UNIVERSIDADE FEDERAL DO PARANÁ

CRISTIANE COLODEL

ISOLAMENTO E CARACTERIZAÇÃO DE POLISSACARÍDEOS DE CUBIU
(S*olanum sessiliflorum* Dunal)

CURITIBA
2015

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências – Bioquímica, da Universidade Federal do Paraná, como requisito para obtenção do grau de mestre em Ciências-Bioquímica.

Orientadora: Profª Drª Carmen Lúcia de Oliveira Petkowicz

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CRISTIANE COLODEL

Isolamento e caracterização de polissacarídeos de cubiu (*Solanum sessiliflorum Dunal*)

Dissertação aprovada como requisito parcial para obtenção do grau de Mestre no curso de Pós-Graduação em Ciências-Bioquímica, Setor de Ciências Biológicas, Universidade Federal do Paraná, pela sequinte banca examinadora:

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Às pessoas mais importantes da minha vida:
meus pais, Sérgio e Andréia; meus irmãos,
Giovani e Juliane; e ao Paulo, incansável
companheiro de aventuras.

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“Quem cresce faz crescer o universo que o rodeia.”
Hamilton Werneck

RESUMO

O cubiu (*Solanum sessiliflorum* Dunal, família Solanaceae) é um fruto nativo da Amazônia. A polpa apresenta sabor ácido característico e é utilizada pela população local no preparo de doces e molhos, além de ser utilizado na medicina tradicional para o controle do diabetes, colesterol e ácido úrico. Neste trabalho, a polpa e a casca do cubiu foram liofilizadas, trituradas e tratadas com etanol. Ambos os materiais foram submetidos a extrações sequenciais com DMSO, água, EDTA, ácido cítrico e NaOH. As extrações com DMSO solubilizaram principalmente amido; as aquosas, com EDTA e as ácidas, principalmente pectinas; e as alcalinas, hemiceluloses. As frações de maior rendimento foram as extraídas da polpa com DMSO 90% (fração DMSO-P, com 9,6% de rendimento) e água a 100°C (fração HW-P, com 4,8% de rendimento) e da casca com água a 100°C (fração HW-S, com 9,6% de rendimento). Análises químicas e espectroscópicas mostraram que a fração HW-S é composta majoritariamente por uma homogalacturonana de alto grau de metil-esterificação (AU = 79,0%; DM = 56,9%), juntamente com uma pequena proporção de arabinogalactana do tipo II. Testes foram realizados para a extração de pectinas da casca, visando a obtenção de pectinas com elevado rendimento e teor de ácido urônico. As extrações foram realizadas a 100°C, utilizando os ácidos cítrico e nítrico. O ácido nítrico forneceu pectinas com maior teor de urônicos, enquanto o rendimento não foi afetado pelo tipo de ácido. Em seguida, as extrações com ácido nítrico foram efetuadas à mesma temperatura, variando-se o pH (1,0; 1,5 e 2,0) e o tempo (2 ou 4h), e as melhores condições testadas foram pH 1,5 e 2h, fornecendo a fração N15t2, com rendimento de ~14% e teor de ácido urônico de 79%. Análises espectroscópicas evidenciaram que esta pectina possui alto grau de metil-esterificação (62%). A capacidade de formação de gel desta fração foi testada. Os géis foram preparados em concentração de 3% (m/m) de N15t2 e 60% (m/m) de sacarose, em pH 1,5; 2,0; 2,5 e 3,0. Análises reológicas demonstraram que a geleificação ocorre apenas em pH<3,0, e que a força do gel aumenta à medida que o pH decresce, mas em pH abaixo de 2,0, o aumento da acidez do meio não afeta consideravelmente a força do gel. A polpa do fruto também foi avaliada quanto à composição centesimal e à composição dos voláteis. O fruto provou ser rico em fibras (27,0% em relação ao peso da polpa seca) e nos minerais: magnésio (172,7mg / 100g), fósforo (293,4mg / 100g) e potássio (2998,7mg / 100g). Foram identificados sete componentes voláteis na polpa liofilizada, sendo o hidroxitolueno butilado (BHT) o composto majoritário (46,8% do total de voláteis), seguido pelo dodecanoato de etila (18,7%) e *E*-β-farneseno (10,0%).

Palavras-chave: cubiu; *Solanum sessiliflorum*; polpa; casca; composição centesimal; compostos voláteis; extrações seqüenciais; extrações ácidas; pectinas; reologia.

ABSTRACT

The cubiu (*Solanum sessiliflorum* Dunal, Solanaceae) is a native fruit from the Amazon. The pulp has characteristic acid flavor and is used by the local population in the preparation of jams and sauces, and is used in traditional medicine to control diabetes, cholesterol and uric acid. In this work, pulp and peel of cubiu were lyophilized, ground and treated with ethanol. Both materials were subjected to sequential extractions with DMSO, water, EDTA, citric acid and NaOH. Extractions with DMSO solubilized mainly starch; aqueous, with EDTA and acid ones, especially pectins; and alkaline, hemicelluloses. The higher yielding fractions were extracted from the pulp with 90% DMSO (DMSO-P fraction, 9.6% yield) and water at 100°C (HW-P fraction, 4.8% yield) and from peel with water at 100°C (HW-S fraction, 9.6% yield). Chemical and spectroscopic analyzes showed that the HW-S fraction is mainly composed of a homogalacturonan high degree of methyl-esterification (AU = 79.0%; DM = 56.9%), together with a small proportion of type II arabinogalactan. Tests were performed for pectin extraction from the peel, in order to obtain pectins with high yield and uronic acid content. The extractions were carried out at 100°C using citric and nitric acids. Nitric acid provided pectins with more uronic acid content, while the yield was not affected by the type of acid. Then, extractions were performed with nitric acid at the same temperature, varying pH (1.0, 1.5 and 2.0) and time (2 or 4 hours) and the best experimental conditions were pH 1.5 and 2h, providing N15t2 fraction with a yield of ~14% and uronic acid content of 79%. Spectroscopic analysis showed that the pectin has a high degree of methyl-esterification (62%). The gel forming ability of this fraction was tested. Gels were prepared at a concentration of 3% (w/w) N15t2, and 60% (w/w) sucrose, at pH 1.5; 2.0; 2.5 and 3.0. Rheological analyzes have shown that gelation occurs only at pH<3.0, and that the gel strength increases as the pH decreases, but at pH below 2.0, the increase in acidity of the medium does not quite affect the strength the gel. The fruit pulp was also evaluated as to their composition and composition of volatile. The result proved high fiber (27.0% based on the weight of dry pulp) and minerals: magnesium (172.7mg / 100 g), phosphorus (293.4mg / 100g) and potassium (2998.7mg / 100g). Seven volatile components were identified in lyophilized pulp, and butylated hydroxytoluene (BHT) was the major compound (46.8% total volatiles), followed by ethyldodecanoate (18.7%) and E-β-farnesene (10, 0%).

Keywords: cubiu; *Solanum sessiliflorum*; pulp; peel; proximate composition; volatile compounds; sequential extractions; acid extraction; pectins; rheology.

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

δ	– Deslocamento químico
$\dot{\gamma}$	– Taxa de cisalhamento
η_{ap}	– Viscosidade aparente
^{13}C NMR	– *Carbon-13 Nuclear Magnetic Ressonance*
AIR	– Resíduo insolúvel em álcool
AIR-P	– Resíduo insolúvel em álcool da polpa de cubiu
AIR-S	– Resíduo insolúvel em álcool da casca de cubiu
AOAC	– Association of Official Analytical Chemists
BHT	– Hidroxitolueno butilado
CA-P	– Fração polissacarídica obtida da polpa dos frutos de cubiu por extração com ácido cítrico em pH 2,5
CA-S	– Fração polissacarídica obtida da casca dos frutos de cubiu por extração com ácido cítrico em pH 2,5
CEAGESP	– Companhia de Entrepostos e Armazéns Gerais de São Paulo
DA	– Grau de acetilação
D_2O	– Óxido de deutério
DM	– Grau de metil-esterificação
DMSO	– Dimetilsulfóxido
DMSO-P	– Fração polissacarídica obtida da polpa dos frutos de cubiu por extração com DMSO 90%
DMSO-S	– Fração polissacarídica obtida da casca dos frutos de cubiu por extração com DMSO 90%
EDTA	– Ácido etilenodiaminotetracético
EDTA-P	– Fração polissacarídica obtida da polpa com EDTA 0,05 M
EDTA-S	– Fração polissacarídica obtida da casca com EDTA 0,05 M
EtOH	– Etanol
FAO	– Food and Agriculture Organization
FDA	– Food and Drug Admnistration
FT-IR	– *Fourier transform infrared*
G'	– Módulo de armazenamento ou módulo de cisalhamento elástico
G"	– Módulo de perda ou módulo de cisalhamento viscoso

GC – Cromatografia gasosa

GC-MS – Cromatografia gasosa acoplada à espectrometria de massa

HA2-P – Fração polissacarídica obtida da polpa dos frutos de cubiu por extração com NaOH 2M após neutralização do extrato

HA2-S – Fração polissacarídica obtida da casca dos frutos de cubiu por extração com NaOH 2M após neutralização do extrato

HA4-P – Fração polissacarídica obtida da polpa dos frutos de cubiu por extração com NaOH 4M após neutralização do extrato

HA4-S – Fração polissacarídica obtida da casca dos frutos de cubiu por extração com NaOH 4M após neutralização do extrato

HB2-P – Fração polissacarídica obtida da polpa dos frutos de cubiu porextração com NaOH 2M após a remoção da fração precipitada na neutralização

HB2-S – Fração polissacarídica obtida da casca dos frutos de cubiu por extração com NaOH 2M após a remoção da fração precipitada na neutralização

HB4-P – Fração polissacarídica obtida da polpa dos frutos de cubiu porextração com NaOH 4M após neutralização do extrato

HB4-S – Fração polissacarídica obtida da casca dos frutos de cubiu por extração com NaOH 4M após remoção da fração precipitada na neutralização

HDL – Lipoproteína de alta densidade

HG – Homogalacturonana

HM – Grau de metil-esterificação superior a 50%

HPSEC – *High-pressure size-exclusion chromatography*

HW-P – Fração polissacarídica obtida da polpa dos frutos de cubiu por extração com água a 100°C

HW-S – Fração polissacarídica obtida da casca dos frutos de cubiu por extração com água a 100°C

INPA – Instituto Nacional de Pesquisas da Amazônia

KI – *Kovats index*

LDL – Lipoproteína de baixa densidade

LM – Grau de metil-esterificação inferior a 50%.

MALLS – *Multi-angle laser-light scattering*

MeOH – Metanol

m/z – Relação massa/carga

N15t2 – Fração de alto rendimento e alto teor de ácido urônico obtida da casca dos frutos de cubiu por extração com HNO_3 a 100°C em pH 1,5 por 2h

$NaBH_4$ – Borohidreto de sódio

NaN_3 – Nitrito de sódio

RDI – *Reference Daily Intake*

RG-I – Ramnogalacturonana do tipo I

RG-II – Ramnogalacturonana do tipo II

RI – *Refraction index*

TMS – Tetrametilsilano

W-P – Fração polissacarídica obtida da polpa dos frutos de cubiu por extração com água a 25°C

W-S – Fração polissacarídica obtida da casca dos frutos de cubiu por extração com água a 25°C

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CAPÍTULO 1

INTRODUÇÃO

1. Introdução

Polissacarídeos de plantas atraem grande atenção para aplicações industriais e biológicas devido à sua grande variedade estrutural, ampla gama de propriedades e baixa toxicidade (SCHEPETKIN; QUINN, 2006). Além disso, são materiais ambientalmente sustentáveis, uma vez que sua síntese utiliza fontes naturais de energia e são completamente biodegradáveis (DIMITRIU, 2005).

Estas biomoléculas encontram ampla aplicação na indústria alimentícia, principalmente por suas propriedades físico-químicas, como emulsificantes, espessantes e estabilizantes (STEPHEN; CHURMS, 2006). O amido é sem dúvidas o polissacarídeo vegetal mais utilizado neste ramo, mas outra classe específica de polissacarídeos merece destaque quanto à sua gama de aplicações alimentícias: as pectinas. Além de terem propriedades funcionais, as pectinas são largamente utilizadas como aditivo alimentar devido à sua grande capacidade de formar géis em diferentes condições (WILLATS; KNOX; MIKKELSEN, 2006).

O cubiu (*Solanum sessiliflorum* D.) é um fruto nativo da região amazônica, pouco conhecido além da sua região de origem, mas que parece estar chamando a atenção de produtores e consumidores devido às suas propriedades nutricionais, o que inclui quantidades consideráveis de pectinas. Porém, esta informação não tem fundamentação científica, pois após ampla revisão, não foram encontrados estudos a respeito dos polissacarídeos deste fruto. O conhecimento disponível a respeito das propriedades do fruto é limitado apenas a alguns estudos sobre sua composição nutricional, focando principalmente no teor de minerais. Assim, neste trabalho os frutos de cubiu foram investigados quanto a sua composição. Os resultados desta pesquisa são apresentados em três partes. A primeira parte apresenta a composição centesimal e os compostos voláteis do fruto, buscando contribuir para os estudos já existentes neste sentido; a segunda parte foi dedicada à caracterização dos polissacarídeos extraídos da casca e da polpa dos frutos de cubiu; a terceira parte relata a investigação sobre a potencialidade da casca do fruto como fonte de pectinas e a caracterização química e reológica da pectina obtida. Uma breve revisão bibliográfica será apresentada previamente a apresentação destes resultados na forma de artigos.

CAPÍTULO 2

REVISÃO DA LITERATURA

2. Revisão da literatura

2.1. Amazônia: um bioma de rica biodiversidade e alto potencial econômico

A Amazônia compreende parte do Brasil, Venezuela, Colômbia, Peru, Bolívia, Equador, Suriname, Guiana e Guiana Francesa (DIAS; SILVEIRA; SCHOFIELD, 2002). É uma região que abriga a maior biodiversidade do planeta (ABRANTES, 2003), com cerca de 60 mil espécies de plantas superiores, somando aproximadamente 22% do total mundial (VALOIS, 1998).

Figura 1 – Mapa da América do Sul destacando a Região Amazônica (contornada em preto). Fonte: adaptado de LINDSAY; SIMMON, 2006.

Na Amazônia, encontram-se inúmeras espécies vegetais que poderiam ser utilizadas em processos produtivos de diferentes setores da indústria. Porém, a falta de informações científicas sobre a maioria destas espécies limita o seu aproveitamento (ABRANTES, 2003).

Frutos e vegetais são fontes de milhares de tipos de substâncias que possuem potenciais aplicações biológicas e industriais (SHIEBER; STINTZING; CARLE,

2001). Considerando a grande quantidade de espécies vegetais existentes nesta região, muitas ainda não foram cientificamente investigadas ou o conhecimento científico disponível ainda é bastante limitado. À medida que pesquisas científicas sejam dedicadas à composição e potenciais usos de substâncias existentes nestes materiais, maior será o interesse na sua produção e preservação e maiores serão os benefícios alcançados com a utilização destas substâncias.

2.2. *Frutos: recurso alimentar e fonte de matérias-primas*

Os frutos têm um importante papel na alimentação humana, pois são uma rica fonte de fibras, vitaminas, minerais e vários compostos biologicamente ativos, além de possuírem baixo teor de sódio (DESAI, 2000; SÁNCHEZ-MORENO *et al.*, 2006). Estudos demonstram que uma dieta rica em frutas e vegetais ajuda a prevenir doenças cardíacas (NESS; POWLES, 1997; DAUCHET *et al.*, 2006), alguns tipos de câncer (STEINMETZ; POTTER, 1996) e outras doenças crônicas (LAMPE, 1999).

A Organização Mundial da Saúde (OMS) e a Organização das Nações Unidas para a Alimentação e Agricultura (FAO) recomendam o consumo diário de 400g de frutas e vegetais (FAO, 2013).

Além de serem consumidos como alimentos, seja na forma *in natura* ou em produtos industrializados, os frutos também podem ser utilizados como fontes de matérias-primas para a indústria alimentícia e cosmética. Bons exemplos disso são o cacau, do qual se extrai a manteiga, principal ingrediente na fabricação de chocolates (JAHURUL *et al.,* 2013), e o cupuaçu, cuja gordura vem sendo explorada como uma boa alternativa para a substituição da manteiga de cacau na fabricação de chocolates, devido ao elevado preço desta última (MEDEIROS *et al.*, 2006; COHEN; JACKIX, 2009), além de ser utilizada como base para produtos cosméticos como batons e cremes (NATURA, 2014). Outro exemplo de matéria-prima obtida a partir de frutos são as pectinas, polissacarídeos com propriedades espessantes, que são comercialmente obtidas de cascas de frutas cítricas e do bagaço de maçãs, geralmente subprodutos da agroindústria (VORAGEN *et al.*, 2009).

Em 2011, a produção mundial de frutas foi de quase 640 milhões de toneladas (FAO, 2013). O Brasil é o terceiro maior produtor mundial, com uma produção de quase 39 milhões de toneladas no ano de 2010, ficando atrás somente da China e da Índia (FAO, 2013). As principais frutas produzidas no país são: laranja (43%),

banana (16%) e abacaxi (7%) (IBRAF, 2013), porém, o Brasil é um grande exportador de frutas cítricas (FAO, 2013). Dentro do país, as frutas mais consumidas são a banana (27%) e a laranja (19%) (SILVEIRA *et al.*, 2011).

Ainda que no Brasil a produção e o consumo se concentrem em frutas mais popularmente conhecidas, a flora brasileira é riquíssima, e existem diversas espécies frutíferas nativas ainda pouco conhecidas pela população, e que são comercializadas apenas local e regionalmente. Apesar da crescente demanda,tanto para o consumo *in natura* quanto para a indústria, existe escassez de conhecimento sobre composição e formas de aproveitamento destas frutas (VIEIRA, 2000). Dentre as frutas ainda pouco conhecidas, destaca-se o cubiu, uma espécie nativa da região amazônica. O cubiu é utilizado localmente na alimentação (SILVA FILHO, 2012), mas vem encontrando mercado como um produto natural ao qual se atribuem propriedades terapêuticas. Contudo, ainda é pouco conhecido pela população e existem poucos estudos a respeito da sua composição.

Se o conhecimento a respeito das propriedades nutricionais de frutos nativos pouco conhecidos pela população já é pequeno, as informações sobre a potencialidade destes frutos como fontes de matérias-primas são ainda mais escassas. Buscando contribuir para um maior conhecimento a respeito de frutos de espécies nativas brasileiras, este trabalho buscou obter informações a respeito da composição química dos frutos de cubiu (*Solanum sessiliflorum* D.), bem como extrair e caracterizar seus polissacarídeos.

2.3. Cubiu (Solanum sessiliflorum D.)

Solanum sessiliflorum Dunal, conhecido como “cubiu”, “maná” ou “tomate-de-índio” nos países de língua portuguesa, “topiro”, “tupiro” ou “cocona” nos países de língua espanhola e “orinoco apple” ou “peach tomato” nos países de língua inglesa (VIEIRA; ROSA, 2010; SILVA FILHO *et al.*, 1999) é uma espécie arbustiva, nativa da Amazônia Ocidental, pertencente à família *Solanaceae*. Tem origem provável na bacia amazônica e foi completamente domesticada pelos povos indígenas da região. Atualmente, apresenta vasta distribuição na Amazônia brasileira, peruana, colombiana, equatoriana, venezuelana e nos Andes do Equador e Colômbia, nos vales interandinos da Colômbia e no litoral Pacífico do Equador e Colômbia (PIRES

et al., 2006; SILVA FILHO *et al.*, 2012; SILVA FILHO *et al.*, 1999; YUYAMA *et al.*, 2007).

A planta é um arbusto, com altura entre 1 e 2 metros. Possui folhas dentadas, que podem chegar a até 58 cm de largura. As flores são estreladas, com pétalas verde-claras e anteras amarelas, que formam inflorescências, localizadas nos ramos, em um grupo de cinco a oito flores, das quais se originam de um a três frutos (SILVA FILHO *et al.*, 1998).

O fruto, quando maduro, possui coloração entre o amarelo-alaranjado e o vermelho (VIEIRA; ROSA, 2010), pesando de 20 a 490g (SILVA FILHO *et al.*, 2012), apresenta sabor ácido e agradável, bastante característico (PIRES *et al.*, 2006).

Os frutos são consumidos na forma de sucos, doces, geléias, compotas e molhos para carnes e peixes; as folhas, galhos e raízes têm usos como medicamentos caseiros para cicatrização de picadas de aranhas e para evitar a formação de bolhas em casos de queimaduras, no tratamento de escabiose e coceiras na pele; o suco é usado contra anemia e no controle dos níveis de colesterol, de ácido úrico e de glucose no sangue, além de ser utilizado como cosmético, dando brilho aos cabelos (PIRES *et al.*, 2006; YUYAMA *et al.*, 2008; SILVA FILHO *et al.*, 2005).

Figura 2 – Fruto de cubiu (*Solanum sessiliflorum*) maduro. À esquerda, o fruto aberto. (Fonte: a autora).

De acordo com Silva Filho (1998), a comercialização do cubiu ocorre em pequena escala, geralmente por produtores rurais em feiras, mercados ou em suas próprias casas, como base de troca entre vizinhos. Nas maiores cidades amazônicas existem pequenas redes de comercialização, nas quais os produtores vendem os frutos a intermediários que repassam estes frutos para feiras e mercados próximos. Restaurantes e hotéis de cidades da Amazônia Ocidental comercializam produtos à base de cubiu, como sucos e sorvetes; no Peru, existem pequenos comércios de néctares industrializados.

Embora não tenha sido encontrada nenhuma referência formal à comercialização do cubiu em outra forma que não *in natura*, sabe-se, através de sites de venda do produto, que a polpa do cubiu vem sendo comercializada em todo o país na forma liofilizada, em cápsulas ou como farinha, sob a alegação de que este produto possui propriedades medicinais. A empresa Mil Grãos (www.milgraos.com.br), uma das empresas que comercializa o cubiu na forma de cápsulas, afirma que o cubiu é recomendado no combate ao colesterol, triglicérides, anemia, diabetes, pressão alta, enxaqueca, depressão, ácido úrico, além de ser digestivo, diurético e tônico sexual, e que entre seus efeitos benéficos está a redução do LDL e aumento do HDL, entre outros.

Os frutos de cubiu são considerados de alto valor nutricional, sendo ricos em ferro, niacina e ácido cítrico (PIRES *et al.*, 2006). Diferentes autores investigaram a composição nutricional do cubiu: Yuyama *et al.* (2007) avaliaram o teor de alguns minerais – ferro, zinco, cobalto, cromo, cálcio, potássio e sódio –, apontando como minerais majoritários potássio e cálcio, com média de 356,4 mg e 12,54 mg, respectivamente, a cada 100g de polpa do fruto, além do teor médio de proteínas (0,5%), fibra solúvel (0,3%) e insolúvel (1,3%)e carboidratos (5,9%), e o valor energético (33,7 kcal) de 8 etnovariedades do fruto. Os conteúdos de amido, ácidos graxos e aminoácidos, determinados em 100g de polpa do fruto, foram descritos por Marx, Andrade e Maia (1998): 2,87g de amido; 1,53g de ácido oléico e 1,00g de ácido palmítico como componentes majoritários entre os ácidos graxos, sendo que os demais apareceram em proporções menores que 0,2g; 366,8 mg de asparagina, 321 mg de serina, 298 mg de ácido γ-aminobutírico e 172,8 mg de glutamina como aminoácidos majoritários, sendo que os demais apareceram em proporções menores que 75 mg. Este estudo traz também o teor de alguns minerais – cálcio,

magnésio, fósforo, ferro, manganês, zinco e selênio -, indicando como minerais majoritários fósforo e magnésio, com valores de 383 mg e 188 mg, respectivamente, a cada 100 g da polpa do fruto.

Embora o cubiu não seja produzido em grande escala, alguns agricultores estão produzindo o fruto em áreas superiores a dois hectares, sendo a produção exportada para o Japão para a extração de pectinas (BRASIL, 2010; INPA, 2014). Apesar da indicação da presença de pectinas nos frutos de cubiu, após ampla revisão bibliográfica não foram encontrados estudos sobre a caracterização destas ou de outros polissacarídeos presentes no fruto.

2.4. Compostos voláteis e óleos essenciais

Os óleos essenciais, também chamados de óleos voláteis ou etéreos, são uma complexa mistura de compostos voláteis, sintetizados por plantas como metabólitos secundários (BAKKALI *et al.*, 2008; BASER; DEMIRCI, 2007). Quimicamente, são compostos por terpenos, alcoóis, ácidos, ésteres, epóxidos, aldeídos, cetonas, aminas e sulfetos. Os componentes dos óleos essenciais são divididos em dois grupos: os compostos terpênicos e os compostos aromáticos (CALO *et al*, 2015).

Na natureza, os óleos essenciais desempenham importante papel nos mecanismos de defesa dos vegetais contra bactérias, vírus, fungos, insetos e também contra animais que possam alimentar-se da planta, reduzindo seu apetite. Além disso, exercem função indireta na reprodução, atraindo insetos polinizadores e animais dispersadores de sementes (BAKKALI *et al*, 2008).

Quando combinados adequadamente com outros compostos, como açúcares e ácidos orgânicos, os compostos voláteis são também responsáveis pelo sabor e aroma das frutas (SPOTO; GUTIERREZ, 2006). Apesar de um único espécime produzir centenas de compostos voláteis, a combinação destas substâncias confere ao vegetal (sendo que cada tecido pode sintetizar diferentes compostos voláteis) aroma e sabor característico (denominado pela literatura como "*fingerprint*"), que auxilia no reconhecimento da planta por animais e também pelos humanos, além de servir como indicador para plantas não apropriadas para a alimentação (GOFF; KLEE, 2006).

Diversos métodos são empregados para obtenção de compostos voláteis de fontes naturais: destilação por arraste a vapor, extração por solventes, extração por dióxido

de carbono líquido, dessorção térmica, fermentação, *enfleurage* (extração em que gorduras são utilizadas como solvente) e prensagem (BURT, 2004; BAKKALI *et al.*, 2008; GODEFROOT; SANDRA; VERZELE, 1981; BIZZO; HOVELL; REZENDE, 2009), mas a técnica mais utilizada para a produção comercial de óleos essenciais é a destilação por arraste a vapor (BURT, 2004). Os óleos essenciais podem ser produzidos a partir de flores, folhas, cascas, frutos, sementes, madeiras e raízes (BURT, 2004; BIZZO; HOVELL; REZENDE, 2009).

Os óleos essenciais são utilizados há muitos séculos, com finalidades aromáticas, preservativas e medicinais. Atualmente, os usos ainda são muito parecidos, porém, há um maior conhecimento a respeito dos seus mecanismos de ação (BURT, 2004; BAKKALI *et al.*, 2008).

A empregabilidade dos óleos essenciais deve-se aos seus efeitos já bastante conhecidos e comprovados por vários estudos. Entre eles, destacam-se as atividades: antiséptica (bactericida, virucida e fungicida), analgésica, anti-inflamatória, sedativa, antioxidante e preservativas, o que confere diversas aplicações dos óleos essenciais na área medicinal e alimentícia, além das propriedades aromáticas, pelas quais são largamente empregados em diversos produtos, principalmente alimentos e cosméticos (BAKALLI *et al.*, 2008; BIZZO; HOVELL; REZENDE, 2009).

2.5. Polissacarídeos de origem vegetal

Os polissacarídeos de origem vegetal se dividem em dois grupos principais, os polissacarídeos de reserva e os polissacarídeos estruturais, presentes na parede celular.

2.5.1. Polissacarídeos de reserva

2.5.1.1. Amido

O amido é o principal polissacarídeo de reserva presente nos tecidos fotossintetizantes e em órgãos de reserva como sementes, tubérculos, raízes e frutos. Ele está entre os principais produtos oriundos de plantas, e também é a principal fonte de energia na dieta humana. Em raízes, tubérculos, frutos, cereais e grãos o conteúdo de amido varia de 40 a 80%, em relação ao peso seco.

(AVÉROUS; HALLEY, 2014; ZOBEL; STEPHEN, 2006; GREENWOOD, 1970; BeMILLER, 1993).

O amido não é uma substância pura, mas uma mistura de dois polímeros distintos: amilose e amilopectina.Estruturalmente, a amilose consiste basicamente em uma glucana linear formada por unidades de α-D-Glc (1→4) ligadas, enquanto a amilopectina difere da amilose pelo fato de ser multiramificada, com cadeias laterais de unidades α-D-Glc (1→4) ligadas por meio de ligações (1→6) (ZOBEL; STEPHEN, 2006). Estas duas glucanas também diferem entre si quanto à massa molecular: enquanto a amilose possui peso molecular média entre 10^5 e 10^6, a massa molecular média da amilopectina fica entre 10^7 e 10^8 (BAMFORTH, 2003).

2.5.2. Polissacarídeos estruturais

2.5.2.1. Parede celular vegetal

As células vegetais são delimitadas por uma parede relativamente delgada, mas mecanicamente forte, que consiste de uma mistura complexa de polissacarídeos e outros polímeros, produzidos pela célula e reunidos em uma rede organizada por meio de ligações covalentes e não-covalentes. Esta parede tem como função regular o volume celular, determinar a forma da célula e proteger o protoplasto. A parede celular constitui uma fonte de matéria-prima para diferentes aplicações como a fabricação de papel, fibras, filmes e produção de agentes espessantes e geleificantes (TAIZ; ZEIGER, 2009).

Embora uma pequena quantidade de proteínas também possa estar presente, os principais componentes da parede celular vegetal são polissacarídeos, sendo que os principais são a celulose, as hemiceluloses e as pectinas (COSGROVE, 2005).

Na maioria das paredes celulares, podem ser identificadas três camadas: a lamela média, a parede primária e a parede secundária, cada uma se desenvolvendo em uma determinada fase do desenvolvimento do vegetal (SMITH, 1993).

A lamela média é uma fina camada que ocorre entre células adjacentes, e é a primeira a ser sintetizada durante o desenvolvimento do vegetal. É composta principalmente por pectinas, e tem função de promover adesão entre as células (COSGROVE, 2005; TAIZ; ZEIGER, 2009).

A parede primária é extensível e formada em células em crescimento. Ela deposita-se sobre a superfície da membrana plasmática após o processo de divisão celular, e

continua a se depositar durante todo o processo de expansão da célula. É composta por celulose, hemiceluloses e pectinas. A celulose é o mais abundante polissacarídeo vegetal, e existe na forma de microfibrilas formadas por cadeias de glucanas β-D-(1→4) unidas entre si por meio de pontes de hidrogênio. Estas microfibrilas associam-se também através de pontes de hidrogênio às hemiceluloses. Esta rede formada pela associação entre as microfibrilas de celulose e as hemiceluloses encontra-se embebida em uma matriz de pectinas (Fig. 3) (BeMILLER, 2001; DAVIES, 2001; COSGROVE, 2005).

Quando a expansão celular cessa, inicia-se o processo de deposição da parede secundária, que difere da parede primária tanto em composição química quanto em função estrutural, pois pode se tornar altamente especializada, refletindo o estado diferenciado da célula (SMITH, 1993; TAIZ; ZEIGER, 2009). A parede celular secundária é composta principalmente por celulose e hemiceluloses, além de grandes quantidades de lignina, um composto aromático que forma ligações cruzadas com a rede polissacarídica, tornando a estrutura da parede secundária mais rígida e mais hidrofóbica (CARPITA; McCANN, 2000; ZHONG; YE, 2009).

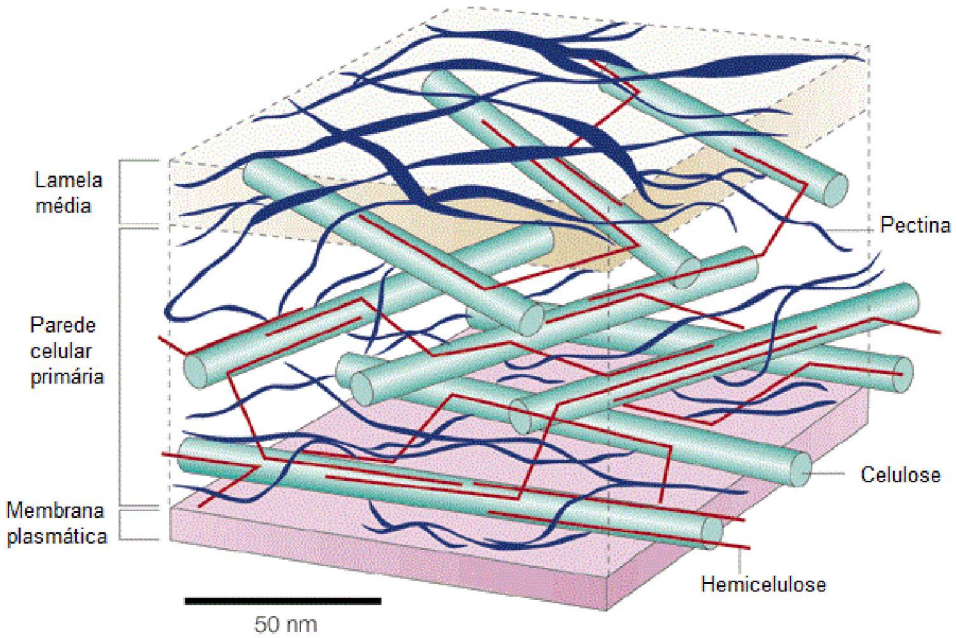

Figura 3 – Estrutura esquemática da parede celular primária, mostrando as microfibrilas de celulose associadas à hemicelulose, embebidas na matriz de pectina (Fonte: adaptado de SMITH, 2001).

2.5.2.2. Hemiceluloses

As hemiceluloses são os polissacarídeos de parede celular vegetal solúveis em soluções alcalinas (CAFFAL; MOHNEN, 2009). O termo hemicelulose designa um grupo de polissacarídeos heterogêneos que corresponde a cerca de 15% a 35% da biomassa vegetal, sendo a segunda mais abundante classe de polissacarídeos na natureza (GÍRIO *et al.*, 2010; SAHA, 2003).

Quimicamente, as hemiceluloses são constituídas por unidades piranosídicas de glucose, manose ou xilose ligadas β(1→4) e que possuem *O*-4 na posição equatorial. Estas características resultam em uma conformação similar à da celulose, favorecendo as interações com as microfibrilas (CAFFALL; MOHNEN, 2009). A composição e estrutura dos polissacarídeos hemicelulósicos dependem da fonte botânica, tipo de tecido e estágio de desenvolvimento (GÍRIO *et al.*, 2010; SAHA, 2003; WYMAN *et al.*, 2004).

As mais abundantes hemiceluloses na parede celular primária das dicotiledôneas são as xiloglucanas. Assim como a celulose, a estrutura das xiloglucanas é formada por uma cadeia principal de unidades de β-D-Glc ligadas (1→4), substituídas por unidades de Xyl na posição *O*-6. As unidades de Xyl podem ser substituída em O-2 por unidades de β-D-Gal, as quais, por sua vez,podem ser substituídas em O-2 por unidades de α-L-fucose. (CAFFALL; MOHNEN, 2009). As xiloglucanas parecem ser o grupo de hemiceluloses que possui mais afinidade e interage mais fortemente com a celulose, tendo grande contribuição na integridade estrutural da parede celular. Por outro lado, esta forte interação dificulta a extração das xiloglucanas (CAFFALL; MOHNEN, 2009; EBRINGEROVÁ; HROMÁDKOVÁ; HEINZE, 2005).

As xiloglucanas são classificadas de acordo com a estrutura das cadeias laterais. Fry *et al.* (1993) sugeriram uma nomenclatura concisa para designar a estrutura das xiloglucanas, com um código de letras representando a configuração de cada tipo de cadeia lateral. Nesta nomenclatura, as unidades de Glc da cadeia principal, substituídas por Xyl são representadas pela letra X, enquanto que unidades de Glc não substituídas são representadas pela letra G. Para as unidades de Gal, Ara e Fuc, presentes como terminais não-redutores, empregam-se as letras L, S e F, respectivamente. Assim, baseando-se no padrão de substituição, Vincken *et al.* (1997) dividiram as xiloglucanas em dois grupos: o primeiro é designado de poli-XXXG, e inclui as xiloglucanas encontradas em muitas gimnospermas e nas angiospermas (O'NEILL; YORK, 2003); o segundo é definido como poli-XXGG, e é

encontrado particularmente nas espécies da família das Solanáceas, à qual o cubiu pertence. Outra particularidade das xiloglucanas de Solanáceas é o fato de conterem unidades terminais de α-L-Ara ligadas (1→2) às unidades de xilose, ao invés de fucose (HAYASHI, 1989).

As xilanas são as principais hemiceluloses da parede celular secundária, compreendendo aproximadamente 20-30% da biomassa das dicotiledôneas. Sua estrutura principal é formada por cadeias lineares de unidades de β-D-xilose ligadas (1→4), e algumas destas unidades podem ser decoradas por grupos acetil em *O*-2 ou *O*-3. Podem ser lineares, sendo denominadas homoxilanas, ou substituídas por outros açúcares caracterizando as heteroxilanas. A maior parte das heteroxilanas é substituída nas posições *O*-2 ou *O*-3, e normalmente se apresentam como arabinoxilanas, glucuronoarabinoxilanas ou glucuronoxilanas (CAFFALL; MOHNEN, 2009; EBRINGEROVÁ; HROMÁDKOVÁ; HEINZE, 2005).

Outras hemiceluloses que ocorrem na parede celular em menores proporções são as mananas, galactomananas, glucomananas e galactoglucomananas (EBRINGEROVÁ; HROMÁDKOVÁ; HEINZE, 2005; MOREIRA; FILHO, 2008).

2.5.2.3. Pectinas

As pectinas compreendem um vasto e heterogêneo grupo de polissacarídeos ácidos, presentes na parede celular vegetal, principalmente na parece celular primária e na lamela média. Suas principais funções nos tecidos vegetais envolvem a hidratação e firmeza dos tecidos, cimentação das redes de celulose, controle da permeabilidade e movimentação de macromoléculas através da parede celular e controle da extensibilidade e crescimento de tecidos (THAKUR; SINGH; HANDA, 1997; VORAGEN *et al.*, 2009; YAPO, 2011).

Assim como o amido, as pectinas também possuem ampla aplicação na indústria alimentícia, como agentes geleificantes, espessantes, estabilizantes e emulsificantes. Suas principais fontes comerciais são o albedo de frutas cítricas e o bagaço de maçã (VORAGEN *et al.*, 2009; YAPO, 2011).

Estruturalmente, o grupo das pectinas é o mais complexo entre os polissacarídeos de parede celular vegetal e inclui quatro classes principais: as homogalacturonanas, ramnogalacturonanas (I e II) e xilogalacturonanas (MOHNEN, 2008; YAPO, 2011).

As homogalacturonanas (HGs) são os principais polissacarídeos pécticos na parede celular vegetal, correspondendo a cerca de 60% do total. Consistem em um polímero formado por unidades de α-D-GalA unidas através de ligações glicosídicas do tipo α(1→4). Estas unidades podem ser metil esterificadas em C-6, e *O*-acetiladas em *O*-2 e/ou *O*-3, dependendo da fonte vegetal. Dependendo do grau de metil esterificação, as pectinas são classificadas em dois grupos: as de baixo grau de esterificação (LM), com grau de esterificação abaixo de 50%, e as de alto grau de esterificação (HM), com grau de esterificação acima de 50% (VORAGEN *et al.*, 2009; YAPO, 2011).

Nas ramnogalacturonanas do tipo I (RG-I), as unidades de α-D-GalA são alternadas por unidades de α-L-Rha, formando uma estrutura repetitiva [→2) α-L-Rha (1→4) α-D-GalA (1→] na cadeia principal. As unidades de α-L-Rha podem ser substituídas na posição *O*-4 por cadeias laterais, formadas principalmente por arabinanas, galactanas ou arabinogalactanas (VORAGEN *et al.*, 2009; YAPO, 2011).

As ramnogalacturonanas do tipo II (RG-II), apesar da sugestão do nome, não contêm unidades de α-L-Rha intercaladas e apresentam uma cadeia principal de HG. No entanto, é o mais complexo dos polissacarídeos pécticos, pois possui cadeias laterais bastante complexas, que contém em sua estrutura até 12 monossacarídeos diferentes (incluindo açúcares raros como a apiose, ácido acérico, DHA e KDO), em 20 tipos diferentes de ligações (MOHNEN, 2008; VORAGEN *et al.*, 2009).

As xilogalacturonanas consistem em cadeias principais de HG substituídas por unidades individuais de β-D-Xyl (1→3) (VORAGEN *et al.*, 2009).

A representação esquemática da estruturas das quatro principais classes de pectinas pode ser visualizada na Fig. 4.

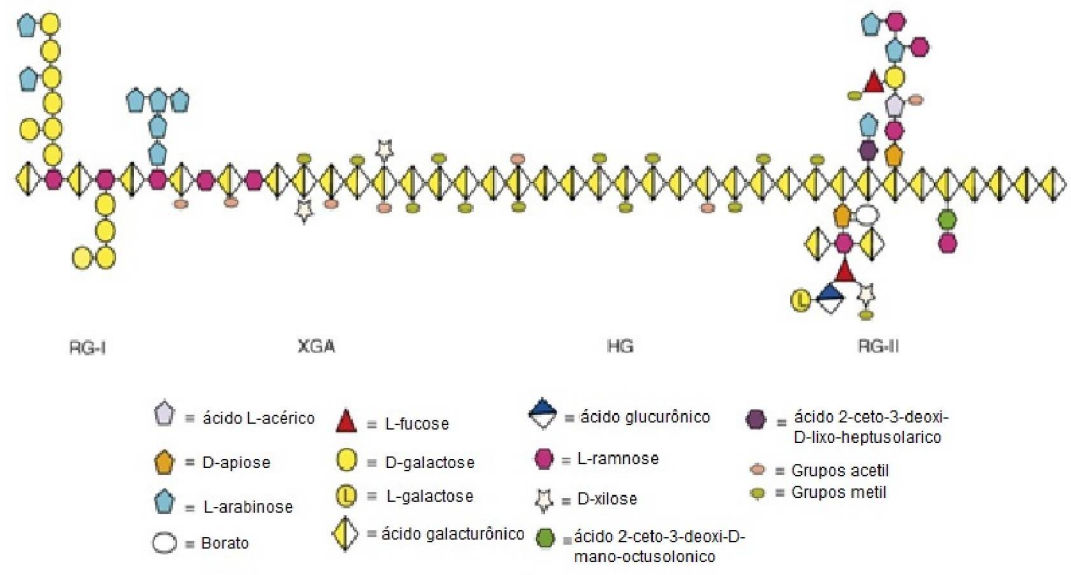

Figura 4 - Estrutura química esquemática dos polissacarídeos pécticos HG, XGA, RG-I e RG-II. (Fonte: adaptado de MOHNEN, 2008).

A propriedade mais notável das pectinas é a sua capacidade de formar géis em condições específicas. As características físico-químicas dos géis de pectina são consequencias da formação de uma rede tridimensional contínua de polímeros unidos por ligações cruzadas e dependem das zonas de junção pelas quais as moléculas são unidas; da interjunção de segmentos de polímeros que sejam relativamente móveis; e de água retida na rede polimérica (THAKUR; SINGH; HANDA, 1997).

Dependendo do grau de metil esterificação as pectinas formam géis em condições diferentes. As pectinas LM formam géis na presença de íons Ca^{2+}, que agem como pontes de junção entre os grupos carboxílicos das moléculas de pectina. Este processo é descrito por um modelo chamado *egg-box*, que envolve a dimerização e subsequente agregação dos dímeros formados. Já as pectinas HM formam géis em pH abaixo de 3.6, sendo necessária a presença de um co-soluto, geralmente sacarose, em concentração maior que 55%. O açúcar dificulta as interações pectina-água, e favorece as interações pectina-pectina, formando zonas de junção que são estabilizadas por meio de interações hidrofóbicas entre os grupos metoxílicos e por pontes de hidrogênio (HWANG; PYUN; KOKINI, 1993; THAKUR; SINGH; HANDA, 1997).

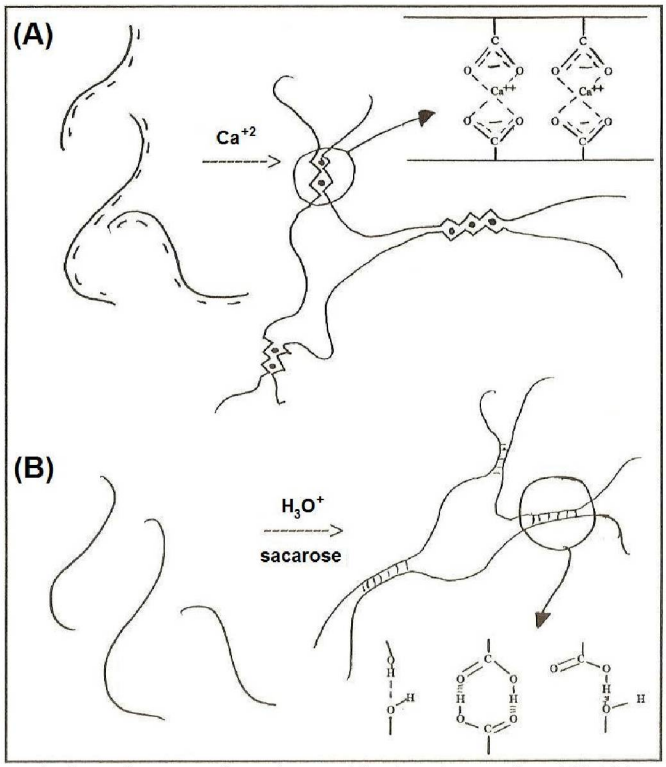

Figura 5 – Mecanismos de ligação cruzada entre moléculas de pectina (A) LM na presença de cálcio e (B) HM em meio ácido na presença de sacarose. (Fonte: adaptado de RINAUDO, 1996).

Além do grau de metil esterificação, outro parâmetro importante nas pectinas é o grau de acetilação. A presença de grupos acetil em pectinas de beterraba, batata e girassol parece inibir a geleificação. Esta inibição ocorre porque a presença dos grupos acetil acarreta na dificuldade de associação das cadeias devido ao impedimento estérico (RINAUDO, 1996; LOPES DA SILVA; RAO, 2006).

Outros fatores que influenciam nas propriedades das pectinas são: o peso molecular, a conformação das moléculas, e a existência, tamanho e distribuição das cadeias laterais (HWANG; KOKINI, 1992; SCHMELTER *et al.*, 2002; DIAZ; ANTHON; BARRET, 2007; PAIVA; LIMA; PAIXÃO, 2009; CANTERI *et al.*, 2012; ENDRESS; MATTES; NORZ, 2006).

Desta forma, considerando que a estrutura química das pectinas é variável conforme a fonte botânica e o método de extração, as propriedades físico-químicas e consequentes aplicações das pectinas podem variar conforme a fonte vegetal e o método através do qual ela é obtida.

CAPÍTULO 3

OBJETIVOS

3. Objetivos

3.1. Objetivo geral

Investigar a composição química dos frutos de cubiu dando ênfase aos seus polissacarídeos.

3.2. Objetivos específicos

- Determinar a composição centesimal da polpa dos frutos de cubiu;
- Determinar a composição dos voláteis da polpa liofilizada;
- Extrair e caracterizar os polissacarídeos obtidos através de extrações sequenciais com diferentes solventes da casca e da polpa dos frutos de cubiu;
- Realizar extrações ácidas para obtenção de pectinas da casca de cubiu visando elevado rendimento e teor de ácido urônico;
- Caracterizar química e reologicamente a pectina com elevado rendimento, maior teor de ácido urônico e maior viscosidade aparente obtida por extração ácida da casca de cubiu.

CAPÍTULO 4

ARTIGO I - PROXIMATE COMPOSITION AND VOLATILE COMPOUNDS FROM LYOPHILIZED PULP OF CUBIU (*Solanum sessiliflorum* D.) FRUITS

PROXIMATE COMPOSITION AND VOLATILE COMPOUNDS FROM LYOPHILIZED PULP OF CUBIU (*Solanum sessiliflorum* D.) FRUITS

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Abstract

Solanum sessiliflorum D., known in Brazil as cubiu, maná or maná-cubiu is a native species from the Amazon region. In the present work, the pulp of cubiu fruits was analyzed for its chemical composition and its volatile compounds. The results proved the edible part of the fruits is rich in fibers and minerals, especially potassium, magnesium and phosphorus. The pulp had a pleasant aroma after lyophilization, and the volatile compounds were extracted from the lyophilized pulpand analyzed by GC-MS. Seven components were identified in the extract from the lyophilized pulp: butylated hydroxytoluene (BHT), ethyl dodecanoate, (*E*)-β-farnesene, ethyl decanoate, decalactone, linalool and α-terpineol.

Keywords: cubiu; *Solanum sesiliflorum*; proximate composition; volatile compounds.

1. Introduction

Solanum sessiliflorum D., known as cubiu, maná or maná-cubiu (Brazil), cocona or topiro (spanish speaking countries) and tomato peach or orinoco apple (english speaking countries), is a shrubby specie native from the Amazon region (MARX; ANDRADE; MAIA, 1998).

It can be cultivated in various soil types, even in acid soils of low fertility and in neutral or alkaline soils of good fertility, with texture from sandy to clayey. It thrives in warm and humid climate, with temperatures between 18 and 30°C and relative humidity of 85%, at altitudes up to 1500 m above sea level and can produce from 30

to 100 tonnes of fruit per hectare. Although it has great nutritional potential, cubiu is a little known fruit, and the production and marketing is almost restricted to the Amazon region (INPA, 2014; SILVA FILHO *et al.*, 2012).

Its fruits are berries, with a thin orange or red skin when ripe and a yellow pulp and its weight can vary from 20 to 490g (PIRES *et al.*, 2006; SILVA FILHO *et al.*, 2012; DUARTE, 2011; MARX; ANDRADE; MAIA, 1998).They have an acid taste quite characteristic and are used in the preparation of many foods by the Amazon people. The fruit juice is used popularly as a cosmetic, to make the hair brighter. It has been pointed that the fruits also have therapeutic properties, such as control of cholesterol, uric acid and blood glucose levels (PIRES *et al.*, 2006; YUYAMA *et al.,* 2005). Sandoval (2004) has demonstrated that cubiu fruits can reduce the serum levels of glucose, cholesterol and triglycerides in humans with dyslipidemia and hyperglycemia. The author found that after the treatment of 100 adult men and women with cubiu extract (40 ml/day for 3 days), the total cholesterol, LDL and triglycerides reached normal values in 61%, 62% and 92% of the subjects, respectively. HDL levels increased in 82% of the subjects treated with cubiu and the glycemia was found in the normal baseline in all treated subjects.

According to the literature, cubiu fruits have high nutritional value, being rich in vitamins A, B_3 and C (SALICK, 1987; PIRES *et al.*, 2006) and in minerals, mainly iron, potassium, calcium, phosphorus and magnesium. However, the reported values are not consensual (MARX; ANDRADE; MAIA, 1998; YUYAMA *et al.*, 2007; SILVA FILHO *et al.*, 2005; PIRES, 2006; GALOZZI; DUARTE, 2007).

The volatile compounds of cubiu was evaluated by Quijano and Pino (2006) and by Marx, Andrade and Maia (1998) but in these studies was used the fresh fruits. Both studies reported nonconsensual results. The first investigated the composition of volatiles in green, mature and ripe fruits and found mainly hexenals in all stages of maturation, whereas methyl salicylate appeared as major component in mature and ripe fruits. The second found safrole and palmitic acid among the volatile constituents of ripe fruits. In this way, this work aimed to contribute to the investigation of the volatile compounds of cubiu fruits. However, in this study, the analyzes were performed with the lyophilized pulp, since this is a fairly common procedure in the food industry, which is already being used in the manufacture of some cubiu based products.

We present now the chemical composition of the pulp of cubiu fruit and the volatile compounds from lyophilized pulp.

2. Materials and methods

2.1. Plant material

Solanum sessiliflorum fruits were acquired from Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP), São Paulo, Brazil. The pulp was separated from the peel and then lyophilized. The dried pulp was ground into powder using an analytical mill IKA A-11.

2.2. Chemical composition of pulp

Moisture was determined by difference after lyophilization. Ash, protein, total fat, minerals and total dietary fiber contents of pulp were determined according to Association of Official Analytical Chemists (AOAC, 2005). The protein content was determined based on total nitrogen value determined by Kjeldahl method; total fat was measured by Soxhlet method. Total dietary fiber was determined by enzymatic-gravimetric method. Ash was determined by ignition of sample in muffle at 600ºC. Minerals was determined from the acid digestion followed by analysis of the ash by atomic absorption spectrophotometry. Phenolic compounds were extracted according to the method described by Velioglu *et al*. (1998), using 80% MeOH with 1% HCl, and quantified by Folin-Ciocalteu method, according to Singleton and Rossi (1965), using galic acid as standard. Total starch was determined by a colorimetric method using a K-TSTA 07/11 kit (Megazyme, Ireland). Total sugar was calculated by difference of others components (except starch). All assays were performed, at least in two independents experiments, done in triplicate, with the exception of total dietary fiber, that was performed in duplicate.

2.3. Volatile compound from the lyophilized pulp of cubiu fruits

Lyophilized pulp was hydrodistilled for 3 hours in a 2 liter flask attached to a glass apparatus according to Stahl and Schild (1981). The volatiles were taken in ethyl ether and the solvent evaporated at room temperature. The volatiles were analyzed in a Varian® CP-3800 Gas Chromatograph using the software Saturn® GC-MS Work

Station 5.51, a capillary column CP-Sil-8 CB Low Bleed/MS 30 m long with a diameter of 0.25 mm and a film of 0.25 µm, and the following conditions: operating in EI mode at 70 eV, mass scan range of 40-650 *m/z* at a sample rate of 1.0 scans^{-1}. The temperature of the injector was kept at 250 °C and the temperature of the interface 240 ºC. A flow rate of 1 mL/min was adopted and helium was the carrier gas. The injection volume was 1.0 µL of sample solution (diluted in ethyl ether). The temperature was programmed as follows: 50 °C in the first 1 minute, going up 3°C/min up to 240°C; Split ratio of 1/50. In order to quantify the chemical compounds in each oil sample, a Gas Chromatograph 14B coupled with a flame ionization detector (GC-FID) and an OV-5 column (30 m x 0.25 mm i. d. x 0.25 µm) was used. Nitrogen was the gas carrier, with a constant pressure of 80 kPa, a split ratio of 1/150 and injection volume of 1 µL of oil (in ethyl ether). The temperature of the detector and the injector was kept at 300 and 250 ºC, respectively. The initial temperature in the column was 50 ºC (3 min), with a heating rate of 5 ºC/min until the temperature reached 270 ºC, with an isotherm of 8 min.

The identification of compounds was performed using data obtained from the analyzes by GC-MS, according to the Kovats index (KI) (ADAMS, 1995) of each component based on retention time provided by injection of a series of n-alkanes (C10 -C25). For the quantification of the components the data obtained with a flame ionization detector (GC-FID) were used, with an identical capillary column and the same conditions.

3. Results and discussion

3.1. Proximate composition of pulp of cubiu fruits

The chemical and nutritional composition of pulp of cubiu fruits is presented in Table 1.

Table 1 – Proximate composition of pulp of cubiu fruits and the contribution of each component for RDI.

Component	Content	%RDI[a]
Moisture (g/100g)	89.4 ± 0.77	-
Ash (g/100g)	6.8 ± 0.07	-
Protein (g/100g)	8.0 ± 0.03	1.7

Total fat (g/100g)	14.3 ± 0.03	2.4
Total dietary fiber (g/100g)	27.0 ± 0.29	11.8
Total starch (g/100g)	7.1 ± 0.37	nd
Total sugar (g/100g)*	43.47	1.2
Total phenolic (mg/100g)	432.2 ± 2.36	nd
Calcium (mg/100g)	104.0 ± 0.28	1.1
Iron (mg/100g)	1.5 ± 0.01	0.9
Magnesium (mg/100g)	172.7 ± 0.28	4.7
Phosphorus (mg/100g)	293.4 ± 0.28	3.2
Potassium (mg/100g)	2998.7 ± 0.56	9.3

The contents are expressed on dry basis, with the exception of moisture. Results represent the average of triplicates ± SD,
* Calculated by difference of the other components.
RDI = Reference daily intake.
[a] Contribution for RDI (FDA, 2013), based on 100g of fresh cubiu pulp.
nd = non-determined.

The pulp of cubiu showed high levels of total dietary fiber, with a content of 27.0 g/100g of dry material. This value is similar to the content obtained by Yuyama *et al.*(2007) (26.5 g/100g) for one of eight etnovarieties of cubiu, but almost twice than the average of all etnovarieties studied in that work. The content of total dietary fiber of cubiu pulp is comparable with pear and sweet lime with 29.45 and 30.34 g/100g (on dry basis), respectively (RAMULU; RAO, 2003), and can be considered a good source of dietary fiber, since 100g of the fresh fruit (an average of 1 ou 2 units, depending on the variety) provides ~2.9g corresponding to about 12% of RDI.

It has been reported that rats fed with dry and sprayed whole cubiu fruit and humans who ingested 40 ml/day of cubiu extract for 3 days had lower blood glucose levels (YUYAMA *et al* 2005; SANDOVAL, 2004). The levels of cholesterol, LDL and triglycerides in humans also decreased after the treatment and the HDL level was increased. Many studies suggest that a fiber-rich diet reduces the blood glucose, triglycerides and cholesterol levels, reduces the absorption of lipids and helps prevent certain types of diabetes and heart diseases (ANDERSON, 1985; IKEDA; TOMARI; SUGANO, 1989; RICARDI; RIVELLESE, 1991; CHANDALIA *et al.*, 2000; WEICKERT; PFEIFFER, 2008). Thus, it is possible that the effect observed by Yuyama *et al.* and Sandoval are due to the high fiber content of cubiu.

The total phenolic content in the cubiu pulp was found to be 432.2 mg/100g (45.8 mg/100f of fresh weight). Contreras-Calderón *et al.* (2011) evaluated the total phenolic content in the edible part and peel of cubiu, and found contents of 70.9 mg and 87.4 mg/100g of fresh weight, respectively. The total phenolic content in the edible part described by the authors is greater than that found in the present work. However, the content of secondary metabolites depends on several factors, such as seasonality, stage of plant development, water and mineral availability, altitude, temperature and interaction with the environment (SPITALER *et al.*, 2008; TAVEIRA *et al.*, 2003; SOLAR *et al.*, 2006; RIIPI *et al.*, 2002; GOBBO-NETO; LOPES, 2007).

The minerals contents in the pulp of cubiu were found to be similar to those reported in the literature (MARX; ANDRADE; MAIA, 1998; PIRES *et al.*, 2006; YUYAMA *et al.*, 2007; GALLOZZI; DUARTE, 2007), as showed in Table 2. Cubiu proved to be rich in calcium, iron, magnesium, phosphorus, and particularly in potassium. The intake of 100g of fresh cubiu fruits provides ~ 318 mg of potassium, about 9% of the RDI of this mineral (3,500 mg/day), and the content is comparable to ripped banana (1480.8 mg/100g on dry basis) (AURORE; PARFAIT; FAHRASMANE, 2009), which is one of the main sources of potassium in the human diet (TUCKER *et al.*, 1999). Iron was the mineral that showed greater divergence between the measured content and levels already reported. The composition of fruits can be influenced by factors such as production area, soil type, climate conditions and water quality available for irrigation, justifying the differences found in the contents of some component (FORSTER *et al.*, 2002).

Table 2– Comparison between minerals contents from pulp of cubiu fruits obtained in the present study with other studies.

Mineral	Present study	Marx, Andrade and Maia (1998)	Pires *et al.* (2006)	Yuyama *et al.* (2007)	Gallozzi and Duarte (2007)
Ca	104.0	99.0	161.1	132.0	139.1
Fe	1.5	2.5	23.3	4.5	13.0
Mg	172.7	188.0	206.0	nd	nd
P	293.4	383.0	250.5	nd	260.9
K	2998.7	nd	4237.3	3751.6	nd

Contents are expressed as mg/100g of dry weight.
nd = non-determined.

1.2. Volatile compounds from the lyophilized pulp of cubiu fruits

Due the pleasant aroma of the pulp of cubiu after lyophilization the composition of its volatile compounds was analyzed.

Volatiles were extracted with ethyl ether under hydrodistillation, resulting on a white oily solid with a pleasant aroma. The yield of the extraction of volatiles was 0.035% on the weight of the lyophilized pulp. The GC-MS analysis of volatile compounds extracted by hydrodistillation from lyophilized pulp of cubiu revealed the identity of these compounds. Eleven components were detected by gas-chromatography (Fig. 1), but only seven of them could have their identity well defined by mass spectrometry (Fig 2) and Kovats index (Table 3). The chemical structure of the identified compounds is presented in Fig. 3.

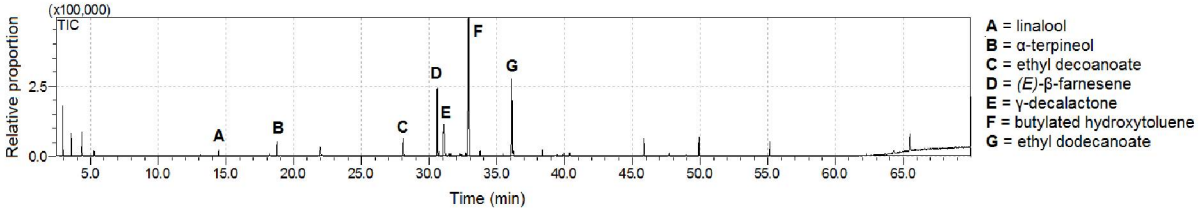

Fig. 1 - Chromatogram of volatile compounds of the lyophilized pulp of cubiu fruits.

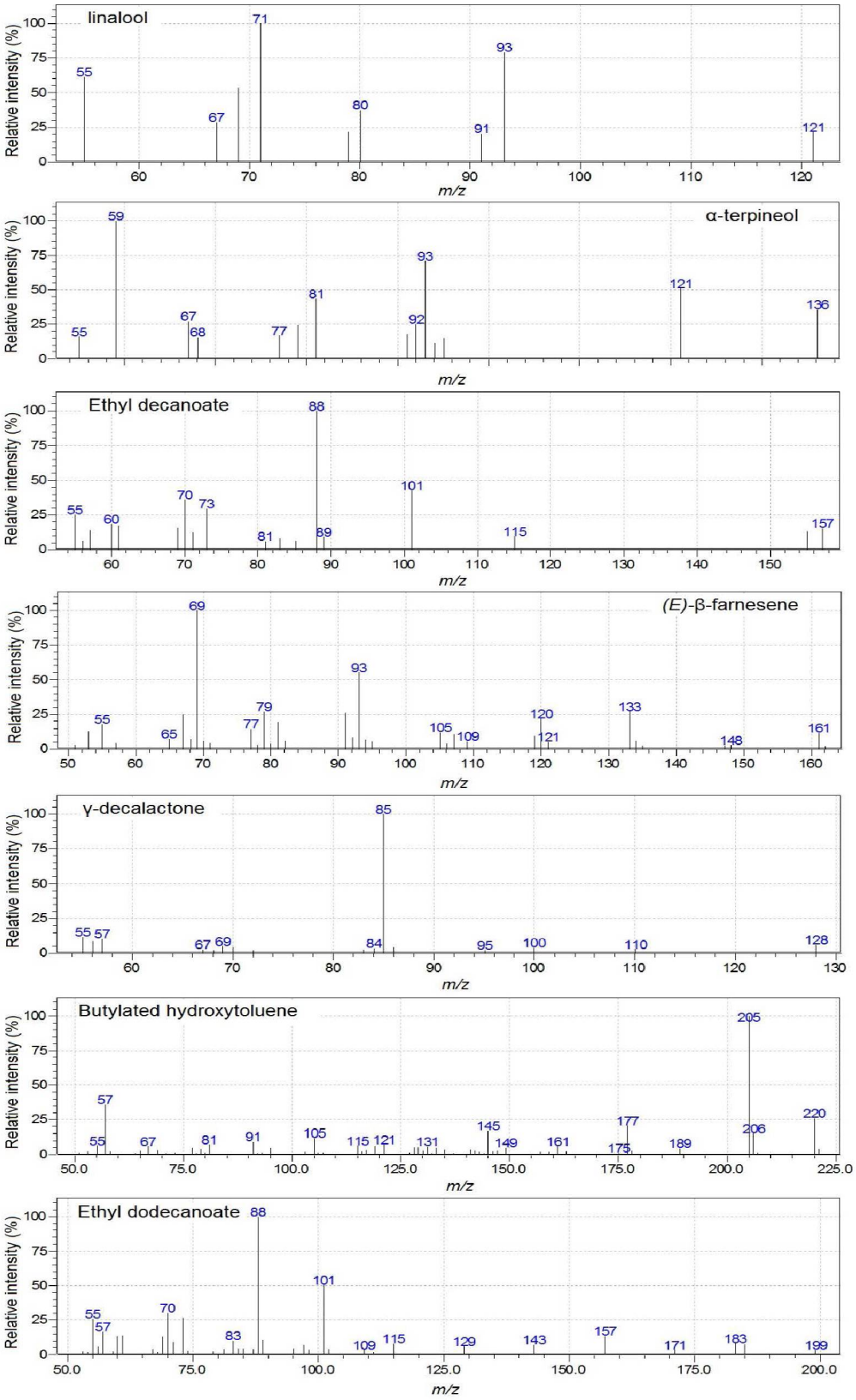

Fig. 2 – Mass spectra of the volatile compounds identified from lyophilized cubiu pulp.

The major component was butylated hydroxytoluene (BHT) (46.8%), followed by ethyl dodecanoate (18.7%) and (*E*)-β-farnesene (10.0%). These results strongly differ from those reported before. Marx, Andrade and Maia (1998) pointed safrole as major component (40.34% of total volatiles), followed by palmitic acid (18.20%), methyl salicylate (8.73%) and 1-dodecanol (6.09%), besides other 16 compounds in minor amounts. Quijano and Pino (2006), evaluating the volatile compounds of green, mature and ripe fruits, found as major components: (*Z*)-2-hexenal (0.90 mg/Kg of fresh pulp), (*Z*)-3-hexenal (0.48 mg/Kg), hexanal (0.36 mg/Kg) and (*E*)-2-hexenal, for the green fruits; methyl salicylate (0.76 mg/Kg), (*Z*)-2-hexenal (0.59 mg/Kg) and (*Z*)-3-hexenal (0.43 mg/Kg) for mature fruits; and methyl salicylate (0.85 mg/Kg), α-terpineol (0.42 mg/Kg), (*Z*)-3-hexenal (0.40 mg/Kg) and (*Z*)-2-hexenal (0.35 mg/Kg), for ripe fruits, along with a wide range of other compounds. However, both these studies used the fresh pulp for extraction of volatile compounds, while this work evaluated the volatile compounds present in lyophilized pulp, and this can lead to loss of the more volatile of lower molecular mass compounds. According to Abascal, Ganora and Yarnell (2005), lyophilization does not change the structure of volatile compounds, but change the relative concentration of them, modifying the aroma characteristics of the material. The authors have compiled a series of studies that demonstrate that the freeze-drying process led to the loss or reduction in the concentration of volatile compounds of certain plant sources, particularly monoterpenes and sesquiterpenes.

Table 3 – Volatile compounds from the lyophilized pulp of cubiu fruits.

Compound	Peak area (%)	$KI_{(cal.)}$	$KI_{(ref.)}$
Linalool	1.1	1100	1098
α-terpineol	1.0	1189	1189
Ethyl decanoate	5.5	1395	1394
(E)-β-farnesene	10.0	1455	1458
γ-decalactone	3.1	1467	1463
Butylated hydroxytoluene	46.8	1511	1512
Ethyl dodecanoate	18.7	1592	1595

Identification was based on mass spectrum and Kovats index (KI) of compounds. $KI_{(cal)}$ refers to calculated KI for the compound, and $KI_{(ref)}$ refers to reported KI for the compound by Adams (1995).

Butylated hydroxytoluene (BHT; 2,6-di-tert-butyl-4-cresol) is an antioxidant used extensively in foods, cosmetic products, insecticides, paints, plastics and petroleum products (ASH; ASH, 2004). Although its use is regulated by agencies responsible

for food safety in some countries (as U.S. FDA) for its suspected carcinogenicity and toxicity (WITSCHI; MORSE, 1983; MADHAVI; SALUNKH, 1995), some studies claim that BHT does not offer significant mutagenic risks to humans (BOMHARD; BREMMER;HERBOLD, 1992), and even may have anticarcinogenic effects in doses commonly used in foods (WILLIAMS; IATROPOULOS; WHYSNER, 1999). Although it is a synthetic antioxidant, it has been reported in some natural sources.

Babu and Wu (2008) reported one green algae (*Botryococcus braunii* Kütz.) and three cyanobacterias (*Cylindrospermopsis raciborskii* (Wołłosz.) Seenaya et Sabba Raju, *Microcystis aeruginosa* (Kütz.) and *Oscillatoria* (sp.)) that are capable of producing BHT. From vegetals, Pripdeevech and Machan (2011) found BHT among volatile compounds in essential oil of manufactured non-fermented and semi-fermented tea (*Camellia sinensis*), and Bessah and Benyoussef (2012) reported the presence of BHT in essential oil from leaves of *Arbutus unedo* L. (6.2%). In fruits, BHT was reported by Siddiqui *et al.* (2004) in *Azadirachta indica* A. Juss. in a fraction extracted with EtOH and eluted with EtOAc on a dry silica column (3.11%), and by Aourahoun *et al.*(2014) in Et_2O/Hexane extract from fruits of *Cytisus triflorus* (24.39%). BHT was also found in extract from leaves, stem and flowers of *Cytisus triflorus* (22.62%, 43.58% and 6.06%, respectively). BHT was also reported by Hanafi *et al.* (2014) as the major component (19.03%) among volatile compounds of pearl millet (*Panicum miliane).* Although the presence of BHT in some plant has already been reported earlier, this seems to be quite rare.

Fatty acid esthers are common components of volatile compounds of fruits, as guava (*Psidium guajava*) (MacLEOD; TROCONIS, 1982), soursop (*Annona muricata*) (JIROVETZ; BUCHBAUER; NGASSOUM, 2008), pawpaw (*Asimina triloba*) (SHIOTA, 1991), sour guava (*Psidium guineense*) (PERALTA-BOHÓRQUEZO *et al.*, 2010), jackfruit (*Artocarpus heterophyllus*) (BICAS *et al.*, 2011) and cupuassu (QUIJANO; PINO, 2007). These compounds also have important contribution to fruit flavour (BICAS *et al.*, 2011). Volatiles from fresh and dried naranjilla (*Solanum quitoense*), a fruit of the same genus of cubiu, also had its volatile analyzed in fresh fruits and lyophilized and showed fatty acid esthers in its composition, and also showed the disappearance of other minor compounds after lyophilization (FORERO *et al.*, 2015). The presence of ethyl dodecanoate in cubiu has previously been

reported by Quijano and Pino (2006), but ethyl decanoate is being reported in this fruit for the first time.

The terpenes linalool and α-terpineol have been reported by Quijano and Pino (2006), and have demonstrated its increased content throughout the fruit maturation. (*E*)-β-farnesene was not present in composition, but they found α-farnesene, which also had gradually increased concentration during ripening.

γ-decalactone is probably the main responsible for the pleasant aroma of the lyophilized pulp, very close to the peach (FISHER; SCOTT, 1997; AN; JOO; OH, 2013). It was among the volatiles described by Quijano and Pino (2006), also increasing its concentration during ripening.

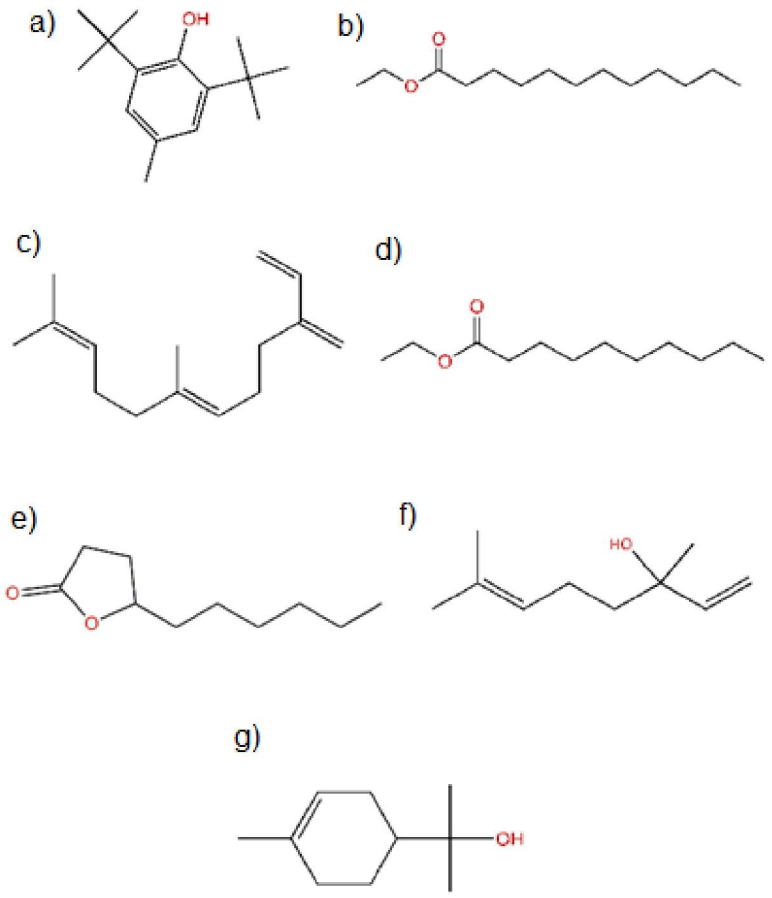

Fig. 3 – Chemical structure of volatile compounds from the lyophilized pulp of cubiu fruits.
a) Buthylated hydroxytoluene; b) ethyl dodecanoate; c) (*E*)-β-farnesene; d) Ethyl decanoate; e) γ-decalactone; f) Linalool; g) α-terpineol.
Structures were taken from www.pherobase.com.

Conclusions

Although cubiu fruits are only consumed in the Amazon region, the results showed they are a rich source of many nutrients, especially fiber and minerals and should be exploited on a larger scale.

The lyophilized pulp cubiu presented a very pleasant aroma and the analysis of their volatile indicated BHT as the main component, followed by ethyl dodecanoate, (*E*)-β-farnesene, ethyl decanoate, decalactone, linalool and terpineol. Comparing the identified volatiles on this study to those reported in the literature from fresh pulp of cubiu, the lyophilization seems to lead to losses of more volatile compounds such as large part of terpenes. This is the first time that butylated hydroxytoluene is reported in fruits of *Solanum sessiliflorum.*

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CAPÍTULO 5

ARTIGO II - CELL WALL POLYSACCHARIDES FROM PULP AND PEEL OF CUBIU (*Solanum sessiliflorum* D.)

CELL WALL POLYSACCHARIDES FROM PULP AND PEEL OF CUBIU (*Solanum sessiliflorum* D.)

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Abstract

Pulp and peel of cubiu (*Solanum sessiliflorum* D.) a native fruit from the Amazon, was subject to sequential extractions with DMSO, water, EDTA, citric acid, 2M and 4M NaOH, to afford pectic and hemicellulosic fractions. Starch was found in the fraction isolated from pulp with DMSO. The peel has proven to be the fruit tissue richest in pectin. Pectins with uronic acid content above 65% (and thus commercial grade) were obtained from the skin of cubiu fruits with water (25°C and 100°C) and EDTA and from pulp using water (100°C). The fraction with the highest yield (9.6%) and uronic acid content (79.0%) isolated from the skin was chemically characterized. The results showed that cubiu is a promising source for the extraction of pectins. Pectins with high uronic acid content can be obtained by using environmentally friendly and cheaper extraction process.

Key words: cubiu; *Solanum sessiliflorum;* peel; pulp; polysaccharides; pectin; hemicelluloses.

1. Introduction

Fruits are well known to be a source of dietary fibers which are mainly made up by cell wall polysaccharides. The cell wall polysaccharides are categorized into three main groups: pectins, hemicelluloses and cellulose (COSGROVE, 2005).

Cellulose is the most abundant polysaccharide in plant cell wall and comprises a linear polymer of β(1→4)-D-Glc forming mechanically strong crystalline microfibrils through interactions between the polymer chains (COSGROVE, 2005; CAFFAL; MOHNEN, 2009).

The hemicelluloses comprise a large group of non-cellulosic polysaccharides, extracted with alkaline solutions with a highly varied composition and structure such as xylans, mannans, β-glucans and xyloglucans (EBRINGEROVÁ; HROMÁDKOVÁ; HEINZE, 2005).

Pectins are the most complex group of plant cell wall polysaccharides and include three main class of acidic polymers: homogalacturonan (HG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II) (MOHNEN, 2008; VORAGEN *et al.*, 2009; YAPO, 2011). HG is the most abundant pectic polysaccharide and have a linear backbone of 1,4-linked α-D-GalA residues, which can be partially methyl esterified at the C-6 carboxyl and *O*-acetylated at *O*-2 or *O*-3 (MOHNEN, 2008). In RG I, α-D-GalA units are interspersed by 1,2-linked α-L-Rha residues, and some of α-L-Rha units contain neutral side chains, mainly of arabinan, galactan or arabinogalactan attached on 4-*O*-Rha (VORAGEN *et al.*, 2009; YAPO, 2011). RG II is the most structurally complex pectin, and is composed of an HG backbone with side branches containing 12 types of sugars, including some rare sugars, in over 20 different linkages (MOHNEN, 2008).

Food industry makes wide use of pectin due to its ability to form gels, being used as gelling agents and stabilizers in jams, jellies and acid milk products (WILLATS; KNOX; MIKKELSEN, 2006). The main sources of commercial pectins are citrus peel and apple pomace (MOHNEN, 2008; VORAGEN *et al.*, 2009; YAPO, 2011).

Cubiu (*Solanum sessiliflorum* D.) is a native species from Amazon which belongs to Solanaceae family. It gives fruits with yellow or orange color, with weight ranging from 20 to 490g, depending on the variety (SILVA FILHO *et al.*, 2012). The fruits have characteristic acid taste and are used in the preparation of many foods by the Amazon people (YUYAMA *et al.*, 2008). It has been pointed that the cubiu juice can be used against anemia and control of cholesterol, uric acid and blood glucose (PIRES *et al.*, 2006; SILVA FILHO *et al.*, 1999; YUYAMA *et al.*, 2008). Some health food stores have been selling fruit cubiu in capsule form or flour, recommending the use of the product in combat and control diabetes, cholesterol, triglycerides, uric acid and hypertension, among others. However, there are few scientific studies that prove the effect of intake of fruits for health.

According to Pires (2006), the fruits of cubiu are rich in pectin and the National Institute for Amazonian Research - INPA (2014) disclosed that the fruits have been

exported to Japan for pectin extraction. However, no study was found in the literature concerning the pectins or other polysaccharides from cubiu. In this way, the aim of this work was to gain information about the cell wall polysaccharides from cubiu fruits.

2. Materials and methods

2.1. Plant material

Fruits of cubiu (*Solanum sessiliflorum* D.) were acquired from São Paulo General Warehousing and Centers Company (CEAGESP), São Paulo, Brazil. The pulp and the peel (skin) were separated and then freeze-dried. The percentage of the pulp and peel was calculated on the fresh weight. The dried materials were ground into powder using an analytical mill IKA A-11. The resulting powders were treated with absolute ethanol (1:10 w/v) for 20 min under reflux affording the alcohol insoluble residue of pulp (AIR-P) and the alcohol insoluble residue of skin (AIR-S) which were used for polysaccharide extractions.

2.2. Starch content

Total starch was determined by a colorimetric method using a K-TSTA 07/11 kit (Megazyme, Ireland).

2.3. Sequential extraction of cell wall polysaccharides

The alcohol insoluble residues AIR-P and AIR-S were subject to sequential extractions. The polysaccharides were sequentially extracted from the alcohol insoluble residues as follows: DMSO 90% for 12h, in order to remove the starch, yielding fractions named DMSO; water extractions, at 25ºC for 15h and then 100ºC for 2h yielding fractions W and HW; EDTA 0.05 M for 4h, yielding fractions EDTA; citric acid, pH 2.5 at 70ºC for 30 min, yielding fractions CA; followed by alkaline extractions using 2 M and 4 M NaOH, in the presence of $NaBH_4$ at 25ºC for 16h. After each extraction, the material was centrifuged and the residue was submitted to the next extraction. After centrifugation, the alkaline extracts were neutralized with acetic acid 50% (v/v), giving rise to a precipitate which yielded fractions HA2 and HA4 for 2 M and 4 M NaOH, respectively. The supernatant was dialyzed (cut-off

12000 Da) for 3 days and precipitated with ethanol 3:1, yielding the fractions HB2 and HB4 for 2 M and 4 M NaOH, respectively. For all other extractions, ethanol (3:1 v/v) was added to the supernatant after centrifugation to precipitate the polysaccharides. After refrigeration (4ºC) overnight, the polysaccharides were isolated by centrifugation at 10,000 rpm for 20 min, washed three times with absolute ethanol and dried under vacuum. The polysaccharides fractions obtained from the pulp (AIR-P), received the letter P at the end of the sample names while those obtained from the skin (AIR-S) received the letter S at the end of the sample name.

2.4. Monosaccharide composition

The composition of neutral monosaccharide was determined after total acid hydrolysis with 2 M trifluoroacetic acid (6 h, 100°C). The monosaccharides, obtained on evaporation to dryness, were reduced with $NaBH_4$ and then acetylated with pyridine-acetic anhydride (1:1 v/v, 16 h, at 25°C). The resulting alditol acetates were extracted with $CHCl_3$, and analyzed by GC–MS (Varian, Saturn 2000R – 3800 gas chromatograph coupled to a Varian Ion-Trap 2000R mass spectrometer), using a DB-225-MS column (0.32 mm internal diameter × 30 m × film thickness 0.25 µm) programmed from 50 to 220°C at 40°C/min, with He as carrier gas at 1 ml/min.

Uronic acids content was determined by colorimetric *m*-hydroxybiphenyl method, according to Blumenkrantz and Asboe-Hansen (1973), using galacturonic acid as standard.

2.5. High Pressure Size Exclusion Chromatography (HPSEC)

Solution of polysaccharides at 1.0 mg/mL in 0.1 M $NaNO_2$ containing NaN_3 (0.5 g/L) were filtered (Millipore, 0.22 µm) and analysed by HPSEC using a Waters 2410 differential refractometer (RI) and a Wyatt Technology Dawn F multi-angle laser light-scattering (MALLS) detector. Data were collected and processed by a Wyatt Technology ASTRA software.

2.6. Nuclear Magnetic Ressonance Spectroscopy (NMR)

For ^{13}C NMR analysis, samples (20 mg) were dissolved in D_2O (400 µl) and analised in Bruker Avance DRX400 spectrometer, at 70ºC. Shifts are expressed in δ (ppm), using acetone (δ30.2) for calibration.

2.7. Degree of methyl esterification (DM)

The Fourier transform-infrared (FT-IR) spectra of pectins were collected at the absorbance mode in the frequency range of 4000–400 cm^{-1} using a Vertex 70 spectrophotometer (Bruker, Alemanha), at 4 cm^{-1} resolution. Spectroscopic grade KBr powder was used and discs were prepared using a 90:10 salt: sample proportion. The DM was determined by quantification of methyl-esterified and free uronic acid band areas of the FT-IR spectra as previously reported by Vriesmann and Petkowicz (2009).

2.8. Degree of acetylation (DA)

Degree of acetylation (DA) was determined by Hestrin (1949) colorimetric method, using galactose pentaacetate as standard, at concentrations of 0.05 – 0.6 mg/ml.

2.9. Protein content

Protein content was measured by Bradford (1976) method, using bovine soroalbumin as standard, at concentrations of 1 – 50 µg/ml.

3. Results and discussion

Mature fruits were used and the peel presented dark yellow color. The pulp corresponded to 81% of the total fresh weight of fruit, while the peel corresponded to 19%.

3.1. Polysaccharides obtained by sequential extraction with different solvents

The polysaccharides from the pulp and peel of cubiu fruits were separated according to their solubility in DMSO, water, chelating agent, dilute acid and alkali. The materials were firstly extracted with DMSO for removal of starch and then sequentially extracted with water, EDTA and citric acid to isolate pectins (CARPITA; KANABUS, 1987; VORAGEN *et al.*, 1995). The residues were treated with 2M and 4M NaOH to solubilize hemicelluloses (LINDBLAD; ALBERTSSON, 2005).

The yield and monosaccharide compositions of polysaccharide fractions obtained by sequential extractions of pulp and skin from cubiu fruits are shown in the Table 1.

Table 1 – Yield and monosaccharide composition of polysaccharide fractions obtained by sequential extractions of pulp and skin from cubiu fruits.

		Fraction																	
		Pulp									Skin								
		DMSO-P	W-P	HW-P	EDTA-P	CA-P	HA2-P	HB2-P	HA4-P	HB4-P	DMSO-S	W-S	HW-S	EDTA-S	CA-S	HA2-S	HB2-S	HA4-S	HB4-S
	Yield (%)	9.6	2.1	4.8	0.7	0.3	3.0	3.0	0.7	0.8	1.6	0.8	9.6	3.2	0.7	2.9	3.8	0.1	4.5
Monosaccharides (%)	**Rha**	0.5	1.2	1.7	5.5	4.2	2.7	1.5	1.3	1.4	2.0	2.3	2.2	3.1	3.4	1.9	3.2	2.4	1.3
	Fuc	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.9	tr	0.4	tr
	Ara	2.6	2.3	2.6	10.9	7.1	2.8	10.0	3.5	4.7	7.4	9.4	4.4	6.8	8.0	6.4	9.4	10.1	8.7
	Xyl	2.5	3.0	1.1	2.5	2.6	62.8	26.5	32.0	4.3	12.7	2.5	0.4	0.9	0.5	61.5	23.1	20.5	17.0
	Man	4.2	0.4	0.6	1.4	1.1	2.2	11.1	21.4	59.0	22.7	0.8	0.9	0.9	0.4	6.3	10.8	9.4	14.1
	Gal	4.8	3.8	4.8	14.4	13.5	4.9	11.6	7.3	9.0	22.7	14.8	11.4	20.0	31.4	6.9	11.3	16.2	16.0
	Glc	83.5	63.2	22.7	22.4	32.8	17.1	34.0	27.0	19.7	29.5	3.2	1.5	1.5	0.8	10.0	38.4	28.9	37.6
	UA[a]	1.7	25.9	66.4	42.6	38.7	7.4	5.1	7.5	1.9	3.0	66.9	79.0	66.6	55.3	6.1	3.7	12.1	5.2
	DM (%)	nd	17.4	47.2	15.4	27.8	nd	nd	nd	nd	nd	43.6	56.9	42.1	48.8	nd	nd	nd	nd

[a]Uronic acid. tr = trace. nd = not determined.

Yields are based on dry weight of AIR. Neutral sugars was determined by GC-MS of derived alditol acetates, and uronic acids was determined by Blumenkrantz and Asboe-Hansen (1973) colorimetric method.

For the pulp, the DMSO fraction had the highest yield (9.6%), while for the skin, the yield was lower (1.6%). This difference is due to the reduced content of starch in the skin (0.77%) compared with the pulp (7.14%),as determined by K-TSTA 07/11 kit (Megazyme, Ireland). The main monosaccharide of DMSO-P was Glc (83.5%) as expected for a starch fraction. However, the DMSO-S fraction had only 29.5% Glc. Gal (22.7%), Man (22.7%), Xyl (12.7%), Ara (7.4%) were also found in DMSO-S suggesting the extraction of hemicelulloses. The Xyl is probably derived from xylans, which are also soluble in DMSO (LAWTHER; SUN; BANKS, 1995; HAIMER *et al.*, 2010; SUN *et al.*, 2011). Man is not a common monosaccharide in DMSO fractions, but was also detected in considerable amount (13.4%) in DMSO extract from tomato which belongs to Solanaceae family as cubiu (SEYMOUR *et al.*, 1990).

Fractions extracted with water at 25°C from the pulp (W-P) and the skin (W-S) had great differences in their yield and composition. W-P had the second highest yield among pectic fractions from pulp (2.1%), but the smallest content of UA (25.9%) and a high content of Glc (63.2%). On the other hand, W-S had one of the lowest yields among all fractions isolated from the skin (0.8%), but the second highest content of UA (66.9%).

The presence of Glc as the main component of fraction W-P suggested that, apart to DMSO, the water also extracted mainly starch from cubiu pulp. This hypothesis was confirmed by the lugol test.

Among the pectic fractions, the highest yield was obtained with hot water extraction for both, the pulp (4.8%) and the skin (9.6%). A by-product of oil processing from the seeds of the Styrian oil-pumpkin (KOŠT'ÁLOVÁ; HROMÁDKOVÁ; EBRINGEROVÁ, 2013) apple (STEVENS; SELVENDRAN, 1984a) and pitaya fruits (RAMÍREZ-TRUQUE; ESQUIVEL; CARLE, 2011) have been also subjected to sequential extractions, and the water and/or hot water extract showed the highest yield compared to other extracts. The yield of HW-P was lower than that found for apple (6.9%) and pitaya (16.4%), but higher than that from the oil-pumpkin fruit biomass (3.7%)

According to Voragen *et al.* (1995), the extractions with water usually provide pectins with higher content of neutral sugars and lower content of uronic acids than chelation extraction. However in the present work the highest UA content was observed for fractions extracted with hot water (66.9% UA for HW-P and 79.0% UA

for HW-S). The HW-P fraction appears to contain mainly an HG together with starch, as evidenced by the presence of Glc 22.7% and confirmed by the Iugol test.

EDTA solubilized little material from pulp (0.7%), however the yield for the same fraction was higher for peel (3.2%). The pectins obtained with EDTA seem to be more branched, with higher contents of typical neutral sugar (Rha, Gal and Ara) than aqueous extractions. Pectins extracted from oil-pumpkin (KOŠŤÁLOVÁ; HROMÁDKOVÁ; EBRINGEROVÁ, 2013), apple (STEVENS; SELVENDRAN, 1984a) and pitaya (RAMÍREZ-TRUQUE; ESQUIVEL; CARLE, 2011) with chelating agents (EDTA, CDTA and ammonium oxalate, respectivelly) generally had less neutral sugars than those extracted with water. However, pectins extracted from peels of papaya and ambarella fruits also showed higher neutral sugar contents in chelate extraction than those from water-soluble pectins (KOUBALA *et al.*, 2008; KOUBALA *et al.*, 2014). The yield of EDTA-S (3.2%) is comparable to those obtained for pectins extracted with chelating agents from peach (4%; ZHOU; BEN-ARIE; LURIE, 2000), grape berry (3.7%; SAULNIER; THIBAULT, 1987) and from carrots (4.0%; STEVENS; SELVENDRAN, 1984b); lower than those from apple fruit (7.4 – 31.9%, RENARD; THIBAULT, 1993), pitaya fruit (13.4%, RAMÍREZ-TRUQUE; ESQUIVEL; CARLE, 2011), ambarella and lime peels (22% and 29.7%, KOUBALA *et al.*, 2008), dragon fruit (20.14%, ISMAIL *et al.*, 2012) and orange albedo (14%, PRABASARI *et al.*, 2011), but higher than pumpkin fruit (2.4%) (KOŠŤÁLOVÁ; HROMÁDKOVÁ; EBRINGEROVÁ, 2013) and yellow passion fruit rinds (2.1%) (YAPO; KOFFI, 2006).

Fractions extracted with citric acid, CA-P and CA-S, had the lowest yield (0.3% and 0.7%, respectively) and UA content (38.7% and 55.3%, respectively) for both, the pulp and the skin. The yield of these fractions is comparable with the pectins extracted from cupuassu pulp with citric acid in concentrations of 0.1-5.0% at 50°C (0.3%-1.5%) (VRIESMANN; PETKOWICZ, 2009).

The skin of cubiu proved to be the part of the fruit richest in pectin, having almost twice pectins than the pulp, with a total yield of 14.3% and 7.9%, respectively. As the pectic fraction HW-S, extracted with water at 100°C from the skin of cubiu fruits, had the highest yield and also the highest uronic acid content, this fraction will be further investigated.

The degree of methyl-esterification of the pectic fractions from cubiu was determined by FT-IR, and is depicted in Table 1. The hot-water soluble fractions

(HW-P and HW-S) had the highest degrees of esterification, while the fractions extracted with EDTA (EDTA-P and EDTA-S) had the lowest DM. Sequential extractions with different solvents are able to extract chemically different pectins. Hot water extracts high methyl-esterified pectins (SRIAMORNSAK, 2003), while chelant agents extract pectins with low degree of methyl-esterification and high GalA content, which are present in the middle lamella, cross-linked with Ca^{2+} ions through the "egg-box" junction zones. According to Van Buren (1991), both water and chelant-soluble pectins have high uronic acids content, with only about 2% of rhamnose and 10-20% of neutral sugars, and water-soluble pectins have higher degree of esterification than chelator-soluble. In the present work, the highest uronic acids contents were found precisely in H-W and EDTA fractions and the DM for HW fractions were higher than those for EDTA fractions, corroborating the literature.

Pectins extracted with citric acid from pulp and peel from cubiu fruits showed differences in the degree of esterification (CA-P had DM of 27.8%, and CA-S, 48.8%), although both were classified as LM. LM pectins (DM of 14.3 – 29.4%) were extracted with citric acid by Marcon *et al.* (2005) from apple pomace, by Vriesmann and Petkowicz (2009) and by Vriesmann, Teófilo and Petkowicz (2012) from cupuassu pulp (39 – 52%) and cacao pod husks (40.3%), respectively. HM pectins were obtained using citric acid from passion fruit peel (average of 59.8%) by Liew, Chin and Yusof (2014), from mango peel (78.1%) and apple (70.7%) by Kermani *et al.* (2015) and from citrus peel (65.1%) by Kurita, Fujiwara and Yamazaki (2008).

The pectins extracted from cubiu fruits showed low degree of methyl-esterification (< 50%), except the fraction HWS, which had DM of 56%. It was noticed that polysaccharides from the skin were more esterified than those from the pulp. This pattern was also observed for pectins from papaya fruits obtained by aqueous extractions. Westerlund *et al.* (1991) extracted pectins with DM of 50%, 49% and 44% from papaya pulp, while Koubala *et al.* (2014) obtained pectins with DM of 91.59% and 82.22% from papaya peel.

The contents of uronic acid found for fractions W-S, HW-P, HW-S and EDTA-S are in agreement with the requirements of European Union for commercial pectins as they have over 65% of uronic acid. However, according to the United States Pharmacopeia, only the fraction HW-S would be considered commercial because it has more than 74% of uronic acid (OVODOV, 2009). This makes cubiu a good

source of pectins, justifying its use as a new source of these polysaccharides (INPA, 2014).

The hemicellulose fractions extracted with 2M NaOH had higher yields (2.9% - 3.8%) than those extracted with 4M NaOH (0.1% - 0.8%), except fraction HB4 which showed the highest yield (4.5%) among hemicellulosic fractions. The hemiceluloses extracted from pulp of cupuassu fruit using 2M and 4M NaOH had similar yields (0.4-3.7%) (VRIESMANN; PETKOWICZ, 2009).

The yields of hemicelluloses extracted from cubiu with 2M NaOH were similar to those reported for other Solanaceae using 1M KOH, as tomato pulp (3%) (SEYMOUR *et al*, 1990) and potato tubers (2.0%) (RYDEN; SELVENDRAN, 1990). However, in the extractions with 4M KOH, only potato yielded a close value (2.4%), while tomato had a higher yield (12%).

In general, there was similarity between the fractions from the skin and pulp extracted with 2M alkali. The presence of ~ 62 % of Xyl in HA2-P and HA2-S suggests that xylans were the main polysaccharide of these fractions. The monosaccharide composition of HB2 fractions from pulp and skin were also similar, being Glc the main component (>30%) followed by Xyl, Gal, Man and Ara, suggesting the presence of xyloglucans and glucomannans. Minor amounts of UA and Rha were also found. Similar monosaccharide composition was found for hemicellulose fractions from papaya fruit (MANRIQUE; LAJOLO, 2004).

It is possible that HB2 fractions had an arabinogalactoxyloglucan as found firstly for a Solanaceae (*Solanum tuberosum*) by Ring and Selvendran (1981). Latter, it was demonstrated that other Solanaceae such as tobacco and tomato also produce atypical xyloglucans which lack fucose and have side chains terminated by arabinosyl residues (YORK *et al.*, 1996; HOFFMAN *et al*, 2005).

Differences were observed in the monosaccharide composition of fractions extracted with 4M NaOH from pulp and skin. HA4-P had Xyl (32.0%), Glc (27.0%) and Man (21.4%) as the main monosaccharides, suggesting the presence of xylans, xyloglucans and glucomannans. A similar result was found for hemicelluloses extracted with 4M NaOH from cupuassu pulp (VRIESMANN; PETKOWICZ, 2009).

HA4-S showed Glc (28.9%), Xyl (20.5%) and Gal (16.2%), besides minor amounts of UA (12.1%) and Ara (10.1%) which probably arise from a mixture of xyloglucans and pectins. This is in agreement with Voragen *et al.* (1995), who pointed out that

pectic substances are generally found in 1-4 M alkali extracts, used classically for hemicelluloses extraction. According to Caffal and Mohnen (2009), the pectins that are strongly associated with other polysaccharides in the cell wall are only extracted with dilute alkali.

Fractions HB4 from pulp and skin of cubiu fruits showed the most important differences in the monosaccharide composition among the hemicellulose fractions. HB4-P had Man (59.0%), followed by Glc (19.7%) and Gal (9.0%) as the main components which could be due to the presence of a galactoglucomannan. A galactoglucomannan was isolated by alkaline extraction from midrib of tobacco, which also belongs to the family of Solanaceae (EDA *et al.*, 1984).

Glc (37.6%), Xyl (17.0%), Gal (16.0%), Man (14.1%) and Ara (8.7%) were the main components of fraction HB4-S. The results suggest the presence of arabinogalactoxyloglucan and galactoglucomannan which were previously isolated from other Solanaceae species (EDA *et al.*, 1984; YORK *et al.*, 1996; HOFFMAN *et al.*, 2005).

3.2. Fraction HW-S

The pectic fraction HW-S which had the highest yield (9.6%) and uronic acid content (79%) was chosen for further investigation. The composition of this fraction is presented in Table 2. In addition to uronic acid, HW-S had Gal (11.4%), Ara (4.4%), Rha (2.2%), Glc (1.5%), Man (0.9%) and Xyl (0.4%) in monosaccharide composition.

Table 2 – Chemical composition of the fraction HW-S, obtained from skin of cubiu fruits by extraction with water (100°C).

	Monosaccharides (%)										
	Rha	**Fuc**	**Ara**	**Xyl**	**Man**	**Gal**	**Glc**	**UA**	**DM (%)**	**DA (%)**	**Protein (%)**
HW-S	2.2	tr	4.4	0.4	0.9	11.4	1.5	79.0	56.9	11.8	7.3

UA = uronic acid; DM = degree of methyl-esterification; DA= degree of acetylation.

Neutral monosaccharides were determined by GC-MS of alditol acetates derived; uronic acid were determined by the colorimetric method of Blumenkrantz and Asboe-Hansen (1973).

Methyl-esterification degree (DM) was determined by the relative area of the peaks for the C-6 esterified and non-esterified in the FT-IR spectrum.

Degree of acetylation (DA) was determined by the colorimetric method of Hestrin (1949).

Protein was determined by the colorimetric method of Bradford (1976).

The proportion of sugars shows that the HW-S fraction is mainly composed by HG regions. This pectin was HM (DM = 56.9%), being suitable for the application on high sucrose foods, since HM pectins form gels in acidic medium with the presence of a co-solute at concentration > 55% (THIBAULT; RALET, 2003). However, it showed a high degree of acetylation (DA=11.8%). According to some studies, a content of acetyl higher than 4% could hinder the gelation of pectins (PILNIK; VORAGEN, 1992). However, it was reported that a pectin with DM = 56.6% and DA = 17.1% isolated from cacao pod husks formed gels through the conventional mechanism of gelling for HM pectin (VRIESMANN; PETKOWICZ, 2013).

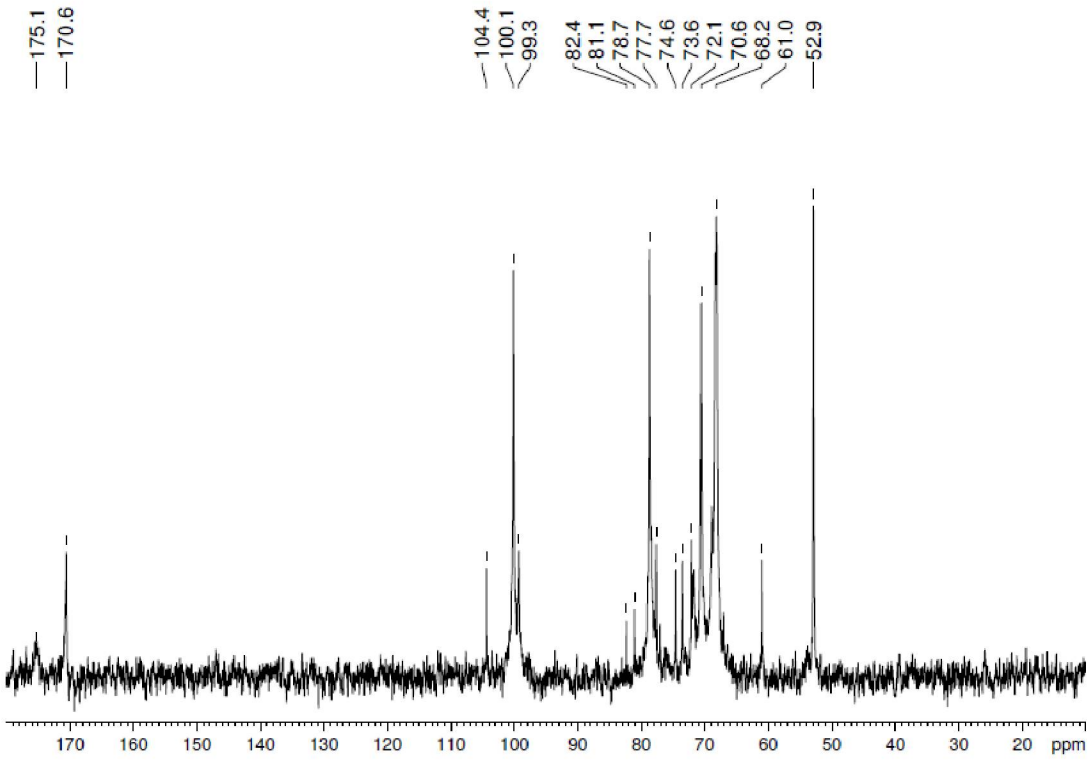

Fig. 1 - ^{13}C NMR spectrum of HW-S in D_2O at 70ºC.

The ^{13}C NMR spectrum of HW-S (fig. 1) showed intense typical signals of →4)-α-D-GalAp-(1→ units: δ100.1(C-1, esterified GalA units), δ99.3 (C-1, non-esterified GalA units), δ68.2 (C-2), δ70.6 (C-3), 78.7 (C-4), δ170.6 and δ175.1 (C-6, methyl esterified and non-esterified) and δ52.9 from methyl groups. All of the assignments were based

on values reported in the literature (VRIESMANN; PETKOWICZ, 2009; VRIESMANN; AMBONI; PETKOWICZ, 2011).

Apart to the signals characteristics of HG, signals from neutral sugars were identified. The signal at δ104.4 was attributed to C-1 of β-D-Gal. Signals at δ81.1 and δ82.4 were attributed respectively to the C-3 and C-6-linked β-D-Gal from type II arabinogalactans (CORRÊA-FERREIRA; NOLETO; PETKOWICZ, 2014; WILLFÖR *et al.*, 2002) which are composed by short (1→3) and (1→6)-D-galactan chains connected to each other by (1→3) and (1→6)-linked branch point residues. The galactosyl residues of the side chains can be substituted with α-L-Araf-(1→3) residues (FINCHER; STONE; CLARKE, 1983). The presence of a type II arabinogalactan agrees with the monosaccharide composition, in which galactose appears with 11.4% among the monosaccharides (Table 2).

When analyzed by HPSEC (Figure 2), HW-S showed a bimodal mass distribution which is in agreement with the presence of two groups of polymers. It is possible to observe one small peak eluting at ~37.5 min detected simultaneously by RI and MALLS. Although, the detection by MALLS that enable calculate the molar mass of the polymer, this determination was not performed since the molecule eluted in the void volume. This peak could arise from the type II arabinogalactan. Type II arabinogalactans are usually covalently linked to proteins, and the protein content is usually between 2% and 10%, while type I arabinogalactans are found linked to RG-I (FINCHER *et al.*, 1983; STEINHORN *et al.*, 2011). However, type II arabinogalactans were found associated to pectins extracted from spent hops (OOSTERVELD *et al.*, 2002). The protein content of 7.3% in fraction HW-S is very similar to that reported by Corrêa-Ferreira, Noleto and Petkowicz (2014) for an arabinogalactan-protein from infusion of aerial parts of *A. Absintum* with protein content of 7%.

The main peak in the chromatogram of fraction HW-S eluted at ~46min and was only detected by RI. This peak probably arises from the HG, which was the main component of the fraction. The polydisperse peak profile also supports its correlation to pectin, since this polysaccharide is very heterogeneous in size and molecular weight of the chains (HOURDET; MULLER, 1991).

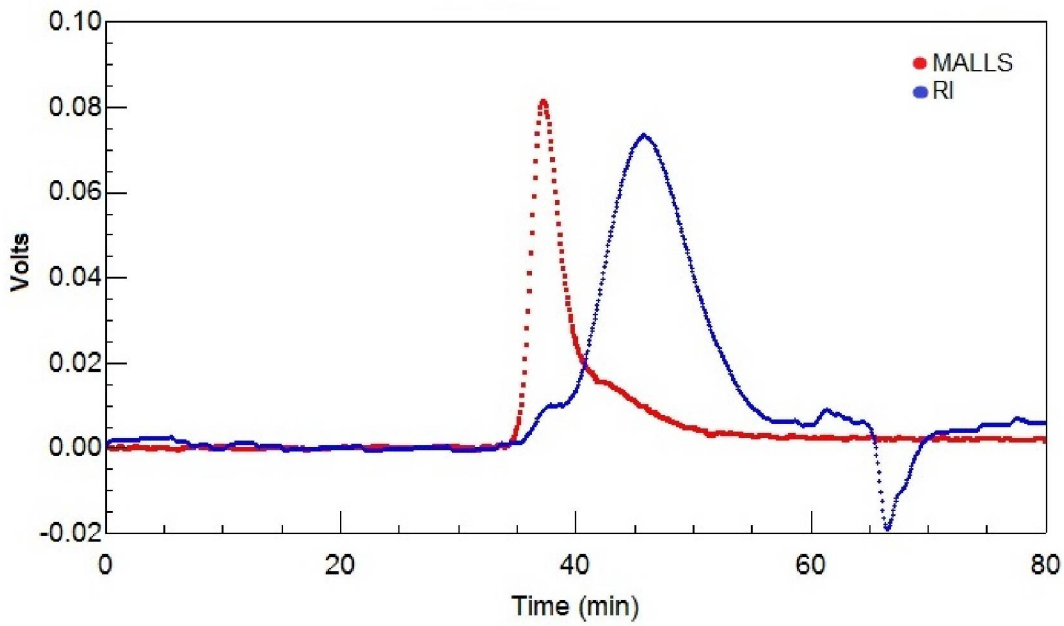

Fig. 2 - Elution profile of HW-S obtained by HPSEC-MALLS/RI.

The ^{13}C RMN data together with the monosaccharide composition and elution profile of HW-S fraction supports the existence of a mixture of an HG and a type II arabinogalactan.

Type II arabinogalactans have been studied as regards their biological properties, and several authors have shown that these molecules have a beneficial effect in the human immunomodulatory system (GÖLLNER *et al.*, 2011; THUDE *et al.*, 2006). The presence of this type of polysaccharide in cubiu fruits could contribute to the beneficial health properties attributed to the lyophilized fruit that is sold as a nutritional supplement.

Considering that it was possible to obtain pectins in agreement with the commercial requirements using hot water, which makes the extraction process cheaper and environmentally friendly, cubiu proved to be a promising source for the extraction of pectins.

Conclusions

Polysaccharides from fruits of *Solanum sessiliflorum* were studied for the first time. Sequential extractions with water, EDTA, citric acid and 2M and 4M NaOH from pulp and skin furnished pectins and hemicelluloses. The monosaccharide composition of hemicelluloses suggested the presence xylans, xyloglucans and

galactoglucomannan. Most of the pectic fractions had low DM and levels of uronic acids which were in agreement with the commercial requirements. The pectic fraction with the highest yield and uronic acid content was isolated from the skin of the cubiu fruits by aqueous extraction and had a high DM. Cubiu proved to be a promising source for the extraction of pectins.

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CAPÍTULO 6

ARTIGO III - ACID EXTRACTION AND PHYSICOCHEMICAL CHARACTERIZATION OF PECTIN FROM CUBIU (*Solanum sessiliflorum* D.) FRUITS PEEL

ACID EXTRACTION AND PHYSICOCHEMICAL CHARACTERIZATION OF PECTIN FROM CUBIU (*Solanum sessiliflorum* D.) FRUITS PEEL

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Abstract

Cubiu (*Solanum sessiliflorum* Dunal) is a fruit from Amazon region. The peel of cubiu fruits were submitted to extractions with a mineral acid and an organic acid at pH 2 for 1h at 100 ºC. The yields and uronic acid content of the polysaccharides obtained using nitric were higher than those with citric acid. Then, new tests were performed with nitric acid at pH 1.0, 1.5 and 2.0, and time of 2 and 4 h. Pectins with high yield (14%), highest uronic acid content (79%) and highest apparent viscosity were extracted from cubiu peel using nitric acid at pH 1.5 for 2 h. The pectin obtained in this condition was characterized by HPSEC, FT-IR and ^{13}C NMR. It was mainly a homogalacturonan with DM 62% and DA 5%.The pectins formed gels in acidic medium (pH<3) and 60% sucrose and the gel strength increased with decreasing of pH, suggesting possible applications as additive in acidic products with high soluble solids content.

Key words: *Solanum sessiliflorum*; cubiu peel; maná-cubiu; pectin; rheological properties.

1. Introduction

Cubiu (*Solanum sessiliflorum* Dunal) is a native fruit from Amazon. When ripe, are yellow-orange to red, and has acid and pleasant taste (PIRES *et al.*, 2006; SILVA FILHO *et al.*, 2012; SILVA FILHO *et al.*, 1999; YUYAMA *et al.*, 2007).

The marketing of cubiu occurs on a small scale, usually only in their origin region. In larger cities there are small Amazonian marketing networks, in which producers sell the fruit to intermediaries that pass these fruits for fairs and markets nearby. Restaurants and hotels on Western Amazon cities market cubiu products, such as

juices and ice cream; in Peru, there are small shops for industrialized nectars (SILVA FILHO, 1998). Although cubiu is not produced in large numbers, some farmers are producing the fruit in areas of more than two hectares, with production exported to Japan for pectin extraction (BRAZIL, 2010). Furthermore, the lyophilized pulp of cubiu has been sold, in capsules or as flour, on the grounds that this product has medicinal properties. According to the site www.milgraos.com.br, which markets the product, it is recommended for the treatment of cholesterol, triglycerides, anemia, diabetes, high blood pressure, migraine, depression, uric acid, besides being digestive, diuretic and sexual tonic". The site www.naturalviver.com.br, that also markets capsules of cubiu, presents the fruit as rich in niacin, pectin, phosphorus and vitamin C. However, no studies regarding the pectins from cubiu were found in the literature.

Pectins are a group of cell wall polysaccharides composed primarily of (1→4)α-D-GalA chains. The GalA units of the backbone can be partially methyl-esterified at the C-6 carboxyl and *O*-acetylated at *O*-2 or *O*-3, and can also contain side chains of neutral sugars, mainly arabinose and galactose (MOHNEN, 2008). Pectins are widely used on food industry as a gelling agent on the production of jams and jellies, fruit juice, and for the stabilization of acidified milk drinks and yogurts (WILLATS; KNOX; MIKKELSEN, 2006), and can be obtained from many raw materials. Only a few sources have been used for commercial production of pectin. Apple pomace and citrus peel are the main source of commercial pectins (VORAGEN *et al.*, 2009). This makes it important to search for new sources of pectins and the study of their properties.

Particulary the ability to form gels in sugar acid systems is a property which has been the main requirement of commercial pectin (MAY, 1999; PINHEIRO *et al.,* 2008). The pectin gelation occurs by different mechanisms depending on their structural characteristics. Low methyl esterified pectins (LM), with degree of esterification below 50%, form gels in the presence of Ca^{2+} ions. High methyl esterified pectins (HM), with a degree of esterification above 50%, are capable of forming gels in acidic media (in pH below 3.6) at high sucrose concentrations (usually over 55%) (THAKUR; SINGH; HANDA, 1997).

Commercial pectins are extracted with hot dilute acid at pH values about 2 (MAY, 1990). Tartaric, malic, citric, lactic, acetic and phosphoric acids can be used for

pectin extraction (CANTERI-SCHEMIN *et al.,* 2005), but commercial pectins have been extracted with nitric acid (THIBAULT; RALET, 2003).

The extraction conditions must be optimized in order to provide good yields of material that also has the desired gelling capacity (THIBAULT; RALET, 2003). On the last decade, many studies have been carried out using citric, nitric and hydrochloric acid for optimization of pectin extraction from different sources (MASMOUDI *et al.*, 2008; PINHEIRO *et al.*, 2008; KLIEMANN *et al.*, 2009; CANTERI *et al.*, 2010; VRIESMANN; TEÓFILO; PETKOWICZ, 2012; CHAN; CHOO, 2013; METHACANON; KRONGSIN; GAMONPILAS, 2014). The pectin extraction is a physicochemical process that involves hydrolysis and solubilization of the polymers from plant tissue. The optimization of extraction process must be conducted by monitoring the influence mainly of time, temperature and pH, and the typical conditions used are in the range of pH 1.5 to 3.0, temperatures between 60 and 100 °C and time of 30 min to 6 h (THIBAULT; RALET, 2003; METHACANON; KRONGSIN; GAMONPILAS, 2014).

In this work, the effect of acid type, pH and time on extraction of pectins from cubiu peel was investigated, as well as the physicochemical properties of the pectin with the highest uronic acid and high yield.

2. Materials and methods

2.1. Preparation of AIR

Solanum sessiliflorum fruits were acquired from Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP), São Paulo, Brazil. The peel (19% of fruits on fresh weight) was separated from the pulp (81% of fruits on fresh weight) and then freeze-dried. The dried peel was ground into powder using an analytical mill IKA A-11.The milled skin was subject to enzyme inactivation with absolute ethanol (1:10 w/v) for 20 min under reflux, and the resulting alcohol insoluble residue (AIR) was used for pectin extraction.

2.2. Extraction of pectins from cubiu peel

Initially, tests were performed with citric acid and nitric acid, in pH 2 for 1h at 100ºC under magnetic stirring. Then, new extractions were carried out with nitric acid, for 2 and 4h and pH of 1, 1.5 and 2 at 100ºC under magnetic stirring.

The AIR (2 g) were suspended in 20 ml of distilled water for 1 h before extraction and then the pH was adjusted with the appropriate acid with a final solid-liquid ratio of 1:25 (w/v). The extract was centrifuged at 5,000 rpm for 20 min at 4 ºC. The polysaccharide fraction was precipitated from the supernatant with addition of ethanol (3:1 v/v) and kept overnight under refrigeration at 4 ºC. The precipitate was separated by centrifuged at 5,000 rpm for 20 min at 4 ºC, washed three times with absolute ethanol and dried under vaccum.

The samples were evaluated for yield, uronic acid content and apparent viscosity (BLUMENKRANTZ; ASBOE-HANSEN, 1973). The conditions that resulted in the highest yield and uronic acid content were used for extraction of pectin in larger amount for physicochemical characterization.

2.3. Chemical characterization of the fraction with the highest yield and UA

The neutral monosaccharide composition was determined after total acid hydrolysis with 2 M trifluoroacetic acid (6 h, 100 °C). The monosaccharides, obtained on evaporation to dryness, were reduced with $NaBH_4$ and then acetylated with pyridine-acetic anhydride (1:1 v/v, 16 h, at 25 °C). The resulting alditol acetates were extracted with $CHCl_3$, and analyzed by GC–MS (Varian, Saturn 2000R – 3800 gas chromatograph coupled to a Varian Ion-Trap 2000R mass spectrometer), using a DB-225-MS column (0.32 mm internal diameter × 30 m × film thickness 0.25 µm) programmed from 50 to 220 °C at 40 °C/min, with He as carrier gas at 1 ml/min. Uronic acids content was determined by colorimetric *m*-hydroxybiphenyl method, according to Blumenkrantz and Asboe-Hansen (1973), using galacturonic acid as standard in concentrations of 5-100 µg/ml, and making up the readings at 520 nm.

The sample was analyzed by HPSEC using a Waters unit coupled to a refractive index (RI) and a Wyatt Technology Dawn-F multi-angle laser light scattering (MALLS) detector. Four Waters Ultra hydrogel columns (2000; 500; 250;120) were connected in series and coupled to the multidetection instrument. A solution of 0.1 M $NaNO_2$ and 0.02% NaN_3 was used as eluent at a flux of 0.6 ml/min. Prior to the analyses, the samples (1.0 mg/ml) were filtered through a 0.22 µm cellulose acetate membrane.

The data were collected and analyzed by a Wyatt Technology ASTRA program. All the analyses were carried out at 25 °C.

The Fourier transform-infrared (FT-IR) spectra of pectins were collected at the absorbance mode in the frequency range of 4000–400 cm^{-1} using a Vertex 70 spectrophotometer (Bruker, Alemanha), at 4 cm^{-1} resolution. Spectroscopic grade KBr powder was used and discs were prepared using a 90:10 salt: sample proportion. The DM was determined by quantification of methyl-esterified and free uronic acid band areas of the FT-IR spectra as previously reported by Vriesmann and Petkowicz (2009).

Degree of acetylation (DA) was determined by Hestrin (1949) colorimetric method, using galactose pentaacetate as standard, at concentrations of 0.05 – 0.6 mg/ml.

Protein content was measured by Bradford (1976) method, using bovine soroalbumin as standard, at concentrations of 1 – 50 µg/ml.

For ^{13}C NMR analysis, samples (20 mg) were dissolved in D_2O (400 µl) and analised in a Bruker Avance DRX400 spectrometer, at 70 ºC. Shifts were expressed in δ (ppm), using TMS (δ0.0) for calibration.

2.4. Rheological measurements

The rheological analysis was performed using a Thermo Scientific Haake Mars III rheometer (Haake GmbH, Germany) coupled to a thermostatized bath HAAKE K15 and a DC5 heating circulator.

Solutions with a concentration of 2% (w/v) were prepared in distilled water under magnetic stirring for 16 h at 25 °C and then rested for at least 1 h. Flow curves were performed in a shear rate range 0.001-500 s^{-1}, at 25 °C using a C60/2° Ti L spindle (cone and plate geometry).

Gels were prepared at concentration of 3% (w/w), sucrose was added to obtain 60% (w/w). Weighed amounts of sample were dissolved in deionized water with agitation over 16 h at 50°C and added to sucrose 70% (w/w) under magnetic stirring until total solubilization. pH was adjusted with 1M HCl or 1M NaOH to 1.5, 2.0, 2.5 or 3.0. Samples were stored under refrigeration at 4°C overnight. Analysis was carried out at 25 °C using a P35 TiL spindle (plate and plate geometry). Frequency sweeps were obtained in the range of 0.01–50 Hz within the linear viscoelastic region (obtained by strain sweep tests at 1 Hz). The software RheoWin 4.3 Data Manager

was used to obtain the rheological and statistical parameters. All experiments were performed at least induplicate and the results are the average values.

3. Results and discussion

3.1. Extraction of pectins from cubiu peel under different conditions

Pectins can be extracted with several acids (CANTERI-SCHEMIN *et al.,* 2005). In order to extract pectins, the peel of cubiu fruits were submitted to extractions with a mineral acid and an organic acid at pH 2 for 1h at 100 ºC under magnetic stirring. The yields and uronic acid content of the polysaccharides obtained using nitric and citric acid are shown in Table 1.

Table 1 – Yield and uronic acid content of pectins obtained from cubiu peel with citric and nitric acids.

Acid type	*Yield (%)	Uronic acid (%)
Nitric	11.5	71.2
Citric	12.4	40.3

*Based on the AIR.
Uronic acid were determined by the colorimetric method of Blumenkrantz and Asboe-Hansen (1973).

According to table 1, the yield of fractions extracted under the same conditions with the two acids were quite similar. Chan and Choo (2013) investigating the effect of pH and time of extraction on yield and chemical characteristics of pectins extracted from cacao husks with citric and hydrochloric acids, also found that acid type has no significant influence on yield. These authors reported yields of 3.58-7.62% with citric acid and 3.62-6.01% with HCl. These values are lower than those obtained for the pectin from cubiu peel with nitric and citric acids. Vriesmann, Teófilo and Petkowicz (2011, 2012) used citric and nitric acid to extract pectins from cocoa pod husks. These authors used an experimental design in which the effect of pH, temperature and extraction time on yield and uronic content were evaluated. The yields were little affected by the kind of acid, being 3.7-9.7% for citric acid and 5.4-11.2% for nitric acid. The yields obtained in the present work were similar to the highest yield found by Vriesmann, Teófilo and Petkowicz (2011, 2012).

However, other authors have reported that the acid type strongly influences the yield of extracted pectins from other plant sources. Canteri-Schemin *et al.* (2005) carried out a practical follow-up to pectin extraction from apple pomace with citric,

phosphoric, nitric, tartaric, hydrochloric, malic and sulphuric acids and obtained yields on the range of 10.4 – 14.7%. The response surface analisys predicted that the theoretical maximum pectin yield (17.82%) would be reached with 6.2 g/ 100 mL of citric acid and a reaction time around 150 minutes. Kliemann *et al.* (2009) performed and experimental design using citric, nitric and hydrochloric acids to extract pectins from passion fruit peel, observing that yields varied from 10% to 70%, and concluded that the theoretical best conditions predicted by response surface method for maximization of pectin yield (70%) were the use of citric acid at 80 °C, pH1 with an extraction time of 10 min. On the other hand, Yapo (2009) used nitric, citric and sulphuric acids to extract pectins also from passion fruit and observed higher yields with nitric than citric acid (6.5-13.9% and 2.8-5.1%, respectively).

Nevertheless, the uronic acid content of the pectins extracted from cubiu peel seem to be affected by the type of acid (Table 1). The pectin extracted with nitric acid had the highest uronic acid content, differing from the results found in other studies in which the uronic acid content was also a variable monitored during optimization of pectin extraction process using citric and nitric acids, and the highest uronic acid contents were observed on pectins extracted with citric acid (VRIESMANN; TEÓFILO; PETKOWICZ, 2011; VRIESMANN; TEÓFILO; PETKOWICZ, 2012).

Considering the yield was not affected by the type of acid and the uronic acid content was higher in pectin obtained with nitric acid, this acid was selected for further testing. The new tests were performed with higher extraction times (2 h and 4 h) at pH1, 1.5 and 2. The results are given in table 2.

The pectin yield ranged from 11.3 – 15.1%. The highest yield was obtained at pH 2.0 for 4 h. The uronic acid content in the pectins ranged from 51.8% to 79.4%. The highest content of uronic acid was obtained when the AIR was extracted at pH 1.5 for 2 h.

Table 2 – Yield, uronic acid content and apparent viscosity of pectins obtained from cubiu peel with nitric acid at different pH and extraction time.

Sample	pH	t (h)	Yield (%)[a]	Uronic acid (%)	η_{ap} (Pa s)[b]
N1t2	1	2	14.8	66.8	0.66 ± 0.018
N15t2	1.5	2	13.9	79.4	0.95 ± 0.097
N2t2	2	2	14.5	52.5	0.38 ± 0.0003
N1t4	1	4	11.3	75.2	0.76 ± 0.12

N15t4	1	4	13.9	51.8	0.48 ± 0.028
N2t4	2	4	15.1	54.1	0.57 ± 0.079

Samples were named according to the extraction conditions: nitric acid, pH (15 = 1.5) and time of extraction. For example, N1t2 was extracted in pH=1, for 2 h.
[a]Based on the AIR.
[b]η_{ap} values are relatives to shear rate of 0.1 s^{-1}.

Fig. 1 shows the relation between the yield and UA content with the pH and extraction time. In general, the yield was slightly affected by pH. However, a decrease in yield and an increase in the uronic acid content was observed for t (4 h) and pH (pH 1), probably due to the hydrolysis of pectins. A reduction in the molecular mass of pectins extracted from tomato was observed by Round *et al.* (2010) when subjected to hydrolysis with HCl at pH 1 and 80 °C. After 1h of hydrolysis, the authors observed a significant reduction in the average molecular weight attributed to the loss of neutral sugars. Since only large molecules are precipitated by addition of ethanol, the side chains of pectins that were hydrolized remain in solution. In this way, the yield is lowered and the uronic acid content is increased at the lower pH values. In agreement with this hypothesis, in the present work, the uronic acid content was strongly affected by pH and the extraction time.

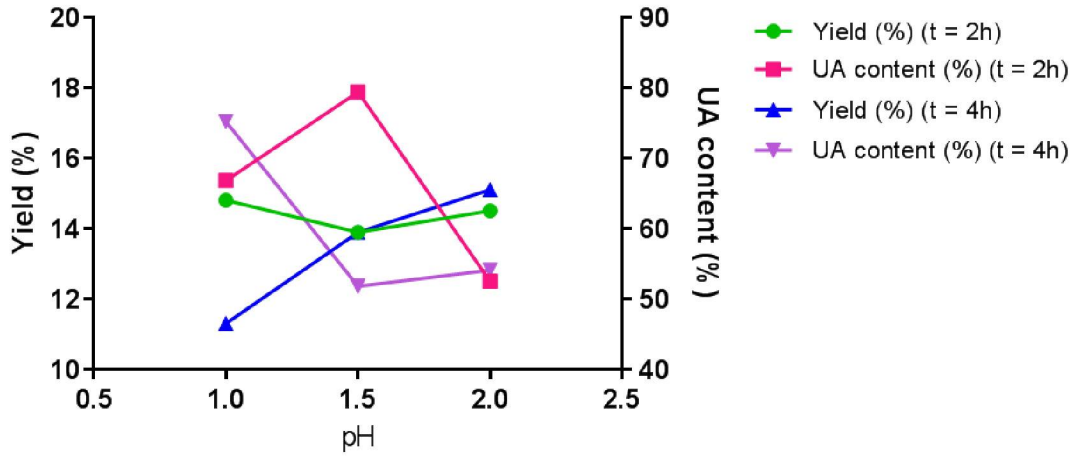

Fig. 1- Variation on yield and uronic acid content of pectins extracted from cubiu peel at different pH and extraction time of 2 h and 4 h.
UA means uronic acid.

The apparent viscosity at shear rate of 0.1 s^{-1} of the aqueous solutions (2.0%, w/v) of pectins obtained from cubiu peel with nitric acid at different pH and extraction time depicted in Table 2. The samples exhibited apparent viscosities between 0.38 and

0.95 Pa s, slightly higher than purified pectins from apple commercial pectin at the same concentration (HWANG; KOKINI, 1992). As can be seen in Table 2, N15t2 fraction showed the highest apparent viscosity among the fractions at the selected shear rate.

Based on these findings, the fraction N15t2, which was had the highest content of uronic, a high yield and the highest apparent viscosity was selected for further chemical and rheological characterization. Similar conditions was found to be the optimum conditions to extract pectin from lemon peel (nitric acid, pH 1.4, 2 h at 75 ºC; YAPO, 2009) and cacao pod husks (nitric acid, pH 1.5, 30 min at 100 ºC; VRIESMANN; TEÓFILO; PETKOWICZ, 2011).

3.2. *Chemical characterization of* fraction N15t2

The chemical composition of fraction N15t2, extracted from the peel of cubiu fruits with nitric acid in pH 1.5 for 2 h at 100 ºC, is given in Table 3.

Table 3 – Composition of the pectic fraction N15t2, extracted with nitric acid in pH 1.5 for 2 h at 100 ºC.

				Monosaccharides[a] (%)						
N15t2	**Protein (%)**	**DM[c] (%)**	**DA[d] (%)**	**Rha**	**Ara**	**Xyl**	**Man**	**Gal**	**Glc**	**UA[b]**
	12	62	5	3	2	2	2	8	4	79

[a] Neutral sugars was determined by GC-MS of derived alditol acetates, and uronic acids was determined by Blumenkrantz and Asboe-Hansen (1973) colorimetric method.
[b] UA = Uronic acid.
[c]DM = Degree of methyl-esterification, determined by the relative area of the peaks for the C-6 esterified and non-esterified in the FT-IR spectrum.
[d]DA = Degree of acetylation, determined by the colorimetric method of Hestrin (1949).
Protein was determined by the colorimetric method of Bradford (1976).

Fraction N15t2 had uronic acid as major component, besides a small amount of neutral monosaccharides, indicating that was a homogalacturonan (HG), with a small proportion of RG-I, evinced by the presence of neutral sugars such Rha (3%), Ara (2%) and Gal (8%). Uronic acid contents above 70% have been reported for pectins obtained by acidic extraction from others vegetal sources, as lemon peel (76.9% of UA) and passion fruit rind (64.9-77.3%) (YAPO, 2009), murta fruits (70.7%) (TABOADA *et al.*, 2010), orange albedo (77%) (FISHMAN *et al.*, 2007) and apple pomace (85.3%) (MIN *et al.*, 2011).

Fig. 2 ilustrates the FT-IR spectrum of the fraction, in which can be seen the two characteristic bands of esterified and non-esterified carboxyl groups at 1749 cm^{-1} and 1630 cm^{-1}, respectively. The relative intensity of these two bands gives a DM of 62%, showing that N15t2 is high methyl-esterified. This DM is similar to pectins from passion fruit rind (53-63%) (YAPO, 2009) and orange albedo (64%) (FISHMAN *et al.*, 2007) also extracted with nitric acid. High methyl-esterified (HM) pectins (DM > 50%) forms gels at pH< 3.6, requiring the presence of a co-solute, usually sucrose on concentration greater than 55% (w/w), being suitable for use in products with high level of sugar and acidic products like jellies, jams and fruit juices (THIBAULT; RALET, 2003; LOPES DA SILVA; RAO, 2006).

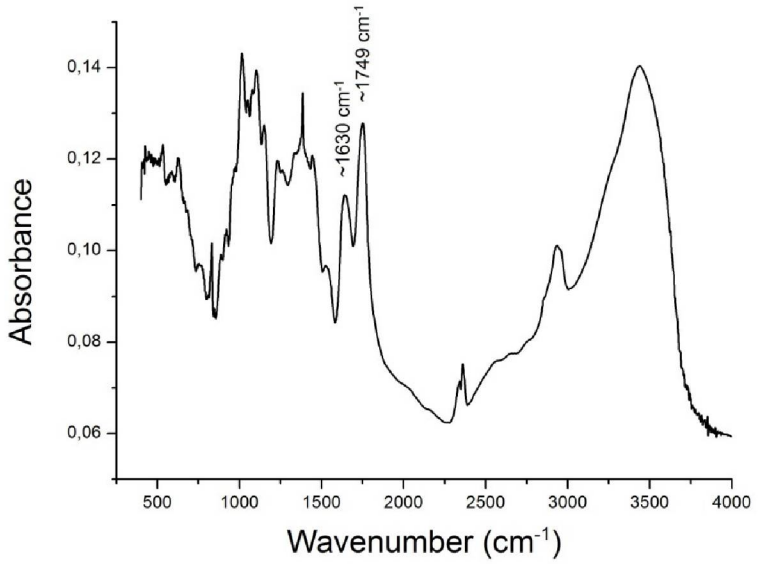

Fig. 2– FT-IR spectrum of N15t2, showing esterified (1749 cm^{-1}) and non-esterified (1630 cm^{-1}) carboxyl bands.

The degree of acetylation (DA) of N15t2 was found to be 5% (table 3). A very similar DA (4.2%) was found for ambarella peel pectin (KOUBALA *et al.*, 2008). Pilnik and Voragen (1992) point out that a content value of 4% acetyl is considered the critical limit for gel formation for previously studied systems. However, few studies have investigated the gelling properties of HM pectins with high acetyl content. Vriesmann and Petkowicz (2013) obtained a highly acetylated pectin from cacao pod

husk (DM = 56.6%; DA = 17.1%), which formed gels through the conventional mechanism of gelling for HM pectin (sucrose 60%; pH 2.5-3.3).

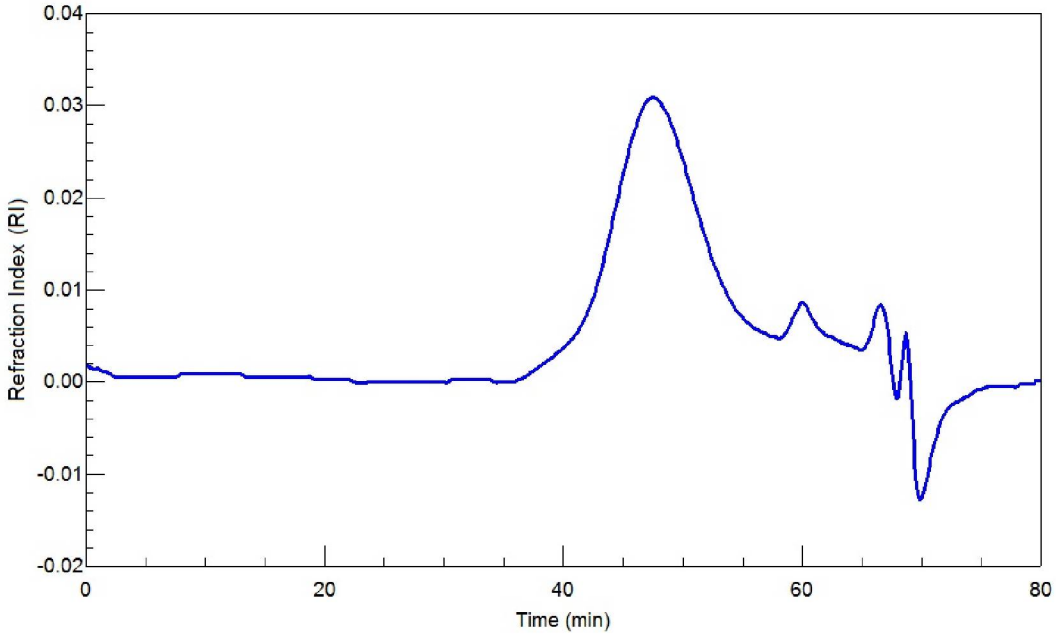

Fig. 3 – Elution profile of fraction N15t2 obtained by HPSEC coupled to refraction index (RI) detector.

Fraction N15t2 was analyzed by HPSEC using RI detector (figure 3.) It is possible to observe a main peak eluted at ~ 47min, probably from the HG which is the main component of the fraction. Another peak was detected with low intensity at ~ 60 min, indicating the presence of small amount of a component with lower molar mass.

^{13}C NMR spectrumof fraction N15t2 is shown in Fig. 4. Characteristic signals of →4)-α-D-GalA-(1→ linkages were found at δ99.9 and δ99.6, corresponding to C-1 and δ170.4 and δ172.0 corresponding to C-6 from esterified and non-esterified GalA*p* units, respectively. The intense signal at δ52.7, assigned to -CH_3O- C-6, confirmed the high degree of esterification of the pectin. Signals at δ68.0, δ70.4, 78.5 and δ71.2 are attributed, respectively, to C-2, C-3, C-4 and C-5 of D-GalA units. Signals at δ104.2, attributed to C-1 of β-Gal, and at δ16.4, corresponding to CH_3- C-6 of α-L-Rha were found, suggesting the presence of rhamnogalacturonan-I mainly branched by galactan. This is in agreement with the monosaccharide composition that showed Gal as the main neutral monosaccharide (8%) and 3% Rha. All of the above assignments were based on values reported in the literature (VRIESMANN; PETKOWICZ, 2009; VRIESMANN; AMBONI; PETKOWICZ, 2011).

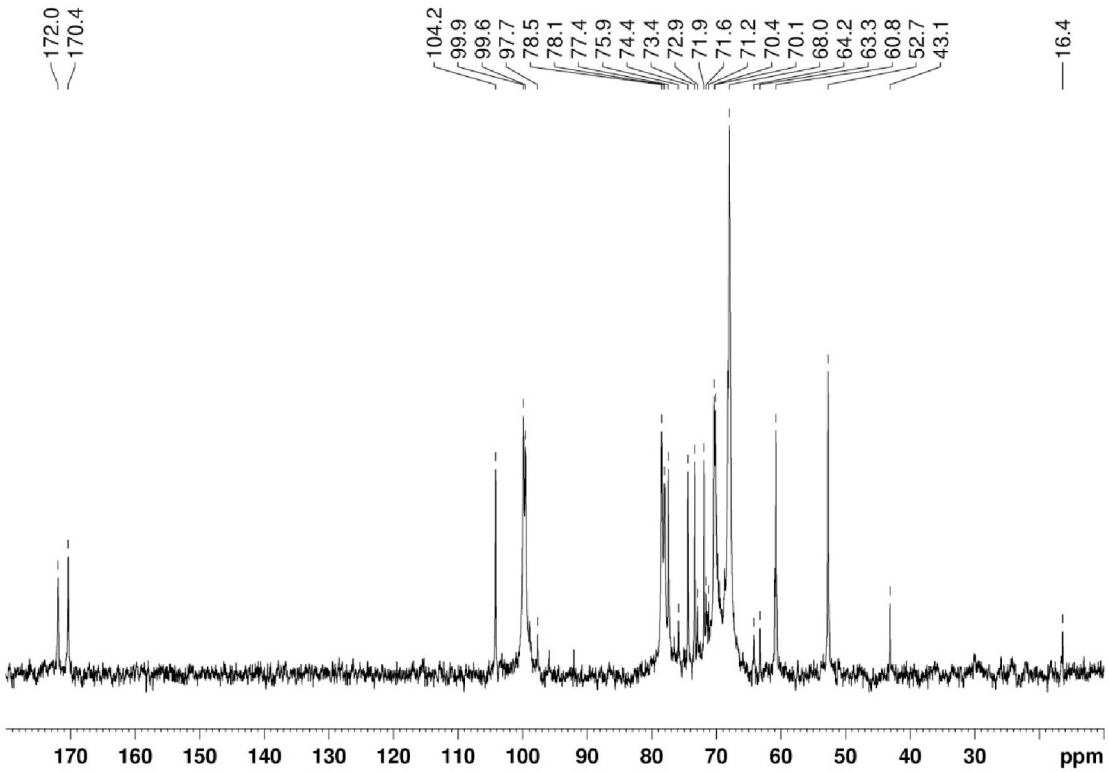

Fig. 4 - ^{13}C NMR spectrum of fraction N15t2. Analysis was carried out at 70 °C and D_2O was used as solvent.

3.3. Rheological measurements

To characterize the viscoelastic behavior of n15t2 gels, dynamic oscillatory tests were performed at 25 °C. Since fraction n15t2 was an HM pectin (DM 62%), gels were prepared at fixed concentration of pectin (3%, w/w) and high sucrose proportion (60%, w/w), varying pH from 1.5 to 3.0. The results are shown in figure 5.

For the gels prepared in pH range of 1.0-2.5, a typical gel-like behavior was observed, where the storage modulus (G') was greater than the loss modulus (G") over the entire examined frequency range. As the pH decreased, lower dependency between the moduli and the frequency was observed indicating increased gel strength. The increase in the gel strength with the decrease of pH were also observed by Vriesmann, Teófilo and Petkowicz (2012) for pectin from cocoa pod husks in the presence of 60% sucrose at pH of 2.5, 2.7 and 3.0 and by Evageliou, Richardson and Morris (2000) for pumpkin pectin with 65% sucrose at pH 3.0 to 4.7.

It is well known that the low pH reduces the negative charges on the carboxylic groups, minimizing electrostatic chain repulsion, favoring interactions between pectin chains (THAKUR; SINGH; HANDA, 1997; VORAGEN *et al.*, 1995; WALKINSHAW; ARNOTT, 1981). In the present work, the gels at pH 2.0 and 1.5 had almost identical viscoelastic behavior indicating that at pH 2.0 the chain to chain interactions were already maximized.

According Rolin and Vries (1990) gelation of HM pectins typicaly occurs at pH 3.1. However, the gelation of pectin from cubiu peel did not occur at pH 3.0, as shown in nearly overlapping the storage modulus (*G'*) and loss modulus (*G"*). Cocoa pod husk pectin also had unsatisfactory gelling properties at pH 3.0 (VRIESMANN; TEÓFILO; PETKOWICZ, 2012).

Fig. 6 shows the effect of pH on the values of G' and G" at frequency of 1 Hz. Reduction on the moduli values and the difference between them was notable when pH was raised and at pH 3.0, G' and G" reached the same value.

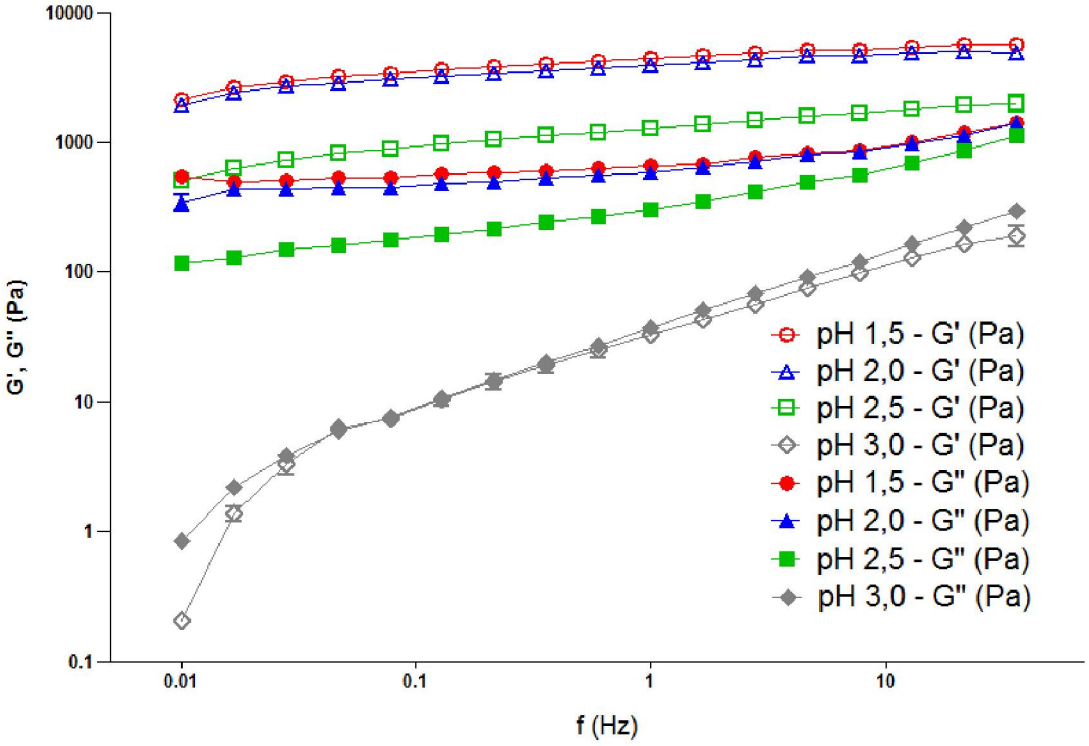

Fig. 5 - Frequency sweep at 25 °C of gels of N15t2 3% (w/w) with sucrose 60% (w/w) varying pH from 1.5 to 3.0.

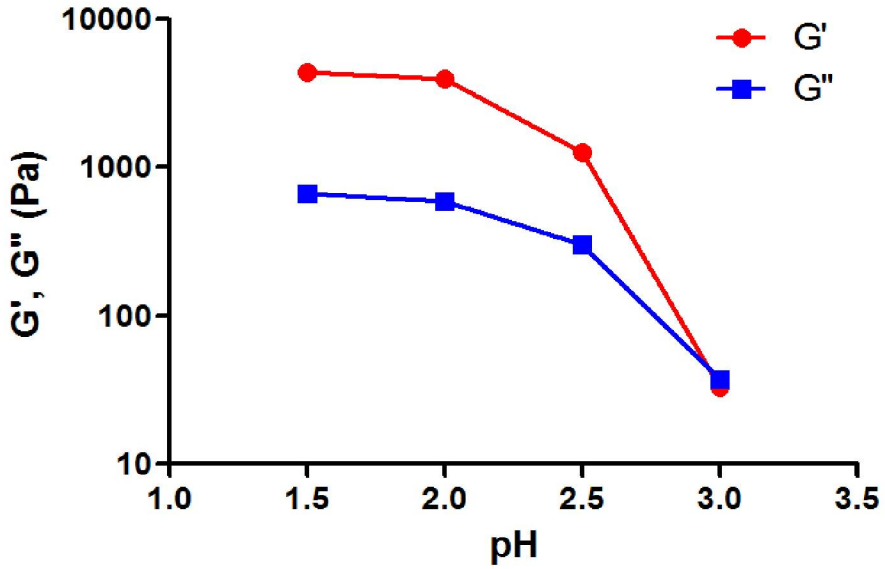

Fig. 6 – pH dependence of storage (G') and loss (G") moduli at frequency of 1 Hz.

Conclusions

Pectins with high yield (14%), uronic acid content (79%) and apparent viscosity can be extracted from cubiu peel using nitric acid at pH 1.5 for 2h. The pectin obtained in this condition was mainly a homogalacturonan with DM 62% and DA 5%. It formed gels in acidic medium (pH<3) and 60% sucrose, suggesting possible applications as additive in acidic products with high soluble solids content.

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CAPÍTULO 7

CONCLUSÕES

Conclusões

A obtenção de pectinas com alto rendimento e que atendem os requisitos comerciais, tanto por meio de extrações ácidas quanto por meio de extrações aquosas a quente, o que torna o processo mais barato e ambientalmente favorável, justifica o interesse pelos frutos de cubiu.

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