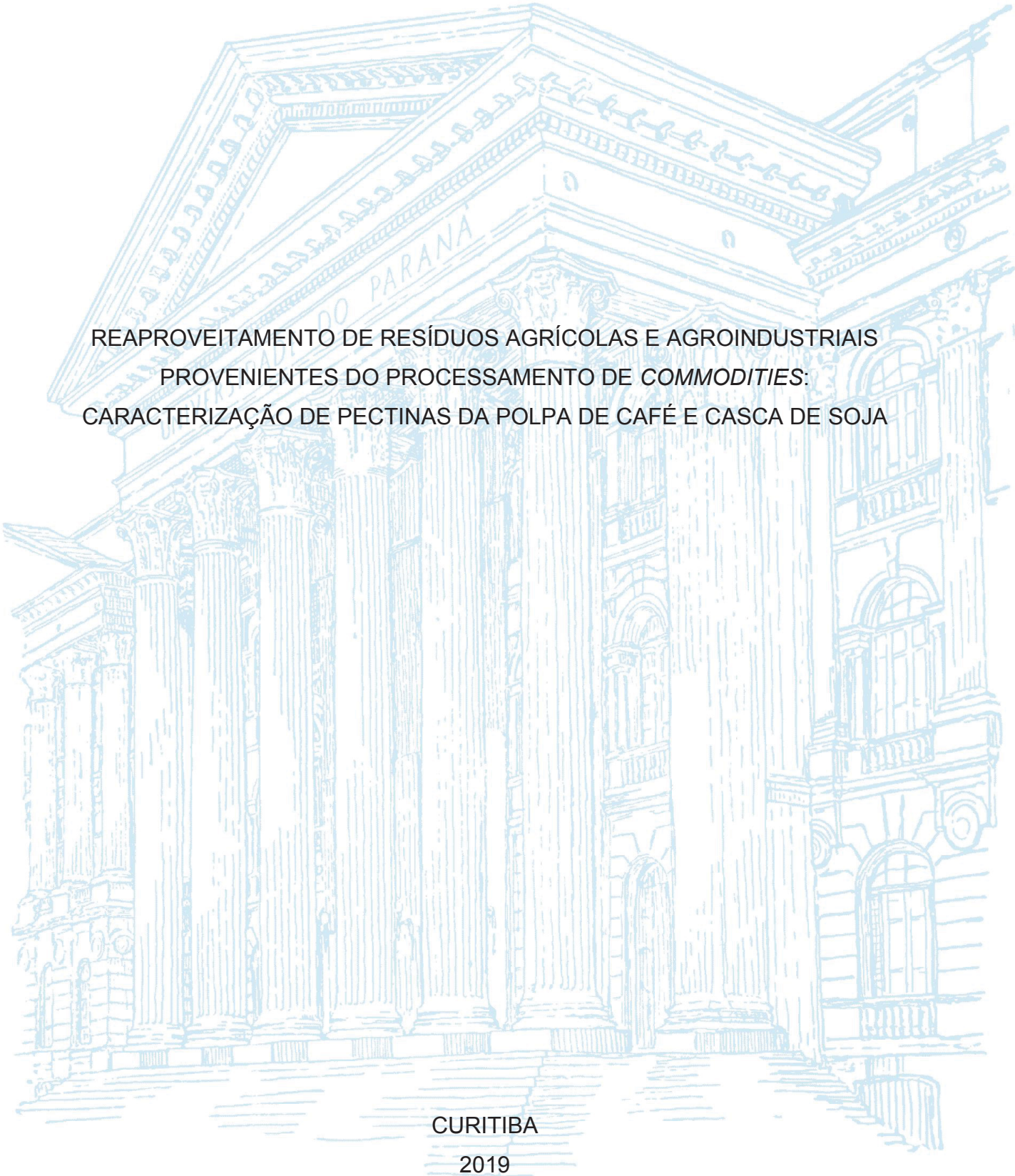
UNIVERSIDADE FEDERAL DO PARANÁ

LUIS HENRIQUE SANTOLIN REICHEMBACH



REAPROVEITAMENTO DE RESÍDUOS AGRÍCOLAS E AGROINDUSTRIAIS PROVENIENTES DO PROCESSAMENTO DE *COMMODITIES*: CARACTERIZAÇÃO DE PECTINAS DA POLPA DE CAFÉ E CASCA DE SOJA

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Dissertação apresentada ao curso de Pós-graduação em Ciências – Bioquímica, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ciências – Bioquímica.

Orientador(a): Profa. Dra. Carmen Lucia de Oliveira Petkowicz

CURITIBA
2019

Universidade Federal do Paraná. Sistema de Bibliotecas.
Biblioteca de Ciências Biológicas.
(Dulce Maria Bieniara – CRB/9-931)

Reichembach, Luis Henrique Santolin
Reaproveitamento de resíduos agrícolas e agroindustriais provenientes do processamento de *commodities*: caracterização de pectinas da polpa de café e casca de soja. / Luis Henrique Santolin Reichembach. – Curitiba, 2019.
121 p.: il.

Orientadora: Carmen Lucia de Oliveira Petkowicz

Dissertação (mestrado) - Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Ciências-Bioquímica.

1. Pectinas 2. Café 3. Soja 4. Resíduos orgânicos – Reaproveitamento 5. Reologia I. Título II. Petkowicz, Carmen Lucia de Oliveira III. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Ciências-Bioquímica.

CDD (20. ed.) 574.192482

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIENCIAS BIOLOGICAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS
(BIOQUÍMICA) - 40001016003P2

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS (BIOQUÍMICA) da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **LUIS HENRIQUE SANTOLIN REICHEMBACH**, intitulada: **REAPROVEITAMENTO DE RESÍDUOS AGRÍCOLAS E AGROINDUSTRIAIS PROVENIENTES DO PROCESSAMENTO DE *COMMODITIES*: CARACTERIZAÇÃO DE PECTINAS DA POLPA DE CAFÉ E CASCA DE SOJA**, sob orientação da Profa. Dra. CARMEN LUCIA DE OLIVEIRA PETKOWICZ, após terem inquirido o aluno e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
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AGRADECIMENTOS

Agradeço à CAPES, CNPq e demais agências financiadoras pelo apoio financeiro e por permitir que a ciência continue se desenvolvendo no Brasil.

À UFPR e ao programa de pós-graduação em Ciências - Bioquímica.

À minha família por ter formado meu caráter: ao meu pai, pela serenidade, à minha mãe, pelo esforço, ao meu irmão, pela liberdade.

À minha orientadora, Profª Carmen, pessoa que admiro muito por sua inteligência e competência. Obrigado por tudo que me ensinou e continua ensinando.

Às minhas amigas por me ensinar o que é cumplicidade e por compartilharmos muitos dos mesmos sonhos.

Ao meu namorado pela parceria e incentivo.

Aos meus colegas do laboratório 259 que participaram desta jornada: Carlos, Kaiany, Cristiane, Giulia e Flávia. Gosto demais de vocês.

Aos técnicos Flávia, Keylla, Rosane, Grazielli e Arquimedes pelas análises realizadas, indispensáveis ao meu trabalho.

Ao pessoal do Sítio São João pela polpa de café, em especial à Rafaela.

Às empresas Copacol, Coopavel, Cargill e Imcopa pelas amostras de casca de soja.

Ao Centro de Microscopia Eletrônica da UFPR pelas análises de SEM-EDS.

Às pessoas do departamento que de alguma forma contribuíram para este trabalho, seja por um favor prestado ou até mesmo um bom dia.

Obrigado a todos!

“E se eu voar ou se eu cair, ao menos posso dizer que dei tudo de mim”

RuPaul Andre Charles

RESUMO

Pectinas são polissacarídeos vegetais ricos em ácido galacturônico com diversas aplicações na indústria de alimentos, principalmente como agente gelificante. As principais matérias-primas utilizadas para a extração industrial de pectina são a casca dos cítricos e o bagaço de maçã. Entretanto, novas fontes vêm sendo estudadas com o intuito de atender à crescente demanda pelo produto. Desta forma, o objetivo deste estudo foi investigar o potencial de utilização da polpa de café e da casca de soja, duas matérias-primas facilmente acessíveis no Brasil para a extração de pectinas com características comerciais. Após extrações ácidas, as pectinas foram caracterizadas utilizando métodos colorimétricos, cromatográficos e espectroscópicos. A polpa de café foi liofilizada, tratada com etanol e extraída sequencialmente com HNO_3 fervente a 0,1 M por 30 min, resultando nas frações CAP-1 e CAP-2. O rendimento total de pectinas foi de ~18% e o teor de ácido galacturônico de 79,5% para CAP-1 e 85,6% para CAP-2, no material livre de umidade e cinzas. As massas molares foram de $3{,}921.10^5$ g/mol para CAP-1 e $2{,}642.10^5$ g/mol para CAP-2. Foram encontradas baixas concentrações de proteínas e fenólicos para ambas as frações, assim como baixo grau de acetilação. Ambas foram classificadas como pectinas HM, com um DM de 63,2% (CAP-1) e 74,1% (CAP-2). Curvas de viscosidade a 25°C de soluções a 5% das pectinas em água mostraram um comportamento pseudoplástico, sendo que CAP-2 demonstrou maior pseudoplasticidade. Varreduras de frequência revelaram que as soluções se comportaram como géis fracos. Soluções a 1% de CAP-1 em água e NaCl 0,1 M e 1,0 M mostraram que quando o pH < pK_a, a viscosidade das soluções aumentou na presença do sal, já quando o pH > pK_a, um efeito oposto foi observado. Géis foram obtidos utilizando CAP-1 como agente gelificante em diferentes pHs (1,5-3,0), concentrações de pectina (0,5-2,5%), sacarose (55-65%) e xilitol (55-60%). O efeito da temperatura na formação desses géis também foi avaliado. As pectinas da casca de soja moída foram extraídas com HCl 0,1 M a 90°C por 45 min (extração A) e com HNO_3 0,14 M fervente por 30min (extração B) e 60 min (extração C). As extrações A, B e C resultaram em pectinas com conteúdo de ácidos urônicos de 32,1%, 37,2% e 41,5%, respectivamente, abaixo dos parâmetros comerciais (>65%). Cascas de soja provenientes de diferentes fornecedores foram utilizadas para a extração de pectinas, corroborando os resultados iniciais. Os conteúdos de manose e galactose foram altos, indicando a presença de galactomananas, o que foi confirmado por RMN. Uma extração aquosa a 40°C por 16 h foi realizada com o intuito de remover a galactomanana e extrações ácidas foram conduzidas com o material residual. O conteúdo de ácidos urônicos do material proveniente da extração ácida aumentou e uma análise de FT-IR indicou um DM de 29,3% para a pectina da casca de soja. Entretanto, a pectina ainda apresentou um teor de ácidos urônicos < 65%. Os resultados obtidos demonstraram que a extração ácida da casca de soja não fornece pectinas com características adequadas para uso comercial. Já a polpa de café, pode ser usada para a obtenção de pectinas com propriedades gelificantes e que atendem aos requisitos comercias.

Palavras-chave: Pectinas. Polpa de café. Casca de soja. Aproveitamento de resíduos. Reologia.

ABSTRACT

Pectins are plant polysaccharides rich in galacturonic acid with several applications in the food industry, mainly as gelling agent. The main raw materials used for industrial extraction of pectin are citrus peel and apple pomace. However new alternative sources have been studied aiming to supply the growing demand for the product. Thus, the objective of the study was to investigate the potential of utilization of coffee pulp and soy hull, both easily accessible in Brazil, for the extraction of pectins with commercial characteristics. After acid extractions, pectins were characterized using colorimetric, chromatographic and spectroscopic methods. Coffee pulp was lyophilized, treated with ethanol and sequentially extracted with boiling 0.1 M HNO_3 for 30 min, giving rise to fractions CAP-1 and CAP-2. The total yield of pectins was ~18% and the galacturonic acid content of 79.5% for CAP-1 and 85.6% for CAP-2, on the ash and moisture-free substances. The molar masses were $3.921.10^5$ for CAP-1 and $2.642.10^5$ for CAP-2. Low concentration of proteins and phenolics were found for both fractions, as well as low degree of acetylation. Both were classified as HM pectins, with DM of 63.2% (CAP-1) and 74.1% (CAP-2). Viscosity curves at 25°C of 5% pectin solutions in water showed a pseudoplastic behavior and CAP-2 demonstrated higher pseudoplasticity. Frequency sweeps revealed that the solutions behaved as weak gels. 1% solutions of CAP-1 in water and 0.1 and 1.0 M NaCl showed that when the pH < pK_a, the viscosity of the solutions increased in the presence of the salt, while at pH > pK_a, the opposite effect was observed. Gels were formed using CAP-1 as a gelling agent in different pHs (1.5-3.0) and concentrations of pectin (0.5-2.5%), sucrose (55-65%) and xylitol (55-60%). The effect of temperature on the formation of the gels was also evaluated. Soy hull pectins were extracted with 0.1 M HCl at 90°C for 45 min (extraction A) and with boiling 0.14 M HNO_3 for 30 min (extraction B) and 60 min (extraction C). Extractions A, B and C resulted in pectins with uronic acid contents of 32.1%, 37.2% and 41.5%, respectively, lower than the commercial parameters (>65%). Soy hulls from different suppliers were used in order to extract pectins and the previous results were corroborated. Mannose and galactose content were high, indicating that a galactomannan was being coextracted in the acid extractions, which was confirmed by NMR. An extraction with water at 40°C for 16h was carried out aiming to remove the galactomannan and then acid extractions were performed with the residual material. The uronic acid content of the material coming from the acid extractions increased and a FT-IR analysis revealed a DM of 29.3% for soy hull pectin. However, the pectin still had an uronic acid content < 65%. The results demonstrated that the acid extraction of soy hull does not provide pectin with suitable characteristics for commercial use. On the other hand, coffee pulp can be used for the obtention of pectin with gelling properties and that fulfill the commercial requirements.

Key words: Pectins. Coffee pulp. Soy hull. Crop residue. Rheology.

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LISTA DE ABREVIATURAS OU SIGLAS

AGA - Apiogalacturonana

AIR - Resíduo insolúvel em álcool (*alcohol insoluble residue*)

BSA - Soroalbumina bovina (*bovine serum albumin*)

CAP-1 - Pectina de *Coffea arabica*-1

CAP-2 - Pectina de *Coffea arabica*-2

CR - Taxa controlada (*controlled rate*)

DA - Grau de acetilação (*degree of acetylation*)

DM - Grau de metil-esterificação (*degree of methyl-esterification*)

FAO - Organização das Nações Unidas para Agricultura e Alimentação (*Food and Agriculture Organization*)

FT-IR - Espectroscopia no infravermelho com transformada de Fourier (*Fourier-transform infrared*)

GalA - Ácido galacturônico (*galacturonic acid*)

GC - Cromatografia gasosa (*gas chromatography*)

HG - Homogalacturonana

HM - Alto grau de metil-esterificação (*high methoxyl*)

HPSEC - Cromatografia por exclusão de tamanho de alta performance (*high performance size exclusion chromatography*)

HSQC - Correlação heteronuclear de quantum único (*heteronuclear single quantum coherence*)

LM - Baixo grau de metil-esterificação (*low methoxyl*)

MALLS - Espalhamento de luz laser com multiângulos (*multi angle laser light scattering*)

NMR - *Nuclear magnetic resonance*

RG-I - Ramnogalacturonana-I

RG-II - Ramnogalacturonana-II

RI - Índice de refração (*refractive index*)

RMN - Ressonância magnética nuclear

SEM-EDS - Microscopia eletrônica de varredura e espectroscopia por dispersão de energia (s*canning electron microscopy and energy dispersive spectroscopy*)

TFA - Ácido trifluoracético (*trifluoracetic acid*)

TGA - Análise termogravimétrica (*thermogravimetric analysis*)

TLC - Cromatografia em camada delgada (*thin-layer chromatography*)

UA - Uronic acids

UTMC - Controlador universal de temperatura (*universal temperature module controller*)

XGA - Xilogalacturonana

LISTA DE SÍMBOLOS

δ	- Deslocamento químico
f	- Frequência
dn/dc	- Incremento do índice de refração
G’	- Módulo elástico
G”	- Módulo viscoso
M_w	- Massa molar média
M_w/M_n	- Polidispersão
η	- Viscosidade
η_∞	- Viscosidade em taxa de cisalhamento infinita
η_0	- Viscosidade em taxa de cisalhamento nula
K	- Constante de tempo de Cross
n	- Índice de comportamento de fluxo
R^2	- Coeficiente de determinação
tan δ	- Tangente de perda

SUMÁRIO

CAPÍTULO 3 – ARTIGO: "Investigation of the potential of soy hull as a source of commercial pectin"

1 INTRODUÇÃO

As pectinas são um grupo de polissacarídeos da parede celular primária e de regiões intercelulares de plantas superiores, amplamente utilizadas como agente gelificante, espessante, emulsificante e estabilizante de diversos produtos alimentícios (FU; RAO, 2001). Atualmente, a casca dos cítricos é a principal matéria-prima utilizada para a produção comercial de pectina (85,5%), seguida pelo bagaço de maçã (14%), ambos resíduos da indústria de sucos (CIRIMINNA; CHAVARRÍA-HERNÁNDEZ; HERNANDÉZ, 2015).

O mercado de pectina cresceu cerca de 16% entre os anos de 2015 e 2018 (GRAND VIEW RESEARCH, 2017; WISE GUY REPORTS, 2019), mostrando uma tendência de crescimento na utilização do produto. A produção deve acompanhar o ritmo de crescimento da demanda e novas fábricas devem ser abertas, como por exemplo a recém confirmada fábrica da Cargill no estado de São Paulo (CARGILL, 2019). É de se esperar que a demanda por matéria-prima aumente, justificando o estudo de novas fontes para a extração comercial de pectina.

Diversos materiais alternativos vêm sendo estudados como prováveis fontes de pectina, tais como a casca do cacau (VRIESMANN; AMBONI; PETKOWICZ, 2011), a casca do maracujá (YAPO; KOFFI, 2006), a casca da melancia (PETKOWICZ; VRIESMANN; WILLIAMS, 2016), o bagaço do fruto de caju (YAPO; KOFFI, 2014), casca de pomelo (LIEW *et al.*, 2018), polpa de batata (YANG; MU; MA, 2018) e a casca da soja (KALAPATHY; PROCTOR, 2001). Dentre os materiais, destacam-se os que possuem grande disponibilidade e baixo custo, especialmente se forem considerados resíduos indesejados. Desta forma, ganham notoriedade os resíduos de *commodities,* pela grande extensão de áreas produtoras e volume de geração de matéria-prima. Enquadrados nessas características estão a polpa de café e a casca de soja, ambas largamente disponíveis no Brasil.

Considerado um resíduo agrícola, a polpa do café possui poucos estudos relacionados a extração de suas pectinas, com pobreza de informações relacionados a sua caracterização química. Não há registros da formação de géis e análises reológicas utilizando esta pectina. Já a casca de soja, um subproduto agroindustrial, costuma ser apontada como um dos

principais potenciais materiais alternativos para a produção de pectinas. Porém, os escassos dados sobre sua caracterização química e propriedades reológicas não são suficientes para implementar a sua utilização.

Os resultados obtidos no presente estudo são apresentados em 3 capítulos, redigidos na forma de artigos. O capítulo 1 descreve a caracterização química e estrutural de duas frações de pectina (CAP-1 e CAP-2) obtidas por extrações ácidas da polpa de café e as propriedades reológicas de solução aquosas a 5%. O capítulo 2 apresenta um estudo reológico da fração CAP-1, focando nos fatores que afetam a viscosidade das soluções e as propriedades gelificantes da pectina. Finalmente, o capítulo 3 relata a obtenção e caracterização da composição monossacarídica de pectinas obtidas da casca de soja usando diferentes condições de extração. Juntos, os capítulos sintetizam o trabalho realizado durante o período de mestrado e propõem uma alternativa para a utilização de dois materiais provenientes do processamento de *commodities* produzidas no Brasil.

1.1 OBJETIVOS

1.1.1 Objetivo Geral

Investigar o potencial de utilização da polpa de café e da casca de soja para a extração de pectinas com características comerciais.

1.1.2 Objetivos Específicos

- Caracterizar a composição monossacarídica das matérias primas utilizadas para a extração de pectina (polpa de café e casca de soja) e do material residual resultante das extrações;
- Obter pectinas da polpa de café e da casca de soja por meio de extrações ácidas;
- Identificar as condições de extração mais apropriadas para a obtenção de pectinas;
- Caracterizar as frações de pectina obtidas quanto a sua composição e estrutura química;

- Investigar as propriedades reológicas da pectina da polpa de café.

1.2 JUSTIFICATIVA

O Brasil é o maior produtor mundial de café, em especial o *Coffea arabica*, chegando a produzir 3,75 milhões de toneladas em 2018 (INTERNATIONAL COFFEE ORGANIZATION, 2019). Quando o processamento da cereja do café se dá pela via úmida, a polpa é produzida em abundância, uma vez que representa entre 39-49% da massa do fruto fresco (DURÁN *et al.*, 2017). A polpa do café é considerada um resíduo, visto que o grão é o produto de interesse. Desta forma o presente estudo se justifica ao propor uma forma de agregar valor a um resíduo agrícola subutilizado, que ainda pode gerar problemas ambientais caso seja disposto de forma incorreta. Não há registros na literatura de estudos envolvendo a formação de géis utilizando a pectina da polpa de café e suas características reológicas.

Outro material muito pouco utilizado e amplamente produzido no Brasil é a casca de soja, subproduto do processamento do grão para a produção de óleo e farelo. É um material vegetal barato, rico em fibras e utilizado, em geral, apenas para a alimentação animal (SILVA, 2004). Estudos mostram que a casca de soja possui um grande potencial como matéria-prima para a extração de pectinas, porém elas ainda não foram caracterizadas quanto sua estrutura e composição.

Análises para determinar a composição e estrutura química das pectinas presentes nos resíduos e subprodutos agrícolas e agroindustriais são indispensáveis para propor aplicações em diferentes segmentos da indústria.

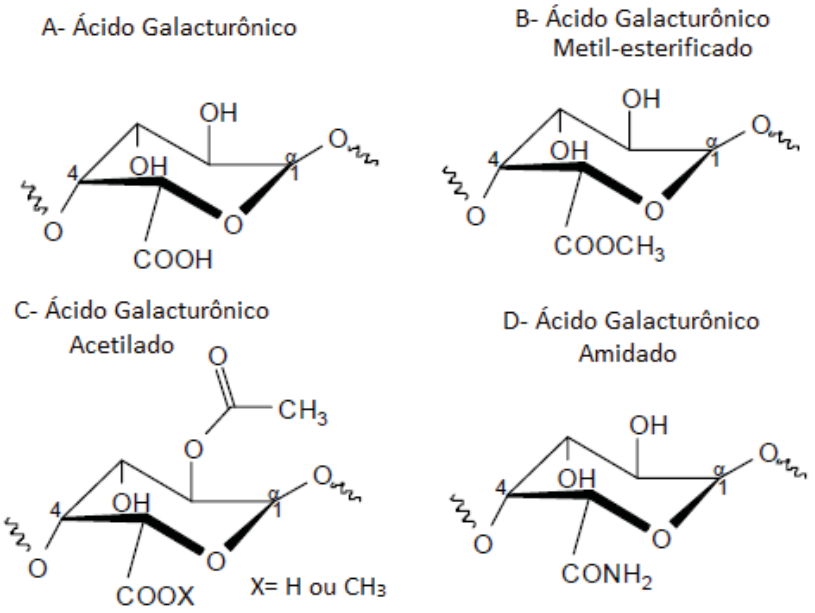
2 REVISÃO DA LITERATURA

2.1 A ESTRUTURA DAS PECTINAS

As pectinas são um grupo de polissacarídeos encontrados na parede celular primária e nas regiões intercelulares de células vegetais, especialmente nas dicotiledôneas, onde estão presentes em concentração de aproximadamente 30-35%. Nas monocotiledôneas e tecidos lenhosos são encontradas em quantidades menores, entre 2-10% e 5%, respectivamente. As pectinas participam de processos fisiológicos complexos, como crescimento e diferenciação celular, mecanismos de defesa contra patógenos, transporte de íons, permeabilidade da parede e capacidade de retenção de água pela célula, muitos destes relacionados a sua natureza aniônica (ASPINALL, 1980; WILLATS, KNOX & MIKKELSEN, 2006; VORAGEN; COENEN, 2009).

As pectinas têm como característica dominante a presença de grandes quantidades de ácido D-galacturônico (GalA) em sua estrutura (FIGURA 1 - A). As unidades de GalA podem conter o grupo funcional metoxil em C-6 (FIGURA 1 - B), e o substituinte acetil pode estar presente em C-2 ou C-3 (FIGURA 1 – C). Se a pectina for tratada com amônia, os grupos metil carboxilato são convertidos em grupos carboxamidas (FIGURA 1 – D) e o produto resultante, a pectina amidada, tem importância comercial (ASPINALL, 1980; ROLIN, 1993).

FIGURA 1 – DIFERENTES FORMAS APRESENTADAS PELAS UNIDADES DE ÁCIDO GALACTURÔNICO NAS PECTINAS



FONTE: Adaptado de Cpkelco (2005)

Além do ácido galacturônico, as pectinas ainda possuem uma certa quantidade de açúcares neutros, sendo a maior parte presente em cadeias laterais. De Vries (1983) identificou na pectina extraída da maçã, um padrão de regiões lisas, contendo apenas o ácido galacturônico, e regiões ramificadas, onde estavam a maior parte dos açúcares neutros. Mais tarde, foram isolados e caracterizados diferentes polissacarídeos pécticos, que incluem a homogalacturonana (HG), ramnogalacturonana I (RG-I), ramnogalacturonana II (RG-II), xilogalacturonana (XGA) e apiogalacturonana (AGA) (SCHOLS; VORAGEN, 1996; RIDLEY; O'NEILL; MOHNEN, 2001).

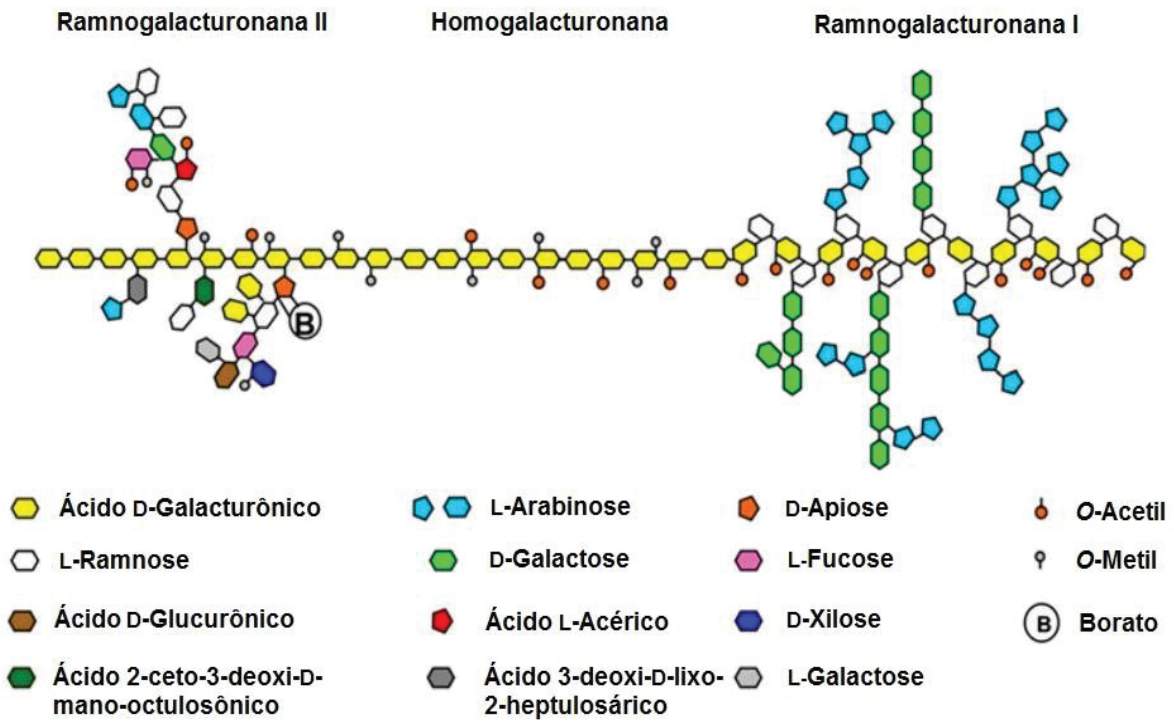
A HG corresponde a um homopolímero linear de unidades de GalA unidas por ligações α-(1→4) sendo a mais abundante das pectinas (~65%). As HG apresentam uma certa porcentagem de suas unidades de GalA metil-esterificadas, a qual é referida como grau de metil-esterificação (DM). Pectinas com um DM maior que 50% são classificadas como pectinas de alto grau de metil-esterificação (HM), enquanto as inferiores a 50%, como pectinas de baixo grau de metil-esterificação (LM) (BEMILLER, 1986).

Presentes em quantidades entre 20 a 35%, as RG-I possuem uma cadeia principal formada por repetições de [→4)α-D-GalA-(1→2)-α-L-Rha-

(1→]$_n$, onde 20-80% das unidades de ramnose possuem cadeias laterais contendo α-L-Ara*f* e/ou β-D-Gal*p*. As unidades de GalA presentes tanto em HG quanto em RG-I podem estar acetiladas na posição O-2 e/ou O-3 do anel.

Correspondendo a apenas 10% das pectinas, as RG-II são altamente conservadas nas células vegetais e têm como característica uma cadeia principal de HG contendo cadeias laterais com 12 tipos diferentes de monossacarídeos (KOUWIJZER; SCHOLS; PÉREZ, 1996, MOHNEN, 2008). A estrutura dos principais polissacarídeos que constituem as pectinas está representada de forma esquemática na FIGURA 2.

FIGURA 2 – REPRESENTAÇÃO ESQUEMÁTICA DA ESTRUTURA DOS PRINCIPAIS POLISSACARÍDEOS PÉCTICOS



FONTE: Adaptado de Harholt, Suttangkakul e Scheller (2010)

Já as XGA e AGA, são homogalacturonanas substituídas que foram encontradas na parede celular de um número restrito de plantas. As XGA contêm unidades de β-D-xilopiranose (Xyl*p*) ligadas ao C-3 de unidades de GalA, podendo ser encontradas na parede de tecidos reprodutivos de plantas, como da maçã, cenoura e algodão. Por outro lado, as AGA são encontradas em algumas monocotiledôneas aquáticas e contêm unidades de β-D-

apiofuranose (Api*f*) ligadas ao C-2 ou C-3 de unidades de GalA da cadeia principal, na forma de uma única unidade ou do dissacarídeo β-D-Api*f*-(1→3')-β-D-Api*f*-(1→ (RIDLEY; O'NEILL; MOHNEN, 2001).

2.2 A IMPORTÂNCIA INDUSTRIAL DA PECTINA

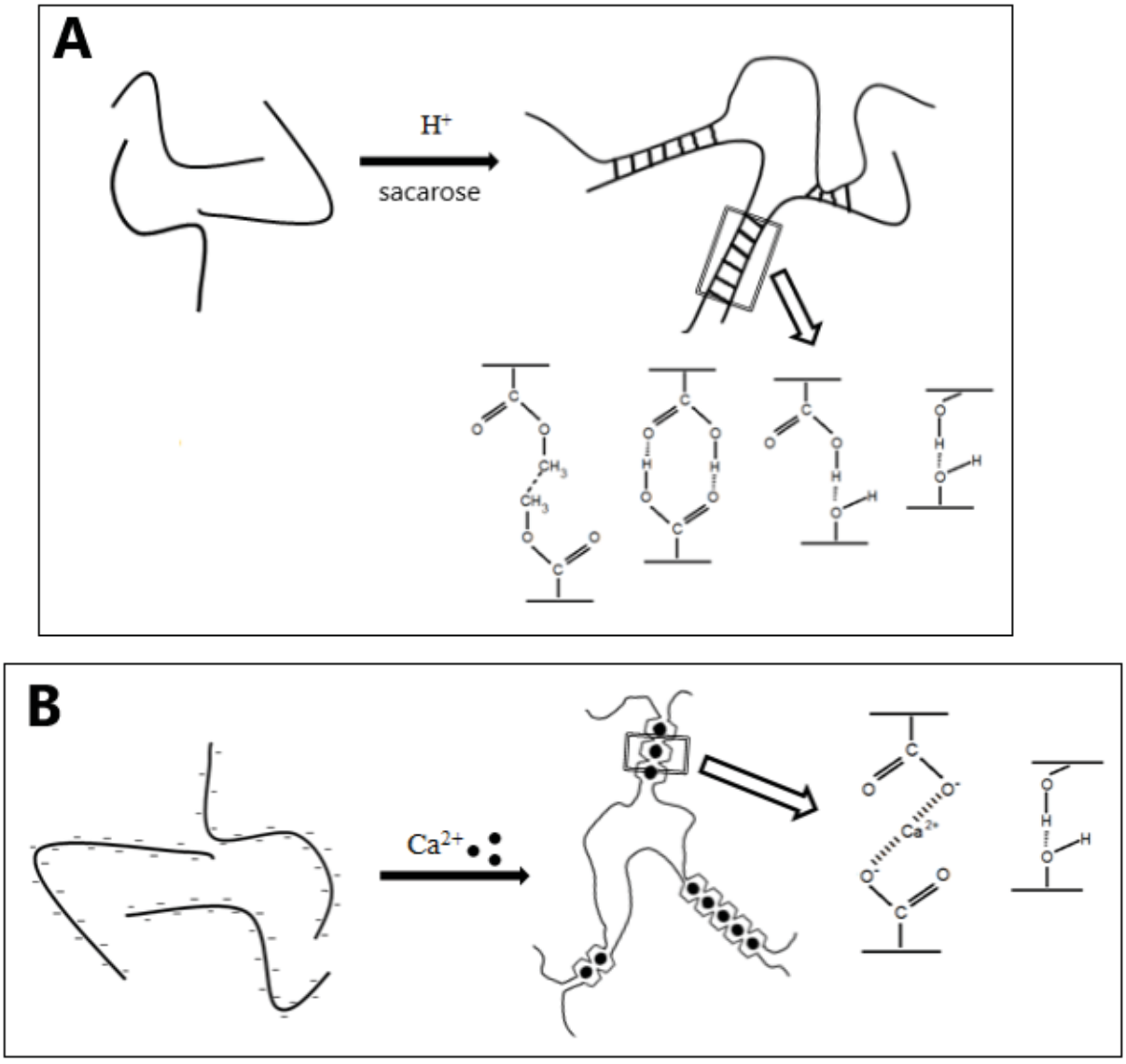
A obtenção da pectina e algumas de suas propriedades foram primeiramente publicadas em 1825 por Henri Braconnot, o qual detectou a presença de um "ácido presente universalmente em todos os vegetais". Ele encontrou este ácido em cenouras, maçãs, peras, grãos e cebolas e utilizou o material extraído das cenouras para a fabricação de geleias (BRACONNOT, 1825; MUZZARELLI, 2012). Entretanto, foi apenas no início do século XX, na Alemanha, que a produção industrial teve início, quando os produtores de suco de maçã começaram a comercializar um agente gelificante proveniente do cozimento do bagaço residual, obtido após a retirada do suco. Atualmente, o bagaço de maçã ainda é utilizado para a produção industrial da pectina, correspondendo a 14% do total produzido, porém deu espaço às cascas dos cítricos, que representam 85,5% da matéria-prima total destinada a este fim (CIRIMINNA; CHAVARRÍA-HERNÁNDEZ; HERNANDÉZ, 2015).

As pectinas com características comerciais são compostas por grandes quantidades de GalA, o que se tornou parte da definição legal para a pectina utilizada como aditivo alimentício ou para propósitos farmacêuticos. A pectina comercial deve conter um mínimo de 65% de GalA em sua massa excluindo umidade e cinzas, conforme especificado pela FAO e União Europeia (MAY, 1990, WILLATS; KNOX; MIKKELSEN, 2006). A característica mais importante que leva ao uso industrial da pectina é a sua habilidade de formar géis. Os géis são formados quando as moléculas de pectina interagem para formar zonas de junção resultando em uma rede que aprisiona moléculas de solvente e soluto (BEMILLER, 1986).

As pectinas HM necessitam uma quantidade mínima de sólidos solúveis e um pH específico para gelificação. Em geral, esses géis são obtidos na presença de sacarose em meio ácido, condição esta que estabiliza as zonas de junção pela promoção de interações hidrofóbicas entre os grupos metil-ésteres e também por pontes de hidrogênio (FIGURA 3-A). Já para as pectinas LM, a

gelificação independe da quantidade de açúcar, mas requer cátions divalentes (em geral o cálcio). Os íons cálcio são usados para indução da associação de cadeias de pectina através de interações eletrostáticas e, assim, formação de gel pelo mecanismo conhecido como *egg-box.* Além de interações eletrostáticas, pontes de hidrogênio entre as hidroxilas contribuem para a estabilização da rede (FIGURA 3-B) (SHARMA *ET AL.* 2006; THAKUR *ET AL.* 1997; WALTER, 1991).

FIGURA 3 – MECANISMOS DE GELIFICAÇÃO DAS PECTINAS HM (A) E LM (B)



FONTE: Adaptado de Vriesmann e Petkowicz (2013)

Além de atuarem com agentes gelificantes, outras propriedades como espessante, emulsificante e estabilizante fazem com que as pectinas sejam amplamente utilizadas em diversos produtos alimentícios. Geleias, confeitos, sucos concentrados, produtos lácteos, sobremesas, pães e molhos são alguns exemplos de alimentos que podem conter pectina. Também pode ser empregada em fármacos, dispositivos médicos e cosméticos (CPKELCO, 2005).

A pectina comercial é extraída a partir de um tratamento do resíduo seco com um ácido mineral diluído e quente em um pH próximo a 2. O extrato, então, é clarificado por filtração ou centrifugação, concentrado e precipitado com o uso de um álcool de cadeia curta. A pectina é prensada, lavada, seca e moída, para obter a pectina em pó (MAY, 1990). Ainda, há o problema de que bateladas individuais dependem do tipo e qualidade do material inicial, porém do ponto de vista do consumidor, uma padronização da pectina é essencial. A padronização é obtida ao se misturar pectinas com características diferentes e ao se “diluir” a pectina em pó com sacarose (ROLIN; DE VRIES, 1990).

As principais matérias-primas utilizadas para extração contêm altas quantidades de pectina, sendo que a casca dos cítricos contêm um teor de 25-35%, enquanto o bagaço de maçã de 10-15% em base seca (CIRIMINNA; CHAVARRÍA-HERNÁNDEZ; HERNANDÉZ, 2015), tornando-os excelentes materiais para este fim. Além disso, são resíduos disponíveis em grandes quantidades provenientes da indústria de sucos, o que os tornam baratos. Considerando que a demanda por pectina vem aumentando nos últimos anos (GRAND VIEW RESEARCH, 2017; WISE GUY REPORTS, 2019), é esperado que novas fontes sejam identificadas para obtenção industrial de pectina. Matérias-primas que estejam disponíveis em grandes quantidades e que sejam consideradas resíduos e, portanto, possuam um baixo custo, são preferíveis para a extração de pectinas.

2.3 RESÍDUOS AGRÍCOLAS E AGROINDUSTRIAIS: IMPACTOS AMBIENTAIS E ECONÔMICOS

Os significativos avanços no desempenho do agronegócio, principalmente a partir da década de 80, proporcionados pela adoção de inovações tecnológicas, possibilitaram ganhos expressivos de produtividade, o que implicou no aumento do consumo de insumos e geração de resíduos nas atividades agrícolas e agroindustriais (ROSA *et al.* 2011).

Uma grande quantidade de resíduos agrícolas é produzida anualmente no mundo, incluindo palhas de cereais, cascas de arroz, bagaços, palhas e espigas de milho, cascas de coco, vagens e serragens (CHANDRA; TAKEUCHI; HASEWAGA, 2012). Os resíduos orgânicos provenientes de atividades agrossilvopastoris, somados aos resíduos urbanos e industriais, chegam a 800 milhões de toneladas (MINISTÉRIO DO MEIO AMBIENTE, 2017).

Infelizmente, grande parte desses resíduos é queimada ainda no campo, por ser um meio barato e rápido de descartar o material e limpar a lavoura. Entretanto, são gerados diversos problemas ambientais decorrentes desta prática: os nutrientes essenciais que mantêm o solo fértil, incluindo carbono, nitrogênio, fósforo, potássio e enxofre, são perdidos com a combustão, as altas temperaturas podem destruir a biota presente no solo, essencial para o desenvolvimento das raízes das plantas, além de causar uma severa poluição do ar (FAO, 2018). A cada tonelada de biomassa queimada são produzidos aproximadamente 1600 kg de dióxido de carbono, 111 kg de monóxido de carbono, 9 kg de metano, 6 kg de hidrocarbonetos e 5 kg de material particulado (CHANDRA; TAKEUCHI; HASEWAGA, 2012).

Além do problema da geração de resíduos na agricultura, há ainda a geração de resíduos no processamento industrial desses materiais. Estima-se que os resíduos acumulados nas diversas etapas da cadeia produtiva da agroindústria cheguem a 30% do material inicial, que podem incluir cascas, sementes, polpas e até mesmo o fruto inteiro caso não seja adequado aos padrões estabelecidos (FILHO; FRANCO, 2015). As agroindústrias investem cada vez mais em sua capacidade de processamento, gerando quantidades enormes de resíduos, tornando necessário reavaliar a maneira com que os

mesmos são gerenciados. Muitas indústrias já realizam manejos internos a fim de reduzir o volume total de resíduos e, quando possível, gerar produtos que incrementem sua receita. A maior parte dos resíduos da agroindústria pode ser utilizada como fonte de ração animal, porém isso pode gerar um custo operacional para a indústria, sendo economicamente mais vantajoso o simples descarte do material (FILHO; FRANCO, 2015; PEREIRA, 2002).

O impacto ambiental gerado pelo descarte a céu aberto de grandes quantidades de resíduos orgânicos inclui a contaminação de águas superficiais e de lençóis freáticos, contaminação do solo pelo contato direto com o resíduo, poluição do ar pela queima de resíduos, propagação de doenças por diferentes vetores, geração de odores e geração excessiva de metano por decomposição anaeróbica da biomassa (NGOC; SCHNITZER, 2009).

Embora seja possível a utilização do lixo orgânico como matéria-prima para alguns processos, restrições sanitárias e custos de transporte limitam a sua utilização e fazem com que esses resíduos sejam encarados como um problema. Entretanto para os resíduos gerados de forma homogênea e concentrada, várias são as oportunidades disponíveis para sua valorização, passando a ser encarados como subprodutos (WOICIECHOWSKI *et al.*, 2013). Os materiais lignocelulósicos são os mais promissores recursos naturais renováveis para o funcionamento da sociedade industrial moderna. As quantidades enormes de biomassa lignocelulósica têm o potencial de serem convertidas em diferentes produtos com alto valor agregado, incluindo biocombustíveis, produtos químicos finos e como matéria-prima barata para fermentação e produção de enzimas (ANWAR; GULFRAZ; IRSHAD, 2014).

2.4 A POLPA DE CAFÉ: UM RESÍDUO DA AGRICULTURA

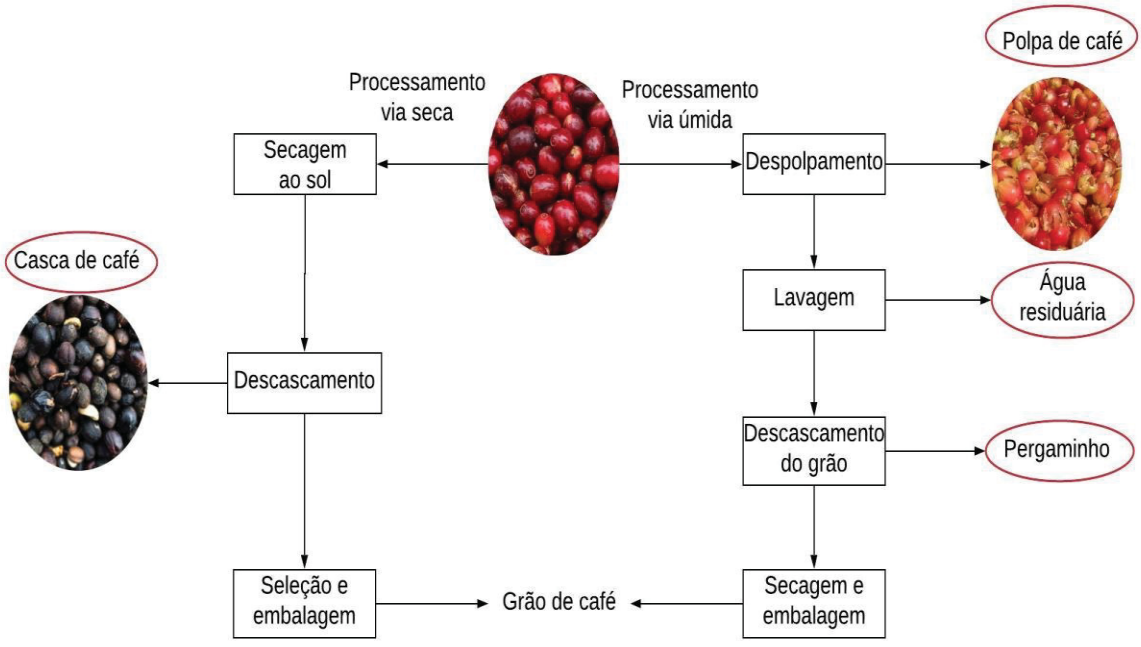
O café é uma *commodity* majoritariamente produzida em países em desenvolvimento, porém consumida em sua maior parte na Europa (~33%). Apenas em 2018 o Brasil foi responsável pela produção de 3,75 milhões de toneladas de grãos de café, sendo o maior produtor e exportador do grão (INTERNATIONAL COFFEE ORGANIZATION, 2019). Em especial, destaca-se a espécie *Coffea arabica* L., mais produzida e valorizada por haver um

consenso que ela produz uma bebida de melhor sabor que a espécie *Coffea canephora* (KEMSLEY; RUAULT; WILSON, 1995).

Durante o processamento do café, diverso resíduos são gerados, sendo considerados resíduos agrícolas, visto que o processamento geralmente ocorre na fazenda onde a colheita foi realizada. Mais de 50% da cereja do café não é utilizada para fins comerciais, sendo descartada durante o processamento (ESQUIVEL; JIMÉNEZ, 2012). Dentre os resíduos produzidos estão as cascas de café, no caso de o beneficiamento ser por via seca, ou a polpa, água residuária e pergaminho caso o beneficiamento se dê por via úmida, apresentados em vermelho na FIGURA 4.

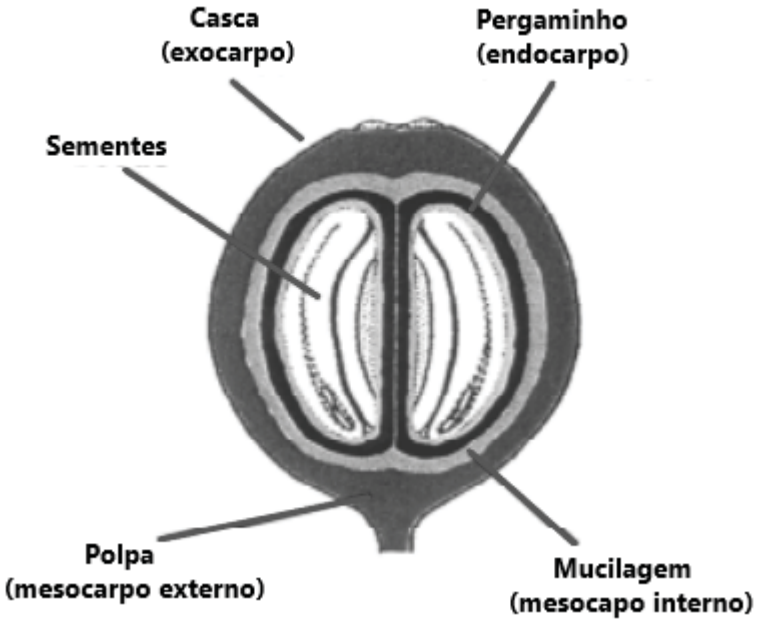
A polpa de café representa a maior parte dos resíduos sólidos produzidos durante o despolpamento, já que para cada 2 toneladas de cerejas de café processadas, aproximadamente 1 tonelada de polpa é gerada (ROUSSOS, 1995). A FIGURA 5 mostra as partes constituintes do fruto do café. O resíduo comumente referido como “polpa de café” é constituído pela casca (exocarpo), polpa (mesocarpo externo) e um pouco da mucilagem (mesocarpo interno), que vem aderida ao material na etapa de despolpamento.

FIGURA 4 – ETAPAS DOS DIFERENTES TIPOS DE PROCESSAMENTO DE CAFÉ E OS RESÍDUOS GERADOS



FONTE: Adaptado de Pandey *et al.* (2000)

FIGURA 5 – CONSTITUINTES DO FRUTO DO CAFÉ



FONTE: Adaptado de Avallone *et al.* (2000)

Os resíduos da via úmida representam um risco maior para o meio ambiente, uma vez que são materiais ricos em matéria orgânica, contendo taninos, cafeína e polifenóis. Além disso, o alto teor de açúcares, proteínas e minerais criam um meio propício para o crescimento de microrganismos, que contribuem para o problema ambiental. A polpa possui entre 21-32% de carboidratos, 7,5-15% de proteínas, 2,0-7,0% de lipídeos, 1,5-2,6% de polifenóis, 14,3-17,5% de lignina, 1,8-8,5% de taninos, 2,4-2,6% de ácido clorogênico e 1,3-1,5% de cafeína. De acordo Durán et al. (2017), as pectinas corresponderiam a 6,5% dos carboidratos.

Poucos estudos foram encontrados na literatura quanto a extração de pectinas utilizando a polpa de café. Garcia *et al.* (1991) estudou as pectinas presentes na mucilagem, no suco da polpa e na polpa de café espremida para a retirada do suco. Especialmente para a polpa espremida, o teor de ácidos urônicos foi de 91,2% quando o material foi extraído com HCl em pH 2 por 1 h e a pectina resultante foi purificada com um sal quaternário de amônio. O grau de metil-esterificação da pectina foi de 23,8%, porém as tentativas de produção de gel com cálcio falharam. Outro estudo encontrado para a pectina da polpa de café é um método patenteado por Otalora (2018), o qual extraiu pectinas da polpa do café e através de um tratamento enzimático obteve uma pectina

funcionalizada com polifenóis. Entretanto, o alto grau de acetilação e metil-esterificação da pectina mostrou-se inadequado para a formação de géis com sacarose e um pH ácido.

A utilização da polpa de café ainda é muito limitada, principalmente devido à natureza tóxica proporcionada pela grande quantidade de taninos, polifenóis e cafeína. Tentativas de uso de uma pequena parcela do material disponível como ração animal e na compostagem já foram descritas, mas com pouco sucesso. Mesmo com as dificuldades apresentadas, a alta disponibilidade da polpa de café a torna uma potencial matéria-prima para a produção de produtos com alto valor agregado (PANDEY *et al.*, 2000).

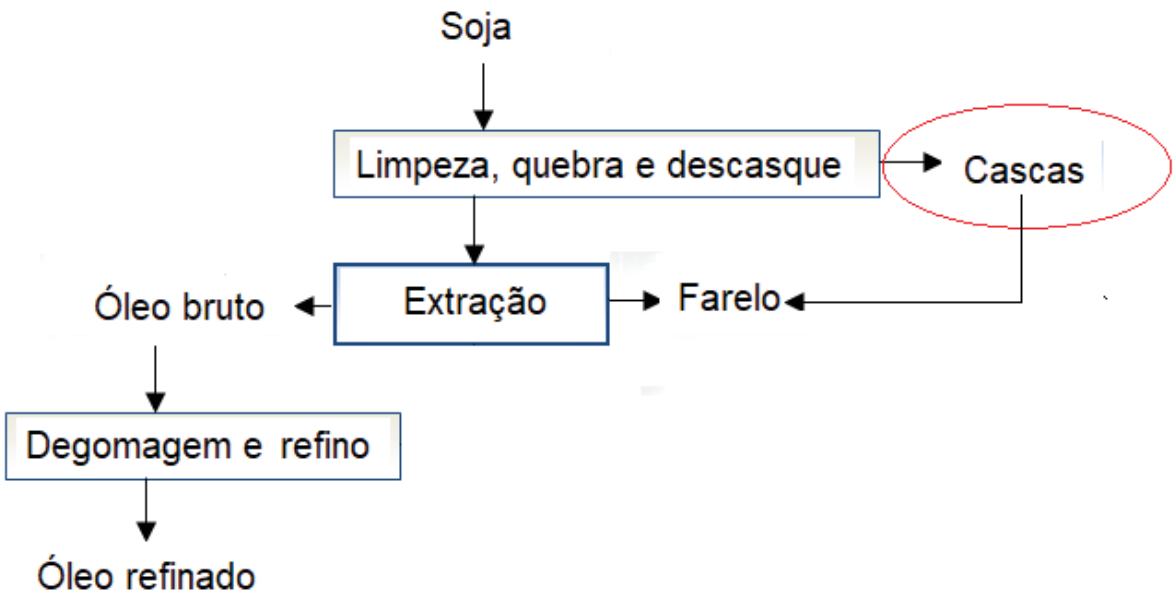
2.5 A CASCA DE SOJA: UM SUBPRODUTO DA AGROINDÚSTRIA

A soja (*Glycine max* L. Merril) é uma das *commodities* mais importantes atualmente, devido aos seus usos na alimentação humana e animal. Ela pertence a família das leguminosas e apresenta grande popularidade pelo fato de conter altos níveis de óleo (20-22%) e proteína (40-42%) (BHANGU; VIRK, 2019). De acordo com dados do USDA e da CONAB, os Estados Unidos foram os principais produtores do grão na safra de 2018/19, chegando a 123 milhões de toneladas. O Brasil aparece em segundo lugar, com aproximadamente 115 milhões de toneladas produzidas (EMBRAPA, 2019). O sucesso da produção de soja no Brasil aconteceu após década de 50, quando pesquisadores estadunidenses desenvolveram variedades de soja adaptadas a climas mais quentes para utilização como fonte de proteína por avicultores da região. Devido a semelhanças no clima, esta variedade também se adaptou ao sul do Brasil (GOLDSMITH, 2008).

A FIGURA 6 mostra o processo de beneficiamento da soja para a produção de óleo e farelo. Ele se inicia com a limpeza dos grãos, seguido de uma quebra com um rolo compressor para que o grão seja reduzido a pedaços menores, facilitando a remoção da casca. A extração do óleo geralmente é feita com solventes orgânicos, usualmente o hexano, que separa o óleo bruto do restante do material. O óleo bruto passa por um processo de degomagem para retirada de fosfolipídeos e etapas de refino para produção do óleo refinado. Já os flocos deslipidificados restantes na parte sólida da extração passam por

etapas de retirada do solvente orgânico e torragem a fim de inativar a enzima urease, para originar, assim, o farelo de soja. A casca retirada no processo de descascamento ainda pode ser adicionada ao farelo a fim de reduzir o teor proteico e aumentar o teor de fibras (BLASI *et al.,* 2000). De fato, a casca de soja é constituída por 8-10% de proteínas e um teor de fibra bruta de 43%, contra 51% de proteínas e 6% de fibras do farelo de soja (PERKINS, 1995; ZAMBOM *et al.* 2001).

FIGURA 6 – ETAPAS DO PROCESSAMENTO DA SOJA



FONTE: Adaptado de Perten (2019)

Totalizando cerca de 7,3% do grão, a casca da soja possui um valor comercial muito menor do que o farelo e o óleo, sendo basicamente utilizada para a ração animal. São vendidas tipicamente na forma de *pellets* e utilizadas na alimentação de bovinos e suínos. Possuem baixo teor de lignina (1-4%) se comparadas a outros resíduos agroindustriais, tornando-as um substrato mais fácil de ser trabalhado em processos biotecnológicos, pois a presença de lignina atrapalha a conversão de materiais lignocelulósicos em açúcares fermentescíveis (LOMAN; JU, 2016; PERKINS, 1995).

A casca de soja possui altos teores de ferro (32% do ferro do grão está na casca), o que a torna uma matéria-prima interessante na fabricação de alimentos que funcionem como um suplemento deste mineral. Outros estudos envolvendo a casca de soja sugerem sua aplicação como um adsorvente e para produção de enzimas, proteínas, etanol, pectina e lipídios (DE PRETTO *et al.*, 2018; ZHANG; HU, 2012). Segundo De Pretto *et al.* (2018) existiam 147 artigos até 2016 descrevendo aplicações não convencionais da casca de soja, as quais excluem o uso como ração animal. Do total, 20 deles estudam as pectinas e fibras dietéticas presentes na casca e seu uso como um aditivo alimentar.

Os estudos envolvendo o uso da casca de soja para extração de pectina com propósito comercial foram iniciados por Gnanasambandam e Proctor (1999) que através de uma extração ácida utilizando HNO_3 0,1 M a 90°C por 40 min, obtiveram uma pectina com alto teor de ácidos urônicos (76,7%) e um rendimento de 15%. Posteriormente, Kalapathy e Proctor (2001) testaram diferentes condições de extração e de precipitação para as pectinas da casca da soja e conseguiram um máximo rendimento de extração de 28% de pectina. Este rendimento foi alcançado quando foi utilizado HCl 0,1 M a 90°C por 45 min para a extração e um pH de 3,5 para a precipitação. O conteúdo de ácidos urônicos e o grau de metil-esterificação da pectina não foram afetados significativamente com mudanças da força do ácido e do pH do extrato previamente à precipitação, mantendo-se entre 68-72% e 56-60%, respectivamente. O rendimento alcançado ficou muito próximo ao rendimento da casca dos cítricos, mostrando que a casca de soja tem um grande potencial como material alternativo para a extração de pectinas. Além disto, em comparação a outras fontes, ela apresenta a vantagem de não necessitar de secagem prévia para transporte e estocagem, como ocorre no caso da casca dos cítricos e do bagaço de maçã (MONSOOR *et al.*, 2001).

CAPÍTULO 1

Extraction and characterization of Brazilian coffee (*Coffea arabica* L.) pulp pectin

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Abstract

Hydrolysis of *Coffea arabica* L. pulp, the main waste from coffee processing, indicated the presence of pectins. The pulp was treated with boiling 80% (v/v) ethanol and then two sequential acid extractions were performed, using boiling HNO_3 for 30 min. The first extraction resulted in CAP-1 and the second one in CAP-2. A total yield of 17.6% pectin was obtained, being 14.6% CAP-1 and 3.0% CAP-2. Chromatographic, colorimetric and spectroscopic methods, including FT-IR, NMR and SEM-EDS, were used to characterize the pectic fractions. CAP-1 and CAP-2 had high contents of galacturonic acid, 79.5% and 85.6%, respectively. Protein and phenolics content of CAP-1 was 1.4% and 0.7%, respectively, while CAP-2 presented contents of 1.5% and 0.6%. Both were classified as high-methoxyl pectins, however CAP-2 had a higher degree of methyl-esterification (74.1%) than CAP-1 (63.2%). CAP-1 and CAP-2 presented low degree of acetylation (5.7% and 3.1%), high molar mass ($3.921.10^5$ g/mol and $2.642.10^5$ g/mol) and low polydispersity (1.560 and 1.789). The viscosity at 25°C of 5% solutions was investigated and both displayed a shear thinning behavior, although CAP-2 was more pseudoplastic. Frequency sweeps showed that CAP-2 behaved as a weak gel. The results demonstrated that coffee pulp may be used as source of commercial pectin.

Keywords: Agricultural waste, Chemical characterization, Hydrogel, Biorefinery and Rheology.

1. Introduction

The growth in population, food production and industrialization have drastically accelerated the generation of waste material, such as crop residues, becoming a big concern (Willy, Muyanga & Jayne, 2019). Increased waste generation creates a series of environmental problems, such as contamination of surface and groundwater, spreading of diseases by different vectors like birds, insects, and rodents, generation of odors, release of methane by anaerobic decomposition of waste, changes in soil pH and microbiome (Ngoc & Schnitzer, 2009). Lignocellulosic biomass arising from agricultural wastes has the potential to be recycled and used for the production of value-added products, such as biofuels, food additives, organic acids, enzymes and others (Naik *et al.*, 2010).

With a production surpassing 10 million tons in 2018, coffee stands out as one of the most traded commodities and consumed beverages (International Coffee Organization, 2019). Thus, it is responsible for the generation of large amounts of different wastes along its production and consumption, such as coffee pulp, coffee husks, coffee silver skin, coffee parchment, coffee wastewater and spent coffee grounds. Coffee pulp accounts for most of the waste generated during coffee wet processing, since for every 2 tons of coffee cherries processed, nearly 1 ton of pulp is generated (Roussos, 1995). Therefore, aiming to find a proper use for this residue, coffee pulp has been already studied as substrate for mushroom cultivation (Salmones, Mata & Waliszewski, 2005) and biogas (Corro *et al.,* 2013), bioethanol (Shenoy *et al.,* 2011), pectinase (Antier *et al,* 1993) and pectin production (Garcia *et al,* 1991).

Pectins are a family of polysaccharides found in plant cell wall, particularly abundant in dicots, playing a central role in the ripening and texture of fruits and vegetables (Willats, Knox & Mikkelsen, 2006). Pectic polysaccharides are characterized by the presence of galacturonic acid and encompass the homogalacturonan (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), xylogalacturonan (XGA) and apiogalacturonan (AGA) (Schols & Voragen, 1996).

Comprising ~65% of the pectin, HG is the most abundant pectic polysaccharide. It is a linear homopolymer of α-1,4-linked galacturonic acid,

partially methyl-esterified at the C-6 carboxyl. Pectins with a degree of methyl-esterification (DM) higher than 50% are classified as high methoxyl (HM) pectins, otherwise they are categorized as low methoxyl (LM) pectins. The second most abundant pectic polysaccharide is RG-I (20-35%), which is formed by a main chain of $[-\alpha\text{-D-GalA-1,2-}\alpha\text{-L-Rha-1,4-}]_n$ repetitions. Linear or branched side chains, mainly of arabinose and/or galactose, are linked to 20-80% of the rhamnosyl residues in the main backbone. RG-II, XGA and AGA are substituted HGs which are minor components of the pectin and in the case of AGA, it was only identified in aquatic monocots (Bemiller, 1986; Mohnen, 2008).

In addition to the methoxyl at C-6, acetyl groups can also be found at O-2 and/or O-3 position of galacturonic acid from HG and RG-I. In general, acetyl groups are present in low amounts in native pectins, with some exceptions, such as sugar beet pectin (Kouwijzer, Schols & Pérez, 1996).

Pectin is largely used in food industry, mainly as gelling, stabilizing or thickening agent. However, the number of sources that has been used for commercial manufacture of pectins is very limited, due to the dependence of molecular and structural features such as molar mass, DM and galacturonic acid content of pectin in the process of gelation. (Thakur, Singh & Handa, 1997). Typical requirement for its use as food additive or pharmaceutical purposes is a minimum of 65% of galacturonic acid on the ash and moisture-free substances (May, 1990).

The main sources for pectin production are citrus peel and apple pomace, both by-products from juice manufacturing (Thakur, Singh & Handa, 1997; Voragen & Coenen, 2009). Usually, commercial pectin production starts with an extraction of the dry material with hot diluted mineral acid. The drying process is very expensive, since the material presents high moisture levels and has to be desiccated until it reaches 10-12% to avoid fermentation. Therefore, the process is only economically feasible if high amounts of unexpansive material are available, making commodities waste preferable in this case (Ciriminna, 2015).

The global pectin market was valued in 964.1 million US$ in 2015 and recently, in 2018, it was estimated to be 1.12 billion US$, representing a growth of 16% in 3 years (Grand View Research, 2017; Wise Guy Reports, 2019). As the demand rises, it is opportune to study new sources of plant material for

pectin extraction. Previous studies have already investigated the use of cacao pod husks (Vriesmann, Amboni & Petkowicz, 2011), yellow passion fruit rind (Yapo & Koffi, 2006), watermelon rind (Petkowicz, Vriesmann & Williams, 2017), dragon fruit peel (Tang, Wong & Woo, 2011), mango peel (Rehman *et al.,* 2004), and many other wastes for pectin extraction. However, only few of them are promising considering its availability and the environmental benefits provided from its utilization. In this sense, coffee pulp might be considered a promising source of pectin.

Previous studies have attempted to obtain pectin from this material. Garcia *et al.* (1991) used boiling HCl at pH 2 for 1 h to extract pectin from *C. arabica* pressed pulp from the Bourbon variety harvested in Guatemala. The pectin, purified using quaternary ammonium and ammonium sulfate salts, had high galacturonic acid content (91.2%) and low DM (23.8%). Otalora (2018) has patented a method for obtaining a polyphenol functionalized coffee pectin using *C. arabica* from Colombia. The procedure was carried out by an acid extraction using HCl at pH 2 for 1 h at 90°C, followed by an alkali extraction of the residual product of the first extraction, using NaOH at pH 12 and room temperature. Both extractions were pooled and then treated with laccase. The resulting pectin was described to have 65.4% galacturonic acid, DM 100% and degree of acetylation of 97%.

As noticed in the reported investigations, the differences in coffee varieties and the approach used for pectin isolation impact directly in the pectin features. Therefore, the aim of the present study was to extract pectin from coffee pulp from Brazilian *Coffea arabica* L., the most produced and exported *arabica* in the world. Two pectin fractions were obtained by sequential extraction with nitric acid and characterized.

2. Material and methods

2.1. Material preparation

Coffee pulp from *Coffea arabica* L. was obtained in a coffee farm located in Ibaiti, Paraná, Brazil (23°54'27.7"S 50°09'01.4"W). Coffee cherries were depulped using the pulped natural process, which consists in the mechanical removal of their outermost layer and the subsequent drying of the coffee beans

in the sun. The stripped pulp, mainly composed of the exocarp and mesocarp (pericarp) of coffee cherries, was collected, immediately frozen and then freeze-dried. The dried pulp was ground in a conventional blender. The resulting powder was boiled in 80% (v/v) ethanol, under reflux, for 20 min, giving rise to the alcohol insoluble residue (AIR). The AIR was separated from the ethanol solution by filtration, washed 3 times with absolute ethanol and left to dry at room temperature. Lastly, it was milled in an analytical mill IKA-A11 (IKAWerke GmbH & Co. KG, Germany) and stored at -20°C for further extraction.

2.2. Pectin extraction

Extraction of pectins from AIR was carried out using boiling 0.1 M HNO_3, with a solid:liquid ratio of 1:25 (w/v), under reflux for 30 min. The extract was filtered with a synthetic fabric and then centrifuged at 5000 rpm for 20 min, to make sure a clean extract was obtained. A second extraction was performed with the residual solid at the same conditions. Then, the extracts were precipitated with 2 volumes of absolute ethanol and stored for 16 h at 4°C. The precipitates were filtered, washed 3 times with absolute ethanol and dried under vacuum. The pectins from the first and second extractions were named CAP-1 (*Coffea arabica* pectin-1) and CAP-2 (*Coffea arabica* pectin-2), respectively. The extractions were performed in triplicate.

2.3. Monosaccharide composition

For the hydrolysis of the exocarp and mesocarp of coffee pulp and the final residue remaining after pectin extraction, fractions were treated with 72% (w/w) sulfuric acid, for 1 h, alternating vortex and ice bath. The solution was then diluted to 8% and kept in a boiling water bath for 6 h (Saeman, Moore, Mitchell, & Millet, 1954). For the pectins, hydrolysis was carried out with 2 M TFA at 120°C for 2 h in autoclave.

For neutral sugars analyses, the monosaccharides resulting from hydrolysis were reduced with sodium borohydride for 16 h at 4°C (Wolfrom & Thompson, 1963b) and then acetylated with pyridine/acetic anhydride for 16 h at room temperature. The alditol acetates were extracted with chloroform (Wolfrom & Thompson, 1963a) and analyzed by gas chromatography (GC)

using a Thermo Scientific Trace GC Ultra and a DB-225 column (internal diameter 0.32 mm x length 30 m x film thickness 0.25 μm), programmed from 100°C to 230°C at a heating rate of 60°C/min, with a mixture of helium and nitrogen as carrier gas at 1 mL/min.

Uronic acid content (UA) was determined by the *m*-hydroxybiphenyl method as described by Blumenkrantz and Asboe-Hansen (1973), using galacturonic acid (GalA) as standard. The experiments were performed in triplicate. The uronic acid was identified by thin-layer chromatography (TLC) after hydrolysis of the pectin. The analysis was carried out with a 20x20 cm silica gel plate (Merck KGaA, Germany), the mobile phase was ethyl acetate:n-propanol:acetic acid:water (4:2:2:1, v/v) and orcinol-sulfuric acid was used as detection reagent (Chaplin & Kennedy, 1994).

2.4. Degree of methyl-esterification (DM)

DM of pectins was estimated by Fourier transform mid-infrared (FT-IR) spectroscopy by the use of a Vertex 70 spectrophotometer (Bruker, Germany), in the range of 400 to 4000 cm^{-1} at 4 cm^{-1} resolution. KBr was dried at 105°C and mixed with pectin in a proportion 99:1. The mixture was grinded, dried for 16 h under vacuum and pelletized using a manual hydraulic press. Its spectra provided peaks corresponding to the methyl-esterified and the free carboxyl group at 1749 cm^{-1} and 1630 cm^{-1}, respectively (Vriesmann & Petkowicz, 2009). The ratio of methyl-esterified peak area and the sum of areas from both peaks provided the DM of the sample. Experiments were performed in triplicate.

2.5. Degree of acetylation (DA)

Acetyl content of the sample was obtained according to the Hestrin (1949) method using penta-O-acetyl-β-D-galactopyranose as standard. The degree of acetylation (DA) was calculated by the proportion between mols of acetyl and mols of galacturonic acid present at the pectin, according to the equation:

$$DA\ (\%) = \frac{mols\ of\ acetyl}{mols\ of\ GalA} \times 100$$

$$DA\ (\%) = \frac{acetyl\ content\ (\%) \div 43.04}{GalA\ (\%) \div \left[\frac{(\%ME \times 190.15) + (\%NE \times 176.12)}{100}\right]} \times 100$$

Where ME for methyl-esterified anhydrogalacturonic acid (M = 190.15 g/mol); NE for non-esterified anhydrogalacturonic acid (M = 176.12 g/mol); and acetyl content is the percentage (w/w) of acetyl group (M = 43.04 g/mol) in the sample. Experiments were performed in triplicate.

2.6. Protein content

Protein content was estimated using the Bradford method (1976) and BSA as standard. Experiments were performed in triplicate.

2.7. Phenolics content

Phenolics content was determined by Singleton and Rossi (1965) method. Gallic acid was used as standard. Experiments were performed in triplicate.

2.8. Ash content

Ash content was obtained by thermogravimetric analysis (TGA) using a Q600 SDT (Ta Instruments, USA). Approximately 100 mg of pectin was heated from 18 to 800°C at a rate of 10°C/min in an atmosphere of synthetic air. The ratio between the final and the initial mass was considered the ash content. Experiments were performed in duplicate.

2.9. Scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS)

SEM-EDS was used to obtain the mineral elements composition of pectin fractions. The samples were lyophilized and placed in a carbon tape mounted on a SEM stub and analyzed in a VEGA3 LMU microscope (Tescan, Czech Republic) using a 15 kV accelerating voltage for 60 seconds to obtain each spectrum.

2.10. Nuclear magnetic resonance spectroscopy (NMR)

Pectin was solubilized in D_2O in a concentration of 40 mg/mL and the spectra of heteronuclear single quantum coherence (HSQC) NMR and ^{13}C NMR were obtained at 70°C, using a Bruker DRX 400 Avance spectrometer (Bruker, Germany). Acetone was used as internal standard (δ=30.2 for ^{13}C and δ=2.22 for ^{1}H). Data were analyzed by TopSpin software, version 3.5 (Bruker, Germany).

2.11. High performance size exclusion chromatography (HPSEC)

Pectins were analyzed by high performance size exclusion chromatography (HPSEC) coupled to a refractive index (RI) (Waters Corporation, USA) and a Dawn-F multi-angle laser light scattering (MALLS) (Wyatt Technology, USA) detectors. Four Ultrahydrogel columns (Waters Corporation, USA) were connected in series (2000; 500; 250; 120) and coupled to the equipment. The eluent was 0.1 M $NaNO_2$ and 0.02% of NaN_3 at a flow rate of 0.6 mL/min. Samples were filtered through a 0.22 µm cellulose acetate membrane before injection. The differential refractive index increment (dn/dc) value of the solvent-solute solution was determined using concentrations of 0.2 to 1.0 mg/mL, in order to obtain the average molar mass (M_w) and polydispersity (M_w/M_n) of the pectins. Data were analyzed using ASTRA software (Wyatt Technology, USA).

2.12. Rheological analyses

Rheological analyses were performed at 25°C with a cone/plate geometry (C60 2º Ti L) using a Thermo Scientific Haake Mars rheometer (Haake GmbH, Germany) coupled to a thermostatic bath (Haake K15), a Haake DC5 heating control and a Haake UTMC unit.

Solutions were prepared by the dispersion of CAP-1 and CAP-2 in ultrapure water at 5% (w/w) concentration. Viscosity curves were obtained in CR mode (0.001-1000 s^{-1}). Frequency sweeps were carried out in the range of 0.01 to 10 Hz under stress within the linear viscoelastic region, obtained by stress sweeps (0.01-10 Pa) at frequency of 1 Hz.

RheoWin Data Manager version 4.82.0002 (Thermo Fisher Scientific, USA) software was used to obtain the fit models and the rheological and statistical parameters. All the experiments were performed in triplicate and error bars are the standard deviation of the averages.

3. Results and Discussion

3.1. Monosaccharide composition of coffee pulp

The exocarp and mesocarp of the coffee pulp were manually separated using tweezers and the monosaccharide composition determined (Table 1).

Table 1. Monosaccharide composition of the exocarp and mesocarp of coffee pulp and the final residue after the extraction of pectins CAP-1 and CAP-2.

Monosaccharide[a]	**Exocarp (%)**	**Mesocarp (%)**	**Final residue (%)**
Rha	2.7±0.2	2.6±0.1	1.2±0.1
Fuc	traces	traces	traces
Ara	25.3±0.9	12.7±0.8	2.4±0.3
Xyl	4.7±0.7	11.2±1.5	29.3±1.9
Man	15.1±2.0	13.0±1.7	13.9±1.0
Gal	8.1±0.1	6.1±0.1	6.6±0.4
Glc	34.0±0.2	46.5±0.8	43.0±3.5
UA	10.1±0.7	7.9±0.3	3.6±0.3

[a] Neutral monosaccharide determined by GC of alditol acetates and uronic acid (UA) determined by colorimetric method.

The UA content of coffee pulp was found to be in the range of 8-10%. The exocarp was richer in pectins than the mesocarp. The UA levels were lower than that described for orange peel (24.1%) (Kaya, 2014), but comparable to lemon peel (13.0%) (Marín *et al.*, 2005) and apple pomace (7.7%) (Albuquerque, 2003). The pectin content of coffee pulp found in the present study was higher than that determined by Wilbaux (1956) who reported a content of 6.5%.

Glucose was the main neutral monosaccharide in the exocarp and mesocarp of coffee pulp, mainly coming from cellulose, which was previously described to account for 28% of the pulp (Wilbaux, 1956) and also as component of xyloglucans. Pericarp also had high contents of arabinose and mannose. According to Urbaneja (1996), the most likely cause of the high levels of arabinose is that mucilage, predominantly composed of pectins, is carried along with coffee pulp during the depulping process. This is in agreement with Avallone *et al.* (2000) who observed that arabinose is the main neutral monosaccharide of the AIR from coffee hand-dissected mucilage (Ara = 34.2%) and also from its crude pectins (Ara = 52.5%).

After acid extractions, the remaining monosaccharides were mainly glucose, xylose and mannose, implying that cellulose and hemicelluloses (probably xyloglucan, xylan and mannan) were the major components of the residue. The amounts of rhamnose, arabinose and uronic acid decreased substantially in the residue, suggesting that most of the pectin was extracted. However, lower contents of pectic monosaccharides were found in the residue after the acid extractions. Saulnier & Thibault (1987) also analyzed the residual material after sequential extractions of the pulp of grape berries with cold water, cold potassium oxalate, hot hydrochloric acid and cold sodium hydroxide. They found 11.6% of uronic acid in the AIR and 2.1% remained in the residue after the extractions along with other monosaccharides typical of pectins. Part of the pectins are recalcitrant to acid extractions and this may be partially due to a covalent linkange between RG-I side chains and xyloglucans (Thompson and Fry, 2000) and also cellulose interactions with arabinans and galactans of pectins (Zykwinska *et al.,* 2005).

3.2. Extraction and chemical characterization of coffee pulp pectin

The dried pulp was treated with ethanol solution resulting in the AIR. This procedure is used for removal of pigments, low molar mass compounds and inactivation of endogenous enzymes. Freezing and drying the raw material prior to the ethanol treatment is also crucial to avoid pectinolytic enzymes activity. It has been reported that in the natural fermentation of coffee cherries, 3.8% of

pectin from *Coffea canephora* mucilage is decomposed within 2 h, while in 10 h, 13.7% is degraded (Agate and Bhat, 1966).

The AIR was subjected to two consecutive extractions with boiling nitric acid for 30 min, giving rise to fractions CAP-1 and CAP-2. Not all pectin extractable in acidic conditions was obtained in the first extraction as shown in table 2. In addition, the insoluble residue after the two consecutive extractions with nitric acid had 3.6% UA (Table 1). These results suggest that adjustments in the conditions of the first extraction could result in higher yields of pectin. The total yield of pectin (~18%) as well as the yield of CAP-1 (~15%) were higher than previously reported for pressed coffee pulp from Guatemala extracted with boiling hydrochloric acid at pH 2 for 1 h, which was ~5% based on the moisture free pressed coffee pulp (Garcia *et al.,* 1991). The pectin yield was also higher than those found for other agricultural wastes such as cacao pod husks (9.5%) (Vriesmann, Teófilo and Petkowicz, 2011), peapods (8.3%) (Müller-Maatsch *et al.,* 2016) and sunflower heads (11.6%) (Iglesias and Lozano, 2004). However, the yield was lower than that described for orange peels (20.6%) which is the main agroindustrial waste used for commercial production of pectin (Ma *et al.,* 1993).

The monosaccharide composition (Table 2) shows typical pectic monosaccharides, with CAP-2 having GalA content considerably higher than CAP-1. Both fractions met the Food and Agricultural Organization (FAO) and the European Union (EU) commercial requirements, in which pectin must consist of at least 65% of galacturonic acid on the ash and moisture-free mass (May, 1990).

The GalA content relative to the polysaccharide portion, obtained excluding moiety, ashes, protein and phenolics, was 81.2% for CAP-1 and 87.4% for CAP-2, in accordance with Garcia *et al* (1991) that found a value of 91.2% of GalA for pressed coffee pulp pectin. Lower amounts of galactose, arabinose and rhamnose were found in CAP-2, indicating that it is less branched than CAP-1. Differently from coffee mucilage pectin, where arabinose is the main neutral monosaccharide of RG-I (Avallone *et al.,* 2000), the pectin from coffee pulp had a predominance of galactose.

The monosaccharide composition was used to estimate the amount of HG and RG-I in CAP-1 and CAP-2 as shown in Table 2. The pectins were

mainly composed of HG, although CAP-2 had a higher percentage (84.6%) than CAP-1 (78.1%). The value of the ratio (Ara + Gal)/Rha revealed short side chain length for both pectins, being CAP-2 side chains shorter. A higher value of this ratio (10.8) was previously reported by Otalora (2018) for a pectin extracted from coffee pulp. The difference is probably due to the milder extraction conditions (acid extraction at 90°C and pH 2 followed by alkali extraction at room temperature and pH 12) that results in less degradation and longer side chains of RG-I. The different origin (Brazil x Colombia) and pretreatment of the raw material might also cause differences in the extracted pectins.

Similar contents of protein and phenolics were found for CAP-1 and CAP-2 (Table 2). Protein contents were in the range of values reported for commercial citrus pectin (0.9-3.3%) (Kravtchenko, Voragen & Pilnik, 1992; Leroux *et al.*, 2002) and lower than found for coffee mucilage pectin (3.4%) (Avallone *et al.,* 2000). Phenolics were higher than found for citrus pectin (0.15-0.18%), but the same of reported for apple pectin (0.6%) (Kravtchenko, Voragen & Pilnik, 1992).

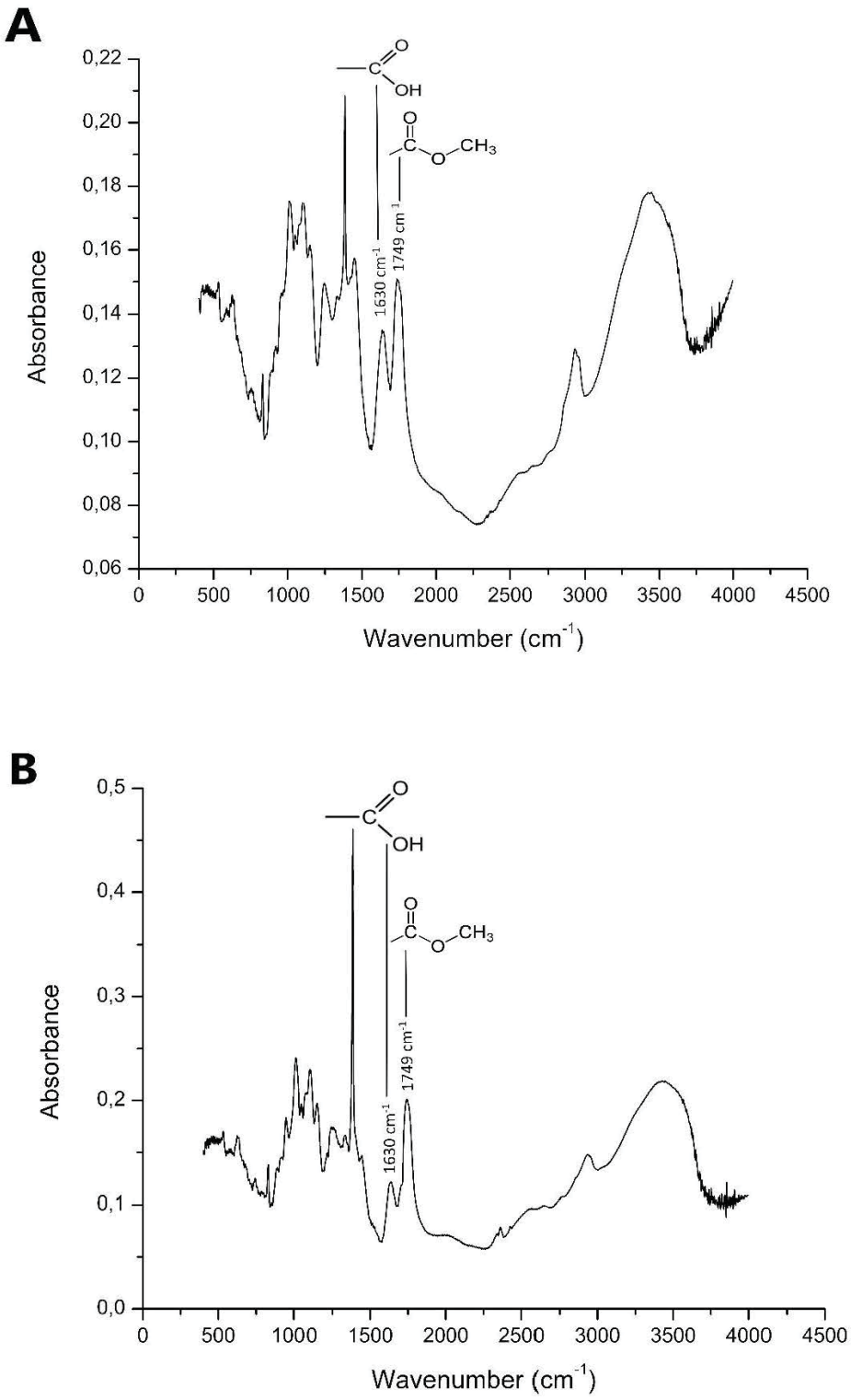
Table 2. Yield and composition of CAP-1 and CAP-2.

	CAP-1	**CAP-2**
Yield[a] (%)	14.6±0.6	3.0±0.4
GalA[b] (%, according to FAO and EU)	79.5±2.5	85.6±1.8
Monosaccharide (relative %)[c]		
Rha	3.1±0.5	2.8±0.1
Fuc	traces	not found
Ara	2.1±0.1	0.8±0.1
Xyl	1.6±0.8	1.9±0.2
Man	1.3±0.3	1.0±0.1
Gal	8.0±0.9	3.8±0.3
Glc	2.7±0.5	2.3±0.1
GalA	81.2±2.6	87.4±1.8
HG[d] (%)	78.1	84.6
RG-I[d] (%)	16.3	10.2
(Ara + Gal)/Rha	3.3	1.6
DM (%)[e]	63.2±0.8	74.1±2.0
DA (%)[f]	5.7±0.2	3.1±0.2
Moisture (%)[g]	13.0±1.2	12.3±0.1
Protein (g/100g)[f]	1.4±0.1	1.5±0.01
Phenolics (g/100g)[f]	0.7±0.03	0.6±0.01
Ashes (g/100g)[h]	3.2±1.0	3.4±1.4

[a] Yield based on the AIR.
[b] Galacturonic acid on the ash and moisture-free mass.
[c] Neutral monosaccharide determined by GC of alditol acetates and GalA determined by colorimetric method and identified by TLC (Fig. S1).
[d] HG = GalA – Rha and RG-I = 2(Rha) + Ara + Gal (M'sakni *et al.*, 2006).
[e] Degree of methyl-esterification (DM) determined by FT-IR.
[f] Degree of acetylation (DA), protein and phenolics content obtained by colorimetric method.
[g] Calculated as loss of mass after lyophilization.
[h] Obtained by thermogravimetric analysis.

The peak areas of methyl-esterified and unesterified carboxyl groups from FT-IR spectra of CAP-1 and CAP-2 (Figure 1) were used to determine the DM (Table 2). Differently from Garcia *et al* (1991), who obtained LM pectins from coffee pulp, in the present study, HM pectins were obtained in the first and the second extraction. CAP-1 had DM of 63.2%, being in the range of slow set pectins (DM between 58-65%), while CAP-2, with DM of 74.1%, may be considered as rapid set (DM around 70%) (May, 1990).

Figure 1. FT-IR spectra showing the methyl-esterified peak (1749 cm^{-1}) and unesterified carboxyl peak (1630 cm^{-1}) of CAP-1 (A) and CAP-2 (B).



The DM of CAP-1 was similar to that described for pectins from coffee mucilage (61.8%) (Avallone *et al.,* 2000) and *Citrus tankan* (63.2%) (Tamaki *et*

al., 2008), while CAP-2 had a DM close to commercial pectins from lemon peel (72.1%) and apple pomace (74.3%) (Kravtchenko, Voragen and Pilnik, 1992).

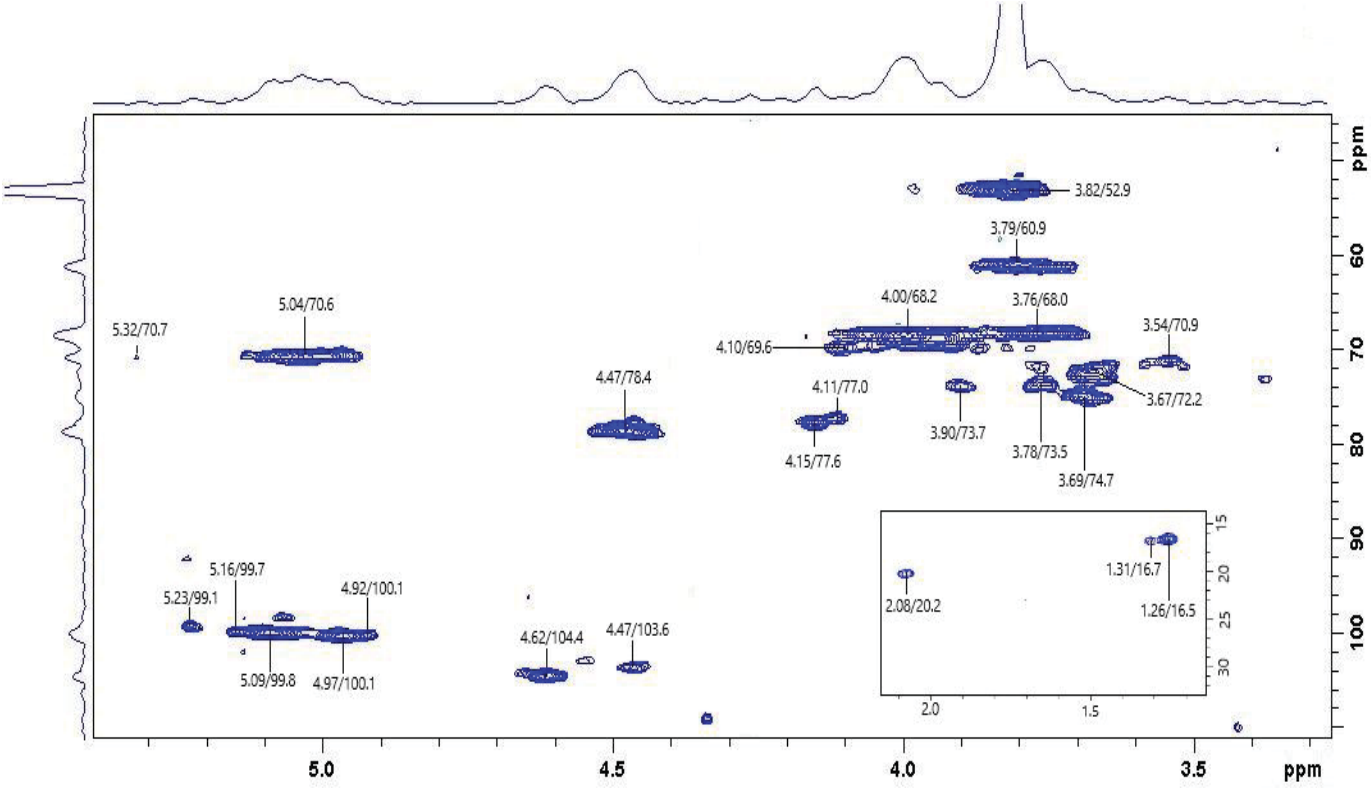
Acetyl content of CAP-1 was estimated to be 1.1% and CAP-2 0.7%, resulting in a DA of 5.7% and 3.1%, respectively (Table 2). Values of DA obtained by GC and HPLC for commercial apple and citrus pectin were 2-5% and 1-2%, respectively (Voragen, Schols & Pilnik, 1986; Kravtchenko, Voragen & Pilnik, 1992), very close to the ones found in the present study for coffee pectin. However, the DA found for Brazilian coffee pectin was much lower than the value of 97%, previously reported for Colombian coffee pulp pectin obtained by sequential extractions with acid (HCl, pH 2, 90°C) and alkali (NaOH, pH 12, room temperature) (Otalora, 2018). The relatively low DA found for Brazilian coffee pectin can be favorable for gel formation, since high acetyl contents has been associated with poor gelling properties of pectins (Pippen, McCready & Owens, 1950; Oosterveld *et al.* 2000; Ralet *et al.* 2003).

SEM-EDS analyses provided the mineral elements composition of the fractions and it was possible to find the presence of elements in a descending order of K > Ca > S > Al for CAP-1 and K > Ca > Na for CAP-2.

Structural information about coffee pectin was obtained by HSQC NMR (Figure 2). Regarding CAP-1, chemical shifts (δ) of homogalacturonan were found for esterified (E) and unesterified (U) galacturonic acid, with the methoxyl group of E appearing at δ 3.82/52.9. H1/C1 signals of →4)α-D-6MeGalA*p*(1→ were found at δ 4.97/100.1 when linked to another esterified unit (EE) and at δ 4.92/100.1 when linked to a unesterified unit (EU). Anomeric H1/C1 signals of →4)α-D-GalA*p*(1→ appeared at δ 5.09/99.8 for UE and δ 5.16/99.7 for UU. Chemical shifts of H2/C2, H3/C3, H4/C4 and H5/C5 of →4)α-D-6MeGalA*p*(1→ were found at δ 3.76/68.0, 4.00/68.2, 4.47/78.4 and 5.04/70.6, respectively, while those of →4)α-D-GalA*p*(1→ at δ 3.76/68.0, 4.10/69.6, 4.47/78.4 and 5.32/70.7. Signals from rhamnosyl residues and galactans evidenced the presence of rhamnogalacturonan I. Unbranched rhamnosyl presented stronger signals than branched rhamnosyl, suggesting that most of RG-I region from CAP-1 was not substituted. Signals from H1/C1 and H3/C3 from both rhamnosyl units were detected at δ 5.23/99.1 and 3.90/73.7. H2/C2 and H6/C6 of →2)α-L-Rha*p*(1→ were respectively found at δ 4.15/77.6 and 1.26/16.5 while H2/C2, H5/C5 and H6/C6 of →2,4)α-L-Rha*p*(1→ appeared at δ 4.11/77.0,

3.54/70.9 and 1.31/16.7. The signals at δ 4.62/104.4, 3.69/74.7, 3.78/73.5, 4.15/77.6 and 3.79/60.9 were assigned to H1/C1, H2/C2, H3/C3, H4/C4 and H6/C6 of →4)β-D-Gal*p*(1→. Terminal galactosyl residues (t-β-D-Gal*p*(1→) were also found in the spectrum, presenting signals of H1/C1 and H3/C3, with respective chemical shifts of δ 4.47/103.6 and 3.67/72.2. Acetyl group was found at δ 2.08/20.2, indicating acetylation at C-3 position of GalA (Renard & Jarvis, 1999). ^{13}C NMR spectrum was used to obtain the signals of carboxylic carbons of methyl-esterified and unesterified galacturonic acid, found at δ 170.6 for →4)α-D-6MeGalA*p*(1→ and at δ 172.2 for →4)α-D-GalA*p*(1→ (data not shown).

Figure 2. ^{1}H-^{13}C HSQC NMR spectrum of CAP-1 in D_2O using acetone as internal standard.



Almost identical signals were found for CAP-2 HSQC NMR spectrum (data not shown), except that less signals for neutral monosaccharides were found, due to the lower amounts of branches in this fraction. Only H6/C6 of rhamnosyl and galactosyl were found at δ 1.26/16.5 for →2)α-L-Rha*p*(1→, δ 1.31/16.7 for →2,4)α-L-Rha*p*(1→, δ 3.79/60.9 for →4)β-D-Gal*p*(1→ and δ 3.75/60.9 for t-β-D-Gal*p*(1→. All the assignments were based on literature

values (Colodel, Vriesmann & Petkowicz, 2018; Ovodova *et al.* 2005; Golovchenko *et al.* 2007).

The results suggest that CAP-1 and CAP-2 are composed mainly of homogalacturonan, with substantial methyl-esterification and RG-I side chains are mainly substituted with short chains of β-(1→4) galactans.

Chromatograms from HPSEC analyses are depicted in Figure 3. Both pectins had a prominent peak eluting around 50 min, detected by both refractive index (RI) and light scattering. The average molar mass (M_w) and polydispersity index (M_w/M_n) of CAP-1 and CAP-2 calculated by light scattering are given in Table 3.

Garcia *et al* (1991) reported M_w of $2.236.10^4$ g/mol for a pectin extracted from pulp of Guatemalan coffee, more than 10 times lower than those found in the present study. The extraction was conducted with pressed pulp, with no pretreatment, using boiling hydrochloric acid for 1 h, at pH 2. The dissimilarities in the experimental protocol and variety of coffee used in that study may explain the difference. The molar mass of coffee pectins was higher than other waste pectins, such as cashew apple pomace ($0.76\text{-}1.42.10^5$ g/mol) (Yapo & Koffi, 2014), melon peel ($6.76.10^4$ g/mol) (Raji *at al.,* 2017) and passion fruit rind ($5.13\text{-}6.37.10^4$ g/mol) (Yapo & Koffi, 2006). The polydispersity index (M_w/M_n) found for CAP-1 and CAP-2 were close, but lower than the values found for commercial citrus (2.30) (Zhang *et al.,* 2015), sugar beet (2.32) (Funami *et al.,* 2007) and apple (2.20) (Zhang *et al.,* 2013) pectins.

Figure 3. Elution profiles of CAP-1 (A) and CAP-2 (B) by HPSEC using RI and MALLS (90°) detectors.

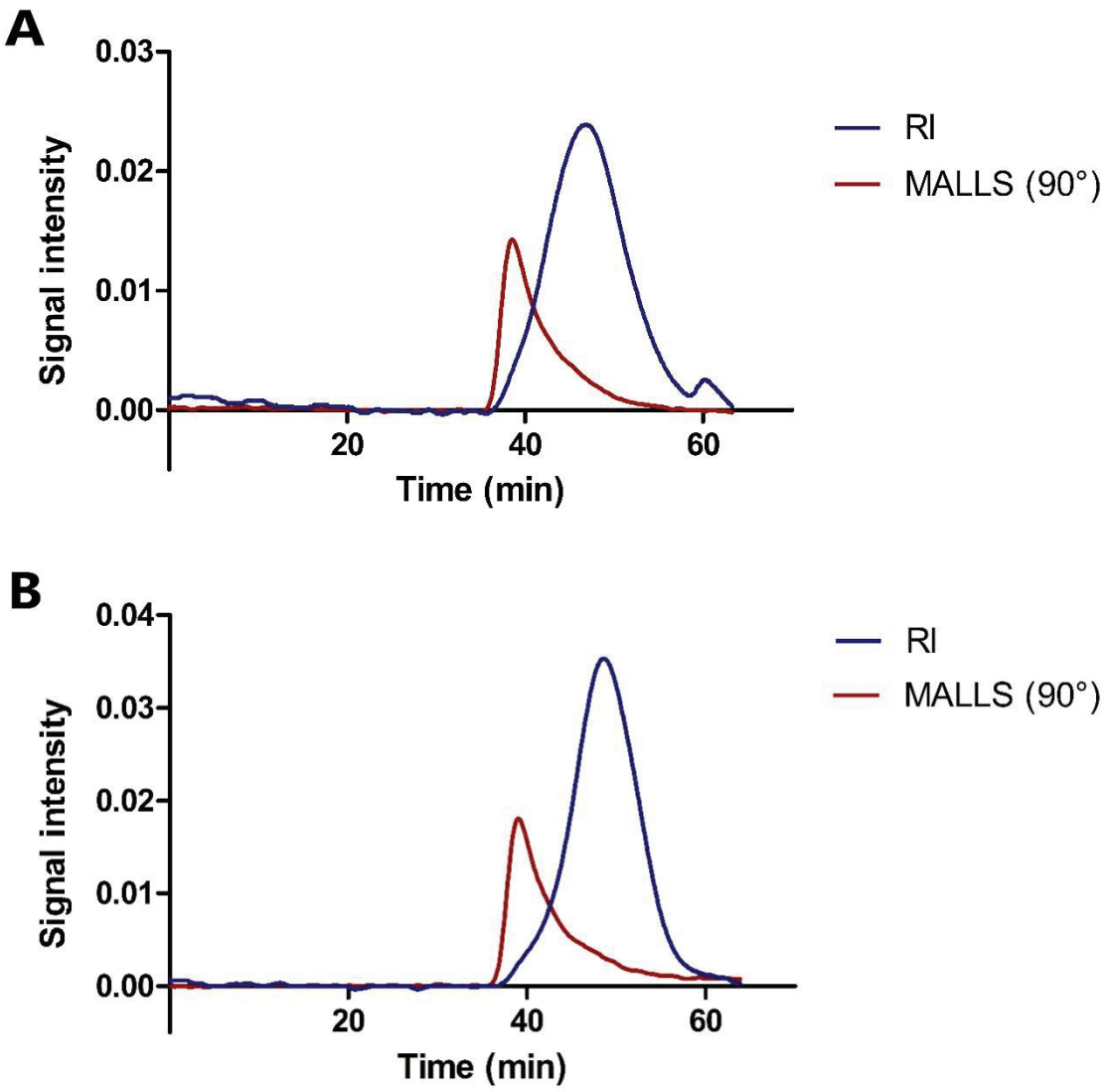


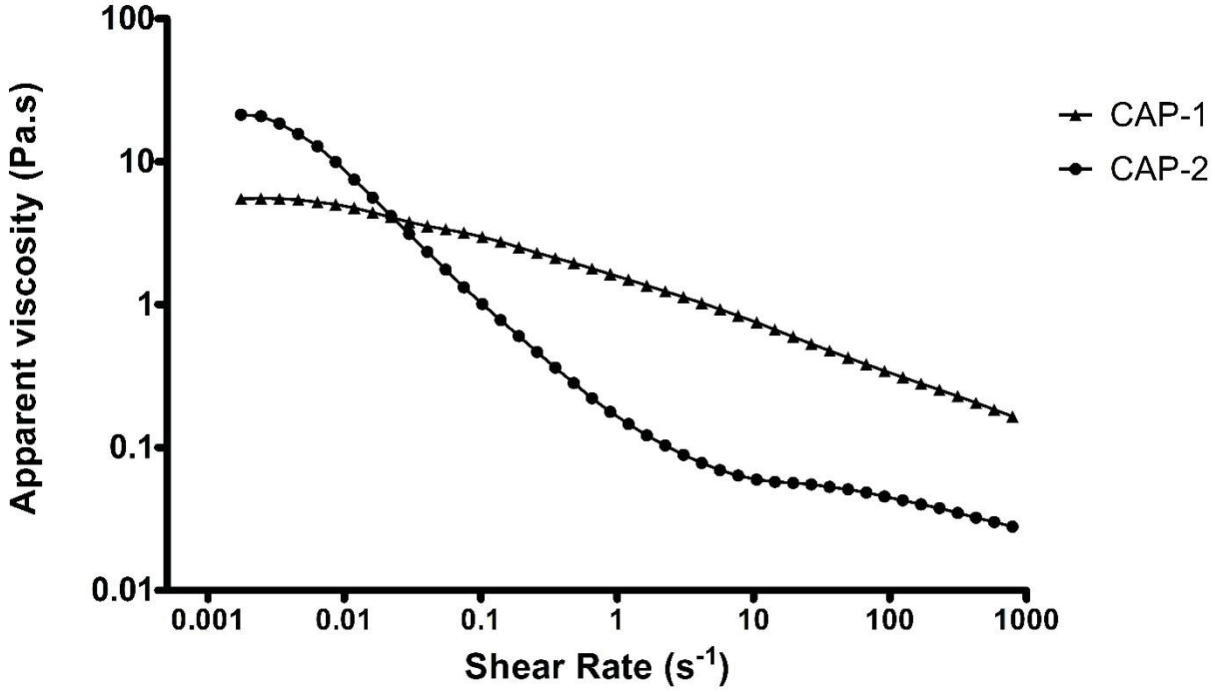
Table 3. Molecular features of CAP-1 and CAP-2.

	CAP-1	**CAP-2**
dn/dc	0.122	0.121
M_w (g/mol)	3.921 x 10^5	2.642 x 10^5
M_w/M_n	1.560±0.027	1.789±0.017

3.3. Rheological analyses

The viscosity curves of 5 % (w/w) CAP-1 and CAP-2 in ultrapure water is given in Figure 4. Both solutions showed a shear thinning behavior, meaning that viscosity decreased with the increase of shear rate, in accordance with other pectin solutions, such as okra (Kontogiorgos *et al.* 2012), jackfruit (Begum *et al.* 2017) and commercial citrus and apple pectins (Chou & Kokini, 1987).

Figure 4. Viscosity curves of 5% CAP-1 and CAP-2 solutions in water at 25°C.



The flow behavior of many solutions can be described by the Cross model (Cross,1965), wherein the equation in the form of $\eta=\eta_\infty+(\eta_0-\eta_\infty)/[1+(K\gamma)^n]$ can relate the viscosity and shear rate value with the parameters of infinite-shear rate viscosity (η_∞), zero-shear rate viscosity (η_0), Cross time constant (K) and the flow behavior index (*n*). The values found for the parameters of cross model are given in Table 4.

Table 4. Parameters of Cross equation found for 5% solutions of CAP-1 and CAP-2.

	CAP-1	**CAP-2**
η_0 (Pa.s)	9.220	41.67
η_∞ (Pa.s)	0.04069	0.06826
K (s)	79.808	359.066
n	0.3625	1.057
R^2	0.9994	0.9998

Data from viscosity curves of CAP-1 and CAP-2 was fitted to the Cross equation. Both curves were well adjusted to the model ($R^2 \approx 0.999$) and presented different characteristics. The zero-shear viscosity of CAP-1 was ~5 times lower than CAP-2. However, both pectins had similar viscosity in the infinite-shear rate, indicating a more pseudoplastic behavior of CAP-2, as confirmed by its higher values of *n*. In non-Newtonian liquids, the most shear-thinning solutions have their *n* value tending to unity, as far as more Newtonian liquids have *n* tending to zero (Barnes, 2000).

At the same concentration, zero-shear viscosity of CAP-1 and CAP-2 were higher than pectin of apple pomace (Min *et al.,* 2011) and similar to solutions of pectin from cacao pod husk (Vriesmann, Teófilo & Petkowicz, 2012), ponkan peel (Colodel, Vriesmann & Petkowicz, 2019) and watermelon rind (Petkowicz, Vriesmann & Williams, 2017).

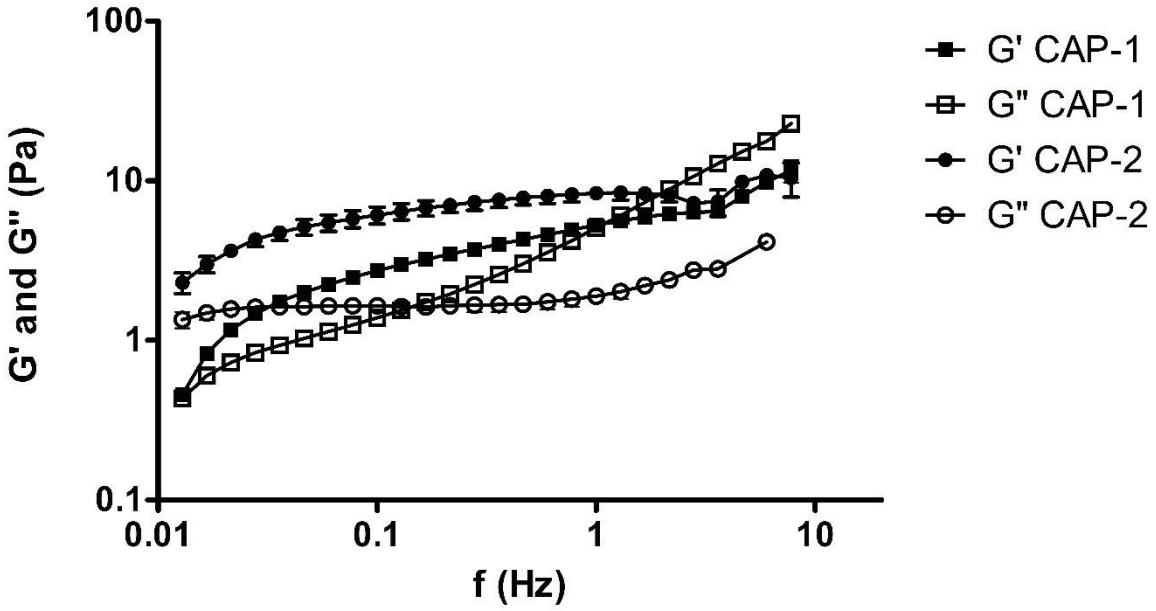
Despite the higher molar mass of CAP-1, it had a lower value of zero-shear viscosity than CAP-2. Therefore, parameters such as the DM, GalA content and the presence of side chains may play an important role in flow behavior of pectins. Kpodo et al. (2017) compared the viscosity of six okra genotypes and concluded that the viscosity of solutions was not a simple function of molar mass, instead it was affected by structural features, such as the galacturonic acid content and the RG-I regions. For coffee pectin, the fraction which had a higher DM, less branches and was richer in GalA presented higher η_0 and showed to be more pseudoplastic. These data are different from the ones of Hwang & Kokini (1992) whose results indicated that the more branched is the pectin, the higher is the η_0 and pseudoplasticity of its

solutions. Petkowicz, Vriesmann & Williams (2017) investigated the flow behavior of two fractions of pectins extracted from fresh (FW) and lyophilized watermelon rind (LW). The viscosity of FW was 3-fold higher than LW, even though its molar mass was lower and their chemical characteristics were very similar, with the only remarkable difference being that the GalA content of FW (74.2%) was higher than LW (68.7%). It was proposed that electrostatic interactions between positively charged side chain of proteins and negatively charged GalA might be responsible for this distinctive behavior.

For pseudoplastic fluids, as the shear rate increases there is a disentanglement of the polymer, leading to the alignment of the molecules with the shear field or the disruption of weak physical interactions (Hosseini, Khodaiyan & Yarmand, 2016). It is suggested that a high DM provides less polymer interactions between pectin chains (Min *et al.*, 2011). Since CAP-2 had a higher DM and less side chains, the molecules might align more easily than CAP-1, which could explain the differences in pseudoplasticity. Colodel, Vriesmann & Petkowicz (2019) compared the flow behavior of an HM pectin extracted from ponkan peel (DM = 85.7% and GalA = 84.5%) and a commercial citrus pectin (DM = 71.4% and GalA = 84.4) and also observed a more shear thinning behavior of the pectin with higher DM.

The viscoelastic behavior of CAP-1 and CAP-2 at 5% (w/w) concentration was investigated by oscillatory experiments (Figure 5). For CAP-2, G’ was higher than G” in the whole frequency range and the moduli increased with the frequency, demonstrating that CAP-2 behaved like a weak gel. On the other hand, for CAP-1, the moduli were more frequency dependent and G’ > G” only at the lower frequencies with a crossover at a frequency of ~1 Hz. This behavior was different from 5% solution of a LM pectin highly acetylated from cacao pod husk (Vriesmann, Teófilo & Petkowicz, 2012) which had G”>G’ over the analyzed frequency range, but similar to the watermelon rind pectin (Petkowicz, Vriesmann & Williams, 2017).

Figure 5. Frequency sweep of 5% (w/w) CAP-1 and CAP-2 in water at 25°C.



Conclusions

Coffee pulp contains pectins that can be partially extracted with boiling HNO_3 for 30 min. Two sequential extractions gave rise to two pectic fractions with more than 75% GalA and low degree of acetylation. They were mainly homogalacturonans, highly methyl-esterified with high molar mass. The RG-I regions are mainly substituted by short chains of β-(1→4) galactans. Solutions of 5% (w/w) pectins displayed thickening properties with shear-thinning behavior. The pectin with higher DM and less side chains showed a higher degree of pseudoplasticity and mechanical spectrum typical of weak gel. The structural and rheological properties of coffee pectin make coffee pulp a promising raw material for commercial production of pectin.

Acknowledgements

The authors are grateful to NMR Center of UFPR for NMR analyses, to Electron Microscopy Center of UFPR for SEM-EDS analyses, to São João farm for providing coffee pulp and to the Brazilian agencies Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior - Brasil (CAPES)-Finance Code 001 and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico

(CNPq) for the financial support. C.L.O.P. is a research member of the CNPq (309159/2018-0).

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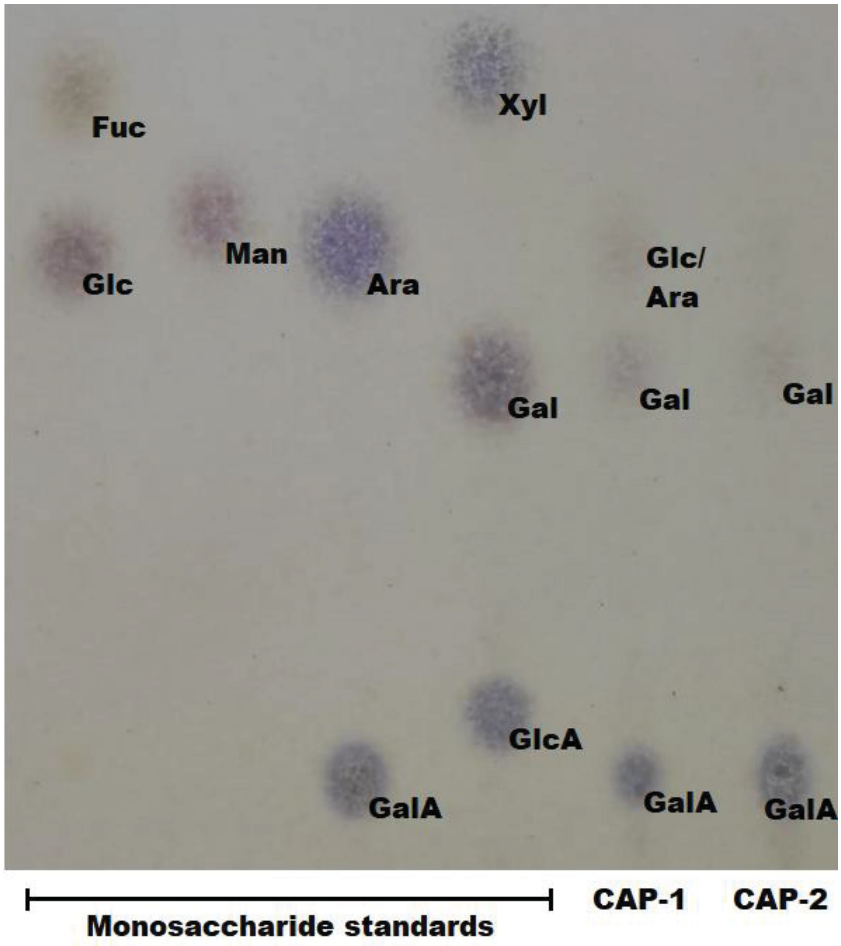
Supplementary data

Figure S1. Thin-layer chromatography of monosaccharide standards (left side) and CAP-1 and CAP-2 (right side).



Rha: Rhamnose, Fuc: Fucose, Ara: Arabinose, Xyl: Xylose, Man: Mannose, Gal: Galactose, Glc: glucose, GalA: Galacturonic Acid.

CAPÍTULO 2

Viscosity and gel formation of Brazilian coffee (*Coffea arabica*) pulp pectin

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Abstract

The rheology of a high-methoxyl pectin (CAP-1) with 79.5% galacturonic acid, extracted from pulp of Brazilian *Coffea Arabica,* was investigated. Viscosity curves of pectin solutions in water and NaCl below and above pK_a showed a shear-thinning behavior. At pH lower than pK_a, the presence of salt in high concentration increased the viscosity of the solutions, independent on pectin concentration, while at pH > pK_a, an opposite effect was observed. Mechanical spectra of 5% pectin solutions at pH > pK_a indicated that a gel was formed in water and 0.1M NaCl solutions, possibly induced by the presence of calcium, revealed by SEM-EDS. A high concentration of soluble solids and low pH successfully produced gels, never before accomplished with a pectin from coffee pulp. Gels prepared with xylitol diplayed similar viscoelastic characteristics to the gels prepared with sucrose, showing to be a possible substituent as a cosolute in low-calorie products. Changes on extrinsic factors were tested on the production of coffee pectin gels. Concentrations of pectin (0.5-2.5%), sucrose (55-65%) and xylitol (55-60%) were evaluated and a positive correlation between pectin and cosolute concentration with gel strength was found. A pH range from 2.0 to 2.87 was set as an ideal range for gel preparation. After heating and cooling, the viscoelastic profile is recovered. Gel structuring was followed with noticeable phases. The results pointed out that the pectin isolated from the pulp of Brazilian coffee is suitable to be used as food additive and coffee pulp is a potential source for commercial pectin extraction.

Keywords: Agricultural waste, Low-calorie food, *Coffea arabica,* Gelation, Viscosity and Rheology.

1. Introduction

Since first publication describing pectin and some of its properties by Henri Braconnot (Braconnot, 1825), the studies involving pectin have been evolved significantly, mostly regarding to its properties as a rheology modifier. The addition of pectin or other hydrocolloids in food systems, helps to modify their sensory properties, such as smoothness, sliminess and thickness, provided by changes in viscosity and texture (Malone, Appelqvist & Norton, 2003). While all hydrocolloids thicken, only a few are able to form gels. In gel formation, there is an association or cross-linking of the polymer chains to form a three-dimensional network that traps the water within it to form a rigid structure that is resistant to flow and it is recognized as a viscoelastic solid (Kavanagh & Ross-Murphy, 1998; Saha & Bhattacharya, 2010).

Pectin is one of the polysaccharides that has the ability to form gels, its most important property (Bemiller, 1986). Other uses include the increase of stability and thickness of jams, yoghurts, fruity milk drinks and ice cream (Voragen *et al.*, 2009). There is also a growing interest on health promotion and pharmaceutical uses of pectin (Sriamornsak, 2003).

Pectins are a family of galacturonic acid-rich plant cell wall polysaccharides. They include four domains: homogalacturonan (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), xylogalacturonan (XGA) and apiogalacturonan (AGA), all containing galacturonic acid linked at the *O*-1 and the *O*-4 position (Mohnen, 2008). The C-6 of galacturonic acid units in the HG regions can be partially methyl-esterified and according to the degree of methyl-esterification (DM), pectins are categorized into two groups: the low-methoxyl (LM pectins), with DM > 50% and high-methoxyl (HM pectins), with DM > 50% (BeMiller, 1986). Acetyl groups can be found at O-2 and/or O-3 position of galacturonic acid residues in the HG and RG-I portions. In general, acetyl groups are present in low amounts in native pectins, with some exceptions, such as sugar beet pectin (Kouwijzer, Schols & Pérez, 1996).

Gel formation is mainly affected by the composition and molecular characteristics of pectin, such as galacturonic acid content, acetyl content, molar mass and DM. The last one controls the gelling mechanism and consequently the conditions required for gel formation. The LM mechanism relies on the *egg-box* model, which involves junction zones between homogalacturonan regions of different chains. In these systems, divalent cations are used to induce association of two polymers into a dimer by electrostatic interaction between the cation and the carboxyl groups. On the other hand, gelation of HM pectins requires the presence of high amount of soluble solids and acidic pH. Usually, sucrose is used as a cosolute in order to stabilize junction zones by promoting hydrophobic interactions between methyl-ester groups. These are called low water activity gels or sugar-acid-pectin gels (Rees, 1981; Sharma *et al.* 2006; Thakur *et al.* 1997).

LM pectins are used in the low-calorie food industry, especially because no sugar is needed to induce gelation. Traditional uses include low-calorie jelly (10 g pectin/kg), strawberry conserve (0.7 g pectin/kg), ice cream fruit syrup (0.3 g pectin/kg) and barbecue sauce (4g pectin/kg) (Hoefler, 1991). However, HM pectins are still little exploited on the manufacture of diet products, due to the need of high content of sugar for gelation.

Pectin is mostly produced from citrus peel and apple pomace, although other wastes have been extensively studied, aiming to find new useful properties and also to cover the increasing demand for pectin (Ciriminna, 2015). In any case, raw material availability and low cost are important factors that should be taken into account. Only with a few studies regarding to its pectins, coffee pulp seems to fit the requirements. A LM pectin (DM 23.81%) with 91.2% galacturonic acid and low molar mass (2.2359×10^4 g/mol) was obtained by extraction of pressed pulp of *Coffea arabica* harvested in Guatemala with boiling HCl at pH 2 for 1 h followed by fractioning with quaternary ammonium and ammonium sulfate salts (Garcia *et al.*, 1991). The extracted pectin did not form gels with sucrose and acidic pH or with calcium salts.

More recently, a polyphenol functionalized coffee pectin was obtained by Otalora (2018). The pectin was extracted from the pulp of *C. arabica* produced in Colombia using sequential acid (HCl pH 2, 1 h, 90°C) and alkaline extraction (NaOH, pH 12, room temperature), followed by a laccase treatment. The

resulting product had 65.4% galacturonic acid, DM 100%, high acetyl content (97%) and did not exhibit gelling properties.

In a previous study (not published) we described the extraction and characterization of two pectic fractions isolated from pulp of coffee fruits harvested in Brazil. The pectins were obtained by sequential extraction with boiling 0.1 M nitric acid for 30 min of the pulp, after a pretreatment with ethanol. The pectins had galacturonic acid content higher than 75% and high degree of methyl-esterification. In the present study, the rheological properties of the pectin with the highest yield (CAP-1, ~15% yield) was investigated. Differently from the pectins obtained from coffee pulp by other authors, it was demonstrated that the pectin previously isolated with nitric acid from Brazilian coffee pulp can be used as a thickener or a gelling agent of regular or low-calorie food.

2. Material and methods

2.1. Pectin extraction and properties

The pectin used for the rheological measurements (CAP-1) was obtained and characterized by Reichembach & Petkowicz (not published), as previously described. CAP-1 had 79.5% of galacturonic acid (GalA) on the ash and moisture-free mass, average molar mass (M_w) of 3.921 x 10^5 g/mol, DM of 63.2% and a degree of acetylation (DA) of 5.7%.

2.2. Pectin solutions

Pectin solutions were prepared by magnetic stirring in the concentrations of 1% and 5% (w/w) in ultrapure water, 0.1 M and 1.0 M NaCl. The pH of pectins were maintained in its natural (~2.9 for 1% and ~2.6 for 5%) or adjusted to ~4.5, in order to deprotonate pectin molecule and evaluate charge interactions. The adjustment of pH of the solutions in 0.1 M NaCl and 1.0 M NaCl was made with 0.1 M NaOH and 1.0 M NaOH, respectively, in order to maintain sodium ion concentration, while for the pectin in water, the adjustment was accomplished by an open dialysis.

2.3. Pectin gels

Pectin gels were prepared using different conditions: pectin concentration varying from 0.5 to 2.5% (w/w), pH from 1.5 to 3.0, sucrose content from 55 to 65% (w/w) and xylitol from 55 to 60% (w/w). Separate solutions of pectins and sucrose or xylitol were prepared in ultrapure water and then mixed under stirring pH was adjusted with 0.1M HNO_3 and the solution was boiled under stirring until the appropriate weight (~ 5 min). The gels were left at 4°C overnight for further analyses.

2.4. Rheological analyses

Rheological analyses were performed at 25°C using a Thermo Scientific Haake Mars rheometer (Haake GmbH, Germany) coupled to a Haake UTMC unit, a thermostatized bath (HAAKE K15) containing water:ethylene glycol (4:1, v/v) and a DC5 circulator. RheoWin Data Manager version 4.82.0002 (Thermo Fisher Scientific, USA) software was used to obtain the fit models and the rheological and statistical parameters and GraphPad Prism version 5.00 (GraphPad Software Inc., USA) software was used to obtain the graphs. Rotational and oscillatory tests were performed with the pectin solutions and gels. Experiments were performed in triplicate and error bars are the standard deviation of the averages.

2.4.1. Rheology of pectin solutions

Viscosity curves of pectin solutions were performed in CR mode, at 25°C, using a plate/cone geometry (C60 2° Ti L) at shear rate of 0.001-1000 s^{-1} for 5% solutions and 0.1-1000 s^{-1} for 1% solutions. The viscoelastic behavior of 5% pectin solutions was assessed using a plate/plate geometry (P35 Ti L) in a frequency range of 0.01-10 Hz, under stress within the linear viscoelastic region.

2.4.2. Rheology of pectin gels

Oscillatory tests of pectin gels prepared in different conditions were carried out using a plate/plate geometry (P35 Ti L). In order to determine the

linear viscoelastic range, stress sweeps were obtained (0.01-10 Pa) at frequency of 1 Hz. Frequency sweeps were performed in the range of 0.01-10 Hz under stress within the linear viscoelastic region.

A temperature ramp was performed using a gel prepared with 2% pectin, 60% sucrose at pH 2.5 in order to evaluate the influence of heating from 4°C to 90°C and then cooling from 90 to 4°C at a rate of 1°C/min. Another temperature ramp (5-95°C/95-5°C, at a rate of 1°C/min) was performed with a 60% sucrose and 1.5% pectin solution at pH 2.87 in order to investigate the gel structure formation. A plate/plate geometry (P35 Ti L) was used in the temperature ramp experiments, in a set frequency of 1 Hz and stress of 1 Pa. The edges of the exposed gel were covered with mineral oil and a solvent trap was used to prevent water loss.

2.5. Scanning Electron Microscopy and Energy Dispersive Spectroscopy (SEM-EDS)

SEM-EDS was used to obtain the calcium content of the dialyzed coffee pectin. The sample was lyophilized and placed in a carbon tape mounted on a SEM stub and analyzed in a VEGA3 LMU microscope (Tescan, Czech Republic) using a 15 kV accelerating voltage for 60 s to obtain the spectrum.

3. Results and Discussion

The pK_a of pectin is around 3.5, meaning that below the pH of 3.5 the majority of GalA units are protonated and above it, are deprotonated (Hoefler, 1999). The presence of counterions in the deprotonated pectin allows to evaluate charge interactions between sodium ions and the negative charge of carboxyl group of GalA units. They modify the stability of the polyelectrolyte solutions through the formation of electrostatic interactions, altering viscosity and other rheological properties. It can also lead to network formation or gelation, in the case of multi-valent ions (Marudova *et al.* 2004). Therefore, the viscosity of pectin solutions at pH below and above pK_a in the concentrations of 1% and 5% (w/w) in ultrapure H_2O, 0.1 M NaCl and 1.0 M NaCl was investigated.

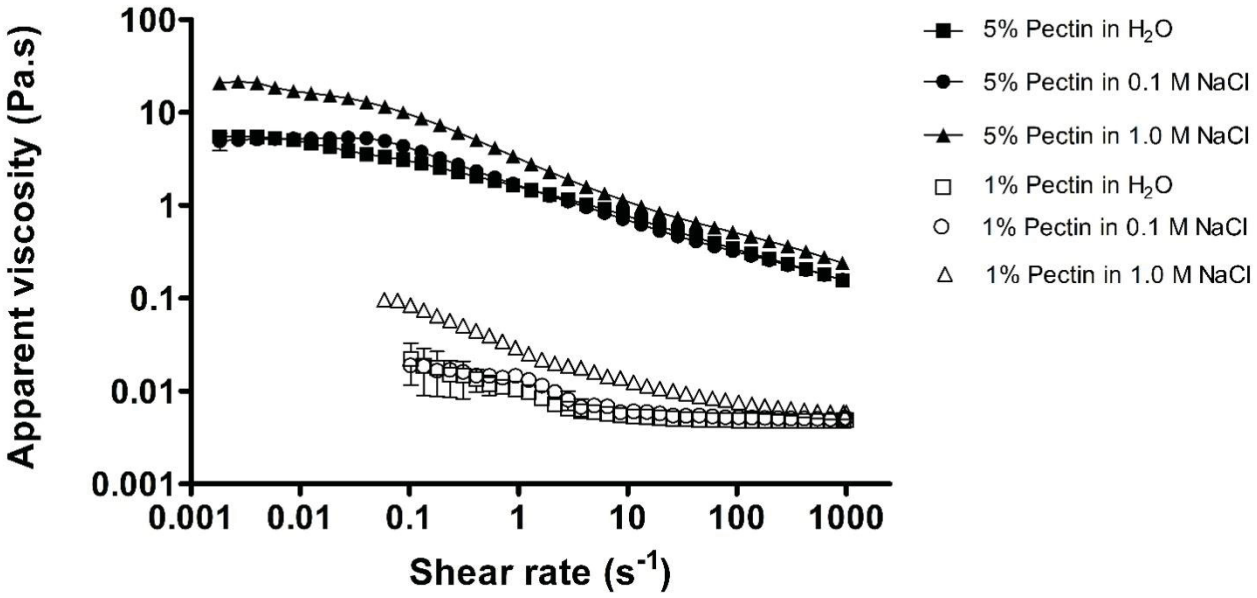
3.1. Rheological measurements of pectin solutions

3.1.1. Rheological measurements of pectin solutions at pH < pK_a

As other hydrocolloids, pectin shows thickening properties when solubilized in aqueous media. Not rarely, it is used in low pH products, such as juices and sauces, allowing pectin to be present in its protonated form (U.S. Food and Drug Administration, 2008). The viscosity of 1% and 5% pectin solutions in ultrapure H_2O, 0.1 M NaCl and 1.0 M NaCl at pH < pK_a is shown in Figure 1. The solutions exhibit shear-thinning behavior, with increase in the viscosity with the increase of pectin concentration. The pectin solutions in 1.0 M NaCl had the highest viscosity when compared to the other solutions in the same polysaccharide concentration. However, in a same concentration superimposed viscosity curves were obtained for pectins in water and 0.1M NaCl, indicating no effect on viscosity at low salt concentration.

Previous studies also identified a positive dependence between salt concentration and the viscosity of polymers. A significative increase in viscosity was also found for solutions of 0.05% welan in 1.0 M NaCl (Campana *et al.,* 1990) and 0.5% hydrophobically modified polyacrylamide derivatives (Feng *et al.* 2002). Doyle, Lyons & Morris (2009) explain this effect by a greater intermolecular association (hyperentanglement) in response to charge screening, where ionic strength is increased by the addition of salt with no accompanying increase in charge density.

Figure 1. Viscosity curves of 1% and 5% (w/w) pectin in water and NaCl solutions at pH< pK_a.

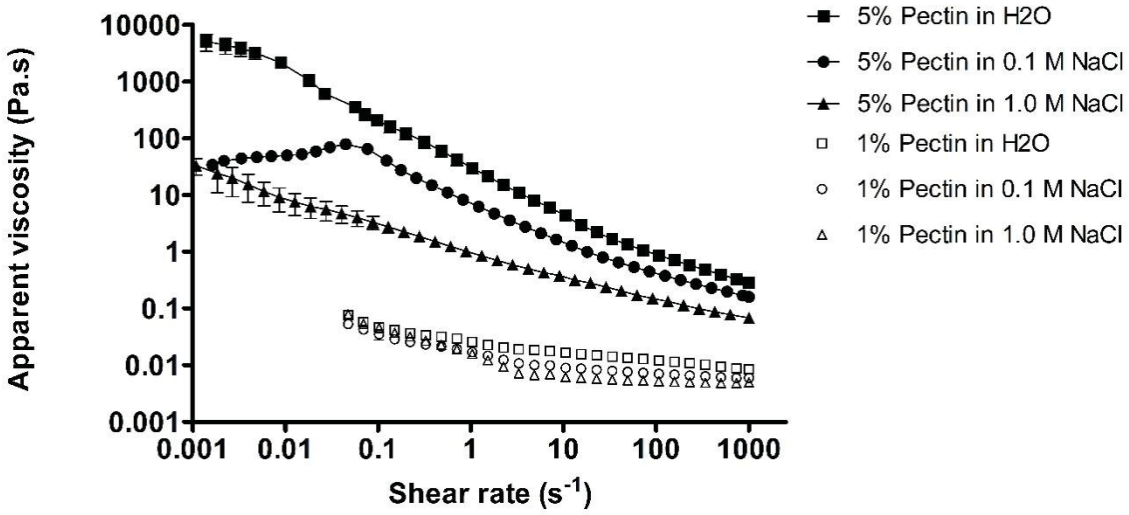


3.1.2. Rheological measurements of pectin solutions at pH > pK_a

When 1% CAP-1 solutions were analyzed at pH > pK_a, it was possible to observe a decrease in the viscosity of in the presence of NaCl compared to the solution in water (Figure 2). In this condition, sodium ions screen electrostatic repulsions between pectin chains, resulting in more flexible conformation and less resistance to flow. Wyatt, Gunther & Liberatore (2011) investigated the flow behavior of polyelectrolytes solutions, including polysaccharides like xanthan, carrageenan, welan and chitosan. The authors demonstrated that above the critical concentration (C*), the presence of monovalent salts increased the viscosity of the solution, while below C*, the viscosity is decreased and the effect is not dependent on the salt concentration. On the other hand, they observe that higher concentrations of the polyelectrolyte tend to enhance entanglement and increase viscosity (Wyatt, Gunther & Liberatore, 2011). The increase in the concentration of pectin from 1% to 5% significantly raised the viscosity values (Figure 2) while the presence of counterions reduced the apparent viscosity as observed for the more diluted solution. At a shear rate of 0.0015, the viscosity of the solution in water showed to be more than 150-fold higher than pectin in salt solutions, having a dependence on the salt

concentration. Mechanical spectra of these preparations were obtained (Figure 3). The elastic modulus (G') was higher than the viscous modulus (G"), therefore both samples showed a gel-like behaviour. Pectin in water showed to be a stronger gel, with G' higher than G" over the whole frequency range. The solution with 0.1 M NaCl was a weaker gel, displaying lower values of moduli and losing its gel characteristic after frequency of 4.6 Hz, where a crossover is observed.

Figure 2. Viscosity curves of 1% and 5% (w/w) pectin in water and NaCl solutions at pH > pK_a.



Wehr, Menzies & Blamey (2004) found that apple and citrus HM pectins can form gels in the presence of alkali (NaOH or KOH), induced by a de-methoxylation of pectin and subsequent shielding of carboxylic charges by sodium or potassium ions. The addition of NaCl boosted gelation, unlike coffee pectin.

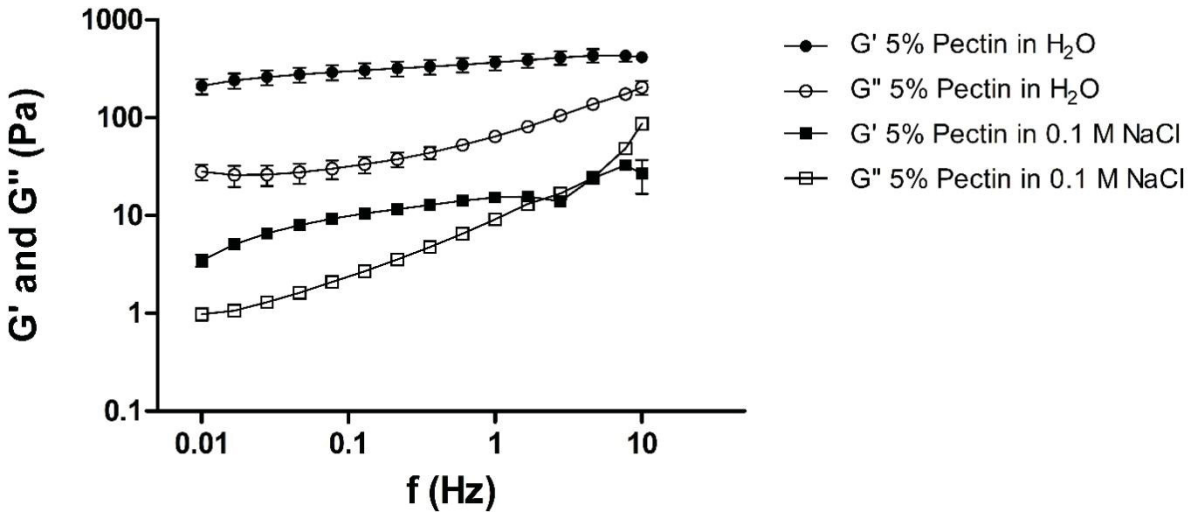
In the present study, it is possible that intrinsic divalent ions might interact with carboxyl groups of pectin chains by *egg-box* mechanism providing the junction zones for gel formation. Although this is not the classical mechanism for gelation of HM pectin, gel formation by addition of divalent ions has been described for a commercial HM pectin (1% w/v) (Yang *et al.* 2013),

pomelo pectin (0.8% w/v) (Gamonpilas *et al.* 2015) and unripe tomato pericarp pectin (1-3% w/w) (Tibbits, MacDougall & Ring, 1998).

The calcium content in the pectin CAP-1 was previously reported by Reichembach & Petkowicz (not published) to be 0.2 mol%. Since the dialyzed pectin in H_2O formed the stronger gel, a SEM-EDS analysis was performed in order to verify the elemental composition of the pectin after dialysis. The spectrum revealed that calcium content was 0.9 mol%, ~4 times higher than before dialysis. The open dialysis may have provided calcium ions from the tap water to the sample and, possibly, the higher amount of calcium in this pectin is related to the formation of the stronger gel.

When NaCl was added, the gelation via calcium cross-linking was probably impaired due to the competition of sodium and divalent ions over the interaction with the charged carboxyl groups. Marudova & Jilov (2003) studied the influence of sodium salts in the gelation of low-methoxyl amidated pectins induced by calcium, including NaCl, and they found that the presence of this salt narrowed the usable range of Ca^{+2} at which gels with satisfactory properties can be formed.

Figure 3. Frequency sweep of 5% (w/w) pectin in water and 0.1 M NaCl at pH > pK_a.



3.2. Rheological measurements of pectin gels

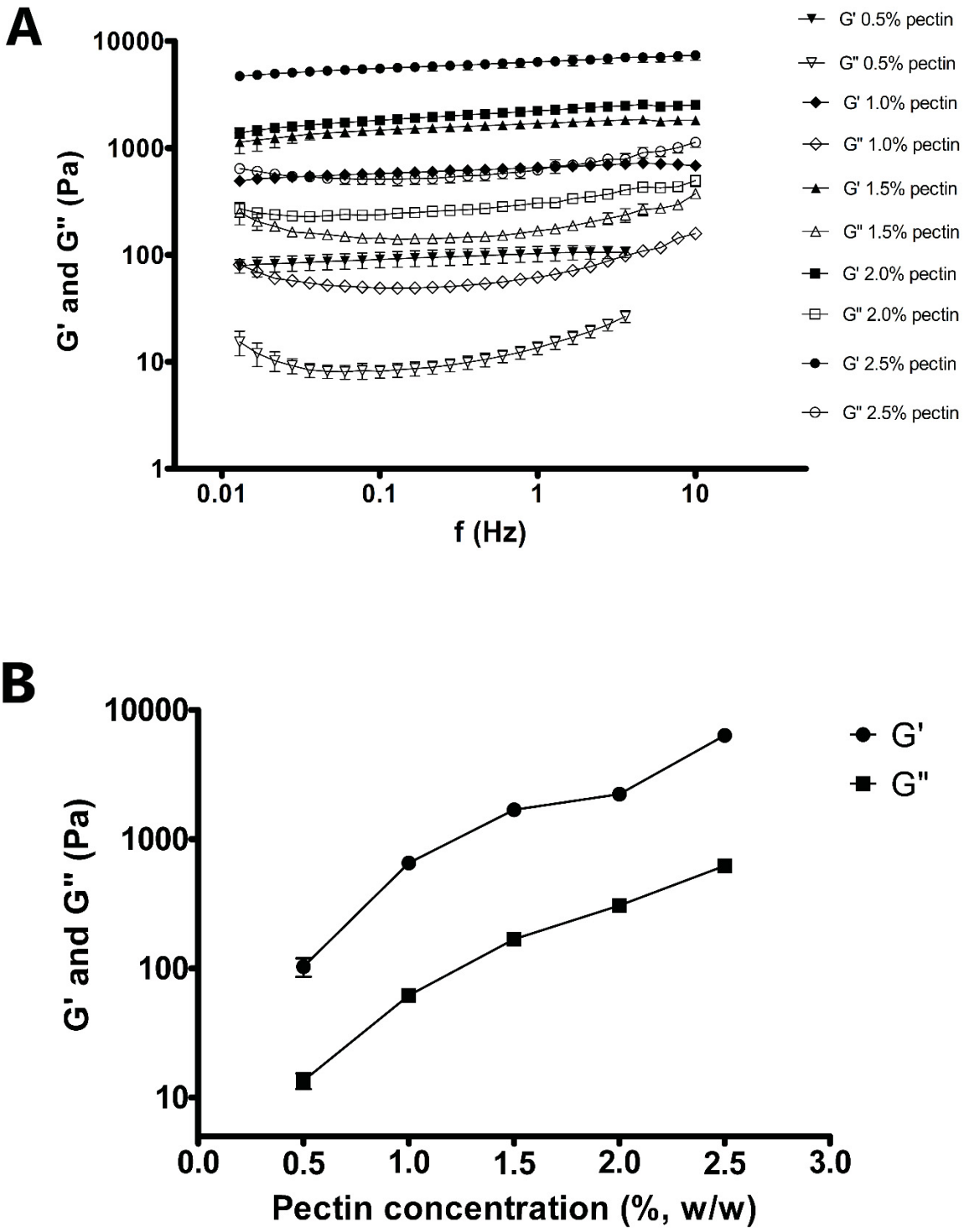
The word pectin is derived from the Greek (πηχτος) meaning ‘to congeal, solidify or curdle’ in reference to its more remarkable property, which is the ability to form gel under specific conditions. However, the pectins isolated from coffee pulp by other authors were not able to form gel. In the present study, the gelling properties of the pectin CAP-1, extracted from Brazilian *Coffea arabica* pulp, was investigated. Initially, the pectin was tested at different concentrations, low pH and high sucrose content.

3.2.1. Influence of pectin concentration on the gelling properties of CAP-1

Frequency sweeps of gels prepared with 60% (w/w) sucrose, pH 2.0 and 0.5-2.5% (w/w) pectin concentration are depicted in Figure 4-A. Pectin gelation occurred for all concentrations tested, given that G’ was higher than G” over the analyzed frequency range. Overall, G’ was less frequency dependent than G”.

There was a clear tendency for stronger gels to be produced in more concentrated pectin solutions (Figure 4-B). A higher concentration of pectin results in increased self-association by hydrogen bonds involving the protonated carboxyl groups and hydrophobic interactions between methoxyl groups (Willats, Knox & Mikkelsen, 2006). Previous reports on pectins from different sources also found that the increase of pectin concentration produces firmer gels, as observed for gels prepared with cacao pod husk pectin with 60% (w/w) sucrose at pH 2.7 (Vriemann & Petkowicz, 2013) and with an HM pectin from apple in the same conditions (Rascón-Chu *et al.* 2009). The stiffness of the gel network of the gels prepared with pectin from apple was accessed by the determination of the gel hardness, which increased from 10.2 to 20.4 g when the pectin concentration was increased from 2% to 3% (w/v). The moduli values were very similar to those obtained for ponkan peel pectin in the same pectin and sucrose concentrations (Colodel, Vriesmann & Petkowicz, 2019). Considering the values of the moduli as well as the difference between the values, the concentration of 1.5% was chosen for the following experiments.

Figure 4. Effect of pectin concentration on the viscoelastic behavior of pectin CAP-1 gels with 60% (w/w) sucrose at pH 2.0 (A) and values of G' and G'' at the frequency of 1 Hz as a function of pectin concentration (B).



3.2.2. Influence of pH on the gelling properties of CAP-1

As the pH is gradually reduced, pectin is able to form gels at high soluble solids concentration (Oakenfull & Scott, 1984). The carboxyl ions become mostly protonated, lowering the attraction between pectin and water molecules and also repulsive forces between pectin molecules (Sriamornsak, 2003). At pH values around 3.0, a rapid setting pectin (DM above 72%) will be capable of forming gel, while a slow-set pectin (DM between 58 and 65%) will require lower pH for gelation (May, 1990). Since CAP-1 presented a DM of 63.2%, it was classified as slow-set and the gels were prepared in the pH values of 1.5; 2.0; 2.5; 2.87 and 3.0. The pH of 2.87 was used since it was the natural pH at the pectin concentration used to prepare the gels (1.5 %, w/w).

The resulting gels had similar viscoelastic behavior (Figure 5 - A and B), except for pH 3.0, which showed a marked decrease in the values of the moduli. According to May (1990), if the sugar content is held constant, the effect of changes in the pH is seen as a loss in strength above a certain critical pH (May, 1990). For coffee pectin, this critical pH is > 2.87 and ≤ 3.0, since the decrease in moduli was seen for the gel prepared at pH 3. Up to pH 2.87, the values of G' were around 10 orders of magnitude greater than G'' at the frequency of 1Hz, indicating that the natural pH of the pectin is suitable to be used for gelation with no need of pH adjustments.

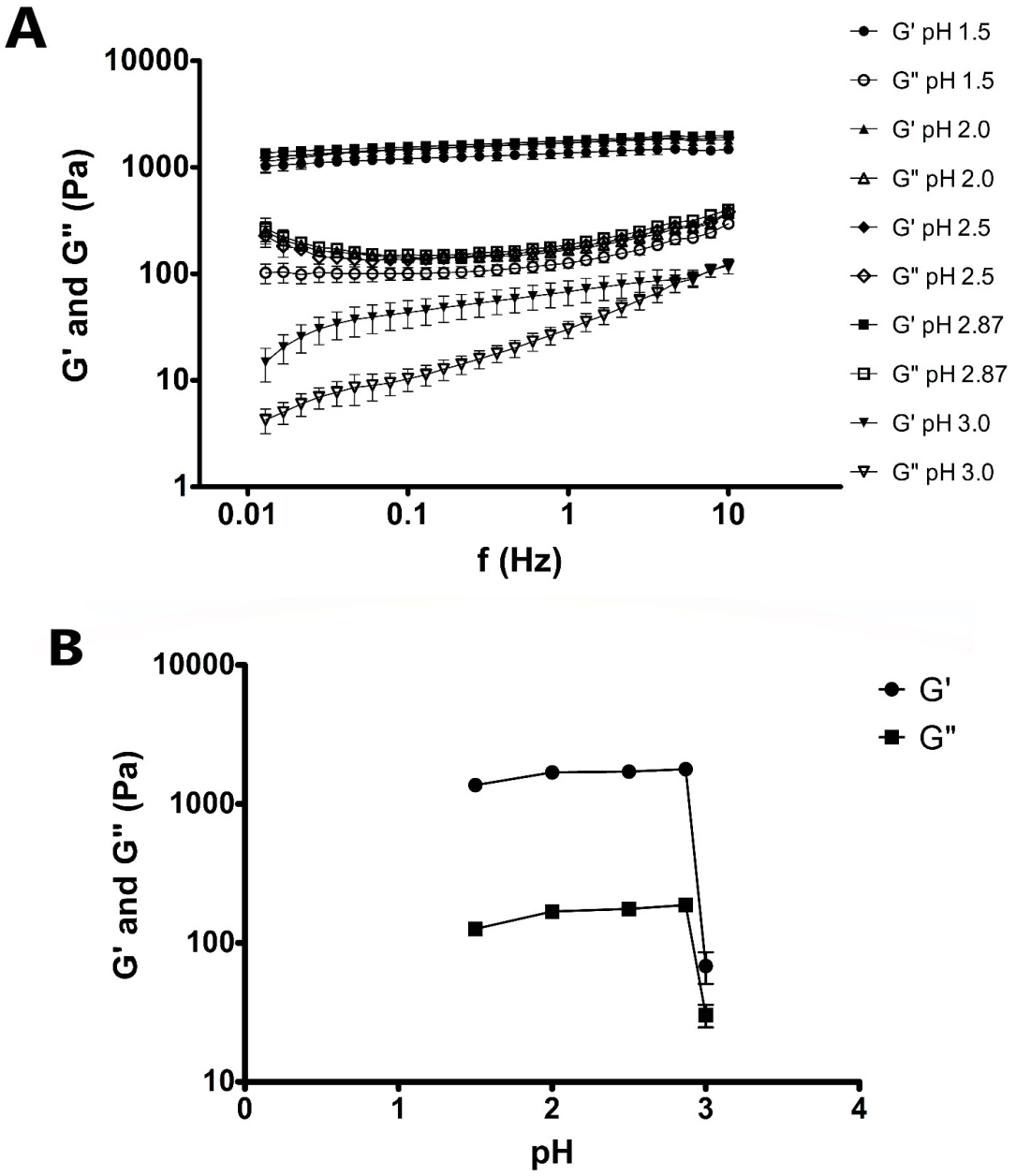
Owens & Maclay (1946) were the first to describe that the maximum pH at which pectin gels could be formed decreased with decreasing methoxyl content. They found that the maximum pH could vary from 2.9 to 3.5, depending on the DM and was not influenced by M_w or pectin concentration. As for coffee pectin, one single optimum pH was not observed in the moduli vs pH curves by these authors for lemon peel and commercial citrus pectins.

El-Nawawi & Heikel (1997) investigated the relationship between the jellying power and pH for pectins with different degrees of methyl-esterification. They found that HM pectins with lower DM produced gels with maximum strength at a narrower range of pH than those with higher DM. The low DM pectins result weaker gels in the higher pHs (2.8-3.1). For a pectin with DM 61%, close to the DM of CAP-1 (63.2%), gels prepared with 55% sucrose had the maximum strength in the pH range from 2.2 to 2.7, with little difference

among the results with at these different pHs, as found for coffee pectin. However, values of pH lower than 2.2 were not tested by the authors.

A pH of 2.5 was chosen to evaluate the effect of the cosolute concentration on the gelling properties of the pectin extracted from Brazilian *Coffea arabica* pulp.

Figure 5. Effect of pH on the viscoelastic behavior of pectin CAP-1 gels with 1.5% (w/w) pectin and 60% (w/w) sucrose (A) and the values of G' and G" at the frequency of 1 Hz as function of pH (B).



3.2.3. Influence of cosolute concentration on the gelling properties of CAP-1

Gelation using HM pectins requires a low water activity that may be achieved either by addition of soluble solids or water-miscible solvent. Almost all applications depend on sucrose as water activity-reducing substance, being the absolute lower limit around 55%, and 65% is usual fulfilled (Rolin & De Vries, 1990). Therefore, gels with concentrations of sucrose from 55 to 65% (w/w) were analyzed (Figure 6 - A). There was an increase in gel strength in higher concentrations due to the optimization of water removal from pectin, enhancing the interactions between chains and the formation of junction zones. Increased gel strength using higher sucrose contents was observed for other HM pectins, such as cupuassu pulp pectin (Vriesmann, Silveira & Petkowicz, 2010) and commercial citrus pectin (Giacomazza *et al.* 2018) and also for LM pectins from sunflowerheads (Sosulski, Lin & Humbert, 1978) and a commercial citrus pectin (Fu & Rao, 2001).

The increasing concern regarding to health problems caused by excessive sugar ingestion enhanced the demand for low-sugar products, still little used in HM pectins formulations. Therefore, gels were prepared with xylitol at concentrations 55 and 60% (w/w) and their viscoelastic properties assessed (Figure 6 - B). The mechanical spectra confirmed that CAP-1 formed gels when sucrose was replaced by xylitol. When the concentration of xylitol was increased from 55 to 60% (w/w), the raise in the values of the moduli was less pronounced than when sucrose was used.

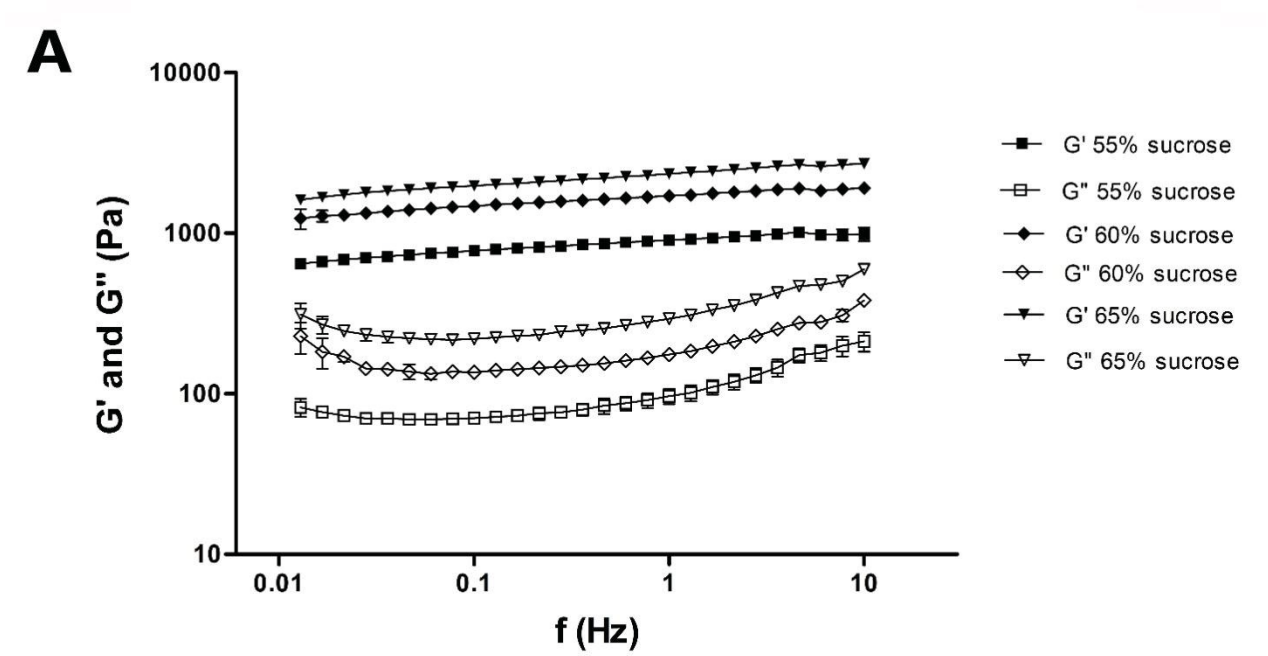
Torres, Raymundo & Sousa (2013) compared the viscoelastic behavior gels prepared with 25% sucrose or xylitol and chestnut or rice flours and found that moduli values were higher when xylitol was used instead of sucrose. For pectin gels this was not the case. However, the substitution of sucrose by xylitol produced gels with similar strength as can be seen in Figure 6 - C.

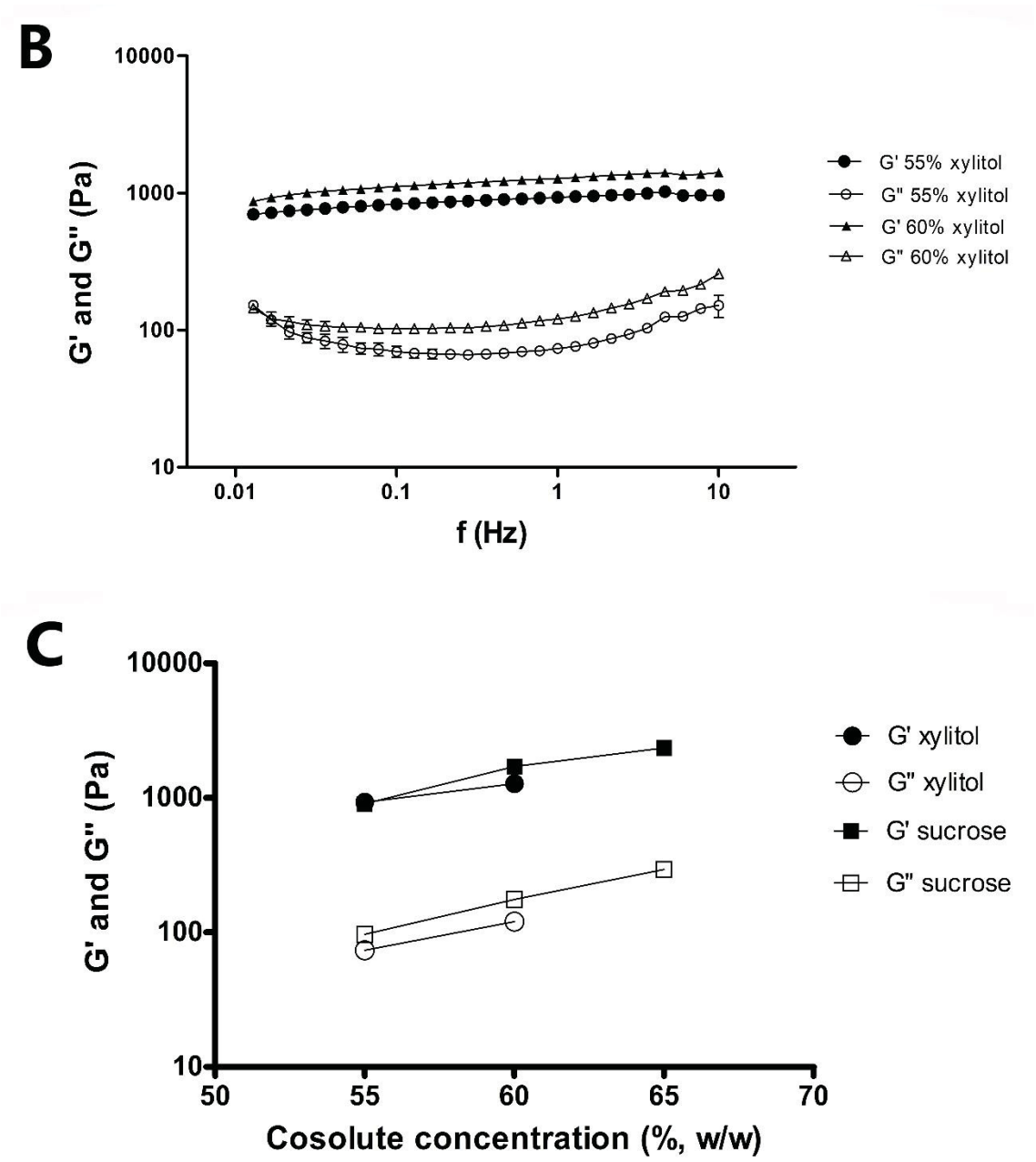
Tsoga, Richardson & Morris (2004) investigated the role of cosolutes in gelation of a commercial pectin with DM 70%. They used different polyols, including sweeteners like xylitol and sorbitol. Sorbitol has two-third calories of sucrose but only 60% of its sweetening activity (PubChem, 2019), making it not worthy as part of a low-calorie diet. On the other hand, xylitol presents the same

sweetening power and one-third caloric content of sucrose, thus it can replace sucrose with no big differences in mouthfeel (Ur-Rehman *et al.*, 2015).

According to our results, xylitol might be used as a sucrose replacer in the manufacture of low-calorie products that use CAP-1 pectin as a gelling agent, with little differences in their viscoelastic properties.

Figure 6. Effect of cosolute concentration on the viscoelastic behavior of pectin CAP-1 gels with 1.5% (w/w) pectin at pH 2.5 for 55-65% (w/w) sucrose (A) and 55-60% (w/w) xylitol (B) and values of G' and G" at the frequency of 1 Hz as function of sucrose and xylitol concentration (C).





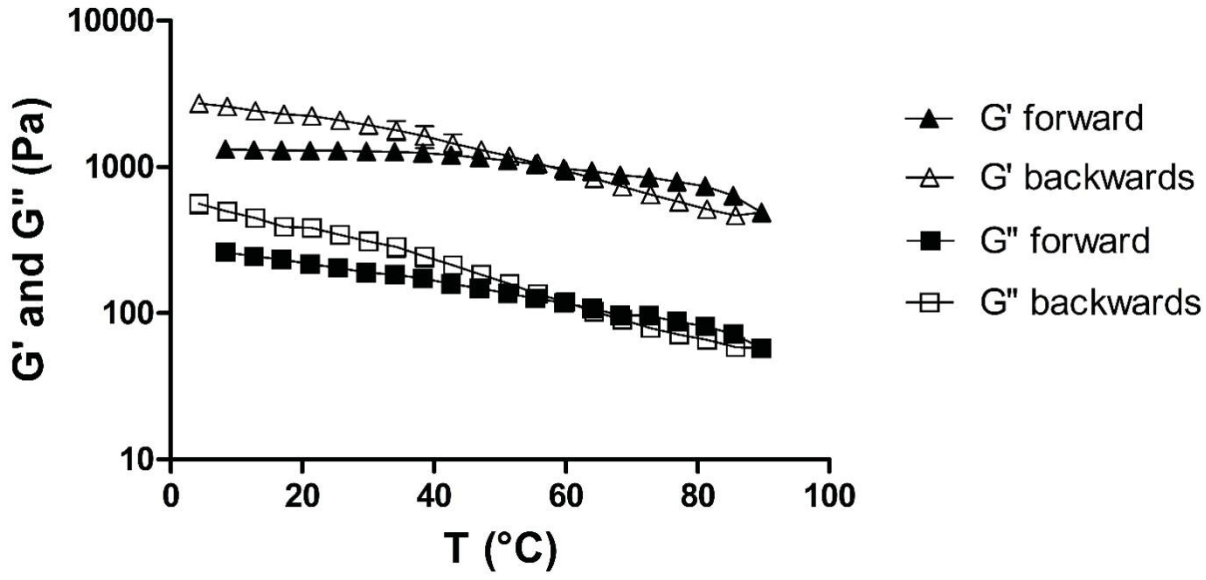
3.2.4. Effect of temperature on pectin CAP-1 gel

The processing and preservation of preparations that use pectin as a gelling agent frequently involve temperature changes, becoming important to assess how gels are affected by this factor (Thakur *et al.* 1997). The gel prepared with 2% pectin at pH 2.5 and 60% sucrose was heated from 5 to 90°C and then cooled (90-5°C). The values of the moduli decreased on heating and increased on cooling (Figure 7).

The heating of pectin-chitosan mixture demonstrated a more modest decrease in moduli on initial temperatures and more pronounced decrease in the final temperatures (Chan *et al.* 2017), similar to coffee pectin.

It is theorized that pectin gels are energetically possible to be formed by an energy contribution of hydrophobic interactions between methyl-ester groups and hydrogen bonds, being that hydrogen bonds between adjacent galacturonan chains have a larger contribution (Rolin & De Vries, 1990). High temperatures disrupt hydrogen bonds responsible to maintain the gel structure, thus weakening polymer interactions. This weakening was evident in 2% cupuassu pectin gels with 60% sucrose, where the value of G' was reduced when heated (5-95°C) and increased when cooled (95-5°C) (Vriesmann, Silveira & Petkowicz, 2010). The sweep shows that G' was higher than G" over the analyzed temperatures, showing that coffee pectin gels have good thermal stability. Even though the moduli decreased with the increase of temperature, their distance was considerably kept, with loss tangents (tan δ) on the initial heating of 0.20, on the maximum temperature of 0.12 and in the final cooling of 0.21. The values of tan δ during the sweep indicate that G" is more dependent on temperature than G'.

Figure 7. Effect of temperature on the viscoelastic behavior of pectin CAP-1 gel with 2.0% (w/w) pectin and 60% (w/w) sucrose at pH 2.5 over heating (4-90°C) and cooling (90-4°C) at 1°C/min.

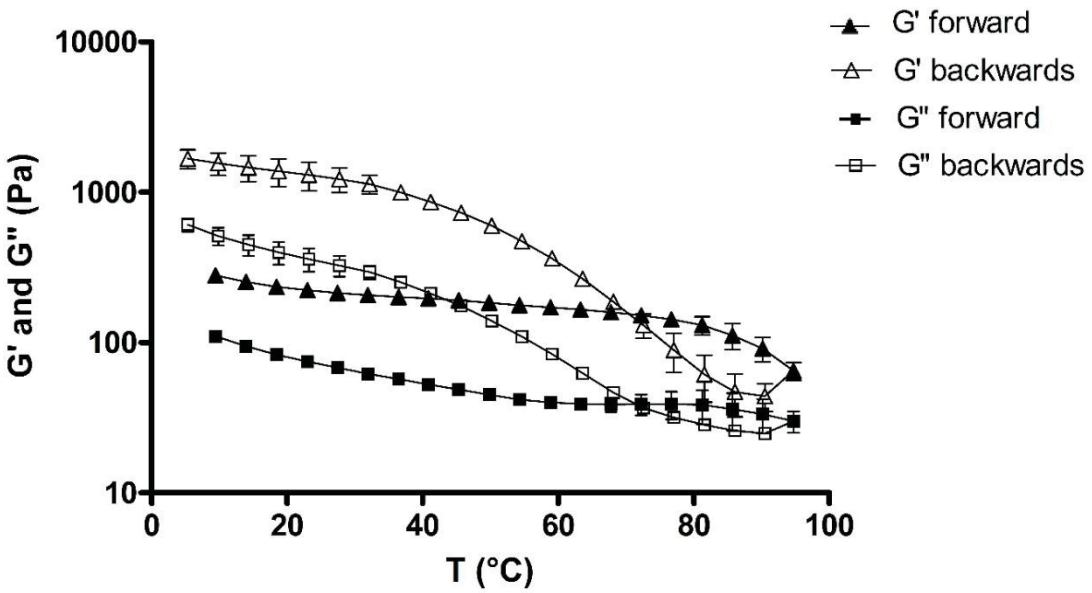


3.2.5. Effect of temperature on the gel formation by CAP-1

Structure formation of pectin gels occurs in a given temperature, the gelling temperature. At this point, chains begin to associate and arrange as a network, which extends throughout the liquid and entraps water. Common convention is to determine the setting temperature when G' overtakes G" (CpKelco, 2005). The definition of the gelling or setting temperature is the temperature where gelation occurs with infinitely slow cooling. This is experimentally assessable after determination of the setting temperature of a gel in different cooling rates and extrapolation of the setting temperature when the cooling rate is zero (Hinton, 1950).

In order to visualize the effect of temperature in the gel formation of coffee pectin, a temperature ramp from 5 to 95°C and 95 to 5°C was performed with a 60% sucrose and 1.5% pectin solution in its natural pH (Figure 8). After heating followed by cooling, the values of the moduli significantly increased after ~60°C. However, G' was already higher than G" in the solution previously to heating, making impracticable to assess the gelling temperature in terms of moduli cross-over. The pectin in the presence of high sucrose concentration was more elastic than viscous, being a pre-gelled system. Despite the difficulties in determining a gelling temperature, it is possible to observe that at the temperature of ~72°C a more solid structure than the pre-gelled mixture was achieved. Since this is the point where the backwards moduli cross the forward moduli, it can be inferred that in this temperature new interactions are being formed besides the already existing ones, that will result in the final network.

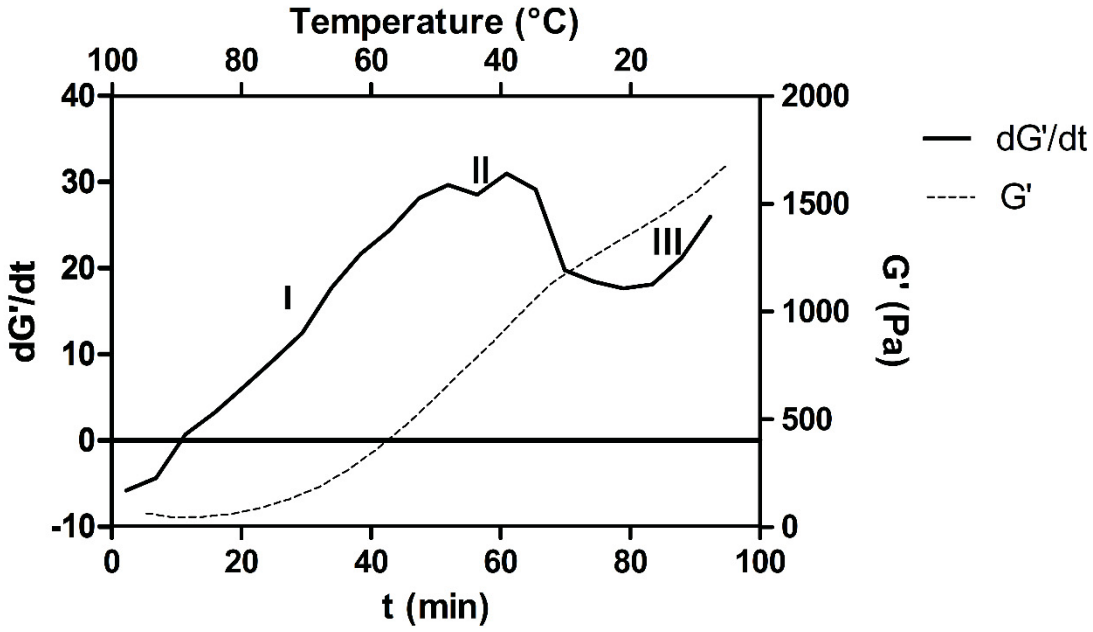
Figure 8. Effect of temperature on the gel formation of a mixture containing 1.5% pectin and 60% (w/w) sucrose at pH 2.87 over the temperatures of 5 to 95°C and 95 to 5°C at 1°C/min.



The study of Kastner *et al.* (2014) already pointed the difficulties in identifying the point of gel formation based only in moduli cross-over for HM pectins. Fu & Rao (2001) demonstrated the correlation between the beginning of formation of junction zones and the structure development rate during cooling (SDR) defined as dG'/dt. Kastner, Einhorn-Stoll & Senge (2012) presented new concepts, based on these previous definitions, which allows the determination of the start of structure formation and detect the phases of gel structuring, also in pre-gelled preparations with no clear gelling point, as for coffee pectin gel. The plot of the first derivation dG'/dt as a function of time and temperature of cooling is given in Figure 9. It was possible to see that the formation of interactions started very early in the sweep with noticeable phases. Kastner, Einhorn-Stoll & Senge (2012) separated the phases of gel structuring as: (I) a first phase at higher temperatures where the hydrophobic interactions are formed, completed at about 40°C; (II) a second phase dominated by hydrogen bonds formation, starting below 50°C; (III) a third phase of dimer aggregations, below 20°C. These ranges of temperature were similar to the ones obtained for coffee pectin, being evident that hydrophobic interactions occurred in a slower and continuous rate. Hydrogen bond contribution was faster and achieved its

maximum rate at 40°C. Dimer interactions and inter-dimer aggregations also occurred below 20°C, increasing its rate as the temperature decreases.

Figure 9. First derivation dG'/dt as a function of time and temperature of cooling during gel structuring of the mixture containing 1.5% pectin and 60% (w/w) sucrose at pH 2.87 over the temperatures of 95 to 5°C at 1°C/min. The phases were represented by hydrophobic interactions (I), hydrogen bonds (II) and dimer aggregation (III).



Conclusions

The pectin from the pulp of Brazilian *Coffeea arabica* had high apparent viscosity in water and salt solutions. At pH < pK_a, the pectin produced more viscous solutions in the presence of a high concentration of NaCl, independent on the pectin concentration. On the other hand, at pH > pK_a, the pectin exhibited opposite behaviour. Solutions prepared with 5% pectin at a pH of ~4.5 in water and 0.1 M NaCl formed gels, possibly through the formation of junction zones with calcium. The pectin formed stronger gels by the classic mechanism of gelation of HM pectin using sucrose as cosolute. To the best of our knowledge, for the first time, a pectin extracted from coffee pulp was able to form gel in the presence of a high concentration of soluble solids and acidic pH and xylitol showed to be a promising substituent of sucrose in the gelation

process. Temperature, pH and pectin and cosolute concentrations were important parameters that modified the viscoelastic properties of the gels. The structure formation of gels suggested typical hydrophobic interactions, hydrogen bonds and dimer interactions until the complete network development. Overall, Brazilian coffee pectin was a suitable ingredient for industrial purposes, making coffee pulp a potential source for pectin extraction.

Acknowledgements

The authors are grateful to Electron Microscopy Center of UFPR for SEM-EDS analysis and to the Brazilian agencies Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior - Brasil (CAPES)-Finance Code 001 and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) for the financial support. C.L.O.P. is a research member of the CNPq (309159/2018-0).

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CAPÍTULO 3

Investigation of the potential of soy hull as a source of commercial pectin

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Abstract

Soy hull was ground and extracted using 0.1 M HCl at 90°C for 45 min, giving rise to fraction A, which presented relatively low content of uronic acid (UA) (32.1%). Two other extractions using boiling 0.14 M HNO_3 for 30 and 60 min were performed in an attempt of increasing the UA content, resulting respectively, in fractions B and C, which reached 37.2% and 41.5% UA. SEM-EDS analysis indicated high amounts of calcium in the extracted pectin that might contribute for the difficulty of pectin extraction. Monosaccharide composition of the initial material and the final residue after sequential extractions with water at 40°C for 16 h and boiling 0.14 M HNO_3 for 1 h showed that soy hull UA content was of 6.2% and 35% of UA remained in the residue. Three other soy hull samples from different industries were used to perform pectin extractions in the same conditions, resulting in fractions with similar monosaccharide compositions. Fraction A was rich in mannose () and galactose, suggesting that galactomannans were being coextracted with the pectins, which was confirmed by HSQC-NMR. Fractions B and C contained less mannose and more UA, rhamnose and xylose, indicating that soy hull pectin is rich in RG-I and xylans, previously reported to be present in soy hulls, were coextracted under the harsher acid extractions. HPSEC analyses showed three modal elution profiles, attributed to the presence of xylan, pectin and galactomannan. A hot water extraction was performed previously to the acid extractions resulting in partial removal of the galactomannan and increasing the UA content in the acid extractions. The fraction that presented the highest UA

content (52.7%) was analyzed by FT-IR and a DM of 29.3% was found. However, soy hull pectin did not reach the requirements to be used as food additive (GalA>65%) and seem to be not a suitable material for commercial extraction of pectin.

Keywords: Agroindustrial waste, Chemical characterization, Soy hull, Galactomannan, Pectin, Xylan.

1. Introduction

Soybean (*Glycine max* (L.) Merril is one of the most cultivated and consumed commodities worldwide with a production surpassing 360 million tons in the 2018/19 crop (USDA, 2019). Soybean oil and meal production generates large amounts of soy hulls as a byproduct, which account for about 8% of the whole seed (Gnanasambandam & Proctor, 1999). The hull is removed from the beans through the cracking of the grain and can either be pelleted or sold as bulk (Blasi *et al.* 2000). Part of the hulls is used in animal feeding due to its high amounts of fiber, reported to be of 42-47% (Kornegay, 1978; Zambom *et al.* 2001). It can also be added to soybean meal in order to reduce its protein content (Shriver *et al.* 2003). However, as a low-price and underused by-product, soy hulls have a high potential for utilization to obtain value added products.

Previous studies have investigated the usage of soy hulls for the production of lipids by solid-state fermentation (Zhang & Hu, 2012), enzymes (Coffman, Li & Ju, 2014; Paradkar & Dordick, 1993), ethanol (Schirmer-Michel *et al.*, 2008; Mielenz, Bardsley & Wyman, 2009) and pectin (Gnanasambandam & Proctor, 1999; Monsoor & Proctor, 2001; Kalapathy & Proctor, 2001). Particularly for pectin, soy hull has been pointed by as one of the most promising alternative material for its extraction (De Pretto *et al.,* 2018; Monsoor & Proctor, 2001).

Pectins are additives used in the food industry, mainly as a gelling agent in the production of jams, jellies, fruit juices, confectionary products and bakery fillings (Willats, Knox & Mikkelsen, 2006). Other uses are as a texturizer, emulsifier, thickener and stabilizer (Hoefler, 1991).

Pectins a complex class of cell wall polysaccharides which contain high amounts of galacturonic acid (GalA) (Schols & Voragen, 1996). The two most abundant ones are homogalacturonan (HG) and rhamnogalacturonan I (RG-I) (Ridley, O'Neill & Mohnen, 2001). HG is composed of linear chain of α-1,4-linked GalA, with partial methyl-esterification at the C-6 carboxyl and possible *O*-acetylation at *O*-2 or *O*-3 (Mohnen, 2008). RG-I is composed of a backbone of repeating units of $[-\alpha\text{-}D\text{-GalA-1,2-}\alpha\text{-L-Rha-1,4-}]_n$ with partial substitution at *O*-4 rhamnosyl with arabinans, galactans and arabinogalactans (Vincken *et al.* 2003).

Typical process for pectin production starts with an extraction of fresh or dried raw material using water acidified by mineral acid at a pH near 2 at a temperature of 70°C for 3 h. Filtration, precipitation, drying, blending and standardization complete the steps for pectin production (Rolin & De Vries, 1990).

The main raw material for pectin extraction is citrus peel which is a by-product of juice production. Citrus peel naturally contains significant amounts of pectin methylesterase, causing it to present a loss in the quality of the final product if the peels are not dried before transportation and extraction (May, 1990). Citrus peel must be dried from the start level of about 82% moisture, down to 10-12%, which increases the costs of pectin production (Ciriminna *et al.*, 2015). Since soy hulls have low moisture levels, this can be seen as an advantage in respect to the other alternative raw materials for pectin extraction.

Former studies related with polysaccharides from soy hulls have shown that galactomannans (Whistler & Saarnio, 1957; Aspinall & Whyte, 1964) and xylans (Aspinall, Hunt & Morrison, 1966) could be obtained by aqueous extractions. Later, Gnanasambandam & Proctor (1999) proposed the use soy hulls for pectin extraction. They reported the isolation of a pectin with GalA content of 76.7% and yield of 15% using 0.1 M HNO_3 at 90°C for 40 min. When the raw material was treated with mannanase prior to the extraction, the GalA increased to 83.3% due to the degradation of the soluble galactomannan. Further studies found similar characteristics for soy hull pectin extracted with diluted acid, with the highest yield of ~28% obtained by Kalapathy & Proctor (2001). This fraction had 72% GalA and was extracted using 0.1 M HCl at 90°C for 45 min and a pre-adjusted pH of 3.5 before precipitation. However, the

pectins from soy hull were not fully characterized and the gelling properties were not investigated. Thus, the aim of the present study was to isolate pectins from soy hull by acid extractions in order to investigate if soy hull indeed has the potential to be used as a source of commercial pectin.

2. Material and methods

2.1. Material preparation

Soy (*Glycine max* (L.) Merrill) hulls were obtained from industries of soybean processing located in Paraná, Brazil. Non-pelleted hulls were provided by the companies Copacol, Coopavel and Cargill and were named as sample number 1, 2 and 3, respectively. Cargill also provided pelleted hulls, named as sample number 4. The hulls were ground in a Wiley mill (Arthur H. Thomas Company, USA) through a 0.5 mm sieve and then milled in an analytical mill IKA-A11 (IKAWerke GmbH & Co. KG, Germany) and stored at -20°C for further extraction.

2.2. Extraction of soy hull galactomannan

Sample 1 was extracted with deionized water at 40°C for 16 h under magnetic stirring in order to extract galactomannans.

2.3. Pectin extraction

Pectin extraction from the ground hulls and from the solid residue after the galactomannan removal were performed with 0.1 M HCl at 90°C for 45 min and with boiling 0.14 M HNO_3 for 30 and 60 min, using a solid:liquid ratio of 1:20 (w/v). The extractions resulted, respectively, in the pectin fractions named as A, B and C if the ground soy hull was used or AR, BR and CR, if the solid residue was used for the extraction. The extracts were filtered through a synthetic fabric and centrifuged at 5000 rpm for 15 min. The pH of the supernatants was adjusted to 3.5, precipitated with 2 volumes of absolute ethanol and stored at 4°C for 16 h. The precipitates were filtered, washed 3 times with absolute ethanol and dried under vacuum. The dried polysaccharides were weighted and the yield was calculated in relation to the soy hull mass used for extraction. The extractions were performed in triplicate.

2.4. Monosaccharide composition

Soy hull sample 1 and the final residue remaining after a sequential extraction in water at 40°C for 16 h, HCl at 90°C for 45 min and HNO_3 for 1 h, were hydrolyzed by Saeman hydrolysis (Saeman, Moore, Mitchell, & Millet, 1954). The hydrolysis of polysaccharides was carried out with 2 M TFA at 120°C for 2 h in autoclave.

For neutral sugars analyses, the monosaccharides resulting from hydrolysis were reduced with sodium borohydride for 16 h at 4°C (Wolfrom & Thompson, 1963b) and then acetylated with pyridine/acetic anhydride for 16 h at room temperature. The alditol acetates were extracted with chloroform (Wolfrom & Thompson, 1963a) and analyzed by gas chromatography (GC) using a Thermo Scientific Trace GC Ultra and a DB-225 column (internal diameter 0.32 mm x length 30 m x film thickness 0.25 µm), programmed from 100°C to 230°C at a heating rate of 60°C/min, with a mixture of helium, nitrogen and compressed air as carrier gas at 1 mL/min. Uronic acid content (UA) was determined by m-hydroxybiphenyl colorimetric method, as described by Blumenkrantz and Asboe-Hansen (1973), using galacturonic acid as standard. The derivatization and colorimetric method were carried out in triplicate.

2.5. Degree of methyl-esterification (DM)

DM of pectin was estimated by Fourier transform mid-infrared (FT-IR) spectroscopy by the use of a Vertex 70 spectrophotometer (Bruker, Germany), in the range of 400 to 4000 cm^{-1} at 4 cm^{-1} resolution. Pectin and KBr, in a proportion of 1:99, were grinded, dried for 16 h under vacuum and transformed in discs. Its spectra provided peaks corresponding to the methyl-esterified and the free carboxyl group at 1749 cm^{-1} and 1630 cm^{-1}, respectively (Vriesmann & Petkowicz, 2009). The ratio of methyl-esterified peak area and the sum of areas from both peaks provided the DM of the sample. Experiments were performed in triplicate.

2.6. Scanning Electron Microscopy and Energy Dispersive Spectroscopy (SEM-EDS)

SEM-EDS was used to obtain the mineral elements composition of pectin fractions. The samples were lyophilized and placed in a carbon tape mounted on a SEM stub and analyzed in a VEGA3 LMU microscope (Tescan, Czech Republic) using a 15 kV accelerating voltage for 60 seconds to obtain each spectrum.

2.7. High performance size exclusion chromatography (HPSEC)

Pectins were analyzed by high performance size exclusion chromatography (HPSEC) coupled to a refractive index (RI) (Waters Corporation, USA) and a Dawn-F multi-angle laser light scattering (MALLS) (Wyatt Technology, USA) detectors. Four Ultrahydrogel columns (Waters Corporation, USA) were connected in series (2000; 500; 250; 120) and coupled to the equipment. The eluent was 0.1 M $NaNO_2$ and 0.02% of NaN_3 at a flow rate of 0.6 mL/min. Samples were filtered through a 0.22 µm cellulose acetate membrane before injection. Data were analyzed using ASTRA software (Wyatt Technology, USA).

2.8. Nuclear magnetic resonance spectroscopy (NMR)

Pectin was solubilized in D_2O in a concentration of 40 mg/mL and the spectra of heteronuclear single quantum coherence (HSQC) NMR was obtained at 70°C, using a Bruker DRX 400 Avance spectrometer (Bruker, Germany). Acetone was used as internal standard (δ=30.2 for ^{13}C and δ=2.22 for ^{1}H). Data were analyzed by TopSpin software, version 3.5 (Bruker, Germany).

2.9. Statistical analyses

The results were expressed in terms of ± SD (standard deviation). Significance was determined by one-way analysis of variance (one-way ANOVA) followed by Tukey's multiple range test using GraphPad Prism version

5.00 (GraphPad Software Inc., USA) software. *P* values < 0.05 were considered to be significant.

3. Results and Discussion

3.1. Extraction and characterization of pectin from soy hull

Initially, soy hull sample 1 was ground and extracted following the protocol used by Kalapathy & Proctor (2001) in order to obtain pectin for structural and rheological characterization. Thus, sample 1 was extracted with 0.1 M HCl at 90°C for 45 min and resulted in fraction A. The extraction yield was 10.1% and the uronic acid (UA) content was 32.1% (Table 1). The values were lower than the yield of 28% and UA content of 72% reported by Kalapathy & Proctor (2001) using the same conditions for extraction and the same method for UA detection. Harsher extraction conditions were tested in order to improve the pectin yield and UA content. Extractions using boiling 0.14 M HNO_3 for 30 and 60 min were performed, resulting in fractions B and C, respectively. Although, a slighltly higher yield was observed for fraction C, statistical analysis showed no differences in the yields of the three fractions. Concerning the uronic acid content, fractions B and C showed higher UA than fraction A, with $p < 0.01$ and $p < 0.001$, respectively. These results were also different from that described by Kalapathy & Proctor (2001) who reported that increasing the temperature to 100°C with longer times of extraction, did not affect the pectin yield.

According to the results, increasing acid strength, temperature and time of extraction improved pectin extraction, resulting in higher yields and UA. However, the UA content was still below the commercial requirements (>65%) (Willats, Knox & Mikkelsen, 2006).

Mannose was the main neutral monosaccharide of fraction A, followed by galactose. Mannose content decreased as the extraction conditions got harsher, while uronic acid, rhamnose and xylose increased. Fraction C showed to have a predominance of pectic monosaccharides, with a noteworthy amount of rhamnose. The high amount of mannose and galactose in fraction A pointed to the presence of galactomannans, previously reported to be extractable with water from soy hulls (Whistler & Saarnio, 1957; Aspinall & Whyte, 1964). In

more severe conditions, mannose content substantially decreased and xylose increased, suggesting that there was degradation of the galactomannan in these conditions and increased extraction of xylans, previously reported to be extractable with aqueous KOH solution (Aspinall, Hunt & Morrison, 1966).

Table 1. Yield and composition of the pectins extracted from soy hull sample 1

Fraction[a]	**A**	**B**	**C**
Yield (%)[b]	10.1±0.3	10.7±0.1	11.4±0.5*
Monosaccharide[c] (relative %)			
Rha	8.7±0.5	17.3±0.2*	22.2±0.3*
Fuc	0.7±0.1	0.2±0.1*	ND*
Ara	1.8±0.5	1.3±0.2	2.4±0.6
Xyl	3.7±0.2	6.3±0.1*	7.3±0.1*
Man	31.2±0.7	16.6±0.2*	8.7±0.5*
Gal	18.2±0.6	18.7±0.1	16.2±1.0
Glc	3.6±1.0	2.4±0.6	1.7±0.1
UA	32.1±1.0	37.2±1.2*	41.5±0.7*
Mineral elements[d] (w/w %)			
Na	0.2	0.6	0.6
Mg	0.1	0.2	0.2
Al	0.2	0.1	0.1
P	0.1	0.2	0.2
S	0.2	-	-
Cl	0.6	-	-
K	0.2	0.5	0.4
Ca	1.1	1.7	2.0

[a] Fraction A: extracted with 0.1 M HCl at 90°C for 45 min; Fraction B: extracted with boiling 0.14 M HNO_3 for 30 min; Fraction C: extracted with boiling 0.14 M HNO_3 for 60 min.

[b] Yield = (polysaccharide mass/initial soy hull mass)*100.

[c] Neutral monosaccharide determined by GC of alditol acetates; UA determined by colorimetric method.

[d] Mineral elements obtained by SEM-EDS.

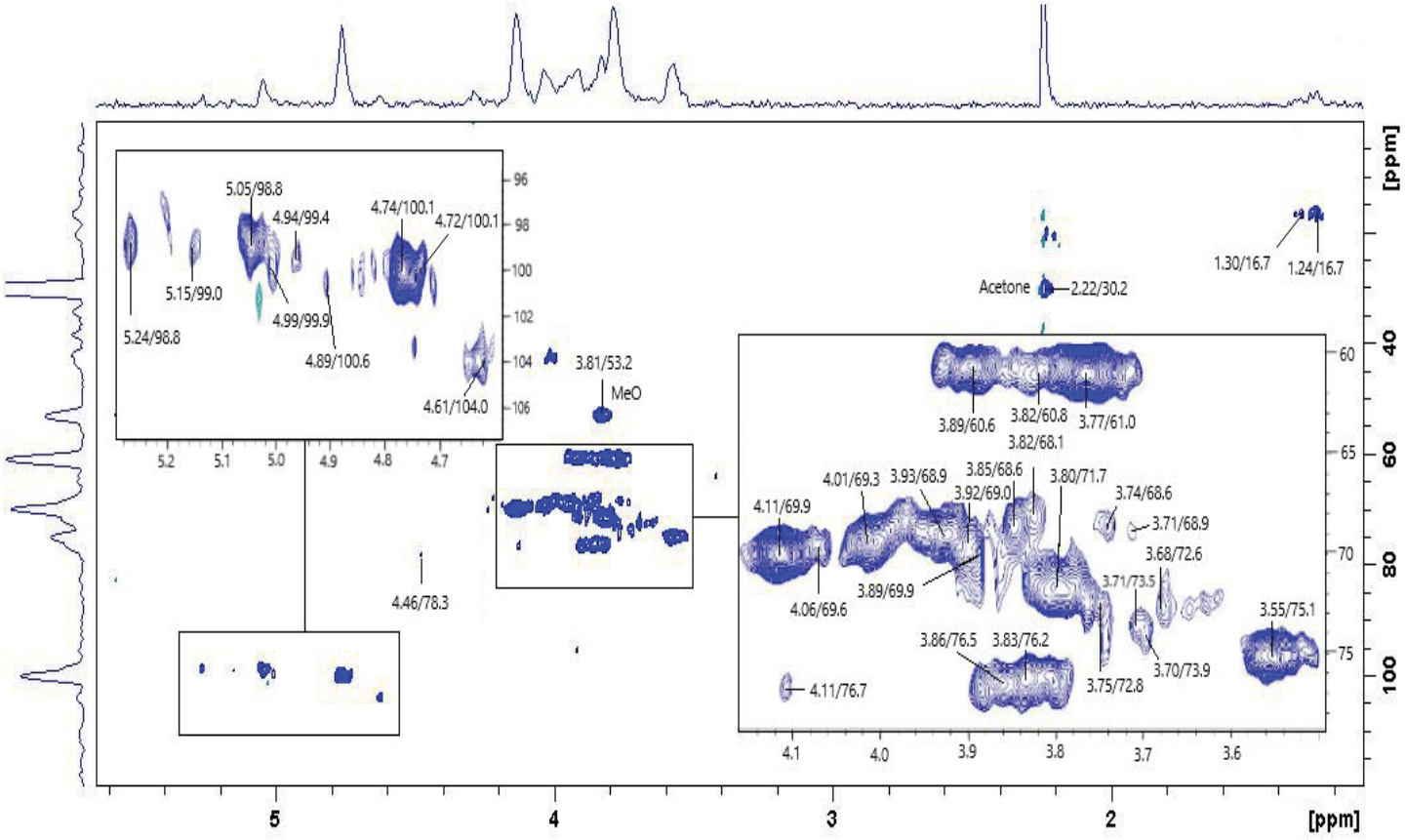
[e] ND = Not detected.

* Statistically different from fraction A.

The most intense signal in the HSQC NMR spectrum of fraction A (Figure 1) were assigned to galactomannans. Chemical shifts (δ) corresponding to H1/C1, H4/C4, H5/C5 and H6/C6 of →4)β-D-Man*p*(1→ were respectively found at δ 4.72/100.1, 3.83/76.2, 3.55/75.1 and 3.89/60.6 and those of →4,6)β-D-Man*p*(1→ at 4.74/100.1, 3.86/76.5, 3.75/72.8 and 3.93/68.9. H2/C2 and H3/C3 of both branched and unbranched mannoses were assigned at δ 4.11/69.9 and 3.80/71.7. H1/C1, H2/C2, H3/C3, H4/C4, H5/C5 and H6/C6 of α-D-Gal*p*(1→ appeared at δ 4.99/99.9, 3.82/68.1, 3.92/69.0, 4.01/69.3, 3.89/69.9, 3.77/61.0, respectively (Albuquerque *et al.,* 2014; Cunha *et al.,* 2009). Signals from HG having esterified (E) and unesterified (U) galacturonic acid were also found, with the methoxyl group of E appearing at δ 3.81/53.2. H1/C1 signals of →4)α-D-6MeGalA*p*(1→ were found at δ 4.94/99.4 when linked to another esterified unit (EE) and at δ 4.89/100.6 when linked to a unesterified unit (EU). Chemical shifts of H2/C2 and H4/C4 of →4)α-D-6MeGalA*p*(1→ were found at δ 3.71/68.9 and 4.46/78.3, respectively. H1/C1 signals of →4)α-D-GalA*p*(1→ appeared at δ 5.05/98.8 for UE and δ 5.15/99.0 for UU. The chemical shifts for H2/C2 and H3/C3 of unesterified GalA were assigned at δ 3.74/68.6 and 4.06/69.6. The detection of rhamnosyl residues and galactans evidenced the presence of rhamnogalacturonan I. Signals from H1/C1 and H3/C3 of both rhamnosyl units were detected at δ 5.24/98.8 and 3.85/68.6. Chemical shifts of H2/C2 and H6/C6 of →2)α-L-Rha*p*(1→ were respectively found at δ 4.11/76.7 and 1.24/16.7 and of →2,4)α-L-Rha*p*(1→ at δ 4.09/76.8 (not shown in the spectrum) and 1.30/16.7. β-1,4-linked galactans signals corresponding to H1/C1, H2/C2, H3/C3, H5/C5 and H6/C6 were found at δ 4.61/104.0, 3.68/72.6, 3.71/73.5, 3.70/73.9 and 3.82/60.8 (Colodel, Vriesmann & Petkowicz, 2018; Ovodova *et al.* 2005; Golovchenko *et al.* 2007).

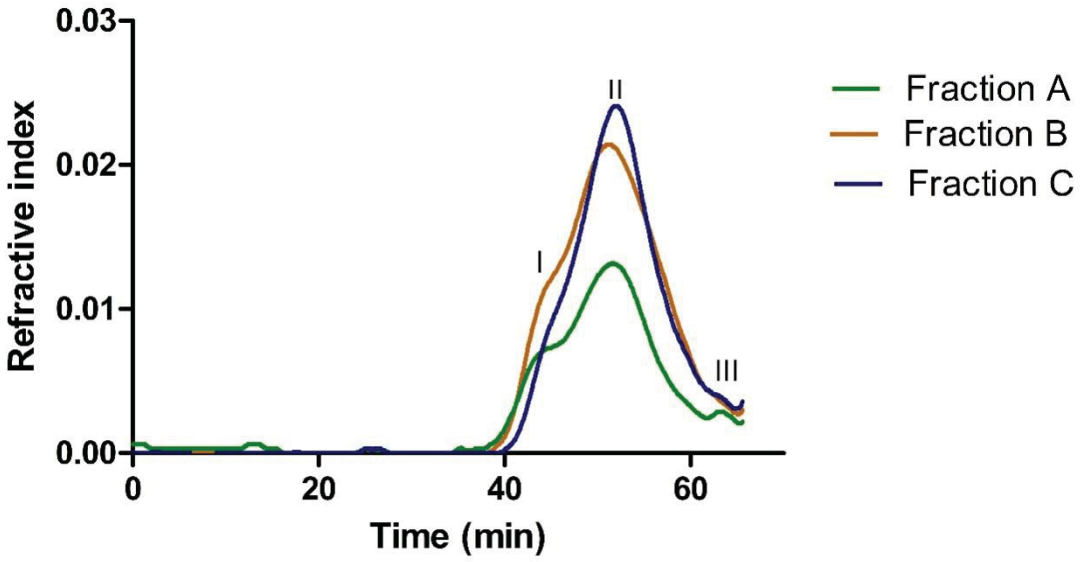
The results indicated the presence of galactomannans, corroborating the high content of mannose and galactose found in the monosaccharide composition of fraction A. HG and RG-I mainly branched by β-1,4-galactans side chains were also found.

Figure 1. ^{1}H-^{13}C HSQC NMR spectrum of extraction A in D_2O at 70°C using acetone as internal standard.



The chromatogram of fraction A, obtained by high performance size exclusion chromatography (HPSEC) using refractive index (RI) detector, showed three main peaks (I, II and III) (Figure 2). Since peak II showed to be more prominent in fractions B and C, which were richer in pectins than fraction A, this peak seems to be related to the pectins. In fraction C, peak I was less defined and it was shifted to a higher elution time, suggesting lower molar mass.

Figure 2. HPSEC elution profiles of fractions A, B and C.



Fraction A: extracted with 0.1 M HCl at 90°C for 45 min; Fraction B: extracted with boiling 0.14 M HNO_3 for 30 min; Fraction C: extracted with boiling 0.14 M HNO_3 for 60 min.

Soy hull pectins showed a diversity of mineral elements (Table 1), with higher amounts of calcium, known for inducing interchain association and formation of junction zones (Walkinshaw & Arnott, 1981) and it was also reported to be involved pectin-protein interaction in middle lamella (Yamauchi, Hara & Sonada, 1986). Chlorine was found in fraction A, possibly as a residue from the acid extraction with HCl.

Calcium levels were in the range of 1.1 to 2.0% and were higher in the fractions with higher UA content. Commercial pectins from lemon had calcium contents of 0.36-0.74% and from apple of 0.31% (Kravtchenko, Voragen & Pilnik, 1991). The higher amount of calcium found for pectins from soy hull suggests that they may be more attached to the primary wall being more difficult to be extracted. Monosaccharide analysis of the soy hull before and after sequential extractions with water at 40°C for 16 h, 0.1 M HCl at 90°C for 45 min and 0.1 M HNO_3 for 60 min were performed in order to evaluate the efficiency of the pectin extraction.

3.2. Monosaccharide composition of soy hull

The relative monosaccharide composition of soy hull before and after acid extractions is shown in Table 2. Initial amount of UA (6.2%) was lower than the contents reported for apple (10-15%), citrus peel (25-35%) and sugar beet (10-20%), the most common sources for industrial pectin production (Ciriminna *et al.*, 2015). However, it was comparable to the UA content of coffee mesocarp (7.9%), described by Reichembach & Petkowicz (not published).

The final residue after the sequential extractions was analyzed and it was observed that the extractions did not remove the total pectin. The remaining rhamnose and UA indicates that 35-37% of pectin remained in the residual material, which could be related with the high levels of calcium found in the present study.

Table 2. Monosaccharide composition of soy hull and the final residue after sequential extractions with water at 40°C for 16 h, 0.1 M HCl at 90°C for 45 min and boiling 0.1 M HNO_3 for 60 min.

Monosaccharide (%)	**Soy hull**	**Final residue**
Rha[a]	1.4±0.1	0.5±0.3
Fuc[a]	0.9±0.1	ND[c]
Ara[a]	21.4±1.0	1.7±0.4
Xyl[a]	30.0±0.3	71.1±2.4
Man[a]	14.6±0.9	8.8±0.3
Gal[a]	7.3±0.1	1.9±0.7
Glc[a]	18.2±0.3	13.7±1.3
UA[b]	6.2±0.3	2.3±0.2

[a] Neutral monosaccharide determined by GC of alditol acetates.
[b] Uronic acid determined by colorimetric method.
[c] ND = Not detected.

Xylose was the major sugar of soy hull, showing that hemicelluloses are the main components of the raw material. The final residue was mainly

composed of xylose and mannose from hemicelluloses and glucose from cellulose.

Fucose, common in lignocellulosic material (Carpita & Gibeaut, 1993), had the lowest content in soy hull. However, it had higher levels than other raw material used for pectin extraction, such as the alcohol insoluble residue from pulp (0.1%) and skin (0.2%) of cubiu (Colodel *et al.* 2017), grape peel (0.07%), apple skin (0.03%) (Arnous & Meyer, 2009) and citrus peel (traces) (Chau & Huang, 2003). Polysaccharides containing Fuc were fully extracted from soy hulls as it was not found in the final residue. The results are in agreement with the study of Aspinall, Hunt & Morrison (1967) who characterized fractions from aqueous extractions of soy hull and found the presence of the oligosaccharide 2-*O*-α-L-fucopyranosyl-D-xylose after partial hydrolysis.

3.3. Acid extraction of pectins from soy hull provided by different companies

Considering the remarkable differences between our results and those reported by Kalapathy & Proctor (2001), soy hull provided by different companies were used in order to check if the sample used in the initial investigation was responsible for the differences. Three other samples of soy hull (2, 3, and 4) from different industries were used to extract pectin using the same conditions described before (extractions of fractions A, B and C). The resulting yields are given in Table 3 and compared with sample 1. The yields of fraction A extracted from the hulls 2, 3 and 4 were slightly higher than that from sample 1 (confirmed by statistical analysis; $p < 0.01$ for samples 2 and 3 and $p < 0.05$ for sample 4). However, they were not significantly different from each other. Fractions B and C have not presented statistically significant differences in yields among the soy hull samples.

The yields were lower than those reported by Kalapathy & Proctor (2001) (18-28%), but they were in the range of yields reported by Monsoor & Proctor (2001) (8-16%) for pectins extracted from soy hull using 0.05 M HCl under different conditions.

The UA contents of pectins extracted from the different samples of soy hull are also presented in Table 3. For soy hull sample 2, extractions A and B

resulted in lower UA contents (21.5% and 31.1%) than those obtained with sample 1, while for fraction C no significant difference was found.

Only minor diferences were observed in the UA contents of pectins extracted from the different samples of soy hull, confirming the initial results. Differently from a previous report by Kalapathy & Proctor (2001), the extraction of soy hull using 0.1 M HCl at 90°C for 45 min and solid:liquid ratio 1:20 (extraction A) did not provide food grade pectins (UA > 65%), whose study indicated an UA content of 72%. Other reports described 77% UA for pectins from soy hull extracted with 0.1 M HNO_3 at 90°C for 40 min (Gnanasambandam & Proctor, 1999) and 69% using 0.05 M HCl at 90°C for 60 min (Monsoor & Proctor, 2001).

In contrast to these studies, Porfiri & Wagner (2018) used the same conditions described by Kalapathy & Proctor (2001), except by the solid:liquid ratio that was 1:15, and found a GalA content of 23.4%, in the range of those obtained in the present study. The monosaccharide composition determined by the authors also revealed high contents of mannose (23.4%), galactose (23.1%), rhamnose (11.4%) and xylose (7.8%).

Fractions C, obtained using boiling 0.14 M HNO_3 for 60 min, had the highest UA contents, in the range of 38 to 42%, which are lower than previous studies described for pectins from soy hulls (Gnanasambandam & Proctor, 1999; Monsoor & Proctor, 2001; Kalapathy & Proctor, 2001). Fractions with the highest UA also had remarkable high amounts of rhamnose, indicating that soy hull pectins had a significant content of RG-I. The presence of unexpected high amounts of RG-I in the pectins from soy hull contribute to the lower amount of UA in the fractions. Rhamnose was more abundant than galactose and arabinose in fractions C, indicating that side chains of RG-I were short.

In order to seek the reasons for the differences between our results and those previously reported in the literature, a new nitric acid was acquired and the extraction B and C of hull sample 3 were performed again using this acid. As the nitric acid used to prepare the solutions for the previous extractions came from a bottle that has been opened for the first time some months before, we hypothesized that hydration of the acid by from atmospheric humidity could have decreased the acid concentration. However, no significant differences were found in the UA contents when the new acid was used (Table 3),

indicating that it was not the reason for the results obtained for the pectins extracted from soy hulls in the present study.

Table 3. Comparison of pectins extracted from soy hull provided by different industries

	Hull sample 2[a]			**Hull sample 3[a]**					**Hull sample 4[a]**		
Fraction[b]	**A**	**B**	**C**	**A**	**B**	**B (n.a.)[e]**	**C**	**C (n.a.)[d]**	**A**	**B**	**C**
Yield (%)[c]	12.2±0.4•	11.9±0.1	11.7±0.3	11.9±0.4•	11.1±0.7	11.6±0.5	11.4±0.3	11.4±0.2	12.7±0.6•	12.4±0.8	12.3±0.5
Monosaccharide[d] (relative %)											
Rha	8.4±2.2	13.0±2.4	16.7±0.9	8.7±1.9	14.7±4.2	14.4	16.1±3.8	15.8	9.2±2.3	15.1±2.5	17.8±3.6
Fuc	1.0±0.2•	1.0±0.7	1.7±1.5	1.1±0.1•	0.3±0.01*	0.3	0.1±0.05*	0.2	0.8±0.1	0.4±0.04*	Tr[g]*
Ara	3.3±0.4	1.4±0.6	2.4±0.6	2.2±0.2	2.7±0.4	1.8	1.7±0.1	2.4	2.7±0.6	3.8±1.0	1.8±0.4
Xyl	7.8±0.8•	10.0±1.7	11.3±2.2	6.2±1.2	8.5±3.0	5.7	9.3±2.5	9.0	7.3±0.1•	10.0±2.3	9.4±2.0
Man	30.7±2.0	18.8±0.1*	11.2±0.4*	31.2±1.1	20.5±1.5*	20.3	14.9±1.2*•	13.7	29.9±1.3	14.8±1.5*	13.1±0.4*•
Gal	22.7±1.1	21.1±1.5	15.2±1.3	19.6±0.6	17.9±2.0	17.4	17.5±0.7	15.3	19.1±1.7	17.0±0.9	15.0±1.3
Glc	4.6±1.2	3.6±0.5	2.8±0.7	3.4±1.1	2.8±0.3	3.3	2.4±0.4	3.0	2.3±0.2	2.0±0.3	1.8±0.5
UA	21.5±0.8•	31.1±0.6*•	38.8±0.9*	27.6±2.9	32.5±0.6	36.8±2.0	38.0±0.4	40.6±3.5*	28.7±0.6	36.9±0.7*	41.1±0.6*

[a] Hull sample 2: unpelleted hull from Coopavel; Hull sample 3: unpelleted hull from Cargill; Hull sample 4: pelleted hull from Cargill;

[b] Fraction A: extracted with 0.1 M HCl at 90°C for 45 min; Fraction B: extracted with boiling 0.14 M HNO_3 for 30 min; Fraction C: extracted with boiling 0.14 M HNO_3 for 60 min.

[c] Yield = (polysaccharide mass/initial soy hull mass)*100.

[d] Neutral monosaccharides determined by GC of alditol acetates; UA determined by colorimetric method.

[e] n.a. = new nitric acid.

[f] ND = Not detected.

[g] Tr = Traces.

* Statistically different from fraction A for the same soy hull sample (1, 2, 3 or 4).

• Statistically different from the same fraction (A, B or C) of soy hull sample 1.

3.4. Water and acid extractions of soy hull

The presence of galactomannan in soybean hulls was first described by Whistler & Saarnio (1957). The polysaccharide was extracted with water at 40°C for 16 h with yield of 2% and Man:Gal ratio of 1.5:1. Aspinall & Whyte (1964) used cold water and obtained galactomannans with different properties. Extraction of soy hull sample 1 at 40°C for 16 h, prior to the pectin extraction, resulted in a fraction rich in galactomannan with a yield of 3.4% (Table 4). Considering only the contents of mannose and galactose a galactomannan yield of 2% was obtained, in close agreement with the reports by Whistler & Saarnio (1957). The Man:Gal ratio was 1.2:1, also close to the value previously reported. The residue from water extraction, was subjected to extractions with 0.1 M HCl at 90°C for 45 min and boiling 0.14 M HNO_3 for 60 min, giving rise to the fractions named AR and CR, respectively. The monosaccharide composition of the resulting fractions is given in Table 4. The pectic fractions obtained by acid extractions (AR and CR) using soy hulls previously extracted with water had noticeable lower contents of mannose compared with those previously observed (Table 3).

Table 4. Monosaccharide composition of fractions extracted from soy hull with hot water and using the conditions of extractions A and C to extract pectin fractions AR and CR from the insoluble residue of water extraction.

Fraction[a]	Hot water	AR	CR
Yield (%)[b]	3.4±0.3	10.7±0.1	10.7±0.1
DM (%)[c]	-	-	29.3±0.1
Monosaccharide[d] (relative %)			
Rha	4.6±0.7	12.1±0.3	19.0±1.2
Fuc	0.6±0.03	3.4±0.2	ND
Ara	8.7±0.2	8.6±0.4	1.6±0.3
Xyl	2.2±0.3	11.1±0.2	12.9±0.6
Man	32.1±0.8	7.0±0.1	3.4±1.1
Gal	26.0±0.2	21.9±0.2	8.9±0.1
Glc	3.1±0.1	2.2±0.1	1.5±0.3
UA	22.7±1.3	33.7±1.2	52.7±3.1

[a] Hot water fraction: extracted with 40°C for 16 h; Fraction AR: extracted with 0.1 M HCl at 90°C for 45 min; Fraction CR: extracted with boiling 0.14 M HNO_3 for 60 min.

[b]Yield = (polysaccharide mass/initial soy hull mass)*100.

[c]Degree of methyl-esterification determined by FT-IR.

[d]Neutral monosaccharide determined by GC of alditol acetates; UA determined by colorimetric method.

[e]ND = Not detected.

Figure 3 gives the chromatograms from HPSEC analyses using RI detector for fractions obtained from soy hull using hot water and the pectic fractions AR and CR isolated from the residual solid after the water extraction.

The elution profile of the hot water fraction showed two main peaks, corresponding to the peaks II and III of the previous HPSEC profile (Figure 2). Since it was suggested that peak II was due to pectins, peak III can now be attributed to the galactomannan, the main polysaccharide of this fraction. Fraction A presented two more intense peaks (I and II) and a minor peak (III), in agreement with the presence of low amount of galactomannan in this fraction. Apart from monosaccharides typical from pectin (peak II from HPSEC) and the galactomannan (peak III from HPSEC), fraction A had xylose (11.1%) and fucose (3.4%), both previously reported to be

covalently linked in soy hull (Aspinall, Hunt & Morrison, 1967) being part of a xylan, which also had 3-4% glucuronic acid (Aspinall, Hunt & Morrison, 1966). Fucosylated xylans are uncommon, however they have been also found in the seed endosperm of prickly pear fruits (Habibi, Mahrouz & Vignon, 2005) and seed-coat mucilage of *Hyptis suaveolens* (Aspinnal *et al.* 1991).

Fraction CR had the highest amount of UA (52.7%), in agreement with the more prominent peak II from HPSEC. The DM of this pectin was determined by FT-IR from bands areas of methyl-esterified (1749 cm^{-1}) and unesterified carboxyl groups (1630 cm^{-1}) (Figure 4). A DM of 29.3% was found, classifying soy hull pectin as low-methoxylated, as did Monsoor & Proctor (2001), who found DM values of 17-21% for 0.05 M HCl extracted pectins using different hull/solvent ratios. Previous report from Kalapathy & Proctor (2001), found that soy hull pectin was high-methoxylated (DM = 53-60%) when extraction using different concentrations of HCl (0.05–0.3 M) were conducted. Despite the increasing in the UA content of fraction CR, it was still lower than the ones reported in the literature. For peak I (xylan), a shift towards to higher elution times was observed in fraction CR compared to AR, suggesting that the harsher extraction conditions resulted in partial degradation of the polysaccharide. In fact, fucose was not present in extraction C, corroborating this possibility.

Figure 3. HPSEC elution profiles using RI detector for fractions obtained from soy hull using hot water and the pectic fractions AR and CR isolated from the residual solid after the water extraction.

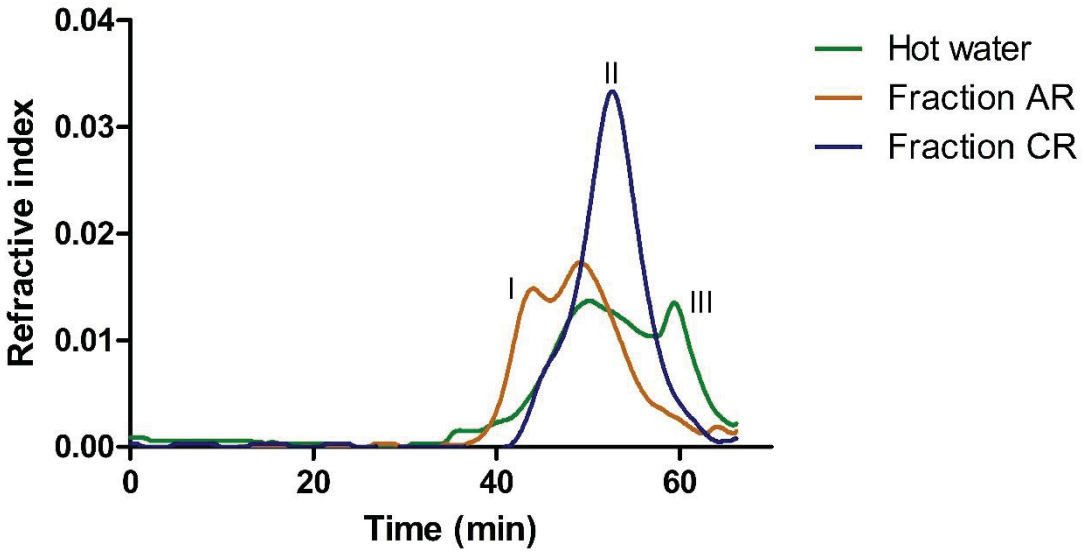
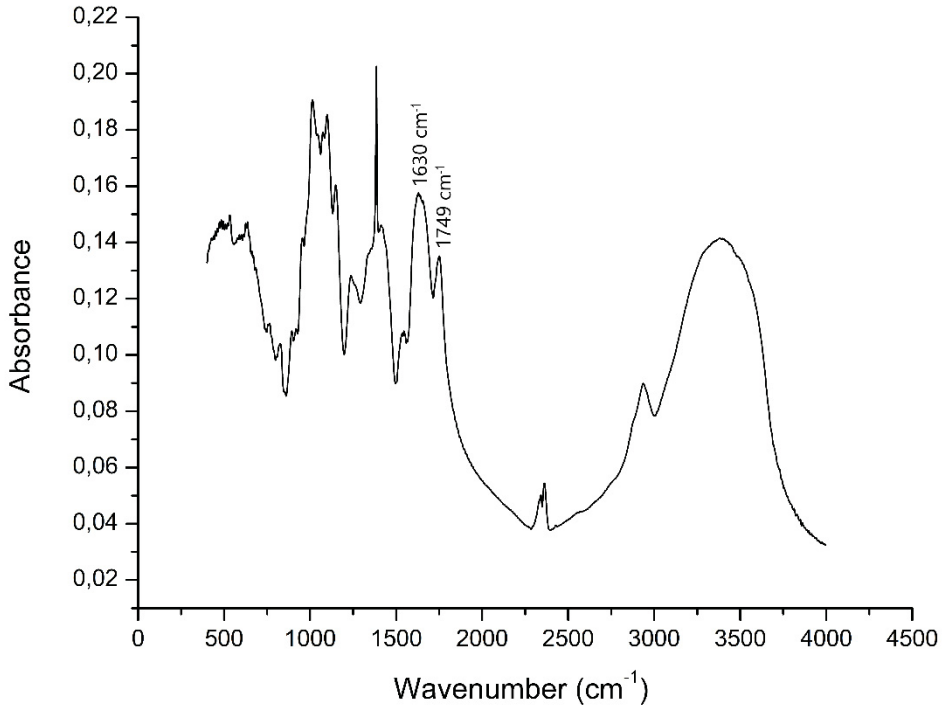


Figure 4. FT-IR spectrum of the pectin fraction CR from soy hull after removal of galactomannans with hot water.



Conclusions

The results previously reported in the literature for pectins from soy hull were not reproducible using soy hull provided by three different companies. Instead of pectins with high yield and galacturonic acid content, the acid extraction of soy hull resulted in a mixture of galactomannans, xylans and pectins, confirming previous findings that demonstrated the presence of water soluble galactomannans and xylans in soy hulls. Taken together, the results showed that soy hull pectin seems to be inappropriate for extraction of commercial pectins since acid extractions usually designed for pectin extraction were not suitable to give food grade pectins.

Acknowledgements

The authors are grateful to Electron Microscopy Center of UFPR for SEM-EDS analyses, to the industries Copacol, Coopavel and Cargill for the soy hull samples and to the Brazilian agencies Coordenacao de Aperfeicoamento de Pessoal de Nivel

Superior (CAPES)-Finance Code 001 and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) for the financial support. C.L.O.P. is a research member of the CNPq (309159/2018-0).

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3 CONSIDERAÇÕES FINAIS

O presente estudo demonstrou que a polpa de café pode ser utilizada como uma fonte para a produção de pectina de alta qualidade. Por ser um resíduo abundante e praticamente sem custo, surge como uma forte alternativa em relação à casca de cítricos e ao bagaço de maçã. Extrações ácidas conduzidas com ácido nítrico resultaram em frações com alto conteúdo de ácido galacturônico e teores de proteínas, fenólicos e acetil próximos às pectinas comerciais. Análises reológicas demonstraram potencial de uso desta pectina como um agente espessante e gelificante. Após ampla revisão da literatura, não foi encontrado nenhum estudo que descrevesse a obtenção de pectina da polpa de café com propriedades gelificantes. Assim, pela primeira vez uma pectina proveniente da polpa de café foi capaz de formar gel na presença de açúcar e um pH baixo, o que a torna adequada à indústria de alimentos.

Já a pectina da casca de soja se mostrou incompatível com os requisitos comerciais, resultados contrários a trabalhos existentes na literatura. A composição monossacarídica e análises cromatográficas e espectrométricas de pectinas extraídas em diferentes condições e a partir de amostras de casca de soja distintas, indicaram que uma galactomanana e uma xilana estavam sendo coextraídas juntamente com a pectina, o que dificulta a obtenção de uma fração com um alto teor de ácidos urônicos. Diferentemente do que vinha sendo proposto, a casca de soja não é um material adequado para a extração de pectinas utilizadas na indústria de alimentos.

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