



FRANCIELE MARIA PELISSARI MOLINA

**PRODUCTION AND CHARACTERIZATION OF BIODEGRADABLE FILMS
OF BANANA STARCH AND FLOUR REINFORCED WITH CELLULOSE
NANOFIBERS**

***PRODUÇÃO E CARACTERIZAÇÃO DE FILMES BIODEGRADÁVEIS DE
AMIDO E FARINHA DE BANANA REFORÇADOS COM NANOFIBRAS DE
CELULOSE***

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FACULDADE DE ENGENHARIA DE ALIMENTOS

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**PRODUCTION AND CHARACTERIZATION OF BIODEGRADABLE FILMS
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Orientadora: Profa. Dra. Florencia Cecilia Menegalli

Co-Orientador: Prof. Dr. Paulo José do Amaral Sobral

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CELULOSE***

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Banca examinadora:

Florencia Cecilia Menegalli [Orientador]

Carlos Raimundo Ferreira Grosso

Caroline Joy Steel

Fabio Yamashita

João Borges Laurindo

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BANCA EXAMINADORA

Profa. Dra. Florencia Cecilia Menegalli
Orientadora

Prof. Dr. Carlos Raimundo Ferreira Grosso
Membro Titular
Universidade Estadual de Campinas

Profa. Dra. Caroline Joy Steel
Membro Titular
Universidade Estadual de Campinas

Prof. Dr. Fabio Yamashita
Membro Titular
Universidade Estadual de Londrina

Prof. Dr. João Borges Laurindo
Membro Titular
Universidade Federal de Santa Catarina

Profa. Dra. Delia Rita Tapia Blácido
Membro Suplente
Universidade de São Paulo

Profa. Dra. Maria Victoria Eiras Grossmann
Membro Suplente
Universidade Estadual de Londrina

Profa. Dra. Rosemary Aparecida de Carvalho
Membro Suplente
Universidade de São Paulo

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Lista de Abreviaturas e Siglas

b.s., d.b. – Base seca

Cg – Concentração de glicerol

D – Diâmetro

DSC – Calorimetria diferencial de varredura

E – Lado do filme seco em contato com o ar

EDS – Dispersão de energia por raios-X

Endo – Endotérmico

Exo – Exotérmico

FC – Filme controle

FFS, SF – Solução filmogênica

FN0 – Filme reforçado com nanofibras N0

FN3 – Filme reforçado com nanofibras N3

FN5 – Filme reforçado com nanofibras N5

FN7 – Filme reforçado com nanofibras N7

FTIR – Espectroscopia de absorção na região do infravermelho

I – Lado do filme seco em contato com a placa

i.e. – Isto é

I_{002} – Intensidade que representa o material cristalino

I_{am} – Intensidade que representa o material amorfo

I_c – Índice de cristalinidade

L – Comprimento

L/D – Relação de comprimento/diâmetro

MEV, SEM – Microscopia eletrônica de varredura

N0 – Nanofibras sem tratamento com o homogeneizador de alta pressão

N3 – Nanofibras com três passagens pelo homogeneizador de alta pressão

N5 – Nanofibras com cinco passagens pelo homogeneizador de alta pressão

N7 – Nanofibras com sete passagens pelo homogeneizador de alta pressão

R – Faixa de temperatura de gelatinização

RH, UR – Umidade relativa

RS – Amido resistente

S – Solubilidade

SP – Poder de inchamento

Td – Temperatura de secagem
TEM – Microscopia eletrônica de transmissão
TEMPO – 2,2,6,6-tetrametilpiperidina-N-óxido
T_f – Temperatura final
T_g – Temperatura de transição vítrea
T_o – Temperatura inicial
T_p – Temperatura de pico
T_p – Temperatura de processo
UV – Radiação ultravioleta
v/v – Relação de volume/volume
w/v – Relação de peso/volume
wt – Peso
WU – Absorção de umidade
WVP – Permeabilidade ao vapor de água
XRD – Difração de raios-X

Lista de Símbolos

a_o^* , b_o^* – Parâmetros de cromaticidade do padrão

a^* , b^* – Parâmetros de cromaticidade da amostra

a_w – Atividade de água

C , k – Constantes do modelo de GAB

D – Difusividade do vapor de água

L^* , L_o^* – Luminosidade

m_0 – Valor da monocamada

M_∞ , X_w – Conteúdo de água no equilíbrio

R^2 – Coeficiente de determinação

S – Coeficiente de sorção

ΔE^* – Diferença de cor

ΔH – Entalpia de gelatinização

Resumo

Este trabalho de doutorado teve como objetivo estudar o potencial de uso do amido, farinha e nanofibras obtidos a partir de bananas verdes da variedade Terra (*Musa paradisiaca*) na elaboração de filmes biodegradáveis. Na primeira etapa do trabalho, o amido e a farinha de banana foram caracterizados quanto às propriedades físico-químicas, funcionais e térmicas. Ambas as matérias-primas apresentaram considerável conteúdo de amido (94,8 e 83,2%, respectivamente) com alto teor de amilose (35,0 e 23,1%, respectivamente) e amido resistente (49,5 e 50,3%, respectivamente), além de fibras, proteínas e lipídios. Numa segunda etapa, filmes a partir de farinha de banana foram elaborados segundo um planejamento experimental. Os resultados obtidos foram analisados estatisticamente empregando a metodologia de superfície de resposta, que juntamente com a função de desejabilidade permitiu a obtenção da formulação e condições de processo ótimas (concentração de glicerol de 19%, temperatura de processo de 81 °C, temperatura de secagem de 54 °C e umidade relativa de 48%). Os filmes produzidos sob essas condições apresentaram tensão na ruptura de 9,2 MPa, alongação de 24,2%, módulo de Young de 583,4 MPa, permeabilidade ao vapor de água de $2,1 \times 10^{-10}$ g/m.s.Pa e opacidade de 51,3%. Na terceira etapa do trabalho, foram elaborados filmes de farinha e amido de banana para determinar o efeito das fibras, proteínas e lipídios sobre as propriedades dos filmes. O filme de farinha de banana foi mais flexível, solúvel em água e opaco, e menos cristalino e resistente mecanicamente quando comparado com o filme de amido de banana. A partir da microestrutura, observou-se que o filme de farinha apresentou imperfeições na sua superfície e uma seção transversal menos densa com pequenas fissuras quando comparado com o filme de amido. Na quarta etapa, a casca da banana (subproduto do processamento da fruta) foi utilizada como matéria-prima para a obtenção de nanofibras de celulose, empregando tratamento químico e mecânico. A influência do número de passagens (0, 3, 5 e 7) das suspensões em um homogeneizador de alta pressão sobre a estrutura das nanofibras foi estudada. Os tratamentos foram efetivos no isolamento de fibras de banana na escala nanométrica (10,9 – 22,6 nm). Conforme o aumento do número de passagens no homogeneizador, nanofibras de celulose mais estáveis, cristalinas e de menor comprimento foram obtidas. Na última etapa, as nanofibras isoladas foram incorporadas na elaboração de nanocompósitos de amido da mesma fonte. As propriedades desses nanocompósitos foram comparadas com as de um filme de amido

sem adição de nanofibras (controle), a fim de estudar o efeito deste reforço. Os nanocompósitos apresentaram uma melhora significativa nas propriedades tensão na ruptura, módulo de Young, resistência à água, opacidade e cristalinidade. Uma homogeneização mais drástica (7 passagens) promoveu a degradação das nanofibras, acarretando numa piora das propriedades do nanocompósito resultante, portanto, a condição de tratamento mecânico mais adequada foi de 5 passagens. As propriedades dos nanocompósitos foram relacionadas com as características físico-químicas das nanofibras incorporadas e também com a boa compatibilidade apresentada entre os biopolímeros amido e nanofibras, uma vez que estes foram obtidos da mesma fonte vegetal.

Palavras-chave: Musa paradisiaca, banana verde, amido, farinha, nanofibras de celulose, embalagens biodegradáveis, nanocompósitos.

Abstract

This doctor thesis aimed to study the potential use of the starch, flour, and nanofibers obtained from unripe bananas of the variety “Terra” (*Musa paradisiaca*) to develop biodegradable films. In the first stage of the work, banana starch and flour were characterized on the basis of their physicochemical, functional, and thermal properties. Both raw materials exhibited considerable starch content (94.8 and 83.2%, respectively) with high amylose (35.0 and 23.1%, respectively) and resistant starch (49.5 and 50.3%, respectively), besides fibers, proteins, and lipids. In the second stage, films based on the banana flour were prepared according to an experimental design. The results were statistically analyzed using the response surface methodology which, along with the desirability function, furnished the optimum formulation and process conditions (19% for glycerol concentration, 81 °C for process temperature, 54 °C for drying temperature, and 48% for relative humidity). Films produced under these conditions presented tensile strength of 9.2 MPa, elongation at break of 24.2%, Young’s modulus of 583.4 MPa, water vapor permeability (WVP) of 2.1×10^{-10} g/m.s.Pa, and opacity of 51.3%. In the third stage of the work, films from banana flour and starch were produced and the effect of fibers, proteins, and lipids on the properties of the flour film was studied. The results showed that the flour film was more flexible, soluble in water and opaque as well as less crystalline and mechanically resistant than the starch film. Compared with the starch film, the microstructure of the flour film has flawed surface, less dense cross-section, and small cracks. In the fourth stage, the banana peel (byproduct from fruit processing) was treated chemically and mechanically, to obtain cellulose nanofibers. The influence of the number of passages (0, 3, 5, and 7) in a high-pressure homogenizer on the structure of the nanofibers was investigated. The treatments were able to isolate the banana fibers in the nanometer scale (10.9 – 22.6 nm). Increasing the number of passages in the homogenizer afforded more stable, more crystalline, and less long cellulose nanofibers. In the last stage, the cellulose nanofibers were incorporated into starch nanocomposites from the same source. The properties of these nanocomposites were compared with those of a starch film without nanofibers (control), in order to study the effect of this reinforcement. The nanocomposites exhibited significantly improved tensile strength, Young’s modulus, water resistance, opacity, and crystallinity. A more drastic homogenization (seven passages) degraded the nanofibers, deteriorating the properties of the resulting nanocomposite. Thus, the most suitable mechanical

treatment condition involved five passages. The properties of the nanocomposites are a function of the characteristics of the nanofibers, such as crystallinity, zeta potential, and aspect ratio; they also depend on the compatibility between the starch and the nanofibers, which were obtained from the same plant source.

Keywords: Musa paradisiaca, unripe banana, starch, flour, cellulose nanofibers, biodegradable packagings, nanocomposites.

Apresentação Geral

Visando atender às demandas ambientais que surgiram a partir do impacto de embalagens produzidas por polímeros convencionais não biodegradáveis, nas últimas décadas tem-se estimulado a pesquisa para viabilizar a substituição destes materiais por produtos que, além de serem biodegradáveis, provenham de fontes renováveis.

Neste enfoque, o grupo de pesquisa de filmes e coberturas comestíveis do Laboratório de Engenharia de Processos (DEA/FEA/UNICAMP) tem se consolidado pelos trabalhos que desenvolve na área de embalagens biodegradáveis, com resultados promissores e de relevância para a pesquisa mundial, demonstrado pela crescente publicação em revistas especializadas e pela formação de alto nível de alunos de pós-graduação na referida área.

Nos últimos anos, as pesquisas realizadas pelo grupo vêm sendo conduzidas para a exploração sustentável do uso da biodiversidade sul-americana como fonte de matérias-primas para a produção de embalagens biodegradáveis. Neste contexto, as fontes promissoras de origem vegetal utilizadas até o momento são amaranto (*Amaranthus caudatus*), biri (*Canna indica* L.), quinoa (*Chenopodium quinoa* Willdenow), cañihua (*Chenopodium pallidicaule*) e banana (*Musa paradisiaca*). Além de trabalhos na área de produção de filmes e coberturas comestíveis, o grupo de pesquisa ainda desenvolve estudos com fibras vegetais de dimensão nanométrica, visando sua utilização como material de reforço para os filmes. Apesar desta linha de pesquisa ser recente, os resultados coletados se mostram muito promissores e abrem precedentes para que esta área continue em evolução.

O presente trabalho de doutorado teve como objetivo realizar um aproveitamento total dos materiais obtidos a partir da banana. Desta forma, foi avaliado o potencial do amido e da farinha (extraídos da polpa) e das nanofibras (extraídas da casca) na elaboração de filmes biodegradáveis. Além disso, o estudo visou o aproveitamento da casca da banana verde, resíduo proveniente do processamento da fruta, como fonte de fibras naturais na escala nanométrica que, depois de isoladas e caracterizadas, foram empregadas para elaboração de filmes reforçados.

Apesar do grande desafio científico e industrial nesta área, os estudos têm demonstrado avanços tecnológicos significativos. A conciliação dos conhecimentos adquiridos por esta pesquisa contribuiu para a elucidação do potencial dos

nanomateriais, como sinônimo de avanços na área de embalagens biodegradáveis. Assim, este trabalho de doutorado está dividido em seis capítulos:

- Capítulo 1: revisão bibliográfica sobre os tópicos abordados na presente pesquisa.
- Capítulo 2: caracterização do amido e da farinha obtidos a partir de bananas verdes da variedade Terra (*Musa paradisiaca*), como matérias primas para a elaboração de filmes biodegradáveis.
- Capítulo 3: estudo sobre a otimização da formulação e condições de processo para produção de filmes de farinha de banana.
- Capítulo 4: estudo comparativo das propriedades dos filmes de amido e farinha de banana.
- Capítulo 5: isolamento de nanofibras de celulose a partir da casca da banana e a caracterização dessas nanopartículas.
- Capítulo 6: estudo sobre o efeito da incorporação de nanofibras de celulose como material de reforço em nanocompósitos a base de amido e a caracterização desses filmes.

Introdução

O crescente interesse mundial no desenvolvimento de processos e produtos que levem a um menor impacto ambiental tem motivado a utilização sustentável de recursos naturais. A grande demanda por novos materiais que atendam a todas as especificações tecnológicas, de qualidade e da legislação ambiental têm conduzido ao estudo e utilização de matérias-primas, processos e produtos amigáveis ao meio ambiente. Os materiais empregados na elaboração das embalagens são cruciais para a preservação dos produtos, por serem determinantes na difusão de gases, umidade, gordura e aromas, entre outros, devendo garantir vida útil prolongada, qualidade e preservação dos produtos. Desta forma, filmes e coberturas, quando empregados como materiais de embalagem, podem impedir a migração de umidade, proteger os produtos durante seu transporte evitando danos mecânicos e conservando sua integridade física. Esses materiais têm demonstrado um grande potencial no controle da respiração de frutas e hortaliças, funcionando como uma embalagem de atmosfera modificada que favorece a conservação das características sensoriais e prolongando o período de armazenamento dos produtos (MALI et al., 2010). Dentro das características desejadas de um filme, deve-se destacar a baixa permeabilidade ao vapor de água, para fornecer uma boa propriedade de barreira, e a alta resistência mecânica.

Durante a fabricação de filmes biodegradáveis e coberturas, é comum o uso de plastificantes como o glicerol, que adicionado à solução formadora do filme é capaz de reduzir a formação de pontes de hidrogênio entre as cadeias poliméricas, diminuindo as forças intermoleculares, aumentando a mobilidade molecular e, conseqüentemente, levando à obtenção de estruturas poliméricas mais flexíveis. Isto favorece o aumento da permeabilidade e reduz a resistência mecânica do filme (MALI et al., 2004). Diante disso, filmes com alta permeabilidade e baixa resistência mecânica têm se tornado o ponto de partida de diferentes pesquisadores, a fim de encontrar materiais de reforço que quando adicionados à formulação dos filmes possam melhorar essas propriedades. Nesta área, destaca-se a utilização de fibras naturais ou lignocelulósicas como material de reforço de matrizes poliméricas. Desde 2009, a Organização das Nações Unidas para a Agricultura e a Alimentação (FAO-ONU) incentiva o estudo das fibras naturais em virtude da enorme variedade de fontes disponíveis, principalmente as de origem vegetal (SILVA et al., 2009). Diante do expressivo número de artigos publicados, patentes e

produtos já comercializados, as fibras vegetais apresentam-se como excelente matéria-prima para a química de polímeros e compósitos.

Os compósitos são misturas preparadas na tentativa de conciliar as distintas propriedades dos componentes puros, procurando interações favoráveis entre estes, que levem a melhores características e desempenho dos materiais resultantes (AZEREDO et al., 2009; CURVELO et al., 2001; LU et al., 2005). Na preparação de nanocompósitos, as fibras vegetais de tamanho nanométrico vêm sendo exploradas como materiais de reforço, tendo em vista que sua elevada área superficial específica por massa de material ($> 100 \text{ m}^2/\text{g}$) lhes permitem interagir de forma mais efetiva com a fase contínua dos compósitos, quando comparadas com aquelas que possuem dimensões micrométricas (FAVIER et al., 1995). Em concordância com tal abordagem, os participantes do Workshop realizado em 2005 pela *American Forest and Paper Association* recomendaram, dentro dos trabalhos de pesquisa, o estudo de compósitos poliméricos reforçados com nanomateriais, apontando o potencial tecnológico do emprego de fibras celulósicas de dimensão nanométrica (HUBBE et al., 2008).

A celulose é o principal componente das fibras vegetais e sua característica de hidrofiliabilidade lhe confere alta afinidade com a maioria dos polímeros naturais (YU et al., 2006). No entanto, as condições de processo para a obtenção das nanofibras, seja na etapa de obtenção das fibras ou de hidrólise para atingir dimensões nanométricas, afetam as características morfológicas desses nanomateriais e, conseqüentemente, seu desempenho como partícula de reforço em compósitos (ARAKI et al., 1998; BONDESON et al., 2006; ELAZZOUI-HAFRAOUI et al., 2008; GEORGE et al., 2001). Desta maneira, a maior dificuldade na obtenção e incorporação de nanocristais de celulose aos compósitos é evitar a degradação das cadeias poliméricas e a aglomeração das partículas nanométricas, esta última devida ao fato de que nanofibras de celulose sem modificação de superfície apresentam fortes interações entre partículas, com notória dificuldade em serem dispersas em água (SILVA et al., 2009; VAN DEN BERG et al., 2007). Assim, diferentes autores têm avaliado o emprego de diversos métodos físicos e químicos, ou a combinação destes, para a separação de nanofibras ou nanocristais de celulose a partir de diferentes fibras vegetais como, por exemplo, algodão, madeira, rami, cânhamo, linho, sisal, casca de ervilha, bagaço de cana de açúcar, beterraba açucareira, entre outras.

O Brasil é detentor de uma grande extensão territorial cultivável e uma ampla biodiversidade pouco conhecida e estudada. Desta forma, devem-se intensificar os

estudos para a utilização dos recursos existentes e a identificação de espécies vegetais com propriedades promissoras, cujas culturas possam ser adaptadas e aproveitadas para novos fins tecnológicos, aumentando a difusão de sua importância, encorajando o aumento do seu plantio e permitindo sua valorização frente à comunidade e indústria. Em virtude da disponibilidade de certas fontes vegetais, existe uma crescente motivação pelo estudo da obtenção de nanocristais de celulose a partir destas matrizes.

Neste enfoque, uma fonte ainda não pesquisada para tal fim é a casca da banana (*Musa* spp.), que pode conciliar sua disponibilidade no país e suas propriedades, tornando-se uma matéria-prima importante para a elaboração de nanocompósitos. A banana é uma pseudobaga de origem Asiática cultivada em praticamente todas as regiões tropicais do planeta (ZHANG et al., 2005). Atualmente, sua produção mundial é de cerca de 90,7 milhões de toneladas, sendo o Brasil o quarto maior produtor mundial (FAO, 2009). Apesar disso, as perdas ao longo da produção da banana são bastante elevadas, chegando a 40% da produção mundial total, devido à sua alta perecibilidade e à tecnologia precária utilizada pelos bananicultores (TADINI e DITCHFIELD, 2006). Estas perdas podem ser reduzidas, uma vez que a banana quando verde torna-se uma matéria-prima interessante para a extração de amido (BELLO-PÉREZ et al., 2000; ESPINOSA-SOLIS et al., 2009) e para elaboração de filmes biodegradáveis (ROMERO-BASTIDA et al., 2005; SOTHORNVIT e PITAK, 2007), indicando a potencialidade industrial desta fonte. No entanto, a casca da banana (subproduto resultante do processamento da fruta), não foi previamente estudada como fonte de material lignocelulósico para a obtenção de nanofibras. Isto permitiria um aproveitamento integral da banana, tornando-se também uma oportunidade de pesquisa interessante para explorar novas alternativas de uso desta fruta, incentivando sua cultura e utilização em diferentes países. O presente trabalho de doutorado visou o estudo e a valorização do amido, farinha e nanofibras, obtidos a partir de bananas verdes como matérias-primas para a elaboração de filmes biodegradáveis. Para tal fim, a cultivar Terra (*Musa paradisiaca*) foi selecionada para a investigação, por possuir alto teor de amido e poucos estudos na literatura trabalhando com esta variedade.

Objetivos e Metas

Objetivo geral

Desenvolvimento de novos nanocompósitos à base de amido e farinha de banana (*Musa paradisiaca*) reforçados com nanofibras de celulose obtidas da casca da banana.

Objetivos específicos

Parte 1: Obtenção das matérias-primas

- Produzir o amido e a farinha a partir de bananas verdes da variedade Terra (*Musa paradisiaca*);
- Caracterizar o amido e a farinha de banana quanto a sua composição química, microestrutura, distribuição de tamanho de partícula, cristalinidade, grupos funcionais, e propriedades de pasta e térmicas.

Parte 2: Otimização do filme de farinha

- Determinar a influência da formulação e condições de processo: concentração de glicerol, temperatura de processo e temperatura e umidade relativa de secagem nas propriedades do filme de farinha de banana, empregando um delineamento composto central rotacional;
- Obter as condições ótimas de formulação e processo, e caracterizar o filme de farinha de banana em relação à microestrutura, propriedades mecânicas, ópticas, térmicas, solubilidade, barreira ao vapor de água, cristalinidade e grupos funcionais.

Parte 3: Comparação do filme de amido e farinha

- Produzir filmes de amido e farinha de banana nas condições ótimas de formulação e processo encontradas para o filme de farinha;
- Caracterizar os filmes de amido e farinha de banana em relação à microestrutura, propriedades mecânicas, ópticas, térmicas, solubilidade, barreira ao vapor de água, cristalinidade e grupos funcionais;

- Determinar a influência do teor de proteínas, lipídios e fibras da farinha de banana sobre as propriedades do filme elaborado a partir dessa matéria-prima quando comparado com filme de amido de banana.

Parte 4: Isolamento de nanofibras de celulose

- Isolar as nanofibras de celulose a partir de cascas de bananas verdes da variedade Terra (*Musa paradisiaca*);
- Caracterizar as nanofibras de celulose quanto sua a composição química, microestrutura, potencial zeta, cristalinidade, grupos funcionais e estabilidade térmica;
- Comparar a ausência e presença de tratamento mecânico, dentro da metodologia desenvolvida, sobre as propriedades das nanofibras de celulose isoladas.

Parte 5: Produção dos nanocompósitos

- Produzir nanocompósitos de amido de banana reforçados com nanofibras de celulose isoladas da casca da banana;
- Caracterizar os nanocompósitos em relação à microestrutura, propriedades mecânicas, ópticas, térmicas, solubilidade, barreira ao vapor de água, cristalinidade e grupos funcionais;
- Avaliar o efeito da adição das nanofibras de celulose sobre as propriedades dos nanocompósitos;
- Comparar as nanofibras de celulose isoladas sem e com tratamento mecânico, frente às propriedades dos nanocompósitos.

Capítulo 1

Revisão Bibliográfica

O conhecimento sobre as matérias-primas que apresentam potencial para serem aplicadas na elaboração de filmes constitui uma importante etapa a ser abordada no desenvolvimento de biomateriais, devendo ser levados em consideração aspectos como sua composição centesimal, microestrutura, propriedades térmicas e funcionais, que podem fornecer informações relevantes sobre as características do produto final.

1. A banana (*Musa spp.*)

A banana é uma pseudobaga da bananeira (Figura 1), uma planta herbácea vivaz acaule pertencente à família Musaceae (gênero *Musa*). A banana é o quarto produto alimentar mais produzido no mundo, após o arroz, trigo e milho. Originárias do sudeste da Ásia, elas são atualmente cultivadas em praticamente todas as regiões tropicais do planeta (ZHANG et al., 2005). Vulgarmente, inclusive para efeitos comerciais, o termo "banana" refere-se às frutas de polpa macia e doce que podem ser consumidas cruas. Contudo, existem variedades de cultivo, de polpa mais dura e de casca mais firme e verde, geralmente designadas por plátanos, banana-pão ou *plantains*, que são consumidas cozidas (assadas, cozidas ou fritas), constituindo o alimento base de muitas populações de regiões tropicais (VILAS BOAS et al., 2001).

As bananas formam-se em cachos na parte superior dos “pseudocaules” que nascem de um verdadeiro caule subterrâneo (rizoma ou cormo) cuja longevidade chega a 15 anos ou mais. Depois da maturação e colheita do cacho de bananas, o pseudocaulo morre (ou é cortado), dando origem, posteriormente, a um novo pseudocaulo. As pseudobagas formam-se em conjuntos com até cerca de vinte bananas (cada conjunto é uma “penca”). Os cachos de bananas, pendentes na extremidade do falso caule da bananeira, podem ter de 5 a 20 pencas e pesar de 30 a 50 kg. Cada banana pesa, em média, 125 gramas, apresentando uma composição de 75% de água e 25% de matéria seca, e uma fonte apreciável de vitamina A, vitamina C, fibras e potássio (VILAS BOAS et al., 2001). A Tabela 1 apresenta a composição da banana da Terra (*Musa paradisiaca*) *in natura*.

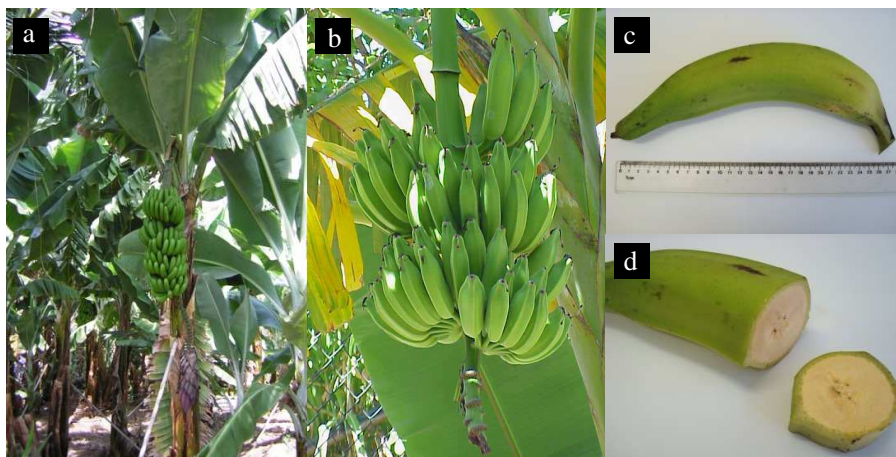


Figura 1. Fotografias de bananas (*Musa* spp.): (a) bananeira, (b) cacho de bananas verdes ainda na bananeira, (c) parte externa da fruta, e (d) parte interna da fruta.

Tabela 1. Composição da banana da Terra madura (*Musa paradisiaca*) em 100 gramas.

Composição	% base úmida
Umidade	63,9
Carboidratos	33,7
Cinzas	0,8
Proteínas	1,4
Lipídios	0,2
Fibra alimentar	1,5
Fibras solúveis	0,1
Calorias (kcal)	128,0
Cálcio (mg)	4,2
Ferro (mg)	0,3
Fósforo (mg)	25,5
Potássio (mg)	328,0
Vitamina C (mg)	15,8

Fonte: ALVES (1999).

2. Amido e farinha de banana

O amido é o carboidrato mais abundante na banana verde (mais de 70% da sua massa seca), apresentando um rendimento de extração entre 12 e 70% (BELLO-PÉREZ et al., 2000). Pesquisas pioneiras desenvolvidas por Chiang et al. (1987) e Flores-Gorosquera et al. (2004) avaliaram em planta piloto o processamento de extração do amido de bananas verdes, bem como sua caracterização físico-química e avaliação da forma e tamanho dos grânulos. Os resultados obtidos tornaram possível sugerir que as

bananas apresentam um grande potencial para uso como matéria-prima amilácea. Em geral, os grânulos de amido de banana são de formato oval elipsóide de diâmetro maior (longitudinal: 35 – 55 μm e radial: 19,5 – 32,8 μm) que os apresentados pelos grânulos de amido de mandioca (5 – 35 μm), batata-doce (10 – 25 μm) e milho (2 – 30 μm), o que facilita o seu processo de extração (BELLO-PÉREZ et al., 2005; HOOVER, 2001).

O amido é um biopolímero constituído por uma mistura de dois polissacarídeos: amilose e amilopectina. No caso do amido de banana, o conteúdo de amilose varia entre 22% e 37%, e o conteúdo de amilopectina entre 63 a 78%, dependendo da variedade (APARICIO-SAGUILÁN et al., 2005; DE LA TORRE-GUTIÉRREZ et al., 2007). Devido ao alto conteúdo de amilose e as longas cadeias de amilopectina, o amido de banana produz uma pasta que desenvolve maior viscosidade e estabilidade quando comparado com outros amidos, cujo conteúdo de amilose e comprimento de cadeia da amilopectina são menores (ESPINOSA-SOLIS et al., 2009). As propriedades térmicas dos amidos, como a temperatura de gelatinização, dependem também do conteúdo de amilose, lipídios e do tamanho dos grânulos. Espinosa-Solis et al. (2009) encontraram temperaturas de gelatinização inicial (T_o), de pico (T_p) e final (T_f), bem como entalpia de gelatinização (ΔH), superiores para o amido de banana em relação aos outros amidos (Tabela 2). Segundo esses autores, estas propriedades refletem as forças intermoleculares mais intensas no interior dos grânulos do amido de banana.

Tabela 2. Propriedades térmicas do amido de milho, manga e banana.

Fonte	Parâmetros de gelatinização *			
	T_o (°C)	T_p (°C)	T_f (°C)	ΔH (J/g)
Milho	63.9 ± 0.2	68.7 ± 0.3	73.2 ± 0.4	13.4 ± 0.3
Manga	66.5 ± 0.2	71.3 ± 0.3	76.1 ± 0.3	14.0 ± 0.7
Banana	70.9 ± 0.6	76.5 ± 0.9	83.3 ± 1.0	16.5 ± 0.7

* Temperatura inicial (T_o), temperatura de pico (T_p), temperatura final (T_f) e entalpia de gelatinização (ΔH). Fonte: ESPINOSA-SOLIS et al., 2009.

A cristalinidade dos grânulos de amido é atribuída principalmente à amilopectina e não à amilose, que embora seja linear, apresenta uma conformação que dificulta sua associação regular com outras cadeias. O amido nativo pode ser classificado em três tipos de estruturas cristalinas a partir das diferenças dos difratogramas de raios-X: amidos de cereais de tipo “A”, amidos de tubérculos de tipo “B” e amidos de raízes e legumes de tipo “C”, uma mistura de “A” e “B” (ZHANG et

al., 2005). A formação de cristais do tipo “B” tem sido frequentemente reportada para o amido de banana (FAISANT et al., 1995; LII et al., 1982; TEIXEIRA et al., 1998), ao mesmo tempo que Bello-Pérez et al. (2005), De la Torre-Gutiérrez et al. (2007), Jane et al. (1999) e Waliszewski et al. (2003) encontraram um padrão do tipo “C”; portanto o tipo de cristalinidade para o amido da banana ainda não está claro. Este comportamento estaria relacionado à variedade da fonte, condições de crescimento e/ou da técnica utilizada para isolar o amido.

O amido de banana verde é altamente resistente à hidrólise enzimática. De acordo com Zhang et al. (2005), o grânulo possui uma espessa camada externa que impede a ação enzimática reduzindo a taxa de hidrólise. Vários fatores podem afetar a formação do amido resistente (AR): o teor de amilose, o tipo de amido, a umidade, o tempo e a temperatura de armazenamento dos géis de amido, os ciclos de autoclavagem/resfriamento, além da presença de lipídios e proteínas (ZHANG et al., 2005). Hernández et al. (2008) pesquisaram a quantidade de AR em diferentes fontes de amido. Os maiores valores foram relatados para os amidos de banana (44,01%) e batata (45,56%), seguido pelos amidos de sagu (34,47%) e milho (0,44%).

Na Tabela 3 é apresentada a composição da farinha e do amido obtidos a partir de bananas verdes de diferentes variedades, mas de mesma espécie (*Musa paradisiaca*). A farinha e o amido de banana são materiais promissores a serem empregados na elaboração de filmes biodegradáveis, dada sua interessante composição centesimal. Cabe ressaltar seu considerável conteúdo de amido com alto teor de amilose, além de fibras, proteínas e lipídios. Em virtude da composição centesimal da farinha e do amido, estas matérias-primas apresentam diferenças quanto às suas características físico-químicas, funcionais e térmicas, o que poderia influenciar nas propriedades dos filmes resultantes.

Tabela 3. Composição centesimal da farinha e do amido de banana (g/100 g em base seca).

Componente	Farinha	Amido
Umidade ^a	11,7 ± 0,1	12,9 ± 0,3
Cinzas	3,6 ± 0,1	1,3 ± 0,3
Proteína	3,3 ± 0,2	2,0 ± 0,2
Lipídios	0,9 ± 0,5	0,2 ± 0,1
Amilose ^b	22,2 ± 1,3	36,2 ± 0,8
Amido	80,2 ± 2,9	97,2 ± 2,4

^a em base úmida.

^b g/100 g de amido seco.

Fonte: farinha (DARAMOLA e OSANYINLUSI, 2006) e amido (BELLO-PÉREZ et al., 1999).

3. Filmes biodegradáveis

Os filmes biodegradáveis constituem materiais finos e flexíveis produzidos a partir de biopolímeros, que podem atuar como coberturas, quando aplicadas diretamente sobre a superfície dos alimentos e como filmes, quando possuem a capacidade de formar estruturas próprias independentes do produto embalado (KROCHTA e DE MULDER-JOHNSTON, 1997).

3.1. Filmes biodegradáveis a base de amido

O amido é considerado a matéria-prima mais promissora na elaboração de filmes, graças à sua capacidade de formar uma matriz apropriada, além do seu baixo custo, disponibilidade e produção a partir de fontes renováveis. As macromoléculas de polissacarídeos como o amido são estruturas compostas pela interação da amilose e amilopectina. Dentre estes dois biopolímeros, a amilose tem sido mais associada com a capacidade para formar filmes e coberturas, devido à sua natureza linear. Um filme pode ser feito de qualquer tipo de amido que contém amilose, quando preparado nas condições requeridas para a formação de filmes (KRAMER, 2009).

Diversos estudos têm sido publicados sobre a influência da proporção amilose/amilopectina (ALVES et al., 2007; LOURDIN et al., 1995), o emprego de plastificantes (MALI et al., 2006; MÜLLER et al., 2008) e condições de fabricação (RINDLAV et al., 1997; STANDING et al., 2001) nas propriedades dos filmes de

amido. Lourdin et al. (1995) estudaram o efeito da proporção amilose/amilopectina usando uma combinação de frações de amidos nativos, reportando que a resistência mecânica e a elongação na ruptura aumentaram com o incremento do percentual de amilose. Hernández et al. (2008) elaboraram filmes comestíveis à base de amidos de banana e biri com e sem adição de plastificante (glicerol), e estudaram a digestibilidade *in vitro* desses filmes, em comparação com filmes produzidos a partir de amidos de milho e batata. Bergo et al. (2008) estudaram o efeito da concentração de glicerol em filmes de amido de mandioca. Dias et al. (2010) desenvolveram filmes biodegradáveis à base de amido de arroz com adição de glicerol ou sorbitol como plastificantes. Araujo-Farro et al. (2010) estudaram o efeito de algumas variáveis de formulação e processo nas propriedades de filmes à base de amido de quinoa, e otimizaram a formulação e as condições de processo para esses filmes.

Embora a utilização de diferentes amidos na produção de filmes tenha sido bastante estudada, estes materiais possuem moderada permeabilidade ao oxigênio, baixa barreira à umidade e baixa resistência mecânica (KROCHTA e DE MULDER-JHONSTON, 1997). Uma alternativa para melhorar tais propriedades consiste na preparação de filmes compostos, obtidos a partir da combinação de polissacarídeos, proteínas e lipídios ou a adição de fibras (GUILBERT et al., 1997; WOLLERDORFER e BADER, 1998). Esta alternativa tem incentivado pesquisas para melhorar as propriedades dos filmes de amido dependendo das características desejadas e em virtude do uso a que se destinam.

3.2. Filmes biodegradáveis a base de farinha

O emprego de misturas naturais de uma mesma origem para elaboração de filmes biodegradáveis é uma tendência recente e compreende o grupo das farinhas de diversas fontes como frutas, grãos de cereais, pseudocereais, tubérculos e rizomas. O interesse em combinar polissacarídeos, proteínas, lipídios e fibras deve-se às vantagens e desvantagens de cada componente e ao fato de estarem em seu sistema original. Assim, as propriedades dos filmes de farinha dependem do tipo de interações formadas por seus biopolímeros (amido, proteína e fibras) e pelo lipídio, da distribuição dessas interações dentro da matriz do filme, do balanço das interações hidrofílicas e hidrofóbicas, bem como da concentração de cada componente dentro do filme (ANDRADE-MAHECHA et al., 2012; TAPIA-BLÁCIDO et al., 2005).

Rayas e Hernández (1997) prepararam filmes comestíveis a partir de três tipos de farinha de trigo, já Mariniello et al. (2003) utilizaram farinha integral de soja e pectina de maçã como matérias-primas para a produção de filmes. Tapia-Blácido et al. (2005, 2011) reportaram que filmes produzidos à base de farinha de amaranto (*Amaranthus caudatus* e *Amaranthus cruentus*), respectivamente, apresentaram ótimas propriedades de barreira ao oxigênio e vapor de água, quando comparados com outros filmes. Dias et al. (2010) verificaram que a farinha de arroz é uma boa matéria-prima para o preparo de filmes, propondo uma nova alternativa para a utilização do arroz. Andrade-Mahecha et al. (2012) otimizaram a formulação e as condições de processo para elaboração de filmes de farinha a partir de rizomas de biri (*Canna indica* L.).

4. Materiais de reforço para filmes

O emprego de fibras como material de reforço começou no início do século XX com a utilização da celulose em resinas fenólicas. Hoje em dia, a indústria dos polímeros reforçados com fibras é um negócio que movimenta bilhões de dólares ao redor do mundo (JHON e THOMAS, 2008). O emprego de fibras em compósitos tem-se incrementado muito nas últimas décadas pelas vantagens que este material oferece como seu baixo custo, boa resistência mecânica e biodegradabilidade, além de ser proveniente de fontes renováveis de matéria-prima disponíveis em todo o mundo.

A eficiência do reforço a partir de fibras naturais está associada à natureza do polímero (celulose) e sua cristalinidade. Geralmente, a resistência mecânica e a rigidez das fibras aumentam com o conteúdo de celulose. O ângulo de orientação das microfibrilas determina a dureza das fibras. Desta maneira, as fibras vegetais são mais flexíveis se suas microfibrilas encontram-se orientadas na forma de espiral em direção ao eixo da fibra, enquanto a orientação paralela das microfibrilas ao eixo está associada a maior rigidez das fibras (EICHHORN et al., 2010). Pesquisas orientadas à melhoria das propriedades de barreira de filmes a base de amido indicam que a adição de fibras naturais reduz a permeabilidade ao vapor de água dos filmes (MÜLLER et al., 2009). No entanto, a melhoria das propriedades de filmes com adição de fibras depende do grau de incorporação das fibras, o qual é limitado em função das dificuldades de dispersão na matriz polimérica. Assim, fatores como a técnica de processamento usada, a natureza físico-química da matriz e o grau de interação matriz-fibra afetam tal incorporação (AVÉROUS et al., 2001; DUFRESNE et al., 2000). A natureza polar e

hidrofílica das fibras lignocelulósicas constitui a maior desvantagem para seu uso na elaboração de filmes, dada sua incompatibilidade com polímeros não polares. Além disso, sua alta capacidade de absorver umidade limita o emprego destas fibras para algumas aplicações. Diante disso, as propriedades dos compósitos dependem das características dos seus componentes individuais e da compatibilidade interfacial entre estes (GEORGE et al., 2001; JHON e THOMAS, 2008).

4.1. Fibras vegetais

As fibras vegetais são estruturas alongadas de secção transversal arredondada, amplamente distribuídas na natureza podendo ser classificadas de acordo com a origem anatômica como fibras de talo (como as de juta, ramí, linho e algodão), fibras de folha (sisal, abacaxi, banana e palma), fibras de lenho (bambu, bagaco de cana) e fibras de superfície que formam a camada protetora de caules, folhas, frutos e sementes (açai e coco) (FAGURY, 2005). São abundantes, renováveis, recicláveis, biodegradáveis e têm um custo mais acessível que as fibras sintéticas. As fibras vegetais podem ser consideradas como compósitos de fibrilas de celulose interligadas por uma rede de moléculas de hemicelulose e lignina permeada por pectinas (Figura 2) (JHON e THOMAS, 2008). Na Figura 3 é apresentada a estrutura comum de uma fibra vegetal, a qual possui camadas constituídas por microfibrilas formadas por longas cadeias de celulose estendidas (entre 30 e 100) que circundam o lúmen ou cavidade central de seção elíptica localizada no interior da fibra (SILVA et al., 2009). O principal componente das microfibrilas é a celulose.

A celulose é a substância orgânica mais abundante na natureza, constitui um terço de toda matéria vegetal e é o principal constituinte da parede celular de vegetais superiores, sendo seu elemento de estrutura mais importante. Do ponto de vista químico, é um homopolissacarídeo estrutural constituído por unidades de β -D-glicopirranose em arranjo linear unidas por ligações glicosídicas do tipo β (1 $\rightarrow$ 4) (SILVA et al., 2009). As cadeias de celulose formam ligações de hidrogênio intra e intermoleculares. As ligações intramoleculares ocorrem entre grupos hidroxila da mesma molécula, enquanto as intermoleculares ocorrem entre grupos hidroxila de cadeias adjacentes. O primeiro tipo de interação é responsável pela rigidez da cadeia e o segundo pela formação da fibra vegetal. Desta maneira, as fibras naturais são de

natureza hidrofílica, pois o grande número de grupos hidroxila incrementa a afinidade da celulose com a água (LAVONE et al., 2012).

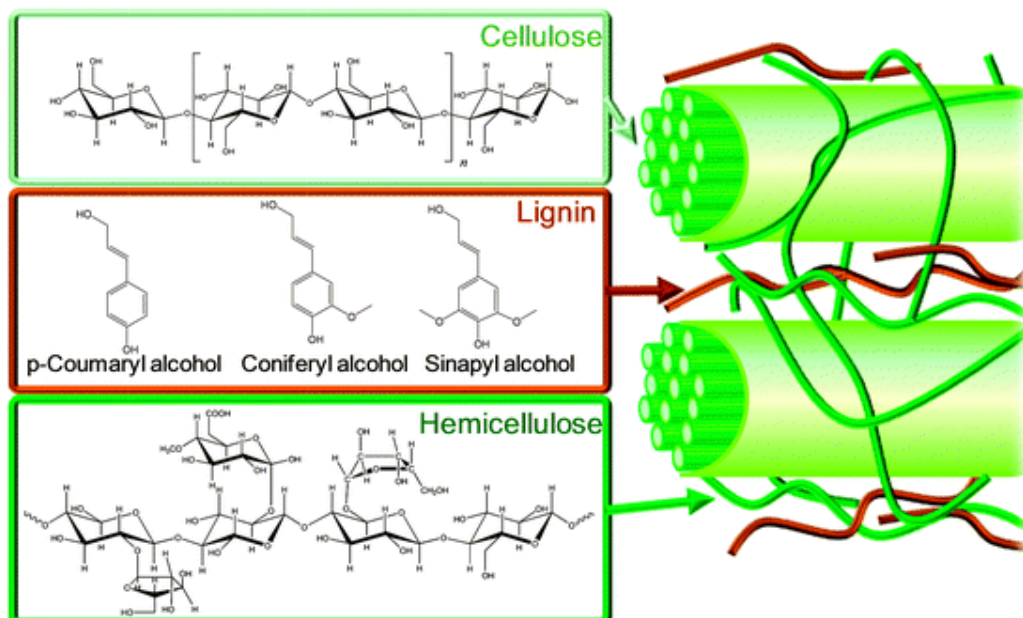


Figura 2. Estrutura de um material lignocelulósico e seus componentes. Fonte: ALONSO et al. (2012).

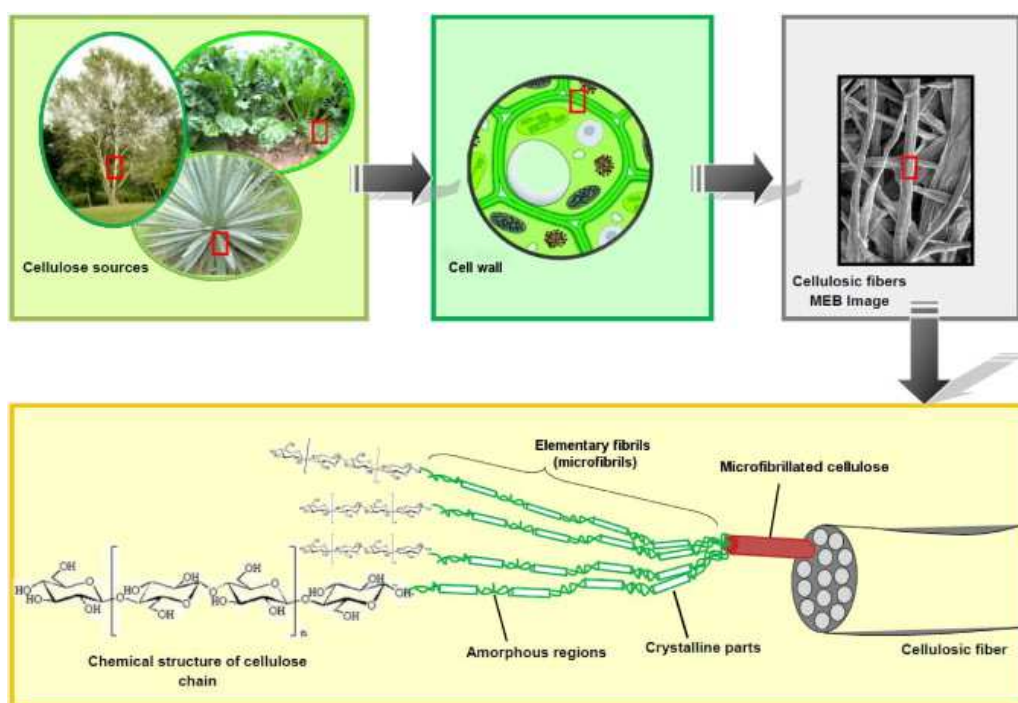


Figura 3. Estrutura de uma fibra vegetal. Fonte: LAVOINE et al. (2012).

As microfibrilas que compõem as fibras, resultantes do arranjo das moléculas de celulose, são formadas por regiões ordenadas tridimensionalmente (cristalitos) que se alternam com regiões completamente desordenadas (amorfas) constituídas de hemicelulose, lignina e pectina. A relação entre regiões ordenadas e desordenadas varia consideravelmente conforme a origem da fibra. O tipo de ligações de hidrogênio que conformam a rede de celulose faz com que este seja um polímero relativamente estável. Esta rede também dá às cadeias de celulose alta rigidez axial e esta rigidez é uma propriedade desejável para uma fibra de reforço em compósitos (EICHHORN et al., 2010).

As propriedades físicas das fibras naturais são influenciadas pela estrutura química da celulose, o grau de polimerização, a orientação molecular e a cristalinidade. Estas, por sua vez, encontram-se influenciadas pela origem e as condições durante o crescimento da planta como também pelos métodos de extração empregados (FAGURY, 2005; GEORGE et al., 2001; SILVA et al., 2009). Diversas pesquisas têm sido orientadas ao aproveitamento de fibras de diferentes fontes vegetais e ao estudo de tratamentos químicos, mecânicos e/ou enzimáticos para a obtenção de celulose.

4.2. Nanofibras

A produção de fibras de celulose em escala nanométrica e sua aplicação em materiais compósitos são temas de pesquisas recentes. Embora haja uma crescente atividade de publicação, o número ainda é modesto quando comparado com publicações que abordam materiais de reforço inorgânico (por exemplo, nanoargilas). A aplicação de nanofibras de celulose pode ainda se encontrar limitada devido à dificuldade de separação das fibras vegetais em componentes nanoestruturados (SIRÓ e PLACKETT, 2010).

O termo “microfibrilas” é geralmente usado para descrever estruturas formadas durante a biossíntese de celulose em plantas superiores, com dimensões de 2 – 10 nm de espessura e várias dezenas de microns de comprimento, dependendo da fonte de celulose (KHALIL et al., 2012). Quando submetidas a tratamentos químicos, físicos e outros, essas microfibrilas de celulose podem ser clivadas transversalmente ao longo das regiões amorfas, resultando em um material com alta relação de aspecto (razão entre comprimento e largura) e alta área superficial específica ($> 100 \text{ m}^2/\text{g}$), conhecido como nanofibras de celulose (GARDNER et al., 2008). Os parâmetros dimensionais para as

várias formas de celulose encontram-se resumidos na Tabela 4. Alguns sinônimos para celulose microfibrilada incluem agregados de microfibrilas, celulose microfibrilar e celulose nanofibrilada; já os *whiskers* de celulose são também reportados na literatura como *nanowhiskers*, *nanorods* e cristais de celulose, entre os mais utilizados recentemente (SIRÓ e PLACKETT, 2010).

Tabela 4. Dimensões de nanocelulose.

Tipo de estrutura de celulose	Diâmetro (nm)	Comprimento (nm)	Relação de aspecto (L/D)
Microfibrilas	2 – 10	> 10000	> 1000
Celulose microfibrilada	10 – 40	> 1000	100 – 150
<i>Whiskers</i> de cellulose	2 – 20	100 – 600	10 – 100
Celulose microcristalina	> 1000	> 1000	~ 1

Fonte: KHALIL et al. (2012).

Devido ao quase perfeito arranjo cristalino das nanofibras, esta forma de nanocelulose tem uma elevada resistência e, portanto, um potencial significativo como um material de reforço (EICHHORN et al., 2010). Diferentes fontes têm sido utilizadas para a obtenção dessas nanofibras de celulose. Entre as fontes vegetais pesquisadas encontram-se: polpa de beterraba açucareira (DINAND et al., 1996), de madeira conífera (SAXENA et al., 2009), de batata (DUFRESNE et al., 2000), eucalipto (CURVELHO et al., 2001), sisal (MORÁN et al., 2008), cânhamo (CAO et al., 2008b), linho (CAO et al., 2008a), farelo de trigo (FAMÁ et al., 2009) e casca de ervilha (CHEN et al., 2009). Existem trabalhos reportados na literatura utilizando o talo da bananeira (ZULUAGA et al., 2007; 2009) e fibras da polpa da banana (CHERIAN et al., 2008) para a obtenção das nanofibras de celulose. No Brasil, podem ser citados alguns estudos utilizando fibra de açaí, coco e juta (FAGURY, 2005), bagaço e palha de cana de açúcar (DA LUZ et al., 2006) e bagaço de mandioca (TEIXEIRA et al., 2009), entre outros.

4.3. Métodos de obtenção de nanofibras de celulose a partir de fibras vegetais

Devido às diferentes procedências e condições de processo de separação, embora constituídas por moléculas de celulose, as nanofibras podem apresentar particularidades nas suas características dimensionais e superficiais e, consequentemente, no seu desempenho como material de reforço (SILVA e

D'ALMEIDA, 2009). Desde 1980, diferentes métodos têm sido desenvolvidos para a separação de fibras de celulose a partir de fontes vegetais, já que quanto menor o tamanho da partícula maior o índice de cristalinidade obtido (BONDESON et al., 2006). Esses métodos podem ser agrupados em: tratamentos químicos, mecânicos e dissolução, sendo utilizados separadamente, em sequência ou combinação (HUBBE et al., 2008). Apesar da diversidade de fontes para se obter nanopartículas, os procedimentos para o isolamento de celulose, geralmente seguem um esquema de três etapas: (1) tratamento químico ou enzimático para eliminar os componentes amorfos das fibras como hemiceluloses e lignina; (2) hidrólise parcial, por ácidos ou enzimas para quebrar a estrutura das fibras em cristais; e (3) desintegração mecânica, por exemplo, pelo uso de forças de cisalhamento, para separar as nanopartículas. Na maioria dos casos, estas etapas estão orientadas em produzir uma suspensão coloidal de nanofibras em água, estabilizada pelas cargas negativas dos grupos aniônicos introduzidas durante uma etapa de hidrólise parcial. Detalhes dos tratamentos, assim como a ordem das etapas (2) e (3), dependem da fonte da celulose (NEWMAN e STAIGER, 2008). Desta maneira, a separação das fibras a partir de matérias-primas celulósicas consiste de várias etapas, começando no pré-tratamento, passando pela hidrólise e finalizando com processos de purificação. Alguns autores consideram que o pré-tratamento das fibras é necessário para garantir a eficácia da hidrólise. Nesta etapa, o material é classificado e purificado por meio de operações de moagem e classificação em peneiras (SILVA e D'ALMEIDA, 2009) e, em alguns casos, a adição de hidróxido de sódio ou de potássio é utilizada com o objetivo de purificar o material antes da hidrólise com ácido (HABIBI et al., 2007).

A remoção de componentes amorfos das fibras, como a lignina, pode ser realizada mediante o emprego de reagentes seletivos e sob condições de processo brandas para evitar a solubilização e degradação de celulose e hemicelulose. A utilização do cloro e seus compostos na remoção da lignina em fibras vegetais é o meio mais utilizado na escala laboratorial. Desta maneira, a lignina é rapidamente oxidada pelo cloro e cloritos, promovendo a sua deslignificação e a formação de grupos hidroxila, carbonila e carboxílico, os quais facilitam a solubilização da lignina em meio alcalino e, portanto, a purificação da celulose (DUFRESNE et al., 2000).

A maioria dos tratamentos químicos para a obtenção de celulose reportados na literatura emprega ácidos fortes. O uso de ácidos deve-se ao fato das regiões cristalinas serem insolúveis em ácidos nas condições em que estes são empregados e que a desorganização natural das moléculas de celulose nas regiões amorfas, favorece a

hidrólise das cadeias presentes nestas regiões (SILVA e D'ALMEIDA, 2009). Na hidrólise ácida pode ser utilizado ácido sulfúrico ou clorídrico. Segundo Bondeson et al. (2006), a hidrólise com ácido sulfúrico foi inicialmente documentada em 1951 e atualmente constitui o ácido mais utilizado para a separação de nanofibras de celulose. Araki et al. (1998) avaliaram o efeito do tipo de ácido empregado sobre as propriedades de dispersão de *whiskers* de celulose, e observaram que estes apresentaram tamanhos e formas de partículas similares para os dois tipos de ácidos (sulfúrico e clorídrico). Entretanto, o emprego de ácido sulfúrico levou à obtenção de dispersões aquosas de nanopartículas de celulose mais estáveis.

De acordo com a literatura, variáveis como concentração de ácido, tempo de tratamento, temperatura e a relação ácido/matéria-prima exercem alta influência nas características das nanofibras obtidas (ARAKI et al., 1998; ELAZZOUI-HAFRAOUI et al., 2008).

Nos últimos anos, o tratamento mecânico com alta pressão tem provado ser um procedimento simples e eficiente para separar as nanofibras obtendo suspensões mais homogêneas e estáveis (DUFRESNE et al., 2000). Os homogeneizadores de alta pressão consistem basicamente em uma bomba de pistões com deslocamento positivo, onde a pressão do sistema é controlada por uma válvula que restringe a passagem do líquido (suspensão) através de um orifício pressurizado (Figura 4). Durante o processo, a suspensão é adicionada em um recipiente de alimentação e bombeada com baixa velocidade. Com o fechamento da válvula, há uma redução da área de passagem do líquido, aumentando desta forma a pressão do sistema. O produto passa por um orifício na ordem de milímetros chocando-se com velocidade extremamente elevada (200 – 300 m/s) com o dispositivo de impacto em volta da válvula (HERRICK et al., 1983). Através da queda brusca de pressão e do impacto do líquido com as aletas, as partículas maiores fracionam-se em partículas menores. A uniformidade e o diâmetro das partículas podem ser ajustados através das variáveis: pressão, número de ciclos (passagem da suspensão pela bomba) e concentração de nanopartículas na suspensão. Vários trabalhos têm mostrado a utilização do homogeneizador de alta pressão como etapa complementar no processo de isolamento de nanofibras (BESBES et al., 2010; DUFRESNE et al., 2000; KAUSHIK e SINGH, 2011; ZULUAGA et al., 2007). Segundo esses estudos, suspensões tratadas com homogeneizador de alta pressão apresentam nanofibras mais dispersas, individualizadas e de tamanho uniforme quando comparadas as suspensões sem tratamento mecânico.

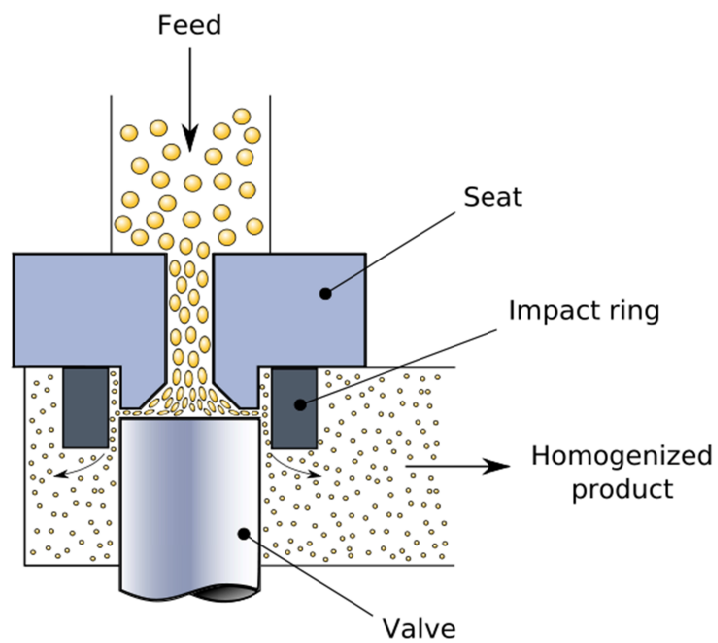


Figura 4. Esquema de funcionamento de um homogeneizador de alta pressão.

Na Tabela 5 são apresentados alguns trabalhos recentes sobre a obtenção de nanofibras empregando diferentes tratamentos.

Tabela 5. Procedimentos empregados para obtenção de nanofibras de celulose.

Fonte	Método	Dimensão da nanofibra	Referência
Casca de amoreira	Pré-tratamento alcalino: NaOH 1%, 80 °C, 2 h; Tratamento alcalino: NaOH 1% com Na ₂ S 1%, 80 e 130 °C, 1,5 h; Branqueamento: NaClO ₂ 0,7%, pH 5, 80 °C, 1,5 h; Hidrólise ácida: H ₂ SO ₄ 64%, 60 °C, 30 min.	Diâmetro: 20 – 40 nm Comprimento: 400 – 500 nm	Li et al. (2009)
Casca de ervilha	Hidrólise ácida: H ₂ SO ₄ 64%, 45 °C, 4, 8, 12, 16 e 24 h; Branqueamento: NaClO 40%; Tratamento: diálise, 3 dias.	Tempo de hidrólise: maior tempo, nanofibras com menores dimensões. Diâmetro: 7 – 12 nm Comprimento: 240 – 400 nm	Chen et al. (2009)
Fibras de pinho, eucalipto e grama esparto branqueada	Oxidação catalítica: sistema com 2,2,6,6-tetrametilpiperidina-N-oxilo (TEMPO), NaBr e NaClO ₂ (pH 7, 60 °C, 2 – 4 h); Tratamento mecânico: homogeneizador de alta pressão com 5 e 10 passagens.	Diâmetro: 5 – 20 nm	Besbes et al. (2011)
Fibras de eucalipto branqueadas	Tratamento mecânico: microfluidizador com 3, 4 e 5 passagens; Oxidação catalítica: sistema com TEMPO, NaBr e NaClO (pH 10, 25 °C, 1 h).	Diâmetro: 25 – 70 nm Comprimento: ~ 300 nm	Bilbao-Sainz et al. (2011)
Fibras da folha do abacaxi	Explosão a vapor com tratamento alcalino: NaOH 2%, em autoclave com pressão 138 kPa, 1 h; Branqueamento: NaOH, CH ₃ COOH glacial e NaClO (6x); Explosão a vapor com hidrólise ácida: C ₂ H ₂ O ₄ 11%, em autoclave com pressão 138 kPa, 15 min (2x); Tratamento mecânico: agitador mecânico, 8000 rpm, 4h.	Diâmetro: 5 – 15 nm Comprimento: > 1 µm	Cherian et al. (2011)
Fibras de pinho	Tratamento mecânico: triturador com 1, 3, 5, 9, 15 e 30 passagens.	Diâmetro: 20 – 50 nm Comprimento: > 1 µm	Iwamoto et al. (2007)

Continuação Tabela 5.

Fonte	Método	Dimensão da nanofibra	Referência
Palha de trigo	Pré-tratamento alcalino: NaOH 2%, durante a noite; Explosão a vapor com tratamento alcalino: NaOH 10-12%, em autoclave com pressão 20 bars, 200 °C, 4 h; Branqueamento: H ₂ O ₂ 8%, durante a noite; Hidrólise ácida: HCl 10%, 60 °C, 5 h; Tratamento mecânico: homogeneizador de alta pressão.	Diâmetro: 10 – 50 nm	Kaushik e Singh (2011)
Polpa de batata	Pré-tratamento: peneira de 0,25 mm; Tratamento alcalino: NaOH 2%, 80 °C, 2,5 h (2x); Branqueamento: NaClO ₂ , pH 5, 70 °C, 2,5 h; Tratamento mecânico: homogeneizador de alta pressão com 15 passagens.	Diâmetro: 2 – 4 nm Comprimento: > 1 µm	Dufresne et al. (2000); Dufresne e Vignon (1998)
Polpa de beterraba	Tratamento alcalino: NaOH 0,5M, 80 °C, 2 h; Branqueamento: NaClO ₂ , pH 5, 70 °C, 2 h; Tratamento mecânico: Ultra-Turrax, 24000 rotações/min; Tratamento mecânico: homogeneizador de alta pressão com 10 – 15 passagens.	Diâmetro: 30 – 100 nm Comprimento: > 1 µm	Leitner et al. (2007)
Polpa de abeto e pinho branqueada	Pré-tratamento: 1ª etapa de refinamento; Tratamento enzimático: endoglucanase; Pré-tratamento: 2ª etapa de refinamento; Tratamento mecânico: homogeneizador de alta pressão.	Diâmetro: 30 nm Comprimento: > 1 µm	Svagan et al. (2007)
Talo da bananeira	Método 1 Tratamento alcalino: H ₂ O ₂ 1,5%, pH 12, 45 °C, 14 h; Hidrólise ácida: ácido nítrico e acético, 120 °C, 15 min. Método 2 Tratamento alcalino: H ₂ O ₂ 1,5%, pH 12, 45 °C, 14 h; Tratamento mecânico: homogeneizador de alta pressão.	Método 1: nanofibras com menores dimensões. Diâmetro: 5 nm Comprimento: 500 – 1000 nm	Zuluaga et al. (2007)

5. Filmes reforçados com nanofibras de celulose

Os nanocompósitos são obtidos pela incorporação física de nanopartículas de celulose em matrizes poliméricas. As propriedades destes nanocompósitos dependem das características dos nanocristais de celulose, da matriz polimérica, da interação entre ambos e das técnicas de processamento.

A obtenção de nanocompósitos homogêneos é o principal desafio no seu processo de preparação. A utilização de matrizes hidrossolúveis facilita a formação de compósitos em virtude da alta dispersabilidade da suspensão coloidal dos nanocristais em meio aquoso. A importância da dispersão das nanofibras em matrizes poliméricas tem sido reportada por vários pesquisadores como pré-requisito para alcançar resultados desejáveis como materiais de reforço (KVIEN et al., 2005). Segundo Bondeson et al. (2006), a repulsão eletrostática de nanofibras em suspensão pode ser alcançada por hidrólise com ácido sulfúrico devido a introdução natural de grupos sulfatos sobre a superfície das nanopartículas durante essa etapa. Van den Berg et al. (2007) verificaram que nanofibras de celulose sem modificação de superfície apresentam fortes interações entre elas, com notória dificuldade em serem dispersas em água. A presença de tratamento mecânico com homogeneizador de alta pressão entre as etapas de obtenção de nanofibras também ajuda a promover uma melhor individualização dessas nanoestruturas, além de contribuir para formação de suspensões homogêneas (DUFRESNE et al., 2000). Na Tabela 6 são apresentadas as características de filmes obtidos em algumas pesquisas recentes, empregando nanofibras vegetais como material de reforço.

Tabela 6. Características de filmes reforçados com nanofibras vegetais.

Referência	Biopolímero / Nanofibras	Resultados
Alemдар e Sain (2008)	Amido de batata / Nanofibras da palha de trigo. Diâmetro: 10 – 80 nm Comprimento: > 1 µm	Dispersão uniforme das nanofibras na matriz polimérica observada pelas imagens de MEV. Aumento significativo da tensão na ruptura e módulo de Young nos nanocompósitos quando comparados com o filme de amido puro. Incremento da temperatura de transição vítrea (Tg) nos filmes adicionados de nanofibras.
Azeredo et al. (2009)	Purê de manga / Nanofibras de celulose comercial. Diâmetro: 7,2 nm Comprimento: 82,6 nm	Boa dispersão das nanofibras na matriz sem presença significativa de aglomerados. Aumento da tensão na ruptura e módulo de Young e, redução da elongação e permeabilidade ao vapor de água nos filmes reforçados com nanofibras. Pequeno incremento também na Tg dos nanocompósitos.
Chen et al. (2009)	Amido de ervilha / Nanofibras de casca de ervilha. Diâmetro: 7 – 12 nm Comprimento: 240 – 400 nm	Dispersão homogênea das nanofibras na matriz polimérica. Maior absorção ultravioleta, transparência, tensão na ruptura, elongação e resistência à água quando comparado com o filme de amido de ervilha sem adição de nanofibras.
Dufresne et al. (2000)	Amido de batata / Nanofibras da polpa da batata. Diâmetro: 2 – 4 nm Comprimento: > 1 µm	Melhora das propriedades mecânicas e térmicas nos nanocompósitos. Diminuição da sensibilidade à água conforme o aumento da concentração de nanofibras nos filmes.
Kaushik et al. (2010)	Amido de milho / Nanofibras da palha de trigo. Diâmetro: 10 – 60 nm	Melhora nas propriedades mecânicas com o aumento da concentração de nanofibras. Redução das propriedades de barreira com a adição de nanopartículas até 10%, porém uma concentração maior deteriorou as propriedades devido à aglomeração das fibras.
Teixeira et al. (2009)	Amido de mandioca / Nanofibras do bagaço de mandioca. Diâmetro: 2 – 11 nm Comprimento: 360 – 1700 nm	Diminuição da hidrofiliabilidade e, aumento da tensão na ruptura e elongação dos nanocompósitos plastificados com glicerol.

6. Conclusões

Os estudos citados nesta revisão bibliográfica sobre o uso de amido, farinha e celulose obtidos a partir de fontes vegetais evidenciaram que estes materiais são promissores para a produção de filmes biodegradáveis, devido a suas propriedades mecânicas, ao melhoramento da barreira ao oxigênio, vapor de água e solubilidade, além do seu caráter renovável e biodegradável. A eficácia da celulose como material de reforço em compósitos depende de vários fatores como a origem, métodos de obtenção, características físico-químicas e preparação dos compósitos. Diante da necessidade de desenvolver suspensões de nanofibras de celulose estáveis e individualizadas a fim de facilitar suas aplicações em diferentes áreas, o emprego de hidrólise ácida (H_2SO_4) e tratamento mecânico (homogeneizador de alta pressão) nas etapas de purificação das fibras vegetais apresenta-se como alternativa potencial para aprimorar a dispersão desses materiais. Estes tratamentos foram avaliados na obtenção de celulose a partir da casca da banana, visando à utilização deste biopolímero como material de reforço na elaboração de nanocompósitos de amido. Desta maneira, a compatibilidade dos materiais (amido, farinha e celulose) obtidos da mesma fonte vegetal (banana verde) foi avaliada a partir das propriedades dos filmes resultantes. Além disso, não há relatos na literatura sobre o emprego de nanocompósitos reforçados com nanofibras de celulose isoladas da casca da banana, o que comprova o grande caráter inovador desta proposta.

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Capítulo 2

Isolation and Characterization of the Flour and Starch of Plantain Bananas (*Musa paradisiaca*)

Abstract

Unripe plantain bananas of the variety “Terra” (*Musa paradisiaca*) may have industrial value due to their high starch content. In this chapter, flour and starch of such unripe fruits were isolated and their chemical, physicochemical and structural characteristics were determined. Banana flour and starch had a dry basis yield of 50.6 and 28.5%, and an average granule size of 31.7 and 47.3 μm , respectively. The starch in both raw materials revealed a C-type pattern and high gelatinization temperatures. The peak viscosity was greater for flour (378.0 RVU) than for starch (252.6 RVU), although the final viscosity was lower. At temperatures above 65 °C, the swelling power of banana flour was lower than that of starch, while the solubility of flour was greater than that of starch at all temperatures. Furthermore, the presence of other components in banana flour influenced its physicochemical properties. In general, the flour and starch processed from unripe bananas have numerous possible uses as ingredients in food systems and for other industrial purposes.

Keywords: plantain banana, Musa paradisiaca, flour, starch, physicochemical properties.

1. Introduction

Banana is a general term embracing a number of species or hybrids of the genus *Musa* of the family Musaceae. Almost all of the known edible-fruited cultivars arose from two diploid species, *Musa acuminata* and *Musa balbisiana* (VILAS BOAS et al., 2001; ZHANG et al., 2005).

Originating in Southeast Asia, bananas are an important food crop and are grown extensively in tropical and subtropical regions. Currently, worldwide production is around 90.7 million tons, and Brazil is the fourth largest world producer after India, the Philippines and China (FAO, 2009). Unfortunately, losses during banana production are very high, reaching 40% of the total, due to their highly perishable nature and inadequate post-harvest handling. These losses can be reduced by the processing of surplus fruits and those unsuitable for fresh consumption (TADINI and DITCHFIELD, 2006). Since unripe bananas contain large amounts of starch (over 70% of dry weight), their processing into flour and starch is of interest as a possible resource for food and/or other industrial purposes. The cultivar “Terra” (*Musa paradisiaca*), a type of plantain, has an exceptionally high starch content and since few studies in the literature deal with this particular variety, it was selected for investigation.

Starch is the principal component of unripe bananas, which undergoes important changes during ripening. Lii et al. (1982) investigated the chemical composition of bananas during the different stages of maturation and showed that as starch is degraded, the sucrose content increases and glucose and fructose accumulate. Moreover, a large portion of the starch found in unripe bananas is resistant, i.e. has a crystallinity that makes it only slightly susceptible to hydrolysis, a property of interest for functional foods (TRIBESS et al., 2009).

Pioneer research developed by Chiang et al. (1987) and Flores-Gorosquera et al. (2004) evaluated the process of starch extraction from unripe bananas on a pilot plant scale. According to these authors, the yield and purity of the banana starch isolated showed that the process was technically feasible on an industrial scale, thus offering an alternative source of starch. Suntharalingam and Ravindran (1993) also observed the potential for conversion of excess and rejected unripe bananas into flour.

The objective of the present study was to produce the flour and starch from the non-conventional source of plantain bananas to investigate their functional,

physicochemical and structural properties and suggest possible utilizations for these products in processed food.

2. Materials and methods

2.1. Materials

Flour and starch were prepared from unripe plantain bananas (mature green) of the variety “Terra” (*Musa paradisiaca*). The fruit was obtained from the harvest occurred in March 2010 in the state of Espírito Santo, Brazil, and was not subjected to any postharvest treatment. All chemicals used were reagent grade.

2.2. Banana flour preparation

Unripe bananas were washed and peeled. They were then cut into 0.5 cm slices and immediately rinsed in potassium metabisulfite solution (1% w/v) for 15 min to inhibit oxidation. The rinsed slices were placed on trays and dried in a forced air convection oven at 45 °C for 48 h. After drying, the material was ground in a knife mill (Marconi, model MA340, São Paulo, Brazil), consecutively sieved through screens (115, 150, 170 and 200 US mesh) to eliminate seed particles, and stored at 4 °C in a sealed container (Figure 1).

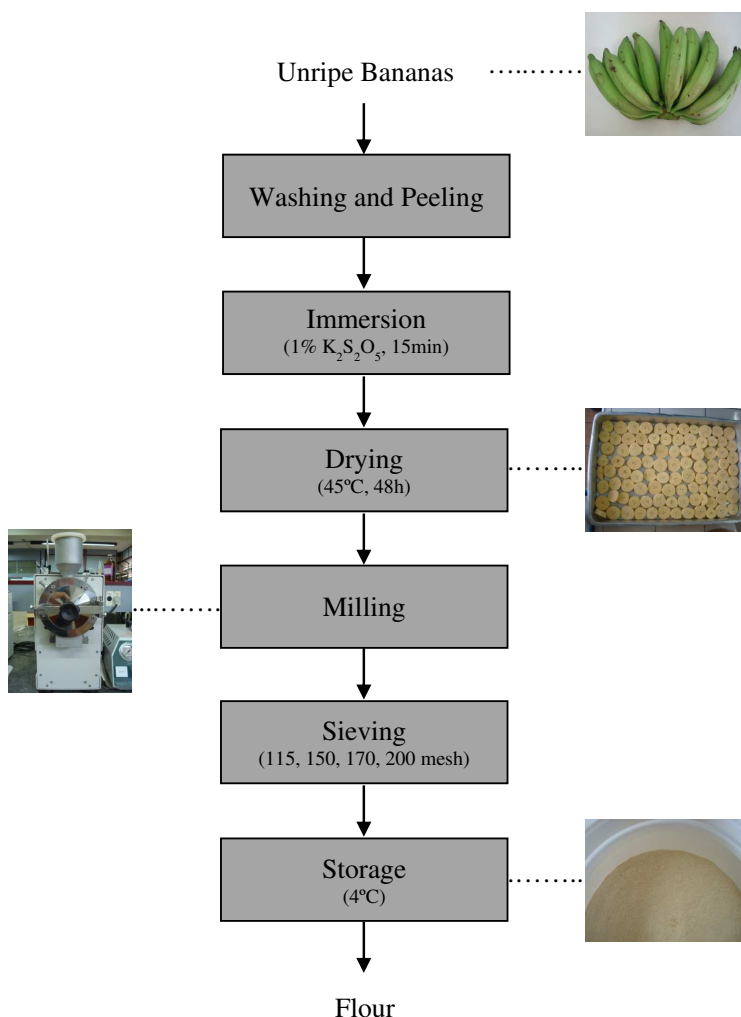


Figure 1. Scheme for preparation of the banana flour.

2.3. Banana starch preparation

Banana starch was obtained using the methodology described by Espinosa-Solis et al. (2009), with some modifications (Figure 2). Unripe bananas were washed, peeled and cut into 5 – 6 cm cubes. These cubes were immediately rinsed in potassium metabisulfite solution (1% w/v) for 15 min to inhibit oxidation. The material was added to a new potassium metabisulfite solution (ratio of 1:2) and macerated at low speed in a Waring blender for 2 min. The homogenate was consecutively sieved through 60 and 100 US mesh screens, resulting in two products: insoluble residue and starch milk. The starch milk was allowed to settle for 3 h and the resulting sediment was washed with hydrated ethyl alcohol (92.8° INPM) until it revealed a uniform white color. The sediment was then washed again for the removal of soluble fiber using a NaOH solution

(0.3% w/v). The cleaned starch sediment was dispersed in distilled water and repeatedly washed until neutrality. The resultant material was then placed on trays and dried in a forced air convection oven at 45 °C for 48 h. The dry starch was ground in a knife mill (Marconi, model MA340, São Paulo, Brazil) and stored at 4 °C in a sealed container.

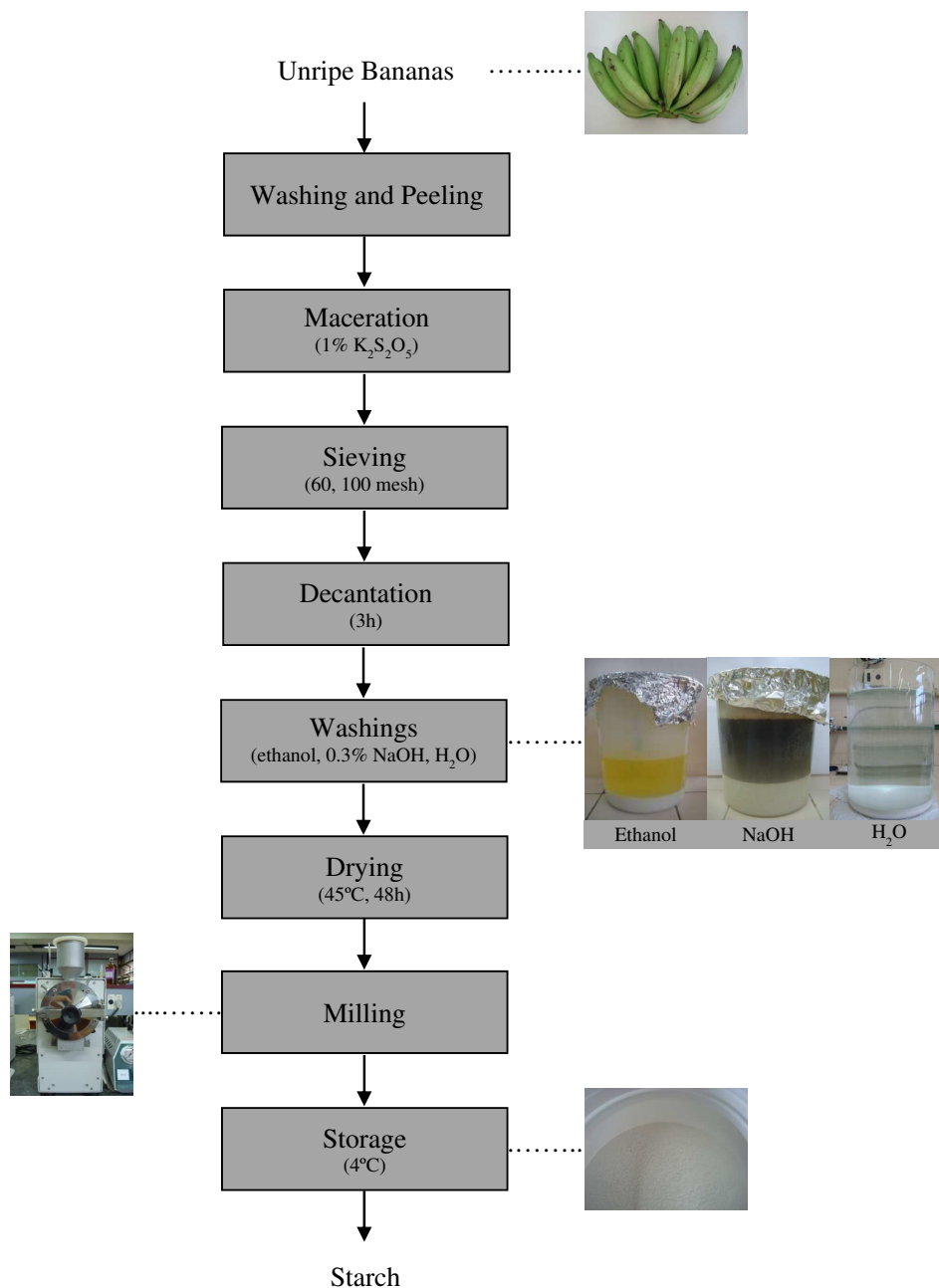


Figure 2. Scheme for preparation of the banana starch.

2.4. Characterization of flour and starch

2.4.1. Chemical and color analysis

The content of moisture, ash, protein and crude fiber was obtained using the standard methods of the AOAC (2005). Lipids were determined by the method of Bligh and Dyer (1959), and the amylose content according to the methodology in ISO 6647 (1987). Resistant starch was identified using the method proposed by Goñi et al. (1996), and total starch according to Diemair (1963). Samples of banana flour and starch were also subjected to color analysis using a colorimeter (UltraScan VIS, HunterLab, Virginia, USA) in the reflectance mode, with the classification system of the CIELab and illuminant D₆₅ (daylight) (HUNTERLAB, 1996). The parameters were classified directly by EasyMatch QC software: L (white = 100, black = 0); a (red = positive, green = negative); and b (yellow = positive; blue = negative). The color difference (ΔE^*) was determined using Equation 1:

$$\Delta E^* = \left[(L^* - L_o^*)^2 + (a^* - a_o^*)^2 + (b^* - b_o^*)^2 \right]^{1/2} \quad [1]$$

where L^* is the degree of lightness of the sample, L_o^* is the degree of lightness of the standard, a^* and b^* are the chroma parameters of the sample, and a_o^* and b_o^* are the chroma parameters of the standard.

The instrument was calibrated against a standard white reference plate ($L_o^* = 100$, $a_o^* = 0$ and $b_o^* = 0$). All the experiments were performed at least in triplicate, and the results are presented as mean values.

2.4.2. Scanning electron microscopy (SEM)

Banana flour and starch were analyzed by scanning electron microscopy for microstructure evaluation. Samples were fixed on aluminum stubs using double-sided tape and coated with a layer of gold (Sputter Coater POLARON, model SCD050) to improve conductivity. The coated samples were then viewed under a scanning electron microscope (JEOL, model JSM-5800LV, Tokyo, Japan) using an acceleration voltage of 10 kV.

2.4.3. Particle size distribution

The size of flour and starch particles was determined in triplicate with a laser diffraction analyzer (Laser Scattering Spectrometer Mastersizer S, model MAM 5005 - Malvern Instruments Ltd., Surrey, England) using ethanol as a solvent. An ultrasound device was coupled to the equipment to increase the dispersibility of the samples.

2.4.4. X-ray diffraction (XRD)

X-ray diffraction analysis was performed using an X-ray diffractometer (Siemens, model D5005, Baden-Württemberg, Germany) operated at a voltage of 40 kV and a current of 30 mA; the target was Cu. The diffraction data of the samples were collected over an angular range from 5 to 70° (2 θ), with a scanning speed of 1.2°/min. The relative crystallinity (%) of the banana flour and starch was quantitatively estimated following the method of Nara and Komiya (1983), as the ratio of crystalline area to total area of the diffractogram using Origin 8.0 software (OriginLab Corporation, Massachusetts, USA).

2.4.5. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra were recorded on a spectrophotometer (Perkin Elmer, model Spectrum One, Ohio, USA) fit with a Universal Attenuated Total Reflectance (UATR) device. Analysis was performed in the infrared region, with 16 scans in wavenumbers ranging from 4000 to 650 cm⁻¹ for a spectral resolution of 4 cm⁻¹.

2.4.6. Differential scanning calorimetry (DSC)

Thermal properties of samples were determined using a differential scanning calorimeter (TA-Instruments, model 2920, Pennsylvania, USA) equipped with a cooling system. Banana flour and starch (1.8 mg, d.b.) were weighed precisely in an aluminum pan and mixed with distilled water in order to obtain a total weight of 6 mg. The pans were sealed hermetically and allowed to equilibrate for 1 h. Samples were scanned at a heating rate of 10 °C/min over a temperature range of 10 to 100 °C. For all DSC runs, a sealed empty aluminum pan was used as reference. The values of onset (T_o), peak (T_p),

and final (T_f) gelatinization temperatures, and enthalpy change (ΔH) were obtained from the thermograms of the samples using Universal Analysis 3.9A software. The DSC measurements were performed in triplicate, and the results are presented as mean values.

2.4.7. Pasting properties

The pasting properties of banana flour and starch suspensions (14% moisture basis) were analyzed using a Rapid Visco Analyzer (RVA, Newport Scientific Pty. Ltd., New South Wales, Australia). The sample slurries were maintained 1 min at 50 °C, then heated from 50 to 95 °C at the rate of 6 °C/min. After holding at 95 °C for 5 min, the sample pastes were cooled to 50 °C at the same rate of 6 °C/min. The pasting temperature, peak viscosity, breakdown point, and final and setback viscosities were recorded for triplicate samples.

2.4.8. Swelling power and solubility

Swelling power (SP) and solubility (S) of the banana flour and starch samples were determined in triplicate according to the method of Yu et al. (2010). Approximately 0.5 g (d.b.) of sample was heated in 20 mL of distilled water at different temperatures (55, 65, 75, 87 and 95 °C) for 30 min. Each sample was cooled to room temperature and centrifuged at 3000 rpm for 15 min. The supernatant was poured off from the tube into a glass dish (of known weight), dried at 105 °C for 24 h and weighed. The material adhering to the wall of the centrifuge tube, considered to be the wet sediment, was weighed for swelling power determination. Swelling power (g/g) and solubility (%) were estimated with Equations 2 and 3, respectively:

$$SP = \frac{W_s}{W - W_d} \quad [2]$$

$$S = \frac{W_d}{W} \times 100 \quad [3]$$

where W_d is the weight of the dried supernatant (g), W_s is the weight of the wet sediment (g), and W is the weight of the sample (g).

2.4.9. Light microscopy

Flour and starch gelatinization was followed by microscopy analysis. The samples heated at 55, 65, 75, 87 and 95 °C were mounted on a microscope slide covered with a coverslip and stained with iodine solution (0.2% I₂ and 2% KI). The stained samples were photographed using a camera attached to an optical microscope (Carl Zeiss, model Jenamed Variant, Jena, Germany) with 40x magnification.

2.5. Statistical analysis

An analysis of variance (ANOVA) and a Tukey test of multiple comparisons with a significance level of 5% were run using Statistica 7.0 software (StatSoft Inc, Tulsa, Oklahoma, USA) to compare the differences between means for the thermal and pasting properties of banana flour and starch.

3. Results and discussion

3.1. Chemical and color analysis

The starch isolated from “Terra” bananas (*Musa paradisiaca*) had a dry basis yield of 28.5%, less than that reported in most of the specialized literature: 46 – 70% for Taiwan bananas (*Musa sapientum*) (CHIANG et al., 1987), 43.8% for “Macho” bananas (*Musa paradisiaca*) (BELLO-PÉREZ et al., 2000), 33.8% for “Valery” bananas (*Musa valery*) (WALISZEWSKI et al., 2003) and 52.3 – 69.6% for square bananas (*Musa balbisiana*) (DE LA TORRE-GUTIÉRREZ et al., 2007). Only one study reported a lower value of 11.8% for “Criollo” bananas (*Musa paradisiaca*) (BELLO-PÉREZ et al., 2000). Although few reports of the yield of banana flour were found in the literature the one presented here (50.6%, d.b.) was greater than those reported by Ormenese (2010) for “Nanicão Jangada” (21.2 – 36.8%) banana, and Suntharalingam and Ravindran (1993) for “Alukehel” (31.3%) and “Monthan” (25.5%) bananas (*Musa paradisiaca*). Different stages of ripening, as well as specific species and extraction processes are likely the main factors behind the differences in yield.

The chemical composition and color parameters of banana flour and starch are presented in Table 1. The contents of ash, protein, lipids and crude fiber of banana

starch are comparable to those reported by other authors, such as Eggleston et al. (1992) and Pérez-Sira (1997). However, higher values were reported for “Macho” and “Criollo” banana starch (BELLO-PÉREZ et al., 1999). Despite the use of different varieties of bananas, the composition of flour was similar to what was found by Da Mota et al. (2000), Kayisu et al. (1981), Ormenese (2010), and Suntharalingam and Ravindran (1993). The lower values of ash, protein, lipids and crude fiber in starch than in flour show that these components are extensively extracted during starch preparation.

Table 1. Chemical composition (g/100 g d.b., except moisture) and color parameters of banana flour and starch.

	Flour	Starch
<i>Components</i>		
Moisture	8.00 ± 0.02	9.27 ± 0.07
Ash	1.92 ± 0.10	0.03 ± 0.01
Protein	3.16 ± 0.10	0.97 ± 0.16
Lipid	0.56 ± 0.03	0.02 ± 0.01
Crude fiber	1.18 ± 0.07	0.28 ± 0.03
Amylose ^a	27.8 ± 0.1	36.9 ± 0.3
Resistant starch ^a	49.5 ± 0.8	50.3 ± 1.0
Total starch	83.2 ± 0.2	94.8 ± 0.1
<i>Color parameters</i>		
L*	86.6 ± 0.1	90.9 ± 0.4
a*	0.6 ± 0.1	0.6 ± 0.1
b*	9.5 ± 0.1	3.9 ± 0.1
ΔE*	16.5 ± 0.1	9.9 ± 0.4

^a g/100 g of dry starch.

Banana starch had an amylose content (35.0%) greater than those reported for square bananas (22.2%) (DE LA TORRE-GUTIERREZ et al., 2007), plantain starches (9.11 – 17.16%) (EGGLESTON et al., 1992), and “Cavendish” variety bananas (19.5%) (LING et al., 1982), but similar to that reported for “Macho” variety bananas (37%, 36.2%) (APARICIO-SAGUILÁN et al., 2005; ESPINOSA-SOLIS et al., 2009). The amylose content affects functional and physicochemical properties, with starches of higher amylose contents producing firmer and more opaque gels, and making them a potential ingredient in sausages and meat emulsions requiring such properties for texture (DE LA TORRE-GUTIERREZ et al., 2007). Materials with high amylose content are also suitable to be used as raw material for edible packaging (MALI et al., 2004).

It is well established that variable amounts of starch in food can escape digestion in the human duodenum and pass into the colon; this fraction is so-called resistant starch (RS). Unripe banana starch is very resistant to digestion in both rats and humans as studied by Faisant et al. (1995a; 1995b). In the present paper, banana flour and starch revealed large fractions of RS (49.5 and 50.3%, respectively), making them interesting for addition to functional foods, since RS has a positive effect in the human colon. The contents obtained here were greater than those reported by Hernández et al. (2008) in their investigation of the amount of RS in different starch sources, where potato had 45.56%, banana 44.01%, sagu 34.47% and corn 0.44%. Tribess et al. (2009) found that drying conditions affected the RS content of unripe banana flour (*Musa cavendishii*), which varied from 40.9 to 58.5%, as a function of treatment.

The total starch (94.8%) obtained here from banana starch is in agreement with the values found by De la Torre-Gutiérrez et al. (2007) for square banana starch (94.5%). However, our result was lower than values reported by other authors (BELLO-PÉREZ et al., 1999; COULIBALY et al., 2006; PÉREZ-SIRA, 1997). These researchers used centrifugation instead of gravitational sedimentation to extract the starch. That former procedure, may have favored the greater recovery of the granules.

The banana flour had a total starch content greater than that reported by Suntharalingam and Ravindran (1993), Kayisu et al. (1981), and Da Mota et al. (2000). This difference may have been the result of maturation stage and diversity in the banana varieties used in flour extraction or a consequence of sieving process (up to 200 mesh), which was carried out to eliminate the seed particles and also caused the elimination of great amount of fibers. Thus, the lower content of crude fiber (1.18%) observed in the banana flour allows its application as raw material for the production of edible films, whereas the fibers with smaller diameters act as reinforcement and avoid the defects usually caused by the presence of course fibers in the polymeric matrix.

The color of the banana flour was darker (lower L^*) and yellowish (higher b^*) than that of the starch, as can be seen in Figure 3, but the red tone (a^*) was similar (Table 1). The measurement of color difference (ΔE^*) resulted in values of 9.9 and 16.5 for the starch and flour, respectively. Based on the ΔE^* values, both raw materials can be recommended for use in products requiring a uniform color (e.g. ice creams, juices, candies); moreover, banana starch may prove especially useful for clear starchy products.



Figure 3. Appearance of banana raw materials.

3.2. Scanning electron microscopy (SEM)

Scanning electron micrographs (Figure 4) of banana flour and starch showed irregularly shaped, compact granules with both elongated and spheroid forms, very similar to those found by Carmona-Garcia et al. (2009), Espinosa-Solis et al. (2009), and Kayisu et al. (1981). The presence of other components in the microstructure of the flour can be seen in Figure 4a. The starch granules revealed no apparent damage, suggesting the adequacy of the techniques of preparation of the flour and starch.

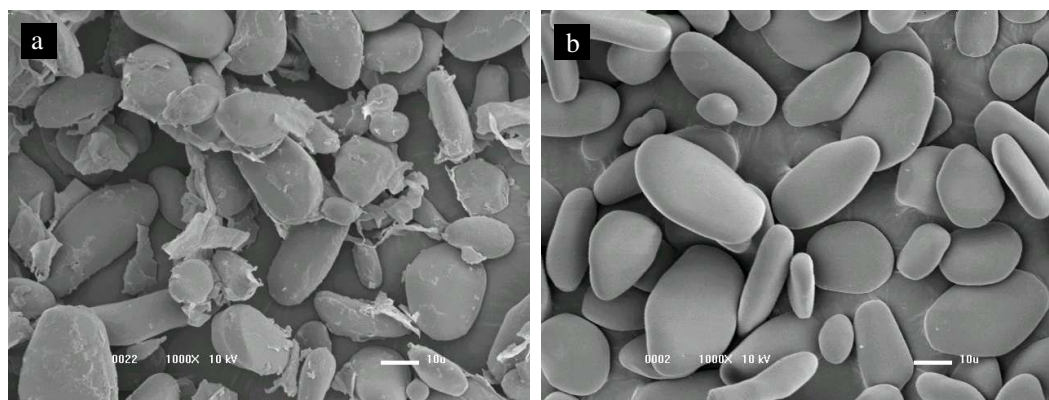


Figure 4. Scanning electron micrographs of banana flour (a) and starch (b) (1000x, scale bar = 10 μ m).

3.3. Particle size distribution

Figure 5 shows the distribution of particle sizes for banana flour and starch. Banana flour exhibited a modal distribution, with an average size of 31.7 μm . Banana starch, however, showed a bimodal distribution, with a principal peak for an average size of 47.3 μm , greater than that of the flour, and a second smaller peak of 150 μm . This second peak probably reflects granule aggregation, caused by the chemical treatment with NaOH to remove fibers during starch isolation. A similar behavior was reported in cross-linked banana starch (CARMONA-GARCIA et al., 2009). The fact that the particles in the starch were larger than those in the flour may be the result of non-settling of small starch granules during starch sedimentation.

Banana starch presented particle size within the range reported by Coulibaly et al. (2006) (3.33 to 56.66 μm) and Eggleston et al. (1992) (3.9 to 76.4 μm) for various bananas, plantain cultivars and hybrids. For banana flour, Suntharalingam and Ravindran (1993) reported an average granule size of 80 μm for the variety “Alukehel” and 90 μm for the variety “Monthan”. Those value differences may be due to variety, growing conditions, maturation stage, and different method used to determine particle size.

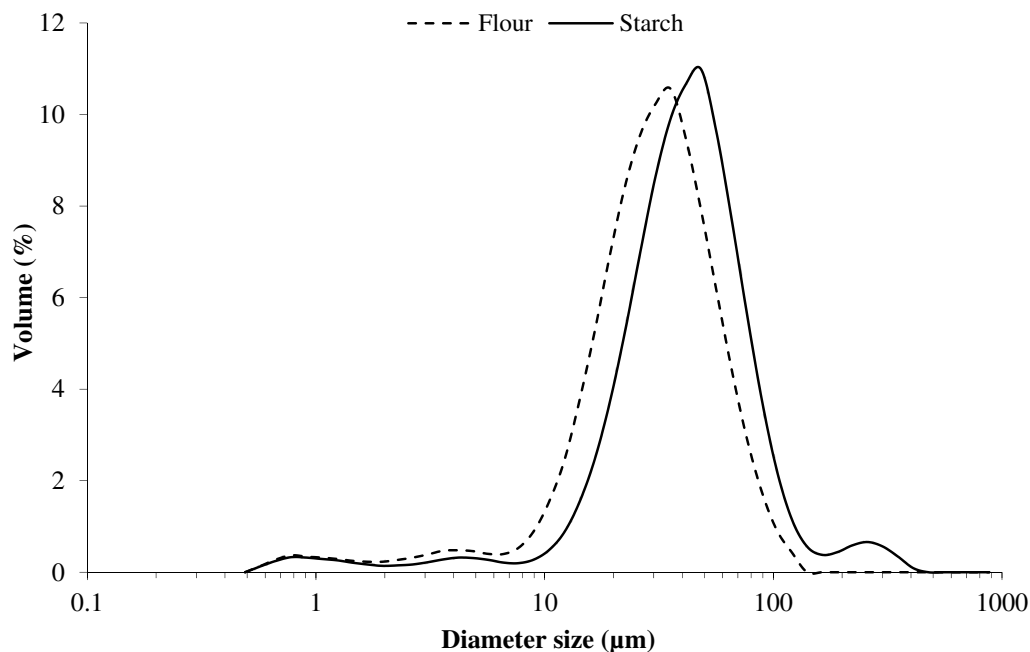


Figure 5. Particle sizes of banana flour and starch.

3.4. X-ray diffraction

Banana flour and starch revealed an X-ray pattern that was a mixture of the A- and B-type polymorphs, a pattern that can be referred to as C-type (Figure 6). Generally, cereal starches have an A-type pattern, whereas tuber starches display the B-type pattern, and certain roots and legumes starches show a C-type pattern (ZHANG et al., 2005). Some of the peaks observed for the plantain banana products were similar to those found for cereal starches, such as the typical A-type pattern peak at $2\theta = 15^\circ$; however, clear differences indicated the presence of B-type crystals, such as the peak observed at $2\theta = 5.6^\circ$, the peak at $2\theta = 17^\circ$ was much more prominent than that at $2\theta = 18^\circ$, and the broad peak at $2\theta = 23^\circ$. Faisant et al. (1995a; 1995b), Lii et al. (1982) and Teixeira et al. (1998) reported a B-type pattern for banana starch, whereas Bello-Pérez et al. (2005), De la Torre-Gutiérrez et al. (2007), Jane et al. (1999) and Waliszewski et al. (2003) all found a C-type pattern, in agreement with the results observed in this paper.

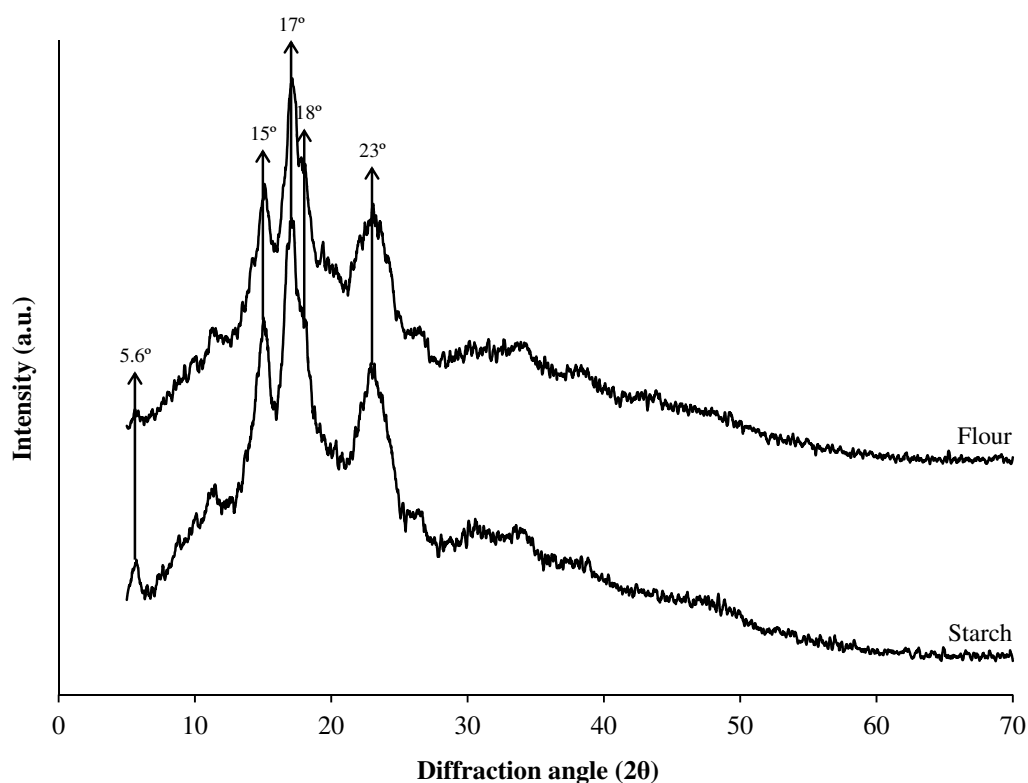


Figure 6. X-ray diffraction patterns of banana flour and starch.

The crystallinity characteristics of banana flour are similar to those of banana starch; although, their intensity is lower, as seen in the relative crystallinity of 18.0% for flour and 22.8% for starch. These results indicate that other components present in flour (ash, protein, lipids and crude fiber) may influence the crystallinity of the granules. Bello-Pérez et al. (2005) reported a much higher relative crystallinity (36%) for starch from bananas of the “Macho” variety. This difference may be related to the specific species used for starch extraction, but it may also depend on the method of calculation of the percent crystallinity.

3.5. Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra of banana flour and starch are shown in Figure 7. Samples presented a band at 3289 – 3298 cm^{-1} corresponding to symmetric and asymmetric stretching of O-H bonds. This region has a broad rounded base that, according to Solomons and Fryhle (2001), indicates the major contribution of the water molecule, theoretically located at 3500 cm^{-1} . The second band, at 2928 – 2931 cm^{-1} , would be the result of the stretching of C-H bonds. The amide I band is found between 1600 – 1720 cm^{-1} ; this is the amide group of proteins and reflects C=O stretching. Another major band relating to proteins is the amide III band, located between 1200 – 1350 cm^{-1} (SINGH, 2000). This band arises from the stretching of the C-N bond and the inflexion of the N-H bond of the amide group. All of these bands were present in both of the materials studied, although, with a greater intensity in the banana flour due to its greater protein content. The region located from 400 to 1200 cm^{-1} originates mainly from carbohydrate vibrations. In this area, the presence of the band at 1149 cm^{-1} represents the C-O stretching of carbohydrates. The bands at 1076 and 1077 cm^{-1} observed in the spectra of banana flour and starch, respectively, were attributed to the stretching of the C-OH bond of the starch molecules, while the band at 995 cm^{-1} is characteristic of partially crystalline materials (VICENTINI et al., 2005).

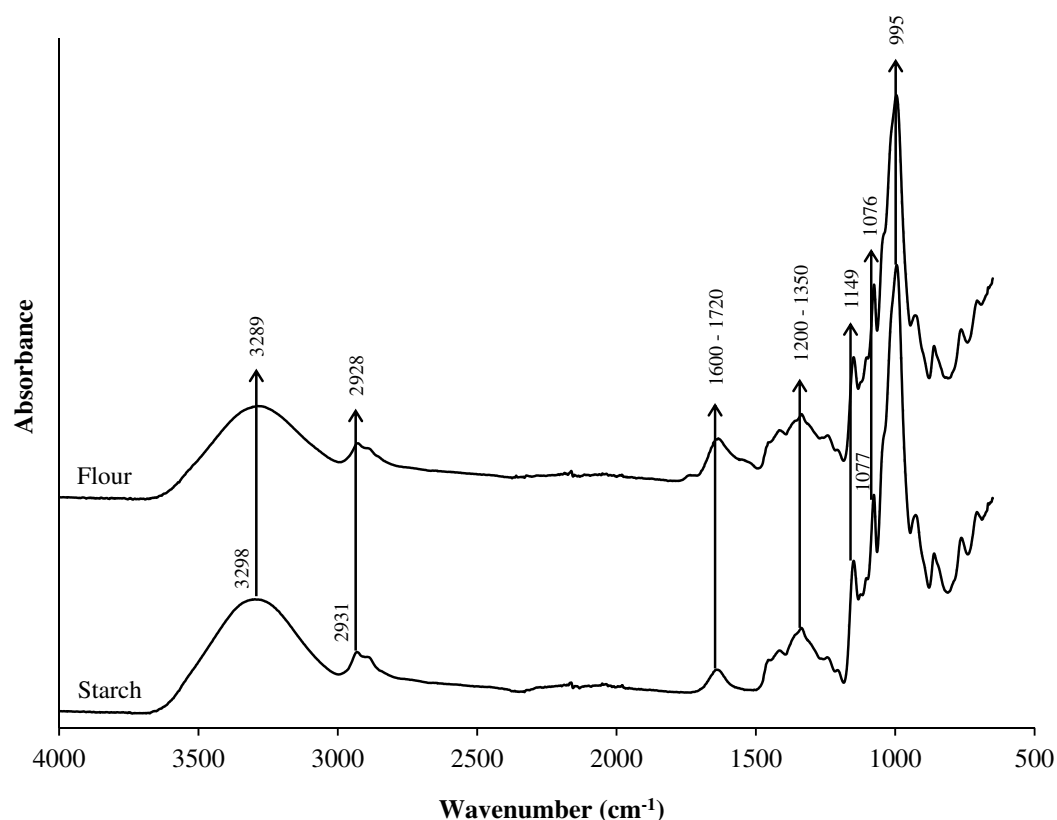


Figure 7. FTIR spectra of banana flour and starch.

3.6. Differential scanning calorimetry (DSC)

The DSC heating curves for suspensions of banana flour and starch are shown in Figure 8, and the resulting data are presented in Table 2. DSC thermograms show an endothermic peak, which is attributed to the gelatinization process. The gelatinization temperature, defined as the peak temperature (T_p), was higher for banana starch (74.9 °C) than for corn (65.7 °C), potato (64.4 °C) and cassava (71.0 °C) starches (LI and YEH, 2001), but similar to those reported for “Macho” (74.5 °C) and “Criollo” (75.0 °C) varieties of bananas (BELLO-PÉREZ et al., 2000). The banana flour had a T_p of 76.2 °C, greater than that reported by Tribess et al. (2009), Da Mota et al. (2000), and Faisant et al. (1995a). The especially high temperatures necessary to ensure complete gelatinization and pasting mean that the raw materials from bananas (flour and starch) should be potentially useful in products in which delayed pasting is desired, such as retorted canned foods.

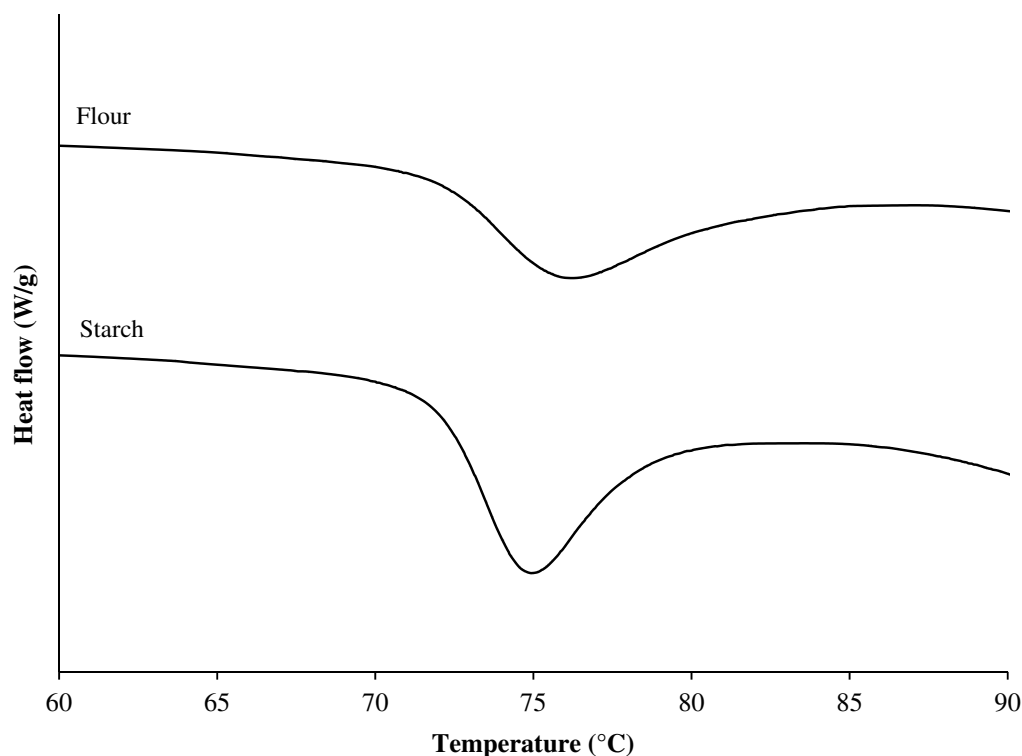


Figure 8. DSC thermograms of banana flour and starch.

Table 2. Thermal properties of banana flour and starch.

Source	Gelatinization parameters *				
	T_o (°C)	T_p (°C)	T_f (°C)	R ($T_f - T_o$)	ΔH (J/g)
Flour	72.3 ± 0.3^a	76.2 ± 0.3^a	80.5 ± 0.1^a	8.2 ± 0.3^a	13.0 ± 0.8^a
Starch	72.1 ± 0.1^a	74.9 ± 0.1^b	78.3 ± 0.2^b	6.3 ± 0.2^b	14.7 ± 0.8^a

* Onset temperature (T_o), peak temperature (T_p), final temperature (T_f), gelatinization range (R) and gelatinization enthalpy charge (ΔH).

^{a,b} Different letter superscripts in the same column indicate a statistically significant difference ($p < 0.05$).

Comparing the values for the temperature of gelatinization in both raw materials, banana flour T_p was significantly higher than that for the starch ($p < 0.05$; Table 2). Moorthy et al. (1996) reported similar behavior for the gelatinization of cassava flour and starch, indicating that the higher fiber content in flour may contribute to the greater T_p from the same plant species. Other authors have suggested that since the lipids, also present in greater amounts in flours, can form stable complexes with amylose, their presence may contribute to the increasing values of T_p in flours (OSMAN, 1967).

Another factor that may increase the gelatinization temperature is the presence of proteins in suspension, which may provide a protective effect and prevent the entrance of water to the starch granules, as noted by Zaidul et al. (2008). The combined presence of these other components in higher proportions in the banana flour may thus explain the differences in thermal properties between flour and starch.

The change in enthalpy of gelatinization (ΔH) was statistically similar for suspensions of the two materials, although that for banana starch was actually slightly higher (Table 2). In this study, banana flour presented a smaller average diameter and a higher gelatinization range (R) than did the banana starch (Table 2). According to Singh and Kaur (2004), such behavior can be explained by the large number of starch granules per unit mass in flour.

3.7. Pasting properties

Pasting properties are influenced by particle size, the amylose/amylopectin ratio, starch molecular characteristics, the volume fraction of suspended solids, and the conditions of the thermal process used to induce gelatinization (ZHOU et al., 1998). Both raw materials showed a gradual increase in viscosity up to the maximum value (Figure 9). Banana starch had a lower peak viscosity (Figure 9), followed by a slight decrease in viscosity and more stability during the holding period at 95 °C, showing that the isolated starch granules are more resistant to mechanical fragmentation than are those of flour. This behavior was confirmed by the breakdown viscosity values which represent the granule fragmentation (Table 3). However, during the cooling process, the viscosities of both flour and starch continued to increase (a tendency more pronounced for the starch), as a result of the more accelerated formation of the network of amylose and amylopectin despite the retention of a certain amount of water. The retrogradation of banana starch was higher than that of banana flour (setback viscosity values in Table 3).

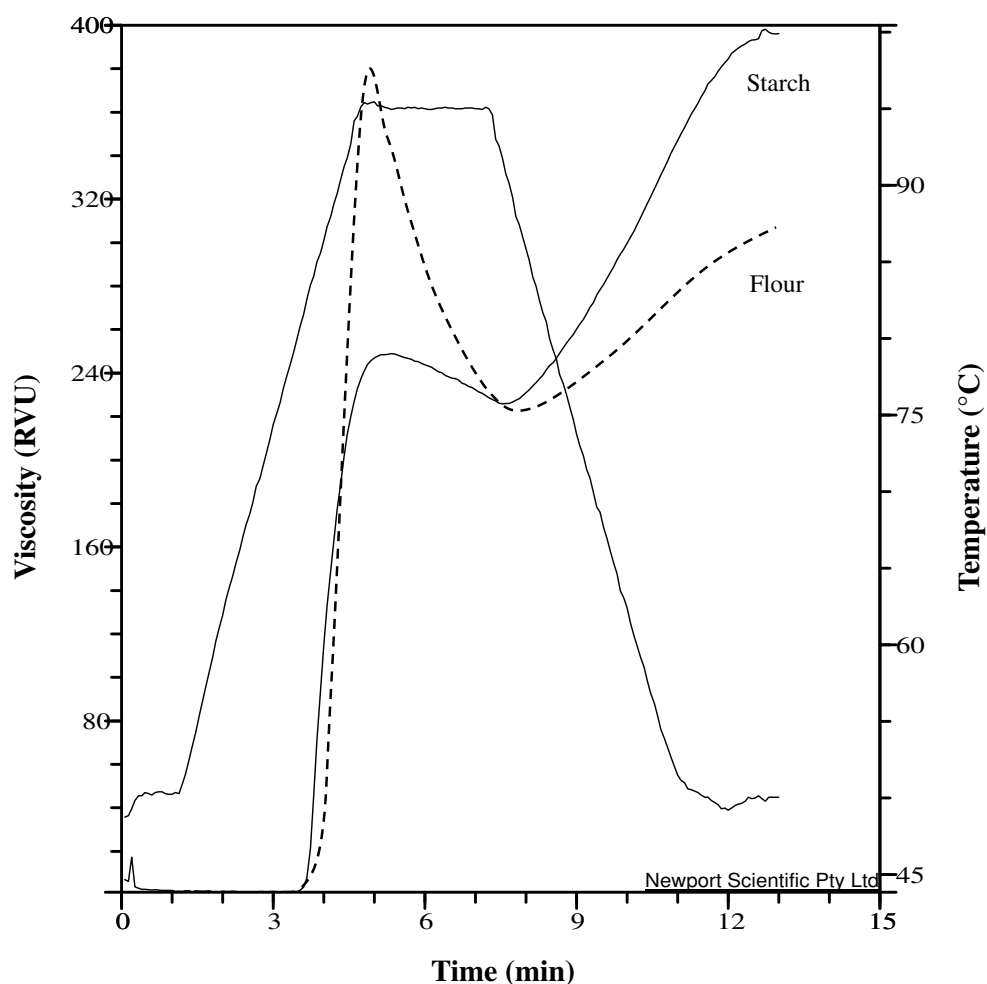


Figure 9. Rapid Visco Analyser profiles of banana flour and starch (14% m.b.).

Table 3. Pasting properties of banana flour and starch.

Source	Pasting temperature (°C)	Viscosity (RVU)			
		Peak	Breakdown	Final	Setback
Flour	85.6 ± 0.1 ^a	378.0 ± 4.1 ^a	154.6 ± 1.9 ^a	306.8 ± 2.1 ^a	83.3 ± 0.1 ^a
Starch	82.8 ± 1.0 ^a	252.6 ± 5.1 ^b	20.3 ± 3.8 ^b	401.4 ± 7.4 ^b	169.1 ± 1.6 ^b

^{a,b} Different letter superscripts in the same column indicate a statistically significant difference ($p < 0.05$).

The significant differences in pasting properties of banana flour and starch ($p < 0.05$) suggest that the presence of other components in flour may be influencing this characteristic, such as the interactions between starch and fibers, amylose and lipids, or protein and starch gel. Similar results have been reported for the flour and starch of bananas (DA MOTA et al., 2000), as well as for rice (YU et al., 2010).

Espinosa-Solis et al. (2009) reported that banana starch had a lower pasting temperature (79.3 °C) than did corn starch (84.0 °C), although it was higher than that of mango starch (71.3 °C). The peak and final viscosities reported by these authors for banana starch (215.8 and 323.8 RVU, respectively) were higher than those of the other starches, and similar to those found in this paper (Table 3). In general, pastes from banana flour and starch reveal higher viscoamylograph viscosities at a temperature of 95 °C than do the common food starches. Therefore, such raw materials from bananas have a potential in applications as thickeners in products requiring sterilization, such as sauces and baby foods (DE LA TORRE-GUTIÉRREZ et al., 2008).

3.8. Swelling power and solubility

The swelling power and solubility of banana flour and starch are presented in Figure 10. Both were directly correlated to increases in temperature. For the two raw materials, these properties show that the granules did not swell appreciably at temperatures below 65 °C. At temperatures above 65 °C, however, they swelled rapidly, due to the breaking of intermolecular hydrogen bonds in amorphous areas, thus permitting irreversible and progressive water absorption, as reported by Bello-Pérez et al. (1999) for “Macho” banana starch. Increases in the swelling power of banana flour and starch were 3.4 to 20.3 and 2.4 to 24.0 g/g, respectively, with that of banana flour being lower at temperatures above 65 °C (Figure 10a). These differences are the result of the presence of other components in flour. According to Ogawa et al. (2003), the presence of fibers, proteins, and lipids can influence water permeation during the gelatinization process.

The solubility of the flour and starch of bananas began to increase at 65 °C (Figure 10b). This increase was linked to their high gelatinization temperatures and is the result of amylose exudation from the swollen starch granules. De la Torre-Gutiérrez et al. (2008) reported the same behavior and similar values for solubility of square banana starch. The solubility of banana flour was higher than that of banana starch at all temperatures investigated, increasing from 8.1 to 16.8% for flour and from 1.5 to 13.7% for starch. These results indicate that solubility is positively correlated to temperature, although, negatively correlated to amylose content. According to Pérez and Bertoft (2010), there is an enrichment of amylose towards the periphery of the granule, and that amylose found near the surface of the granule has a smaller chain length than amylose

located nearer the center of the granule. Amylose chains are believed to be in a single helical state, although a small proportion may be involved in lipid complexes. Some of the larger (non-leachable) amylose chains may be involved in double-helical interactions with amylopectin. It is clear, however, from the evidence that amylose can only be completely extracted from granules at temperatures above 90 °C, that some large amylose molecules are present within the starch granule, and it is further hypothesized that these larger amylose chains which are not easily leached may either participate in double helices with amylopectin or be entangled within the intricate architecture of the starch granule. Therefore, the greater the amylose content, the more compact the starch granules, making it more difficult for amylose to escape from the granules which favors lower solubility values. In this research, banana starch had an amylose content higher than that of flour (36.9 g of amylose/100 g of starch versus 27.8 g of amylose/100 g of starch, respectively), in agreement with the results of solubility. Higher content of amylose in purified starch must be related to the granule size distribution because amylose content may increase with granule size. The higher solubility of flour might also be due to the presence of these small starch granules that failed to sediment during centrifugation in the dried supernatant used for the calculation of solubility. In the starch, these small granules would not be present because they did not settle during extraction. Moreover, other soluble compounds present in the flour contribute to the increase of this property.

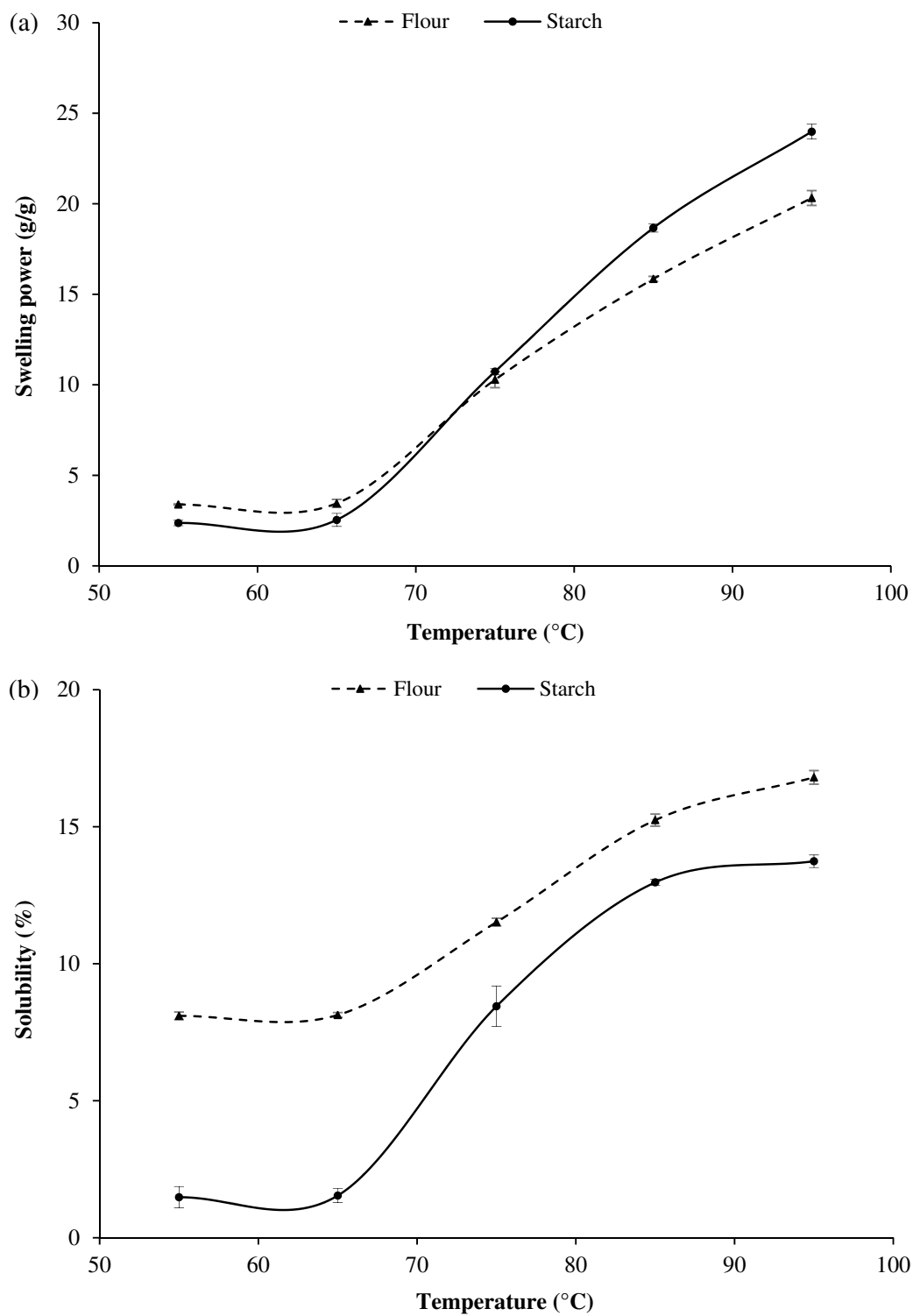


Figure 10. Effect of temperature on swelling power (a) and solubility (b) of banana flour and starch.

Figure 11 permits the visualization of the behavior of starch granules in water with increasing temperatures in the range studied. In these photographs, the granule structures have been colored with iodine solution. The increase in temperature results in swelling of the granules, followed by their disruption and the release of amylose. This release can be seen in Figures 11a3 to 11a5 and 11b3 to 11b5, as the blue regions surrounding the swollen granules. Figures 11a3 and 11b3 show a more pronounced swelling of the granules at 75 °C, in agreement with the results found for swelling power and solubility, with a greater increase in the values of both properties observed at temperatures above 65 °C.

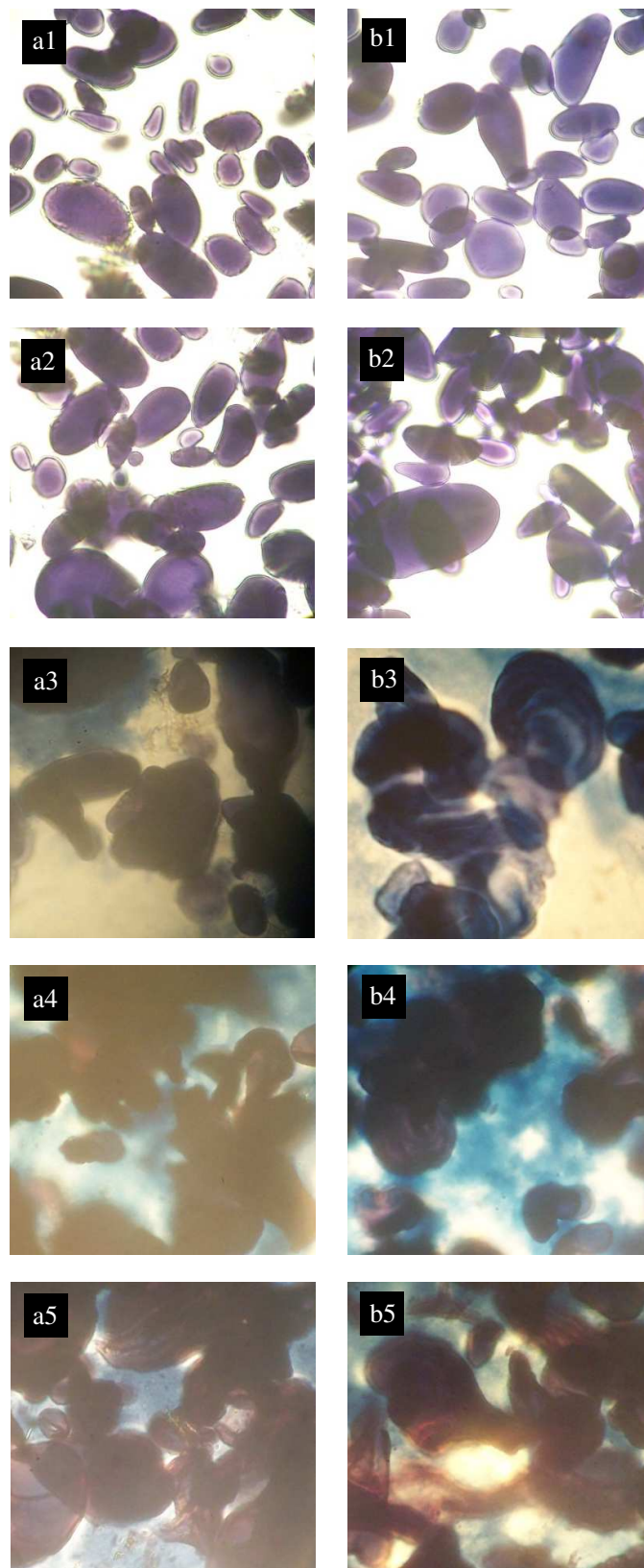


Figure 11. Photomicrographs of banana flour (a) and starch (b) granules heated to temperatures of: 1) 55 °C, 2) 65 °C, 3) 75 °C, 4) 85 °C, 5) 95 °C (40x).

4. Conclusions

The functional and physicochemical properties of flour and starch isolated from plantain bananas suggest that they may have numerous possible uses as ingredients in food systems and other industrial applications. Moreover, the high amylose content and resistant starch provide these materials with appropriate properties to be used as raw materials for films and coatings used in food products. The use of banana pulp for flour and starch production can lead to the elaboration of competitive products on the market, thus improving the financial situation of banana producers, and also contributing to the elimination of the problem of what to do with excess or rejected bananas.

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Capítulo 3

Optimization of Formulation and Process Conditions for the Production of Films based on the Flour from Plantain Bananas (*Musa paradisiaca*)

Abstract

In this chapter, the casting process has been employed for the production of flour films from unripe plantain bananas (*Musa paradisiaca*); glycerol has been used as plasticizer. The influence of formulation and process conditions such as the glycerol concentration (Cg), the process temperature (Tp), the drying temperature (Td), and the relative humidity (RH) on the mechanical, barrier, and optical properties of the prepared banana flour films has been evaluated by means of a central composite design. The results have been statistically analyzed by the response surface methodology and desirability function (multi-response analysis), and the optimal formulation and process conditions for film have been determined. All the obtained films are light yellow and exhibit homogeneous surface and good handling characteristics. The formulation and process variables have a significant impact on the mechanical properties, water vapor permeability (WVP), and opacity of the films, but these features are mostly affected by the Cg parameter. It was not possible to obtain mathematical models that can describe the correlation of the solubility in water and the color of the films with the formulation and process conditions. The desirability function employed here has allowed for the establishment of the optimal conditions, which are Cg = 19 g of glycerol/100 g of flour, Tp = 81 °C, Td = 54 °C, and RH = 48%. This function has proven to be an effective tool for this type of study. Films produced under these conditions presented tensile strength of 9.2 MPa, elongation at break of 24.2%, Young's modulus of 583.4 MPa, WVP of 2.1×10^{-10} g/m.s.Pa, and opacity of 51.3%.

Keywords: banana flour, biodegradable films, experimental design, desirability function, mechanical properties, barrier properties.

1. Introduction

Polysaccharides and proteins of animal and vegetable origin are natural biodegradable polymers that have traditionally been used to produce environmentally-friendly films (CHANDRA and RUSTGI, 1998; KROCHTA and DE MULDER-JOHNSTON, 1997). Starch is the most widely employed polysaccharide for film production, because it is naturally abundant and inexpensive (ALVES et al., 2007; MALI et al., 2004). Starch films present good mechanical and oxygen barrier properties, but their sensitivity to moisture is a major drawback. To improve the characteristics of these materials, some authors have designed films based on starch and protein mixtures (COUGHLAN et al., 2004; JAGANNATH et al., 2003). Researchers have also added lipids to the formulation of some films, to enhance the water vapor barrier (BRAVIN et al., 2004; GARCÍA et al., 2000).

When developing films, another alternative is to use flours, which are naturally occurring complex blends of starch, protein, lipids, and fibers. Some authors have reported on the potential application of flours obtained from whole materials such as amaranth, soy, and wheat for film production (MARINIELLO et al., 2003; RAYAS et al., 1997; TAPIA-BLÁCIDO et al., 2005a, 2007, 2011). The excellent characteristics of these films stem from the natural and intrinsic molecular interactions taking place between their starch, protein, lipid, and fiber components.

An interesting renewable raw material for the preparation of edible and biodegradable films is the unripe banana fruit. Originating in Southeast Asia, bananas (genus *Musa*) are an important food crop that is extensively grown in tropical and subtropical regions. Unfortunately, post-production losses are huge due to the highly perishable nature and inadequate post-harvest handling of banana fruit. Processing both the surplus fruit and the fruit that is inappropriate for fresh consumption reduces these losses. Starch is the major constituent of unripe bananas and comprises over 70 g/100 g of their dry weight. Moreover, this fruit contains a significant amount of protein (1.0 – 2.5 g/100 g), lipids (0.2 – 0.5 g/100 g) and fiber (1.5 – 2.5 g/100 g) (ZHANG et al., 2005), which could be interesting for the production of biodegradable films. In this sense, the flour of unripe bananas may be an attractive alternative for the attainment of a continuous matrix.

Romero-Bastida et al. (2005) used starches isolated from banana, okenia, and mango to produce edible films. Okenia and banana films were the most (32%) and the

least (23%) soluble in water, respectively. The banana film had the highest tensile strength value (25 MPa) compared with mango (18 MPa) and okenia (17 MPa) films. A group of researchers studied the effect of banana starch modified by oxidation and acetylation on the properties of films (ZAMUDIO-FLORES et al., 2009; ZAMUDIO-FLORES et al., 2006). The oxidation level increased but acetylation decreased the solubility and WVP of the film. Oxidation enhanced the tensile strength of the film, and acetylation of the oxidized starch improved this property. Elongation at break diminished when the oxidation level rose.

More recently, Pitak and Rakshit (2011) employed banana flour/chitosan composite film bags to preserve freshly cut vegetables. The composite yellowish film exhibited great water permeability of $4.5 - 4.8 \times 10^{-10}$ g/m.s.Pa. Tensile strength and elongation were in the range of 5.2 – 14.2 MPa and 1.6 – 2.6%, respectively, while solubility ranged between 40.9 and 64.2%. The presence of starch in the composite film furnished water soluble and sealable bags or wraps, while the presence of chitosan provided them with the antimicrobial property. In another work, Sothornvit and Pitak (2007) prepared films from the banana flour developed; however, they evaluated the oxygen permeability and the mechanical properties of these films, but they did not determine solubility or the water vapor barrier and the optical properties.

Among the different varieties of banana, the cultivar “Terra” (*Musa paradisiaca*), a type of plantain, exhibits excellent features for the preparation of biodegradable films (Chapter 2). Because a few studies reported in the literature have dealt with this cultivar, we have selected it for the present investigation.

Considering that the several parameters employed during film formulation and production influence the film properties, a study was carried out to verify the effect of glycerol concentration (Cg), process temperature (Tp), drying temperature (Td), and relative humidity (RH) on film features, so that banana flour films with the desired characteristics can be obtained. In this context, the present study aimed to determine the optimal formulation and process conditions of banana flour film by using response surface methodology and multi-response analysis, in order to obtain films with low water vapor permeability, moderate elongation, and high resistance to break.

2. Materials and Methods

2.1. Materials

The flour was prepared from unripe plantain bananas (mature green) of the variety “Terra” (*Musa paradisiaca*), according to the methodology described in Section 2.2 of Chapter 2. All the chemicals used in this work were reagent grade.

2.2. Chemical analysis of the banana flour

The moisture, ash, protein, and crude fiber contents were analyzed by means of the AOAC standard methods (2005). Lipids were assayed by the method of Bligh and Dyer (1959). Amylose content was obtained according to the methodology reported in ISO 6647 (1987), and total starch was identified by using the method proposed by Diemair (1963). All the experiments were performed at least in triplicate, and the results are presented as mean values.

2.3. Film production

Films were produced by the casting method. This process consists in drying a film-forming suspension (FFS) that has been applied onto a support. The procedure developed herein involved the homogenization of a water solution of 4 g/100 g (d.b.) of banana flour by mechanical stirring for 30 min, followed by heating to the process temperature (T_p : 75 to 95 °C) under gentle stirring. Glycerol (C_g : 15 to 30 g of glycerol/100 g of flour) was added at this point, and the solution was maintained at this temperature for 15 min. Next, the FFS was sonicated for 10 min, and 70 g of the solution was poured onto acrylic plates (18 x 21 cm), so as to obtain a constant thickness of $85 \pm 5 \mu\text{m}$. Preliminary tests of grammage were performed to determine the exact amount of FFS to be poured onto acrylic plates (see Appendix 1). The films were dried in a chamber with air circulation under controlled temperature ($T_d = 35$ to 55 °C) and relative humidity ($RH = 30$ to 70%).

Before the characterization of the films in terms of moisture content, mechanical properties, and water vapor permeability, they were conditioned in desiccators under 58% RH, at 25 °C, for 48h.

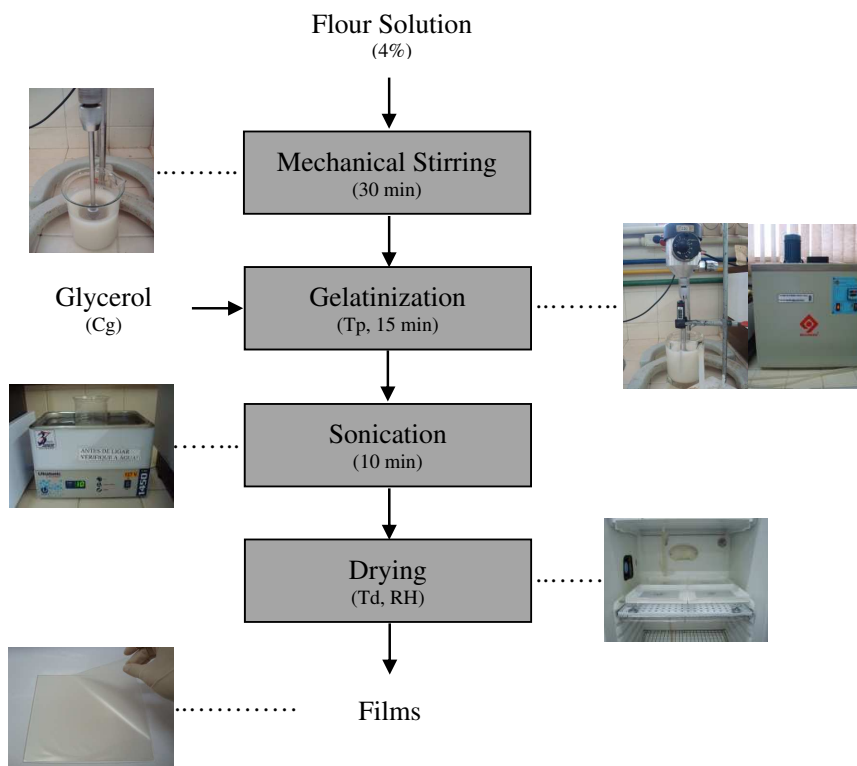


Figure 1. Scheme describing the procedure for the production of the banana flour films.

2.4. Film characterization

2.4.1. Thickness and density

The thickness of the films was measured using a manual micrometer (Fowler, model FOW52-229-001, Pennsylvania, USA) with an accuracy of 0.1 μm . The mean thickness of each film was determined from an average of 10 random measurements.

To determine the density, samples of each film were cut into 20 x 20 mm squares, and the thickness of these films was measured (3 random measurements). The film samples were dried at 105 $^{\circ}\text{C}$ for 24 h and weighed, and the density was calculated as the ratio between the weight and volume (thickness x area) of the film. The density experiments were accomplished in triplicate, and the data are reported as mean values.

2.4.2. Moisture content

The moisture content of the films was analyzed gravimetrically, in triplicate, according to the standard method D644-99 (ASTM, 1999), by drying the samples at 105 °C for 24 h.

2.4.3. Mechanical properties

The tensile properties were investigated with the aid of a texture analyzer (Stable Micro Systems, model TA.TXplus, Surrey, England) according to the standard method D882-02 (ASTM, 2002), by taking an average of six determinations in each case. The samples were cut into 25 mm wide and 115 mm long strips by means of a scalpel and mounted between the tensile grips. The initial grip separation and the crosshead speed were set at 80 mm and 1.0 mm/s, respectively. The tensile strength (force/initial cross-sectional area) and the elongation at break were computed directly from the strength x elongation curves using the Texture Exponent 32 software, and Young's modulus was calculated as the slope of the initial linear portion of this curve.

2.4.4. Solubility in water

The solubility (S) values were determined by employing the methodology described by Gontard et al. (1992). To this end, three discs (diameter = 20 mm) of each film were stored in a desiccator containing silica gel (~0% RH) for 48 h. The samples were weighed, to obtain the initial dry weight (W_i), and they were then immersed into 50 mL water containing sodium azide (0.2 g/L) at 25 °C for 24 h, under sporadic agitation. After this period, the solution containing the film discs was filtered, the insoluble matter was dried at 105 °C for 24 h, and the resulting material was weighed for determination of the final dry weight (W_f). Analyses were carried out in quadruplicate, and the solubility in water (%) of the films was calculated according to Equation 1:

$$S = \frac{W_i - W_f}{W_i} \times 100 \quad [1]$$

where W_i is the initial dry weight of the sample (g), and W_f is the final dry weight of the sample (g).

2.4.5. Water vapor permeability (WVP)

The WVP was determined gravimetrically by following the standard method E96-00 (ASTM, 2000) with modifications. For this purpose, the film (diameter = 0.06 m) was placed on the circular opening (area = 0.00196 m²) of a permeation cell and was sealed with sealant ring, to ensure that humidity migration would occur through the film only. The interior of the cell was filled with silica gel (~0% RH), and the system was stored in a desiccator containing distilled water (100% RH) at 25 °C. After steady state conditions were reached (about 2 h), the weight gain of the cells was monitored every 30 minutes, for 8 h. Analyses were conducted in triplicate, and the WVP was calculated on the basis of Equation 2 and expressed in g/m.s.Pa:

$$WVP = \frac{w}{t} \times \frac{\delta}{A \cdot \Delta P} \quad [2]$$

where w/t is the slope of the line of weight gain (w) as a function of time (t) graph (g/s), δ is the mean sample thickness (m), A is the sample permeation area (m²), and ΔP is the difference in water vapor pressure through the sample for pure water at 25 °C (Pa).

2.4.6. Optical properties

Banana flour films were subjected to color and opacity analyses using a colorimeter (UltraScan VIS, HunterLab, Virginia, EUA) in the transmittance mode, with classification system of the CIELab and illuminant D₆₅ (daylight) (HUNTERLAB, 1996). The parameters were classified directly by EasyMatch QC software: L (white = 100, black = 0); a (red = positive, green = negative); b (yellow = positive; blue = negative); and haze (opacity). The color difference (ΔE^*) was determined using Equation 3:

$$\Delta E^* = \left[(L^* - L_o^*)^2 + (a^* - a_o^*)^2 + (b^* - b_o^*)^2 \right]^{1/2} \quad [3]$$

where L^* is the degree of lightness of the sample, L_o^* is the degree of lightness of the standard, a^* and b^* are the chroma parameters of the sample, and a_o^* and b_o^* are the chroma parameters of the standard.

The instrument was calibrated against a standard white reference plate, and for the calculation of the ΔE^* a polyethylene film was used as standard ($L_o^* = 96.33$, $a_o^* = 0.06$ and $b_o^* = 0.28$). All the experiments were performed at least in triplicate, and the results are presented as mean values.

2.5. Experimental design

To determine the influence of the formulation and process conditions (Cg, Tp, Td, and RH) on the properties of the films, a 2^4 central composite design was performed, with four replicates at the central point and eight axial points, totaling 28 experiments (RODRIGUES and IEMMA, 2005). The dependent variables (responses) were the tensile strength, the elongation at break, Young's modulus, the solubility in water, the WVP, the color difference (ΔE^*), and the opacity. The experimental design and the coded and real values of the independent variables are given in Table 1. The study ranges had been defined in preliminary tests.

A second order model (Equation 4) was adopted, in order to fit the response variables:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_i^2 X_i^2 + \sum \beta_{ij} X_i X_j \quad [4]$$

where Y is the dependent variables, X_i and X_j are the coded independent variables, β_0 is the constant, β_i is the linear coefficient, β_i^2 is the quadratic coefficient, and β_{ij} is the interaction coefficient.

Statistical analysis of the experimental data and the response surface methodology were performed using the Statistica 7.0 software (StatSoft Inc, Tulsa, Oklahoma, USA). The optimal values of the formulation and process conditions were obtained by the desirability function, a multi-response analysis, proposed by Derringer and Suich (1980).

Table 1. Central composite design matrix and some characteristics of banana flour films.

Test	Independent variables ^a				Drying time (h)	Density (g/cm ³)	Moisture content (%) ^b
	X ₁ (Cg)	X ₂ (Tp)	X ₃ (Td)	X ₄ (RH)			
1	-1 (18.75)	-1 (80)	-1 (40)	-1 (40)	4.2	1.12 ± 0.04	15.0 ± 0.1
2	1 (26.25)	-1 (80)	-1 (40)	-1 (40)	4.2	1.09 ± 0.02	20.4 ± 0.7
3	-1 (18.75)	1 (90)	-1 (40)	-1 (40)	4.2	1.11 ± 0.04	14.3 ± 0.1
4	1 (26.25)	1 (90)	-1 (40)	-1 (40)	4.5	1.05 ± 0.04	19.9 ± 0.9
5	-1 (18.75)	-1 (80)	1 (50)	-1 (40)	3.8	1.25 ± 0.02	15.8 ± 0.5
6	1 (26.25)	-1 (80)	1 (50)	-1 (40)	3.8	1.16 ± 0.01	19.0 ± 0.1
7	-1 (18.75)	1 (90)	1 (50)	-1 (40)	3.3	1.12 ± 0.04	14.1 ± 0.2
8	1 (26.25)	1 (90)	1 (50)	-1 (40)	3.8	1.07 ± 0.04	18.5 ± 0.5
9	-1 (18.75)	-1 (80)	-1 (40)	1 (60)	6.0	1.10 ± 0.02	14.3 ± 0.4
10	1 (26.25)	-1 (80)	-1 (40)	1 (60)	6.5	1.05 ± 0.03	19.5 ± 0.9
11	-1 (18.75)	1 (90)	-1 (40)	1 (60)	6.2	1.09 ± 0.03	14.4 ± 0.2
12	1 (26.25)	1 (90)	-1 (40)	1 (60)	6.5	0.96 ± 0.04	20.3 ± 0.5
13	-1 (18.75)	-1 (80)	1 (50)	1 (60)	4.8	1.19 ± 0.01	15.7 ± 0.4
14	1 (26.25)	-1 (80)	1 (50)	1 (60)	5.0	1.14 ± 0.03	19.1 ± 0.5
15	-1 (18.75)	1 (90)	1 (50)	1 (60)	4.8	1.00 ± 0.02	14.3 ± 0.3
16	1 (26.25)	1 (90)	1 (50)	1 (60)	5.2	1.06 ± 0.03	19.6 ± 0.7
17	-2 (15.00)	0 (85)	0 (45)	0 (50)	4.3	1.09 ± 0.04	13.1 ± 0.1
18	2 (30.00)	0 (85)	0 (45)	0 (50)	5.0	1.04 ± 0.03	22.3 ± 0.9
19	0 (22.50)	-2 (75)	0 (45)	0 (50)	5.0	1.22 ± 0.03	17.8 ± 0.2
20	0 (22.50)	2 (95)	0 (45)	0 (50)	5.0	0.94 ± 0.01	16.8 ± 0.3
21	0 (22.50)	0 (85)	-2 (35)	0 (50)	6.3	1.11 ± 0.03	17.2 ± 0.7
22	0 (22.50)	0 (85)	2 (55)	0 (50)	3.8	1.10 ± 0.01	15.7 ± 0.3
23	0 (22.50)	0 (85)	0 (45)	-2 (30)	3.5	1.17 ± 0.02	17.5 ± 0.3
24	0 (22.50)	0 (85)	0 (45)	2 (70)	8.0	1.06 ± 0.03	17.7 ± 0.4
25	0 (22.50)	0 (85)	0 (45)	0 (50)	5.0	1.06 ± 0.04	16.5 ± 0.3
26	0 (22.50)	0 (85)	0 (45)	0 (50)	5.0	1.07 ± 0.07	16.2 ± 0.2
27	0 (22.50)	0 (85)	0 (45)	0 (50)	5.0	1.07 ± 0.04	16.3 ± 0.3
28	0 (22.50)	0 (85)	0 (45)	0 (50)	5.0	1.06 ± 0.02	16.3 ± 0.2

^a Cg = glycerol concentration (g/100 g of flour), Tp = process temperature (°C), Td = drying temperature (°C), RH = relative humidity (%).

^b After conditioning at 58% RH for 48h.

3. Results and Discussion

3.1. Chemical analysis of the banana flour

The flour prepared from “Terra” bananas (*Musa paradisiaca*) had the following chemical composition, on dry basis (except moisture): 8.0 ± 0.0 of moisture/100 g; 1.9 ± 0.1 g of ash/100 g; 3.2 ± 0.1 g of protein/100 g; 0.6 ± 0.0 g of lipids/100 g; 1.2 ± 0.1 g

of crude fiber/100 g; and 83.2 ± 0.2 g of starch/100 g (among which 27.8 ± 0.1 g/100 g of dry starch was amylose). The composition of this flour is similar to those obtained by Da Mota et al. (2000), Kayisu et al. (1981), and Suntharalingam and Ravindran (1993). The protein and lipids present in the banana flour exert an important and well-known plasticizer effect, which reduces the mechanical resistance and increases the flexibility of the biopolymer films. Also, the presence of hydrophilic components such as protein and fiber in the flour may give rise to a greater number of interactions with water molecules. The amylose content is an important feature for the film-forming capacity of the starch. Compared with other raw materials used in the production of biodegradable films, the banana flour presents higher amylose content than the amaranth flour (7.6 g/100 g of dry starch) (TAPIA-BLÁCIDO et al., 2005a) and the cassava flour (17 g/100 g of dry starch) (SUPPAKUL et al., 2012), but lower than the achira flour (30.7 g/100 g of dry starch) (ANDRADE-MAHECHA et al., 2012).

3.2. Film characterization

All the prepared films displayed homogeneous surface with no bubbles or cracks, as well as good handling characteristics. This means that the films could be easily detached from the plates without tearing, and that they were not sticky or too brittle. The banana flour films were transparent, with light yellow coloration (Figure 2) and an average thickness of 87 ± 3 μm .

Table 1 lists the drying time, density, and moisture content of the films obtained in the different formulation and process conditions tested in the experimental design. The density of the films ranged from 0.94 to 1.25 g/cm³. The drying conditions (drying temperature and relative humidity) influenced the drying time. The combination of high Td and low RH diminished the drying time of the films by about 50% on average, as can be noticed when one goes from a less drastic (lower Td and higher RH) (assays 21 and 24) to a more drastic (higher Td and lower RH) condition (assays 22 and 23), considering the same Cg and Tp. On the other hand, the Cg variable affected the moisture content of the films after the conditioning period (58% RH, 48 h) the most. Indeed, films with greater plasticizer content exhibited higher moisture values as compared with those with lower Cg, a result of the highly hygroscopic character of glycerol.

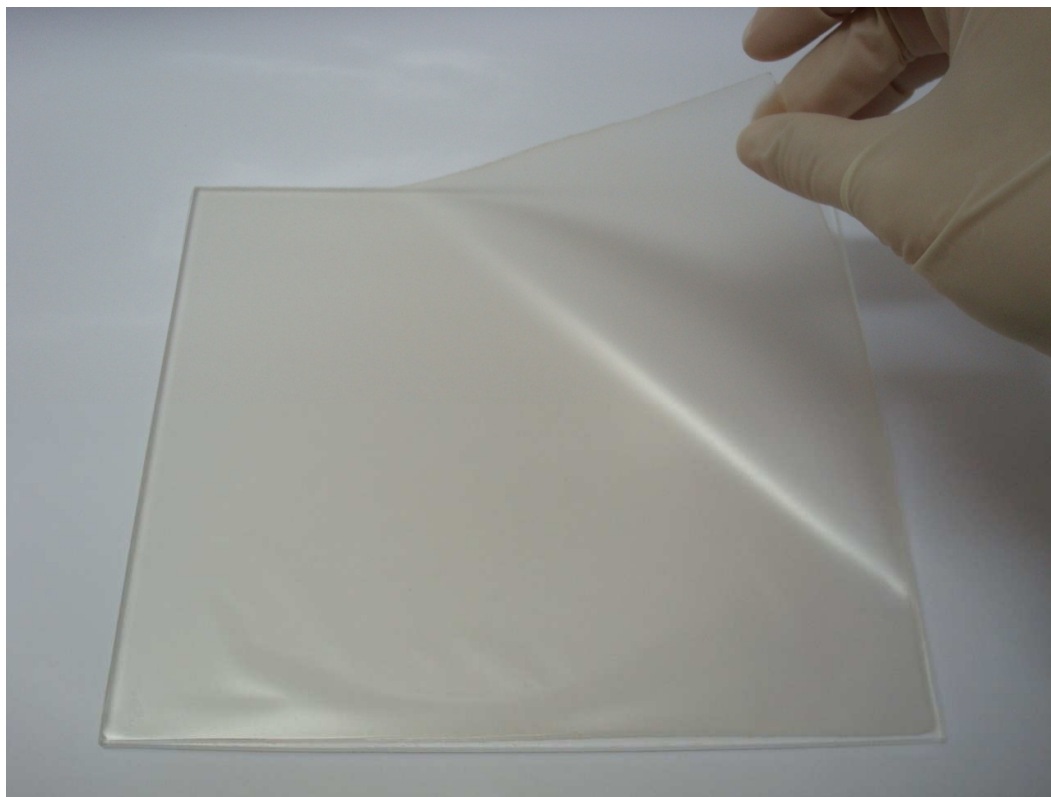


Figure 2. Banana flour film.

3.3. Statistical analysis

Table 2 brings the film properties obtained according to the experimental design. These data were submitted to statistical analyses, including fitting to Equation 4, followed by an analysis of variance (ANOVA) at 90% confidence level. Only the statistically significant parameters have been used for analysis of the behavior of the fitted mathematical models:

$$Y_1 = 6.85 - 2.71X_1 - 0.40X_4 + 0.94X_1^2 - 0.51X_1X_2 + 0.46X_1X_3 + 0.67X_3X_4 \quad [5]$$

$$Y_2 = 26.07 + 6.98X_1 - 1.68X_2 + 1.99X_3 - 2.57X_1^2 - 2.07X_1X_4 - 1.74X_2X_3 - 2.60X_2X_4 \quad [6]$$

$$Y_3 = 334.95 - 324.52X_1 + 124.27X_1^2 - 57.44X_1X_2 + 45.34X_2X_3 \quad [7]$$

$$Y_4 = 26.69 + 1.33X_1^2 + 1.64X_2^2 - 2.01X_2X_3 \quad [8]$$

$$Y_5 = 1.49 + 0.18X_1 + 0.26X_4 + 0.24X_2^2 - 0.21X_1X_2 \quad [9]$$

$$Y_6 = 2.60 + 0.06 X_1 - 0.09 X_3 + 0.06 X_2^2 + 0.08 X_1 X_4 \quad [10]$$

$$Y_7 = 49.20 + 2.18 X_1 - 1.66 X_2 + 3.04 X_4 + 2.26 X_2^2 - 1.33 X_4^2 + 2.22 X_2 X_4 \quad [11]$$

where Y is the dependent variable (Y_1 = tensile strength, Y_2 = elongation at break, Y_3 = Young's modulus, Y_4 = solubility in water, Y_5 = WVP, Y_6 = color difference, and Y_7 = opacity), and X are the coded independent variables (X_1 = Cg, X_2 = Tp, X_3 = Td, and X_4 = RH).

Table 3 summarizes the results of the ANOVA, including the regression coefficients for the coded second order polynomial equation, the coefficients of determination (R^2), and the F and p values. Finally, to determine whether the fitted equations are predictive, they must satisfy a certain criterion based on the F_{test} values ($F_{\text{calculated}}$ and F_{listed}), which in turn depends on the calculated F ratio value for the regressions related to the residuals ($F_{\text{calculated}}/F_{\text{listed}}$). The criterion is that the value of this ratio must be higher than that of F_{listed} (KHURI and CORNELL, 1996), thereby enabling plotting of the response surfaces.

Except for solubility and color difference, the fitted equations are predictive for all the studied properties, since their F ratio values ($F_{\text{calculated}}/F_{\text{listed}}$) are higher than the corresponding F_{listed} values (Table 3). These results suggest that the models fitted for the mechanical properties (tensile strength, elongation at break, and Young's modulus), WVP, and opacity are suitable (significant and predictive) and lead to significant regression, low residual values, no lack of fit, and satisfactory coefficients of determination. As for solubility in water and color difference, the coefficients of determination (R^2) are 0.36 and 0.46, respectively, indicating that the models explain only 36 and 46% of the observed data variation. The lower $F_{\text{calculated}}/F_{\text{listed}}$ value in relation to F_{listed} proves that the models are not valid for these variables, so they cannot be considered predictive in these cases (Table 3). Because these properties do not correlate with the formulation and process conditions, it is not possible to generate the response surfaces and contour curves.

Table 2. Results of the central composite design.

Test	Tensile strength (MPa)	Elongation (%)	Young's modulus (MPa)	Solubility (%)	WVP (10 ⁻¹⁰ g/m.s.Pa)	ΔE*	Opacity (%)
	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅	Y ₆	Y ₇
1	12.4 ± 0.1	12.8 ± 3.4	947.9 ± 59.9	27.6 ± 1.8	1.65 ± 0.10	2.90 ± 0.09	46.2 ± 0.3
2	7.2 ± 0.2	22.2 ± 1.9	256.9 ± 10.7	32.9 ± 2.5	2.89 ± 0.10	2.79 ± 0.06	51.0 ± 0.4
3	11.6 ± 0.9	12.9 ± 1.1	871.2 ± 79.0	34.1 ± 1.3	2.26 ± 0.08	2.69 ± 0.07	44.0 ± 0.3
4	5.4 ± 0.2	30.6 ± 1.3	160.2 ± 13.1	27.4 ± 2.6	1.88 ± 0.05	2.75 ± 0.06	51.7 ± 0.2
5	10.6 ± 0.8	15.9 ± 1.7	767.8 ± 64.7	34.9 ± 1.6	1.68 ± 0.02	2.65 ± 0.05	48.6 ± 0.2
6	5.6 ± 0.3	39.3 ± 3.7	132.4 ± 10.7	26.8 ± 2.6	2.11 ± 0.05	2.32 ± 0.04	48.2 ± 0.4
7	10.5 ± 0.7	11.9 ± 2.1	938.2 ± 60.0	27.8 ± 1.7	1.47 ± 0.03	2.31 ± 0.08	35.8 ± 0.4
8	4.4 ± 0.3	36.4 ± 2.1	114.8 ± 12.0	25.0 ± 0.4	1.77 ± 0.06	2.39 ± 0.04	43.8 ± 0.4
9	9.0 ± 0.6	19.3 ± 1.0	644.7 ± 70.3	26.0 ± 1.4	2.71 ± 0.38	2.60 ± 0.10	48.9 ± 0.4
10	4.2 ± 0.2	31.9 ± 2.5	95.3 ± 1.1	32.4 ± 2.4	3.50 ± 0.45	2.69 ± 0.15	50.0 ± 0.5
11	10.1 ± 0.6	13.5 ± 1.1	798.2 ± 49.2	33.6 ± 1.3	2.26 ± 0.14	2.74 ± 0.13	51.2 ± 0.2
12	2.4 ± 0.4	23.9 ± 1.0	55.6 ± 7.4	33.1 ± 2.4	2.19 ± 0.02	2.92 ± 0.11	59.9 ± 0.4
13	8.3 ± 0.2	27.4 ± 1.5	423.0 ± 20.4	37.5 ± 1.5	2.22 ± 0.20	2.37 ± 0.08	48.0 ± 0.9
14	7.1 ± 0.6	32.9 ± 1.5	226.2 ± 33.1	34.1 ± 2.8	3.15 ± 0.18	2.78 ± 0.04	52.1 ± 0.2
15	10.0 ± 0.7	10.1 ± 1.2	938.4 ± 107.1	24.9 ± 1.9	2.22 ± 0.09	2.46 ± 0.04	48.6 ± 0.1
16	5.7 ± 0.2	24.0 ± 1.0	223.8 ± 25.7	32.9 ± 2.6	2.44 ± 0.15	2.78 ± 0.05	56.2 ± 0.3
17	16.9 ± 0.2	4.6 ± 1.1	1482.2 ± 54.3	25.1 ± 2.3	1.48 ± 0.10	2.43 ± 0.07	47.5 ± 0.2
18	4.5 ± 0.2	29.7 ± 1.7	120.1 ± 15.4	34.7 ± 1.7	1.89 ± 0.05	2.75 ± 0.07	52.9 ± 0.1
19	7.3 ± 0.7	27.8 ± 2.7	309.1 ± 67.1	28.9 ± 0.7	1.98 ± 0.04	3.19 ± 0.27	70.0 ± 0.4
20	5.6 ± 0.5	26.8 ± 2.0	253.9 ± 16.7	33.5 ± 0.9	2.22 ± 0.03	2.62 ± 0.08	50.9 ± 0.4
21	8.1 ± 0.9	26.5 ± 2.1	439.3 ± 71.5	24.8 ± 0.3	1.67 ± 0.03	2.74 ± 0.04	48.1 ± 0.2
22	5.4 ± 0.4	35.0 ± 2.0	176.5 ± 41.8	28.9 ± 2.2	1.53 ± 0.03	2.71 ± 0.09	52.3 ± 0.4
23	6.4 ± 0.2	27.6 ± 2.3	247.2 ± 22.0	27.1 ± 1.4	1.36 ± 0.07	2.75 ± 0.15	39.3 ± 0.2
24	6.9 ± 0.3	22.3 ± 3.0	293.4 ± 15.9	26.0 ± 0.2	2.02 ± 0.13	2.68 ± 0.07	52.9 ± 0.3
25	7.1 ± 0.7	24.1 ± 1.6	367.1 ± 22.3	24.8 ± 0.8	1.51 ± 0.06	2.57 ± 0.02	50.4 ± 0.2
26	7.3 ± 0.6	25.4 ± 1.6	361.1 ± 12.0	24.4 ± 0.9	1.46 ± 0.01	2.53 ± 0.06	50.2 ± 0.3
27	7.3 ± 0.4	28.0 ± 2.9	359.5 ± 27.5	24.6 ± 1.0	1.52 ± 0.05	2.54 ± 0.04	50.8 ± 0.3
28	7.4 ± 0.4	25.6 ± 3.3	356.9 ± 30.4	24.7 ± 2.6	1.48 ± 0.05	2.58 ± 0.06	50.4 ± 0.4

Table 3. Results for regression coefficients and analysis of variance (ANOVA) for the dependent variables of the central composite design.

Parameter ^a	Coefficient	Tensile strength (MPa)	Elongation (%)	Young's modulus (MPa)	Solubility (%)	WVP (10 ⁻¹⁰ g/m.s.Pa)	ΔE*	Opacity (%)
		Y ₁	Y ₂	Y ₃	Y ₄	Y ₅	Y ₆	Y ₇
Mean	β ₀	6.85	26.07	334.95	26.69	1.49	2.60	49.20
<i>Linear</i>								
X ₁	β ₁	-2.71	6.98	-324.52	—	0.18	0.06	2.18
X ₂	β ₂	—	-1.68	—	—	—	—	-1.66
X ₃	β ₃	—	1.99	—	—	—	-0.09	—
X ₄	β ₄	-0.40	—	—	—	0.26	—	3.04
<i>Quadratic</i>								
X ₁	β ₁ ²	0.94	-2.57	124.27	1.33	—	—	—
X ₂	β ₂ ²	—	—	—	1.64	0.24	0.06	2.26
X ₃	β ₃ ²	—	—	—	—	—	—	—
X ₄	β ₄ ²	—	—	—	—	—	—	-1.33
<i>Interaction</i>								
X ₁ X ₂	β ₁₂	-0.51	—	-57.44	—	-0.21	—	—
X ₁ X ₃	β ₁₃	0.46	—	—	—	—	—	—
X ₁ X ₄	β ₁₄	—	-2.07	—	—	—	0.08	—
X ₂ X ₃	β ₂₃	—	-1.74	45.34	-2.01	—	—	—
X ₂ X ₄	β ₂₄	—	-2.60	—	—	—	—	2.22
X ₃ X ₄	β ₃₄	0.67	—	—	—	—	—	—
F _{calculated}		40.17	21.44	70.94	4.45	13.40	5.09	8.09
F _{listed} ^b		2.08	2.04	2.21	2.33	2.21	2.33	2.08
F _{calculated} ·F _{listed}		19.31	10.51	32.10	1.91	6.06	2.18	3.89
p-value		< 0.00001	< 0.00001	< 0.00001	< 0.05	< 0.00001	< 0.05	< 0.00015
R ² ^c		0.92	0.88	0.93	0.36	0.70	0.46	0.70

^a X₁ = glycerol concentration, X₂ = process temperature, X₃ = drying temperature, X₄ = relative humidity.

^b Values of F_{listed} at p < 0.10.

^c R² = coefficient of determination.

3.4. Response surface and contour curve

3.4.1. Mechanical properties

The desired property of a food packaging material will depend on the application. In general, food packaging must not be deformed and must provide structural integrity to the food or reinforce food structure. In other situations, a deformable film is desirable (GONTARD et al., 1992).

Figures 3 to 5 depict the response surfaces and contour curves obtained for the mechanical properties of banana flour films. Cg affected the tensile strength and Young's modulus negatively and the elongation at break positively (Equations 5 – 7; Figures 3 to 5a – b). This behavior of the glycerol content agrees with other studies and stems from its plasticizing effect (ARAUJO-FARRO et al., 2010; BERGO et al., 2010; SOBRAL et al., 2001; TAPIA-BLÁCIDO et al., 2011). According to Mali et al. (2004) and Mali et al. (2005), films containing glycerol as plasticizer experience weaker intermolecular forces or attractive forces between the adjacent polymer chains, which culminates in enhanced molecular mobility throughout the film structure. Consequently, the elongation at break increases, reducing the tensile strength and Young's modulus. In addition, as demonstrated by Myllärinen et al. (2002) in their work on the influence of glycerol on edible amylose and amylopectin films, the employed amylose/amylopectin ratio, plasticizer concentration, and/or the moisture content and storage conditions can affect the mechanical properties of the film. Alves et al. (2007) reported that cassava starch films with 20 g of glycerol /100 g of starch presented a tensile strength of 21.7 MPa, elongation at break of 5.2%, and Young's modulus of 40.5 MPa, while those with higher glycerol concentration (45 g of glycerol/100 g of starch) had values of 5.4 MPa, 153.2%, and 0.2 MPa, respectively. In their work on the development of potato starch films, Talja, Helén, Roos, and Jouppila (2008) also observed a rise in the tensile strength and Young's modulus values and a reduction in the elongation at break values when they used a low plasticizer content (20 g of plasticizer/100 g of starch), due to the improved crystallinity of the films.

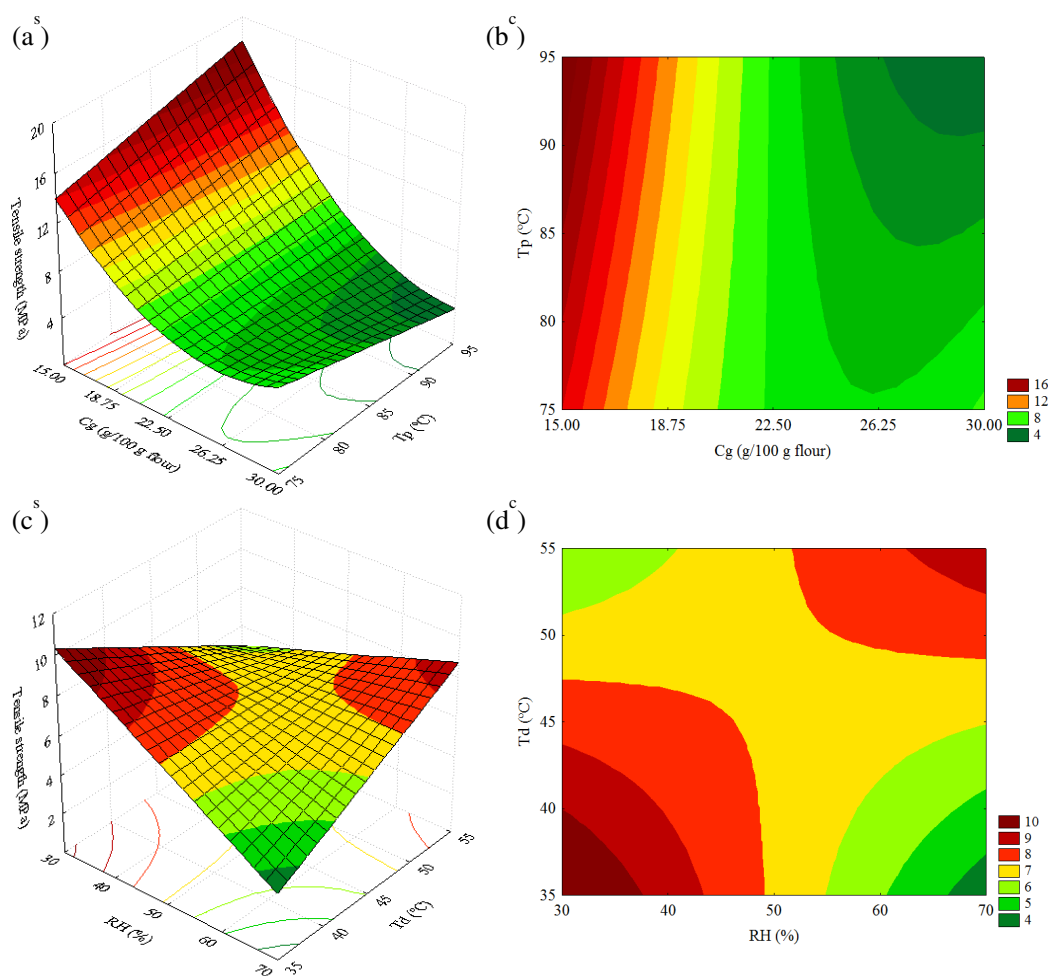


Figure 3. Tensile strength of banana flour films as a function of (a – b) Cg and Tp (Td = 45 °C, RH = 50%), (c – d) Td and RH (Cg = 22.50 g/100 g of flour, Tp = 85 °C). The upper indices s and c denote the response surface and the contour curve, respectively.

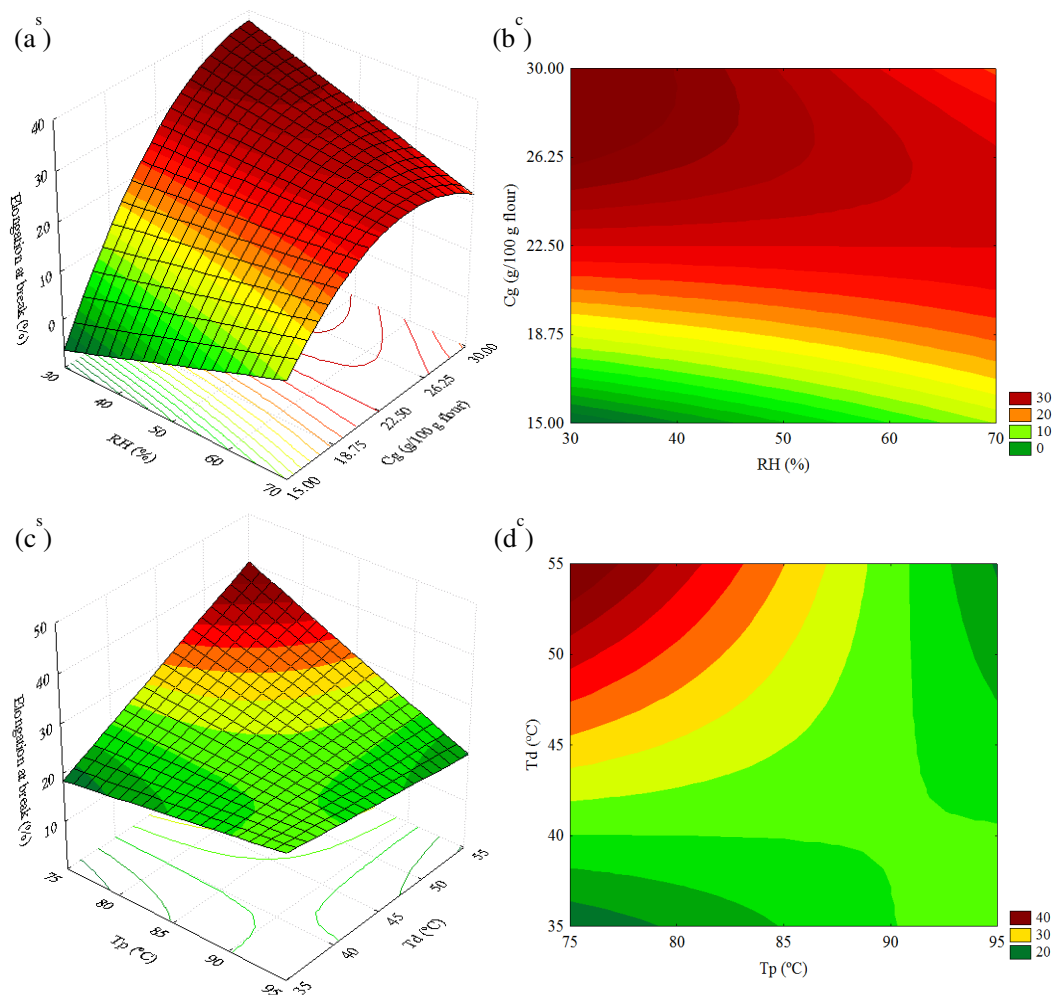


Figure 4. Elongation at break of banana flour films as a function of (a – b) Cg and RH (Tp = 85 °C, Td = 45 °C), (c – d) Tp and Td (Cg = 22.50 g/100 g of flour, RH = 50%). The upper indices s and c denote the response surface and the contour curve, respectively.

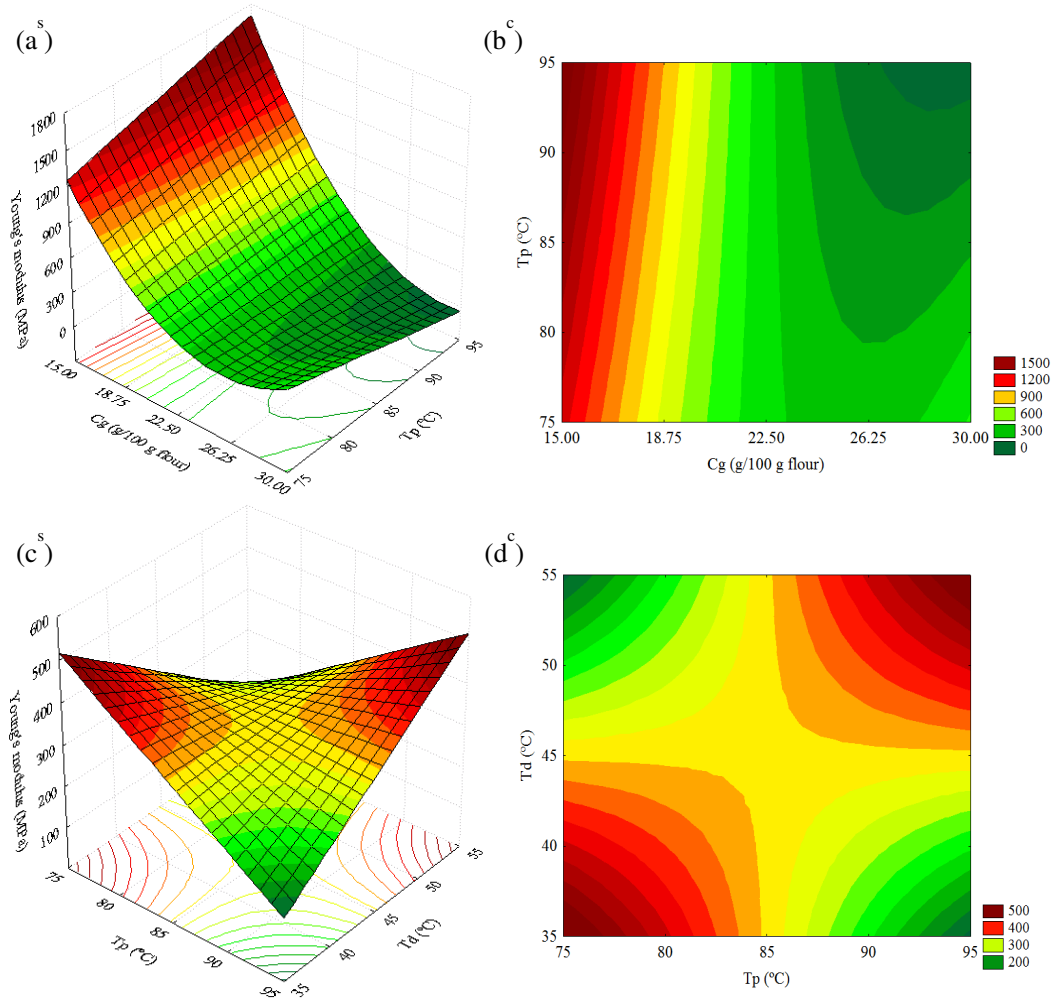


Figure 5. Young's modulus of banana flour films as a function of (a – b) Cg and Tp (Td = 45 °C, RH = 50%), (c – d) Tp and Td (Cg = 22.50 g/100 g of flour, RH = 50%). The upper indices s and c denote the response surface and the contour curve, respectively.

The RH (X_4) process variable did not exert a remarkable effect on the elongation at break or Young's modulus properties. However, this parameter had an interesting negative effect on tensile strength (Equation 5); that was, larger RH values resulted in lower tensile strength values (Figure 3c – d). This behavior is probably due to changes in the sample macrostructure as a function of the air RH during drying. Transmission electron microscope (TEM) micrographs have attested to this behavior and suggested that it is related to the use of higher RH during film formation, which alters the pore size distribution and the non-homogeneity of the amylose network (STADING et al., 2001). Therefore, amylose generates homogenous films at lower RH values, but higher RH affords denser polymer zones with larger pores, probably causing the lower tensile

strength (Figure 3c – d) and the higher elongation at break (Figure 4a – b) values obtained for the banana flour films. Tapia-Blácido et al. (2005b) reported that several phenomena may occur during the drying of the film-forming solution, because of its complex composition (biopolymers and plasticizers). This in turn would influence the structure and properties of the film by prompting changes in the microstructure or large-scale alterations in the macrostructure, thereby affecting the entire network.

ANOVA (Table 3) confirmed that T_p (X_2) and T_d (X_3) have significant negative and positive effects on the elongation at break, respectively (Equation 6). According to Figure 4c – d, high elongation at break values correspond to the minimum T_p and maximum T_d values. Thus, longer drying times (lower T_d) favor the molecular interactions between the compounds present in the film solution, contributing to the lower elongation of the banana flour films found in this research.

The effect of T_p on the mechanical properties of the banana flour films is associated with the gelatinization process. During gelatinization, amylose leaches to the aqueous phase between the granules, and the polymer chains approach each other more closely because of the high amylose content. This forms a denser polymer matrix with greater mechanical strength and lower flexibility (Figure 4c – d). High T_p may also favor denaturation of the proteins present in the flour and increase the number of interactions between the different biopolymers (DENAVI et al., 2009). Some authors reported that the high temperature of the prepared films should be understood in terms of a closely packed state where extensive intermolecular bonding occurs, which inhibits further orientation and better alignment of the protein and starch chains (ARVANITOYANNIS et al., 1998).

According to Equation 7, the interaction between T_p and T_d (X_2X_3) has a significant, positive and relevant effect on the Young's modulus property. In the search for conditions that allow for the achievement of rigid films, two combinations for these process variables are possible: higher T_p and T_d or lower T_p and T_d . Figure 5c – d illustrates the two regions, in red, where the highest Young's modulus values can be obtained. This behavior offers more possibilities to adjust process conditions that favor the production of banana flour films with the desired characteristics within the studied limits.

3.4.2. Water vapor permeability (WVP)

Since the main function of a food packaging material is to control the moisture transfer between the food and the surrounding atmosphere, or between two components of a heterogeneous food product, the WVP should be as low as possible (GONTARD et al., 1992). In general, the use of low C_g (15 – 22.5 g/100 g of flour), intermediate T_p (80 – 85 °C), high T_d (45 – 55 °C), and low RH (30 – 40%) yields banana flour films with lower WVP values, as shown in Figure 6.

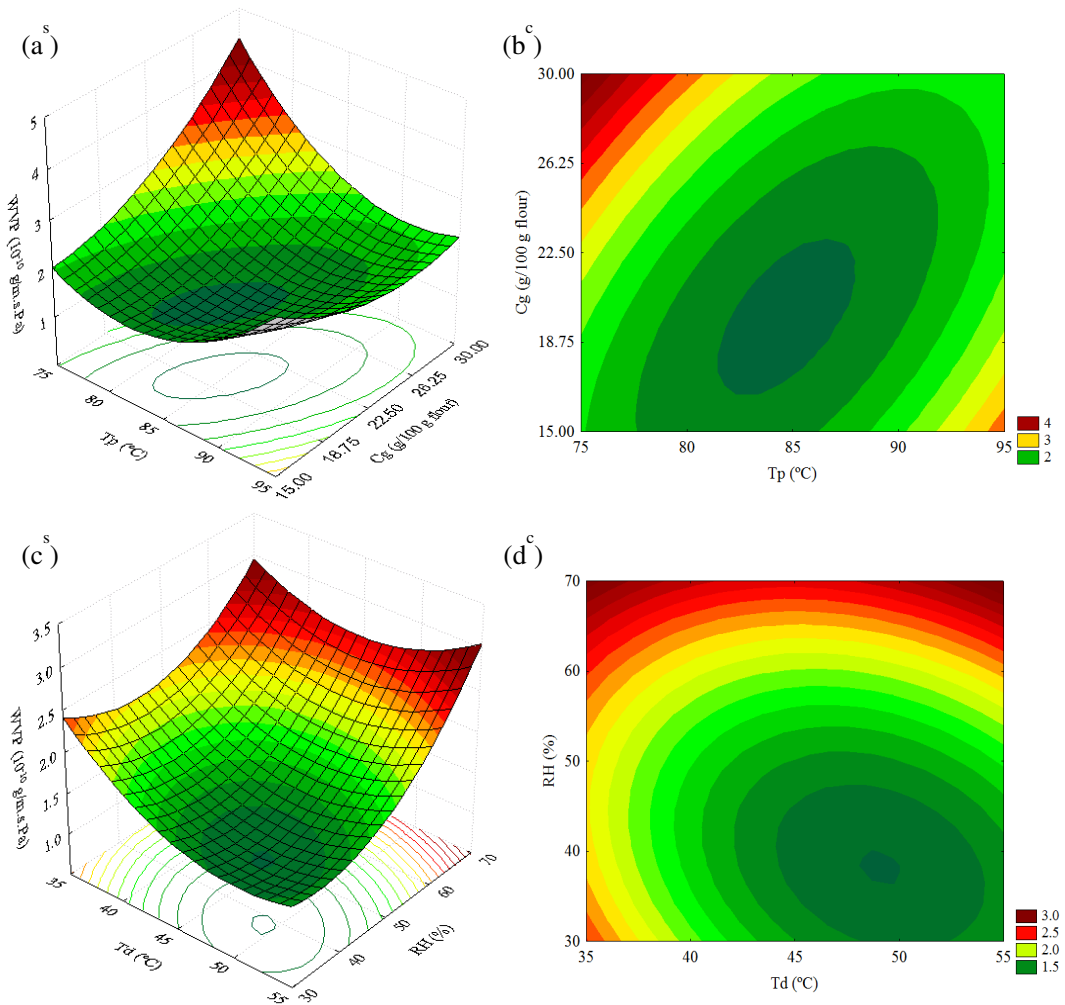


Figure 6. WVP of banana flour films as a function of (a – b) C_g and T_p ($T_d = 45$ °C, RH = 50%), (c – d) T_d and RH ($C_g = 22.50$ g/100 g of flour, $T_p = 85$ °C). The upper indices s and c denote the response surface and the contour curve, respectively.

Many authors have extensively reported the effect of Cg on the WVP of films (CUQ et al., 1997; GARCÍA et al., 1999; MALI et al., 2006). The increase in WVP values with rising plasticizer content (Figure 6a – b) is due to structural modifications occurring in the starch network in combination with the hygroscopic character of glycerol. Moreover, glycerol molecules are small and present high capacity to interact with starch chains, thereby enhancing the molecular mobility and augmenting the free volume in the film matrix (SOTHORNVIT and KROCHTA, 2001). Tapia-Blácido et al. (2011) carried out the optimization of amaranth flour films plasticized with glycerol and sorbitol by multi-response analysis. These authors reported that the film prepared with the optimal formulation and using glycerol as plasticizer was more hygroscopic, less resistant to break, flexible, and more permeable to oxygen as compared with the film containing sorbitol as plasticizer.

Other components in the structure of the banana flour may behave in different ways depending on the film formulation and processing conditions. Heating promotes the denaturation of proteins, and a more drastic thermal treatment modifies the three-dimensional structure of globular proteins. This exposes the sulfhydryl (SH) groups, which may culminate in hydrophilic interactions during drying because of the hydrophilic nature of this group (TAPIA-BLÁCIDO et al., 2005a). Thus, increasing the Tp from 75 to 85 °C/15 min leads to reduced WVP values in these films. However, more intense temperatures (above 85 °C) furnish more permeable films (Figure 6a – b).

Figure 6c – d evidences that drastic drying conditions (high temperature and low relative humidity) result in lower WVP values and thus less permeable films. As observed by ANOVA (Table 3), the RH (X₄) process variable influences the WVP, exerting a positive effect on this property (Equation 9). The presence of higher RHs could contribute to greater moisture contents in the films, thereby improving their hydrophilicity.

3.4.3. Opacity

Packages are often the only way of exposing products to consumers before purchase (MARSH and BUGUSU, 2007). Thus, the materials chosen to produce packages play a vital role in product commercialization. The optical properties of polymer films are related to opacity (transparency), color, and brightness. Opacity has

an important part, since certain very attractive products, especially foodstuffs, must be well visible across the film by consumers (GONTARD et al., 1992).

The parameters T_p (X_2) and RH (X_4) as well as the interaction between them (X_2X_4) strongly influence opacity (Equation 11). Based on ANOVA results (Table 3), T_p has a negative effect while RH exerts a positive effect on this property. According to Figure 7, lower Cg (15 – 18.75 g/100 g of flour), higher T_p (85 – 95 °C), and lower RH (30%) values give less opaque films.

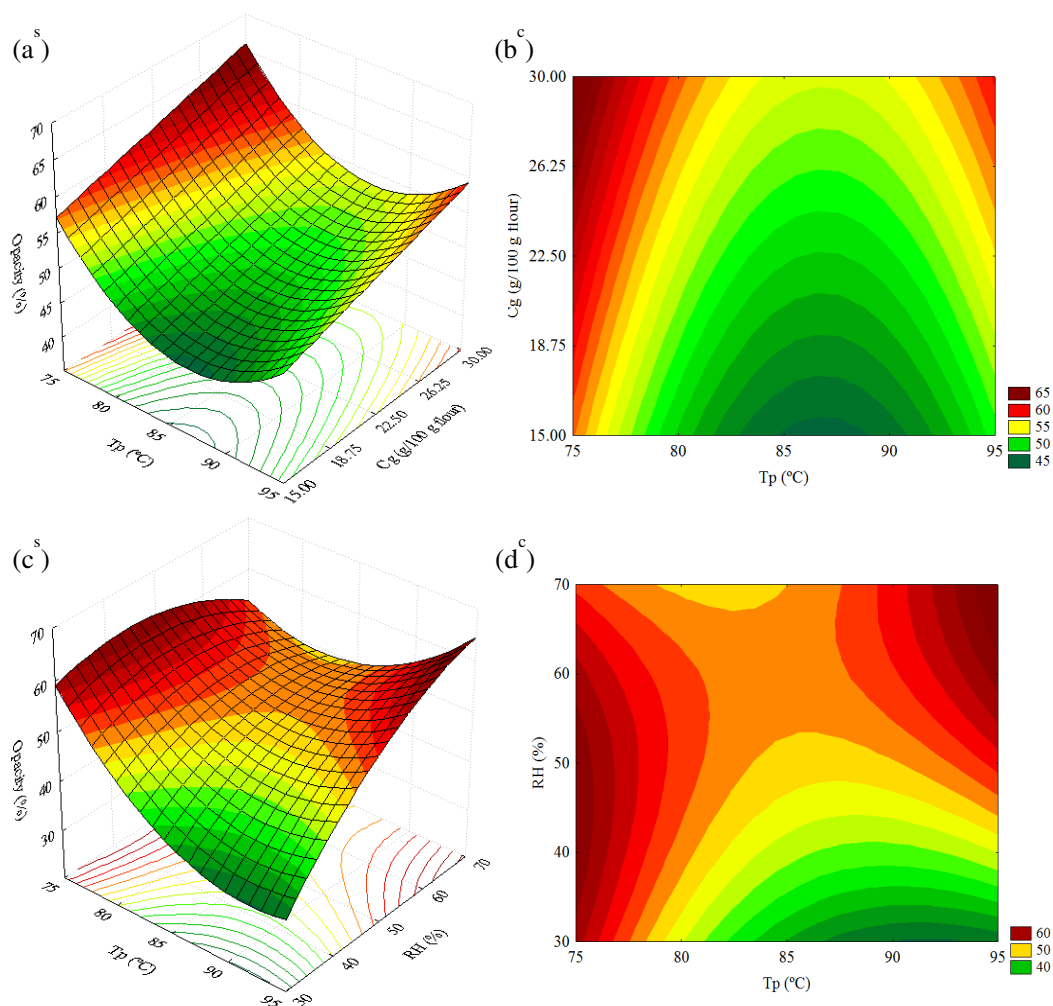


Figure 7. Opacity of banana flour films as a function of (a – b) Cg and T_p ($T_d = 45$ °C, RH = 50%), (c – d) T_p and RH (Cg = 22.50 g/100 g of flour, $T_d = 45$ °C). The upper indices s and c denote the response surface and the contour curve, respectively.

A higher T_p condition is expected to suffice for the crystalline-to-amorphous transition of starch (gelatinization) to happen. At the same time, a milder thermal

treatment leads to incomplete material melting and favors the presence of crystalline residues and the consequent formation of more opaque banana flour films (Figure 7).

Concerning the RH variable, wetter conditions afford higher opacity values (Figure 7c – d). Longer drying times (higher RH) favor the molecular interactions between the compounds present in the film solution, thereby culminating in more compact structures and increased opacity values.

The experimental opacities of banana flour films range between 35.8 and 70.0%, which are higher as compared with those of achira flour films (17.5 – 44.1%) (ANDRADE-MAHECHA et al., 2012), amaranth flour films (6.5%) (TAPIA-BLÁCIDO et al., 2005a), and quinoa flour films (5.3%) (ARAUJO-FARRO, 2008). These differences might be related to starch retrogradation and amylose content of the different botanical sources used in the production of the films but will also depend on the method used to measure opacity.

3.5. Optimization and validation

The optimization of the formulation and process conditions for the production of banana flour films was accomplished using a multi-response method called desirability (Derringer and Suich, 1980). This method involves the transformation of each response variable (Y_i) to an individual function of desirability (d_i) (Equations 12 and 13); the desirability scale ranges from 0 to 1. If the response fell outside an acceptable region, it was set to $d_i = 0$; if the response was fully desirable (at its goal or target), it was set to $d_i = 1$.

$$d_i = \frac{Y_i - Y_{\min}}{Y_{\max} - Y_{\min}} \quad [12]$$

where $Y_{\min}$ is the response minimum value, and $Y_{\max}$ is the response maximum value. In the case of WVP and opacity, Equation 12 had to be redesigned, so that the minimum values for these responses could be obtained (Equation 13).

$$d_i = \frac{Y_{\max} - Y_i}{Y_{\max} - Y_{\min}} \quad [13]$$

The individual desirability functions from the considered responses were then combined, to obtain the overall desirability (D), defined as the geometric average of the individual desires (Equation 14). D was later maximized using the Statistica 7.0 software.

$$D = \left(d_1^{n_1}, d_2^{n_2}, \dots, d_k^{n_k} \right)^{1/k} \quad [14]$$

where k is the number of considered responses and n_i is the weight of each response. Thus, since $0 \leq D \leq 1$, a high D value shows that all d_{ks} are toward the target value, which is considered as the optimal solution of the system.

Figure 8 shows the multi-response optimization of the predicted profiles for the response variables that presented valid mathematical models (tensile strength, elongation at break, Young's modulus, WVP, and opacity) together with their respective desirability function profiles, for each of the investigated variables of formulation and process (Cg, Tp, Td, and RH). Therefore, the optimal formulation and process conditions leading to the maximum global desirability value (0.96) for the production of banana flour films are Cg = 19 g/100 g of flour, Tp = 81 °C, Td = 54 °C, and RH = 48%, with predicted response variables of 8.7 MPa for the tensile strength, 25.0% for the elongation at break, 590.5 MPa for Young's modulus, 2.0×10^{-10} g/m.s.Pa for the WVP, and 50.0% for the opacity. Other authors also found the optimal formulation and process conditions for their films using the multi-response analysis (ANDRADE-MAHECHA et al., 2012; ARAUJO-FARRO et al., 2010; TAPIA-BLÁCIDO et al., 2011).

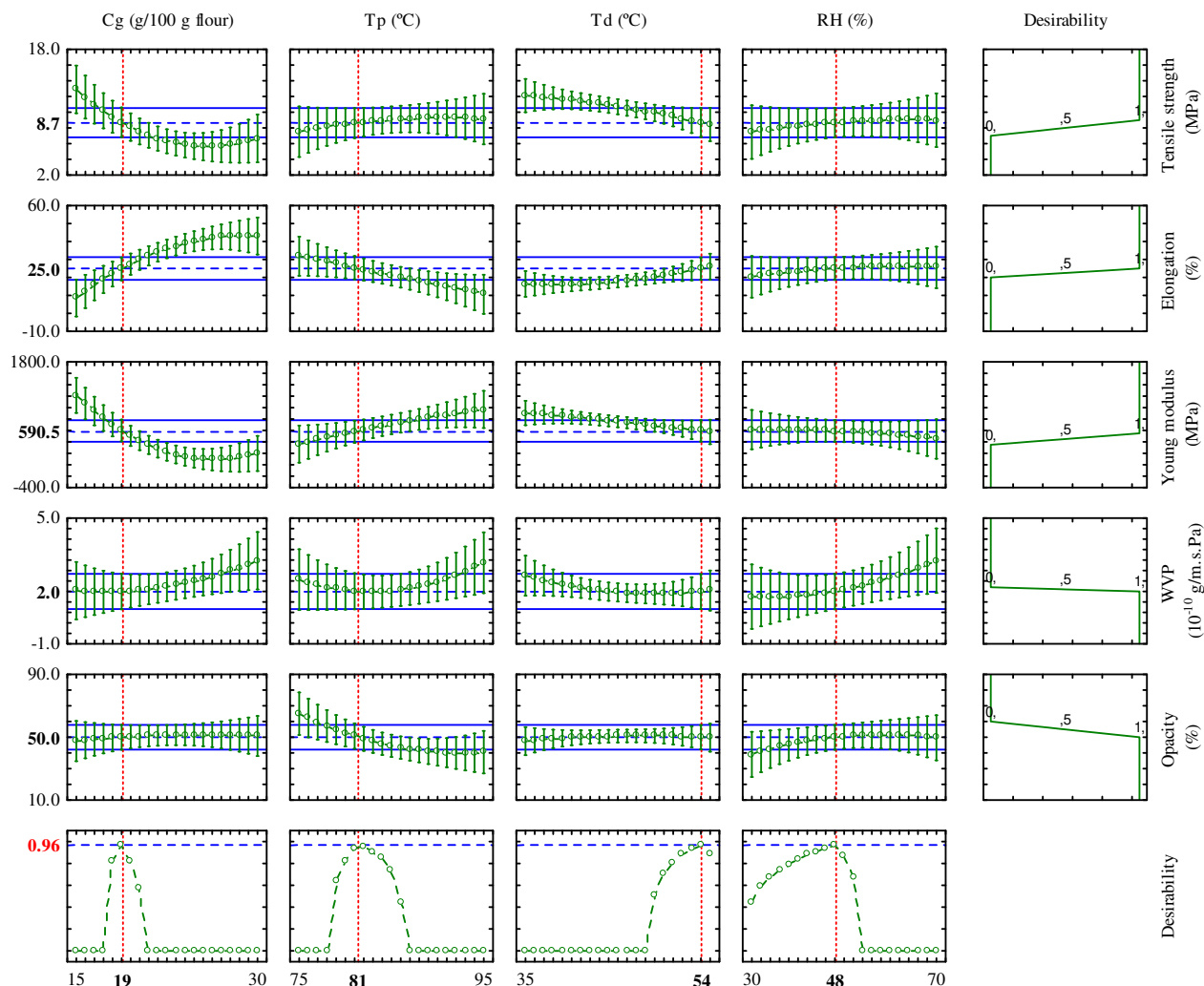


Figure 8. Simultaneous optimization of formulation and process conditions for the production of banana flour films as function of formulation and process variables, predicted response variables, and desirability profiles.

Experimental validation has been performed in triplicate, and the results are given as mean values in Table 4. Based on the relative deviation values obtained for each response variable, the optimization methodology employed here is satisfactory. The experimental value of the tensile strength variable is higher than the value predicted by the desirability function, culminating in a film with increased mechanical resistance. This is associated with the lower experimental elongation at break as compared with the predicted value. The experimental values achieved for the banana flour film properties are very similar to the expected ones, which is highly satisfactory.

Table 4. Experimental validation under the optimized conditions for the production of banana flour films.

Property	Predicted value	Experimental value ^a	Relative deviation ^b (%)
Tensile strength (MPa)	8.7	9.2 ± 0.2	5.9
Elongation at break (%)	25.0	24.2 ± 1.9	-3.4
Young's modulus (MPa)	590.5	583.4 ± 46.4	-1.2
WVP (10 ⁻¹⁰ g/m.s.Pa)	2.0	2.1 ± 0.2	4.3
Opacity (%)	50.0	51.3 ± 0.3	2.5

^a Values obtained in optimal conditions: Cg = 19 g/100 g of flour, Tp = 81 °C, Td = 54 °C, and RH = 48%.

^b Relative deviation = [(experimental value – predicted value)/experimental value] x 100.

Since the banana flour is a natural blend composed of starch, protein, lipids, and fiber (Table 1 of Chapter 2), the film produced from this raw material has mechanical, barrier, and optical properties comparable to those of other films made from the flour of other plant species: achira (ANDRADE-MAHECHA et al., 2012), amaranth (TAPIA-BLÁCIDO et al., 2005a), quinoa (ARAUJO-FARRO, 2008) and rice (DIAS et al., 2010), as shown in Table 5.

Compared with other biodegradable films (Table 5), the banana flour film exhibits excellent mechanical properties; it has higher tensile strength than amaranth, achira, and quinoa flour films and similar tensile strength to the rice flour film. The film produced in this study also presents good flexibility, as seen by the elongation at break value. The presence of protein and lipids in the flour could collaborate with the plasticizing effect. According to Table 5, the higher Young's modulus value attained for the banana flour film confirms the proximity between the polymer chains induced by the high amylose content (27.8 g/100 g of dry starch), which in turn facilitates the formation of a denser polymeric matrix with greater strength and rigidity. Moreover, the polysaccharide and the protein present in the banana flour provide additional hydrogen bonding interactions between the polymer chains, accounting for the higher film strength (SOTHORNVIT and PITAK, 2007).

Table 5. Properties of films prepared with different types of flour.

Property	Film matrix ^a				
	Achira flour	Amaranth flour	Quinoa flour	Rice flour	Banana flour
Plasticizer (g/100 g of flour)	17	22.5	21	20	19
Amylose (g/100 g of starch)	30.7 ± 0.1	7.6 ± 0.4	–	–	27.8 ± 0.1
Thickness (μm)	84 ± 2	83 ± 5	80 ± 2	100 ± 40	87 ± 3
<i>Mechanical properties</i>					
Tensile strength (MPa)	7.0 ± 0.3	1.5 ± 0.1	4.1 ± 0.5	10.3 ± 1.0	9.2 ± 0.2
Elongation at break (%)	14.6 ± 1.1	83.7 ± 5.1	88.4 ± 8.9	2.7 ± 0.5	24.2 ± 1.9
Young's modulus (MPa)	231.7 ± 19.9	21.5 ± 1.4	138.0 ± 40.0	560.7 ± 64.3	583.4 ± 46.4
<i>Barrier properties</i>					
Solubility in water (%)	38.3 ± 1.9	42.3 ± 1.8	18.7 ± 0.1	–	27.9 ± 1.3
WVP (10 ⁻¹⁰ g/m.s.Pa)	5.3 ± 0.2	0.7 ± 0.2	0.6 ± 0.1	1.1 ± 0.1	2.1 ± 0.2
<i>Optical properties</i>					
ΔE*	14.4 ± 0.7	8.9 ± 0.6	18.1 ± 0.4	–	2.7 ± 0.1
Opacity (%)	18.0 ± 0.3	6.5 ± 0.9	5.3 ± 0.8	–	51.3 ± 0.3

^a References: ANDRADE-MAHECHA et al., 2012 (achira flour), TAPIA-BLÁCIDO et al., 2005a (amaranth flour), ARAUJO-FARRO, 2008 (quinoa flour), DIAS et al., 2010 (rice flour), this work (banana flour).

The solubility in water of the banana flour film is relatively low as compared with the other films (Table 5), a consequence of the high level of cohesion within the matrix, which results in a compact structure. This specific matrix structure is associated with the composition of the banana flour and also to the film-forming technique employed in this study. Regarding permeability, the banana flour film has higher WVP than the amaranth, quinoa, and rice flour films (Table 5). However, its permeability is lower than those of other biodegradable films, such as the whey protein isolate (13.4×10^{-10} g/m.s.Pa) (SHAW et al., 2002), amylose (11.9×10^{-10} g/m.s.Pa), amylopectin (14.4×10^{-10} g/m.s.Pa) (RINDLAV-WESTLING et al., 1998), achira flour (5.3×10^{-10} g/m.s.Pa) (ANDRADE-MAHECHA et al., 2012), and corn starch (2.6×10^{-10} g/m.s.Pa) (GARCÍA et al., 2000).

With respect to the optical properties (Table 5), the banana flour film displays a more uniform color, as observed by the lower color difference value (ΔE^*). Furthermore, it is more opaque than the other films prepared from other types of flour, which indicates that banana flour film can be used when protection against incident light is necessary, especially for products that are sensitive to degradation reactions catalyzed by light.

4. Conclusions

The banana flour seems to be a very promising material to formulate coatings and edible films. The presence of native protein, lipids, and fiber, allied with the high content of amylose in its composition, favors the production of films with better properties than those of other biodegradable films. For the first time, a central composite design together with the desirability function (multi-response analysis) has been described for the optimization of the formulation and process conditions involved in the production of banana flour films. This optimization technique has been very useful for the full understanding of the process, since the models allowed for the experimental design of the production of films with the desired characteristics within the studied limits.

5. References

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Capítulo 4

Comparative Study on the Properties of Flour and Starch Films of Plantain Bananas (*Musa paradisiaca*)

Abstract

Biodegradable films were prepared by using the flour and starch isolated from unripe plantain bananas of the variety “Terra” (*Musa paradisiaca*). Since the non-starchy fraction present in the banana flour represents 29.4% (on dry basis) of its composition, we considered it would be interesting to compare the properties of the film elaborated from this natural blend with that of the film produced from the banana starch only. Both films were characterized on the basis of their mechanical, barrier, optical, structural, and thermal properties. The banana flour film was less mechanically resistant but more flexible than the banana starch film. Despite the differences in the microstructure of the flour and starch films, the former was slightly soluble in water, and its water vapor permeability was similar to that of the starch film. Regarding the optical properties, the flour film was yellowish, which can be attributed to its protein content and the presence of phenolic compounds. The starch film, on the other hand, was lighter and less opaque. The FTIR spectra revealed the presence of the amide I group related to proteins only in the case of the flour film. Both plantain banana films displayed a C-type X-ray pattern and one glass transition temperature each, which was higher for the starch film (46.4 °C) as compared to the flour film (30.2 °C). The presence of other components (protein, lipids, and fiber) in the flour film had important effects on its properties. In general, the banana flour and starch are very promising materials for the formulation of coatings and films.

Keywords: plantain banana, flour, starch, biodegradable films, mechanical properties, barrier properties.

1. Introduction

The increase in non-biodegradable waste material and the difficulty in recycling most of the available synthetic packaging have been pushing research toward the development of new biodegradable materials that are suitable for packaging (DAVIS and SONG, 2006; MARSH and BUGUSU, 2007).

Various natural biodegradable polymers such as proteins and polysaccharides have potential application in the production of environmentally-friendly packaging (CHANDRA and RUSTGI, 1998; KROCHTA and DE MULDER-JONHSTON, 1997). Among polysaccharides, starch is the most widely employed in the elaboration of films, due to its low cost and abundance in nature. Several studies have reported on the use of starch from different sources in the preparation of films and coatings with different properties. Such works have indicated that these carbohydrates are promising materials in this regard (ALVES et al., 2007; AVÉROUS et al., 2001; MALI et al.; MÜLLER et al., 2008). However, few studies on the utilization of flour as raw material for the production of films have been conducted over the last decade. The interest in combining polysaccharides, proteins, lipids, and fibers lies on the advantages and disadvantages of these components (BALDWIN et al., 1995). The use of natural blends directly obtained from agricultural sources takes advantage of each component in the original system and appears to be a new source of material in the area of biodegradable films (TAPIA-BLÁCIDO et al., 2005). Rayas and Hernández (1997) have prepared films from three types of wheat flours. More recently, Andrade-Mahecha et al. (2012) have employed the flour from achira, a perennial plant native to the Andes in South America. Also, Colla et al. (2006) and Tapia-Blácido et al. (2005, 2011) have produced films using amaranth flour as raw material.

An interesting renewable source for the production of edible and biodegradable materials is the unripe banana fruit. Originated from Southeast Asia, bananas (genus *Musa*) are extensively grown in tropical and subtropical regions, and this fruit is considered an important food crop (ZHANG et al., 2005). Unripe bananas are a good source of starch (over 70% of the dry weight) for the preparation of films. This is because their native starch contains about 36.2% amylose (ESPINOSA-SOLIS et al., 2009), which is the starch component that is mainly responsible for the film forming capacity of starches. Starches isolated from banana, okenia, and mango have been used for the production of edible films (ROMERO-BASTIDA et al., 2005). In another work,

films prepared from the banana flour were developed; however, only the oxygen permeability and the mechanical properties of these films were evaluated (SOTHORNVIT and PITAK, 2007).

A study on the properties of films obtained from natural blends and their comparison with the characteristics of films produced from a component of the same botanical source would provide relevant information about the type of interactions taking place in the polymer matrix. Because a comparative study on the features of flour and starch films made from the same variety of banana has not yet been conducted, the objective of the present study was to investigate the influence of protein, lipids, and fiber of the banana flour on the properties of the film produced from this raw material and compare the characteristics of this film with those of the banana starch film. To this end, the flour and starch were isolated from unripe bananas of the variety “Terra” (*Musa paradisiaca*), a type of plantain that has exceptionally high starch, amylose, and resistant starch contents and which has been little investigated. The obtained films were characterized with respect to their mechanical, barrier, optical, structural, and thermal properties.

2. Materials and Methods

2.1. Materials

The raw materials were prepared from unripe plantain bananas (mature green) of the variety “Terra” (*Musa paradisiaca*), according to the methodologies described in Sections 2.2 and 2.3 of Chapter 2. All the chemicals employed in this work were reagent grade.

2.2. Film production

The films were produced by the casting method (see Section 2.3 of Chapter 3) using the optimal formulation and process conditions that had been established for the banana flour film (Figure 1). Preliminary tests of grammage were performed in the starch film to determine the exact amount of FFS to be poured onto acrylic plates (see Appendix 2).

The films were conditioned in desiccators under 58% RH, at 25 °C, for 48 h before being characterized in terms of the moisture content, mechanical properties, and water vapor permeability.

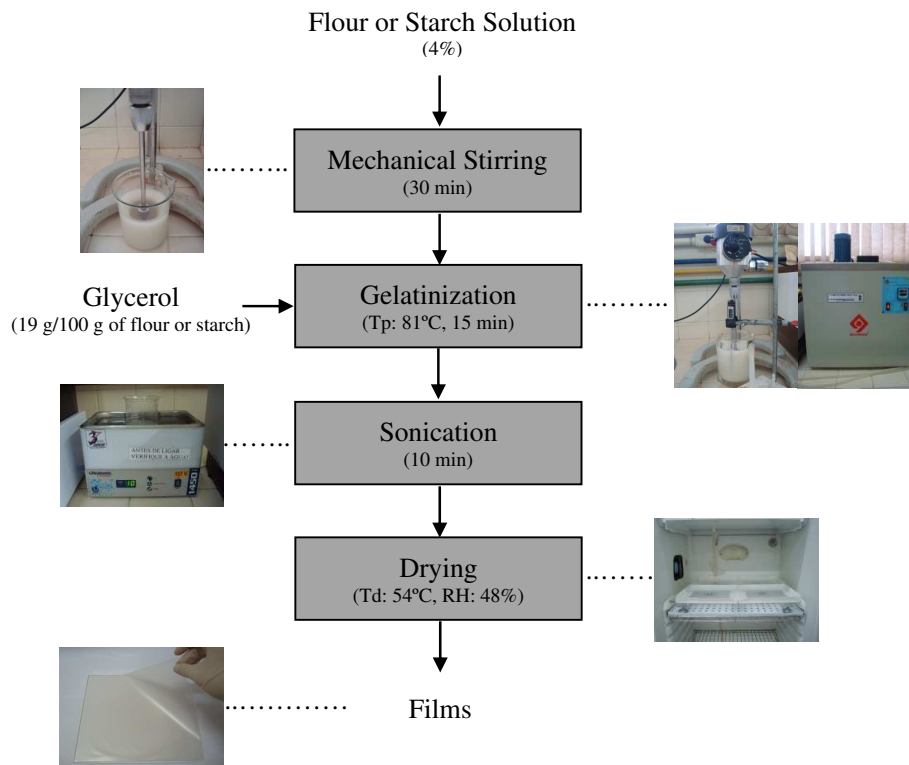


Figure 1. Scheme describing the procedure for the production of the plantain banana films.

2.3. Film characterization

The thickness, density, moisture content, tensile properties, color and opacity analyses, solubility in water and water vapor permeability were determined as described in Section 2.4 of Chapter 3. The other analyses performed in the films are detailed below:

2.3.1. Scanning electron microscopy (SEM)

The surface and cross-section of the films were analyzed by SEM for microstructure evaluation. The film samples were cut into small pieces (20 mm x 20

mm) and dehydrated in a desiccator with silica gel (~0% RH) for 3 weeks. After this period, the dried samples were fractured with the help of tweezers, and small fragments were obtained. Samples of these fragments were fixed on aluminum stubs by means of a double-sided tape and were then coated with a layer of gold (Sputter Coater POLARON, model SC7620), to improve conductivity. The coated samples were viewed under a scanning electron microscope (LEO, model LEO 440i, Cambridge, England) operating at an acceleration voltage of 15 kV.

2.3.2. Mechanical properties

The puncture properties were determined by using a texture analyzer (Stable Micro Systems, model TA.TXplus, Surrey, England), following the methodology described by Gontard et al. (1994). Samples were cut into discs with a diameter of 60 mm and fixed onto a capsule with a circular opening of 34 mm diameter. A cylindrical probe with a diameter of 3 mm was moved perpendicularly to the film surface at a constant speed of 1 mm/s, until the probe passed through the film (rupture point). The puncture force was obtained directly from the force x probe displacement curves using the Texture Exponent 32 software, and the puncture deformation was calculated with the aid of Equation 1:

$$D = \frac{(d^2 - l_0^2)^{\frac{1}{2}} - l_0}{l_0} \times 100 \quad [1]$$

where D is the puncture deformation (%), d is the distance penetrated by the probe (mm), and l_0 is the radius of the film surface (mm).

Analyses of the puncture properties were performed by taking an average of six determinations for each sample, and the results are presented as mean values.

2.3.3. Water sorption isotherms

Sorption isotherms were determined in triplicate, according to the procedure described by Labuza and Ball (2000). Film samples were cut into small pieces (20 mm x 20 mm) and dehydrated in a desiccator with silica gel (~0% RH) for 3 weeks. The

dried samples were precisely weighed to the nearest 0.0001 g into pre-weighed bottles and were equilibrated in hermetically sealed flasks containing different saturated salt solutions of known equilibrium relative humidities (RH, water activities) at 25 °C: LiCl = 0.11, MgCl₂ = 0.33, K₂CO₃ = 0.43, Mg(NO₃)₂ = 0.53, NaBr = 0.58, NaNO₂ = 0.65, NaCl = 0.75, KCl = 0.84, BaCl₂ = 0.90. After the samples had reached equilibrium (21 days), the moisture content was determined by the oven-drying method at 105 °C for 24 h (AOAC, 2005). Isotherms were obtained by plotting the moisture content of the samples in equilibrium versus the water activity (a_w). The Guggenheim, Anderson, and Boer model (GAB), given by Equation 2, was used for data adjustment:

$$X_w = \frac{C.k.m_0.a_w}{[(1 - k.a_w)(1 - k.a_w + C.k.a_w)]} \quad [2]$$

where X_w is the equilibrium moisture content (g water/g dry solid), a_w is the water activity (RH/100), m_0 is the monolayer moisture content (g water/g dry solid), C is the constant related to the monolayer heat sorption, k is the constant related to the multilayer heat sorption. GAB model parameters were determined by non-linear regression, using the Statistica 7.0 software (StatSoft Inc, Tulsa, Oklahoma, USA).

2.3.4. Gloss

The gloss was measured with the aid of a glossmeter (Rhopoint, model Novo-Gloss LiteTM, São Paulo, Brazil), following the standard method D523-89 (ASTM, 1999). The gloss values (in gloss units or GU) were obtained in both surfaces of the film; *i.e.*, the side exposed to air during drying (E) and the side in contact with the support plate (I), directly from the equipment in two angles of measurement, namely 20° and 60°. Results are expressed as the arithmetic mean of five measurements on each surface.

2.3.5. X-ray diffraction (XRD)

Film samples were cut into small pieces (10 mm x 10 mm) and dehydrated in a desiccator with silica gel (~0% RH) for 3 weeks. X-ray diffraction analysis was performed using an X-ray diffractometer (Siemens, model D5005, Baden-Württemberg,

Germany) operated at a voltage of 40 kV and a current of 30 mA; the target was Cu. The diffraction data of the samples were collected over an angular range from 5 to 70° (2 θ), at a scanning rate of 1.2°/min. The index crystallinity (%) of the films was quantitatively estimated as the ratio of crystalline area to the total area of the diffractogram, by following the method of Nara and Komiya (1983) and using the Origin 8.0 software (OriginLab Corporation, Massachusetts, USA).

2.3.6. Fourier-transform infrared spectroscopy (FTIR)

Film samples were cut into small pieces (10 mm x 10 mm) and dehydrated in a desiccator containing silica gel (~0% RH) for 3 weeks. After this period, the FTIR spectra were recorded on a spectrophotometer (PerkinElmer, model Spectrum One, Ohio, USA) fit with a Universal Attenuated Total Reflectance (UATR) device. Analysis was accomplished in the infrared region, with 16 scans, covering wavenumbers ranging from 4000 to 650 cm⁻¹, with a spectral resolution of 4 cm⁻¹ (VICENTINI et al., 2005).

2.3.7. Differential scanning calorimetry (DSC)

The thermal properties of the films were analyzed on a differential scanning calorimeter (TA-Instruments, model 2920, Pennsylvania, USA) equipped with a cooling system. Prior to the determination, the samples were weighed (7 – 8 mg) in aluminum pans and conditioned in hermetically sealed flasks containing a saturated NaBr solution (58% RH) at 25 °C, for seven days. For the analysis, the sample pans were hermetically sealed and scanned at a heating rate of 10 °C/min over a temperature range of - 60 to 150 °C. A sealed, empty aluminum pan was used as reference for all the DSC runs. The glass transition temperature (T_g) was considered to be the inflexion point of the base line, caused by the discontinuity of the specific heat of the sample. This property was obtained from the thermograms of the samples by using the Universal Analysis 3.9A software. The DSC measurements were performed in triplicate, and the results are presented as mean values.

2.4. Statistical analysis

An analysis of variance (ANOVA) and a Tukey test of multiple comparisons with a significance level of 5% were run using the Statistica 7.0 software for comparison of the differences between means of the properties of the banana flour and starch films.

3. Results and Discussion

3.1. Film characterization

Both the flour and starch films from plantain banana presented homogeneous surface with no bubbles or cracks, as well as good handling characteristics. This means that the films could be easily detached from the plates without tearing, and that they were not sticky or too brittle.

The chemical composition of the films is shown in Table 1. Plantain banana films had high starch and amylose contents, and the flour film presented an interesting amount of protein, lipids, and fiber.

Table 1. Chemical composition of the banana flour and starch films (g/100 g dry film).

Component ^a	Flour film	Starch film
Glycerol	17.1	17.1
Ash	1.7	0.03
Protein	2.8	0.9
Lipid	0.5	0.02
Crude fiber	1.1	0.3
Starch	74.9	85.3
Amylose	20.8	31.5

^a Calculated by mass balance from the chemical composition of the banana flour and starch (Table 1 of Chapter 2).

3.1.1. Scanning electron microscopy (SEM)

The scanning electron microscopy of the surfaces and cross-sections of the plantain banana films can be seen in Figure 2. Regarding the surface microstructure, the flour film (Figure 2a) presents a non-homogenous structure; *i.e.*, less smooth surface with imperfections, while the starch film displays a more uniform surface (Figure 2b). The irregularities on the surface of the banana flour film may be related to the presence of more than one macromolecule in the polymer matrix (starch, protein, lipids, and fiber), as well as to the interactions between these components such as starch-protein and starch-cellulose (fiber) interactions, not to mention the stable complexes formed by amylose and lipids. Although the protein, lipids, and fiber contents are lower than 5% (Table 1), they play an important role in the final film structure. In addition, some micro-segregation of the phases may occur.

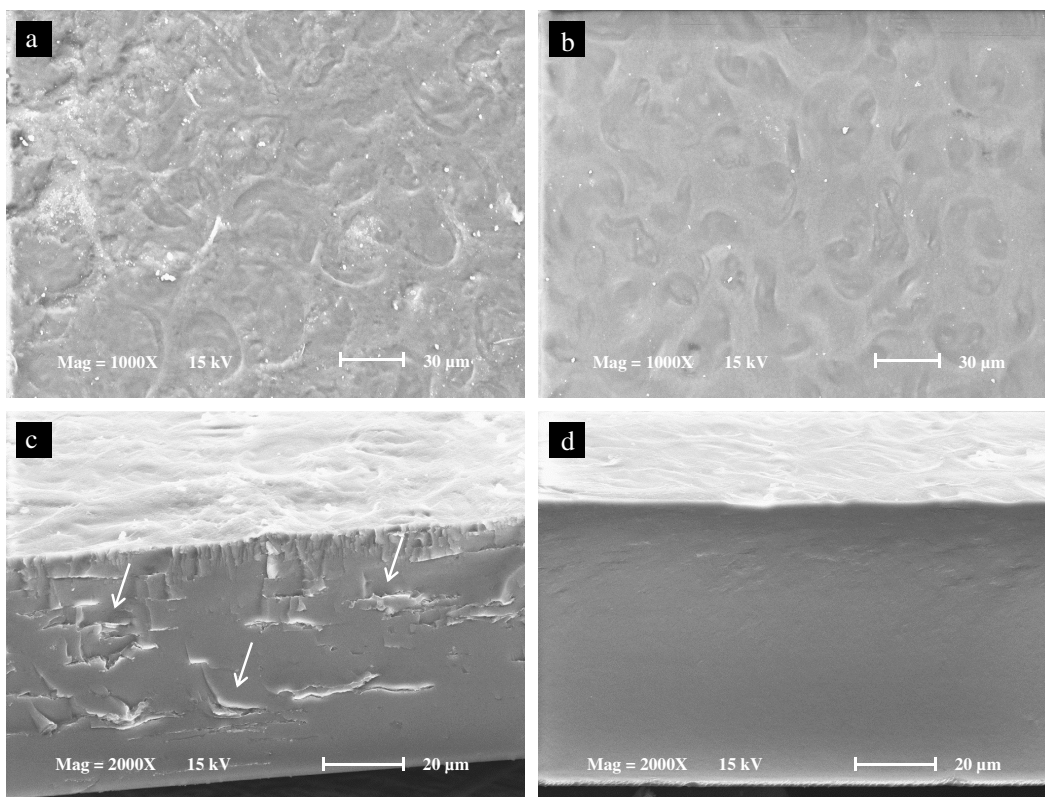


Figure 2. Scanning electron micrograph surfaces and cross-sections of the banana flour (a – c) and starch (b – d) films.

In terms of the cross-section, the flour film looks more like a laminated material and exhibits a less dense and less homogeneous polymeric structure with small cracks (see arrows in Figure 2c), as compared to the starch film (Figure 2d). The greater roughness of the flour film may explain the lower gloss values and the higher opacity obtained for this film (Table 2). Despite these differences, the plantain banana films generally have a homogeneous matrix, which is a good indication of their structural integrity, and thus good mechanical properties, as corroborated by the results.

Table 2. Properties of the banana flour and starch films.

Property	Flour film	Starch film
Thickness (μm)	87 ± 3^a	86 ± 4^a
Density (g/cm^3)	1.18 ± 0.04^a	1.34 ± 0.02^b
Moisture content (%)	15.1 ± 0.3^a	12.2 ± 0.2^b
<i>Mechanical properties</i>		
Puncture force (N)	6.0 ± 0.1^a	8.1 ± 0.3^b
Puncture deformation (%)	6.8 ± 0.4^a	1.3 ± 0.1^b
Tensile strength (MPa)	9.2 ± 0.2^a	19.3 ± 1.9^b
Elongation at break (%)	24.2 ± 1.9^a	4.5 ± 0.7^b
Young's modulus (MPa)	583.4 ± 46.4^a	2268.7 ± 96.8^b
<i>Barrier properties</i>		
Solubility in water (%)	27.9 ± 1.3^a	21.3 ± 1.7^b
WVP ($10^{-10} \text{ g/m.s.Pa}$)	2.1 ± 0.2^a	2.0 ± 0.2^a
<i>Optical properties</i>		
L*	94.50 ± 0.05^a	95.59 ± 0.03^b
a*	-0.08 ± 0.01^a	-0.01 ± 0.01^b
b*	2.20 ± 0.05^a	0.76 ± 0.01^b
ΔE^*	2.65 ± 0.07^a	0.89 ± 0.02^b
Opacity (%)	51.3 ± 0.3^a	29.5 ± 0.5^b

^{a,b} Different letter superscripts in the same line indicate a statistically significant difference ($p < 0.05$).

3.1.2. Thickness and density

The similar thickness values (Table 2) achieved for the plantain banana films are the result of a strict control of the dry mass content per unit area of plate of the film-forming suspensions employed during the casting procedure. The difference in terms of the density values obtained for the films were statistically significant, with the starch film presenting higher density than the flour film ($p < 0.05$; Table 2). The type of polymeric structure that results from the composition, molecular weight, and interactions between the components present in the films accounts for this behavior. The

structure of the flour film was more open, more porous, and less dense than the structure of the starch film, as depicted in Figure 2. Compared with other biodegradable films, the density values obtained for the plantain banana films were lower than that found by Müller et al. (2008) for cassava starch films plasticized with 25 g glycerol/100 g starch ($1.98 \pm 0.31 \text{ g/cm}^3$), but higher than those reported by Moore et al. (2006) for keratin films plasticized with different glycerol concentrations (0.92 to 1.10 g/cm^3). Famá et al. (2009) have reported density values around 1.38 g/cm^3 for cassava starch films and cassava starch-wheat bran composites, similar to the ones found in this work.

3.1.3. Moisture content

The moisture content of the banana flour film was higher than that of the banana starch film ($p < 0.05$; Table 2). Although starch is present in the flour film at a smaller proportion as compared to the starch film, the largest proportion of other also hydrophilic components (protein and fiber) in the flour, as calculated in Table 1, may give rise to a greater number of interactions with water molecules. These other components in the flour also induce the formation of a more porous structure, which improves water retention by capillarity.

3.1.4. Mechanical properties

All the differences between the flour and starch films in terms of mechanical properties were statistically significant ($p < 0.05$; Table 2). The banana flour film had lower values of puncture force, tensile strength, and Young's modulus as compared to the banana starch film. This indicates that the flour film was more flexible; *i.e.*, higher elongation at break and puncture deformation, and that the starch film was more resistant and rigid, as can be verified in the typical curves of mechanical properties shown in Figure 3. These results confirm that the protein and lipids present in the flour films could collaborate with the plasticizing effect, and that the protein did not contribute to the formation of a stronger network in this case. Shellhammer and Krochta (1997) and Batista et al. (2005) have reported that lipid-protein interactions improved film flexibility. The moisture content also accounts for these different properties. In fact, the larger moisture content in the flour films exerts an important and well-known plasticizer effect, which reduces the mechanical resistance and increases the flexibility

of biopolymer films (DELVILLE et al., 2002; GODBILLOT et al., 2006; SOBRAL et al., 2001). Similar behaviors have been detected by other authors in studies on the mechanical characteristics of flour and starch films elaborated from the same botanical source, namely achira (ANDRADE-MAHECHA, 2009), amaranth (TAPIA-BLÁCIDO, 2006), and quinoa (ARAUJO-FARRO, 2008) (Table 3). Meanwhile, Dias et al. (2010) have found no significant differences in the tensile properties of rice flour and starch films plasticized with glycerol. A similar behavior was noted only when sorbitol was used as plasticizer.

Young's modulus, which is an indicator of film rigidity, of the banana flour and starch films prepared under the optimal formulation and process conditions were calculated as being 583.4 and 2268.7 MPa, respectively. Again, the existence of higher protein, lipids, and fiber contents in the flour significantly influenced film rigidity ($p < 0.05$; Table 2).

Compared to other biodegradable films (Table 3), the plantain banana films exhibited excellent mechanical resistance, with higher Young's modulus than those found for achira, amaranth, quinoa, and rice films. This behavior could be explained by the differences in the amylose content of the films, since it is known that films rich in amylose exhibit good mechanical strength but little flexibility (LOURDIN et al., 1995). Water evaporates during the drying of film-forming suspensions, thereby allowing for the formation of a starch network. In this stage, the proximity of starch chains induced by higher amylose contents could facilitate the formation of a matrix with more polymer content per area (ALVES et al., 2007). According to Rindlav-Westling et al. (1998), the amylose network structure is very stable, with strong molecular orientation, which culminates in films that are denser than amylopectin films. Indeed, the banana starch displayed higher amylose content than the amaranth, quinoa and rice starches, and similar to the achira starch. Amaranth and quinoa starches are waxy and their amylose content is much lower, which can be the reason for the poor mechanical resistance of the resulting films. Despite the considerable amylose content in achira flour, the presence of thick fibers in its composition hinders the formation of a denser structure in the films produced from this raw material (ANDRADE-MAHECHA, 2009; ARAUJO-FARRO, 2008; TAPIA-BLÁCIDO, 2006).

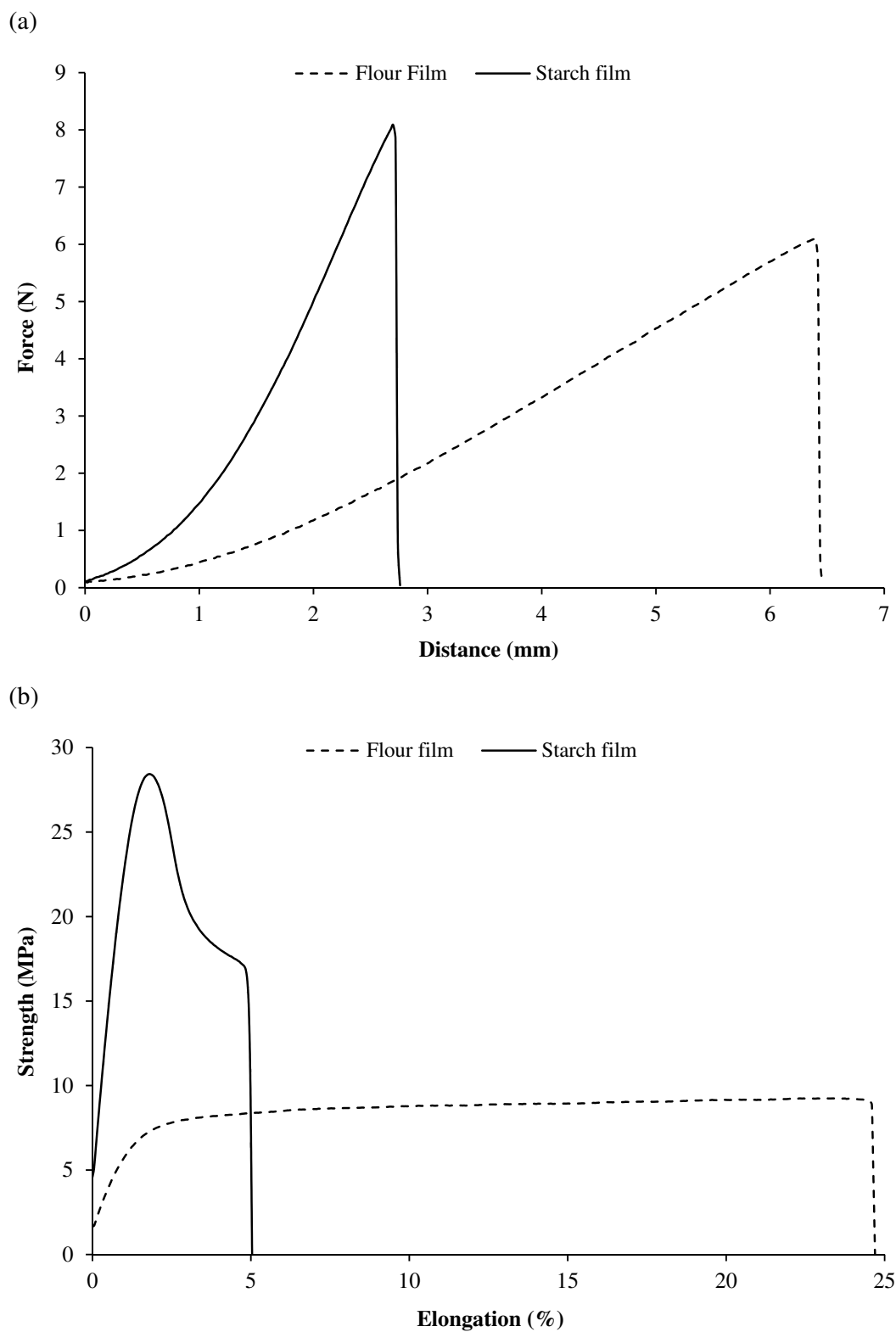


Figure 3. Typical curves for the puncture (a) and tensile (b) tests of the banana flour and starch films.

Table 3. Properties of the films prepared with different matrices.

Matrix/Plasticizer ^a	Thickness (μm)	Puncture force (N)	Puncture deformation (%)	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)	Solubility in water (%)	WVP (10^{-10}g/m.s.Pa)
Achira flour / 17% glycerol	84 ± 2	5.8 ± 0.3	7.8 ± 0.4	7.0 ± 0.3	14.6 ± 1.1	231.7 ± 19.9	38.3 ± 1.9	5.3 ± 0.2
Achira starch / 17% glycerol	83 ± 2	8.0 ± 0.7	1.8 ± 0.4	18.6 ± 0.3	1.4 ± 0.1	1772.0 ± 10.1	28.4 ± 1.7	3.2 ± 0.1
Amaranth flour / 22.5% glycerol	83 ± 5	–	–	1.5 ± 0.1	83.7 ± 5.1	21.5 ± 1.4	42.3 ± 1.8	0.7 ± 0.2
Amaranth starch / 22.5% glycerol	80 ± 3	–	–	3.1 ± 0.5	18.9 ± 7.0	276.9 ± 6.3	62.5 ± 2.5	2.7 ± 0.5
Quinoa flour / 21% glycerol	80 ± 2	7.0 ± 1.5	2.2 ± 0.2	4.1 ± 0.5	88.4 ± 8.9	138.0 ± 40.0	18.7 ± 0.1	0.6 ± 0.1
Quinoa starch / 21.2% glycerol	80 ± 2	7.1 ± 0.4	1.0 ± 0.2	7.6 ± 1.0	58.1 ± 3.2	459.0 ± 106.0	15.9 ± 0.1	0.6 ± 0.1
Rice flour / 20% glycerol	100 ± 40	–	–	10.3 ± 1.0	2.7 ± 0.5	560.7 ± 64.3	–	1.1 ± 0.1
Rice starch / 20% glycerol	110 ± 40	–	–	10.9 ± 1.2	2.8 ± 0.7	532.8 ± 115.6	–	0.5 ± 0.1
Banana flour / 19% glycerol	87 ± 3	6.0 ± 0.1	6.8 ± 0.4	9.2 ± 0.2	24.2 ± 1.9	583.4 ± 46.4	27.9 ± 1.3	2.1 ± 0.2
Banana starch / 19% glycerol	86 ± 4	8.1 ± 0.3	1.3 ± 0.1	19.3 ± 1.9	4.5 ± 0.7	2268.7 ± 96.8	21.3 ± 1.7	2.0 ± 0.2

^a References: ANDRADE-MAHECHA, 2009 (achira, *Canna indica* L.), TAPIA-BLÁCIDO, 2006 (amaranth, *Amaranthus caudatus*), ARAUJO-FARRO, 2008 (quinoa, *Chenopodium quinoa* Willdenow), DIAS et al., 2010 (rice, *Oryza sativa*), this work (banana, *Musa paradisiaca*).

3.1.5. Solubility in water

The solubility in water of the banana flour film (27.9%) was higher ($p < 0.05$) than that of the banana starch film (21.3%). This result agrees with the type of microstructure observed by SEM analysis (Figure 2), which revealed a more open and porous structure for the flour film as compared to the starch film. Moreover, more amylose-amylose, amylopectin-amylopectin, and amylose-amylopectin interactions take place during drying of the starch film, which reduces the amount of hydrophilic groups available for interaction with water.

Several authors have compared the solubility of flour and starch films from other botanical sources such as achira, amaranth, and quinoa. They have observed that this property is linked to the type of raw material used for formation of the polymer matrix, the kind of interactions that occur in the matrix, the use of plasticizers, and the process conditions, among other factors (ANDRADE-MAHECHA, 2009; ARAUJO-FARRO, 2008; TAPIA-BLÁCIDO, 2006). As can be seen in Table 3 for the same raw material (starch or flour), plantain banana films were less water-soluble than the achira and amaranth films, but they were more soluble than the quinoa films.

The desired value for the solubility of a film will depend on its application or intended use. Hence, according to the results, biodegradable films can act as food protection when the water activity is high, or they can avoid exudation of fresh or frozen products when the film must be in contact with water during the processing of the coated food (GONTARD et al., 1992). Therefore, the study of formulation and process conditions during the development of films is important, so that the effects of different conditions on the final properties of the films can be better understood and thus maximized or minimized as necessary.

3.1.6. Water vapor permeability (WVP)

The water vapor permeability (WVP) values obtained for the banana flour and starch films were similar, around 2.0×10^{-10} g/m.s.Pa ($p < 0.05$; Table 2), in spite of the net difference in their microstructures (Figure 2) and the higher water retention by the flour film. Therefore, the larger amount of protein and fiber present in the flour matrix responsible for the increase in moisture content was not enough to cause greater water vapor diffusion through the film. This result is probably the effect of the strong

interaction of water with the components of the flour matrix, or a consequence of the greater tortuosity of the structure of this film, which hinders water diffusion.

The plantain banana films had good WVP; these values were lower than those of the amaranth starch and achira films, but higher than those of the amaranth flour and the quinoa and rice films, as can be observed in Table 3.

3.1.7. Water sorption isotherms

Experimental data for the water sorption isotherms of the banana plantain films are given in Figure 4, together with the GAB model fitted for each film. The GAB model parameters and coefficients of determination are summarized in Table 4. The values of the coefficient of determination ($R^2 > 0.99$) evidenced that the GAB model was adequate for the fitting of the experimental data, as previously reported by other authors (DIAS et al., 2010; GODBILLOT et al., 2006; MALI et al., 2002; MALI et al., 2005; MÜLLER et al., 2008). Both isotherms displayed a sigmoidal shape (Figure 4), which is typical of starchy materials. This shows a slower increase in the equilibrium moisture content until a_w 0.6; thereafter, a steep rise in the moisture content, which is associated with enhanced solubilization, can be detected. A closer look at all the water activities shows that the banana flour film was more hygroscopic than the banana starch film. Therefore, the presence of higher proportions of other components with hydrophilic nature (protein and fiber) in the flour film exerts an important positive effect on the equilibrium moisture content. This result is consistent with those found in terms of moisture content and solubility. Indeed, the banana flour film had greater moisture and solubility as compared to the banana starch film. However, the biopolymer matrix did not seem to be affected by the monolayer value (m_0) or the parameter k of the GAB model (Table 4). This behavior has also been reported by other authors (COUPLAND et al., 2000; DIAS et al., 2010; MALI et al., 2005; MÜLLER et al., 2008). The parameter C varied significantly between the banana films and is related to the enthalpy energy of water sorption at the monolayer. Based on these values, the interactions between water and the components of the flour matrix are stronger as compared to the pure starch matrix, in agreement with the results of water vapor permeability.

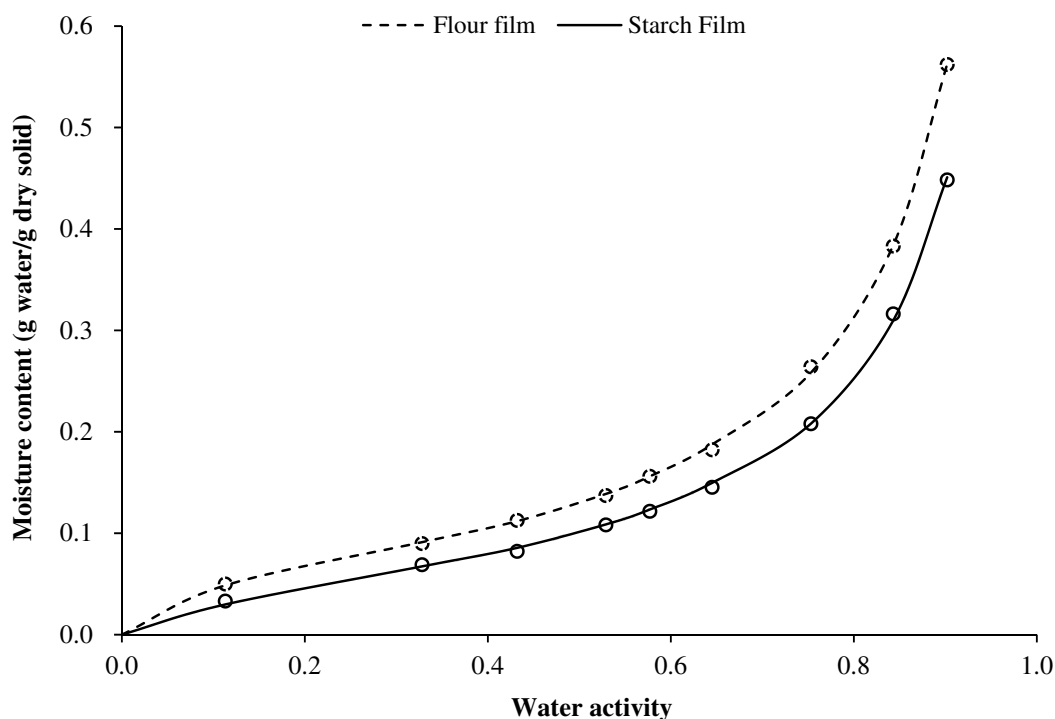


Figure 4. Water sorption isotherms of the banana flour and starch films fitted with the GAB model (symbols are the experimental data and lines are the GAB fitted curves).

Table 4. GAB model parameters for the water sorption isotherms of the banana flour and starch films.

Parameter ^a	Flour film	Starch film
m_0 (g water/g dry solid)	0.07 ± 0.00	0.06 ± 0.00
k	0.97 ± 0.00	0.96 ± 0.00
C	12.44 ± 2.02	6.54 ± 1.10

^a m_0 : monolayer value, C : Guggenheim constant related to the monolayer heat sorption, k : constant related to the multilayer heat sorption.

3.1.8. Optical properties

Based on the L^* , b^* , and ΔE^* values (Table 2), the banana flour film was darker and more yellowish and had less uniform color than the banana starch film (Figure 5). These results reflect the characteristic color of their raw materials. The values of the parameter a^* were negative and close to zero for both films, indicating the absence of characteristic tones of red color.

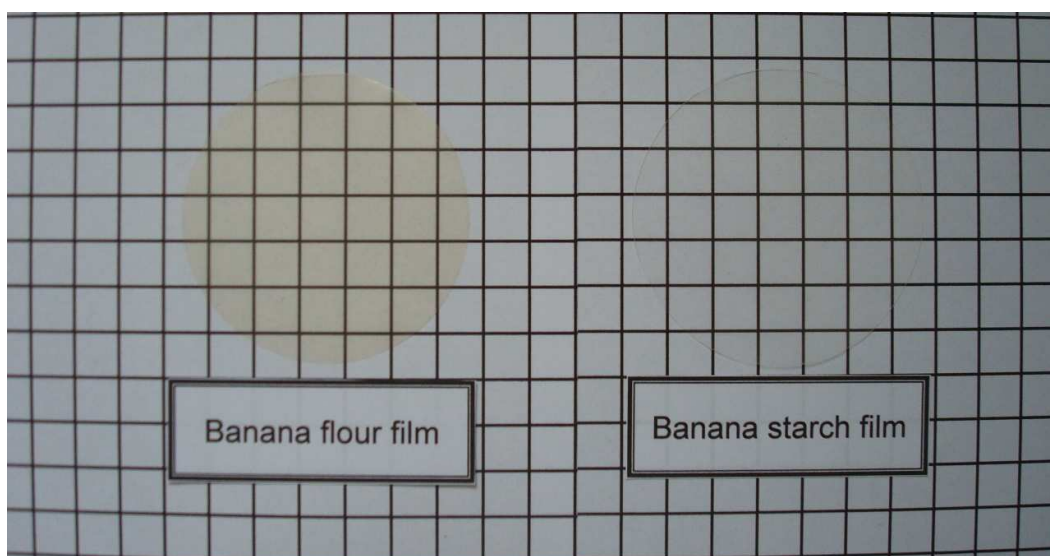


Figure 5. Plantain banana films.

Results on the color of the flour and starch films of the same species obtained by other authors have revealed the tendency toward yellowish among the materials made from flour as well as the higher luminosity of the materials obtained from starch (ANDRADE-MAHECHA, 2009; ARAUJO-FARRO, 2008; TAPIA-BLÁCIDO, 2006). Tapia-Blácido et al. (2007) have compared the optical properties of flour and protein-based films from two amaranth species and found that the yellow color is related to the presence of proteins.

Regarding the opacity of the investigated materials, the banana flour film was more opaque than the banana starch film (Table 2). This property is related to the composition of the raw materials. In this case, the higher levels of protein, lipids, and fiber (Table 1) might contribute to the greater opacity of the flour film. Several authors have associated the presence of protein and lipids with increasing opacity in films (BATISTA et al., 2005; GONTARD et al., 1994; TAPIA-BLÁCIDO et al., 2007). In general, the plantain banana films were more opaque than the achira films (18.0% for flour and 14.7% for starch) (ANDRADE-MAHECHA, 2009), the amaranth films from the species *Amaranthus caudatus* (15.24% for flour and 12.31% for starch) (TAPIA-BLÁCIDO, 2006), and the quinoa films (5.32% for flour and 1.52% for starch) (ARAUJO-FARRO, 2008), which indicates that these films can be used when protection against incident light is necessary, especially for products that are sensitive to degradation reactions catalyzed by light. According to Fakhouri et al. (2007), the

opacity of films can vary depending on the content of amylose, whose molecules of linear nature tend to favor tight hydrogen bonds between the hydroxyl groups of adjacent chains. This phenomenon diminishes the biopolymer-water interactions, promoting the formation of an opaque polymer matrix. However, other factors such as the presence of lipids in the employed raw material may contribute to film opacity. Hence, the opacity values achieved for the films examined in this paper could be explained in terms of the composition of the banana flour and the presence of phenolic compounds, but it will also depend on the method used for measurement of the optical properties.

Banana flour film presented lower gloss values, which were more pronounced for the surface exposed to air drying (E), as compared to the banana starch film (Figure 6). The gloss is a property that is strictly related to surface texture; *i.e.*, to the polishing degree of the analyzed surface (MORAES et al., 2008), and it is influenced by the particle size distribution employed in the film formulation. Thus, a more uniform particle size distribution yields more polished, and consequently brighter surfaces (TREZZA and KROCHTA, 2001). Such consideration may account for the gloss results obtained for the plantain banana films evaluated in this study, if one bears in mind that the flour has a larger amount of components of different sizes, as compared to the starch. This affords rougher films, as observed during the surface microstructural analysis (Figure 2).

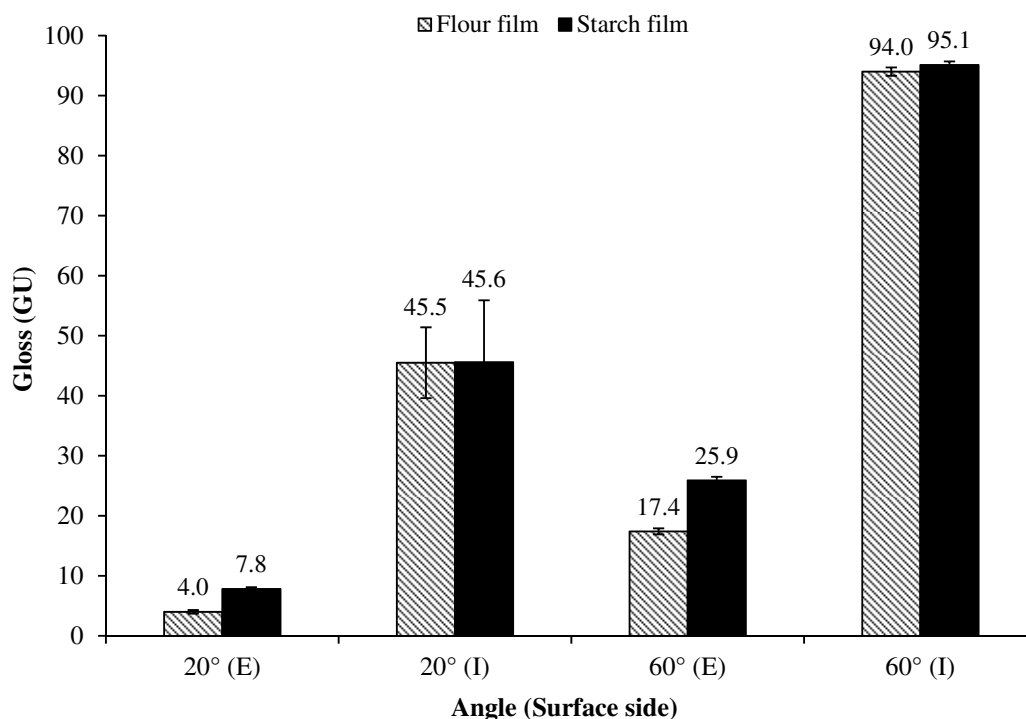


Figure 6. Gloss values for the banana flour and starch films, where E is the surface side exposed to air drying and I is the surface side in contact with the support plate.

3.1.9. X-ray diffraction (XRD)

The X-ray patterns of plantains banana films evidenced amorphous areas and a mixture of the A- and B-type polymorphs, which can be referred to as C-type, characteristic of certain roots and legumes starches (Figure 7). Some of the detected peaks, such as the typical A-type pattern peak at $2\theta = 12^\circ$ and the peak at $2\theta = 5.6^\circ$ only present in B-type starches, suggest that these films include a mixture of the A- and B-type polymorphs. The films also revealed the presence of a V_H -type crystal, as indicated by the peaks at $2\theta = 12^\circ$, 19° , and 22° . The V_H is a processing-induced crystallinity caused by the rapid recrystallization of single-helical amylose structures during cooling after processing (retrogradation) (VAN SOEST and VLIEGENTHART, 1997). According to Van Soest et al. (1996), the V_H structure is formed below 180°C and is frequently found in starch-based films made with more than 10% water.

The crystallinity of starch-based films is influenced by the amylose/amylopectin ratio, the degree of branching and the length of the amylopectin outer chains, the starch source, the conditions of the drying process (rate and temperature), and the final

moisture content of the samples (VAN SOEST et al., 1996; VAN SOEST and VLIEGENTHART, 1997). According to Figure 7, the banana flour film presented slightly larger peak widths and lower peak intensities, which shows a tendency toward less crystalline structures. This can be attributed to the presence of other components in the flour; *e.g.*, protein, lipids, and fiber, which inhibit amylose retrogradation. This behavior is evidenced by the greater crystallinity index of the banana starch film (11.6%) as compared to the flour film (5.2%).

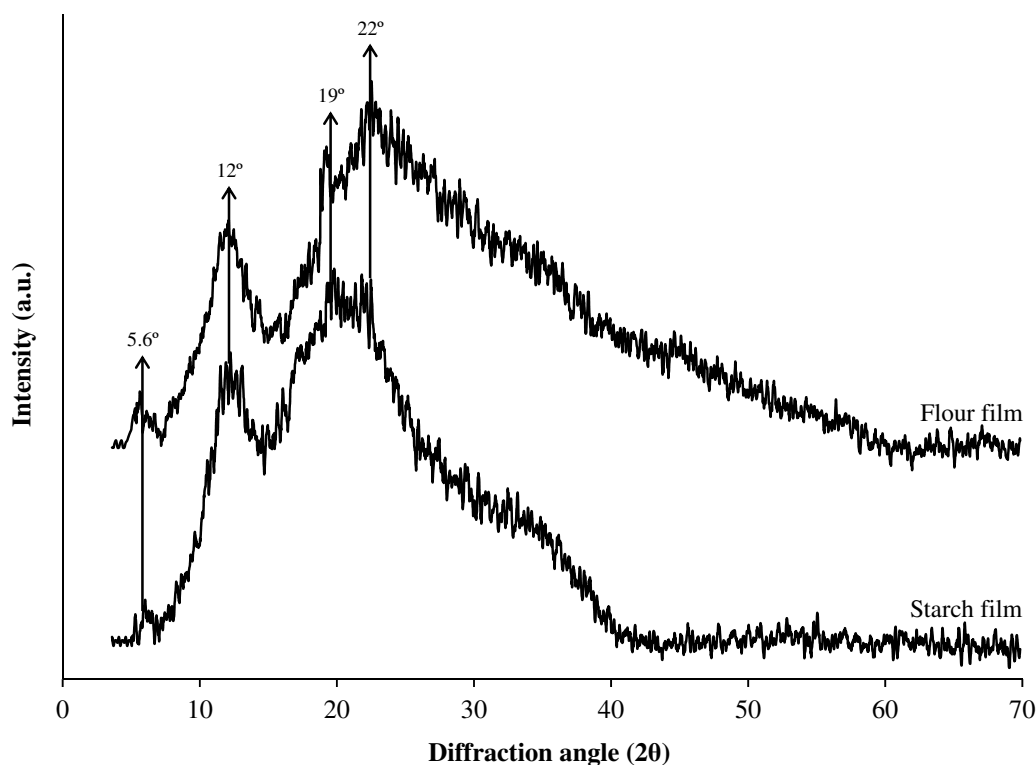


Figure 7. X-ray diffraction patterns of the banana flour and starch films.

3.1.10. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra were recorded for the banana flour and starch films, in order to compare the types of interactions taking place in the film structures. The spectra are illustrated in Figure 8.

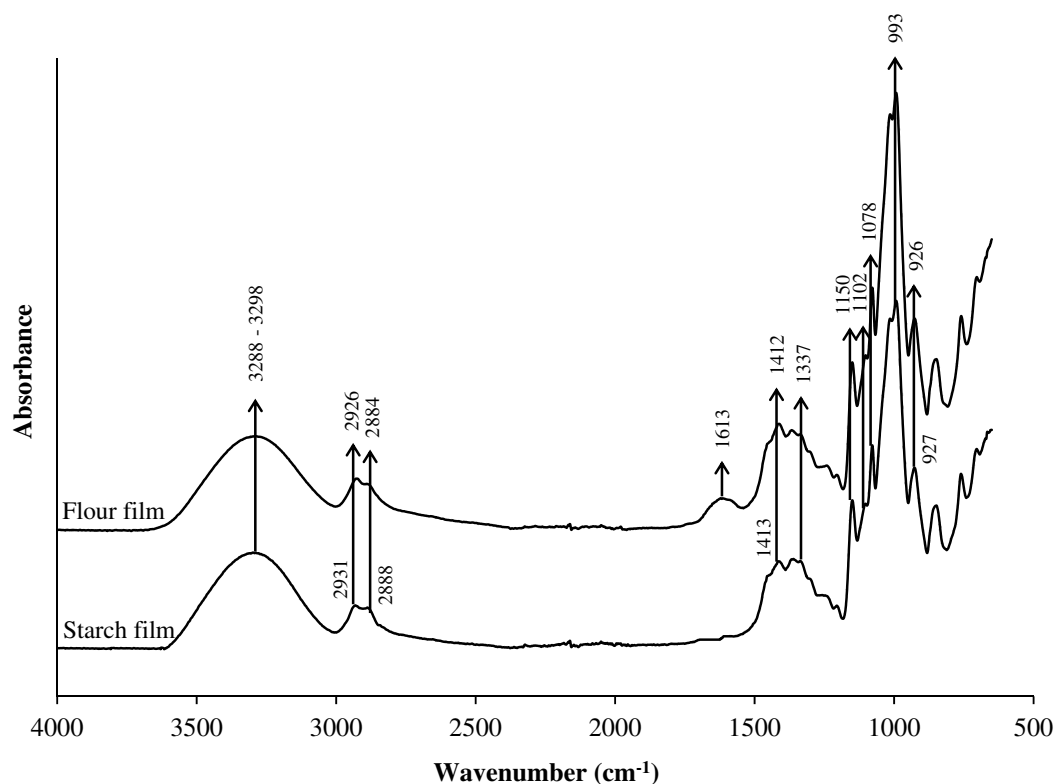


Figure 8. FTIR spectra of the banana flour and starch films.

Based on the fact that the free hydroxyl groups absorb energy between $3650 - 3584 \text{ cm}^{-1}$, the bands observed in the region $3288 - 3298 \text{ cm}^{-1}$ in the spectra of the films can be assigned to the stretching of the -OH groups, caused by formation of hydrogen bonds in the studied matrices (SILVERSTEIN et al., 2007). The values of the second and third bands found for the flour (2926 and 2884 cm^{-1}) and starch films (2931 and 2888 cm^{-1}) are similar and indicate the existence of CH_2 groups. Kizil et al. (2002) have mentioned that the different intensities of the bands located between $2800 - 3000 \text{ cm}^{-1}$ can be attributed to variation in the amounts of amylose and amylopectin. According to our observations, the bands detected in the spectrum of the banana flour film were less intense, as compared to the banana starch film, which may be associated with the lower amylose content in the former film.

The band at 1613 cm^{-1} appeared in the spectrum of the flour film only. This band corresponds to amide I and is related to the amide group of proteins, particularly to the C=O stretching. Andrade-Mahecha (2009) have also identified a band at 1633 cm^{-1} for achira flour films, while Tapia-Blácido (2006) have found a band at 1631 and 1634 cm^{-1} for protein-based films of the species *Amaranthus caudatus* and *Amaranthus cruentus*,

respectively. Both plantain banana films displayed a band at 1337 cm^{-1} , which corresponds to the band relative to amide III and is normally located between 1200 and 1350 cm^{-1} (SINGH, 2000). The presence of this band in the starch film is probably due to protein traces that remained in the raw material.

The band at 1412 and 1413 cm^{-1} detected in the spectra of the flour and starch films, respectively, is associated with the symmetric stretching of the carboxyl group ($-\text{COO}$) (KIZIL et al.; 2002). According to Van Soest et al. (1995) and Huang et al. (2006), the region relative to the stretching of $\text{C}-\text{C}$, $\text{C}-\text{O}$, and $\text{C}-\text{O}-\text{H}$ bonds of starch can be analyzed through the absorption bands lying between 800 and 1300 cm^{-1} . For both films, these bands can be noted at 1078 , 1102 , and 1150 cm^{-1} . Other authors have also described these bands during the FTIR analysis of the quinoa flour and starch films (ARAUJO-FARRO, 2008) as well as modified starches (HUANG et al., 2006).

The band located at 993 cm^{-1} in the spectra of the banana flour and starch films is related to the amount of amorphous structure and thus to the content of water interacting with intramolecular hydrogen bonds, thereby revealing differences in crystallinity between these two films (VAN SOEST et al., 1995).

The bands at 926 and 927 cm^{-1} observed for the banana flour and starch films, respectively, are attributed to the glycosidic bonds of starch. Small differences in the location and intensity of this band may be due to the presence of amylopectin $\alpha - 1,6$ bonds, as described by Kizil et al. (2002). The absorptions between 703 and 762 cm^{-1} indicate the presence of aromatic structures (GUILARDUCI et al., 2006; LOPES and FASCIO, 2004). The existence of this group could be associated with the presence of phenolic compounds in the bananas from which the flour and starch employed in the preparation of the investigated films were obtained. In addition, the phenolic compounds have a plasticizing effect, thus making the films more flexible. On the other hand, the films have less mechanical resistance and weaker water barrier properties (ARCAN and YEMENICIOĞLU, 2011).

3.1.11. Differential scanning calorimetry (DSC)

DSC analysis provides knowledge about the possible interactions occurring in the target matrix, thereby furnishing information that allows for analysis of features such as stability and applicability of the biopolymer. Figure 9 depicts the thermograms of the plantain banana films.

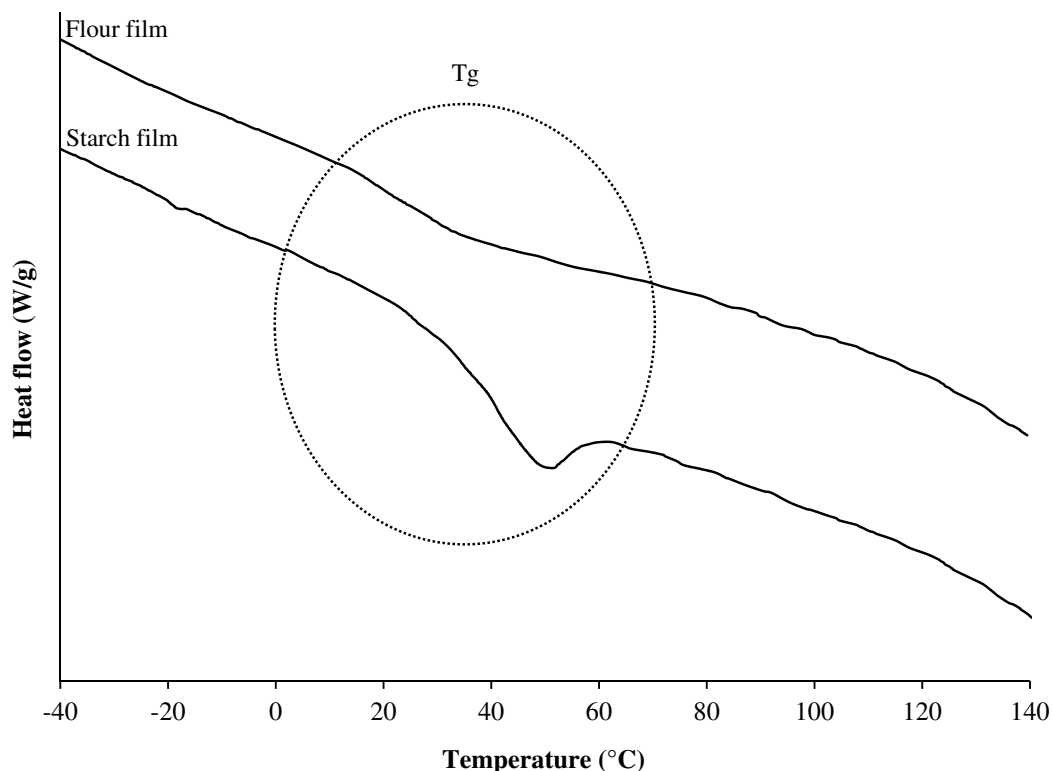


Figure 9. DSC thermograms of the banana flour and starch films.

The films exhibited only one glass transition temperature (T_g) (Figure 9). The attainment of a single value of T_g is characteristic of polymer matrices without phase separation and indicates the homogeneous mixture of the compounds present in the material (BILIADERIS et al., 1999). The starch film displayed higher T_g (46.4 °C) than the flour film (30.2 °C). This is probably due to the plasticizing effect of water, considering that the flour films have higher moisture content as compared to the starch films (Table 2). According to Mali et al. (2002), plasticization weakens the intermolecular forces between polymer chains and consequently diminishes the overall cohesion, thus reducing T_g .

The T_g values imply that both films still are in the glassy state in all the characterization tests performed herein (25 °C). The water present in the investigated films is nonfreezing in nature, as indicated by the absence of an endothermic peak associated with the melting of ice in the DSC thermograms (CHANG et al., 2006).

4. Conclusions

The flour and starch isolated from plantain bananas (cultivar “Terra”) seem to be very promising materials for the formulation of coatings and films. However, the flour is a much more interesting raw material because it can be cheaper to obtain than commercial starches. This is due to the fact that flour production process is simpler and more efficient. The banana flour film prepared here was less mechanically resistant but more flexible than the banana starch film. Both films had low solubility in water and similar water vapor permeability. The flour film presented a discontinuous microstructure that was rougher and more porous, as compared to the starch film, which was more compact. The FTIR spectra revealed the presence of OH, CH₂, and amide III groups in both films, while the amide I group was observed only for the flour film. Both plantain banana films displayed a C-type X-ray pattern and one T_g each. The presence of other components in the banana flour (protein, lipids, and fiber) influenced the properties of the resulting film.

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Capítulo 5

Isolation and Characterization of Cellulose Nanofibers from Banana Peels

Abstract

Cellulose nanofibers were isolated using a combination of chemical treatments, such as alkaline treatment, bleaching, and acid hydrolysis. The suspensions of chemically treated fibers were then passed through a high-pressure homogenizer 3, 5, and 7 times, to investigate the effect of the number of passages on the properties of the resulting cellulose nanofibers. The transmission electron microscopy (TEM) showed that all treatments effectively isolated banana fibers in the nanometer scale. Increasing number of passages in the homogenizer furnished more stable aqueous suspensions of the cellulose product while shortening the cellulose nanofibers. X-ray diffraction studies revealed that homogenized nanofiber suspensions were more crystalline than non-homogenized suspensions. Fourier transform infrared spectroscopy (FTIR) confirmed that alkaline treatment and bleaching removed most of the hemicellulose and lignin components present in the banana fibers. Differential scanning calorimetry (DSC) revealed that the developed nanofibers exhibit enhanced thermal properties. The higher thermal stability of the prepared samples stems from the higher crystallinity of the cellulose obtained after removing hemicellulose and lignin components from the fiber. In general, the nanoparticles isolated from the banana peel have potential application as reinforcing elements in a variety of polymer composite systems.

Keywords: banana peel, cellulose nanofibers, high-pressure homogenizer, morphology, crystallinity studies.

1. Introduction

Growing concern about the environment, rapid depletion of petroleum resources, and new environmental regulations have prompted the search for new environmentally compatible materials obtained from natural resources, including cellulose. Cellulose nanofibers are abundant in plants and wood, and their composites comprise an attractive contemporary research line. Plant-based cellulose nanofibers are important sources of nanometric fillers. Compared with commercial fibers, they are sustainable and abundant, and they display large surface to volume ratio, high tensile strength, stiffness, and flexibility, and good dynamic mechanical, electrical, and thermal properties (Abe et al., 2007; Fahmy and Mobarak, 2008; Kvien et al., 2005; Roohani et al., 2008; Siqueira et al., 2009). The use of nanoreinforcements in the polymer matrix has been shown to give improved properties compared to the neat polymer and micron (sized, filled) composites based on the same fibers (Lee et al., 2009). Therefore, it is of great interest to examine the possibilities of using cellulose-based nanofibers as reinforcing elements.

Cellulose nanofibers can be extracted by different approaches, all of which lead to different types of nanofibrillar materials, depending on the cellulose raw material, pre-treatment, and disintegration (Chen et al., 2011; Dong et al., 1998; Rosa et al., 2010). Mechanical treatments such as high-pressure homogenization generate homogeneous nanofiber suspensions via the breakdown and deagglomeration of biopolymer particles by simultaneous shear and pressure (Bouaouina et al., 2006; Kang and Min, 2010; Thiebaud et al., 2003). The key element to manufacture a good biodegradable film might be reducing particle size, to obtain more uniform particle distribution.

Originating in Southeast Asia, bananas are an important food crop that is extensively grown in tropical and subtropical regions. Banana peel waste is a byproduct of banana processing during the production of food such as banana chips and baby foods. The edible part of banana constitutes only 12 wt% of the plant; the remaining parts become agricultural waste and cause environmental problems (Elanthikkal et al., 2010). Since the banana peel is rich in cellulose, this material is potentially applicable as reinforcing component in high-performance composites, increasing its commercial value and providing a purpose for this byproduct.

Researchers have isolated cellulose nanofibers from various sources such as sugar beet (Dufresne et al., 1997), potato pulps (Dufresne et al., 2000), wheat straw

(Kaushik and Singh, 2011), sugarcane bagasse (Teixeira et al., 2011), and coconut husk fibers (Rosa et al., 2010). Even banana agricultural residues such as rachis (Zuluaga et al., 2007; 2009) and plant wastes (Cherian et al., 2008; Deepa et al., 2011; Elanthikkal et al., 2010) can be used to produce nanoreinforcements. However, the isolation of cellulose nanofibers from the banana peel has not been reported.

To investigate the potential use of cellulose nanofibers isolated from the banana peel bran as reinforcement in composites, this study examined the effect of mechanical treatment on the structure of the nanofibers using a high-pressure homogenizer. Both bran and cellulose nanofibers were characterized by size distribution, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC). The morphological features of the bran and nanostructures were evaluated by scanning and transmission electron microscopy, SEM and TEM, respectively.

2. Materials and Methods

2.1. Materials

The bran was prepared from the peel of unripe plantain bananas (mature green) of the variety “Terra” (*Musa paradisiaca*). All the chemicals used in this work were reagent grade.

2.2. Bran preparation

Unripe bananas were washed and peeled; the peels were then immediately rinsed in potassium metabisulfite solution (1% w/v) for 24 h, to inhibit oxidation. The rinsed peels were placed on trays and dried in a forced air convection oven at 60 °C for 24 h. After drying, the material was ground in a knife mill (Marconi, model MA340, São Paulo, Brazil), sieved through a 200 US mesh screen (to eliminate the larger particles), and stored at 4 °C in a sealed container (Figure 1). The resulting banana peel bran had a dry basis yield of 56.5% and a moisture content of 5.2%.

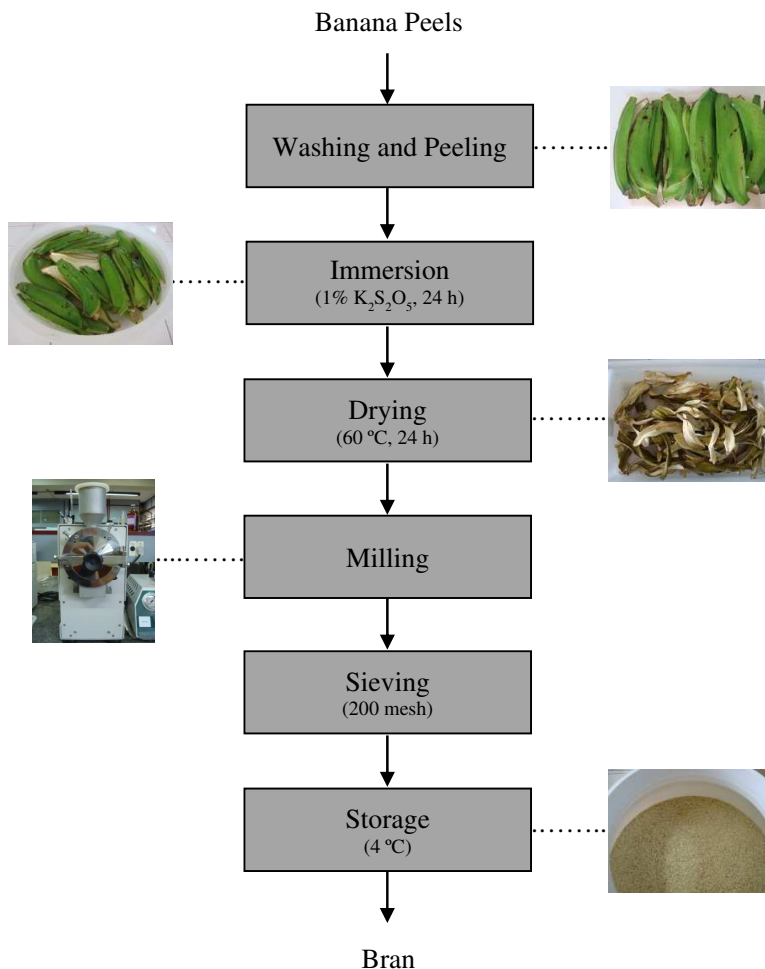


Figure 1. Scheme for preparation of the banana peel bran

2.3. Isolation of cellulose nanofibers

Cellulose nanofibers were isolated from the banana peel bran using the chemical treatment described by Zuluaga et al. (2009), with some modifications; this treatment eliminates non-cellulosic components such as pectins, hemicelluloses, and lignin. Firstly, the bran was treated with 5% w/v KOH solution (ratio of 1:20) under mechanical stirring at room temperature, for 14 h, which should solubilize the pectins and the hemicelluloses. The insoluble residue was delignified with 1% w/v $NaClO_2$ at pH 5.0, adjusted with 10% v/v acetic acid at 70 °C, for 1 h. The bleaching treatment broke down the phenolic compounds or molecules displaying chromophoric groups present in the lignin and removed the byproducts of such breakdown, whitening the pulp. The residue was submitted to the bleaching process using $NaClO_2$ again, to ensure

effective discoloration. A second alkaline treatment with KOH solution under the same conditions as in the first step was performed. Finally, the insoluble residue was subjected to acid hydrolysis with a 1% v/v H₂SO₄ solution at 80 °C, for 1 h, to remove mineral traces and to hydrolyze amorphous cellulose providing the required nanofibers. After each step of the chemical treatment, the insoluble residue was neutralized with alkaline or acid solution (5% KOH or 10% acetic acid, respectively), depending on the pH, extensively washed with deionized water, and centrifuged (10,000 rpm; 5 °C; 20 min), until neutral pH was reached. The final residue was diluted with deionized water, and the suspension was stored at 4 °C in a sealed container.

To tailor and reduce the size of the nanofibers, the latter were submitted to mechanical treatment in a two-stage high-pressure homogenizer (GEA Niro Soavi, model NS 1001L – Panda 2K, Parma, Italy). The pressure in the first and second stage was 500 and 50 bar, respectively. Aliquots of the suspension were passed through the homogenizer 3, 5, or 7 times, and the effect of the number of passages on the structure of the nanofibers was evaluated. Figure 2 summarizes the procedure used to prepare the nanofibers with and without homogenization.

For XRD, FTIR, and DSC analysis, 50 g of the nanofiber suspension obtained after each treatment was dried in a freeze-dryer (Equipamentos Terroni, model LS 3000, São Paulo, Brazil). The freeze-dried samples were stored at 4 °C in sealed containers.

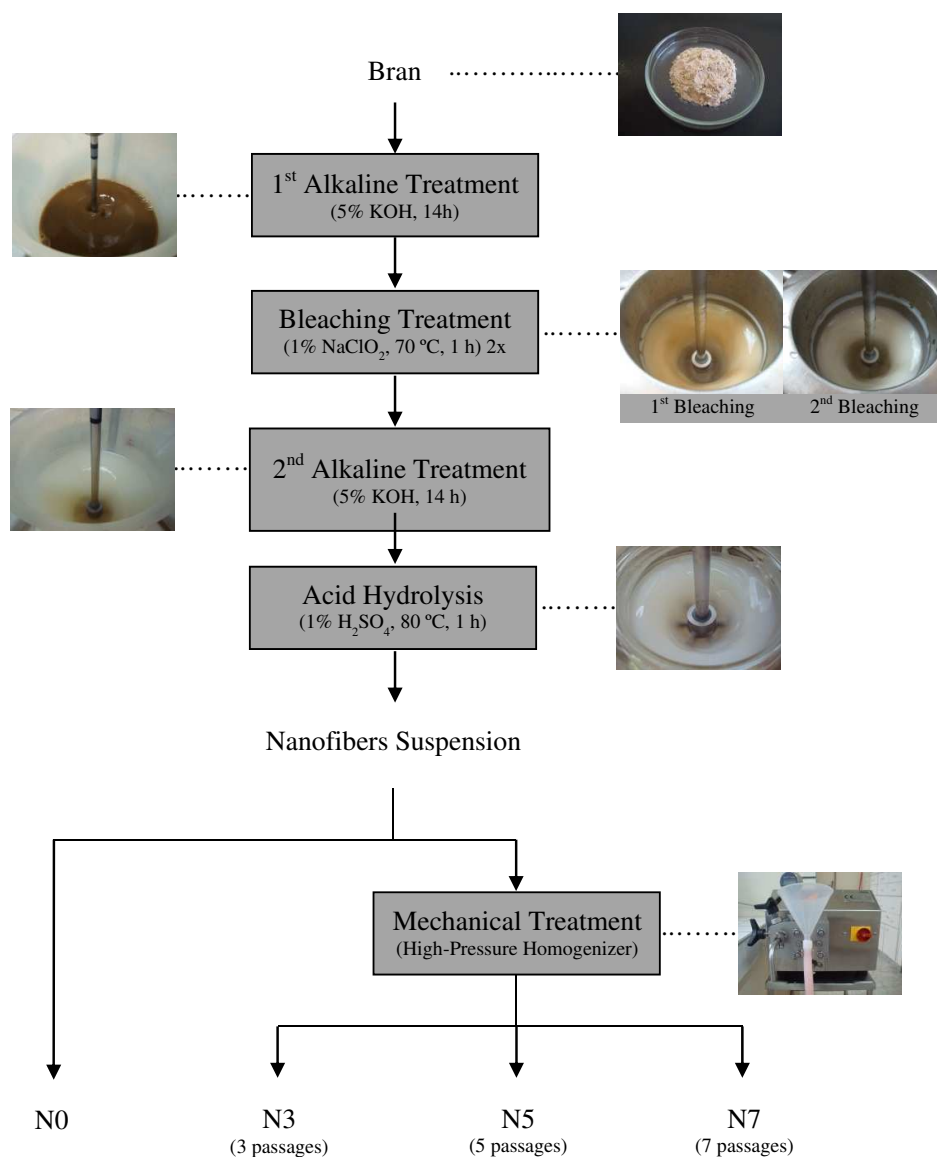


Figure 2. Scheme describing the procedure used to isolate cellulose nanofibers from the banana peel bran.

2.4. Characterization

2.4.1. Concentration of nanofiber suspensions

The concentration of nanofiber suspensions in the solid was performed in triplicate; 2 g of each suspension, previously homogenized by mechanical stirring for 30 min, were dried at 105 °C for 24 h.

2.4.2. Scanning electron microscopy (SEM)

For microstructural evaluation by SEM, the bran sample was fixed on an aluminum stub using double-sided tape and coated with a gold layer (Sputter Coater POLARON, model SCD050), to improve conductivity. The coated sample was visualized under a scanning electron microscope (JEOL, model JSM-5800LV, Tokyo, Japan) using an acceleration voltage of 10 kV.

2.4.3. Transmission electron microscopy (TEM)

The morphology and diameter of cellulose nanofibers were investigated by TEM. Nanofiber suspensions were diluted with deionized water and placed on the ultrasound for 5 min. A drop of each sample was deposited on a microgrid covered with a thin carbon film (200 nm) and allowed to dry at room temperature. The images were obtained using a transmission electron microscope (Zeiss, model CEM 902, Oberkochen, Germany) and an acceleration voltage of 80 kV.

2.4.4. Particle size distribution and zeta potential

The size of the bran particles was determined using a laser diffraction analyzer (Malvern Instruments Ltd., Mastersizer 2000 – model APA2000, Worcestershire, England) equipped with a dispersion unit (Hydro 2000S, model AWA2001), to improve the dispersibility of the sample; ethanol was used as solvent. Zeta potential and length size distribution of the cellulose nanofibers were measured with a light scattering analyzer for nanoparticles (Malvern Instruments Ltd., Zetasizer Nano Series – model Nano ZS, Worcestershire, England). All the experiments were performed in triplicate, and the results are presented as mean values.

2.4.5. X-ray diffraction (XRD)

X-ray diffraction analysis was performed using an X-ray diffractometer (Siemens, model D5005, Baden-Württemberg, Germany) operated at a voltage of 40 kV and a current of 30 mA; the target was Cu. The diffraction data of the samples were collected over an angular range from 2 to 50° (2 θ), with a scanning speed of 1.2° / min.

The crystallinity index (I_c) of the samples was quantitatively estimated from the diffraction intensity data using the method of Segal et al. (1959). The crystallinity index (%) was obtained by means of Equation 1:

$$I_c = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad [1]$$

where I_{002} is the diffraction intensity close to $2\theta = 22^\circ$ and represents a crystalline material; I_{am} is the diffraction intensity close to $2\theta = 18^\circ$ and represents amorphous material in cellulosic fibers.

2.4.6. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra were recorded on a spectrophotometer (PerkinElmer, model Spectrum One, Ohio, USA) fit with a Universal Attenuated Total Reflectance (UATR) device. Analysis was performed in the infrared region, with 16 scans in wavenumbers ranging from 4000 to 650 cm^{-1} and spectral resolution of 4 cm^{-1} .

2.4.7. Differential scanning calorimetry (DSC)

The thermal properties of the samples were determined using a differential scanning calorimeter (TA-Instruments, model 2920, Pennsylvania, USA) equipped with a cooling system. Bran and lyophilized nanofibers were weighed (5 – 6 mg) in aluminum pans and sealed hermetically. Samples were scanned at a heating rate of 10 $^\circ\text{C} / \text{min}$ over a temperature range of - 50 to 400 $^\circ\text{C}$. A sealed empty aluminum pan was used as reference for all the DSC runs. The degradation characteristics were obtained from the thermograms of the samples using the software Universal Analysis 3.9A. The DSC analyses were performed in triplicate, and the results are presented as mean values.

2.5. Statistical analysis

An analysis of variance (ANOVA) and a Tukey test of multiple comparisons with a significance level of 5% were run using the Statistica 7.0 software (StatSoft Inc,

Tulsa, Oklahoma, USA), to compare the differences between means for the properties of cellulose nanofibers treated with and without high-pressure homogenization.

3. Results and Discussion

3.1. Appearance and concentration of nanofiber suspensions

Figure 3 depicts the sequence of steps used to isolate the cellulose nanofibers from the banana peel. Along the chemical treatment steps, the initial brown color of the material (first alkaline treatment step) changed to light yellowish brown (first bleaching treatment step), until finally obtain a white material (second bleaching treatment, second alkaline treatment, and acid hydrolysis steps). During the alkaline treatment, the alkaline solution hydrolyzed constituents like starch, pectins, and hemicelluloses. As for the bleaching treatment, sodium chlorite (NaClO_2) removed lignin and tannins, which are responsible for the brown color of the banana peel bran. This way is the most widespread technique at the laboratory scale to remove lignin from plants. During the bleaching treatment, chlorine and chlorites rapidly oxidize lignin, generating hydroxyl, carbonyl, and carboxylic groups; these groups facilitate lignin solubilization in alkaline medium and thus cellulose purification (DUFRESNE et al., 1997). The treatment using sodium chlorite solution at pH 5.0 for 1 h removes most of the lignin; however, a further treatment is often required to fully bleach the suspension.

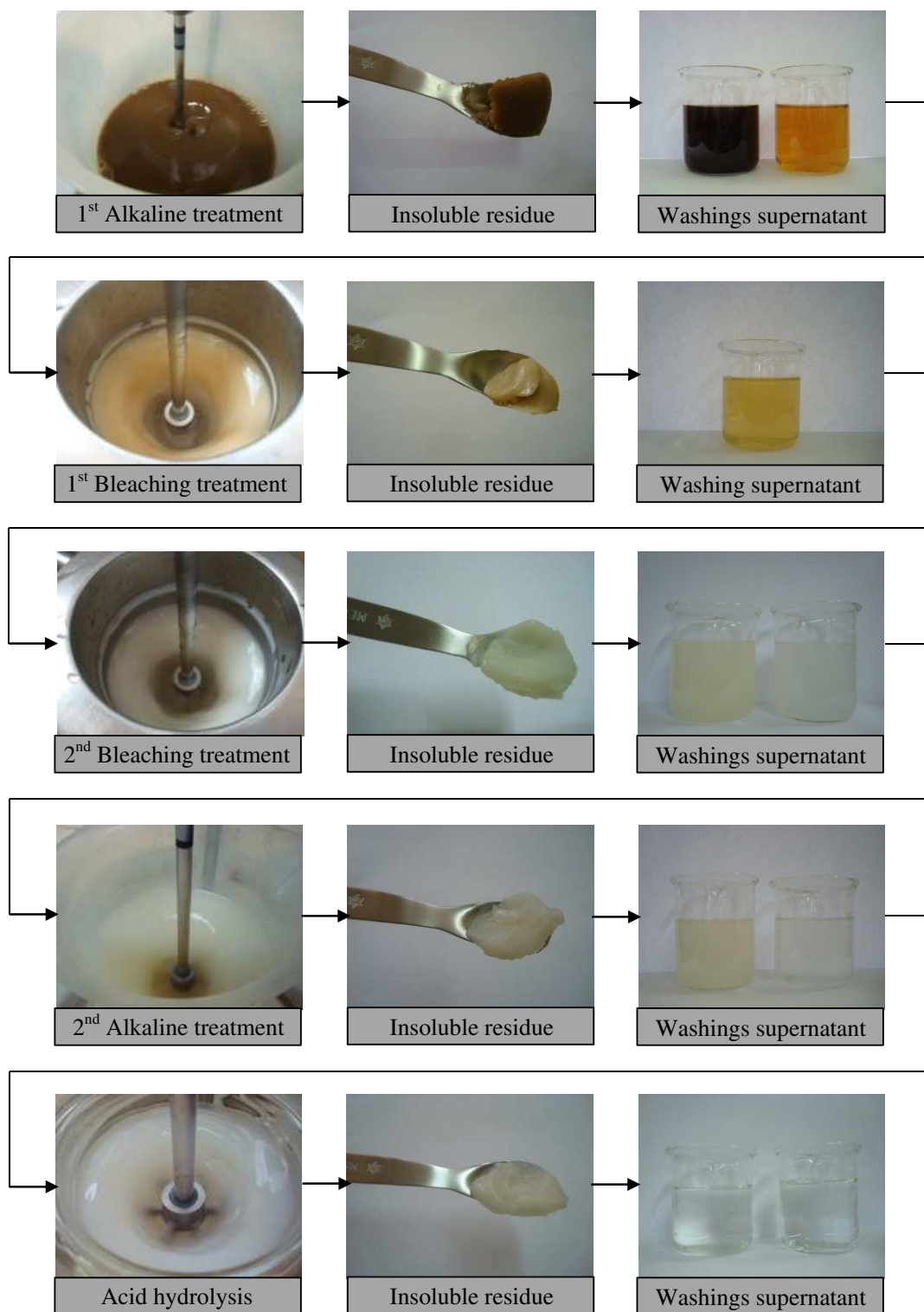


Figure 3. Photographs of the steps involved in the chemical treatment for the isolation of cellulose nanofibers from banana peels.

Cellulose nanofibers from different vegetable sources have been isolated using chemical and mechanical treatments (BHATNAGAR and SAIN, 2005; DUFRESNE et al., 1997; DUFRESNE and VIGNON, 1998). Chemical treatment effectively delignifies cellulose and solubilizes hemicellulose (SUN et al., 2005). However, authors like Dufresne et al. (1997) have suggested a homogenizing mechanical treatment that disrupts the coarse bundles of cellulose nanofibers and improves nanofiber individualization. In the present study, the different number of passages in the high-pressure homogenizer resulted in four nanofiber suspensions. Figure 4a and 4b shows the physical and colloidal aspects of these suspensions, respectively. Compared with suspensions N3, N5, and N7, which underwent mechanical treatment, suspension N0 (obtained without homogenization) presents phase separation (Figure 4a). Thus, the mechanical treatment favored homogeneous suspensions. The concentration of nanofibers in the suspensions (g of nanofibers/100 g of suspension) was 0.54, 0.48, 0.52, and 0.46 for N0, N3, N5, and N7, respectively.

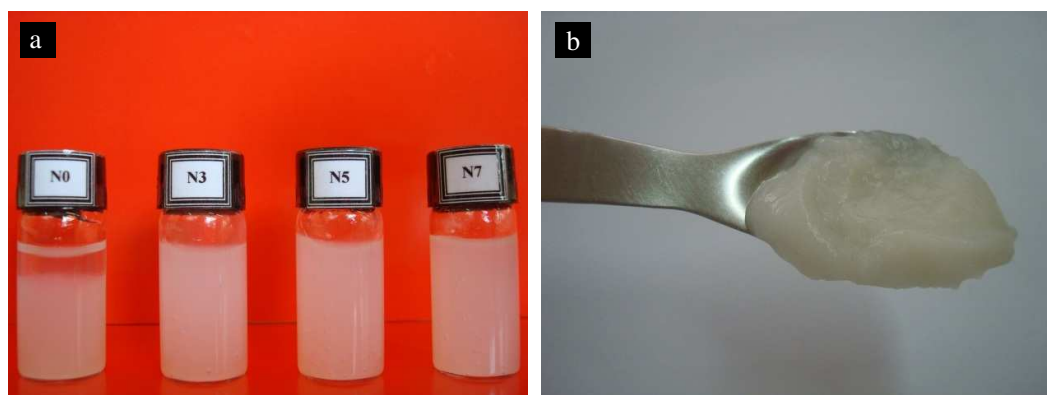


Figure 4. Final appearance of the cellulose nanofibers from banana peels (a) in suspension and (b) in the colloidal state.

3.2. Scanning (SEM) and transmission electron microscopy (TEM)

The chemical and mechanical treatments elicit structural and chemical changes in fibers. A SEM micrograph was recorded of the banana peel bran, to investigate the structure of these fibers (Figure 5a). The bran exhibited an irregular surface and some residues of the grinding. Starch granules from the contact of the peel with the banana pulp are present in the microstructure.

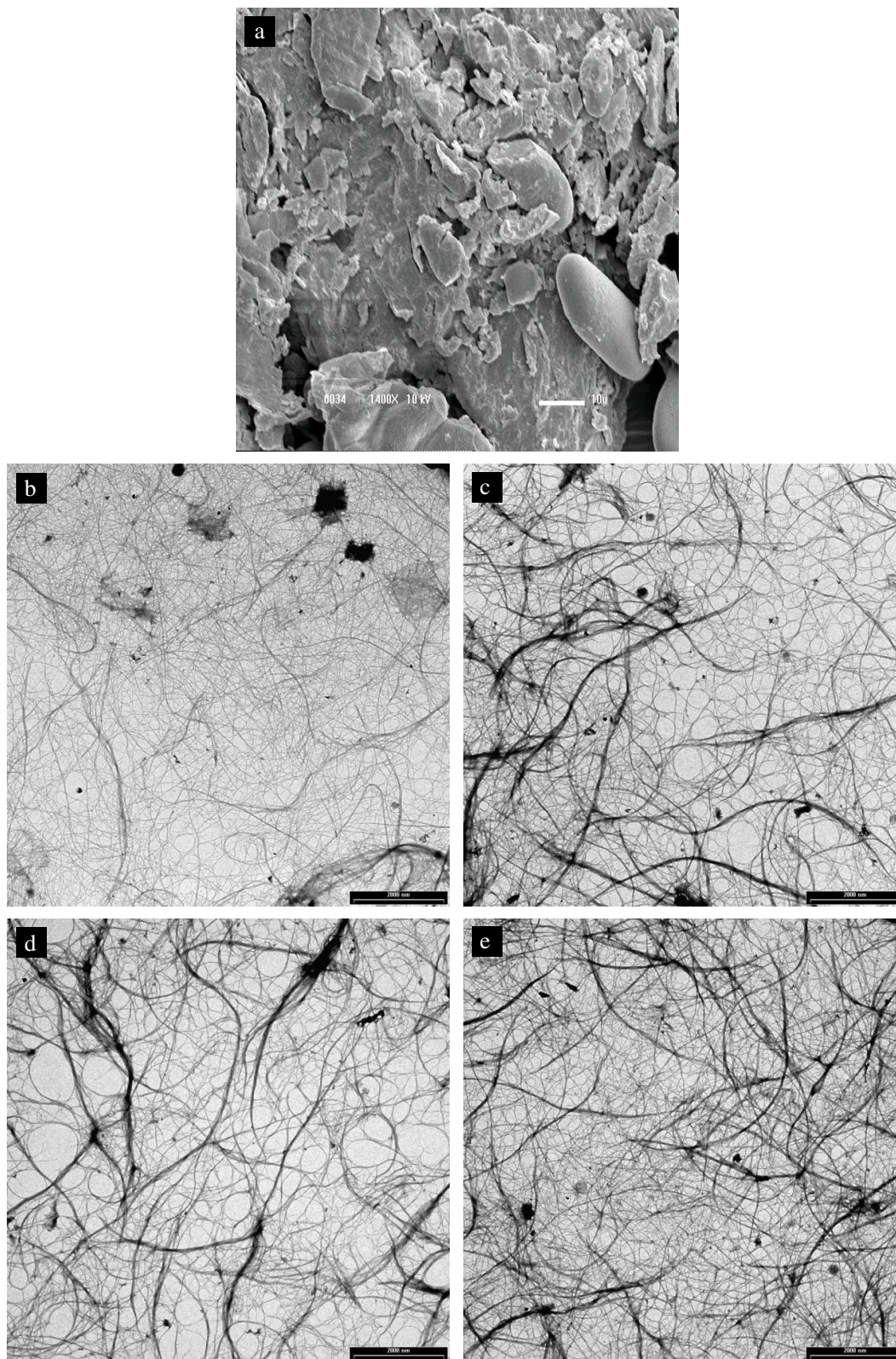


Figure 5. (a) SEM image of the banana peel bran (1000x, scale bar = 10 μm). TEM images of cellulose nanofibers obtained by different number of passages in the high-pressure homogenizer: (b) N0, (c) N3, (d) N5, and (e) N7 (1400x, scale bar = 2000 nm).

The presence of nanofibers was confirmed by TEM (Figures 5b, 5c, 5d, and 5e). These images suggest that hemicelluloses, lignin, and pectin (the cementing materials around the fiber-bundles) were removed after the chemical and mechanical treatments. The diameters of the fibers were determined by an image processing analysis software (ImageJ) using the TEM images. The cellulose nanofibers had diameters between 10.9 and 22.6 nm, showing that all the treatments effectively isolated the banana fibers in the nanometer scale (Table 1). The diameters were similar to the nanosized structures derived from other sources such as nanofibers from rice straw (12 – 35 nm) (ABE and YANO, 2009) and wheat straw (15 – 35 nm) (CHEN et al., 2011), and smaller than nanofibers from sugarcane bagasse (30 nm) (BHATTACHARYA et al., 2008) and sugar beet (30 – 100 nm) (LEITNER et al., 2007).

Table 1. Dimensions and zeta potential of cellulose nanofibers obtained by different number of passages in the high-pressure homogenizer.

Sample	Diameter, D (nm)	Length, L (nm)	Aspect ratio (L/D)	Zeta potential (mV)
N0	10.9 ± 2.3 ^a	454.9 ± 6.6 ^a	42.7 ± 7.5 ^a	-16.1 ± 0.7 ^a
N3	14.0 ± 3.7 ^{ab}	395.6 ± 11.1 ^b	29.4 ± 6.9 ^{ab}	-28.4 ± 1.7 ^b
N5	18.8 ± 4.6 ^{ab}	375.2 ± 9.3 ^b	20.8 ± 5.5 ^b	-36.6 ± 1.3 ^c
N7	22.6 ± 4.1 ^b	335.1 ± 6.6 ^c	15.1 ± 2.3 ^b	-44.1 ± 2.1 ^d

^{a,b,c,d} Different letter superscripts in the same column indicate a statistically significant difference (p < 0.05).

TEM micrographs of the suspensions revealed that cellulose nanofibers displayed a classical web-like network structure and occurred as very long entangled cellulosic filaments. The mechanical treatment resulted in the individualization of the fibers, culminating in more separate network (Figures 5c, 5d, and 5e). Increased number of passages in the high-pressure homogenizer did not alter the structure of the homogenized nanofibers.

The TEM images of all the nanofiber suspensions subjected to high-pressure homogenization (N3, N5, and N7) evidenced greater presence of nanofibers with thicker diameter and bundles as compared with the nanofiber suspension obtained without homogenization (N0). According to De Souza Lima and Borsali (2004), the cellulose nanofibers have no regular surface because they consist of crystalline and amorphous regions. The cellulose chains present in the amorphous regions are randomly oriented in a spaghetti-like arrangement, leading to lower density in these non-crystalline regions

and are susceptible to acid attack. The hydrogen ions can penetrate the cellulose chains in these amorphous domains, promoting the hydrolytic cleavage of the glycosidic bonds and releasing individual crystallites. Because of the large freedom of motion after mechanical treatment, these crystallites can grow in size, which explains the larger dimensions of the homogenized nanofibers as compared with the non-homogenized nanofibers. Incomplete removal of hemicelluloses during chemical treatment and the formation of interfibrillar hydrogen bonds account for the presence of residual nanofiber bundles (ZULUAGA et al., 2007).

Dufresne et al. (2000) used mechanical treatment at high pressure (15 passages at 500 bar) to obtain cellulose nanofibers from potato. These authors verified the individualization of these fibers by TEM and reported diameters of 5 nm and a practically infinite aspect ratio. However, they also noted that some nanofibers associated with each other, forming bundles. Zuluaga et al. (2007) also studied how the homogenizing treatment affects the isolation of cellulose nanofibers from banana rachis and detected long and slender nanofibers, individualized or associated into bundles with an estimated diameter of 5 nm. Teixeira et al. (2009) obtained cellulose nanofibers with diameters between 2 and 11 nm from cassava bagasse. These authors reported the individualization of the nanoparticles after ultrasonic treatment and the association of the nanofibers into long and tangled bundles.

3.3. Particle size distribution

Table 1 shows the effect of the high-pressure homogenizer on the dimensions of cellulose nanofibers. These nanofibers were characterized by particle diameter (D), length (L), and aspect ratio (L/D). Nanofibers isolated from banana peel had diameters between 10.9 and 22.6 nm and lengths ranging from 335.1 to 454.9 nm. The aspect ratio ranged from about 15.1 to 42.7, which lies in the range of long nanofibers with potential use as reinforcing agents in composites. These results are consistent with other reports involving cellulose nanofibers extracted from different sources (BONDESON et al., 2006; ELAZZOUZI-HAFRAOUI et al., 2008; ROSA et al., 2010).

Figure 6 illustrates the particle size distribution for the banana peel bran (diameter) and cellulose nanofibers (length). All the samples exhibited a modal distribution; the bran had an average diameter of 24.5 μm . The peak position of length distributions of cellulose nanofibers shifted to lower values with increasing number of

passages in the high-pressure homogenizer; i.e., successive passages through the homogenizer degraded amorphous zones and partly destroyed the crystalline zones of the fibers, resulting in shorter nanofibers. De Moura et al. (2011) also reported that successive passages through a microfluidizer reduced the length size distribution of cellulose fibers.

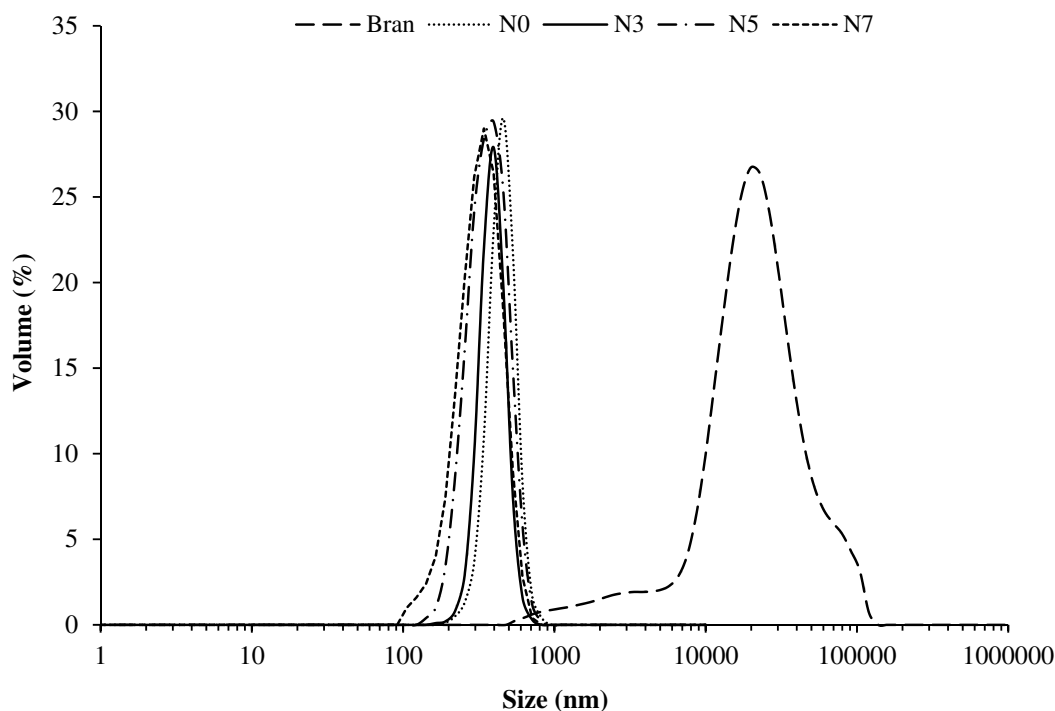


Figure 6. Particle sizes of the banana peel bran (diameter) and cellulose nanofibers (length) obtained by different number of passages in the high-pressure homogenizer.

3.4. Zeta potential

The zeta potential is that its value can be related to the stability of colloidal suspensions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in a dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability; i.e., the solution or dispersion will resist aggregation. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate (SEJERSEN et al., 2007). Nanofibers suspensions with high zeta potential (absolute value) are electrically stabilized, while those with low zeta potentials tend aggregate, compromising their ability to act as reinforcing agents in

polymer matrices. According to Bondeson et al. (2007), the electrostatic repulsion of nanofibers in suspension can be achieved by hydrolysis with sulfuric acid due to the introduction of natural sulfate groups in the nanofiber surface during this step.

Table 1 lists the zeta potential measurements. In neutral water, all the nanofiber suspensions exhibited negative zeta potential. Similar values have been reported for cellulose nanofibers isolated from banana plant wastes (-20.72 to -41.32 mV) (ELANTHIKKAL et al., 2010). Among the cellulose nanofibers, the sample N0 (produced without homogenization) gave the lowest zeta potential value. The zeta potential increased when the number of passages in the high- pressure homogenizer rises. Therefore, the homogenizing mechanical treatment has a significant effect on this property ($p < 0.05$; Table 1) and helps prevent the formation of nanofiber aggregates, thereby yielding a more stable colloidal suspension.

3.5. X-ray diffraction (XRD)

The effect of the homogenizing mechanical treatment on the crystalline nature of the resulting cellulose nanofibers was investigated. Figure 7 shows that the cellulose subjected to treatment was more crystalline than the initial banana fibers. The X-ray diffraction pattern reveals that the bran contains a large amorphous portion. The typical B-type pattern peak at $2\theta = 17^\circ$ evidences the presence of starch. The increased crystallinity of the cellulose nanofibers is due to partial removal of the hemicellulose and lignin during the chemical treatment and is associated with higher tensile strength (ALEMDAR and SAIN, 2008).

The diffraction patterns of the nanofiber samples are similar –they exhibit two broad peaks. These sharper diffraction peaks at $2\theta = 16^\circ$ and $2\theta = 22^\circ$ are typical of cellulose I and indicate the higher crystallinity of the nanofibers (ROSA et al., 2010). Mulberry branch barks led to similar results (LI et al., 2009).

The crystallinity index (I_c) of the samples calculated by Equation 1 is 15.0, 58.6, 61.8, and 62.4%, and $N7 = 64.9\%$ for the bran, N0, N3, N5, and N7, respectively. These results confirm that the chemical and mechanical treatments affected the degree of crystallinity of the cellulose nanofibers. The bran sample had 300% higher crystallinity after the treatments. Increasing the number of passages from 0 to 7 in the homogenizer slightly raised the crystallinity index of the cellulose nanofibers; sample N7 displayed

the highest value. Therefore, prolonged mechanical treatment removes part of the amorphous portion of cellulose nanofibers.

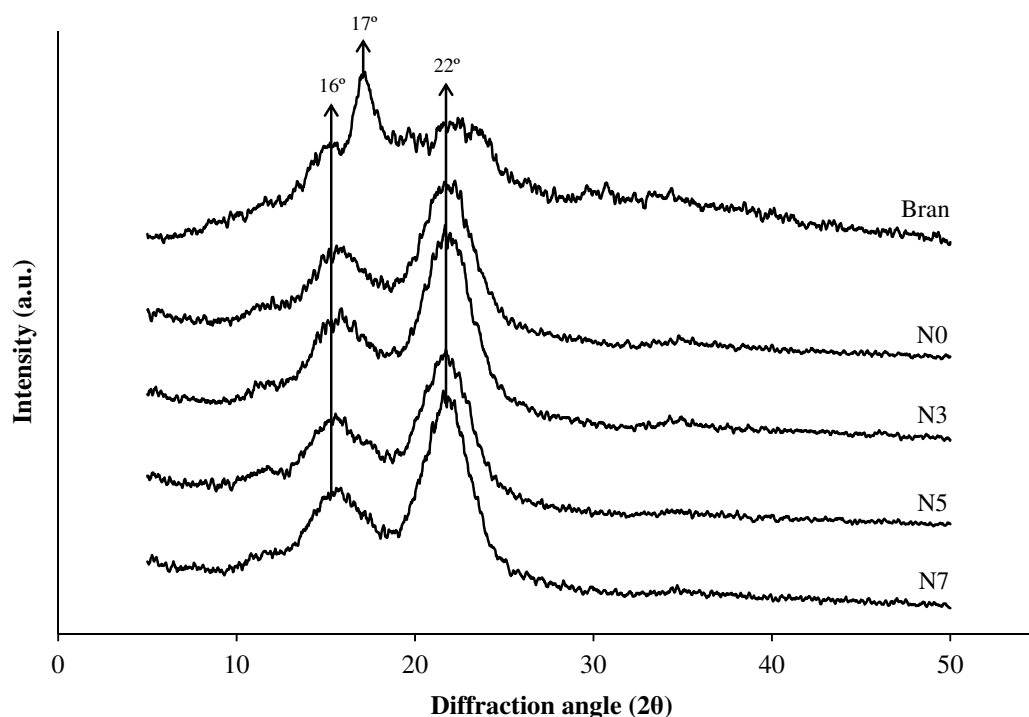


Figure 7. X-ray diffraction patterns of the banana peel bran and cellulose nanofibers obtained by different number of passages in the high-pressure homogenizer.

3.6. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectroscopy analyses of the samples revealed compositional changes in the structure of banana fibers during the chemical and mechanical treatments. Figure 8 depicts the FTIR spectra of the banana peel bran and cellulose nanofibers. All the nanofibers samples presented similar spectral patterns. The broad absorption band in the $3650 - 3000 \text{ cm}^{-1}$ region, related to the $-\text{OH}$ groups present in the components, reflects the hydrophilic tendency of the natural banana fiber and cellulose nanofibers. The two small bands at 3340 and 3287 cm^{-1} correspond to intramolecular hydrogen bonding in cellulose II (ZULUAGA et al., 2009). The peak at 2918 cm^{-1} is due to the aliphatic saturated C-H stretching vibration in cellulose and hemicellulose.

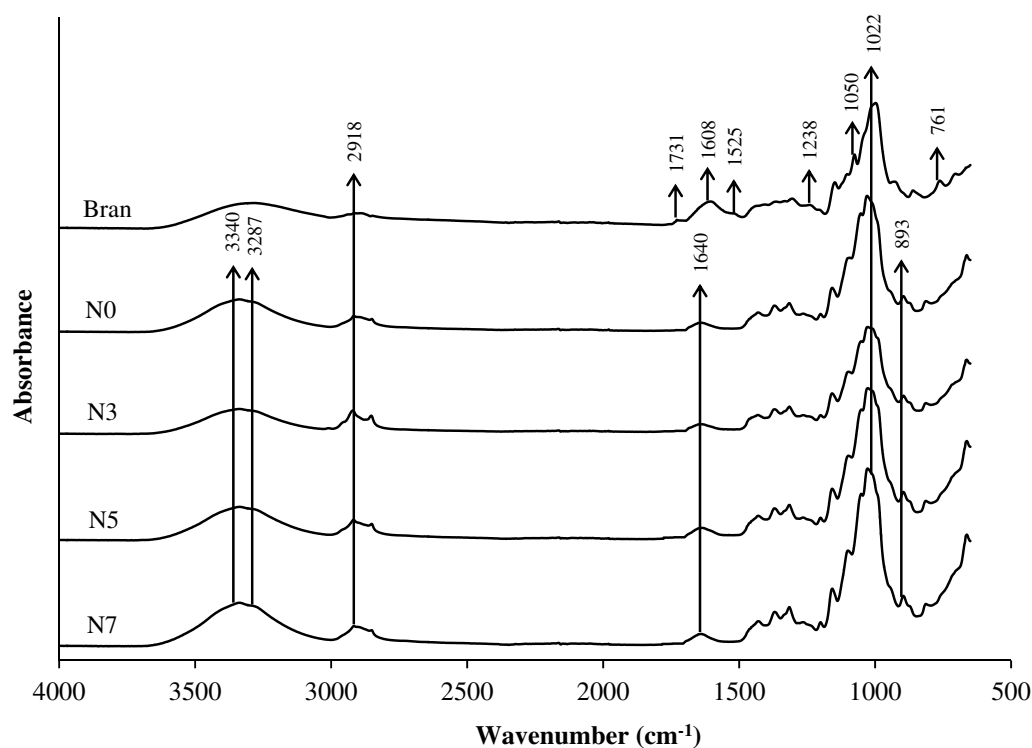


Figure 8. FTIR spectra of the banana peel bran and cellulose nanofibers obtained by different number of passages in the high-pressure homogenizer.

The shoulder at 1731 cm^{-1} in the spectrum of the bran represents vibrations of acetyl and uronic ester groups of hemicelluloses or ester linkage of carboxylic group of the ferulic and *p*-coumaric acids of lignin (CHERIAN et al., 2008). This peak is absent in the spectra of all the nanofiber samples, because the chemical process dissolved the hemicellulose and lignin components of the bran. The band at 1608 cm^{-1} in the spectrum of the bran shifted to 1640 cm^{-1} and became less intense for the cellulose nanofibers; this indicates partial reaction of the C=O bonds of hemicelluloses (WANG et al., 2007).

Figure 8 demonstrates that the bleaching step removed most of the lignin from the cellulose nanofibers; indeed, the vibrations at 1525 cm^{-1} (aromatic ring vibrations), 1238 cm^{-1} (guaiacyl ring breathing with stretching C=O), and 761 cm^{-1} (C–H deformations) disappeared after bleaching (ZULUAGA et al., 2009). Sun et al. (2004) reported that the sodium chlorite method removes lignin more efficiently than the hydrogen peroxide method.

The band near 1050 cm^{-1} , related to the presence of xylans associated with hemicelluloses, was significantly less intense for the nanofiber samples. The C–O–C pyranose ring skeletal vibration gives a prominent band at 1022 cm^{-1} ; a more intense

band at this wavenumber evidences higher cellulose content. The small sharp band at 893 cm^{-1} in the FTIR spectra of nanofiber samples is typical of the structure of cellulose and represents glycosidic C–H deformation, with a ring vibration contribution from O–H bending. These features evidence β -glycosidic linkages between the anhydroglucose units in cellulose (ALEMDAR and SAIN, 2008).

3.7. Differential scanning calorimetry (DSC)

Banana fiber samples were thermally analyzed to compare the degradation characteristics of the treated fibers with those of the untreated fibers. Figure 9 contains the heating DSC curves for banana peel bran and cellulose nanofibers; Table 2 lists the corresponding data. For all the samples, water evaporates around $110 - 150\text{ }^{\circ}\text{C}$, hemicellulose degrades around $240 - 345\text{ }^{\circ}\text{C}$, and cellulose decomposes around $290 - 375\text{ }^{\circ}\text{C}$. The differences in the inherent structures and chemical nature of the sample components account for the observed decomposition behavior. Hemicellulose consists of various saccharides (xylose, mannose, glucose, galactose, etc.) arranged in a random amorphous structure, rich in branches; they are easily removed from the main stem and degrade to volatiles (CO , CO_2 , and some hydrocarbon, etc.) that evolve at low temperatures. Cellulose comprises long glucose polymers without branches; these polymers constitute a strong and ordered structure of high thermal stability. Lignin is full of aromatic rings and displays various branches. The activity of the chemical bonds covered an extremely wide temperature range, so lignin degraded over $500 - 900\text{ }^{\circ}\text{C}$ (YANG et al., 2007). Unfortunately, the lignin decomposition was not able to determine due to limitations of the DSC heating system.

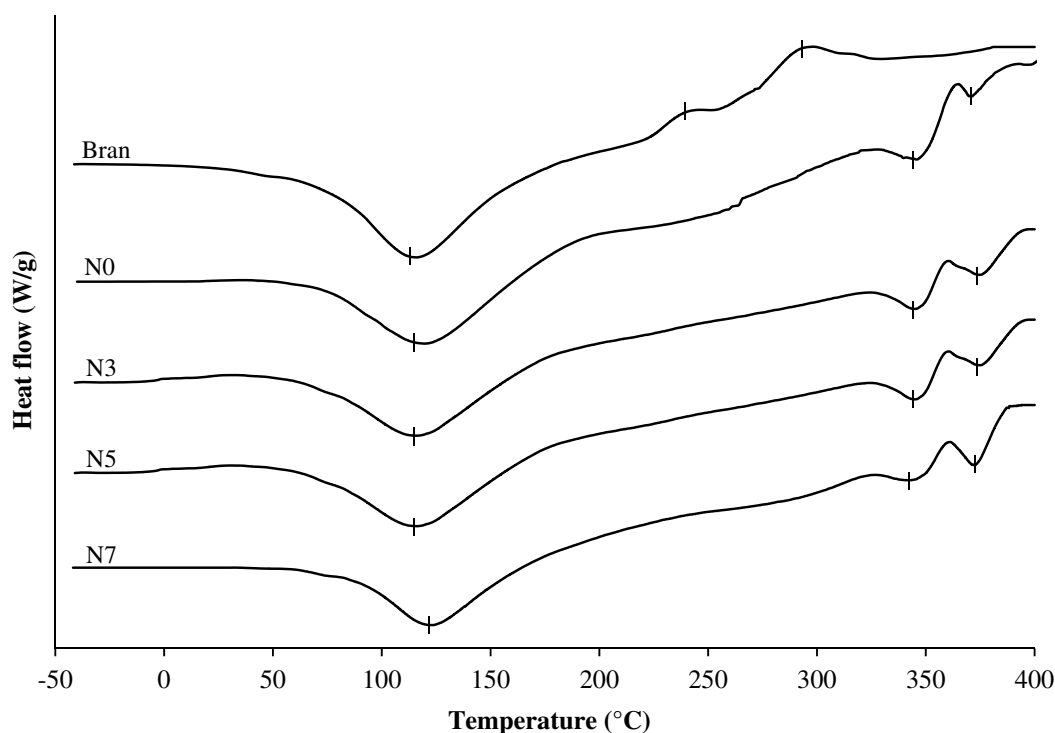


Figure 9. DSC thermograms of the banana peel bran and cellulose nanofibers obtained by different number of passages in the high-pressure homogenizer.

Initially, all the DSC curves evidenced endothermic reactions at 100 °C, corresponding to the removal of moisture during sample heating. Further temperature increase (>200 °C) led to two exothermic peaks in the DSC profile of the bran, which contrasts with the endothermic peaks of cellulose nanofibers and is associated with different reactions or mechanisms involved in the pyrolysis of the components. Ball et al. (2004) pointed out that the charring process is highly exothermic, whereas volatilization is endothermic. The solid residues generated from bran decomposition and evidenced by the exothermic peaks in the case of hemicellulose and cellulose degradation could be attributed to charring, while full decomposition of the nanofibers might result from the fast devolatilization reactions, culminating in a small amount of solid residues and endothermic peaks.

Table 2 contains the enthalpy values associated with moisture desorption, and hemicellulose and cellulose decomposition. Treated fibers exhibit higher enthalpy values than untreated fibers. The enthalpy value is minimum for the banana peel bran and maximum for the cellulose nanofibers passed seven times through the high-pressure homogenizer. Chemical and mechanical processes such as alkaline treatment,

bleaching, acid hydrolysis, and high-pressure homogenization increase the amount of crystalline cellulose, as confirmed by the XRD analyses. The increased percentage crystallinity of the fibers and the increased H-bonding between the closely packed cellulose chains during each treatment tend to stabilize the structure and increase the enthalpy values (DEEPA et al., 2011). Thus, the thermal stability of the banana fibers increases during each processing step; N7 cellulose nanofibers are the most thermally stable. The developed nanofibers have enhanced thermal properties, making them less prone to degradation. Hence, we can process these nanofibers at higher temperatures than their untreated counterparts.

Table 2. Thermal parameters of the banana peel bran and cellulose nanofibers obtained from DSC analysis.

Sample	Identification	Thermal parameters ^a		
		T _o (°C)	T _p (°C)	ΔH (J/g)
Bran	Water evaporation	64.2 ± 6.3	113.5 ± 4.7 (endo)	178.7 ± 4.9
	Hemicellulose	224.9 ± 0.6	241.1 ± 0.7 (exo)	6.4 ± 1.7
	Cellulose	274.0 ± 0.7	294.4 ± 1.0 (exo)	9.8 ± 2.4
N0	Water evaporation	72.4 ± 1.3	132.7 ± 3.5 (endo)	187.8 ± 8.3
	Hemicellulose	327.6 ± 0.5	341.4 ± 0.9 (endo)	16.1 ± 1.2
	Cellulose	361.5 ± 0.8	366.3 ± 1.0 (endo)	10.4 ± 3.8
N3	Water evaporation	81.2 ± 0.1	142.2 ± 5.0 (endo)	208.8 ± 6.9
	Hemicellulose	327.8 ± 0.6	343.5 ± 0.8 (endo)	24.2 ± 0.9
	Cellulose	357.7 ± 0.2	371.8 ± 1.4 (endo)	15.1 ± 0.6
N5	Water evaporation	82.6 ± 2.1	144.6 ± 1.5 (endo)	215.4 ± 2.4
	Hemicellulose	328.1 ± 0.4	345.8 ± 0.6 (endo)	26.2 ± 1.1
	Cellulose	360.9 ± 3.6	373.5 ± 2.1 (endo)	18.0 ± 4.3
N7	Water evaporation	89.8 ± 0.2	145.6 ± 2.8 (endo)	218.2 ± 0.4
	Hemicellulose	328.8 ± 0.8	345.9 ± 1.0 (endo)	27.8 ± 1.7
	Cellulose	362.1 ± 0.1	375.0 ± 0.7 (endo)	20.1 ± 0.2

^a Onset temperature (T_o), peak temperature (T_p), enthalpy of degradation (ΔH), endothermic (endo), and exothermic (exo).

4. Conclusions

Nanofibers suspensions prepared with homogenization had improved properties compared with untreated nanofibers. The number of passages in the high-pressure homogenizer affected the dimensions and the surface charge of the resulting

nanoparticles. The crystallinity of the treated banana fibers increased by 300%. FTIR spectra of the cellulose nanofibers confirmed removal of most of the hemicellulose and lignin components and attested to the efficiency of the applied chemical treatment. The nanofiber samples exhibited enhanced thermal properties; thermal stability increased as a function of the number of passages in the homogenizer.

We successfully isolated cellulose nanofibers from the banana peel bran using chemical and mechanical treatments. This stimulates the use of banana peels, an agroindustrial residue, as a renewable source of nanofibers potentially applicable as reinforcing agents in composites.

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Capítulo 6

Nanocomposites based on Banana Starch Reinforced with Cellulose Nanofibers Isolated from Banana Peels

Abstract

Cellulose nanofibers were isolated from banana peel using a combination of chemical and mechanical treatments with different number of passages through the high-pressure homogenizer (0, 3, 5, and 7 passages). New nanocomposites were then prepared from a mixed suspension of banana starch and cellulose nanofibers using the casting method and the effect of the addition of these nanofibers on the properties of the resulting nanocomposites was investigated. The cellulose nanofibers homogeneously dispersed in the starch matrix increased the glass transition temperature, due to the strong intermolecular interactions occurring between the starch and cellulose. The nanocomposites exhibited significantly increased the tensile strength, Young's modulus, water-resistance, opacity, and crystallinity as the number of passages through the homogenizer augmented. However, a more drastic mechanical treatment (seven passages) degraded the nanofibers, deteriorating the nanocomposite properties. The most suitable mechanical treatment condition for the preparation of cellulose nanofibers and the corresponding nanocomposite was five passages through the high-pressure homogenizer. In general, the cellulose nanofibers improved the features of the starch-based material and are potentially applicable as reinforcing elements in a variety of polymer composites.

Keywords: banana starch, cellulose nanofibers, high-pressure homogenizer, biodegradable films, mechanical properties.

1. Introduction

Researchers have sought several alternatives to minimize the environmental impact of conventional polymers, including the use of biodegradable polymers. Because starch is abundant, inexpensive, and degradable, scientists have employed it to produce biodegradable films. However, to technologically apply starch it is necessary to improve its mechanical and barrier properties, because starch films are water-soluble, brittle, and difficult to process. Using nanotechnology to develop films based on polymer nanocomposites containing nanometric fillers might be a new way to improve the mechanical and barrier properties of a given polymer. The reinforcing agent constitutes a nanoparticle when at least one of its dimensions is lower than 100 nm. This particular feature provides unique nanocomposites with outstanding properties that are not seen in conventional composites (KHALIL et al., 2012; SIQUEIRA et al., 2009).

Among the nanometer-sized fillers, cellulose materials have attracted attention and offer a highly contemporary research line. Cellulose is an abundant polymer in nature and occurs in various plants and living organisms. It is environmentally-friendly, cheap, and biodegradable. Plant-based cellulose nanofibers have generated a great deal of interest because they display large surface to volume ratio, high tensile strength, stiffness, and flexibility, and good dynamic mechanical, electrical, and thermal properties as compared with other commercial fibers (SAMIR et al., 2005; SIRÓ and PLACKETT, 2010). The use of cellulose nanofibers as reinforcing elements in matrices improves thermo-mechanical properties, decreases the sensitivity of polymers to water, and preserves biodegradability. Indeed, mixing cellulose nanofibers with polysaccharides (such as starch) improves mechanical properties (DUFRESNE and VIGNON, 1998; DUFRESNE et al., 2000).

Because of their annual renewability, agricultural crop residues can be a valuable source of cellulose nanofibers. Researchers have isolated nanostructures from various agricultural residues such as the pineapple leaf (CHERIAN et al., 2011), wheat straw (KAUSHIK and SINGH, 2011), coconut husk (ROSA et al., 2010), and sugarcane bagasse (TEIXEIRA et al., 2011). The banana peel waste is a byproduct of the processing of bananas for food products such as banana chips and baby foods. The edible part of the fruit constitutes only 12 wt% of the plant; the remaining parts become agricultural waste and cause environmental problems (ELANTHIKKAL et al., 2010). Since the banana peel is rich in cellulose, this material is potentially applicable as

reinforcing component in high-performance composites, increasing its commercial value and providing a purpose for this byproduct. Agricultural residues from banana crops such as rachis (ZULUAGA et al, 2007; 2009) and plant wastes (CHERIAN et al., 2008; DEEPA et al., 2011; ELANTHIKKAL et al., 2010; MO et al., 2010) can be used to produce nanoreinforcements. However, the isolation of cellulose nanofibers from banana peels has not been reported yet.

Several methods based on successive chemical and mechanical treatments can extract highly pure cellulose nanofibers from plant and wood. In high-pressure homogenization, chemically treated dilute slurries of cellulose fibers are pumped at high pressure and fed through a spring-loaded valve assembly. As this valve opens and closes rapidly, the fibers undergo a large pressure drop with shearing and impact forces. This combination of forces promotes high degree of fibrillation of the cellulose fibers, resulting in homogeneous suspensions with more individualized fibers (HERRICK et al., 1983).

Our previous study showed that chemical treatment followed by high-pressure homogenization successfully isolates cellulose nanofibers from the banana peel. To investigate the potential use of cellulose nanofibers as reinforcing agents in polymers, starch polymer nanocomposites containing these nanofibers were produced and characterized. In order to take full advantage of banana, the pulp was used to obtain the starch that served as matrix for the films; the peel was employed to isolate the cellulose nanofibers. The resulting nanocomposites were characterized with respect to their mechanical, barrier, optical, structural, and thermal properties.

2. Materials and Methods

2.1. Materials

The starch was isolated from unripe plantain bananas (mature green) of the variety “Terra” (*Musa paradisiaca*), according to the methodology described in Section 2.3 of Chapter 2. The extracted banana starch reached a purity of 94.8%; 35.0 and 50.3% corresponded to amylose and resistant starch, respectively, in dry basis. All the chemicals employed in this work were reagent grade.

2.2. Isolation of cellulose nanofibers

Cellulose nanofibers were isolated from the banana peel bran using the method described in Section 2.3 of Chapter 5. Four nanofiber suspensions were obtained at the end of the procedure; the nanofibers not submitted to homogenization (N0) and those that were passed through the high-pressure homogenizer 3 (N3), 5 (N5), and 7 (N7) times. The concentration of nanofibers in the suspensions (g of nanofiber/100 g of suspension) was 0.54, 0.48, 0.52, and 0.46 for N0, N3, N5, and N7, respectively. These data were used to prepare the formulation of the nanocomposite films.

2.3. Nanocomposites production

The nanocomposite films were produced by the casting method under the optimal process conditions established for the banana flour film in Chapter 3. The process consisted of drying a film-forming suspension (FFS) that was then applied onto a support. The procedure developed herein (Figure 1) involved the dispersion of nanofiber suspension (5 g of nanofibers/100 g of starch) by magnetic stirring for 20 h, followed by homogenization with an Ultra-Turrax (IKA® Works Inc., model DI 18 Basic, Wilmington, USA) at a speed of 8,000 rpm for 15 min. The nanofiber suspension was added in a water solution of 4 g/100 g (d.b.) of banana starch previously stirred for 15 min. The resulting suspension was homogenized under mechanical stirring for 30 min and heated to the process temperature of 81 °C. Glycerol (25 g of glycerol/100 g of starch) was added at this point, and the solution was maintained at this temperature for 15 min. Next, the FFS was sonicated for 10 min, and 70 g of the solution was poured onto acrylic plates (18 x 21 cm), to achieve a constant thickness of 85 ± 5 µm. Preliminary tests of grammage were performed, to determine the exact amount of FFS to be poured onto acrylic plates (see Appendix 3). The nanocomposite films were dried in a chamber with air circulation under controlled temperature (54 °C) and relative humidity (48%). A control film (without nanofibers) was produced, to evaluate the effect of the addition of cellulose nanofibers.

The nanocomposite films were conditioned in desiccators under 58% RH, at 25 °C, for 48 h before being characterized for moisture content, mechanical properties, and water vapor permeability.

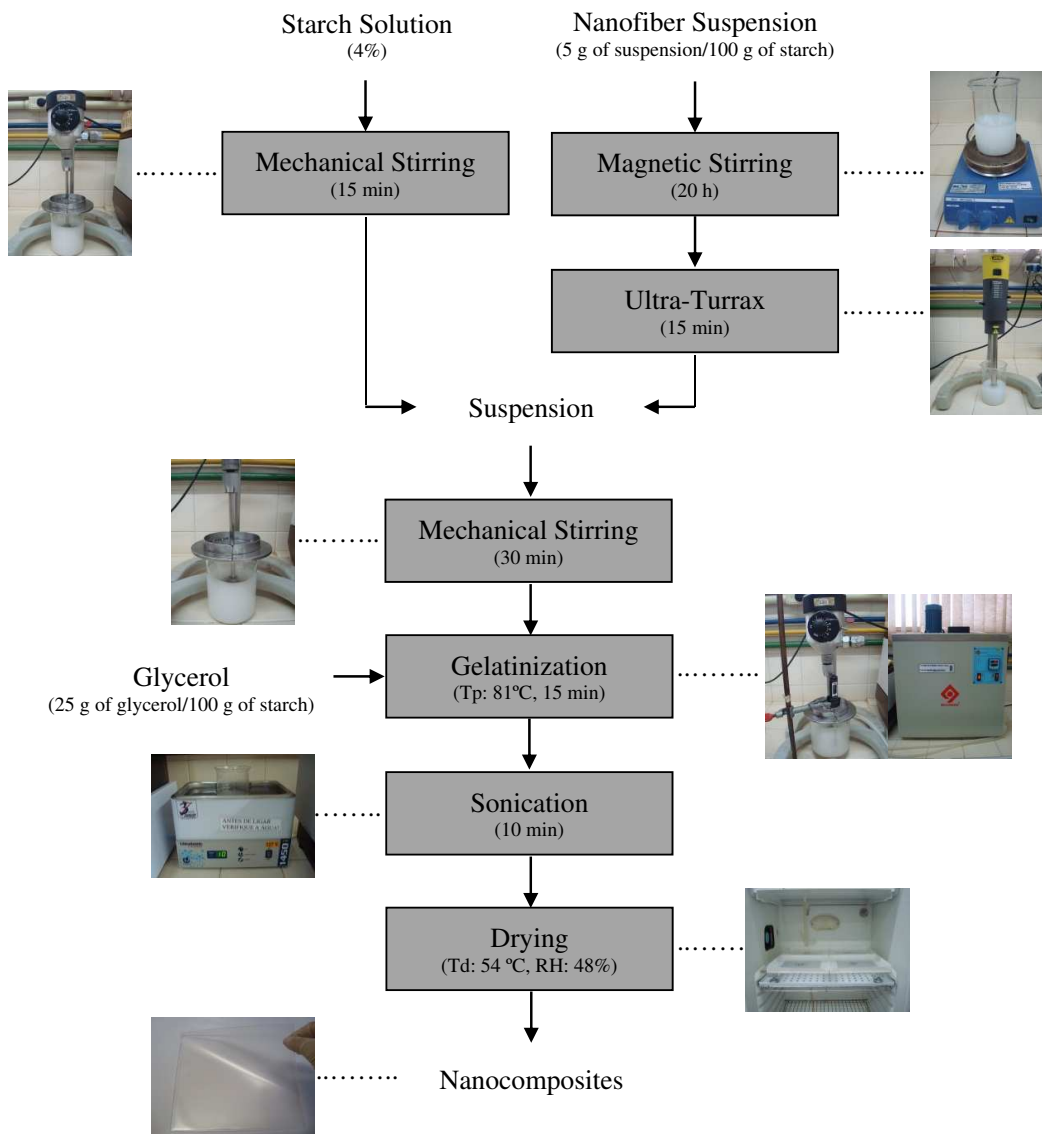


Figure 1. Scheme describing the procedure used to produce nanocomposite films.

2.4. Nanocomposites characterization

The thickness, density, moisture content, tensile properties, color, opacity, and solubility in water were determined as described in Section 2.4 of Chapter 3. Scanning electron microscopy, gloss analysis, X-ray diffraction, Fourier-transform infrared spectroscopy, and differential scanning calorimetry were carried out as described in Section 2.3 of Chapter 4. The other analyses performed in the films are detailed below:

2.4.1. Energy dispersive X-ray spectroscopy (EDS)

To identify the chemical elements present in the nanocomposites, analyses were carried out using energy dispersive X-ray spectroscopy (Oxford, model 6070, Cambridge, England) attached to a field emission scanning electron microscope (LEO, model LEO 440i, Cambridge, England).

2.4.2. Water vapor permeability (WVP)

The WVP was determined gravimetrically by following the standard method E96-00 (ASTM, 2000) with modifications, using three different ranges of relative humidity (2 – 33%, 33 – 64%, and 64 – 90%). The film was placed on the circular opening (diameter = 50 mm) of a permeability capsule with silicone grease, to ensure that humidity migration would occur through the film only. The interior of the capsule was filled with a salt solution that provided the lower relative humidity (2, 33, or 64%, depending on the case), and the system was stored in a desiccator containing a salt solution that provided higher relative humidity (33, 64, or 90%, depending on the case), at 25 °C. The weight gain of the capsules was monitored every 30 min, for 8 h. Analyses were conducted in triplicate, and the WVP was calculated on the basis of Equation 1 and expressed in g/m.s.Pa:

$$WVP = \frac{w}{t} \times \frac{\delta}{A \cdot \Delta P} \quad [1]$$

where w/t is the angular coefficient determined graphically by plotting the weight gain (w) as a function of time (t) (g/s), δ is the mean sample thickness (m), A is the sample permeation area (m²), and ΔP is the difference in water vapor pressure through the sample for pure water at 25 °C (Pa).

2.4.3. Water uptake

The water absorption kinetics was determined according to the methodology described by Dufresne et al. (2000). Nanocomposite films of 20 mm x 20 mm and known thickness, dried at 105 °C for 24 h, were weighed and conditioned at 25 °C in

hermetically sealed flasks containing Na₂SO₄ saturated solution, to ensure a RH of 95%. The samples were removed at desired intervals and weighed until the equilibrium state was reached. The water uptake of the samples was calculated using Equation 2:

$$WU = \frac{W_t - W_0}{W_0} \times 100 \quad [2]$$

where WU is the water uptake (%), W_t is the weight of the sample after time t of exposure to 95% RH (g), and W_0 is the initial weight of the sample (g).

2.5. Statistical analysis

An analysis of variance (ANOVA) and a Tukey test of multiple comparisons with a significance level of 5% were run using the Statistica 7.0 software (StatSoft Inc, Tulsa, Oklahoma, USA), to compare the differences between the mean values of the properties of the banana starch films reinforced with cellulose nanofibers or not.

3. Results and Discussion

3.1. Nanocomposites characterization

The nanocomposites based on banana starch and reinforced with cellulose nanofibers isolated from the banana peel presented homogeneous surface with no bubbles or cracks, as well as good handling characteristics. This means that the films could be easily detached from the plates without tearing, and that they were not sticky or too brittle.

3.1.1. Scanning electron microscopy (SEM)

Figure 2 brings the SEM of the surfaces and cross-sections of the films. Incorporation of cellulose nanofibers significantly changed the microstructure of the nanocomposites (FN0, FN3, FN5, and FN7) as compared with the control film (FC). Nanocomposites presented non-homogenous structures; i.e., irregular surfaces with imperfections, while the control film displayed a more uniform and smooth surface.

Regarding the cross-section, the nanocomposites exhibited less dense and less homogeneous polymeric structures with small cracks, as compared with the control film.

Liu et al. (2011) studied the structure of the cellulosic nanocrystals, to find out whether the cellulose microstructure affected the properties of the ordered nanocrystalline phase. The authors rapidly froze a suspension of cellulose nanocrystals in liquid nitrogen and freeze-dried the sample, which furnished foam consisting of cellulose nanocrystals; they also produced a dried film of cellulose nanocrystals using the casting process and characterized both the foam and the film by SEM. The authors verified that the cellulose nanocrystals were self-aligned layer by layer, which afforded an ordered layered structure. When the authors sublimated water from the fibrous network, they obtained foam with ordered, layered, fine micro-sized pore or tube structure.; when the authors evaporated water instead of sublimating it, the pore tube structure in the cellulose nanocrystals gel networks collapsed, leading to compact nanofiber layers with ordered fibrous arrangement. Therefore, the cast sample of cellulose nanocrystals became a solid film rather than porous foam. The microstructures of the banana nanocomposites displayed a similar behavior, but the presence of other components such as starch and glycerol in the film formulation culminated in slightly less organized structures consisting of multilayers compacted along the nanocomposite cross-sections.

Each nanofiber had a different aspect ratio, surface charge, and crystallinity (see Table 1 and Section 3.5 of Chapter 5), as a result of the treatments they underwent during their isolation. These characteristics may account for the type of interactions between cellulose and polymer matrix components (starch, glycerol, and water) and culminate in distinct microstructures. Despite the different structures, the cellulose nanofibers dispersed well in the matrix and did not agglomerate significantly, indicating structural integrity. This is probably a consequence of hydrogen bonding interactions between the hydroxyl groups of the components. The starch matrix coated the nanofibers even after fracture (cross-section), implying that the nanoparticles adhered to the polymer matrix well.

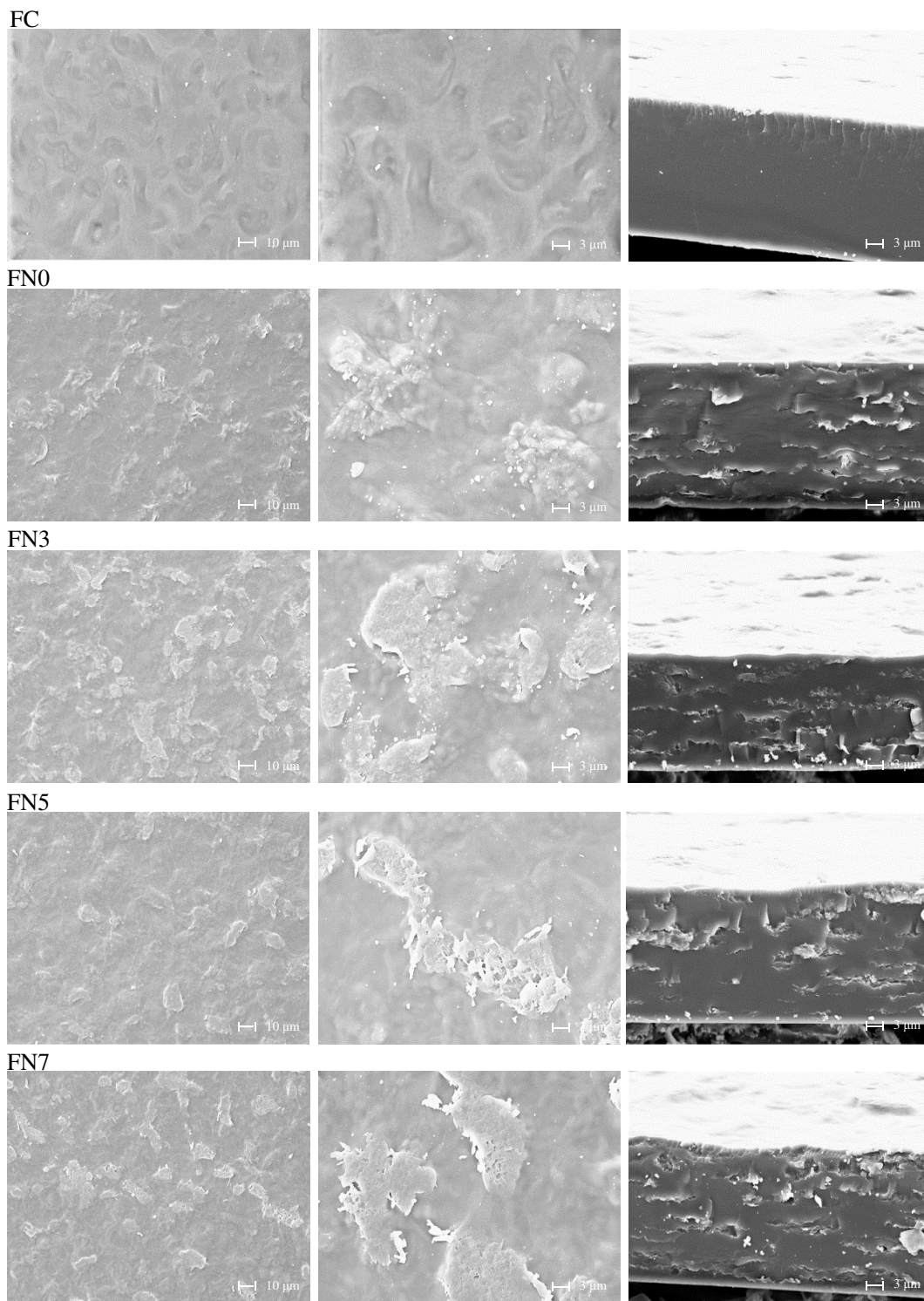


Figure 2. SEM surfaces (500x, scale bar = 10 μm; 2000x, scale bar = 3 μm) and cross-sections (2000x, scale bar = 3 μm) of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

The mechanical treatment performed on the cellulose nanofibers produced nanocomposites with rougher areas (FN3, FN5, and FN7) in the region of nanoparticle incorporation. The nanofibers became shorter with increasing number of passages through the high-pressure homogenizer ($N0 = 454.9 \text{ nm} > N3 = 395.6 \text{ nm} > N5 = 375.2 \text{ nm} > N7 = 335.1 \text{ nm}$, Table 1 of Chapter 5). The larger number of cellulose nanofibers per unit area in the films explain the rougher structures. Also, these nanoparticles might be better dispersed in the matrix due to their smaller size. De Moura et al. (2011) also reported similar results for nanocomposites reinforced with cellulose fibers that were successively passed through a microfluidizer.

To determine the chemical elements present in the nanocomposites the spectroscopy analysis by energy dispersive X-ray (EDS) was conducted. The spectra of all the films (Figure 3) confirmed the presence of carbon and oxygen, as expected (the structures of the polymeric matrix and reinforcing material consist of these elements). Small amounts of calcium were detected in the spectrum of the nanocomposites (FN0, FN3, FN5, and FN7), a result of the chemical treatment employed during isolation of the nanofibers. Thus, calcium salts can be formed during the neutralization steps with chemical reagents, and traces of salts may remain after successive washings of the material.

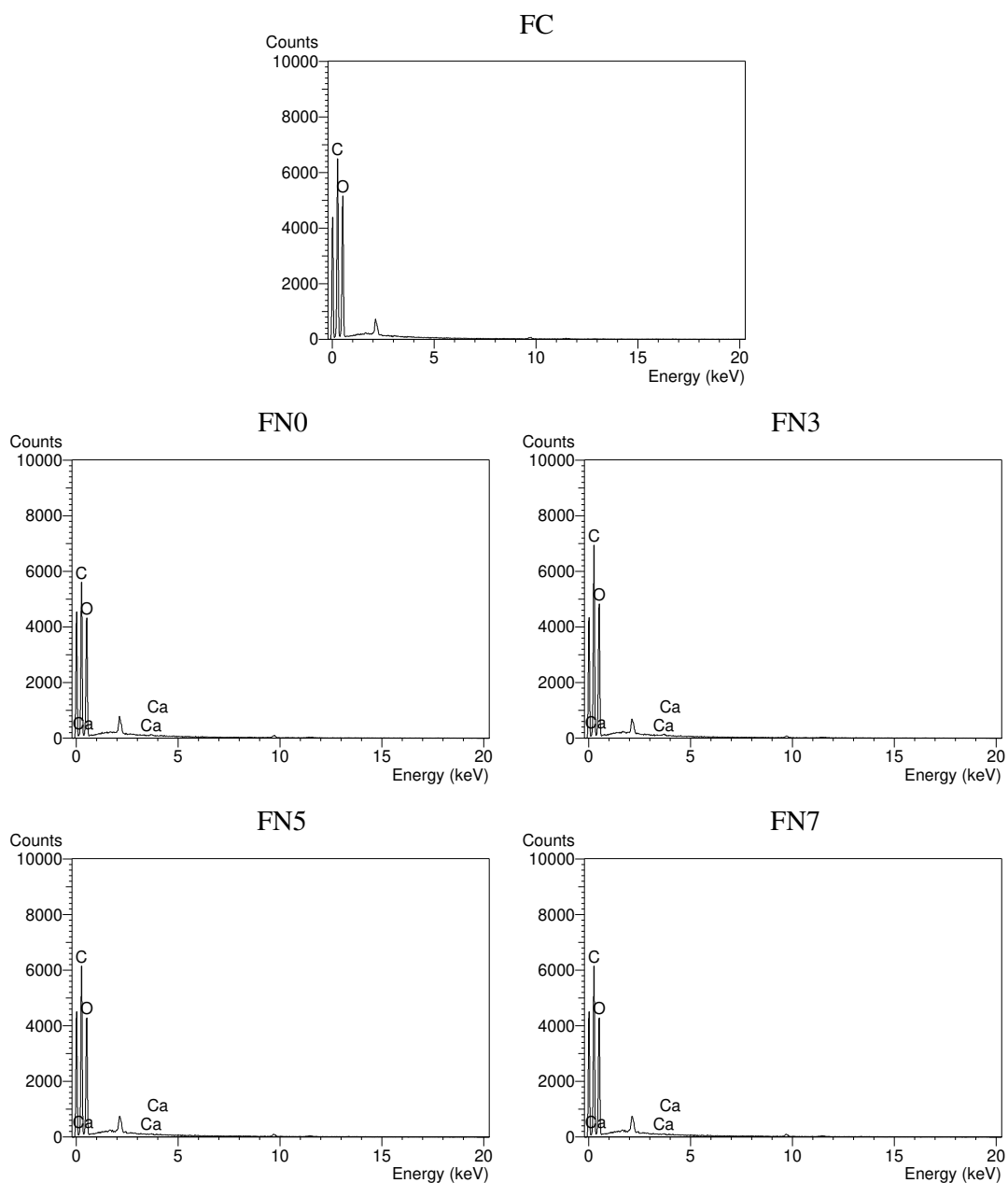


Figure 3. EDS spectra of the (a) control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

3.1.2. Thickness, density, and moisture content

The similar thickness values achieved for the films resulted from the strict control of the dry mass content per unit area of plate of the film-forming solutions employed during the casting procedure (Table 1). On the other hand, the incorporation

of cellulose nanofibers elicited a statistically significant difference in the density values obtained for the films, with the control film presenting higher density than the nanocomposites ($p < 0.05$; Table 1). The addition of cellulose nanofibers to the nanocomposites affected the density, as a result of the type of microstructure observed in the SEM analysis (Figure 2). The micrographs proved that the structure of the nanocomposites is more open, more porous, and less dense than the structure of the control film. The incorporation of cellulose nanofibers also reduced the moisture content in the nanocomposites (Table 1), because the cellulose nanofibers have lower affinity for water as compared with starch. Müller et al. (2009a) reported that the moisture content of composites decreased upon addition of cellulose fibers, as observed in this work.

Table 1. Thickness, density, and moisture content of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

Sample	Thickness (μm)	Density (g/cm^3)	Moisture content (%)
FC	85 ± 1^a	1.21 ± 0.01^a	15.9 ± 0.4^a
FN0	85 ± 1^a	1.15 ± 0.01^b	15.6 ± 0.2^{ab}
FN3	85 ± 4^a	1.17 ± 0.01^b	15.2 ± 0.3^b
FN5	86 ± 1^a	1.17 ± 0.01^b	14.9 ± 0.2^{bc}
FN7	86 ± 3^a	1.15 ± 0.01^b	14.5 ± 0.1^c

^{a,b,c} Different letter superscripts in the same column indicate a statistically significant difference ($p < 0.05$).

3.1.3. Mechanical properties

Table 2 lists the mechanical properties tensile strength, elongation at break, and Young's modulus of the films; Figure 4 illustrates the corresponding stress-strain curves. Compared with the control film, the tensile strength and Young's modulus of the nanocomposites increased upon addition of cellulose nanofibers, whereas the elongation at break decreased. This means that incorporating cellulose crystallites into the starch matrix results in strong interactions between cellulose crystallites and between cellulose crystallites and the starch matrix, which restrict the chain motion of the starch matrix (LU et al., 2005). The typical curves of tensile properties confirmed

that the control film was more flexible; i.e., it had higher elongation at break, and that the nanocomposites were more mechanically resistant. Other authors detected similar behavior for the mechanical characteristics of biodegradable films reinforced with cellulose nanofibers (AZEREDO et al., 2009; CHANG et al., 2010; LU et al., 2005; WANG et al., 2006).

Table 2. Mechanical properties of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

Sample	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)
FC	7.3 ± 0.5^a	32.2 ± 1.4^a	478.6 ± 51.3^a
FN0	8.9 ± 0.5^b	25.9 ± 0.9^b	768.6 ± 80.9^b
FN3	10.1 ± 0.3^{bc}	21.6 ± 1.3^c	966.8 ± 76.0^c
FN5	11.1 ± 0.5^c	21.4 ± 0.4^c	1047.7 ± 13.4^c
FN7	9.9 ± 0.4^{bc}	20.7 ± 1.3^c	663.1 ± 76.7^b

^{a,b,c} Different letter superscripts in the same column indicate a statistically significant difference ($p < 0.05$).

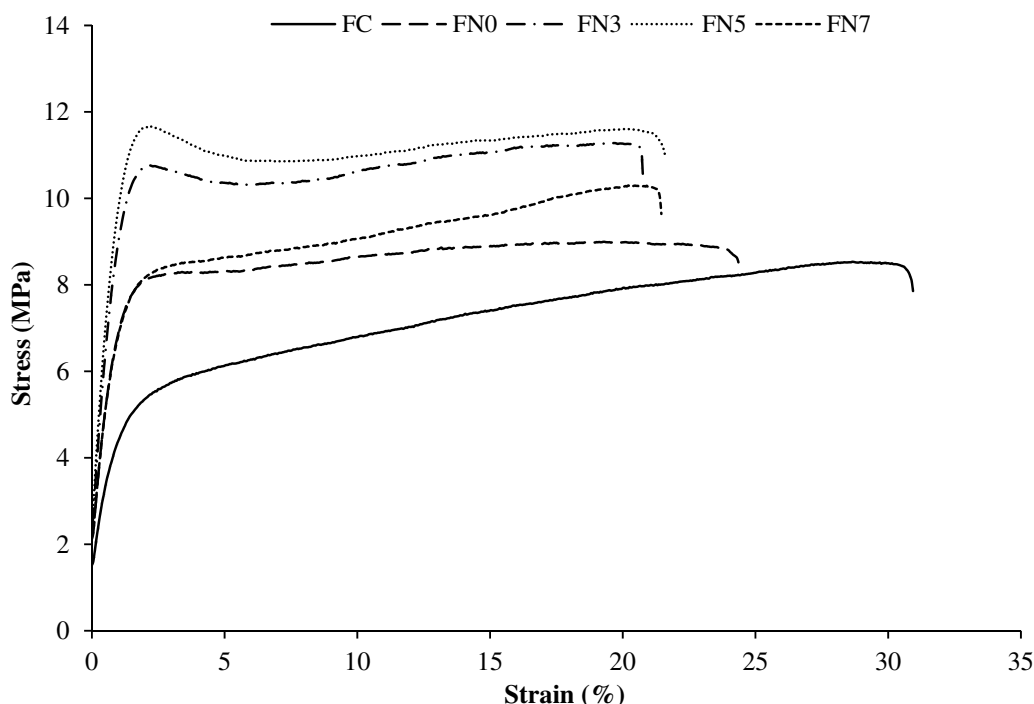


Figure 4. Typical stress-strain curves obtained for the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

In this study, incorporation of the cellulose nanofibers affected the Young's modulus the most; this parameter increased more than 100% upon addition of the N3 and N5 nanofibers. Helbert et al. (1996) attributed this effect to the formation of a fibrillar network within the polymer matrix, the cellulose nanofibers probably interact through hydrogen bonds.

The tensile strength and Young's modulus of the nanocomposites improved with increasing number of passages of nanofibers through the homogenizer, from 0 to 5 passages. This is because the zeta potential of the nanofiber suspensions rose with mechanical treatment ($N0 = -16.1 \text{ mV} < N3 = -28.4 \text{ mV} < N5 = -36.6 \text{ mV} < N7 = -44.1 \text{ mV}$), preventing the formation of aggregates, giving more stable colloidal suspensions with individualized cellulose fibers, and promoting a strong network of nanofibers inside the material (BESBES et al., 2011; DUFRESNE et al., 2000). However, after seven passages through the homogenizer, the mechanical resistance of the nanocomposites reinforced with these nanofibers diminished. This result indicates that more severe mechanical treatment degraded the homogenized fibers.

Fibrillated nanofibers play a role in halting cracks in the nanocomposites and contribute to high elongation and strength (SAMIR et al., 2005). In this study, the elongation at break was supposed to be affected by the aspect ratio of the homogenized nanofibers, because nanofibers with low aspect ratio are rigid and easy to pull out from the aggregated fibers. The nanocomposites containing homogenized nanofibers (lower aspect ratio; $N3 = 29.4$, $N5 = 20.8$, $N7 = 15.1$) were more brittle than the nanocomposite containing non-homogenized nanofiber (higher aspect ratio; $N0 = 42.7$). Therefore, mechanical treatment shortens the fibers and reduces the elongation at break (IWAMOTO et al., 2007; NAKAGAITO and YANO, 2004).

3.1.4. Solubility in water

As well known, starch films are hydrophilic due to the chemical composition of starch (amylose and amylopectin), where hydroxyl groups and oxygen bonds with water are formed (KUCIEL and LIBER-KNEC, 2009). Also, the addition of a plasticizer increases the affinity of the material for moisture (MATHEW and DUFRESNE, 2002). Table 3 shows that, except for FN7, all the nanocomposites were less water-soluble (16 – 26% reduction) as compared with the control film. This may be due to (a) better interfacial bonding between the nanofibers, as observed in the SEM studies, (b) greater

affinity of the matrix for water as compared with the nanofibers, and (c) formation of the nanofiber network, which hinders water diffusion through the matrix (ALVAREZ et al., 2003; RAMÍREZ et al., 2011).

The incorporation of nanofibers N7 into the starch matrix did not diminish the solubility in water. The degradation of nanofibers during the drastic mechanical treatment probably weakened the nanofiber network inside the material.

Table 3. Solubility in water and water vapor permeability of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

Sample	Solubility in water (%)	WVP (10^{-11} g/m.s.Pa)		
		2 – 33%	33 – 64%	64 – 90%
FC	32.3 ± 1.5^a	10.7 ± 0.1^a	27.5 ± 0.2^a	39.5 ± 0.6^a
FN0	27.2 ± 1.4^{bc}	5.7 ± 0.5^b	16.4 ± 0.4^b	17.2 ± 0.2^b
FN3	25.4 ± 1.4^{bc}	4.0 ± 0.1^c	13.0 ± 0.6^c	12.0 ± 0.6^c
FN5	23.9 ± 0.9^c	3.5 ± 0.5^c	11.2 ± 0.1^d	8.9 ± 0.7^d
FN7	29.0 ± 1.6^{ab}	6.0 ± 0.1^b	18.8 ± 0.1^e	25.4 ± 0.4^e

^{a,b,c,d,e} Different letter superscripts in the same column indicate a statistically significant difference ($p < 0.05$).

3.1.5. Water vapor permeability (WVP)

Table 3 presents the water vapor permeability (WVP) of films in three different gradients of relative humidity (RH): 2 – 33%, 33 – 64%, and 64 – 90%. Unlike conventional plastics, biodegradable films are hygroscopic. Different factors affect film permeability; for instance, the relative humidity to which they are exposed. Studying the WVP of films in different gradients of RH helps assess how well the films promote or inhibit exchange between the product and the environment. In other words, we can identify whether the films are potentially applicable as packaging material (MOORE et al., 2006). The RH conditions used for film conditioning modify their crystallinity as well as their mechanical and barrier properties (MÜLLER et al., 2009b).

The nanocomposites had lower WVP than the control film in all gradients ($p < 0.05$; Table 3). Reduced WVP elicited by cellulose nanofiber incorporation improved the characteristics of the nanocomposites, considering the hydrophilicity of the matrix. The lower WVP in the presence of cellulose nanofibers agrees with results reported in the case of nanocomposites for packaging applications (BILBAO-SAINZ et al., 2011;

CHANG et al., 2010; SVAGAN et al., 2009; WANG et al., 2006). Regarding hydrophilic films, WVP is associated with water adsorption and the water diffusion rate (KESTER and FENNEMA, 1986). Considering the tortuous pathways theory of composite materials (NIELSEN, 1962; PINNAVAIA and BEALL, 2000), the diffusivity rate should decrease in the presence of the nanofibers, explaining the lower WVP of the nanocomposites. However, the nanoparticles must be fully dispersed in the biopolymer phase and the fibers, and the biopolymer must interact strongly. Thus, the lower WVP of nanocomposites with increasing number of passages through the homogenizer must be due to the fact that the smaller nanofibers were more properly dispersed and entangled inside the matrix than the longer nanofibers. Lower WVP results from the better dispersion and formation of a network of interwoven fibers, because the water molecules have to go through a more tortuous path in the nanocomposites.

The nanocomposite FN5 was less permeable, with WVP reductions of 67, 59, and 77% compared to the control film, for the three RH gradients (Table 3). FN3 and FN5 became less permeable when exposed to the high RH gradient (64 – 90%), as compared with the intermediate RH gradient (33 – 64%). Therefore, these films can be used in products with high moisture content.

Changes in WVP stemming from the effect of the added filler depend on the size and aspect ratio of the filler (PINNAVAIA and BEALL, 2000). The micrographs in Figure 2 reveal that the nanocomposites have a homogeneous matrix, which probably enhanced the ability of the nanofibers to diminish WVP.

3.1.6. Water uptake

The water absorption behavior of nanocomposites was studied at 25 °C and 95% RH. Figure 5 shows the water uptake for the control film and nanocomposites reinforced with cellulose nanofibers as a function of time. Two well-separated zones are observed: at lower times (zone I: $t < 150$ h), the water absorption kinetics is fast; at times longer than 150 h the water absorption kinetics is slow, resulting in a plateau (zone II). In both zones I and II, the nanocomposites presented lower water uptake as compared with the control film. Water uptake at equilibrium, M_{∞} , decreased with increasing number of passages through the homogenizer; the nanocomposite FN5 had the lowest M_{∞} (24.91%).

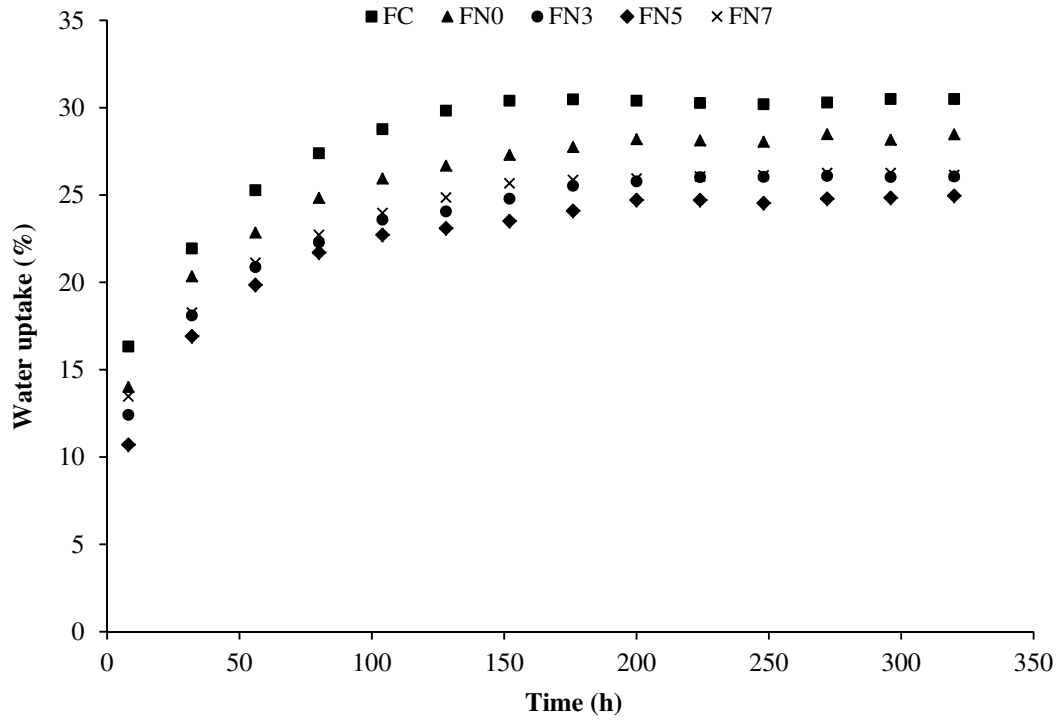


Figure 5. Water uptake during conditioning at 95% RH as a function of time for the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

Theoretically, Fick's second law of diffusion (Equation 3) describes water absorption processes in polymer composites; this law has been employed by several authors (BILBAO-SAINZ et al., 2011; DUFRESNE et al., 2000; LU et al., 2005; KAUSHIK et al., 2010):

$$\frac{\partial C}{\partial t} = D \left(\frac{\partial^2 C}{\partial x^2} \right) \quad [3]$$

where C is the concentration at time t , D is the diffusion coefficient, and x is the coordinate in the thickness direction. The boundary and initial conditions are:

$$\begin{aligned} C &= 0, & 0 < x < L, & & t &= 0 \\ C &= C_{\infty}, & x &= 0, & & t > 0 \\ C &= C_{\infty}, & x &= L, & & t > 0 \end{aligned}$$

Equation 4 gives the general series solution of Fick's second law for plane sheet geometry assuming molecular migration by diffusion, negligible shrinkage, and constant diffusion at short times:

$$\frac{M_t}{M_\infty} = \frac{4}{L} \left(\frac{Dt}{\pi} \right)^{1/2} \quad [4]$$

where M_t and M_∞ are the water contents in the sample at time t and at equilibrium, respectively; L is the sample thickness; and D is the diffusion coefficient that can be calculated from the slope of the linear portion of the curve M_t/M_∞ vs $t^{1/2}$ (Figure 6), where $R^2 \geq 0.99$.

Equation 5 furnishes the sorption coefficient (S):

$$S = \frac{m_\infty}{m_p} \quad [5]$$

where m_∞ represents the mass of solvent taken up at the equilibrium and m_p represents the initial dry mass of the sample.

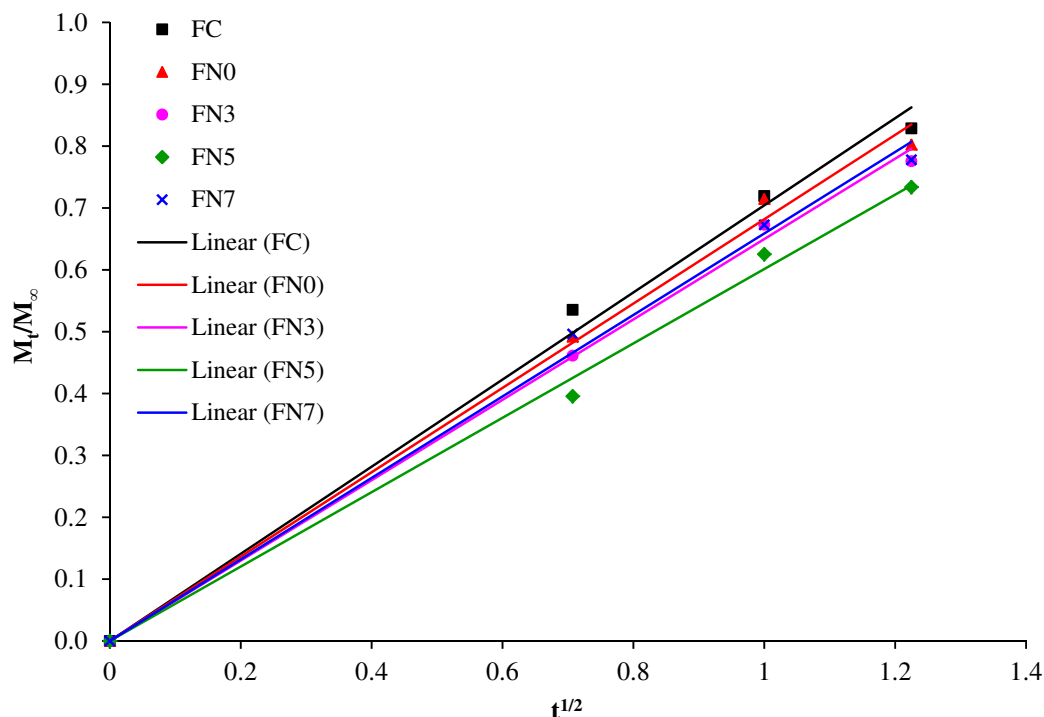


Figure 6. Initial part of water absorption curves of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

Table 4 summarizes the typical kinetic parameters (M_∞ , D , and S). The diffusivity decreases from $2.73 \times 10^{-7} \text{ mm}^2/\text{s}$ for the control film to $1.71 \times 10^{-7} \text{ mm}^2/\text{s}$ for the nanocomposite FN5. The high diffusivity of pure starch film stems from its hygroscopic nature, which makes it prone to water absorption. The diffusivity decreases as a function of the addition of nanofibers, reducing water diffusion due to hydrogen bonding between the matrix and the fiber phases within the nanocomposite and increasing tortuosity (SREEKALA et al., 2008). Strong hydrogen bonding interactions between starch and cellulose crystallites decrease diffusivity, because these interactions tend to stabilize the starch matrix when the nanocomposites are submitted to high humidity. Reduced water absorption by the film is strongly related to the diminished diffusion coefficient imposed by the nanofibers and to decreased sorption of the penetrant. However, increasing the number of passages of the nanofibers through the homogenizer to above 5 slightly augments diffusivity and the amount of absorbed water (see values for the nanocomposite FN7). Drastic mechanical treatment may degrade the nanofibers, reducing matrix cohesion. The interactions between starch and cellulose

become weaker, which is detrimental to the barrier properties (SANCHEZ-GARCIA et al., 2008). Nakagaito and Yano (2004) also observed a similar behavior.

Table 4. Barrier properties of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

Sample	M_{∞} (%)	D (10^{-7} mm ² /s)	S
FC	30.50 ± 0.27	2.73 ± 0.10	0.42 ± 0.02
FN0	28.48 ± 0.26	2.44 ± 0.05	0.27 ± 0.01
FN3	26.06 ± 0.42	2.05 ± 0.08	0.27 ± 0.01
FN5	24.91 ± 0.33	1.71 ± 0.09	0.16 ± 0.01
FN7	26.15 ± 0.25	2.20 ± 0.12	0.27 ± 0.01

Water content at equilibrium (M_{∞}), diffusion coefficient (D), and sorption coefficient (S).

3.1.7. Optical properties

All the differences between the control film and the nanocomposites in terms of optical properties were statistically significant ($p < 0.05$; Table 5). Based on the L^* , b^* , and ΔE^* values, the incorporation of cellulose nanofibers produced darker and more yellowish nanocomposites with less uniform color than the control film. The parameter a^* values were negative and close to zero for all the films, indicating the absence of characteristic tones of red.

Table 5. Optical properties of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

Sample	L^*	a^*	b^*	ΔE^*	Opacity (%)
FC	95.66 ± 0.05^a	-0.02 ± 0.01^a	0.72 ± 0.01^a	0.81 ± 0.04^a	26.1 ± 0.5^a
FN0	95.14 ± 0.08^b	-0.04 ± 0.01^b	0.95 ± 0.01^b	1.37 ± 0.07^b	45.5 ± 0.2^b
FN3	95.14 ± 0.04^b	-0.04 ± 0.01^b	1.01 ± 0.01^c	1.39 ± 0.03^b	46.2 ± 0.2^c
FN5	95.12 ± 0.04^b	-0.04 ± 0.01^b	1.01 ± 0.01^c	1.42 ± 0.04^b	49.1 ± 0.4^d
FN7	94.99 ± 0.01^c	-0.04 ± 0.01^b	1.05 ± 0.01^d	1.55 ± 0.01^c	49.6 ± 0.2^d

^{a,b,c,d} Different letter superscripts in the same column indicate a statistically significant difference ($p < 0.05$).

The control film was more transparent than the nanocomposites (Table 5), implying that reinforcement with nanofibers increases opacity (74 to 90%). This phenomenon may result from: (a) nanometer size effects due to the presence of solid particles, (b) strong interactions between the cellulose nanofibers and the starch matrix, (c) high aspect ratio of the cellulose nanofibers, and (d) random distribution of the nanoparticles in the matrix. Increasing number of passages through the homogenizer from 0 to 7 raised the opacity values of the nanocomposites slightly, probably because the homogeneous dispersion of the nanofibers within the composite diminished film transparency. Bilbao-Sainz et al. (2011) found that hydroxypropyl methylcellulose films reinforced with cellulose nanofibers behave in the same way; Besbes et al. (2011) reported reduced transparency degree for nanofiber suspensions from alpha fibers with increasing number of passages through the high-pressure homogenizer.

Figure 7a depicts the appearance of the nanocomposites reinforced with cellulose nanofibers isolated from the banana peel. Compared with the nanocomposites (Figure 7b), the control film presented higher gloss values, which were more pronounced for the surface exposed to air drying (E). The gloss property is strictly related to surface texture; i.e., to the polishing degree of the analyzed surface (MORAES et al., 2008). The particle size distribution employed in the film formulation influences this property: more uniform particle size distribution yields more polished, brighter surfaces (TREZZA and KROCHTA, 2001), justifying the gloss results obtained for the evaluated biodegradable films (remember that cellulose nanofibers have different diameters and length). The higher roughness of the nanocomposites surfaces (Figure 2) also explains the lower gloss values and the higher opacity of these films.

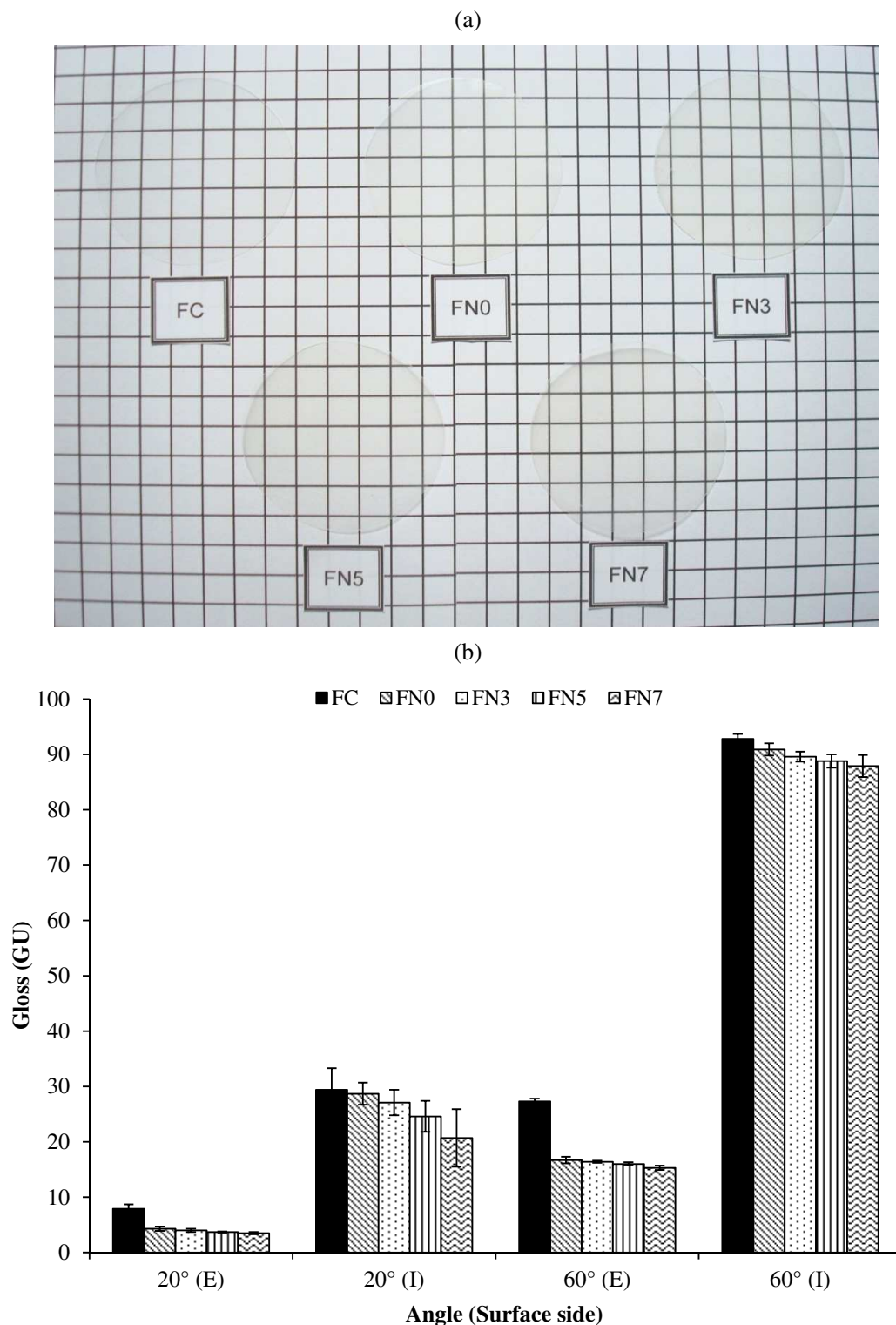


Figure 7. (a) Appearance of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times. (b) Gloss values of the films, where E is the surface side exposed to air drying and I is the surface side in contact with the support plate.

3.1.8. X-ray diffraction (XRD)

The XRD of the nanocomposites was analyzed as a function of the number of passages of the cellulose nanofibers through the high-pressure homogenizer; Figure 8 contains the corresponding diffractograms. The XRD diffractogram of the control film (FC) displayed a typical C-type crystallinity pattern with peaks at $2\theta = 5.6^\circ$, 12° , 17° , and 20° , typical of B-type, A-type, both A- and B-type, and B-type polymorphs, respectively, corroborating the results obtained for the banana starch film in Section 3.1.9 of Chapter 4. The crystalline structure results from spontaneous recrystallization or retrogradation of starch molecules after gelatinization. Researchers have frequently detected this phenomenon in starch-based thermoplastic materials (CAO et al., 2008).

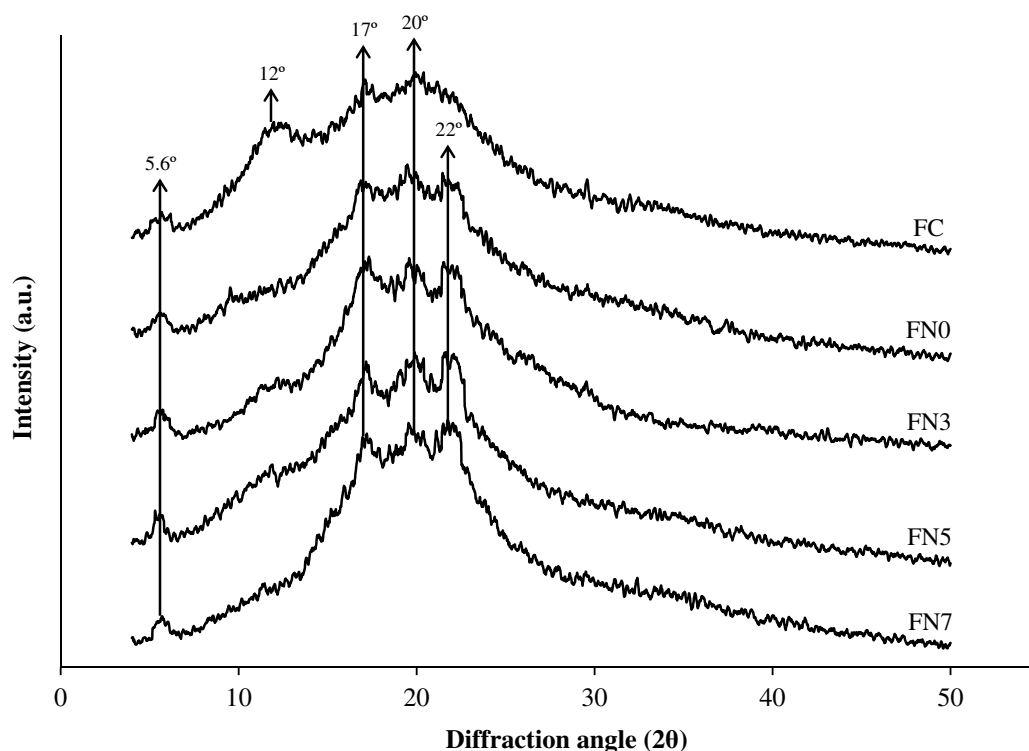


Figure 8. X-ray diffraction patterns of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

Addition of cellulose nanofibers to the starch matrix furnished a peak at $2\theta = 22^\circ$, relative to the XRD pattern of cellulose I. Hence, the cellulose nanofibers retained

their crystallinity within the matrix (CHEN et al., 2009; NISHINO et al., 2004; LI et al., 2009). The broad peak at $2\theta = 12^\circ$ was absent, which suggests changes in the structure of the nanocomposites.

The presence of cellulose nanofibers altered the XRD patterns of the nanocomposites slightly. The peaks at around $2\theta = 5.6^\circ$, 17° , and 20° seemed sharper than those of the control film. Increasing the number of passages through the homogenizer from 0 to 7 raised the relative intensity of the peaks, indicating that longer mechanical treatment removed more amorphous regions in the cellulose nanofibers, corroborating the crystallinity indices (I_c) found for cellulose nanofibers in Chapter 5 ($N0 = 58.6\%$, $N3 = 61.8\%$, $N5 = 62.4\%$, and $N7 = 64.9\%$). This fact is consistent with the I_c of the films obtained in this study ($FC = 13.5\%$, $FN0 = 16.7\%$, $FN3 = 24.4\%$, $FN5 = 26.2\%$, and $FN7 = 21.8\%$). Incorporation of cellulose nanofibers increased the I_c of nanocomposites from 24 to 94% compared with the control film, suggesting that the incorporation of highly crystalline fillers enhances the crystallinity of the nanocomposites. Guimarães et al. (2010) reported crystallinity values between 20 – 21% for composites based on starch and banana/sugarcane bagasse fibers plasticized with 30 g of glycerol/100 g of starch; Müller et al. (2009b) obtained crystallinity values of about 50% for cassava starch-cellulose fiber composites (30 g of glycerol/100 g of starch); and Famá et al. (2009) found I_c between 11.0 – 13.3% for cassava starch films reinforced with wheat fibers (25 g of glycerol/100 g of starch). Factors such as the relative humidity of composite storage, film formulation, chemical composition, and size and crystallinity of the fibers used as reinforcement affect the crystallinity of these materials.

3.1.9. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded for the control film and nanocomposites, to compare the types of interactions taking place in the film structures; Figure 9 depicts the spectra. Due to the chemical similarities between starch and cellulose, and to the relatively low content (5 wt%) of nanofibers within the matrix, the nanocomposites exhibited almost the same FTIR spectra as the control film. Increasing number of passages in the high-pressure homogenizer did not influence the spectra of the nanocomposites.

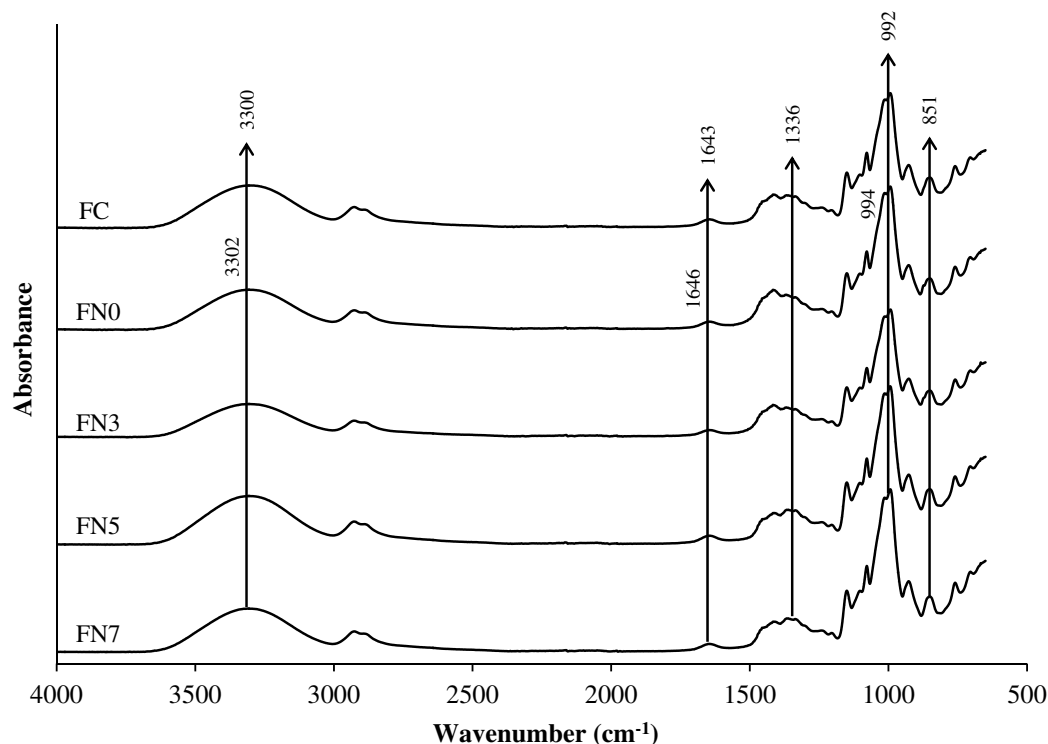


Figure 9. FTIR spectra of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

All the films displayed bands at 2927 and 2891 cm^{-1} , corresponding to C–H stretching. The bands at 3300, 1643, and 992 cm^{-1} in the spectrum of the control film refer to stretching vibrations of the hydrogen bonding –OH groups, bending vibrations of the –OH groups, and stretching vibrations of the C–O in starch, respectively (CHEN et al., 2009). These bands shifted to 3302, 1646, and 994 cm^{-1} in the spectra of the nanocomposites, suggesting that new hydrogen bonding interactions between cellulose and starch molecules result from the addition of cellulose nanofibers to the starch.

The band at 1336 cm^{-1} is attributed to bending of the –COH and –CH groups of cellulose in the spectra of the nanocomposites; in the spectrum of the control film, this band is associated with the presence of the amide III group, which was also identified in the spectrum of the banana starch film in Section 3.1.10 of Chapter 4. The presence of C–O and C–C groups gives a band at 1150 cm^{-1} . The 1077 cm^{-1} band observed in all the spectra is related to stretching of C–OH bond in the starch (VAN SOEST et al., 1995) and stretching vibrations of C–O in cellulose (ZULUAGA et al., 2007).

The characteristic absorption of cellulose at around 893 cm^{-1} in the spectrum of the nanofibers represents β -glycosidic linkages between the anhydroglucose units in cellulose (ALEMDAR and SAIN, 2008); this band was absent in the spectra of the nanocomposites (see Section 3.1.10 of Chapter 4). Instead, a band at 851 cm^{-1} was detected and ascribed to the symmetrical stretching of C–O–C bond and CH and CH₂ deformation in the starch. Chen et al. (2009) reported similar results for nanocomposites based on pea starch reinforced with cellulose nanofibers from pea hull fiber.

3.1.10. Differential scanning calorimetry (DSC)

Figure 10 shows the DSC curves of the control film and the resulting nanocomposites conditioned at 58% RH. Table 6 displays the corresponding glass transition temperatures (T_g). The control film exhibits T_g at about 20.1 °C. The formulation used here consists of a complex heterogeneous system containing glycerol-rich domains dispersed in a starch-rich continuous phase, and each component should exhibit its own T_g. The T_g at 20.1 °C was associated with the glass transition of the starch-rich phase. However, the T_g of the glycerol-rich phase, located between -80 and -50 °C, could not be determined because refrigerated cooling of the DSC systems is limited. Incorporation of the cellulose nanofibers into the starch matrix shifted the T_g of the starch-rich phase to higher temperature; from 26.8 to 34.5 °C. Increased T_g ($p < 0.05$; Table 6) is consistent with the mechanical properties (more rigid and less flexible) and the decreased moisture content of the films upon addition of the nanofiber (Table 1). Water is a plasticizer; diminished water content in the film reduces the plasticizing effect and favors the formation of more thermally stable materials. The mechanical treatment of the cellulose nanofibers also improved the T_g of the nanocomposites; FN5 presented the highest T_g value, probably because the homogenized nanofibers are better distributed in the matrix, promoting more cellulose-starch and cellulose-glycerol interactions.

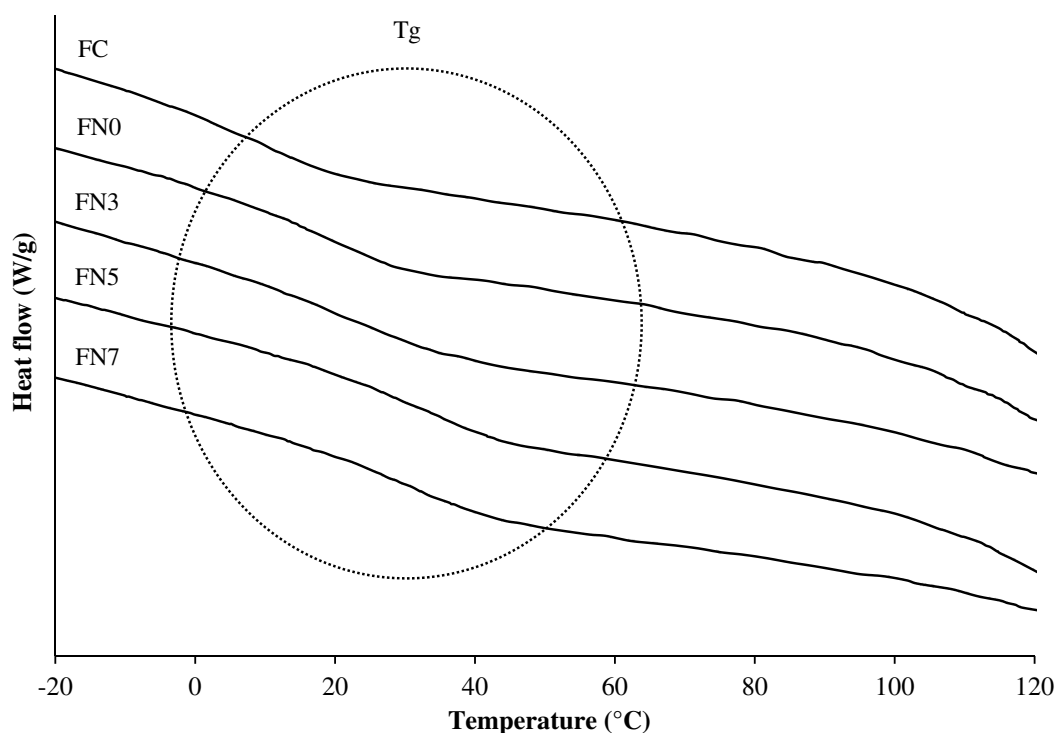


Figure 10. DSC thermograms of the control film (FC) and nanocomposites reinforced with cellulose nanofibers obtained by different number of passages through the high-pressure homogenizer (FN0, FN3, FN5, and FN7).

Table 6. Glass transition temperature (T_g) of the control film (FC) and nanocomposites reinforced with cellulose nanofibers that were passed through the high-pressure homogenizer 0 (FN0), 3 (FN3), 5 (FN5), and 7 (FN7) times.

Sample	T_g (°C)
FC	20.1 ± 0.1^a
FN0	26.8 ± 0.9^b
FN3	29.6 ± 0.3^c
FN5	34.5 ± 0.1^d
FN7	32.8 ± 0.4^e

^{a,b,c,d,e} Different letter superscripts in the same column

indicate a statistically significant difference ($p < 0.05$).

Several authors have reported increased T_g for films upon addition of cellulose nanofibers (CAO et al., 2008; LI et al., 2009; LU et al., 2005). This is a result of certain factors that occur simultaneously, such as:

- (i) Cellulose-water interactions guide the redistribution of water molecules on within the polymer matrix, reducing the plasticizer effect of water (KUMAR and SINGH, 2008).
- (ii) Strong interactions between the matrix and cellulose nanofibers, mainly between the hydroxyl groups of starch and cellulose, reduce matrix mobility. According to Anglès and Dufresne (2000), the amylopectin molecules and reactive cellulose surface have a strong affinity, because both components contain a high density of hydroxyl groups. This coupling effect could culminate in restricted molecular mobility of amylopectin molecules in contact with the nanofibers surface. Owing to the very high specific surface of cellulose nanofibers, this hindered mobility could be strong enough to affect the global flexibility of the starch matrix.
- (iii) Selective partitioning of glycerol within the material in the presence of cellulose nanofibers, with glycerol presenting higher affinity for the cellulose surface than for the starch-based matrix. The main plasticizer migrates from the starch-rich domains toward the filler/matrix interface, decreasing the plasticizing efficiency of glycerol for the starch matrix. This phenomenon should result in increased of T_g and could be more pronounced emphasized in moist conditions (ANGLÈS and DUFRESNE, 2000).

4. Conclusions

Compared with the control film, the nanocomposites reinforced with cellulose nanofibers present higher tensile strength, Young's modulus, water-resistance, opacity, crystallinity, and thermal stability. The improved performance of the nanocomposites were attributed to (a) the chemical similarities between starch and cellulose, (b) the fact of these components are from the same source, (c) the nanometric size effect of the nanofibers, and (d) the hydrogen bonding interactions between the fillers and the matrix. Regarding the mechanical treatment, as the number of passages through the high-pressure homogenizer increased, the nanocomposites containing homogenized nanofibers (FN3 and FN5) displayed better properties than the nanocomposite produced with the non-homogenized nanofibers (FN0). On the other hand, the drastic mechanical treatment involving seven passages through the homogenizer degraded the cellulose nanofibers, deteriorating the properties of nanocomposite FN7. Hence, we considered that five passages through the homogenizer was the most suitable condition for the mechanical treatment of cellulose nanofibers, because we obtained nanocomposites with improved features.

Starch and cellulose nanofibers isolated from unripe bananas are potentially applicable as matrix and reinforcing material in biodegradable films, respectively. Furthermore, any agricultural residue, in this case banana peel, can be a source for processing cellulose nanofibers. The nanocomposites produced in this work could find applications in the food packaging industry because they are biocompatible, biodegradable, and non-toxic.

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Conclusões Gerais

A realização deste estudo demonstrou a potencialidade da banana (*Musa paradisiaca*) bem como o seu aproveitamento integral através da utilização da farinha e do amido (extraídos da polpa) e das nanofibras de celulose (extraídas da casca) como matérias-primas para a elaboração de embalagens biodegradáveis, propondo uma alternativa para o uso comercial desta fruta e uma finalidade para a casca.

A farinha e o amido foram obtidos a partir de bananas verdes da variedade Terra com rendimento de 50,6% e 28,5% respectivamente, em relação ao peso seco da fruta. As metodologias empregadas demonstraram-se eficientes na obtenção das matérias-primas cujas características físico-químicas e funcionais foram satisfatórias para o desenvolvimento de filmes biodegradáveis.

As propriedades dos filmes de farinha de banana foram influenciadas pela formulação e condições de processo estudadas (concentração de glicerol, temperatura de processo e temperatura e umidade relativa durante a secagem). A metodologia de otimização empregada neste estudo mostrou-se eficaz na obtenção da formulação e condições de processo que permitiram maximizar a resistência e a flexibilidade dos filmes em termos de tensão na ruptura, alongação e módulo de Young, e minimizar sua permeabilidade ao vapor de água. Tais condições otimizadas foram: concentração de glicerol de 19 g de glicerol/100 g de farinha, temperatura de processo de 81 °C, temperatura de secagem de 54 °C e umidade relativa na secagem de 48%.

A farinha e o amido de banana são materiais promissores a serem empregados na elaboração de filmes biodegradáveis, dada sua interessante composição centesimal. Cabe ressaltar seu considerável conteúdo de amido (83,2 e 94,8% respectivamente) com alto teor de amilose (23,1 e 35,0% respectivamente) e amido resistente (49,5 e 50,3%, respectivamente), além de fibras, proteínas e lipídios. Em função dos tipos de interações formadas pelos componentes da fração não amilácea (fibra, proteína e lipídio) presente em maior proporção na farinha do que no amido de banana, os filmes de farinha apresentaram características de maior conteúdo de umidade, flexibilidade e solubilidade em água quando comparados com os filmes de amido da mesma espécie.

Neste trabalho foram isoladas fibras de celulose de tamanho nanométrico a partir da casca da banana. O processo desenvolvido para a obtenção dessas nanofibras se mostrou satisfatório na remoção de componentes amorfos presentes na casca, aumentando a cristalinidade do material e levando também à obtenção de cargas

superficiais eletronegativas nas nanofibras. Essas características de tamanho, cristalinidade e carga superficial são de extrema importância para conseguir uma boa dispersão e adesão do reforço (nanofibras) na matriz polimérica.

A etapa do tratamento mecânico com diferente número de passagens pelo homogeneizador de alta pressão influenciou as características físico-químicas das nanofibras isoladas. Conforme o aumento do número de passagens (0, 3, 5 e 7), suspensões de nanofibras mais estáveis, de menor comprimento, maior cristalinidade e estabilidade térmica foram obtidas. Estes resultados são promissores para o estudo de materiais lignocelulósicos que podem ser aproveitados a partir de fontes similares a casca da banana, como alguns resíduos agroindustriais ainda não explorados neste campo.

A incorporação de nanofibras de banana na matriz de amido da mesma fonte vegetal levou ao incremento da tensão de ruptura e módulo de Young favorecendo a obtenção de nanocompósitos com melhor resistência mecânica quando comparados com o filme controle (sem adição de nanofibras). As propriedades que melhor evidenciaram o efeito benéfico das nanopartículas na matriz de amido foram: a permeabilidade ao vapor de água, solubilidade e absorção de umidade, uma vez que a incorporação de 5% de nanofibras de celulose levou a uma diminuição significativa das três propriedades. Com relação ao tratamento mecânico, uma homogeneização mais drástica (7 passagens) promoveu a degradação das nanofibras acarretando numa piora das propriedades do seu nanocompósito resultante. Para a preparação das nanofibras de celulose e seu nanocompósito correspondente, a condição de tratamento mecânico mais adequada foi de 5 passagens. Os resultados encontrados neste estudo foram associados às características físico-químicas das nanofibras, concentração na matriz polimérica, eficácia do método de incorporação das mesmas e, sobretudo, a boa compatibilidade entre biopolímeros de uma mesma fonte para a elaboração de nanocompósitos.

Sugestões para Trabalhos Futuros

O uso de materiais biodegradáveis é o desafio do futuro. Entre eles, as nanofibras de celulose têm demonstrado um potencial interessante como reforço em compósitos. A partir dos resultados obtidos com a realização do presente trabalho foram identificadas as seguintes linhas de ação para futuras pesquisas nesta área:

- Avaliar as propriedades mecânicas e de barreira dos nanocompósitos em função da concentração de nanofibras, abordando também o estudo de isoterma de sorção;
- Determinar a permeabilidade ao oxigênio dos nanocompósitos a fim de identificar possíveis aplicações como materiais de embalagem e/ou coberturas comestíveis;
- Estudar a degradação em solo e de estabilidade dos nanocompósitos durante seu armazenamento e/ou utilização, submetendo-os a diferentes condições de radiação UV, luz solar, oxigênio e umidade;
- Isolar as nanofibras de celulose empregando oxidação catalítica e tratamento enzimático, bem como determinar suas propriedades, e compará-las com as propriedades das nanofibras de celulose obtidas no presente estudo. Nesta abordagem, avaliar a eficiência na produção das nanofibras por esses tratamentos e possíveis vantagens como reduzir os resíduos gerados pelo tratamento químico e o uso de energia, ganhariam maior interesse na utilização dessas nanofibras;
- Avaliar a toxicologia das nanofibras de celulose frente à saúde humana;
- Aplicar coberturas comestíveis à base de amido de banana reforçadas com nanofibras de celulose em produtos frescos;
- Estudar o efeito das coberturas sobre algumas características de qualidade de produtos frescos, bem como estimar a vida útil desses produtos;
- Estudar a migração das nanofibras de celulose presente na cobertura para o alimento.

Apêndices

Apêndice 1: Ensaio de Gramatura do Filme de Farinha de Banana

Para determinar a quantidade de solução filmogênica (SF) a ser depositada nas placas de suporte foram realizados ensaios preliminares de gramatura a fim de garantir uma espessura constante de $85 \pm 5 \mu\text{m}$ para todos os experimentos. Para isto, gramaturas na faixa de 0,15 a 0,35 g de SF/cm² foram avaliadas para soluções filmogênicas com concentração fixa de 4 g de farinha seca/100 g de SF. Foram testadas as condições dos pontos axiais da variável concentração de glicerol (15 e 30 g de glicerol/100 g de farinha), e as outras variáveis de processo foram fixadas na condição do ponto central (temperatura de processo de 85 °C, temperatura de secagem de 45 °C e umidade relativa na secagem de 50%). Após a secagem foi realizada a medição da espessura dos filmes biodegradáveis.

Os resultados do estudo de gramatura vs espessura dos filmes de farinha de banana contendo diferentes concentrações de glicerol (15 e 30 g de glicerol/100 g de farinha) estão apresentados na Figura 1. Observou-se uma relação diretamente proporcional entre o incremento do conteúdo de plastificante e o aumento da espessura dos filmes. Assim, para garantir o controle da espessura na faixa de $85 \pm 5 \mu\text{m}$ em todos os experimentos, escolheu-se a gramatura de 0,185 g de SF/cm² para ambas as situações, uma vez que nesta faixa a mesma espessura foi obtida nos filmes.

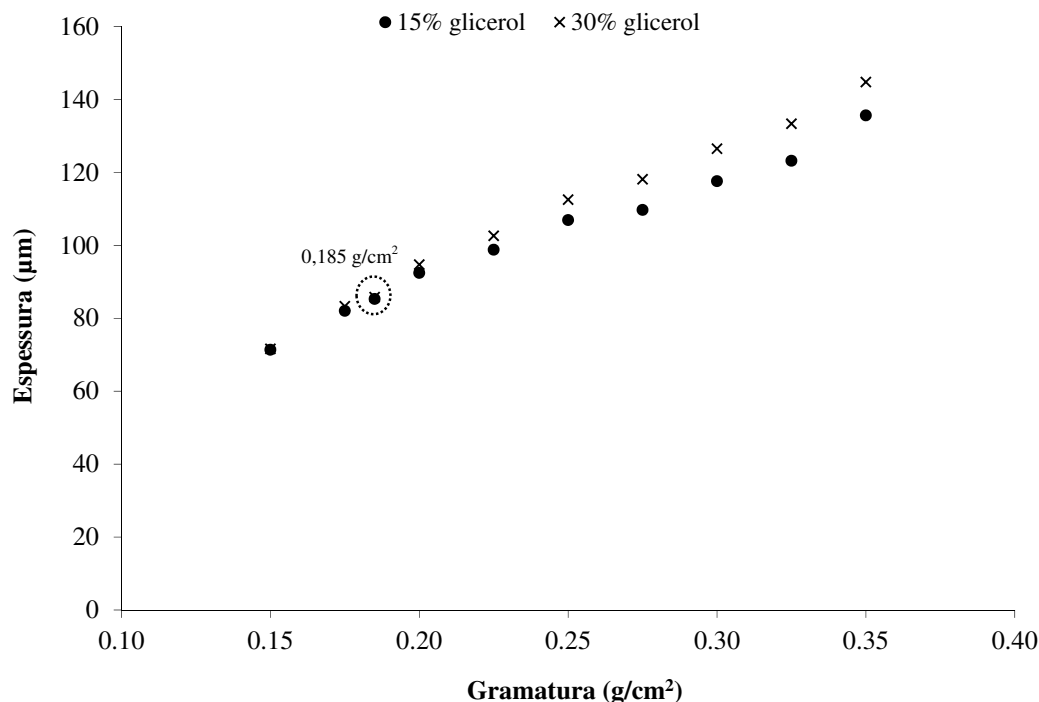


Figura 1. Espessura média dos filmes de farinha de banana contendo diferentes concentrações de glicerol em função da gramatura.

Apêndice 2: Ensaio de Gramatura do Filme de Amido de Banana

Para determinar a quantidade de solução filmogênica (SF) a ser depositada nas placas de suporte foram realizados ensaios preliminares de gramatura a fim de garantir uma espessura constante de $85 \pm 5 \mu\text{m}$ para os filmes de amido e farinha de banana. Para isto, gramaturas na faixa de 0,15 a 0,35 g de SF/cm² foram avaliadas para soluções filmogênicas com concentração fixa de 4 g de amido seco ou farinha seca/100 g de SF elaboradas nas condições ótimas de formulação e processo encontradas no Capítulo 3 para o filme de farinha de banana: concentração de glicerol de 19 g de glicerol/100 g de amido ou farinha, temperatura de processo de 81°C, temperatura de secagem de 54 °C e umidade relativa na secagem de 48%. Após a secagem foi realizada a medição da espessura dos filmes biodegradáveis.

Os resultados do estudo de gramatura vs espessura dos filmes de amido e farinha de banana nas condições ótimas de formulação e processo estão apresentados na Figura 2. Observou-se que o filme de amido é mais espesso do que o filme de farinha para faixas de gramatura superiores a 0,20 g de SF/cm². Assim, para garantir o controle da

espessura em torno de $85 \pm 5 \mu\text{m}$, escolheu-se a gramatura de $0,185 \text{ g de SF/cm}^2$ para ambos os filmes, uma vez que nesta faixa a mesma espessura foi obtida.

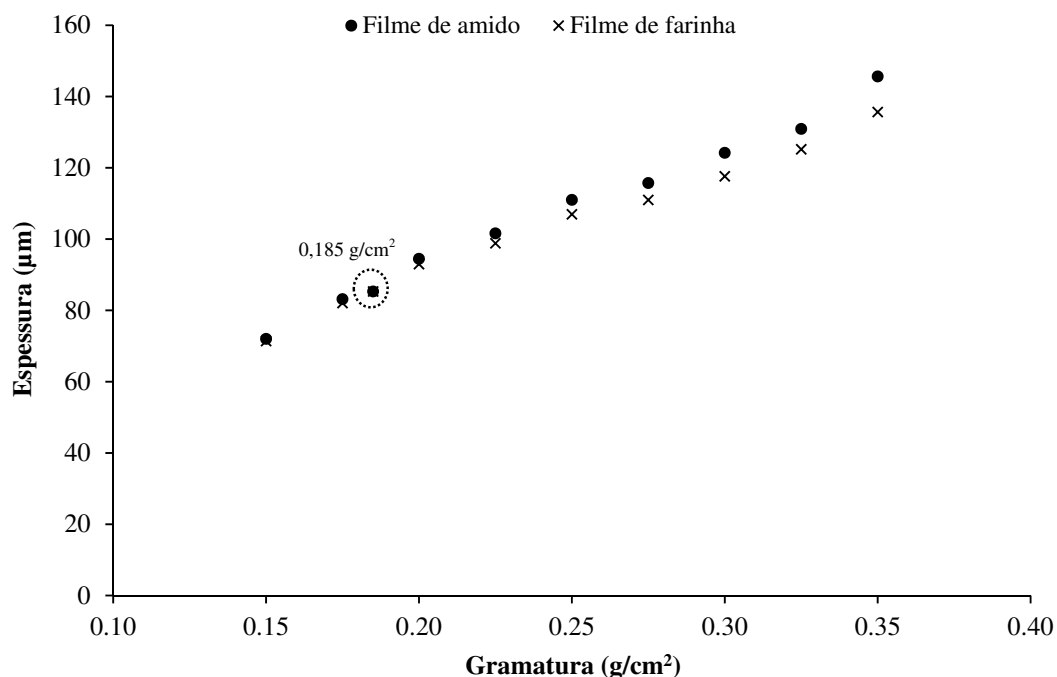


Figura 2. Espessura média dos filmes de amido e farinha de banana nas condições ótimas de formulação e processo em função da gramatura.

Apêndice 3: Ensaio de Gramatura do Nanocompósito

Para determinar a quantidade de solução filmogênica (SF) a ser depositada nas placas de suporte foram realizados ensaios preliminares de gramatura a fim de garantir uma espessura constante de $85 \pm 5 \mu\text{m}$ para os nanocompósitos. Para isto, gramaturas na faixa de 0,15 a 0,35 g de SF/cm² foram avaliadas para soluções filmogênicas com concentrações fixas de 4 g de amido seco/100 g de SF e 25 g de glicerol/100 g de amido. As condições de processo utilizadas foram: temperatura de processo de 81 °C, temperatura de secagem de 54 °C e umidade relativa na secagem de 48%. Foram feito ensaios com as formulações do filme controle (FC; sem adição de nanofibras) e dos nanocompósitos reforçados com 5 g de nanofibras/100 g de amido (FN0 e FN5; sem e com homogeneização). Após a secagem foi realizada a medição da espessura dos filmes biodegradáveis.

Os resultados do estudo de gramatura vs espessura do filme controle e dos nanocompósitos estão apresentados na Figura 3. Observou-se um aumento na espessura dos filmes com a adição das nanopartículas e a presença do tratamento mecânico. Assim, para garantir o controle da espessura na faixa de $85 \pm 5 \mu\text{m}$ nos experimentos, escolheu-se a gramatura de $0,185 \text{ g/cm}^2$ para todas as situações, uma vez que nesta faixa a mesma espessura foi obtida nos filmes.

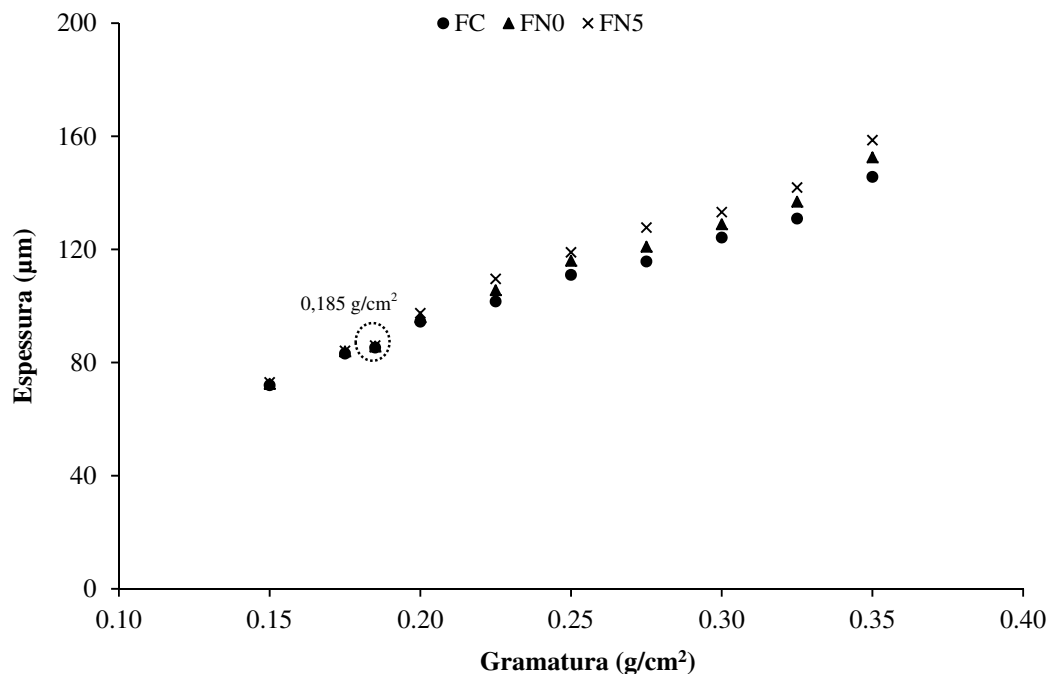


Figura 3. Espessura média do filme controle e dos nanocompósitos em função da gramatura.

Apêndice 4: Artigos Publicados

Até o presente momento três artigos baseados nos resultados coletados nesta Tese de Doutorado foram publicados em revistas internacionais de impacto, abordando a caracterização do amido e da farinha de banana, a otimização da formulação e condições de processo do filme de farinha de banana, e a comparação dos filmes produzidos a partir do amido e da farinha de banana. A seguir são listadas as referências destas publicações:

- PELISSARI, F. M.; ANDRADE-MAHECHA, M. M.; SOBRAL, P. J. A.; MENEGALLI, F. C. Isolation and characterization of the flour and starch of plantain bananas (*Musa paradisiaca*). **Starch - Stärke**, v. 64, n. 5, p. 382-391, 2012.
- PELISSARI, F. M.; ANDRADE-MAHECHA, M. M.; SOBRAL, P. J. A.; MENEGALLI, F. C. Comparative study on the properties of flour and starch films of plantain bananas (*Musa paradisiaca*). **Food Hydrocolloids**, v. 30, n. 2, p. 681-690, 2013.
- PELISSARI, F. M.; ANDRADE-MAHECHA, M. M.; SOBRAL, P. J. A.; MENEGALLI, F. C. Optimization of process conditions for the production of films based on the flour from plantain bananas (*Musa paradisiaca*). **LWT - Food Science and Technology**,doi: <http://dx.doi.org/10.1016/j.lwt.2013.01.011>, 2013.