 42 to 45 %. In another work, Bicu and Mustafa[14] employed a NaOH and ethylenediaminetetraacetic acid (EDTA) treatment followed by a bleaching sequence in three steps: hypochlorite in an alkaline medium, then in an acid medium and finally hydrogen peroxide; the obtained material had 85.8 % cellulose and 8.6 % hemicellulose with a processing yield of 26.2 %.

4.3.3 Scanning electron microscopy (SEM)

Lignocellulosic materials generally show a compact structure covered by an outer layer, as can be seen in the SEM analysis of OB (Fig. 1). According to Ahmadzadeh *et al.*,[46] hemicellulose, lignin, pectin and waxes are components of the thick outer layer of the fiber surfaces that protect the cellulose inside.

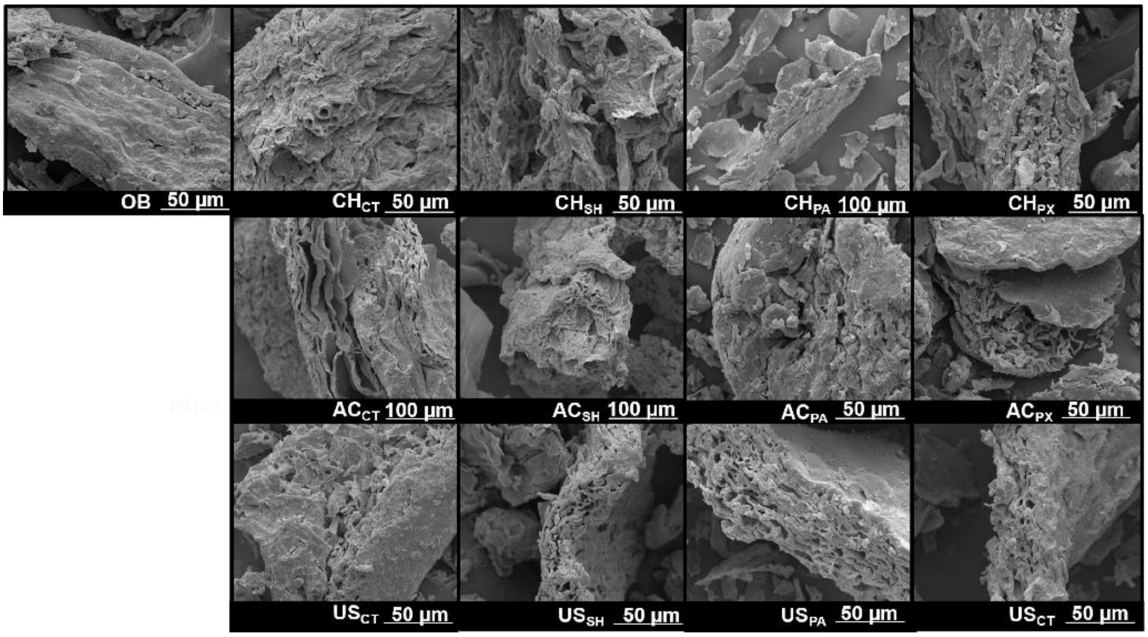

Figure 1 - Morphology of raw orange bagasse (OB) and OB subjected to different treatments obtained by scanning electron microscopy (SEM).

In the control group (CH_{CT}, AC_{CT} and US_{CT}) processed without chemical reagents, there was no large removal of lignin and hemicellulose (Table 2), but some disorganization of the lignocellulosic structure was observed, mainly for the autoclaving-processed sample (AC_{CT}). The samples presented an overall size reduction after extraction processes.

The chemical and physical treatments were able to promote changes in the morphology of the OB, resulting in irregular and porous outer surfaces and indicating a partial removal of the noncellulosic layer. It was possible to observe that the samples subjected to AC presented a more disorganized structure, but the cellulosic fibers were not completely individualized, which can be explained by the composition of these samples, which presented lignin that was not totally removed by the employed processes (Table 2). Lignin acts as a stiffening and impermeabilization agent of the cell wall, and the higher pectin content of raw OB may disfavor its delignification.

4.3.4 X-ray diffraction (XRD)

The crystalline structure of the samples was analyzed by XRD (Fig. 3). The raw OB diffractogram (Fig. 2a) is typical of an amorphous material, with a CI of 18 % (Table 2), with only a discrete peak at 2θ = 22°, which can be attributed to native cellulose. Considering the control group (processed with water), the same peak appeared at 2θ = 22°, and the CI values were very close to the raw OB value, with values of 18, 20 and 21 % for CH_{CT}, AC_{CT} and US_{CT}, respectively.

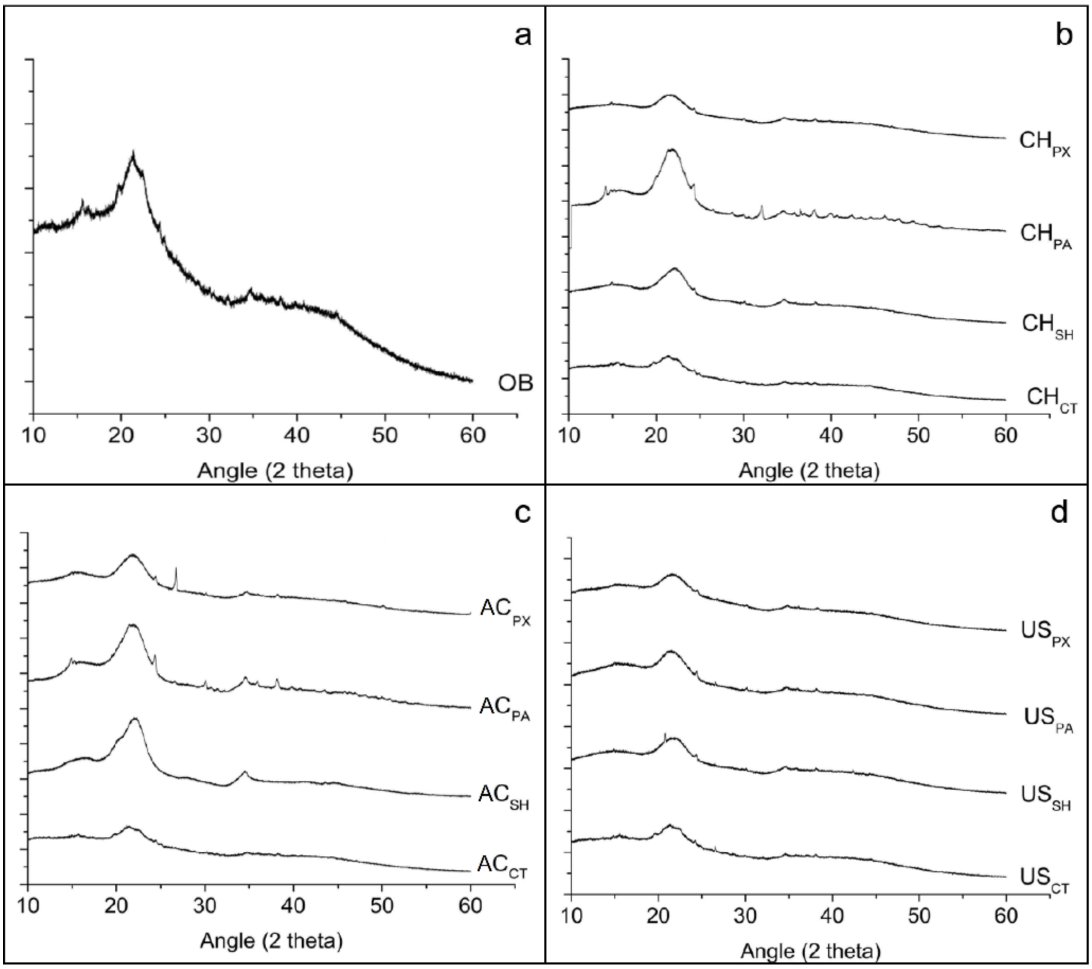

Figure 2 - X-ray diffractograms of raw OB and OB subjected to different treatments: (a) raw OB; (b) OB subjected to chemical treatments with NaOH (CH_{SH}), peracetic acid ($_{CHPA}$), alkaline peroxide (CH_{PX}) and water – control treatment (CH_{CT}); (c) Chemical treatment combined with high-pressure steam in an autoclave employing NaOH (HP_{SH}), peracetic acid (HP_{PA}), alkaline peroxide (HP_{PX}) and water – control treatment (HP_{CT}); (d) Chemical treatment combined with ultrasonication employing NaOH (US_{SH}), peracetic acid (US_{PA}), alkaline peroxide (US_{PX}) and water – control treatment (US_{CT}).

In all processed samples, a new peak appeared at 2θ = 34.5° (Figs. 2b, c, d), which can also be attributed to native cellulose. The samples subjected to chemical treatment combined with autoclaving presented higher CI values (Table 2), and these samples also presented high cellulose contents. The major components of OB are pectin, cellulose, hemicellulose and lignin, and between them, cellulose corresponds to the crystalline fraction. According to Ahmadzadeh *et al*.,[46] cellulose is embedded in amorphous hemicellulose and pectin, and the whole is surrounded by a matrix of amorphous lignin; thus, the removal of these components results in increased crystallinity. Lignin (amorphous compound) and other non-lignocellulosic components were not completely eliminated in any sample, thus influencing the CI (%) value. In a study with orange peel, the authors found similar IC (%), 43.7 and 41.8, for samples with cellulose content (%) of 80.8 and 75.5, respectively.[13]

4.3.5 Fourier transform-infrared spectroscopy (FTIR)

FT-IR spectra (Fig. 3) showed characteristic bands of cellulose in all samples, indicating that the treatments preserved the cellulose fiber. Bands at 3400 cm^{-1} and 2920 cm^{-1} were observed for all samples and were attributed to O–H (including intramolecular and intermolecular hydrogen bonds) and C–H group stretching vibrations, respectively.[47]

The band at 1740 cm^{-1} appeared in raw OB, in all control samples (CH_{CT}, AC_{CT} and US_{CT}) and in samples treated with PA (Figs. 3a, b, c, d). In raw OB and control samples, this band was related to the ester and acetyl groups in hemicellulose and lignin.[15] In the samples treated with PA, this band was more evident and was attributed to the oxidation of C-OH groups from the terminal glucose rings.[48]

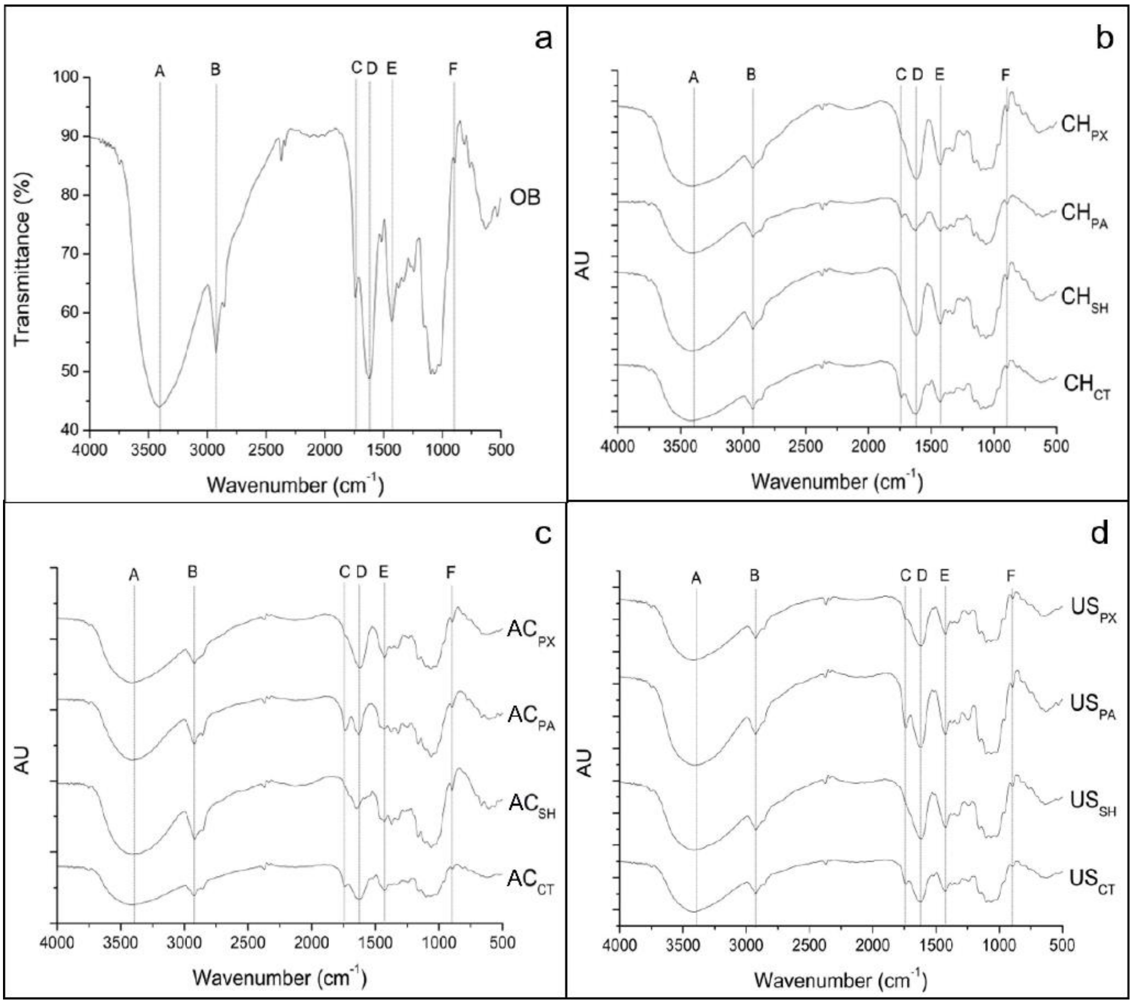

Figure 3 - FTIR spectra of raw OB and OB subjected to different treatments: (a) raw OB; (b) OB subjected to chemical treatments with NaOH (CH_{SH}), peracetic acid (CH_{PA}), alkaline peroxide (CH_{PX}) and water – control treatment (CH_{CT}); (c) Chemical treatment combined with high-pressure steam in an autoclave employing NaOH (HP_{SH}), peracetic acid (HP_{PA}), alkaline peroxide (HP_{PX}) and water – control treatment (HP_{CT}); (d) Chemical treatment combined with ultrasonication employing NaOH (US_{SH}), peracetic acid (US_{PA}), alkaline peroxide (US_{PX}) and water – control treatment (US_{CT}). Bands: A: 3400 cm^{-1}, B: 2920 cm^{-1}, C: 1620 cm^{-1}, D: 1430 cm^{-1}, E: 1320 cm^{-1}, F: 895 cm^{-1}.

The band observed at 1620 cm^{-1} appeared in all samples and was characteristic of cellulose–water absorption [41]. The band at 1430 cm^{-1} was lignin-related and also found in all samples.[47]

A band at 890 cm^{-1} appeared in all samples (Figs. 3a,b,c,d), which seemed to be more defined in the processed samples when compared to raw OB and control samples; it can be attributed to cellulose β-glycosidic linkage.[15]

4.3.6 Differential scanning calorimetry (DSC)

DCS thermograms of OB and OB-treated samples are presented in Fig. 4. For all samples, an endothermic peak was observed in the 60 to 100 °C range due to water evaporation. Raw OB presented a discrete endothermic peak at 284 °C and a second one more pronounced at 431 °C (Fig. 4a), and they could be related to the scission of the glycosidic bonds presented in cellulose, hemicellulose and pectin and to lignin decomposition,[49] respectively.

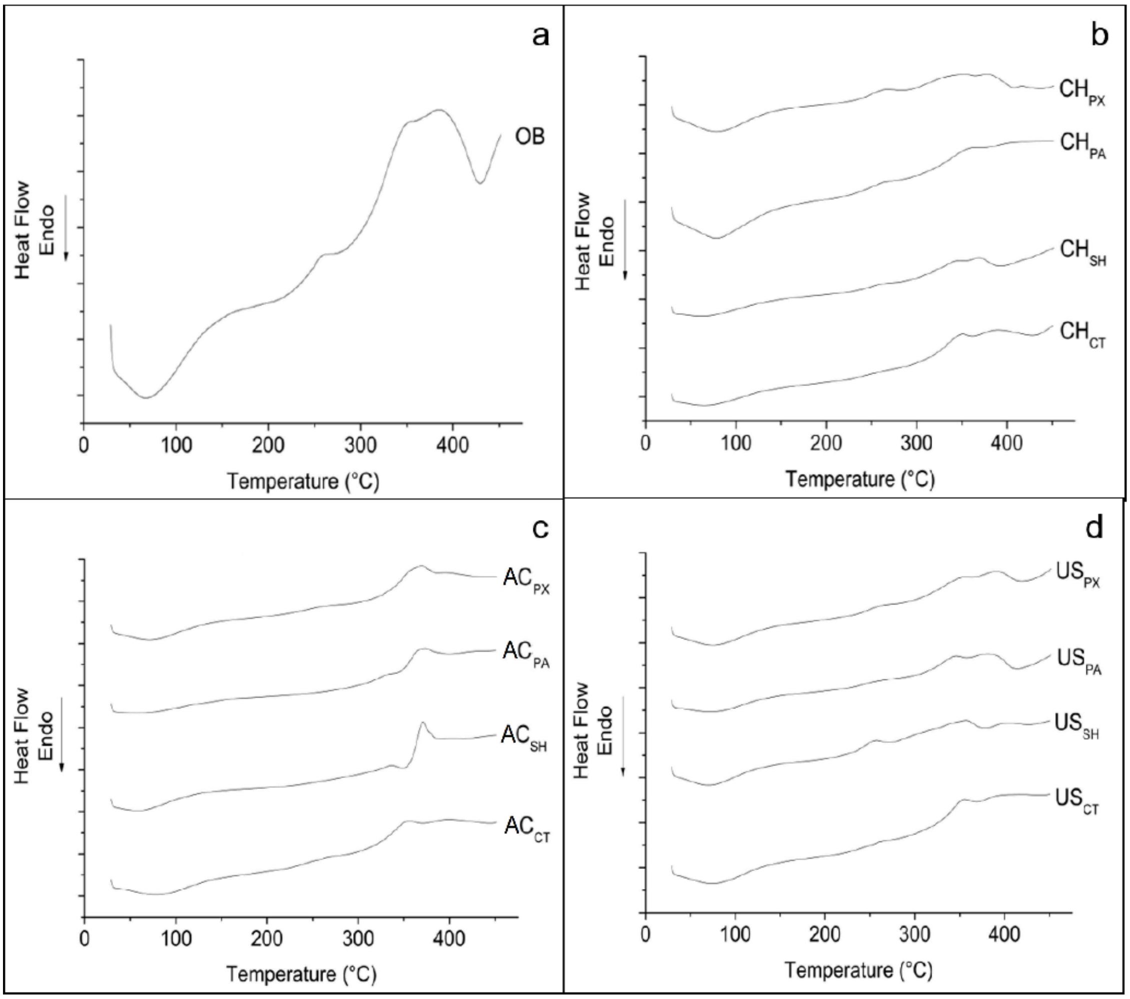

Figure 4 - DSC thermograms of raw OB and OB subjected to different treatments: (a) raw OB; (b) OB subjected to chemical treatments with NaOH ($\mathrm{CH_{SH}}$), peracetic acid ($\mathrm{CH_{PA}}$), alkaline peroxide ($\mathrm{CH_{PX}}$) and water – control treatment ($\mathrm{CH_{CT}}$); (c) Chemical treatment combined with high-pressure steam in autoclave employing NaOH ($\mathrm{HP_{SH}}$), peracetic acid ($\mathrm{HP_{PA}}$), alkaline peroxide ($\mathrm{HP_{PX}}$) and water – control treatment ($\mathrm{HP_{CT}}$); (d) Chemical treatment combined with ultrasonication employing NaOH ($\mathrm{US_{SH}}$), peracetic acid ($\mathrm{US_{PA}}$), alkaline peroxide ($\mathrm{US_{PX}}$) and water – control treatment ($\mathrm{US_{CT}}$).

Samples subjected to chemical treatments (Fig. 4b) and to the combination of chemical treatments and ultrasonication (Fig. 3d) showed the presence of endothermic peaks in the region from 350 to 450 °C, indicating thermal degradation of cellulose, which was also reported by other authors.[50] Other endothermic peaks appeared at 400 to 430 °C, which might be related to lignin degradation.

In samples subjected to the chemical treatments combined with high pressures in autoclaves (Fig. 4c), the presence of exothermic peaks at 345 to 400 °C was observed, attributed mainly to exothermic reactions due to cellulose depolymerization and the cleavage of carbon-carbon bonds between lignin structural units because lignin degradation occurs between 385 and 585 °C. Ray *et al*.[49] and Orozco *et al*.[51] also reported the same peak in cellulosic-rich materials extracted from lignocellulosic residues.

It was interesting to observe that any endothermic or exothermic phenomenon was observed until 270 °C (except for the desidratation), indicating that all these samples could be processed between this temperature range with relative thermal stability.

4.4 Conclusion

Orange bagasse is an abundant residue whose industrial utilization deserves more attention. The present study successfully explored the application of bagasse to the production of a cellulosic-rich material. The use of combined chemical treatments with autoclaving (AC) was more efficient at obtaining high cellulose contents, and NaOH resulted in better results between the chemical reagents. Therefore, AC_{SH} was the most effective treatment for obtaining a high cellulose content (71.1 %). This sample showed total elimination of hemicellulose and partial lignin with a satisfactory cellulose yield of 82 %.

The one-step treatments, allowing energy and solvent savings, were able to result in materials with higher cellulose contents and yields, partial crystallinity and relative thermal stability until 270 °C. Cellulose-rich materials can be used for further purification to produce high-purity cellulose or to be used as a source of dietary insoluble fibers in functional foods or as substrate in fermentation processes because the obtained materials are more accessible to enzymatic hydrolysis.

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5. CAPÍTULO III – ARTIGO II: CELLULOSE-BASED MATERIALS FROM ORANGE BAGASSE EMPLOYING ENVIRONMENTALLY FRIENDLY APPROACHES

Artigo publicado na revista “Biomass Conversion and Biorefinery”.
Referências e citações conforme a revista.

Cellulose-based materials from orange bagasse employing environmentally friendly approaches

Abstract

Orange bagasse (OB) is an abundant lignocellulosic residue in Brazil, and few studies have explored this raw material for cellulose and nanocellulose obtainment. The objective of this study was to obtain cellulose-based materials from OB through three one-step alkaline extraction processes using NaOH (chemical (CH), autoclaving (AC), and ultrasonication (US)). The obtained samples were characterized according to their composition, morphology, crystallinity, thermal stability, water and oil absorption capacity, solubility, and swelling capacity. Nanocellulose was also produced and characterized according to its composition, morphology (atomic-force microscopy), and crystallinity. The cellulose content of materials obtained from OB ranged from 33.7 to 85.4%, with a cellulose recovery of 65.3 to 100%. The combination of alkaline and autoclaving treatments was more efficient in obtaining a material with higher cellulose content, higher crystallinity, and thermal stability. Alkaline treatment combined with ultrasonication was less effective in obtaining a material with higher cellulose contents. Crystallinity and surface morphology of samples affected their functional properties. An increase in crystallinity of samples led to a decrease in water and oil holding capacities, swelling in water, and solubility, while an increase in porosity resulted in higher water and oil holding capacities, swelling in water, and solubility. The nanocellulose obtained had 60% crystallinity with a yield of 4.4% (each 100 g of raw OB subjected to the sequential treatments yielded 4.4 g of nanocellulose). Cellulose-based materials obtained in this study can be used as fibers source in food products, as substrates in fermentation processes, and it can also be used to obtain nanocellulose as performed in this study.

Keywords: one-step pretreatments; autoclaving; ultrasonication; alkaline treatment; nanocellulose.

5.1 Introduction

Cellulose is the most abundant natural polymer on Earth. It is a linear homopolysaccharide of β-1,4-linked anhydroglucose units, which is organized into microfibrils with amorphous and crystalline regions [1, 2]. Several agroindustrial residues have a high potential to be used for the production of cellulose and nanocellulose without the competition with human and animal food chains [3].

The disruption of the cellulose-hemicellulose-lignin complex to obtain cellulose from lignocellulosic materials generally requires the use of multiple-step processes, including physical, chemical, biological methods, or their combination [2, 4–6]. Alkaline treatments can be very efficient in removing lignin, hemicellulose, and pectin from these materials, with the advantage of low cost and eco-friendliness [1, 7, 8]. In conventional approaches, higher amounts of reagents are required, including multiple-step acid and alkaline pretreatments and bleaching, generally using chlorites and hypochlorites, which result in higher amounts of toxic effluents [5, 6]. The use of one-step processes combining physical and chemical treatments employing totally chlorine-free reagents can be consider a strategy to minimize environmental impacts of cellulose production by reducing the energy consumption and overall processing costs [4, 5, 7].

For nanocellulose isolation from a cellulose-rich material, other sequential steps are required, which can involve controlled acid hydrolysis of amorphous regions to obtain cellulose nanocrystals, and mechanical or chemical defibrillation to obtain nanofibrillated cellulose [2, 3, 9].

Cellulose and nanocellulose characteristics can vary depending on the type of extraction method and plant source [10]. The number of anhydroglucose units in the molecule, chain length, and degree of polymerization determine many properties of cellulose. Long cellulose chains show more resistance to rupture than short chains, and other components, such as pectin, can interfere with the extraction process [11]. Several agroindustrial residues have been employed to obtain cellulose and nanocellulose, such as sugarcane bagasse [12], oat husk [10, 13], rice husk [14], corn stover [15], and soybean hull [2, 16]. Several applications have been proposed for cellulose and nanocellulose, including the food sector, as fat replacers and/or binding agents to produce healthier meat products [17], in food packaging systems [18], or as thickeners, stabilizers, texture modifiers, and as dietary fiber source [19].

Brazil is responsible for 60% of the worldwide production of orange juice, accounting for over three-quarters of global orange juice exports [20, 21]. The industrial processing of oranges (Citrus × sinensis (L.) for juice extraction results in remaining bagasse formed by peel (approximately 60%), albedo (about 30%), and seed (up to 10%), which corresponds to 50% of the fruit weight [22, 23]. The chemical composition of OB generally contains varying amounts of cellulose (9–37%), hemicelluloses (7–26%), lignin (1–7%), and pectin (18–20%) [22, 24], and the low lignin content makes OB an interesting alternative for the extraction of cellulose.

Orange bagasse is a lignocellulosic residue with high value-added [22]. However, there are few reports in the literature describing the extraction of cellulose and nanocellulose from orange bagasse, and most of them employing several sequential processes [6, 8, 22, 25–28]. The wide availability and economic importance of orange bagasse in Brazil make this residue an attractive raw material, justifying the objective of the present study, which was to obtain different cellulose-based materials from OB through three one-step alkaline extraction processes using NaOH (chemical (CH), steam explosion in an autoclaving (AC), and ultrasonication (US)). The obtained samples were characterized according to their composition, morphology, crystallinity, thermal stability, water and oil absorption capacity, solubility, and swelling capacity. Nanocellulose was also produced and characterized according to its composition, morphology (atomic-force microscopy), and crystallinity. Environmentally friendly approaches were chosen in this study to minimize environmental and economic impacts.

5.2 Material and methods

5.2.1 Material

An orange-juice processing industry from Paraná - Brazil (Integrada Cooperativa) provided the orange bagasse (OB), which was dried at 50 °C in an air-circulating oven (Marconi MA 415, Piracicaba, Brazil) and milled to yield particles < 0.3 mm (IKA-A 11 Basic Mill, Germany). The chemical reagents used were sodium hydroxide PA (NaOH beads, Synth, Brazil), glacial acetic acid PA-ACS (99%, Synth, Brazil), and hydrogen peroxide PA (H_2O_2 35%, Synth, Brazil).

5.2.2 Methods

5.2.2.1 Cellulose-based materials production

Cellulose was extracted from raw OB through three one-step alkaline extraction processes using NaOH (10% $w.v^{-1}$): chemical (CH), autoclaving (AC), and ultrasonication (US) based on a previous study [4]. In all treatments, a 1:15 ratio of OB:NaOH solution was employed. The chemical treatment (CH) was performed in a magnetic stirrer (IKA RCT basic model), the sample was dispersed in a NaOH solution for 60 min at 60 °C under continuous agitation. Autoclaving treatment (AC) was performed in a vertical autoclave (Prismatec, model CS, São Paulo, Brazil), and the sample was dispersed in a NaOH solution for 30 min at 121 °C and 1.5 atm. Ultrasonication (US) was performed in a Fisher Scientific Sonicator model 505 (Pittsburgh, PA - USA) coupled with a probe with a tip diameter of 1.27 cm (Fisher Scientific model FB 4219, Pittsburgh, PA – USA). The sample was dispersed in a NaOH solution at a temperature range of 25-60 °C for 60 min.

Then, the residual solid fraction of each treatment was washed with distilled water until a pH between 6 and 7, dried for 24 h at 50 ºC in a ventilated oven (035 Marconi MA - São Paulo, SP, Brazil) and milled to particles < 0.3 mm.

For nanocellulose preparation, the cellulose sample obtained from chemical treatment (CH_{NaOH}) was subjected to an additional bleaching process with peracetic acid for 24 h at 60 °C under stirring [13]. After washing and drying as described in the previous section, the resulting fraction was labeled $CH_{NaOH/PA}$, and this sample was treated with sulfuric acid to obtain cellulose nanofibers (CNF_{OB}). The bleached fibers (2.5 g) were suspended in 50 mL of H_2SO_4 solution (60% $w.w^{-1}$) and kept under vigorous stirring for 30 min at 45 °C. The suspension was diluted with the addition of 250 mL of cold alkaline solution (NaOH 0.1 $mol.\ L^{-1}$) to stop the reaction and centrifuged (10000 rpm, 10 °C for 10 min). The precipitate was dialyzed with a cellulose membrane to approximately pH 6 and then centrifuged applying cycles (10000 rpm, 10 °C for 10 min) until there was no sign of complete precipitation.

5.2.2.2 Cellulose-based materials composition

Cellulose and hemicellulose contents were determined by the Van Soest method [26], and lignin content by the standard method of the Technical Association of Pulp and Paper Industry (TAPPI) T222 om-88 [27].

The pectin content of raw OB was determined according to the Kratchanova *et al*. method [28] with some modifications: a dry OB sample (20 g) was mixed with 800 mL of extraction solvent (HCl 0.05 mol/L) and then heated to 80 °C under continuous stirring for 1 h. The solid fraction was separated by filtration, and pectin was precipitated from the liquid fraction using an equal volume of 96% ethanol and kept for 2 h in a refrigerator at 10 °C. The coagulated pectin was filtered and washed using 70% acidic ethanol (0.5% HCl), followed by 70% and then 96% ethanol. Samples were dried at 50 ºC for 12 - 24 h in a ventilated oven (035 Marconi MA - São Paulo, SP, Brazil). Pectin content was calculated from the dry weight of pectin related to the starting OB sample. All experiments were conducted in triplicate.

5.2.2.3 Cellulose-based materials characterization

5.2.2.3.1 Scanning electron microscopy (SEM)

SEM analyses were performed with a FEI Quanta 200 microscope (Oregon, USA) with an accelerating voltage of 30 kV. Dried samples were deposited in bronze stubs using double-sided tape, and the surfaces were then coated with a thin gold layer (40-50 nm).

5.2.2.3.2 X-ray diffraction (XRD)

The crystallinity of each sample was investigated using X-ray diffraction (XRD). The analysis was performed using a PANalytical X´Pert PRO MPD diffractometer (Netherlands) with copper Kα radiation (λ=1.5418 Ǻ) and operational conditions of 40 kV and 30 mA. The relative crystallinity index (CI) was calculated according to the Segal *et al*. method [29] as follows: CI (%): $([I_{002}-I_{am}]/I_{002})$*100, where I_{002} is the intensity of the 002 peak (at approximately 2θ = 20- 22°), and I_{am} is the intensity corresponding to the peak at 2θ = 18°.

5.2.2.3.3Thermogravimetric analysis (TGA)

The specimens were scanned from 30 °C to 500 °C at a rate of 20 °C min^{-1} under a nitrogen gas atmosphere using a Shimadzu TGA 50 thermogravimetric analyzer (Japan). T_{10} was obtained from TGA curves, and it was considered the temperature corresponding to a 10% mass loss.

5.2.2.3.4 Moisture sorption isotherms

Samples (500-800 mg) were dried at 60°C in an air-circulating oven (Marconi MA 415, Piracicaba, Brazil). The isotherms were generated by the dynamic dew point method through an isotherm generator (AquaSorp, Decagon Devices Inc., USA). Isotherms were obtained by plotting the equilibrium moisture content versus water activity, which ranged from 0.1 to 0.8 at 25 °C. The GAB (Guggenheim-Anderson-de-Boer) model [30] was calculated by Sorptrack 1:14 software (Decagon Devices, USA) and can be expressed as follows: $M = (m_0CKaw)/((1 – Ka_w)(1-Ka_w + CKa_w))$, where M is the equilibrium moisture (g water / 100 g solids), a_w is water activity, m_0 is the monolayer value (g water/ 100 g solids), and C and K are GAB constants.

5.2.2.3.5 Functional properties

Water-holding capacity (WHC) and oil-holding capacity (OHC) were determined according to Chau *et al*. [31] with modifications. Samples (2 g) were mixed with 20 mL of distilled water (WHC) or soybean oil (OHC) for 30 min at 75 rpm in a shaker (Quimis Q 225M, Brazil). The resulting suspensions were centrifuged at 2200 x g for 30 min, and the supernatant volumes were measured. WHC and OHC were expressed as g of water or oil held per g of sample, respectively. Solubility in water was calculated from the difference between the weight of undissolved residue and the original weight of the sample. All tests were conducted in triplicate.

The swelling capacity of samples was determined according to Robertson *et al*. [32]. Each sample (1 g) was stirred for 2 h with 20 mL of distilled water in a 25 mL graduated cylinder. The suspension was allowed to stand for 18 h to achieve complete hydration and sedimentation equilibrium. The swelling capacity was expressed as the volume occupied by the sample per g of original sample dry weight. All tests were conducted in triplicate.

5.2.2.3.6 Atomic force microscopy (AFM)

AFM was performed using a NanoSurf FlexAFM microscope (Liestal, Switzerland) for nanocellulose morphology analysis. The analysis was conducted in air, and the images were obtained in intermittent contact mode. The scan was performed in the free oscillation frequency with different amplitudes, and the setpoint was 30–50% of the amplitude.

5.2.2.3.7 Statistical analysis

Analyses of Tukey's mean comparison test ($p \leq 0.05$) were performed using R software (S R Core Team R, Austria, 2016).

5.3 Results and discussion

5.3.1 Cellulose-based materials production

Cellulose, hemicellulose, and lignin contents of raw OB were 12.4, 7.5, and 8.9%, respectively (Table 1), and the pectin content was 12.3%. OB had a lower lignin content than other lignocellulosic materials, such as sugarcane bagasse (20%) [12], oat husk (26.1%) [10], corn stover (19.6%) [15], and rice husk (20%) [14], which can be considered an advantage. Lignin plays a role in reinforcing and waterproofing plant cell walls, and lignin-carbohydrate complexes restrict polysaccharide accessibility. Hence, high lignin contents increase the recalcitrance of biomass [36], making it more challenging to extract cellulose.

All one-step alkaline treatments were effective in obtaining cellulose-based materials, resulting in higher cellulose contents when compared to raw OB (Table 1). A significantly (Tukey test, $p \leq 0.05$) higher cellulose content was obtained for the sample processed by autoclaving (AC_{NaOH}), which presented 71.1% cellulose, followed by the sample subjected to the chemical (CH_{NaOH}) treatment (54.7% cellulose) and ultrasonication (US_{NaOH}) (33.7% cellulose). The alkaline treatment promotes solubilization and dissolution of lignin, and the removal of hemicellulose and pectin by breaking the ester linkages between carboxyl groups of pectin and hydroxyl groups of hemicellulose without significantly affecting the cellulose structure [1, 7, 25].

Combining physical and chemical processes can be considered an excellent approach to reduce energy consumption, environmental impacts, and overall processing costs [37]. Debiagi et al. [4] reported that the combination of autoclaving with an acid or alkaline medium is efficient in the treatment of lignocellulosic materials to obtain cellulose-rich materials. According to Abdullah et al. [38], autoclaving treatment is considered an effective method for deconstructing the three-dimensional structure of lignocellulosic materials, resulting in cellulose-rich materials. The steam pretreatment modifies the lignocellulose structure by hydrolyzing the hemicelluloses and modifying the lignin [39].

Table 1 - Cellulose-based materials obtained from raw orange bagasse (OB) by one-step alkaline treatments and nanocellulose production: cellulose, hemicellulose and lignin contents, cellulose yield and crystallinity index (CI).

One-step alkaline treatments	Cellulose content (%)	Hemicellulose content (%)	Lignin content (%)	Cellulose recovery (%)*	Process yield (%)**	CI (%)
Raw OB	12.4 ± 0.4^{d}	7.5 ± 0.1^{a}	8.9 ± 0.5^{c}	-	-	18
CH_{NaOH}	54.7 ± 1.0^{b}	0.0 ± 0.0^{b}	9.1 ± 0.1^{c}	100	25	26
AC_{NaOH}	71.1 ± 1.0^{a}	0.0 ± 0.0^{b}	19.0 ± 0.5^{a}	82	14	42
US_{NaOH}	33.7 ± 1.0^{c}	7.8 ± 0.1^{a}	10.0 ± 0.1^{b}	97	36	21
Stages for nanocellulose production						
$CH_{NaOH/PA}$	85.4	0.0	3.3	65.3	9.5	43
CNF_{OB}	100	0.0	0.0	35.5	4.4	60

$^{a-i}$- Different letters in the same column indicate significant differences ($p \leq 0.05$) between means (Tukey's test) in materials obtained from OB by one-step alkaline treatments.

*Cellulose recovery: cellulose content in relation to the original cellulose content of raw OB (dry basis).

**Process yield: the amount of cellulose-based material obtained for each 100 g of raw OB (dry basis).

Complete hemicellulose removal was observed for CH_{NaOH} and AC_{NaOH} samples (Table 1), but total lignin removal was not possible in any treatment employed in this study. According to Bhowmick et al. [40], cellulose and lignin are more resistant to degradation than hemicellulose, which can be attributed to the less organized structure of hemicellulose.

As observed in Table 1, the cellulose recovery (% of cellulose in relation to the original cellulose content of raw OB) in one-step alkaline treatments ranged from 82 to 100%, indicating that there was little loss of the original cellulose during the employed processes, which possibly occurred in the washing steps. The process yields were lower as the cellulose content in samples increased (Table 1), which agreed with Bicu and Mustafa [8], who reported that maximum cellulose contents result in minimum yields.

Table 1 also shows the sample yields and cellulose content obtained after each step to obtain nanocellulose. Nanocellulose was obtained from OB in three sequential steps. Initially, the CH_{NaOH} sample was subjected to an additional bleaching step with peracetic acid ($CH_{NaOH/PA}$), resulting in a material with higher cellulose (85.4%) and lower lignin (3.3%), and complete removal of hemicellulose. Peracetic acid is an efficient chlorine-free bleaching reagent, and according to Liang and Wang [41], it is considered a highly selective delignifying agent due to its capacity to oxidize electron-rich structures, such as aromatic rings of lignin.

To obtain nanocellulose, the $CH_{NaOH/PA}$ sample was treated with sulfuric acid, and every 100 g of raw OB yielded 4.4 g of nanocellulose (Table 1). Tsukamoto et al. [28] obtained nanocellulose from orange bagasse using an enzymatic treatment and reported yields of 3%. Cypriano et al. [22] reported a yield of 1.4% nanocellulose with 98% purity using orange bagasse as raw material.

5.3.2 Cellulose-based materials characterization

5.3.3 Scanning electron microscopy (SEM)

Figure 1 shows the surface morphology of raw OB and cellulose-based materials. Raw OB presented a compact aspect with the surface covered with hemicellulose and lignin constituents, and according to Debiagi *et al*. [4], the presence of H-bonding, covalent O-H bonds and Van der Waal interactions between cellulose, hemicellulose and lignin results in a compact structure observed by SEM. The materials changed from a compact surface structure in raw OB to irregular and porous outer surfaces after one-step alkaline treatments, indicating a partial removal of the non-cellulosic layer.

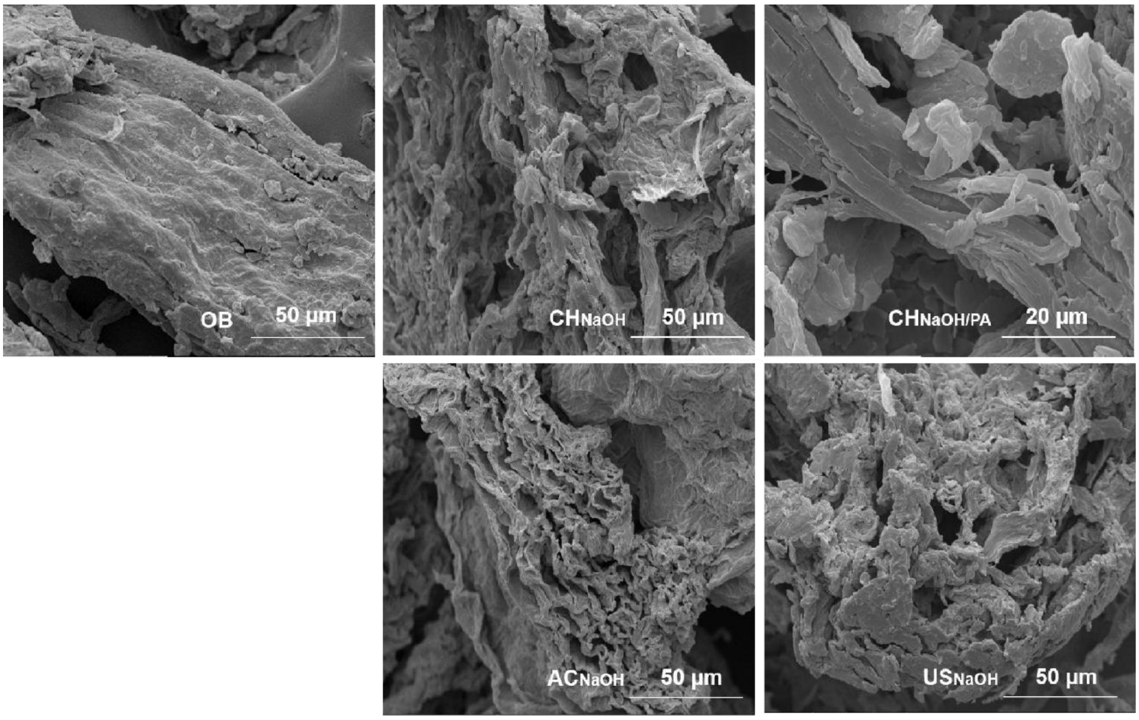

Figure 1 - Surface morphology obtained by scanning electron microscopy (SEM) of raw orange bagasse (OB) and cellulose-based materials from OB.

The sample subjected to the two sequential chemical treatments with NaOH and PA ($CH_{NaOH/PA}$) resulted in major differences when observed by SEM, and cellulosic fibers appeared as individualized bundles, confirming that lignin and hemicellulose removal was more effective in this sample.

5.3.4 X-ray diffraction (XRD)

The crystalline structure of the samples was analyzed by XRD (Fig. 2). All samples exhibited crystalline peaks at 2θ = 22°, and for cellulose-based materials, a new peak appeared at 2θ = 34.5°. The crystallinity index (CI) (Table 1) increased from 18% in raw OB to 43% in $CH_{NaOH/PA.}$ The highest CI occurred in samples with higher cellulose contents (AC_{NaOH} and $CH_{NaOH/PA}$). The increase in crystallinity is correlated with the increase in cellulose content and the reduction in the non-cellulosic fraction (lignin and hemicellulose), which corresponds to amorphous components of lignocellulosic residues [4, 9, 42–44].

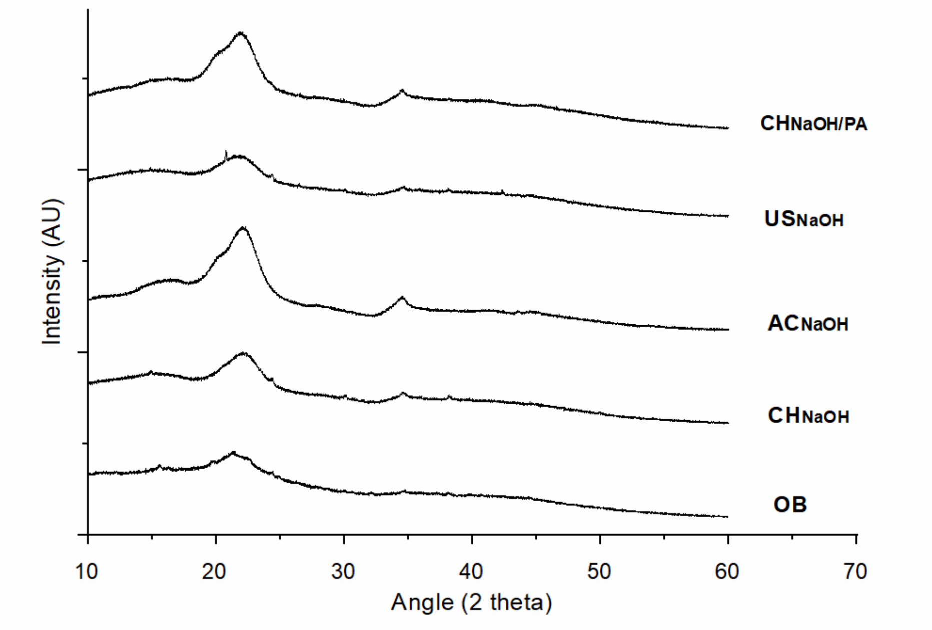

Figure 2 - X-ray diffractograms of raw orange bagasse (OB) and cellulose-based materials from OB.

5.3.5 Thermogravimetric analysis (TGA)

TGA curves of raw OB and OB subjected to alkaline treatments are presented in Fig. 3. All samples showed a small loss weight (< 10%) in a range from 50 to 100 °C caused by evaporation of water and other low molecular weight compounds [16]. Ibrahim *et al*. [39] reported that the first stage of weight loss (temperature below 200 °C) in lignocellulosic residues corresponds to the evaporation of water or low molecular weight compounds, which results in mass losses < 10%.

A remarkable slope of the TG curves was observed in the thermograms, which corresponded to the second degradation stage, which showed a double-peak distribution at 256-273°C and 313-329 °C (Fig. 3) to raw OB, US_{NaOH} and $CH_{NaOH/PA}$ samples. The first set of peaks corresponded to hemicellulose decomposition and the second set of peaks to cellulose decomposition. According to Debiagi *et al*. [2] and Jacquet *et al*. [46], cellulose decomposition is attributed to the reactions of depolymerization, dehydration, and decomposition of glycoside units. A double-peak distribution in the second degradation stage of lignocellulosic materials was also observed by Abdullah *et al*. [35], who reported degradation temperatures with similar values to those found in this study.

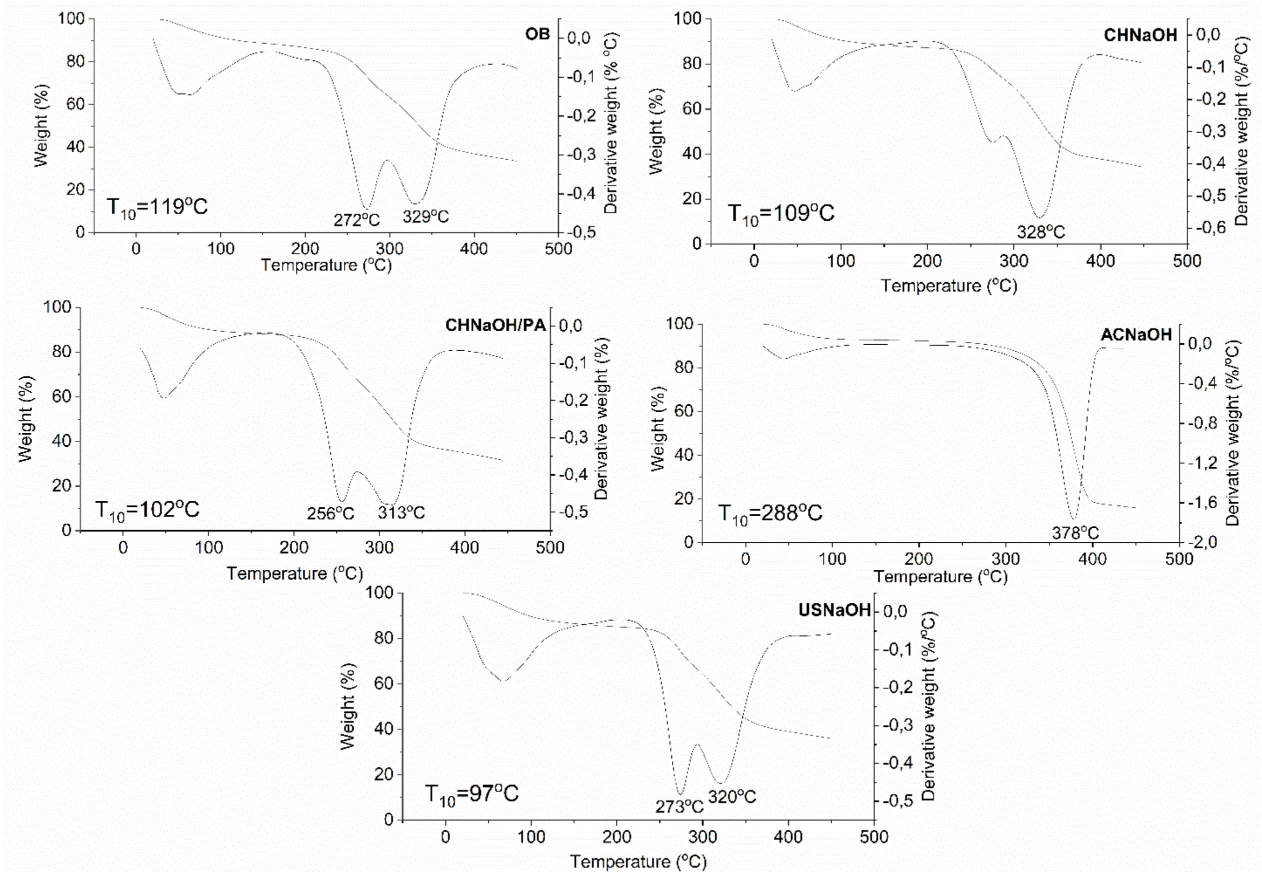

Figure 3 - TGA curves of raw orange bagasse (OB) and cellulose-based materials from OB.

AC_{NaOH} and CH_{NaOH} samples also presented an important slope in their thermograms; however, a single-stage was observed for these samples at 378 and 328 °C (Fig. 4), respectively. Debiagi *et al*. [4] also reported that after bleaching, oat hulls presented a single peak in TGA curves, while the raw oat hull presented a double-peak distribution, which was attributed to the more complex material composition when compared to bleached samples.

T_{10} is the initial decomposition temperature at 10% degradation of the sample. The T_{10} values were obtained from the TGA curves (Fig. 3), and all samples presented values ranging from 97-119 °C (Fig. 3), except for the AC_{NaOH} sample, which showed a value of 288 °C, which possibly may be related to its higher lignin content (Table 1), which resulted in a more thermally stable sample. According to Poletto *et al*. [47], lignin is thermally more stable than cellulose or hemicellulose.

5.3.6 Sorption isotherms of cellulose

Fig. 4 shows moisture sorption isotherms, and the GAB model parameters are shown in Table 2. All samples presented the typical Type II (sigmoidal) isotherm reported in the literature for other cellulosic materials [16, 48], and the GAB model provided for all samples had a good fit ($R^2 = 0.99$). The equilibrium moisture content of all samples increased according to the increase in water activity (a_w). The values of equilibrium moisture content were close to each other, except for the AC_{NaOH} sample, which presented the lowest equilibrium moisture contents at all a_w values.

The monolayer value (m_0) can be considered indicative of the hygroscopicity of the sample, representing the maximum amount of water that can be adsorbed on a single layer per gram of dry matter [49]. In this study, m_0 ranged from 3.51 to 7.74 g water/100 g solids (Table 2), and the AC_{NaOH} sample showed the lowest m_0 value among all samples, while the highest value was observed for CH_{NaOH}. Possibly, the lowest m_0 value observed for AC_{NaOH} can be explained by the higher crystallinity of this sample and also by its higher lignin content. Nair and Yan [50] reported that the strong crosslinking between lignin with cellulose and hemicellulose is generally an obstacle to water absorption.

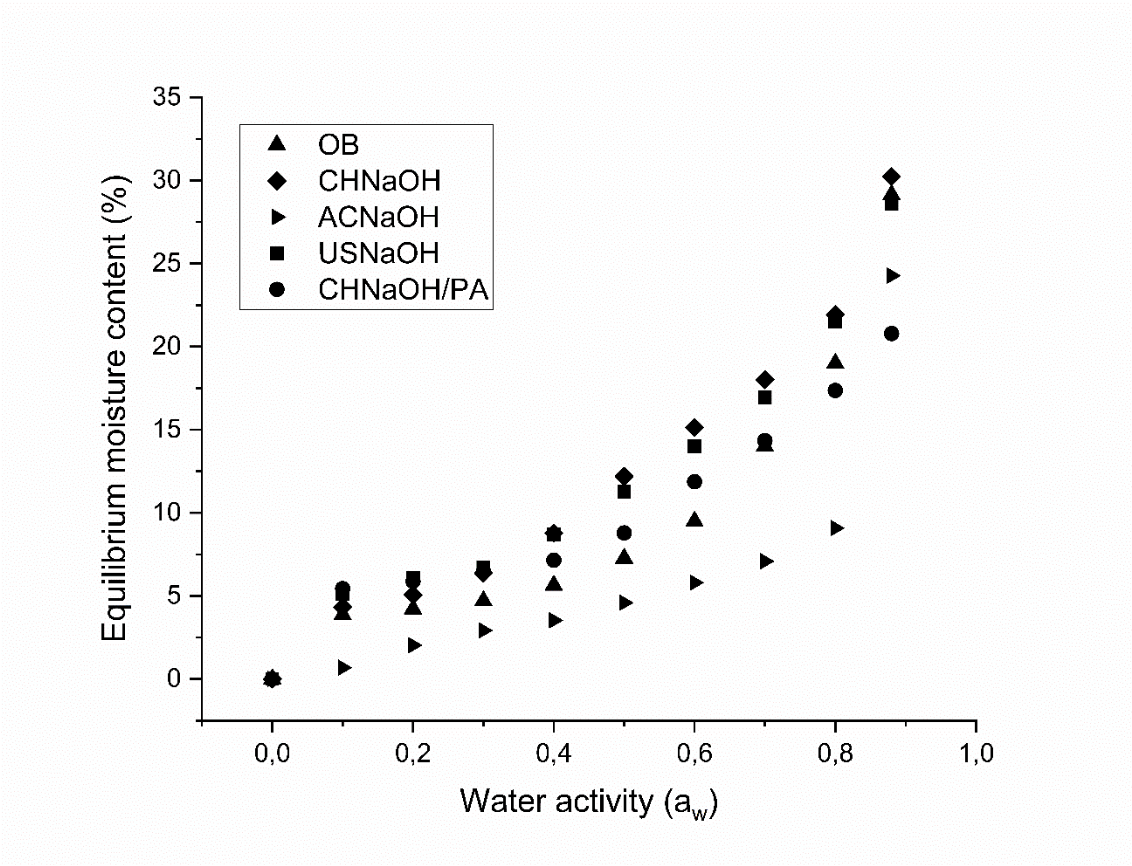

Figure 4 - Water sorption isotherms of raw orange bagasse OB and cellulose-based materials obtained from raw OB.

Table 2 - GAB model* parameters of raw orange bagasse (OB) and cellulose-based materials obtained from raw OB.

Samples	Monolayer (M_0) (g/100 g)	C	K	R^2
OB	4.25	13.40	0.98	0.99
CH_{NaOH}	7.74	6.35	0.86	0.99
AC_{NaOH}	3.51	4.32	0.82	0.99
US_{NaOH}	6.54	22.62	0.88	0.99
$CH_{NaOH/PA}$	5.64	45.34	0.84	0.98

$M = m_0CKa_w / (1 - Ka_w) (1 - Ka_w + CKa_w)$, 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 constants.

The raw OB had the second-lowest m_0 value (4.25 g/100 g), possibly because of its compact surface structure without pores. According to Cunha *et al.* [51], the mechanisms involved in cellulose-water interactions at different water activities are not completely clear. Mihranyan *et al*. [52] reported that the understanding of the mechanisms of moisture sorption of cellulosic materials is complicated because of the complexity of their structure. The moisture sorption in cellulosic materials depends on their composition and structural properties, such as surface area, pore volume and crystallinity. Higher porosities and surface areas result in higher moisture adsorptions, while higher crystallinity indicates lower hydroxyl groups available to interact in the material surface, resulting in lower adsorption of water.

Both US_{NaOH} (6.54 water/100 g solids) and CH_{NaOH} (7.74 g water/100 g solids) samples presented the highest m_0 values (Table 2), and also very close CI values, 21 and 26%, respectively (Table 1). However, their CI values were higher than the CI of raw OB (CI = 18%). Possibly the most critical factor for the increased water adsorption capacity of these samples was their surface morphology, which was characterized by higher porosity and surface area observed by SEM (Fig. 1) when compared to raw OB.

The $CH_{NaOH/PA}$ sample showed a m_0 value of 5.64 g/100 g (Table 2), and despite its CI (43%) being very close to the CI of the AC_{NaOH} sample (42%), the higher surface area observed by SEM (Fig. 1) possibly controlled the adsorption process. Additionally, its lower lignin content when compared to AC_{NaOH} influenced the water adsorption process.

5.3.7 Functional properties

Several factors can affect the functional characteristics of cellulosic materials, such as their non-cellulosic fraction content, particle size, porosity, and crystallinity [53-55]. In this study, the cellulose extraction methods resulted in differences in the chemical composition, morphology, and crystallinity of the obtained materials, which consequently affected their functional characteristics (Table 3).

Table 3 - Functional characterization of raw orange bagasse (OB) and cellulose-based materials obtained from raw OB.

Sample	WHC (g water / g solid)	OHC (g oil / g solid)	Swelling (mL water / g solid)	Solubility (%)
OB	4.56 ± 0.18^{c}	1.30 ± 0.12^{b}	8.83 ± 0.29^{bc}	12.21 ± 0.12^{b}
CH_{NaOH}	5.85 ± 0.36^{b}	0.90 ± 0.18^{c}	9.27 ± 0.25^{b}	13.05 ± 0.23^{a}
AC_{NaOH}	3.10 ± 0.11^{d}	0.78 ± 0.03^{c}	8.43 ± 0.12^{c}	2.05 ± 0.31^{e}
US_{NaOH}	8.96 ± 0.14^{a}	1.67 ± 0.14^{a}	17.17 ± 0.29^{a}	5.85 ± 0.14^{c}
$CH_{NaOH/PA}$	2.56 ± 0.06^{d}	0.81 ± 0.04^{c}	4.83 ± 0.29^{d}	4.65 ± 0.22^{d}

$^{a\text{-}i}$- Different letters in the same column indicate significant differences ($p \leq 0.05$) between means (Tukey's test) in materials obtained from OB by one-step alkaline treatments.

It can be observed from Table 3 that the US_{NaOH} sample presents the highest values of WHC (8.96 g/ g), OHC (1.67 g/ g), and swelling (17.17 mL/ g) when compared to raw OB and other treatments. According to Hassan *et al*. [56], ultrasonication is an efficient physical process to remove hemicellulose and lignin from residues. Its mechanism is attributed to cavitation, in which the impact produced by the collapse of bubbles results in the alteration of the surface structure of lignocellulosic materials, favoring the porosity of materials, which may have contributed to the increase in WHC, OHC, and swelling. Besides, US_{NaOH} was the only sample that contained hemicellulose (7.8%) after treatment (Table 1). Hemicelluloses have branched and amorphous structures, which contribute to a high-water holding capacity [57].

WHC values in this study were very close to the values obtained by other authors. Zain *et al*. [58] extracted cellulose from *Citrus grandis* via alkali treatment followed by $NaClO_2$ bleaching and obtained a WHC of 8.9 g/g. Zhang *et al*. [55] obtained cellulose from orange peel and they reported WHC values of 8.64 and 6.24 g/g after two different pectin extraction processes and reached 13.25 g/g after alkaline treatment. Alkaline chemical treatment could increase the citrus fibers water holding capacities, according to Zhang *et al*. [55].

The lowest values of WHC, OHC, swelling, and solubility were obtained by AC_{NaOH} (CI = 42%) and $CH_{NaOH/PA}$ (CI = 43%) samples, which presented the highest cellulose contents and crystallinity. In cellulosic materials, higher crystallinity results in lower hydration capacity [53]. According to Mihranyan *et al*. [52], the ability of water to penetrate the structure and cause swelling will depend on the number of available hydroxyl groups or, in other words, the degree of crystallinity.

Cellulosic materials with high WHC and swelling capacity values can be interesting to incorporate into food products as dietary fiber sources, such as baked products, jam or fruit juices, meats, sauces, dressings, and dairy products [17, 18]. Fibers with higher oil adsorption capacity can help the processing of fatty foods [59]. Dietary fiber for pet food is also a possibility [60]. Amorphous materials can be used in fermentation processes because they were more accessible to enzymatic attack than crystalline materials. AC_{NaOH} and $CH_{NaOH/PA}$ showed a higher degree of purification, and they can be considered interesting alternatives of low-cost cellulose to obtain nanocellulose, as performed in this study.

All treated samples presented reduced solubility values when compared to raw OB (Table 3), except for the CHNaOH sample, which mainly occurred because the alkaline treatments were able to remove water-soluble pectin from OB [25].

5.3.8 Characterization of nanocellulose: X-ray diffraction (XRD) and atomic force microscopy (AFM)

The nanocellulose was obtained in three successive stages in this study, and it was possible to observe the progressive changes that occurred from the raw material to nanofibers from OB, labeled as CNFOB. Removal of the amorphous non-cellulosic fraction at each step resulted in increased crystallinity with chemical (Table 1) and morphological modifications (Fig. 1).

After the first chemical treatment (CH_{NaOH} sample), a considerable disruption of the rigid structure of the lignocellulosic complex was observed (Fig. 1). The resulting sample showed a moderate increase in cellulose content (54.7%) and crystallinity (26%), total elimination of hemicellulose, and partial removal of lignin (Table 1). Sodium hydroxide can break ester bonds and release hemicelluloses [8].

The following treatment with peracetic acid ($CH_{NaOH/PA}$ sample) promoted more severe changes with a significant cellulose content increase (85.4%), exposing the cellulosic fibers individually (Fig. 1). The role of peracetic acid is to delignify by oxidizing aromatic rings of lignin [41, 61]. After this treatment, the crystallinity increased to 43% (Table 1), and the surface area of the material increased. These previous steps were necessary for reducing the noncellulosic fraction, allowing an effective acid attack to obtain nanofibers. Hideno et al. [26] also reported that the elimination of layer compounds around cellulose microfibrils is crucial for the preparation of nanofibers from orange peels.

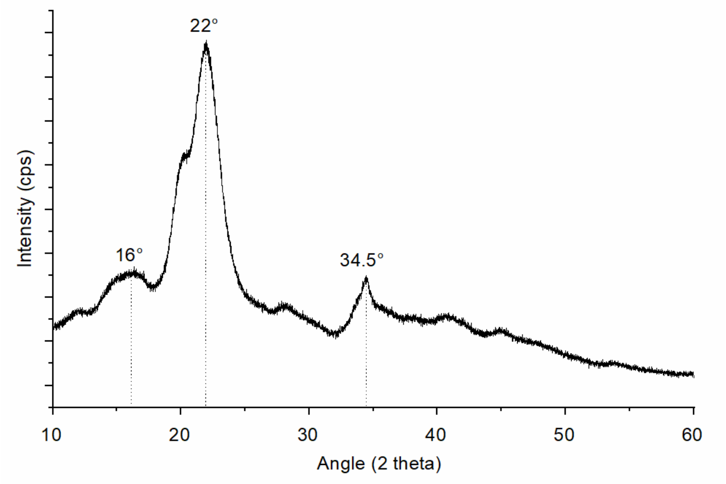

Figure 5 - X-ray diffractogram of nanocellulose from orange bagasse (CNF$_{OB}$).

After acid hydrolysis, a sample with 100% cellulose and 60% crystallinity was obtained (Table 1). The nanocellulose sample exhibited crystalline peaks at 2θ = 16°, 22°, and 34.5° (Fig. 5). All processes employed in this study to obtain nanocellulose resulted in any variation in the polymorph type of cellulose compared to the raw material.

It can be observed from the atomic force microscopy (AFM) images (Fig. 6) that the nanocellulose sample (CFN$_{OB}$) obtained in this study can be classified as nanofibrillated cellulose, with the aspect of interconnected webs of tiny nanofibers with diameters between 25 and 45 nm and lengths of several micrometers (3–4 μm), resulting in materials with aspect ratios between 66 and 160 nm, which can be considered high values [27]. Mariño et al. [27] studied the obtainment of cellulose nanofibers from orange bagasse, resulting in a material with 55% crystallinity, an average diameter of 10 nm, and a length of 458 nm after alkaline, bleaching, and enzymatic processes. Hideno et al. [26] produced cellulose nanofibers from orange peel with CI values ranging from 36.4 to 59.2% using three treatments (boiling, alkaline, and pectinase) combined with subsequent grinding treatment. Mariño et al. [6] employed several steps to obtain nanocellulose from industrial orange bagasse: acidic pectin extraction, alkaline cellulose extraction, sodium chlorite followed by alkaline peroxide bleaching, and cellulose nanocrystal preparation by different alkali or acid methods. The CI varied from 55 to 77%, and cellulose nanofibers showed mean diameters of 20.5 nm ± 7.0 nm. Cypriano et al. [22] found the same CI value (60%) for nanocellulose obtained from orange juice residue obtained by enzymatic hydrolysis.

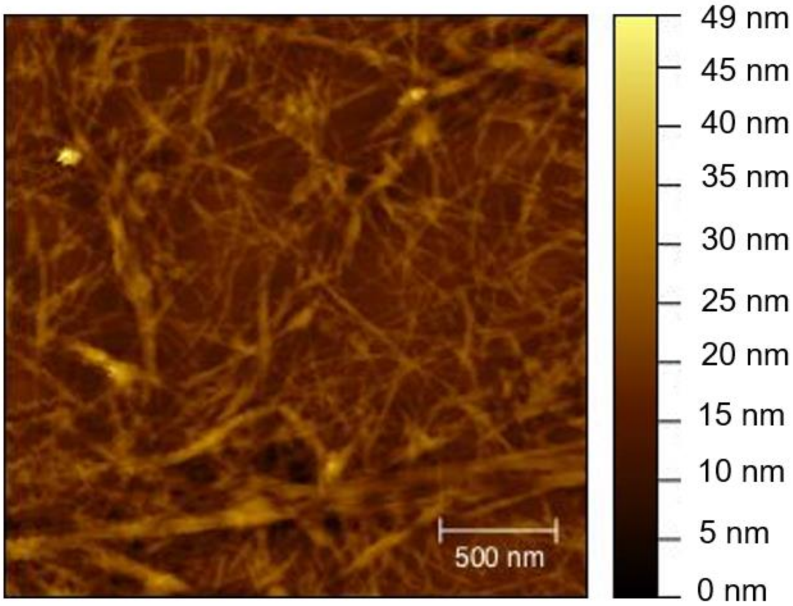

Figure 6 - Morphology of nanocellulose from orange bagasse (CNFOB) obtained by atomic force microscopy (AFM).

5.4 Conclusion

Orange bagasse (OB) was able to supply various cellulose-based materials through less polluting treatments with a reduced number of steps. Among the one-step treatments, alkaline combined with autoclaving was more efficient, resulting in the highest cellulose content (71.1%) and crystallinity index (42%).

Crystallinity indexes and the surface morphology affected the moisture sorption capacity and the functional properties of the samples. It was observed a decrease in water and oil holding capacities, swelling in water, and solubility with an increase in crystallinity. Porous surfaces resulted in higher water and oil holding capacities, swelling in water, and solubility.

Nanofibrillated cellulose was prepared in three stages, resulting in a material with 100% cellulose and 60% crystallinity, with a yield of 4.4%. Each 100 g of raw OB subjected to the sequential treatments yielded 4.4 g of nanocellulose.

Cellulose-based materials obtained in this study can be incorporated into food products as dietary fiber sources, such as baked products, jam or fruit juice, meats, sauces and dressings, dairy products, substrates in fermentation processes or even in animal food. The highest cellulose content samples can be marketed as low-cost sources of cellulose to be used in nanocellulose obtainment, as performed in this study.

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6. CAPÍTULO IV – ARTIGO III: MODIFICATION OF ORANGE BAGASSE BY REACTIVE EXTRUSION PROCESS TO OBTAIN CELLULOSE-BASED MATERIALS

Modification of orange bagasse by reactive extrusion process to obtain cellulose-based materials

ABSTRACT

Orange bagasse (OB) could be considered a sustainable, renewable and low-cost biomass for extraction of cellulose. In this context, reactive extrusion can be considered an excellent eco-friendly process alternative to obtain cellulose from lignocellulosic materials. Thus, the present study aimed to obtain cellulose-based materials by reactive extrusion process, and also to investigate the impact of pectin on the delignification process. For this, two samples were submitted to extrusion, OB and removed-pectin OB. Combinations with sulfuric acid and sodium hydroxide in one-step processes were tested. It was observed that the cellulose content of extrudate materials were highly affected by pectin content in the raw material; the thermal profile (TGA curves) and crystallinity also changed. The cellulose content of materials obtained from OB ranged from 18.8 to 58.4%, with a process yield of 30.6 to 79.2 %. The alkaline reagent provided the highest cellulose content among all treatments tested, mainly for OB without pectin. Extrusion process was considered efficient and sustainable for cellulose obtainment from orange bagasse. Materials obtained in this study can be used as cellulose fiber source for various products and processes, such as in food industry, fermentation substrates or refined applications after subsequent treatments.

Keywords: One-step process; eco-friendly; pectin; alkaline treatment.

6.1 Introduction

In general, each agro-industrial residue requires specific studies to optimize its use for cellulose production due to the great variation in its chemical composition, and preferably meeting the current demands for sustainable and competitive technological solutions. Conventional processes use a lot of energy and reagents, including chlorinated agents, which results in toxic effluents (Mantovan *et al*., 2020; 2021).

In this context, reactive extrusion can be considered an excellent alternative process to obtain cellulose from lignocellulosic materials because it promotes high productivity, lower effluents generation, lowest overall processing cost and short-duration processes (Debiagi *et al*., 2020a; 2020b; Merci *et al*., 2015). Extrusion is an important technique in polymers and food industry, being efficiently employed to cereal brans modification, but few explored for obtention of cellulose-based materials from fruit wastes, mainly orange bagasse (Garcia-Amezquita *et al*., 2019; Huang *et al*., 2019).

By-products are generated in large amounts by the orange (*Citrus* × *sinensis* (L.) processing industry for juice extraction followed by essential oil extraction. It contains peel, albedo and seed, which corresponds to approximately 50% of the fruit weight (Cypriano *et al*., 2018; Ricci *et al*., 2019). The most popular citrus fruit has a well-established global consumer market where Brazil is the leading country in oranges and orange juice production (USDA, 2019). Usually, it is destined for animal feed supplement after a pelletize process or dumped directly in landfill. However, the citrus pulp-pellets production requires expensive energy resources and show few nutritional benefits without previous treatment. Furthermore, the improper disposal generates greenhouse gases and risk of pollution for water courses due to the citrus residue characteristics, as low pH (between 3 and 4), water content about 80 and 90% and high organic matter concentration (about 95% of total solids) (Negro *et al*., 2016; Siles *et al*., 2016).

Orange biorefinery proposal is an alternative to economic and environmental challenges and gains importance from the abundance and varied composition of the orange bagasse. Thus, several studies propose applications to this residue, like production of adsorbent biomaterials for pollutants (Tovar *et al*., 2019), biogas and bioethanol obtaining (Lohrasbi *et al*., 2010), hesperidin extraction (Cypriano *et al*., 2018), source of pectin and soluble sugar (López *et al*., 2010). The orange bagasse lignocellulosic structure also provides cellulose fibers and nanocellulose through specific extraction methods, but few studies explore this function (Bicu and Mustata, 2011; 2013; Tsukamoto *et al*., 2013 Hideno *et al*., 2014; Mariño *et al*., 2015; Cypriano *et al*., 2018; Mariño *et al*., 2018).

The cellulose-based materials have many applications, acting as precursors for obtaining various products of industrial interest, such as membranes, hydrogels, and nanocellulose. Besides that, they can also can be applied as dietary fibers and as a substrate in fermentation processes (Mariño *et al*., 2018; Weng *et al*., 2020; Xie *et al*., 2020). Cellulose-based materials, including nanocellulose and dietetic fibers, obtained by extrusion showed promising characteristics, such as thermal stability, higher crystallinity indexes (Debiagi *et al*., 2018), hypoglycemic, cholesterol-lowering and fermentation capacities. The chemical and functional modification caused by the extrusion is capable of adapting the raw material to various applications, thus producing materials with high added-value (Huang *et al*., 2019).

In this research work, orange bagasse and pectin-removed orange bagasse were processed by reactive extrusion to obtain cellulose based-materials. Composition, crystallinity and thermal stability were analyzed. The use of an abundant waste as raw material and the one-step extrusion process are the key to producing economically competitive and eco-friendly materials. In addition, the extracted pectin can be directed to future food applications. Thus, the field of application can be extended to areas such as food, health and the environment, contributing to a proposal for biorefinery and circular economy.

6.2 Material and methods

6.2.1 Material

Orange bagasse (OB) was provided by an orange-juice processing industry from Paraná, Brazil (Integrada Cooperativa), dried at 50 °C in an air-circulating oven (Marconi MA 415, Piracicaba, Brazil) and milled to yield particles < 0.3 mm (IKA-A 11 Basic Mill, Germany). The chemical reagents used were: sodium hydroxide PA (NaOH beads, Synth, Brazil), sulfuric acid PA-ACS (H_2SO_4, 99 %, Synth, Brazil) and chloride acid PA-ACS (HCl, 36 %, Synth, Brazil).

6.2.2 Methods

6.2.2.1 Extraction of cellulose-based materials by reactive extrusion

Two initial materials were employed to study the production of cellulose-based materials: raw OB (OB) and depectinizated OB (OB-P). The reagents employed were NaOH (10% w/w) and H_2SO_4 (2% w/w); water was used as control. The samples were labeled respectively as EX_{NaOH}, $EX_{H2SO4,}$ EX_{H2O} for OB samples, and EXP_{NaOH}, $EXP_{H2SO4,}$ EXP_{H2O} for OB-P samples subjected to reactive extrusion.

The reactive extrusion steps were performed using a single-screw extruder constructed from special grade of corrosion-resistant stainless-steel (AX Plastics, Diadema, SP, Brazil) with a 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 120 °C, and the screw speed was 50 rpm. One hour before extrusion of the samples, the reagents were dissolved in distilled water and homogenized with the raw OB in sealed plastic bags. All samples (120 g) were extruded with an initial moisture content of 40% (w/w). After, the samples were washed with water and neutralized to pH 5–6, dried in a ventilated oven at 40 °C (035 Marconi MA, Brazil) and milled (particles 0.3 mm).

6.2.2.2 Cellulose, hemicellulose, lignin and pectin contents

The chemical composition of raw OB and cellulose-based materials was analyzed for cellulose, hemicellulose and lignin contents. Cellulose and hemicellulose were measured by the Van Soest (1965) method, and lignin content was measured by the standard method of the Technical Association of Pulp and Paper Industry (TAPPI T222 om-88).

The pectin content of raw OB was extracted and determined in the raw OB according to Kratchanova *et al*. (2004) method with some modifications: the dry OB sample (20 g) was mixed with 800 mL of extraction solvent (HCl 0.05 M) and then heated to 80 °C in continuous stirring for 1 h. The solid fraction (fibers) was separated by filtration, and pectin was precipitated from the liquid fraction using an equal volume of 96 % ethanol and kept for two hours in a refrigerator. The coagulated pectin was filtrated and washed using 70 % acidic ethanol (0.5 % HCl), followed by 70 % ethanol and then 96 % ethanol. Samples were dried at 50 ºC for 12 - 24 h in a ventilated oven (035 Marconi MA - São Paulo, SP, Brazil). Pectin content was calculated from the dry weight of pectin related to the starting OB sample. All experiments were conducted in triplicate.

6.2.2.3 Extruded cellulose-based materials characterization

6.2.2.3.1 Fourier transform-infrared spectroscopy (FTIR)

FTIR analysis was carried out in a Shimadzu FT-IR - 8300 equipment (Japan), with a spectral resolution of 4 cm^{-1} and a spectral range of 4000 - 500 cm^{-1}. Dried samples were mixed with potassium bromide and compressed into tablets.

6.2.2.3.2 X-ray diffraction (XRD)

The crystallinity of each sample was investigated using X-ray diffraction (XRD). The analysis was performed using a PANalytical X´Pert PRO MPD diffractometer (Netherlands) with copper Kα radiation (λ=1.5418 Å) and operational conditions of 40 kV and 30 mA. The relative crystallinity index (CI) was calculated as follows: CI (%): $([I_{002}-I_{am}]/I_{002})*100$, where I_{002} is the intensity of the 002 peak (at approximately 2θ = 20- 22°) and I_{am} is the intensity corresponding to the peak at 2θ = 18°.

6.2.2.3.3 Thermogravimetric analysis (TGA)

The thermal stability of samples was determined using a Shimadzu thermogravimetric analysis (TGA) 50 thermogravimetric analyzer (Japan). The specimens were scanned from 30 °C to 900 °C at a rate of 20 °C min^{-1} under a nitrogen gas atmosphere. T10 was obtained from TGA curves, and it was considered the temperature corresponding to a 10% mass loss.

6.2.2.4 Statistical analysis

Analyses of Tukey's mean comparison test ($p \leq 0.05$) were performed using R software (S R Core Team. R, Austria, 2016).

6.3 Results and discussion

6.3.1 Extraction of cellulose-based materials by extrusion

The orange bagasse (OB) was selected as a sustainable, renewable and low-cost biomass. In this study, the raw OB showed 12.4 ± 0.6% cellulose, 7.5 ± 0.1% hemicellulose, 8.9 ± 0.5% lignin and 12.3 ± 0.7% pectin, totaling 28.8% of the insoluble dietary fiber content (cellulose, hemicellulose and lignin). The cellulose, hemicellulose, lignin and pectin contents related in literature are approximately 18-20, 6-14, 4-6 and 18 %, respectively (Cypriano *et al*., 2018; De la Torre *et al*., 2017). The cellulosic fraction of the orange bagasse has great potential for exploration, as the residue has high availability in Brazil. Pectin, as an abundant polymer in orange fruits, can contribute strongly to the sustainable proposal for use of orange bagasse. It has been widely used in food industry as a thickener, emulsifier, and stabilizer. In addition, this soluble fiber shows many benefits for human health (Beukema *et al*., 2020; Jridi *et al*., 2020). Thus, we suggest that the pectin fraction extracted in this study can be employed to future applications, although it is not the focus of this article.

Orange bagasse (OB) and pectin-removed orange bagasse (OB-P) were modified by extrusion conditions to obtain cellulose based-materials. Table 1 shows the modifications on the lignocellulose fractions caused by extrusion. Extrusion processing significantly increased the cellulose content in all samples tested, including water treatment. Extrusion process is an energy-efficient mechanical treatment for lignocellulose. Processing occurs by heat, means of compression, and shear forces, increasing the surface area and pore size leading to physical and chemical disruption (Liu *et al*., 2013).

In the previous work of this group (Mantovan *et al*., 2020), the orange bagasse processed by an isolated alkaline treatment had a similar cellulose content (54.7%) compared to EXP_{NaOH} (58.4%), however the process yield was 25%, and in the present study the yield was 35% (Table 1), in addition, the pectin could be recovered for future application and the extrusion process provides a drastic reduction of effluents. The combined processes (physical and chemical) in one-step are promising approaches to reduce environmental impacts and costs (Mantovan *et al*., 2021; Marim *et al*., 2020).

The hemicellulose contents of extruded samples were close to OB sample, ranging from 7.4 to 10.6%, except for EXP_{NaOH} sample (OB-P extruded with

NaOH), which presented the lower hemicellulose content (3.5%) (Table 1). Lignin content for all samples increased in relation to raw OB. The elimination of pectin increased the relative proportion of lignin and hemicellulose in extruded samples, even though mass loss had occurred, which resulted in the decrease of the process yields for these samples (Table 1). Possibly, a certain amount of cellulose is also degraded during reactive extrusion, only samples extruded with water (EX_{H2O} and EXP_{H2O}) did not show losses in its cellulose yields, the both samples presented 100% of cellulose yield (Table 1). Generally, treatments do not achieve absolute selectivity for the non-cellulosic fraction (Bicu and Mustata, 2013; Mantovan *et al*., 2020), resulting in decreased in cellulose yields. The alkaline treatment combined with pectin extraction (EXP_{NaOH}) resulted in the sample with the higher cellulose content without compromising cellulose yield.

Table 1 - Cellulose, hemicellulose, lignin contents, process yield, cellulose yield and crystallinity index (CI) of raw OB and cellulose-based materials from OB obtained by extrusion.

Treatment	Cellulose content (%)	Hemicellulose content (%)	Lignin content (%)	Process Yield (%)*	Cellulose yield (%)**	CI (%)
OB	12.4 ± 0.4^{e}	7.5 ± 0.1^{c}	8.5 ± 0.4^{d}	-	-	18
EX_{H2O}	18.8 ± 1.6^{d}	10.6 ± 0.6^{a}	22.5 ± 1.2^{a}	79.2	100	38
EX_{H2SO4}	19.9 ± 0.5^{cd}	7.4 ± 0.2^{c}	23.7 ± 0.8^{a}	37.7	60.5	37
EX_{NaOH}	21.2 ± 0.3^{c}	8.8 ± 0.9^{bc}	20.1 ± 0.2^{bc}	43.5	73.4	45
EXP_{H2O}	31.8 ± 0.5^{b}	10.4 ± 1.1^{ab}	18.6 ± 0.2^{c}	68.5	100	42
EXP_{H2SO4}	33.3 ± 0.6^{b}	8.1 ± 0.4^{c}	20.8 ± 0.3^{b}	30.6	82.3	43
EXP_{NaOH}	58.4 ± 0.6^{a}	3.7 ± 0.2^{d}	19.4 ± 0.1^{bc}	35.5	91.3	51

$^{a-e}$- Different letters in the same column indicate significant differences ($p \leq 0.05$) between means (Tukey's test). *Process yield: the amount of cellulose-based material obtained for each 100 g of raw OB or raw OB-P (dry basis). **Cellulose recovery: cellulose content in relation to the original cellulose content of raw OB (dry basis).

The impact of pectin (soluble fiber) on the extrusion process was evaluated. The pectin-removed samples (EXP) showed a significant increase in cellulose content after being subjected to treatments with water, acid or alkali. Thus, the presence of the soluble fibers fraction (EX samples) resulted in a decrease in cellulose content and cellulose yields. In samples without pectin (EXP), extrusion favored the enrichment of the cellulosic fraction. According to Garcia-Amezquita *et* *al*. (2019) and Larrea *et al*. (2005), extrusion modifies the fibers composition of orange peel by increasing soluble fibers at expenses of insoluble fibers decrease. This same trend was also reported by other authors for soybean hulls (Jing and Chi, 2013) and oat bran (Zhang *et al*., 2011) subjected to extrusion.

The alkaline treatment provided the highest cellulose content among all treatments tested, mainly for OB-P sample. According to the cellulose yield data (Table 1), it is possible to verify that the alkaline treatment was more selective in degradation of non-cellulosic fractions, preserving the cellulose fibers. Alkaline treatments have low cost and show excellent results in removing lignin and hemicellulose, without causing major environmental impact (Batista-Meneses *et al*., 2020; Bicu and Mustata *et al*., 2013). Melikoğlu *et al*. (2019) also reported the efficiency of this reagent for extracting cellulose from apple pomace. For both EX and EXP materials, the use of sulfuric acid (H_2SO_4) did not affected significantly the cellulose and lignin contents when compared to water treatment; hemicelluloses content showed a slight decrease (Table 1).

6.3.2 Extruded cellulose-based materials characterization

6.3.2.2 Fourier transform-infrared spectroscopy (FTIR)

The FTIR spectra of the cellulose-based materials are shown in Figure 1. As can be seen, all spectra appeared to be rather similar, which indicates that the cellulose, hemicellulose and lignin fractions were preserved. This corroborates the chemical composition showed in Table 1.

The samples showed bands at 3400 cm^{-1} for intramolecular and intermolecular hydrogen bonds (O–H), and 2920 cm^{-1} for C–H group stretching vibrations. The band at 1740 cm^{-1} is probably due to the ester and acetyl groups, and was less evident in the spectra of materials treated with NaOH. This suggests that alkali treatment removed a higher content of hemicelluloses and lignin (Ahuja *et al*., 2018).

The prominent band at 1620 cm^{-1} is attributable to cellulose–water absorption. The band at 1320 cm^{-1} represents CH_2 stretching vibration. Cellulose β-glycosidic linkage was observed at 1060 and 895 cm^{-1}. The peak 1430 cm^{-1} lignin-related was found in all samples (Ibrahim *et al*. 2010; Merci *et al*. 2015).

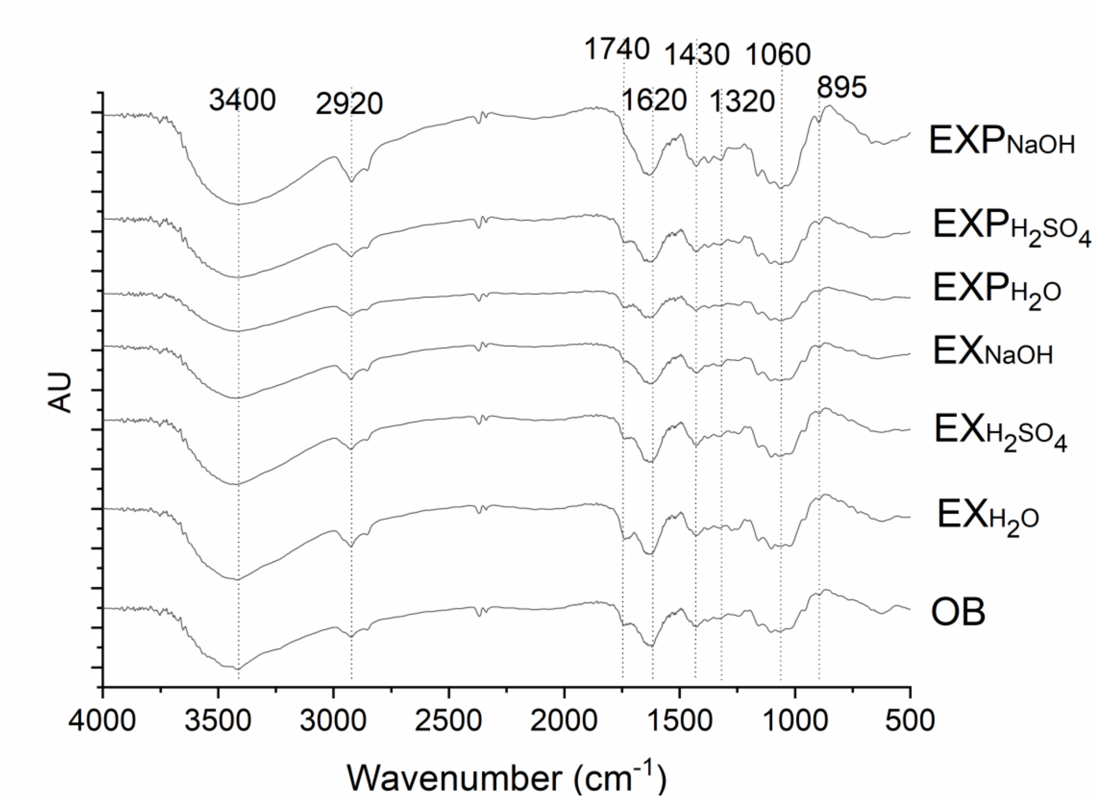

Figure 1 – FTIR analyzes of raw orange bagasse (OB) and cellulose-based materials from OB obtained by extrusion.

6.3.2.3 X-ray diffraction (XRD)

In XRD analysis (Figure 2) it was possible to observe that all the tested samples presented the typical patterns of semicrystalline materials with the main crystalline peaks at 2θ = 22º and an amorphous peak at 2θ = 18º. In addition, it was possible to observe a new peak at 2θ = 34.5º for the extruded samples.

The crystalline peak at 2θ = 22º increased for all extruded material due to the physical and chemical treatments, consequently, the crystallinity index (CI) also increased (Table 1), indicating the partial removal of the amorphous regions from the residue (Ahmadzadeh *et al*., 2018). The CI was 18.1% for OB and changed according to the treatment employed (Table 1). The EXP_{NaOH} sample showed the highest CI (50.57 %) due to its higher cellulose content (58.4 %), while EX_{NaOH} sample showed the higher CI among the EX samples. The pectin-removed materials (EXP) showed higher CI values when compared to the respective EX treatments (samples containing pectin). Pectin is an amorphous polysaccharide, and it also provides some resistance to the disruption of the lignocellulosic matrix.

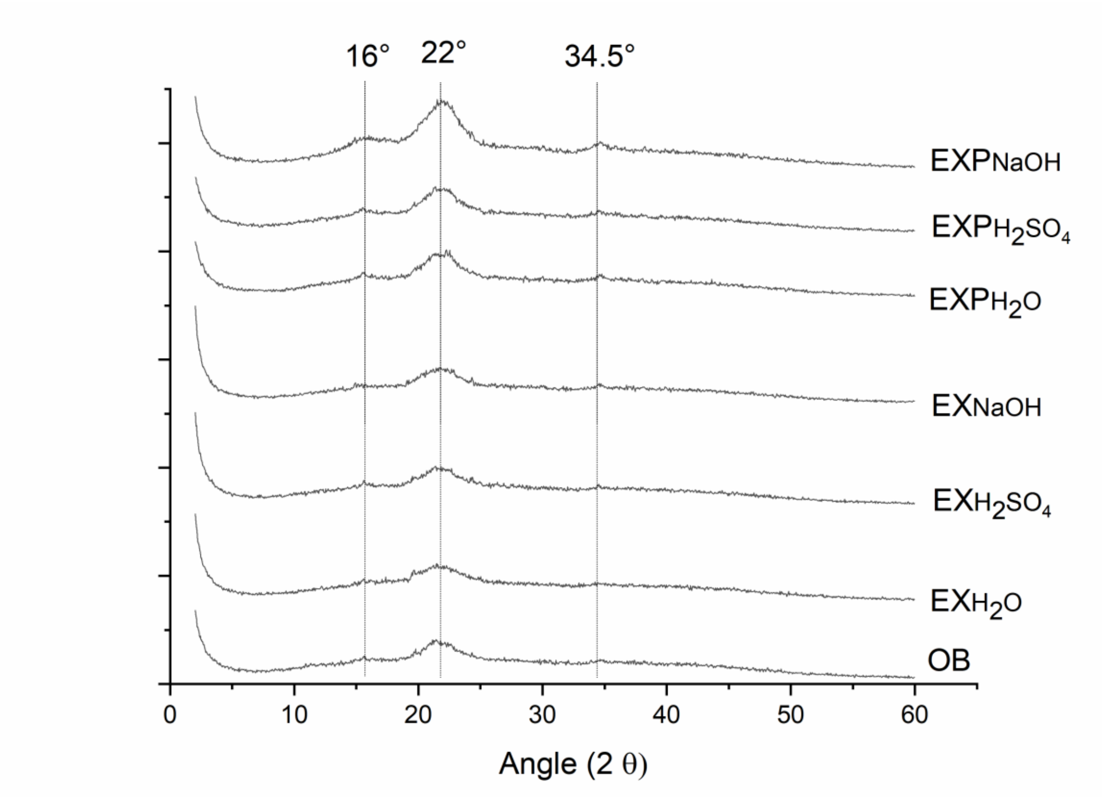

Figure 2 - X-ray diffractograms of raw orange bagasse (OB) and cellulose-based materials from OB obtained by extrusion.

6.3.2.4 Thermogravimetric analysis (TGA)

In Figure 3, thermograms of cellulose-based materials were analyzed. The small weight changes up to 100 °C is assigned to the evaporation of water from the samples. All samples showed a double-peak at 268 - 295 °C and 335 - 372 °C. Similar two-stage decomposition was observed for orange bagasse treated with different alkaline extraction methods (Mantovan *et al*., 2021). A third distinct decomposition stage was observed at temperatures above 450 °C for EX samples, and it could be associated with lignin degradation (Ferrer *et al*., 2016).

Materials that are not subjected to the pectin extraction process have a more complex chemical composition, which resulted in a more complex peak distribution, the same trend was also observed by other authors (Debiagi *et al*., 2020b). The decomposition of lignocellulosic products can be grouped into three stages: (1) corresponding to hemicellulose degradation (110 to 290 °C), (2) corresponding to degradation of cellulose (290 to 390 °C) and (3) lignin degradation (above 390 °C) (Ferrer *et al*., 2016).

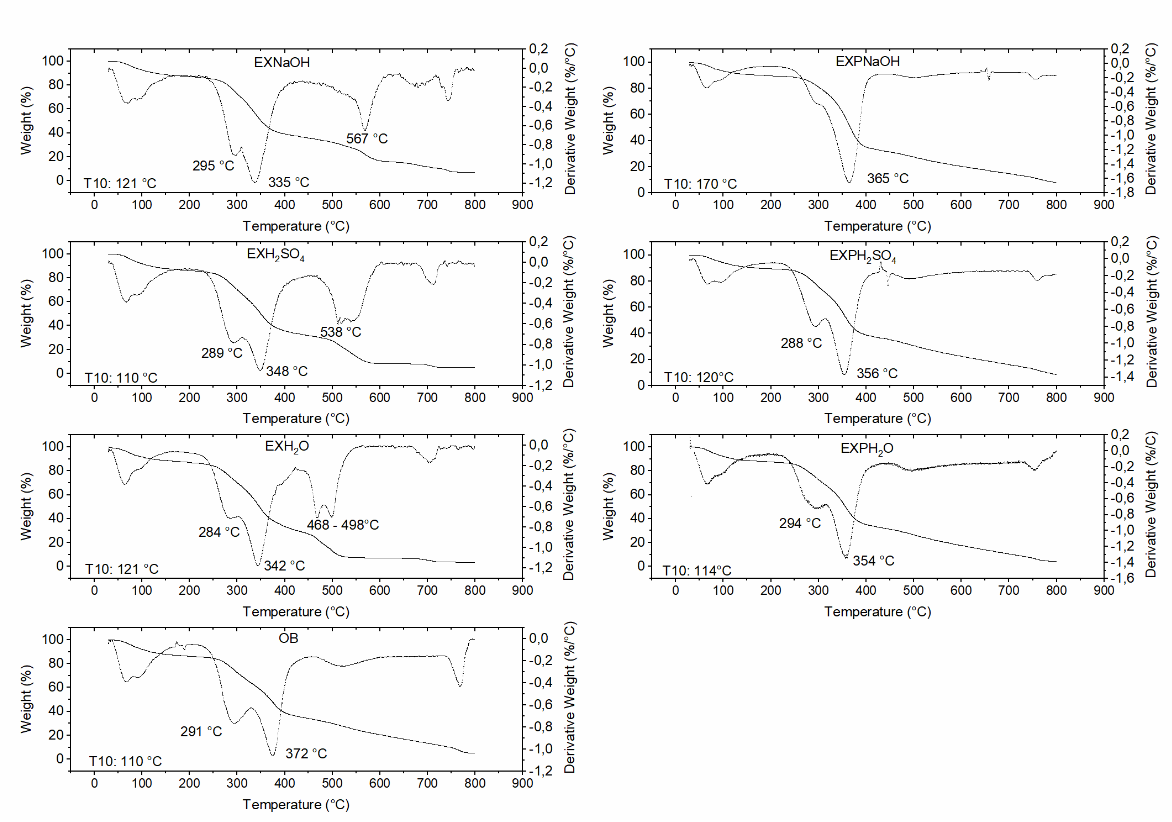

Figure 3 – TGA curves of raw orange bagasse (OB) and cellulose-based materials from OB obtained by extrusion.

T_{10} value corresponds to the initial decomposition temperature at 10% degradation of the sample and were obtained from the TGA curves. All samples showed T_{10} values in the range of 110 to 121 °C, except the EXP_{NaOH} sample that showed a higher value (170 °C, Figure 3), which probably occurred due to its more homogeneous chemical composition, with a higher cellulose content, and also to its higher CI.

6.4 Conclusion

In this study, reactive extrusion was used as a green and short route to prepare cellulose-based materials from orange bagasse. The modification of OB by extrusion process was successfully accomplished and resulted in materials with 18.8 to 58.4% cellulose. The previous pectin extraction from OB effectively improved the cellulose extraction. The alkaline treatment was more efficient in removing the non-cellulosic fraction from OB samples. The materials showed partial crystallinity and relative thermal stability.

One-step treatments were crucial to determine the economic viability and sustainable character of this study. Pectin and cellulosic fraction can be used for several applications, which contributes to a proposal for biorefinery and circular economy.

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CONSIDERAÇÕES FINAIS

O bagaço de laranja é um resíduo abundante e economicamente importante no Brasil cuja utilização industrial merece mais atenção. Assim, o presente estudo explorou a utilização do resíduo na produção de diversos materiais ricos em celulose por meio de tratamentos menos poluentes, preferencialmente, em etapa única.

A utilização dos tratamentos químicos combinados com explosão a vapor em autoclave foi mais eficiente na obtenção de altos teores de celulose, sendo que o uso do tratamento alcalino com NaOH resultou em melhores resultados entre os reagentes químicos empregados, gerando um material com teor de celulose de 71,1 e índice de cristalinidade de 42%.

Quando comparados aos tratamentos em autoclave e químicos isolados, os tratamentos em ultrassom foram menos eficazes na obtenção de materiais com alto teor de celulose.

A extrusão reativa mostrou ser um excelente tratamento para obter materiais à base de celulose com pouca geração de efluentes, além disso permitiu a possibilidade de recuperar previamente a pectina para aplicações futuras.

Os tratamentos de etapa única foram considerados eficientes, resultando em diferentes materiais com maiores teores de celulose em relação à matéria-prima e os rendimentos foram satisfatórios, o que significa um bom custo-benefício com economia de tempo, energia e solvente, portanto, considerados ecologicamente amigáveis.

Os índices de cristalinidade e a morfologia da superfície afetaram a capacidade de sorção de umidade e as propriedades funcionais das amostras. A celulose nanofibrilada, preparada em três etapas, resultou em um material com 100 % de celulose e 60 % de cristalinidade, com rendimento de 4,4 % (g/100 g de bagaço de laranja).

Os materiais, de acordo com suas características, podem ser usados para produzir celulose de alta pureza e nanocelulose, como uma fonte de fibras insolúveis na dieta em alimentos funcionais ou como substrato em processos de fermentação, já que os materiais obtidos são mais acessíveis à hidrólise enzimática.

ANEXO I

Os seguintes artigos foram publicados a partir dos dados obtido nesta Tese:

1. MANTOVAN, J.; GIRALDO, GINA A. G.; MARIM, B.M; GARCIA, P.S.; BARON, A.M.; MALI, S. Cellulose-based materials from orange bagasse employing environmentally friendly approaches. **Biomass Conversion and Biorefinery**, v. 1, p. 1-12, 2021. https://doi.org/10.1007/s13399-021-01279-2.

2. MANTOVAN, J.; GIRALDO, GINA A. G.; MARIM, B.M; KISHIMA, J.O.F.; MALI, S. Valorization of orange bagasse through one-step physical and chemical combined processes to obtain a cellulose-rich material. **Journal of the Science of Food and Agriculture**, v. 1, p. 1-9, 2020. https://doi.org/10.1002/jsfa.10859

A seguinte patente está em fase de depósito pela AINTEC – Agência de Inovação Tecnológica da Universidade Estadual de Londrina:

1. “Método de obtenção de aditivo funcional contendo fibras oriundas do bagaço da laranja para compor rações de animais domésticos”

Campo da invenção

O bagaço da laranja é um resíduo agroindustrial com grande potencial de aplicação em diferentes setores industriais em função da sua elevada disponibilidade, porém pouco explorado. A modificação desse resíduo por processos menos poluentes e rápidos é uma alternativa que amplia suas aplicações nas diferentes áreas industriais, incluindo a indústria de rações para pets (animais domésticos de estimação). O setor de alimentos para animais domésticos ou pet food encontra-se em forte expansão e necessita de novos produtos que atendam questões nutricionais e ambientais. Esta invenção descreve um processo de modificação do bagaço da laranja para gerar um novo produto funcional para compor alimentos para animais domésticos empregando-se o processo de ultrassonicação, que pode ser considerado ecologicamente correto, simples e conveniente, com tempos de reação curtos. O material obtido apresenta um aumento dos seus teores de fibras insolúveis totais, com aumento nos teores de celulose e hemicelulose em relação ao bagaço de laranja bruto, além do aumento na capacidade de absorção de água, óleo e intumescimento em relação ao bagaço de laranja bruto, apresenta ainda capacidade de adsorção de glicose.