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PROPRIEDADES FUNCIONAIS DA FIBRA DE MAÇÃ MODIFICADA QUÍMICA E FISICAMENTE

Santa Maria, RS

2017

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Tese apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Área de Concentração em Qualidade de Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Doutora em Ciência e Tecnologia dos Alimentos.**

Orientadora: Profª. Drª. Luisa Helena Hecktheuer

Coorientadora: Profª. Drª. Leila Picolli da Silva

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RESUMO

Tese de Doutorado
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Universidade Federal de Santa Maria

PROPRIEDADES FUNCIONAIS DA FIBRA DE MAÇÃ MODIFICADA QUÍMICA E FISICAMENTE

AUTORA: Fernanda Teixeira Macagnan
ORIENTADORA: Luisa Helena Hecktheuer
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Este trabalho objetivou estudar a influência da hidrólise ácida e da extrusão e sobre as propriedades físico-químicas e biológicas da fibra do bagaço de maçã, buscando potencializar seus efeitos benéficos como ingrediente funcional. Aliado a isso, investigou-se também, os compostos bioativos associados a parede celular vegetal e a sua relação com a maior proteção antioxidante do organismo. As diferentes condições de hidrólise ácida da fibra da maçã promoveram incremento em todas as propriedades físico-químicas avaliadas, aumentando a capacidade da fibra em ligar ácidos biliares (55%), inibir a atividade da enzima lipase pancreática (206%), reter água (11%) e gordura (20%) na sua estrutura. A fibra hidrolisada (H_2SO_4 1.5N/ 3h) estimulou significativamente o crescimento *in vitro* das culturas de *Bifidobacterium lactis* e *Lactobacillus acidophilus*, sendo o crescimento superior à fibra não tratada para ambas as culturas probióticas e similar ao controle positivo de inulina para a cultura de *B.lactis*. Já o tratamento de extrusão provocou redistribuição entre as frações da fibra, aumentando o teor de fibra solúvel em 112,6%. A desintegração da parede celular foi confirmada pela redução nos teores de celulose e hemicelulose, e pelo aumento no teor de pectina solúvel. Condições experimentais mais agressivas melhoraram a capacidade da fibra de maçã de ligar ácidos biliares (13%), gordura (22%) e inibir a atividade da lipase pancreática (mais de 70%). A energia gerada pela extrusão também possibilitou a liberação parcial dos polifenois associados a fibra, aumentando o teor de polifenois extraíveis. A extrusão da fibra (90ºC/ 33% umidade) provocou maior estímulo ao crescimento da cultura de *L. acidophillus*, mas não influenciou na cultura de *B. lactis*. Para estudar o efeito *in vivo* da extrusão e hidrólise ácida da fibra de maçã, foi selecionada a melhor condição experimental de cada tratamento. Para o ensaio biológico, conduzido por 38 dias, utilizou-se 32 ratos Wistar machos (21 dias de idade) distribuídos entre os seguintes tratamentos: CEL (controle com celulose), AP (com fibra do bagaço de maçã) HAP (com fibra do bagaço de maçã hidrolisada) EAP (com fibra do bagaço de maçã extrusada). O processo de extrusão aumentou a solubilidade e a fermentabilidade da fibra, intensificando a produção do ácido butírico e a proteção antioxidante do ceco; aumentou a excreção fecal de gordura e colesterol; reduziu os triglicerídeos séricos e diminuiu o pico glicêmico pós-prandial. Já a hidrólise ácida se destacou por potencializar o efeito prebiótico da fibra, causando redução do pH fecal, aumentando a fermentabililidade e a produção de AGCCs, em particular do propiônico e butírico. O consumo das fibras de maçã modificadas e não modificada aumentou o teor de polifenois no soro e no intestino dos animais experimentais, proporcionando maior proteção antioxidante ao organismo. Esse fato revela que essas fibras apresentaram também, importante função fisiológica como carreadoras de compostos bioativos através do trato gastrointestinal. Os métodos de modificação da fibra alimentar da maçã avaliados no presente trabalho foram eficazes, intensificando algumas de suas propriedades benéficas e melhorando seu efeito protetor da saúde intestinal. Fundamenta-se, assim, que os métodos propostos produziram ingredientes ativos diferenciados para serem explorados na nutrição funcional humana.

Palavras-chave: Bagaço de maçã. Hidrólise ácida. Extrusão. Propriedades físico-químicas. Efeito prebiótico. Polifenois não-extraíveis.

ABSTRACT

Doctoral Thesis
Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos
Universidade Federal de Santa Maria

FUNCTIONAL PROPERTIES OF APPLE FIBRE MODIFIED CHEMICAL AND PHYSICALLY

AUTHOR: Fernanda Teixeira Macagnan
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This work aimed to study the influence of acid hydrolysis and extrusion on the physicochemical and biological properties of apple pomace fibre, seeking to enhance its beneficial effects as a functional ingredient. Allied to this, it was investigated also bioactive compounds associated with plant cell wall and its relationship with the highest antioxidant protection of the organism. The different acid hydrolysis conditions of the apple fiber promoted increase in all the physicochemical properties evaluated, increasing the capacity of the fibre to bind bile acids (55%), to inhibit pancreatic lipase activity (206%), and to retain water (11%) and fat (20%) in its structure. The hydrolysed fibre (H_2SO_4 1.5N for 3 h) significantly stimulated the growth of *Bifidobacterium lactis* and *Lactobacillus acidophilus* after 48 h of *in vitro* incubation, and the growth was larger ($p < 0.05$) than that detected for the untreated fibre for both probiotic bacteria and similar ($p > 0.05$) to that seen in the positive control with inulin for *B. Lactis*. Regarding extrusion, this treatment caused redistribution among fibre fractions, increasing the soluble fiber content by up to 112.6% (90 ºC/ 33% of moisture). Partial degradation of cell walls was confirmed by the reduction in cellulose and hemicellulose contents and by the increase in soluble pectin levels. more aggressive experimental conditions improved the capacity of apple fibre to bind bile acids (13%), fat (22%), and to inhibit pancreatic lipase activity (by over 70%). The energy generated from extrusion led to the partial release of fibre-associated polyphenols, increasing the extractable polyphenol content. Extrusion (90 ºC/ 33% of moisture) further stimulated the growth of *L. acidophilus,* but it did not influence the growth of *B. lactis*. For the biological assay, conducted for 38 days, were used 32 male Wistar rats (21 days of age), distributed among the following treatments: CEL (control with cellulose), AP (with apple pomace fibre) HAP (with hydrolyzed apple pomace fibre) EAP (with extruded apple pomace fibre). The extrusion process increased solubility and fermentability of the fibre, boosting butyric acid production and antioxidant protection of the caecum; increased fecal excretion of the fat and cholesterol; reduced serum triglycerides, and decreased of postprandial glycemic peak. Acid hydrolysis was highlighted by potentiating the prebiotic effect of the fiber, improved the prebiotic effect of the fibre, reducing faecal pH and increasing the production of SCFAs, especially of propionic and butyric acids. Consumption of modified and unmodified apple fibres increased the concentration of polyphenols in the serum and in the intestine of experimental animals, resulting in better antioxidant protection. This shows that these fibres play an important physiological role as carriers of bioactive compounds through the gastrointestinal tract. The methods of modifying the dietary fibre of the apple evaluated in the present work were effective, intensifying some of its beneficial properties and improving its protective effect of intestinal health. It is therefore argued that the proposed methods produced differentiated active ingredients to be exploited in human functional nutrition.

Keywords: Apple pomace. Acid hydrolysis. Extrusion. Physicochemical properties. Prebiotic effect. Non-extractable polyphenols.

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1 INTRODUÇÃO

A industrialização da maçã, em particular do suco, tem como principal subproduto o bagaço (WASZCZYNSKYJ et al., 2001), o qual é gerado em grandes quantidades e normalmente dispensado de forma inadequada no meio ambiente. Entretanto, o bagaço de maçã é boa fonte de compostos antioxidantes e de fibra alimentar (FA) (mais de 70% em base seca) (MACAGNAN et al., 2015), ambos fatores dietéticos reconhecidos na prevenção de doenças crônicas.

Mais de 50% dos alimentos funcionais disponíveis no mercado tem como componente ativo a FA (GIUNTINI & MENEZES, 2011). Os benefícios associados ao seu consumo incluem a regulação do trânsito intestinal e a prevenção ou tratamento de diabetes, doenças cardiovasculares e câncer de cólon (KACZMARCZYK et al., 2012; KENDAL et al., 2010). Contudo, vale ressaltar que tais benefícios e a intensidade de seus efeitos estão diretamente relacionados à composição da fibra, suas propriedades físico-químicas e aos compostos antioxidantes associados (ELLEUCH et al., 2011; TUNGLAND & MEYER, 2002). Baseado na solubilidade de seus componentes em água, a FA pode ser de dois tipos: solúvel e insolúvel, as quais apresentam propriedades fisiológicas e funcionais diferenciadas. Algumas dessas fibras podem auxiliar na menor absorção intestinal de ácidos biliares, glicose, colesterol e lipídeos, por meio de interações físicas e químicas. Entretanto, grande parte dos efeitos hipolipidêmicos e hipoglicêmicos das fibras alimentares está relacionada à sua fração solúvel (ELLEUCH et al., 2011; KENDAL et al., 2010; MUDGIL & BARAK, 2013). Além disso, essa fração da fibra é mais facilmente fermentável, contribuindo no aumento do balanço microbiótico e na produção de ácidos graxos de cadeia curta (AGCCs), os quais são fundamentais para a saúde intestinal (LÓPEZ et al.,1997; MUDGIL & BARAK, 2013).

Estudos científicos comprovam que os compostos fenólicos associados à parede celular de vegetais são componentes relevantes da fibra alimentar e constituem aproximadamente 50% do total de antioxidantes dietéticos (GOÑI et al., 2009; SAURA-CALIXTO, 1998; SAURA-CALIXTO, 2011; SAURA-CALIXTO & DÍAZ-RUBIO, 2007). A presença de compostos bioativos associados a FA exerce efeitos consideráveis sobre as suas características físico-químicas e contribui nas suas propriedades biológicas em humanos. Contudo, existem poucos estudos que investigaram seu conteúdo e sua ação biológica, em comparação com centenas de trabalhos com foco em polifenois biodisponíveis no intestino delgado. Sabe-se que os compostos fenólicos associados não são absorvidos no intestino delgado e, assim, chegam intactos ao cólon juntamente com os carboidratos indigeríveis, onde se tornam substratos

fermentáveis para a microbiota, produzindo metabólitos e um ambiente antioxidante (GOÑI et al., 2009; MANACH et al., 2005; SAURA-CALIXTO, 2011; SAURA-CALIXTO & DÍAZ-RUBIO, 2007).

A fibra solúvel encontra-se menos reticulada que a fibra insolúvel, o que facilita a liberação dos polifenois associados pela ação da microbiota intestinal, e assim, torna-os mais bioacessíveis ao consumidor (VITAGLIONE et al., 2008). As frutas são os alimentos que mais apresentam quantidade de compostos associados à fibra, contudo a maior proporção encontra-se associada à sua fração insolúvel (GOÑI et al., 2009). O bagaço de maçã é fonte de fibra solúvel, entretanto mais de 70% de sua fibra alimentar é do tipo insolúvel (MACAGNAN et al., 2015). Essa característica química pode limitar seus efeitos hipolipidêmicos e hipoglicêmicos, como também seu potencial prebiótico e sua proteção antioxidante no organismo, pois tal fração apresenta-se bastante reticulada o que dificulta a ação da microbiota intestinal e a liberação dos polifenois no intestino.

Tratamentos físicos e químicos podem causar alterações estruturais na FA e melhorar suas características físico-químicas e de fermentabilidade, mas são poucas as informações científicas sobre os efeitos biológicos de tais tratamentos. Nesse sentido, este trabalho objetivou estudar a influência da hidrólise ácida e do processo de extrusão sobre as propriedades físico-químicas e biológicas da fibra da maçã, buscando potencializar seus efeitos benéficos como ingrediente funcional. Aliado a isso, investigou-se também os compostos bioativos associados a parede celular vegetal e a sua relação com a maior proteção antioxidante do organismo.

1.1 OBJETIVOS

1.1.1 Objetivo geral

Estudar a influência da hidrolise ácida e da extrusão sobre as propriedades físico-químicas e biológicas da fibra do bagaço de maçã, buscando potencializar seus efeitos benéficos como ingrediente funcional.

1.1.2 Objetivos específicos

- Avaliar o efeito de diferentes condições de hidrólise ácida sobre a razão fibra insolúvel e fibra solúvel e sobre as propriedades físico-químicas da fibra de maçã;

- Avaliar o efeito de diferentes condições de extrusão sobre a composição química da fibra e sobre as propriedades físico-químicas da fibra do bagaço de maçã;

- Determinar os compostos fenólicos totais e a capacidade antioxidante total do bagaço de maçã antes e após a extrusão (incluindo os compostos fenólicos associados à fibra e a capacidade antioxidante relacionada a eles);

- Avaliar o efeito prebiótico *in vitro* da fibra de maçã não modificada e das fibras modificadas pela hidrólise ácida e extrusão nas melhores condições experimentais;

- Selecionar a melhor condição experimental de hidrólise ácida e de extrusão dos testes *in vitro* para serem analisados *in vivo;*

- Verificar as propriedades biológicas das fibras de maçã modificadas e não modificada por meio da realização de ensaio biológico com ratos *Wistar* em crescimento, avaliando o consumo de dieta, ganho de peso, digestibilidade aparente da fibra, parâmetros bioquímicos sanguíneos, curva glicêmica, peso do fígado e gordura epididimal; colesterol e gordura hepática; produção intestinal de ácidos graxos de cadeia curta; pH fecal; teor de polifenois e atividade antioxidante do conteúdo intestinal e do soro; e excreção fecal de lipídeos e colesterol.

2 REVISÃO BIBLIOGRÁFICA

2.1 FIBRA ALIMENTAR

De acordo com os métodos oficiais clássicos da AOAC (985.29 e 991.43) e recomendados para análise de FA nos alimentos, existem dois tipos de fibras que coexistem em proporções variadas no alimento e são classificadas quanto à solubilidade de seus componentes em água, em fibras solúveis e fibras insolúveis (AOAC, 1995).

A fração insolúvel é predominante em farelos, vegetais folhosos e grãos e é composta pelos componentes insolúveis em água da parede vegetal, os quais incluem celulose, hemiceluloses insolúveis, lignina e taninos. A fibra solúvel é formada por polissacarídeos não amiláceos hidrossolúveis estruturais, como β-glicanas, pectinas, algumas hemiceluloses, gomas e mucilagens; sendo encontrada em maior quantidade em leguminosas secas, aveia, cevada e frutas (ELLEUCH et al., 2011; KENDAL et al., 2010; MUDGIL & BARAK, 2013).

2.1.1 Propriedades físico-químicas

Como há uma grande diversidade estrutural de parede celular das diferentes fontes vegetais de fibra, as características químicas e estruturais dos polímeros que a constituem irão definir as suas propriedades físico-químicas. O conhecimento das propriedades da fibra vegetal pode ser relacionado com o comportamento fisiológico no intestino humano, pois elas se caracterizam por influir sobre o trânsito digestivo das dietas, sobre a absorção de nutrientes e de sais biliares e sobre o metabolismo dos lipídeos e carboidratos (CHAU & HUANG, 2003; RETORE, 2009; ZARAGOZA et al, 2001). Entre as propriedades físico-químicas da fibra, destacam-se a capacidade de ligação catiônica, a capacidade de retenção de água, a capacidade de absorção de moléculas orgânicas e a capacidade de inibição da lipase pancreática.

A capacidade de ligação catiônica está relacionada com a habilidade da fibra em ligar-se a íons através de grupos situados em sua superfície (ANNISON & CHOCT, 1994). Certos tipos de fibra são capazes de formar complexos insolúveis com compostos inorgânicos ou orgânicos que apresentam cargas, e assim incrementam sua excreção fecal. Carboxilas, aminas, hidroxilas alifáticas e fenólicas são os principais grupos funcionais capazes de exercer ligação catiônica na parede celular e estão presentes em maior quantidade nas pectinas, ligninas e taninos (CERQUEIRA, 2006; JERACI & VAN SOEST, 1990; RETORE, 2009). A propriedade das fibras exercerem ligação catiônica pode ser relacionada à capacidade do alimento em ligar

ácidos biliares, impedindo-os de serem reabsorvidos pelo epitélio intestinal. Esse potencial de ligação depende da presença de fitonutrientes (como polifenois), bem como da estrutura física e química da fibra, hidrofobicidade, natureza iônica e grau de interação com sítios de ligação ativos (KAHLON & SMITH, 2007).

A capacidade de retenção de água pela fibra, além de ser importante no processamento de alimentos, também exerce função fisiológica positiva (CERQUEIRA, 2006; ZARAGOZA et al., 2001). A água é fundamental na atividade bioquímica das fibras porque fluidifica as fezes e auxilia na evacuação. Além disso, a ação anticarcinogênica no cólon, atribuída às fibras, em parte, é devida à diluição dos carcinógenos intestinais na densa hidrosfera que elas geram (RIEGEL, 2012).

As propriedades de hidratação da fibra referem-se à habilidade desta em reter água dentro da sua matriz. Essa propriedade depende da presença de grupamentos hidrofílicos na parede celular, bem como da área de superfície das moléculas e dos espaços intracelulares (ANNISON & CHOCT, 1994). Normalmente, a capacidade de hidratação é maior entre os polissacarídeos, sendo que as pectinas, hemiceluloses, gomas e mucilagens se hidratam mais facilmente. A capacidade de retenção de água dos polímeros insolúveis como a celulose é mais dependente do tamanho de partícula do que da superfície de contato com a água (VAN SOEST, 1994). Já as fibras solúveis possuem estruturas com mais ramificações e com grande quantidade de grupos hidrofílicos, aumentando assim a superfície de contato e a capacidade de hidratação (ANNINSON e CHOCT, 1994). A capacidade da fibra solúvel de reter água está fortemente relacionada ao aumento da viscosidade do conteúdo intestinal, cuja intensidade é variável (FIETZ & SALGADO, 1999). Fibras com fortes propriedades de hidratação podem aumentar o volume fecal e diminuir a taxa de absorção de nutrientes no intestino. Processos como moagem, secagem, aquecimento e extrusão, podem afetar significativamente tais propriedades (GUILLON & CHAMP, 2000).

O potencial da fibra em absorver moléculas orgânicas pode ser relacionado a sua habilidade em ligar-se a moléculas como triglicerídeos, colesterol, ácidos biliares, agentes cancerígenos e compostos tóxicos no trato intestinal (ZARAGOZA et al., 2001). Segundo López et al. (1997), a capacidade da fibra em ligar-se à gordura depende das suas propriedades de superfície, densidade, espessura e da natureza hidrofóbica das suas partículas.

A lipase pancreática é uma enzima chave na hidrólise e na absorção de lipídeos (NAKAY et al., 2005). A fibra alimentar pode diminuir a atividade dessa enzima digestiva e, assim, contribuir na prevenção e/ou tratamento das dislipidemias. Esse potencial está relacionado a características próprias da matriz fibrosa, tais como a porosidade, o grau de

exposição de substâncias inibitórias presentes na superfície, a capacidade de encapsulação do óleo e da enzima pela fibra, e a redução da acessibilidade da lipase ao óleo (CHAU; WANG; WEN, 2007).

2.1.2 Efeitos biológicos

As fibras constituem fator preventivo e contribuem no tratamento dietoterápico de várias patologias, como a constipação intestinal crônica, dislipidemias, obesidade, aterosclerose e diabetes (KACZMARCZYK et al., 2012; KENDALL et al., 2010).

As propriedades físico-químicas dos componentes que constituem cada uma das frações solúvel e insolúvel da fibra alimentar irão contribuir para os diferentes efeitos fisiológicos e metabólicos no organismo humano (MOMM, 2007). Esses efeitos podem ser decorrentes de alterações em funções fisiológicas, como a taxa de excreção endógena e a passagem do alimento pelo trato gastrintestinal; alterações no bolo alimentar, tais como a capacidade de hidratação, o volume, o pH e a fermentabilidade; ou, ainda, alterações nas populações e na atividade da microbiota intestinal (RETORE, 2009).

A fibra insolúvel é pouco fermentável, forma misturas de baixa viscosidade e está mais relacionada à melhora da motilidade intestinal. Essa fração fibrosa apresenta efeito mecânico no trato gastrointestinal, retendo grandes quantidades de água, aumentando o volume e o peso fecal e melhorando a consistência das fezes. Por fornecer a massa necessária para a ação peristáltica do intestino, a fibra insolúvel contribui para o aumento da frequência da evacuação. Dessa forma, essa fração da fibra está relacionada à regulação das funções digestivas e, por tornar a eliminação mais fácil e rápida, diminui o risco de aparecimento de hemorroidas, diverticulites e câncer de cólon (CAMBRODÓN & MARTÍN-CARRÓN, 2001; LÓPEZ et al.,1997)

Grande parte dos benefícios diretos das fibras nas doenças cardiovasculares está relacionada a sua fração solúvel. Seus componentes solúveis apresentam mecanismos hipolipidêmicos mais eficientes, podendo diminuir o colesterol total e LDL em até 20% (RIEGEL, 2012). A fibra solúvel tem a propriedade de se ligar à água, formando gel, sendo responsável por aumentar a viscosidade do conteúdo gastrointestinal. Tal propriedade reflete na capacidade de retardar o esvaziamento gástrico (causando maior saciedade) e o trânsito intestinal (maior resistência ao peristaltismo). O aumento da viscosidade dificulta ou impede a ação enzimática sobre o substrato, como também limita a absorção do material que já foi digerido, pelo fato de criar uma barreira física. Por isso, essa fração solúvel da fibra apresenta

grande capacidade de diminuir a absorção de lipídeos e de colesterol, assim como a circulação enteropática dos ácidos biliares, contribuindo para a redução dos níveis séricos de colesterol e de triglicerídeos (GALISTEO; DUARTE; ZARZUELO, 2008; LÓPEZ et al.,1997; MOMM, 2007; RIQUE et al., 2002; TOPPING, 1991). O aumento da viscosidade também reduz a velocidade de absorção da glicose, retardando o metabolismo dos açúcares e facilitando a estabilização do metabolismo energético, controlando aumentos bruscos na taxa de glicemia pós-prandial (GALISTEO et al., 2008; KENDALL et al., 2010; OU et al., 2001).

A fibra solúvel é altamente fermentável, podendo ainda aumentar o balanço microbiótico e a produção de AGCCs, principalmente os ácidos acético, propriônico e butírico (90 a 95% dos AGCCs produzidos), fundamentais para o metabolismo intestinal, e capazes de exercer influência sobre o metabolismo de lipídeos e carboidratos (CAMBRODÓN & MARTÍN-CARRÓN, 2001; LÓPEZ et al.,1997). O acetato alcança a circulação sistêmica e pode ser utilizado como fonte de energia e na lipogênese. O propionato é metabolizado no fígado e é o único gliconeogênico que pode influenciar algumas fases do metabolismo hepático, como a síntese do colesterol. O butirato parece ser o AGCC de mais acentuado efeito tópico sobre a saúde do epitélio colônico. É a principal fonte de energia dos colonócitos e exerce efeito trófico sobre o epitélio intestinal, estimulando o crescimento e a proliferação das células epiteliais, que resulta em aumento no peso do intestino (REYES & AREAS, 2001; RIEGEL, 2012; RUIZ-ROSO et al., 2001). Além disso, o butirato tem recebido atenção especial por parte dos pesquisadores por apresentar efeitos benéficos em relação ao câncer de cólon (PACHECO & SGARBIERI, 2001). Segundo Riegel (2012), a insuficiente produção de butirato ocasiona lesões no cólon, tais como inflamações, polipose e câncer.

Os AGCC também podem potencializar a secreção pancreática de insulina e afetar a gliconeogênese hepática e a sensibilidade dos tecidos à insulina, contribuindo, então, para reduzir a hiperglicemia normalmente observada em pacientes diabéticos (REYES & AREAS, 2001).

A porosidade e a área superficial podem influenciar a fermentação da fibra. Quanto maior o diâmetro do poro, mais rápido ocorrerá o processo de digestão do substrato, uma vez que as enzimas poderão atuar não só na superfície da partícula, como também entre seus espaços (GUILLON & CHAMP, 2000). Quanto mais suscetível à ação das bactérias colônicas for a fibra, maior o seu grau de fermentação e, assim, maior será o estímulo à atividade e ao desenvolvimento da microbiota do ceco. Com a fermentação da fibra também ocorre a diminuição do pH e a produção de nutrientes necessários para o bom desenvolvimento da microbiota intestinal, promovendo a inibição do crescimento de bactérias patogênicas e atuando

na prevenção de infecções gastrointestinais e de câncer de cólon (LÓPEZ et al., 1997; RAUPP et al., 2000; RIQUE et al., 2002; ZARAGOZA et al., 2001). Dos constituintes da fibra alimentar, a lignina, por exemplo, é resistente ao processo fermentativo, enquanto pectinas e gomas parecem ser completamente fermentadas; já a celulose e a hemicelulose apresentam fermentação parcial no cólon (CERQUEIRA, 2006; RIEGEL, 2012).

Percebe-se que nas últimas décadas, devido à importância da fibra alimentar na proteção e promoção da saúde humana, tem-se aumentado consideravelmente o conhecimento sobre esse componente dos alimentos, tanto na área nutricional quanto analítica. Muita informação foi sendo descoberta sobre os diferentes compostos presentes na fibra alimentar, com destaque, aos prebióticos (devido ao perfil de fermentabilidade de substâncias específicas e sua interação com a microbiota colônica) (GIUNTINI & MENEZES, 2011) e aos compostos bioativos associados (por conferirem um *status* antioxidante ao organismo) (SAURA-CALIXTO, 2011).

2.2 BAGAÇO DE MAÇÃ

A cultura macieira mostrou expansão significativa nos últimos 30 anos (NOGUEIRA et al., 2007). Segundo dados do Instituto Brasileiro de Geografia e Estatística (IBGE), o Brasil apresenta produção expressiva de maçãs, a qual concentra-se principalmente na Região Sul do País, responsável por 99,4% da produção no ano de 2012. O Rio Grande do Sul é o segundo maior Estado produtor de maçã, atrás apenas de Santa Catarina. Na safra de 2012, o Estado produziu 620.841 toneladas, ou seja, 46,6% da produção do País, estimada em 1.339.771 toneladas (IBGE, 2015).

Percebe-se crescente consumo tanto da maçã *in natura* como nos processados (sucos, vinagres e sidras) (SOARES et al., 2006). Dentre os produtos da agroindústria da maçã, os sucos (tipo néctar, clarificado e concentrado) são considerados mais nobres. No Brasil, grande parte do descarte da fruta, de 115 a 150 mil toneladas, é transformado em suco de maçã, pouco comercializado internamente pela falta de hábito do consumidor brasileiro, sendo na sua maioria (90%) concentrado e destinado para exportação (NOGUEIRA et al., 2007).

A industrialização da maçã, em particular do suco, gera, como principal subproduto o bagaço (WASZCZYNSKYJ et al., 2001), o qual é constituído pela mistura heterogênea de casca, semente, cálice, haste e polpa, podendo representar 20 a 40% do peso das maçãs processadas, dependendo da tecnologia empregada na extração (COELHO, 2007). Esse subproduto industrial geralmente é utilizado como ração animal, ou é simplesmente dispensado no solo, o que pode causar riscos de contaminação ambiental (VILLAS-BÔAS & ESPOSITO,

2001) e perda da qualidade do ar nas regiões de processamento, uma vez que o resíduo fermenta e exala odores pouco agradáveis (RAUPP et al., 2000). Entretanto, esse material é excelente para fins biotecnológicos (como para a extração de pectina) e nutricionais (FERTONANI, 2006; MARCON et al., 2005; PAGANINI et al. 2005; RIBEIRO, 2007).

O incentivo ao uso do bagaço de maçã na alimentação humana provém de estudos que comprovam a ação benéfica do consumo da fruta *in natura*, cujos constituintes, principalmente fibras e compostos fenólicos, têm características hipolipidemiantes e hipoglicemiante (BOYER & LIU, 2004). Nawirska e Kwasniewska (2005) analisaram a composição de fibras alimentares em subprodutos do processamento de vegetais e obtiveram para o bagaço de maçã 12% de pectina, 24% de hemicelulose, 44% de celulose e 20% de lignina.

Trabalhos científicos envolvendo ensaios clínicos e biológicos vêm mencionando o elevado valor nutricional do subproduto do processamento da maçã (COELHO, 2007; LEONTOWICK et al., 2001; MOMM, 2007). Outros estudos vêm demonstrando também que o bagaço de maçã, além de ser fonte de fibras alimentares e de polifenois (SUDHA; BASKARAN; LEELAVATHI, 2007; SOARES et al., 2008), possui propriedades tecnológicas e sensoriais aplicáveis à indústria de alimentos (RAUPP et al., 2000; SUDHA; BASKARAN; LEELAVATHI, 2007; WASZCZYNSKYJ et al., 2001).

No Brasil, poucas pesquisas tecnológicas foram desenvolvidas com o intuito de explorar o potencial do bagaço de maçã (WASZCZYNSKYJ et al., 2001). Atualmente, existe grande preocupação com a contaminação ambiental pelo descarte incorreto de subprodutos agroindustriais e também, com o acentuado desperdício de alimentos frente à notável carência de alimentação de qualidade e acessível à população de baixa renda. A indústria brasileira de processamento da maçã mostra interesse em alternativas econômicas e tecnologicamente viáveis para a utilização do descarte sólido produzido. Apesar disso, e enquanto a comunidade científica não responder satisfatoriamente aos anseios dessas indústrias, esses materiais continuarão a ser considerados, embora quantitativamente relevantes, apenas como resíduos, ou seja, produtos sem valor econômico (RAUPP et al., 2000).

Nesse contexto, há necessidade de melhor aproveitar as matérias sólidas produzidas durante o processamento do suco de maçã no Brasil. Para isso, são necessários estudos mais aprofundados e precisos, que demonstrem as suas propriedades biológicas, ou, então, que as potencializem por meio de tratamentos diferenciados, buscando um direcionamento eficiente do seu uso para uma finalidade mais nobre como a promoção e a proteção da saúde humana.

2.3 MÉTODOS QUÍMICOS E FÍSICOS PARA MODIFICAÇÃO DA FIBRA ALIMENTAR

A porosidade e a superfície disponível da fibra alimentar podem influenciar a sua fermentabilidade (disponibilidade para degradação microbiana no cólon), enquanto a regioquímica da camada superficial pode desempenhar papel em algumas propriedades físico-químicas (de adsorção ou de ligação de algumas moléculas) sendo responsável por alguns efeitos fisiológicos da fibra alimentar no organismo (GUILLON & CHAMP, 2000). Dessa forma, as propriedades físico-químicas da fibra podem ser manipuladas por meio de tratamentos: químicos, enzimáticos, mecânicos (moagem), térmicos ou termomecânicos (extrusão), entre outros, para melhorar a sua funcionalidade (GUILLON e CHAMP, 2000).

A hidrólise dos polissacarídeos da parede celular pode levar ao aumento da concentração de fibras solúveis. Além disso, o volume de intumescimento das fibras insolúveis tende a aumentar, provavelmente pelo aumento da porosidade, pois o mesmo depende do tamanho e da distribuição dos poros (GUILLON e CHAMP, 2000). Nesse sentido, as hidrólises ácidas são capazes de produzir alterações na fibra alimentar dos alimentos, modificando a sua solubilidade, sua fermentabilidade e suas propriedades físico-químicas. Esses tratamentos químicos são os que apresentam menor custo e os mais fáceis de serem realizados, mas, algumas vezes, são inadequados para alguns tipos de alimentos, pois podem alterar as propriedades sensoriais do produto final e serem percebidos como não naturais por parte dos consumidores (VITAGLIONE et al., 2008).

Olson et al. (1988) reportaram que a hidrólise ácida do farelo de alguns cereais resultou no aumento do valor nutricional e fermentabilidade da fibra alimentar devido a sua maior solubilidade. A hidrólise ácida também contribuiu para o aumento da capacidade de ligação a ácidos biliares (38%) da fibra alimentar de tremoço (*Lupinus angustifolius* L.) (CORNFINE et al., 2010). Nesse estudo, observou-se relação direta entre o tempo de incubação (4, 18, 48 h) e a degradação da parede celular, em particular dos compostos de pectina e hemicelulose. Isso indica que a desintegração dos componentes da parede celular e, simultaneamente, a formação de fragmentos menores de fibra têm impacto considerável sobre a capacidade do material fibroso se ligar a ácidos biliares.

O cozimento por extrusão, que envolve aquecimento em combinação com a homogenização, também é capaz de alterar as propriedades físico-químicas e fisiológicas da fibra alimentar. Na extrusora, o material é submetido a intenso cisalhamento mecânico e o cozimento ocorre com temperatura e pressão elevada e com baixo teor de água. O tratamento

mecânico desorganiza completamente a estrutura original do material (DAOU & ZHANG, 2012). Durante a extrusão pode ocorrer solubilização da fibra de algumas fontes alimentares dependendo da severidade do processo (alta temperatura, alta velocidade do parafuso e baixo teor de umidade). O rompimento mecânico das ligações glicosídicas durante o processo de cozimento por extrusão poderia levar ao aumento da fração solúvel da fibra. De fato, a tensão mecânica durante o processo pode ocasionar a degradação das ligações glicosídicas dos polissacarídeos, levando à liberação de oligossacarídeos e, consequentemente, o aumento da fração solúvel da fibra (VITAGLIONE et al., 2008). Gualberto et al. (1997) verificaram que o processo de extrusão provocou diminuição do teor de fibra insolúvel de aveia e de farelo de arroz, mas não afetou o conteúdo dessa fração da fibra no farelo de trigo e, em geral, o conteúdo de fibra solúvel aumentou após a extrusão de todos os farelos de cereais analisados no estudo. A fibra extrusada de farelo de arroz desengordurado mostrou superior capacidade de adsorção da glicose (40,73%), solubilidade e viscosidade no estudo de Daou e Zhang (2012). Isso demostra que esse tratamento físico é um processo interessante de ser investigado quando se quer potencializar os efeitos nutricionais das fibras de alimentos.

Nesse sentido, tratamentos físicos e químicos como a hidrólise ácida e a extrusão podem causar alterações estruturais e físico-químicas na fibra alimentar, as quais poderiam ser relacionadas com as suas propriedades funcionais *in vitro* e *in vivo*.

3 ARTIGOS CIENTÍFICOS

3.1 ARTIGO 1

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DIETARY FIBRE: THE SCIENTIFIC SEARCH FOR AN IDEAL DEFINITION AND METHODOLOGY OF ANALYSIS, AND ITS PHYSIOLOGICAL IMPORTANCE AS A CARRIER OF BIOACTIVE COMPOUNDS.

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Review

Dietary fibre: The scientific search for an ideal definition and methodology of analysis, and its physiological importance as a carrier of bioactive compounds

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ABSTRACT

There is a growing need for a global consensus on the definition of dietary fibre and the use of appropriate methodologies for its determination in different food matrices. Oligosaccharides (prebiotic effect) and bioactive compounds (antioxidant effect) are important constituents of dietary fibre, which enhance its beneficial effects in the body, such as those related to maintaining intestinal health. These dietary components need to be quantified and addressed in conjunction with fibre in nutritional studies due to the close relationship between them and their common destiny in the human body. This review discusses updates to the concept of dietary fibre, with an emphasis on biological and methodological aspects, and highlights the physiological importance of fibre as a carrier of bioactive compounds.

Contents

1. Introduction

Dietary fiber (DF) is an essential component of a healthy diet and its positive relationship with human health has been established by the scientific community (Kaczmarczyk, Miller, & Freund, 2012). The benefits associated with its adequate consumption include the regulation of the intestinal transit, and the prevention or treatment of diabetes, cardiovascular diseases and cancer of the colon (Kaczmarczyk et al., 2012; Kendall, Esfahani, & Jenkins, 2010). More than 50% of functional foods available in the market have DF as an active component (Giuntini & Menezes, 2011). Because of its importance in protecting and promoting human health, there has been a great increase in knowledge about DF in recent decades, in both the nutritional and analytical areas.

Initially, research focused mainly on the chemical composition of the DF. After the attention was directed to classification based on solubility

Abbreviations: AMG, amyloglucosidase; AOAC, Association of Official Analytical Chemists; DF, dietary fibre; EPA, extractable proanthocyanidins; EPP, extractable polyphenols; HMWDF, high molecular weight dietary fibre; LMWDF, low molecular weight dietary fibre; NEPA, non-extractable proanthocyanidins; NEPP, non-extractable polyphenols.

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in water. In this context, appeared the soluble fibre (SDF) and insoluble fibre (IDF) denominations, and the relationship between these fractions of fibre important for both, dietary and functional properties. These denominations have provided a simple and useful classification for the DF with different physiological properties, according to an understanding at the time (Giuntini & Menezes, 2011).

The beneficial effects and effectiveness of DF depend not only on fibre intake but also on fibre composition, its organisational structure and physicochemical characteristics, and associated bioactive compounds, which are directly related to its plant source and preparation methods (Chau & Huang, 2004; Elleuch et al., 2011; Tungland & Meyer, 2002). Plant fibres show some functional properties, which have been more useful for understanding the physiological effect of DF, than the chemical composition alone. The properties that are nutritionally relevant are mainly the particle size and bulk volume, the surface area characteristics, the hydration and rheological properties, and the adsorption or entrapment of minerals and organic molecules. Among these properties, the viscosity and ion exchange capacity (attributed to the components such as pectin) are the main contributors to metabolic effects (glucose and lipid metabolisms) whereas fermentation pattern, bulking effect and particle size are strongly involved in effects on colonic function (Guillon & Champ, 2000). Functional properties of plant fibre are involved in the biological effects and depend on the IDF/SDF ratio, particle size, vegetable source, as well as the different preparation methods (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005).

In fact, the DF is made up of a complex and heterogeneous group of substances that have different physical, chemical and physiological properties, making it difficult to define and to develop methodologies capable of efficiently reflecting its biological effects (Westenbrink, Brunt, & Van der Kamp, 2013). According to Saura-Calixto (2011), the need for an up-to-date, well-accepted concept of DF has been raised, mainly through studies of the substrates in the human colon and also through studies about bacterial mass and energy balance.

Over the last few decades much information has been discovered about the different compounds present in DF, such as prebiotics (due to the profile of fermentability of specific substances and their interaction with colonic microflora) (Giuntini & Menezes, 2011) and associated bioactive compounds (which can confer antioxidant status to the body) (Saura-Calixto, 2011). These findings triggered conceptual changes and also changes in relation to the analytical methodology of DF.

There is a common interest within the scientific community regarding the need for specific methods to quantify indigestible carbohydrates of low molecular weight (oligosaccharides) because they behave in a similar way to DF in the human body and/or because they have numerous beneficial effects on intestinal health (prebiotics) (Giuntini & Menezes, 2011). However, bioactive compounds associated with polysaccharides in the cell walls (phenolic compounds, carotenoids and phytosterols) deserve greater attention from researchers because they can impart specific properties to DF, such as those derived from their antioxidant capabilities (Saura-Calixto, 2011). Furthermore, the presence of these associated compound influences the physicochemical properties of DF due to increased reactivity with components in complex food matrices (Jakobek, 2015; Sun-Waterhouse, Bekkour, Wadhwa, & Waterhouse, 2014) and may significantly affect the physiological properties and health effects of DF (Saura-Calixto, 2011).

This review discusses updates to the concept of DF, with an emphasis on biological and methodological aspects, combined with the physiological importance of studying the bioactive compounds that are present in this fraction of food.

2. Definition of dietary fibre

The definition of dietary fibre first appeared in 1953 (Hipsley, 1953) in the context of describing the food components from the cell walls of plants. With analytical progress and advances in research in relation to the nutritional and physiological role of DF, this definition evolved significantly until the current definition was arrived at, which was suggested by the Codex Alimentarius Commission (Codex Alimentarius, 2015). A Codex definition is particularly important since Codex sets standards for food for the world and this definition will be used as the basis for analytical methods, food labeling, setting of nutrient reference values and health claims (Mann & Cummings, 2009).Codex defines DF as carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories: (1) edible carbohydrate polymers naturally occurring in the food as consumed; (2) carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities; and (3) synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities (Codex Alimentarius, 2008).

In the early 1970s, cellulose, hemicelluloses and lignin, the named fraction of crude fibre, were known as being important for bowel function and having zero energy value (Giuntini & Menezes, 2011). The first definition of DF that was essentially nutritional in nature was created by Trowell (1976), who considered this fraction of food, such as polysaccharides and lignin, which are resistant to hydrolysis by human digestive enzymes. This definition was adapted to include other components, in addition to that made up of crude fibre, and it prevailed for many years, driving the development of analytical methods for DF that complied with this definition. These methods included Prosky's enzymatic–gravimetric method, which was the basis for the official AOAC 985.29 method, which was approved in 1985. Later, in 1991, there was an extension and optimisation of this method for the determination of DF in foods; the so-called AOAC 991.43 method. Based on scientific findings of nutritional relevance, new constituents were being discovered, and new concepts and classifications of DF were developed over the years (Devries, 2010; Hollmann, Themeier, Neese, & Lindhauer, 2013; Westenbrink et al., 2013).

It is more than 50 years since Hipsley first used the term DF for the non-digestible constituents of plant cell walls and more than 30 years since Trowell adopted the term and suggested a definition for DF. Since this time there has been no accepted international regulatory definition until Codex adopted a final definition. In order to harmonise the concept of DF among all member countries, Codex Alimentarius held several discussions about the definition of DF and analytical methods for its determination. This debate was completed in 2008–2009, and agreed with the definition described previously (Codex Alimentarius, 2008). In this definition, it is recognized that there are three categories of DF, which are not necessarily equivalent. In general, this definition includes all carbohydrate polymers that are neither digested nor absorbed in the human small intestine. The first category recognises intrinsic carbohydrates of the plant cell wall, characteristic of healthy diets, as the major form of fibre. The second and third categories describe extracted and synthetic carbohydrate polymers and clearly state that for such categories to be included as DF competent authorities need to be satisfied that physiological effect of benefit to health has been demonstrated by generally accepted scientific evidence (Mann & Cummings, 2009). Nevertheless, these latter two categories raise important questions for which answers have not been provided, such as: What are the significant physiological effects that may benefit human health? Who determines the level of evidence? Is it necessary that the extension of physiological benefit and the criteria for the evidence base are defined.

Added to this, analytical complications may also arise from the statement in the definition that added carbohydrates, when analyzed as fibre, can only qualify as fibre if they proven its beneficial effect.

However, this differentiation cannot be made analytically. Food producers with knowledge of product formulation can subtract for labeling purposes the added fibre from the total dietary fibre level analytically measured. However, laboratories, food inspection agencies or food composition database compilers without the knowledge of the product recipe cannot, which makes it impossible for them to correctly identify the total dietary fibre content according to the definition (Westenbrink et al., 2013). This author suggest that, from the analytical perspective a preferred option would be to acknowledge as a benefit to health the fact that dietary fibres have a lower caloric value than other carbohydrates.

It was also established that when DF is derived from plant origin, it may include fractions of lignin and/or other compounds associated with polysaccharides in the cell walls, and these compounds can also be quantified by the method(s) specified for DF. These substances are included in the definition of fibre insofar as they are actually associated with the non-digestible oligo- and polysaccharides. However, when extracted or even re-introduced into a food containing non-digestible carbohydrates they cannot be defined as DF. When associated with polysaccharides, these substances may provide additional beneficial effects (Codex Alimentarius, 2008).

The main controversy about the actual definition of DF adopted by the Codex Alimentarius refers to the inclusion of low molecular weight carbohydrates, oligosaccharides, consisting of 3 to 9 monomer units. This question was not resolved, and it was determined that such a decision would be at the discretion of national authorities (Codex Alimentarius, 2009). Thus, a footnote was added to the definition to allow national authorities to include these carbohydrates.

Institutions from various countries such as Canada, China, Australia, New Zealand and some European Union countries, as well as reference groups in the area (e.g. AOAC, AACC and IOM), have been chosen to include oligosaccharides in their definitions (de Menezes, Giuntini, Dan, Sardá, & Lajolo, 2013; Westenbrink et al., 2013). The American Association of Cereal Chemists (AACC, 2001) chose to include oligosaccharides in its definition of DF because these substances exhibit some of the same physiological properties as their larger counterparts. The AACC defined DF as "the edible part of plants, or analogous carbohydrates, that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. DF includes polysaccharides, oligosaccharides, lignin and associated plant substances." This was the first time that substances associated with DF, such as phenolic compounds, were included in the definition of DF (Vitaglione, Napolitano, & Fogliano, 2008).

Some researchers argue that although these carbohydrate polymers have unique potentially beneficial properties, these are not characteristic of traditional DF. Furthermore, consumers may be misled and tempted to get their recommended DF intake from such products instead of fruits, vegetables and cereal products (Codex Alimentarius, 2008; Mann & Cummings, 2009). However, a strong tendency of the scientific community to include the oligosaccharides in the definition of DF is noticed. In fact, it is known that some components of DF have no direct relationship with some beneficial effects associated to the consumption of DF. Or also some of these benefits are more specific or more pronounced for some substance or group of substances, as the several beneficial properties attributed to the soluble components of DF (e.g. pectin). In this sense, it is known that oligosaccharides are not associated with all beneficial effects of DF, however they are also unavailable carbohydrates and have important physiological characteristics similar DF in the body.

de Menezes et al. (2013) argue that there are no scientific, methodological or physiological reasons that indicate that unavailable carbohydrates behave differently when they have more or less than 10 monomer units. Both types of carbohydrate are resistant to hydrolysis by human digestive enzymes and provide beneficial health effects that are commonly associated with fibre. Some of the physiological benefits related to oligosaccharides include: stimulation of intestinal microflora, with the production of short-chain fatty acids and reduction of intestinal pH; the reduction of gastrointestinal infections; a decrease in insulin response and glucose uptake; and improved blood lipid profile. Additionally, oligosaccharides are low in calories and are anticarcinogenic; they prevent and ameliorate the symptoms of diarrhea, as well as stimulate the absorption of minerals such as calcium, magnesium and iron (Moura, Macagnan, & da Silva, 2015).

Besides that, according to (Westenbrink et al., 2013), carbohydrate oligomers with 2 and 3 monomeric units can easily be differentiated analytically, whereas differentiation between 9 and 10 units tends to be more complicated. From the analytical perspective, the choice to include non-digestible carbohydrates from 3 monomeric units in the DF definition is the preferred choice.

Therefore, due to the lack of a clear reason to justify the exclusion of oligosaccharides from the definition of DF a consensus arose within the scientific community about the inclusion of these carbohydrates in the definition, and consequently in the methodologies for DF analysis and in food databases. This would bring about a universally accepted definition throughout many countries, allowing, for example, a comparison of DF consumption in different geographic regions, the reduction of barriers to international trade and, above all, a better interpretation of the results of studies that evaluate the beneficial physiological effects of foods. Moreover, such inclusion would boost the supply of healthier products, which would be directly reflected in terms of consumer health (de Menezes et al., 2013).

3. Official methods to analyse dietary fibre

In general, the establishment and the improvements observed in the methods developed for the analysis of DF, have been based on analytical methods for the isolation of DF focusing on its physiological aspects. In this context, various classifications of DF fractions can be found in the scientific literature and these are based on the analytical methods that are used. The classic methods until about 2005 were AOAC 985.29 and 991.43, which were the official methodologies recommended for DF analysis in food. Both these methods quantify only components of high molecular weight DF (High Molecular Weight Dietary Fibre — HMWDF). The first method directly determines the total dietary fibre of a food and the second method distinguishes the soluble and insoluble fractions of fibre (Devries, 2010). However, such methods are inadequate to determine a new category of DF referred to in Codex definition, that of low molecular weight (Low Molecular Weight Dietary Fibre — LMWDF), such as inulin, fructooligosaccharides, galactooligosaccharides and polydextrose (Westenbrink et al., 2013). The new definition of DF introduced by Codex Alimentarius in 2008 includes resistant starch and the option to include non-digestible oligosaccharides. Therefore, the implementation of this definition required new methodology.

Due to the increased use of soluble low molecular weight fibre in food products, the official AOAC methods 985.29 and 991.43 have become inappropriate to quantify the total dietary fibre in foods (Brunt & Sanders, 2013). Furthermore, they may overestimate the energy value in food composition tables and nutrition labels, especially in the case of foods that are rich in oligosaccharides (de Menezes et al., 2013). An additional drawback is that the conventional methods quantify only one of the four categories of resistant starch i.e. RS3 (retrograded starch), which is prevalent in most food products (Brunt & Sanders, 2013; Westenbrink et al., 2013).

Thus, several specific AOAC methods were developed to measure the different components of DF separately, which made it extremely complex to choose a correct measurement of this fraction in an unknown sample. Unfortunately, according to Westenbrink et al. (2013), the application of both methods (classical and specific) is not a solution because there is considerable overlap between the methodologies.

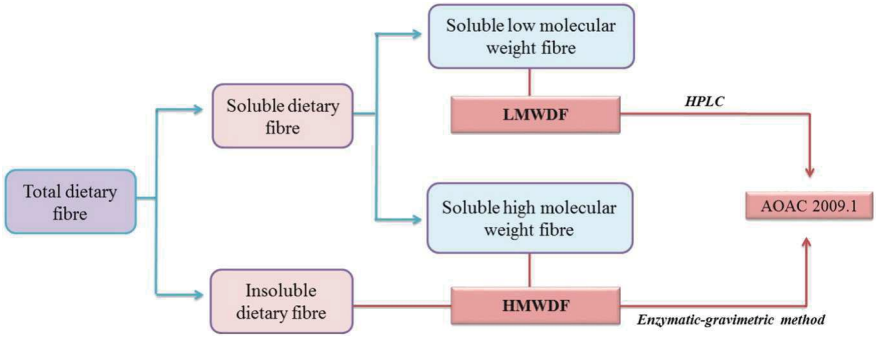

Fig. 1. Classification of the fractions of dietary fibre according to the official AOAC 2009.01 method. HMWDF — high molecular weight dietary fibre; LMWDF — low molecular weight dietary fibre.

In 2007, an integrated method for the determination of total DF, including non-digestible oligosaccharides, was described by McCleary (2007). This process is now known as a method for the determination of total dietary fibre (AOAC 2009.01) and it measures the fraction of high molecular weight dietary fibre (HMWDF) by enzymatic–gravimetric techniques, and low molecular weight dietary fibre (LMWDF) by high-performance liquid chromatography (HPLC) (Fig. 1). The sample is first incubated with α-amylase at 37 °C and then the protein is digested by the protease at 60 °C. The insoluble fibre and soluble high molecular weight fibre (which is precipitated with 78% ethanol) are determined gravimetrically. The non-digestible oligosaccharides are measured in ethanol filtrate by HPLC. This method (AOAC 2009.01) is especially valuable for measuring the DF content in foods enriched with prebiotics because it eliminates the need to apply both the AOAC 985.29 method and a specific method for the analysis of non-digestible oligosaccharides (Brunt & Sanders, 2013).

An extension of the AOAC 2009.01, which is known as the 2011.25 method, was developed (McCleary et al., 2012). In this method, the fraction of high molecular weight of dietary fibre (HMWDF) is divided into soluble and insoluble, which together represent the total contents of HMWDF. Fig. 2 shows the analytical methods used to determine DF. It can be seen that there is overlapping of some techniques when the classic and specific methods are applied and, therefore, the integrated methods (AOAC 2009.01 and 2011.25) are advantageous because they include all the fractions of DF.

Recent studies have compared the dietary fibre values of some foods obtained by the traditional AOAC methods (985.29 and 991.43) with values obtained by the current integrated method (AOAC 2009.01) (Brunt & Sanders, 2013; Hollmann et al., 2013; Westenbrink et al., 2013). Some of the results of this comparison are summarised in Table 1. It can be seen that there was concordance between the values obtained for total dietary fibre by the classic methods, and the values of HMWDF fraction using the integrated method. However, the results obtained using the AOAC 2009.01 method showed that the analysed foods also contained significant quantities of the LMWDF fraction (ranging from 0.9% to 5.1%, depending on the type of food), which causes an increase in the total dietary fibre value, and which had not been previously quantified by the classic methods. This proves that the classic AOAC methods underestimate the total dietary fibre in foods, which influences their final energy value. This difference is

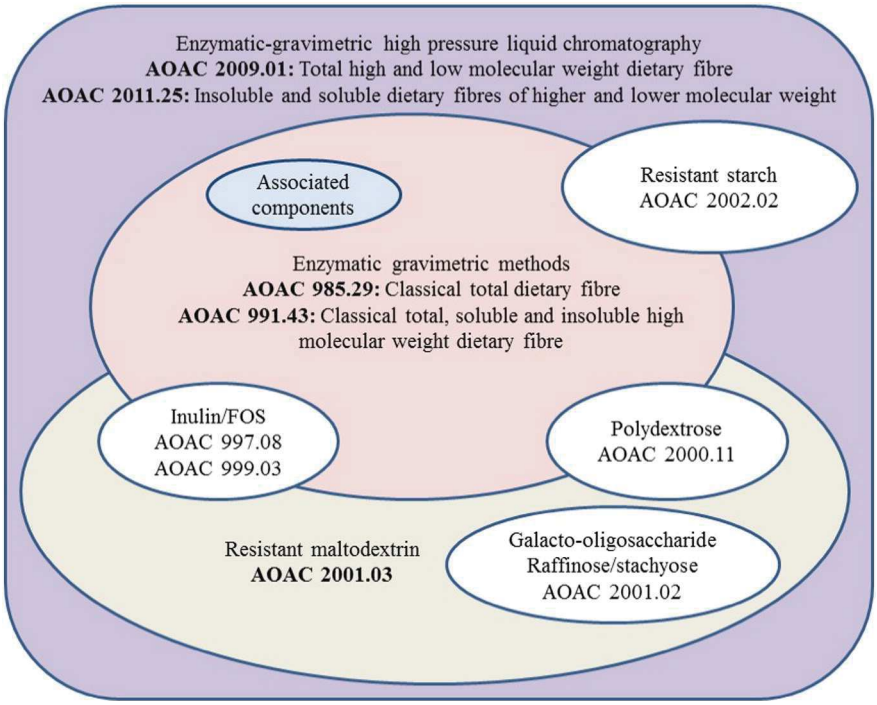

Fig. 2. Schematic view of analytical methods for dietary fibre fractions.
Adapted from Westenbrink et al. (2013) and DeVries (2010).

Table 1
Comparison of level of dietary fibre quantified by classic methods (AOAC 985.29 and 991.43) and by the new method (AOAC 2009.01). TDF, total dietary fibre; SDF, soluble dietary fibre; IDF, insoluble dietary fibre; HMWDF, high molecular weight dietary fibre; LMWDF, low molecular weight dietary fibre.

	Content of dietary fiber (g/100 g)						
	Classical methods				Integrated method		
	AOAC 985.29		AOAC 991.43		AOAC 2009.01		
Foods	TDF	IDF	SDF	TDF	HMWDF	LMWDF	TDF
White bread[a]	3.0	–	–	–	3.0	1.1	4.1
Wholemeal bread[a]	7.5	–	–	–	7.7	0.9	8.6
Currant loaf[a]	3.2	–	–	–	3.5	1.3	4.8
Crude pasta[a]	6.7	–	–	–	6.5	2.1	8.6
Wheat flour[a]	2.4	–	–	–	3.4	2.9	6.3
Wheat grain[a]	12.8	–	–	–	12.4	2.8	15.2
Wheat middlings[a]	46.6	–	–	–	45.6	3.5	49.1
Rye whole meal bread[b]	–	6.3	2.7	9.0	8.9	2.1	11.0
Wheat and rye bread[b]	–	3.2	2.2	5.4	5.5	1.4	6.9
Butter cookies with inulin[b]	–	2.5	1.4	3.9	2.1	5.1	7.2
Rice wafers[b]	–	2.6	0.9	3.5	2.8	1.9	4.7
Salt sticks[b]	–	3.4	1.6	5.0	4.0	2.3	6.3
Currant bread[c]	6.6	–	–	–	6.8	4.7	11.5
Orange juice[c]	0.7	–	–	–	1.0	1.4	2.4

[a] Brunt and Sanders (2013). Obs: values obtained for AOAC 2009.01 method without modification.
[b] Hollmann et al. (2013).
[c] Westenbrink et al. (2013).

particularly significant for foods that contain high levels of resistant starch and low molecular weight fibres, or food products made from such substances.

However, it is noteworthy that the AOAC 2009.01 method has a flaw in relation to starchy food matrices. This is due to incomplete hydrolysis of the available starch, which results in small residual amounts of malto-oligosaccharides in the LMWDF fraction. This error can be easily controlled by introducing an extra amyloglucosidase (AMG) hydrolysis step in the analytical protocol, as shown in a study by Brunt and Sanders (2013). These researchers tested the modification of the technique using samples of wheat grains, wheat flour and products derived from this cereal. They obtained an average level of 33.7% of LMWDF, which was lower when compared to the levels found using the original AOAC 2009.01 method.

Recently, other modifications and adaptations to integrated method (AOAC Method 2009.01 and 2011.25) have been defended and proposed by researchers (Kleintop, Echeverria, Brick, Thompson, & Brick, 2013; McCleary, 2014; McCleary, Sloane, & Draga, 2015; Tanabe,

Table 2
Recommended official methods for the analysis of dietary fibre in foods.

Groups	Methods	Principle/description
General methods that do not measure the lower molecular weight fraction (DP ≤ 9)[a]	AOAC 985.29	Enzymatic gravimetric.
	AOAC 991.43	Dietary fibre quantitated as resistant insoluble and soluble polysaccharides,
	AOAC 992.16	lignin, and plant cell wall.
	AOAC 993.21	Non-enzymatic gravimetric.
		Dietary fibre in food and food products with less than 2% starch.
	AOAC 994.13	Enzymatic chemical.
		Dietary fibre quantitated as component neutral sugars, uronic acids, plus Klason lignin.
General methods that measure both the higher (DP > 9) and the lower molecular weight fraction (DP ≤ 9)	AOAC 2001.03	Enzymatic gravimetric and liquid chromatography. This method includes resistant insoluble and soluble polysaccharides, resistant maltodextrins, lignin, and plant cell wall and has quantification loss for resistant starch. The method is applicable in food where resistant starches are not present.
	AOAC 2009.01	Enzymatic-gravimetric high pressure liquid chromatography.
	AOAC 2011.25	These methods include resistant insoluble and soluble polysaccharides, lignin, resistant starch and oligosaccharides. The method is applicable in food that may, or may not, contain resistant starches.
Methods that measure individual specific components	AOAC 991.42	Enzymatic gravimetric; insoluble dietary fibres.
	AOAC 992.28	Enzymatic; (1 → 3)(1 → 4) β-D-glucans.
	AOAC 993.19	Enzymatic gravimetric; soluble dietary fibres.
	AOAC 995.16	Enzymatic; (1 → 3)(1 → 4) β-D-glucans.
	AOAC 997.08	Enzymatic and HPAEC-PAD[b]; fructans.
	AOAC 999.03	Enzymatic and colorimetric; fructans.
	AOAC 2000.11	HPAEC-PAD[b]; polydextrose.
	AOAC 2001.02	HPAEC-PAD[b]; galacto-oligosaccharides.
	AOAC 2002.02	Enzymatic; resistant starch (RS2 and RS3)

Source: Codex Alimentarius (2009) with the inclusion of AOAC Method 2011.25 (McCleary et al., 2012).
[a] These methods have quantitation loss for inulin, resistant starch, polydextrose and resistant maltodextrins.
[b] High performance anion exchange chromatography with pulsed amperometric detection.

Nakamura, & Oku, 2014), as additional incubation with AMG (McCleary, 2014), and optimization of enzyme levels allowing an incubation time (4 h) with α-amylase and AMG consistent with transit time in the human ileum (McCleary et al., 2015).

The summary of the description of the official methods recommended by the Codex Alimentarius Commission (Codex Alimentarius, 2009) for determination of DF, its fractions and individual components is shown in Table 2.

4. Bioactive compounds as relevant constituents of dietary fibre

There is a common interest among researchers for up-to-date methods for measuring concentrations of resistant starch and oligosaccharides non-digestible in foods, which are important components in human nutrition. However, bioactive compounds with antioxidant properties that are linked to carbohydrates, such as polyphenols, are still omitted from the usual methods of determining DF (Goñi, Díaz-Rubio, Pérez-Jiménez, & Saura-Calixto, 2009). Currently, several studies have been calling attention to the phenolic compounds linked to the food matrices, especially for those associated with cell wall components (Ayoub, de Camargo, & Shahidi, 2016; Cuervo et al., 2014; Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011; Pérez-Jiménez, Elena Díaz-Rubio, & Saura-Calixto, 2015; Pérez-Jiménez & Saura-Calixto, 2015; Pozuelo et al., 2012; Quirós-Sauceda et al., 2014).

DF and antioxidant compounds are two dietary factors recognised to have an effect in the prevention of chronic diseases and in reducing the risk of developing cancer. Because of structural, physical and biological differences between these two components, there is a perceived tendency on the part of the scientific community to treat them separately, i.e. as unrelated compounds. However, both DF and a considerable amount of antioxidant compounds follow a common physiological process, producing synergistic effects within the gastrointestinal tract (Quirós-Sauceda et al., 2014; Saura-Calixto, 2011).

Phenolic antioxidants are most abundant in the human diet and they comprise a complex group of substances (flavonoids, hydroxycinnamic acids, tannins, etc.) with different molecular weights, which can be free or bound to the cell wall in plants and foods (Goñi et al., 2009). Scientific evidence has proved that the phenolic compounds associated with the cell wall of plants are components that are relevant to DF (Goñi et al., 2009; Saura-Calixto, 1998; Saura-Calixto & Díaz-Rubio, 2007) and they constitute approximately 50% of total dietary antioxidants (Pérez-Jiménez & Saura-Calixto, 2015; Pérez-Jiménez et al., 2015; Saura-Calixto, 2011). This interaction can occur during fruit ripening, food processing or during the gastrointestinal process and can be attributed to the ability of polysaccharides to bind and trap phenolic compounds at several sites. Phenolic compounds have both hydrophobic aromatic rings and hydrophilic hydroxyl groups with the ability to bind to polysaccharides and proteins at several sites on the cell wall surface (Quirós-Sauceda et al., 2014).

The results obtained by Goñi et al. (2009) show that polyphenols are significant constituents of DF, representing from 1.4 to 50.7% of the dry weight of insoluble dietary fibre in plant foods and 2.9 to 62.8% of the soluble fibre in common beverages. In this study, fruits and vegetables were analysed as eaten (raw, soaked, boiled, fried, etc.), and wet foods were dried before grinding. The beverages were concentrated at vacuum to get suitable indigestible polysaccharides concentration for further chemical analysis and also to remove alcohol in alcoholic drinks, and beverages containing gas (beer, soft drinks) from previously sonicated. Saura-Calixto and Díaz-Rubio (2007) analyzed the total polyphenols in wines and in corresponding dietary fibre solutions obtained after enzymatic treatments (with pepsin, α-amylase and amyloglucosidase) and dialysis. These authors reported that the polyphenols associated with DF accounted for 30 to 60% of total polyphenols in red wine, and about 9% in white wine. These results showed that these polyphenols associated are not detected by the usual HPLC analytical techniques and consequently may be ignored in most chemical and biological studies.

The largest concentrations of polyphenols associated with DF are found in fruits, largely consisting of tannins. The tannins also are present in high concentrations in vegetables and nuts. Cereals are important dietary sources of hydrolysable polyphenols (Saura-Calixto, 2011), which are primarily associated (95%) with cell wall polysaccharides (Vitaglione et al., 2008). In simplified form, the results obtained in the study by Goñi et al. (2009) show that fruits have the highest proportion of associated polyphenols followed by vegetables and cereals. These polyphenols appear in both fractions of fibre, but in a greater proportion in the insoluble portion.

5. Physiological action of bioactive compounds in relation to dietary fibre

The presence of bioactive compounds associated with the cell wall exerts profound effects on the physicochemical properties of DF and also influences its physiological properties in humans. Dietary fibre contains polyphenols, and these constituents may have a significant role in the DF properties that generally are attributed to polysaccharides, the major constituents of DF. Consequently, the definition of DF restricted to non-digestible polysaccharides and lignin could be extended to include polyphenols (Saura-Calixto, 2012).

Studies report that the use of ingredients rich in fibre and phenolic compounds in the production of functional foods could result in a loss of absorption of the antioxidant, because fibre may trap these substances, reducing the functionality of the proposed food. However, these interactions with DF could give polyphenols a very different role. Some evidence suggests that antioxidant compounds entrapped in DF have a role in maintaining intestinal health (Quirós-Sauceda et al., 2014). In the last years, several authors have studied the health related properties of phenolic compounds present in the dietary fibre, using either isolated polyphenols or polyphenol-rich matrices, combining in vitro models, animal models or humans trials. Some health effects related to these important constituents of dietary fibre are shown in Table 3.

Some dietary antioxidants (vitamins, low molecular weight polyphenols, and carotenoids) are bioaccessible in the small intestine and promptly absorbed by the intestinal mucosa. Others (non-bioaccessible) pass unchanged through the upper gastrointestinal tract in association with DF, reaching the colon, where they can be fermented by the action of bacterial enzymes. Antioxidants in the non-bioaccessible group are mainly phenolic compounds (polymeric polyphenols and low molecular weight polyphenols associated with DF) along with minor amounts of carotenoids and others (Saura-Calixto, 2011). These antioxidants, which are linked to indigestible food compounds, have generally been ignored in most studies, probably because it is believed that they are irrelevant from the quantitative and physiological points of view.

Associated phenolic compounds are not absorbed in the small intestine and, therefore, they reach the colon intact, where they become fermentable substrates for bacterial microflora, along with indigestible carbohydrates and proteins, producing metabolites and promoting an antioxidant environment (Goñi et al., 2009; Manach, Williamson, Morand, & Scalbert, 2005; Quirós-Sauceda et al., 2014; Saura-Calixto, 2011, 2012; Saura-Calixto & Díaz-Rubio, 2007).

The contribution of polyphenols to the antioxidant status of the digesta is relevant for the health of the intestine. The digesta constitutes a separate and important component of the overall antioxidant status of humans, and strategies for reducing oxidative stress should be modified or expanded to include the digesta in addition to blood and tissues. The digesta is a highly active biological system where epithelial cells, microbiota, non-digestible dietary components and a large number of metabolic products interact and comes into direct contact with the very large surface area of the digestive system. Many of these metabolic

Table 3
Health effects related to phenolic compounds presents in the dietary fibre.

Type of study	Material tested	Main results	Reference
Animal models	Bound ferulic acid from bran	Plasmas of rats fed with bran show a better antioxidant activity than the control group and the pure ferulic acid supplemented group, increasing the resistance of erythrocytes to hemolysis by a factor 2 and 1.5, respectively. These results show the good bioavailability of ferulic acid from bran and its potential efficiency to protect organism	Rondini et al. (2004)
	GADF, grape antioxidante dietary fibre (concentrate from grape seeds)	The polyphenols, extractable and non-extractable, exhibited considerable antioxidant status in the large intestine (89% and 80% higher in animals fed with GADF, respectively)	Goñi and Serrano (2005)
	GADF, grape antioxidant dietary fibre (natural product obtained from red grapes)	GADF intake stimulates proliferation of *Lactobacillus* (showed a roughly one-log increase compared with control group) and slightly affects the composition of *Bifidobacterium* species in the cecum of rats	Pozuelo et al. (2012)
	Non-extractable proanthocyanidins (NEPAs) from grapes (preparation free from any extractable polyphenols)	Once subjected to colonic fermentation, NEPAs are a source of absorbable and bioactive metabolites and are in contact with the intestinal tract and bioavailable for at least 24 h after ingestion	Mateos-Martín, Pérez-Jiménez, Fuguet, and Torres (2012)
	Grape antioxidant dietary fibre (obtained from red grapes)	Reduced intestinal tumorigenesis in mice with colon cancer, significantly decreasing the total number (76%) and size of polyps (65 to 87%)	Sánchez-Tena et al. (2013)
In vitro models	Extractable (EPPs) and non-extractable (NEPPs) polyphenols isolated from industrial apple waste	NEPPs at the concentration of 1 mg/mL had significant inhibitory effects against all tested cancer cells (46.2% to 95%), where EPP showed lower effect (3.9% to 22.2%). These results clearly indicated that NEPPs from industrial apple waste could be a good source of natural antioxidants with significant antiproliferation efficacy against human cancer cells	Tow, Premier, Jing, and Ajlouni (2011)
	Cell wall components in some fruits (Chinese quince, quince, hawthorn, apple, pear and blueberry)	Non-extractable procyanidins and lignin are important fruit cell wall components that contribute to the bile acid binding (ranging from 4.5 to 15.9 μmol/g) and radical scavenging activities of the fruit. In the DPPH assay, the activities expressed as 1/EC50 values ranged from 0.34 (hawthorn) to 5.63 (quince)	Hamauzu and Mizuno (2011)
Human Trials	Grape antioxidant dietary fibre (natural product obtained from red grapes peels and seeds)	An acute intake of a dietary fiber rich in associated phenolic antioxidants increased antioxidant capacity of plasma in relation to a control group, becoming significant 8 h after the intake (area under the curve in ABTS assay 3.6% lower in a control group). No significant changes were observed after long-term intake	Pérez-Jiménez, Serrano, Tabernero, Arranz, and Díaz-Rubio (2009)
	GADF, grape antioxidant dietary fibre	GADF (7.5 g/d) showed significant reducing effects in lipid profile and blood pressure (reduced 6% for systolic and 5% for diastolic blood pressures). Greater reductions in total cholesterol (14.2%) and LDL cholesterol (11.6%) were observed in hypercholesterolemic subjects. No changes were observed in the control group subjects.	Pérez-Jiménez, Serrano, et al. (2008)

products are free radicals produced by metabolism of food components and by interaction of bacteria with the contents of the colon. Thus, the presence of antioxidants can protect the exposed surface or may enter the cells lining the digestive system and provide protection inside the cell (Goñi & Serrano, 2005). According to (Saura-Calixto, 2011), it has been hypothesized that the association between dietary fibre and polyphenols is able to exert a considerable reducing activity in both the small and large intestines before fermentation. Because this insoluble material remains in the gastrointestinal tract for a long time, it has the capacity to quench the soluble radicals formed in the gastrointestinal tract by a surface interaction and protein.

It is currently known that only the intestinal microflora can naturally disrupt this fibrous matrix and release the associated antioxidant compounds in soft physiological conditions. Intestinal bacteria are able to hydrolyse, reduce, decarboxylate, dehydroxylate and demethylate polyphenols, and thus produce various metabolites. This enormous capacity to extract and transform the indigestible polyphenols, which greatly outstrips physicochemical and biotechnological treatments, is a consequence of the genomic complexity and variety of the human microflora (Saura-Calixto, 2011). Phenylacetic, phenylpropionic and phenylbutyric acids are examples of absorbable metabolites formed by the colonic fermentation of polyphenols, which can have systemic effects (Rechner et al., 2004; Saura-Calixto et al., 2010). Saura-Calixto et al. (2010) investigated the colonic fermentation of non-extractable proanthocyanidins (NEPAs) associated with DF, using a model of in vitro small intestine digestion and colonic fermentation. The main metabolite identified after 24 h of colonic fermentation from *Ceratonia siliqua L. proanthocyanidin* (CSPA) and indigestible fraction of grape antioxidant (IF-GADF) was hydroxyphenylacetic acid with concentrations of 8.7 and 8.3 ng/mg of dry sample, respectively. This metabolite may be the result of dehydroxylation of dihydroxyphenylacetic acid, which is typically formed during in vitro colonic fermentation of flavonols. Hydroxyphenylvaleric acid was the second main metabolite detected in the samples (2.7 ng/mg for IF-GADF and 2.1 ng/mg for CSPA). Another aim of this work was therefore to determine proanthocyanidins (PAs) fermentation metabolites in plasma of healthy subjects. The results show that the main PA metabolite in human plasma was also the hydroxyphenylacetic acid (was also detected in its methylated and sulphated forms, indicating probable conjugation in the liver), followed by hydroxyphenylvaleric and hydroxyphenylpropinic acids. According to the authors, the presence in human plasma of the same metabolites as were detected after in vitro colonic fermentation of NEPAs means that included these compounds in a regular diet would undergo colonic fermentation, releasing absorbable metabolites with potential healthy effects.

Pérez-Jiménez et al. (2009) measured the antioxidant capacity of plasma to demonstrate that the phenolic compounds associated with DF are partially bioavailable in humans, although the fibre appears to delay their absorption. Vitaglione et al. (2008) argued that the slow, continuous release of polyphenols associated with the DF of cereals in the intestinal lumen can exert in vivo antioxidant activity, allowing the constant protection of those who consume it against diseases whose etiology and progression are governed by a state of redox imbalance. This slow and continuous release may be more advantageous than very high peaks of antioxidants in the blood, which in some cases also provides a pro-oxidative environment. According to the aforementioned researchers, free polyphenols, which are mainly released from soluble fibre (less cross-linked) by the action of intestinal and microbialesterase, are absorbed in different degrees, passing through the bloodstream where they can exert their health benefits by reducing,

for example, the oxidation of low-density lipoprotein (LDL).For these reasons, it is advisable to convert insoluble fibre into soluble fibre in order to maximise the health benefits of the polyphenols associated with the fibre in cereals, which can be accomplished through chemical, physical or enzymatic treatments (Vitaglione et al., 2008).

Regarding non-absorbable metabolites and unfermented polyphenols, these remain in the lumen of the colon, where they can contribute to the formation of an antioxidant environment through the elimination of free radicals and the neutralisation of pro-oxidant effects in the diet (Goñi & Serrano, 2005; Goñi et al., 2009; Saura-Calixto, 2011, 2012; Vitaglione et al., 2008).

Dietary polyphenols are substrates for colonic microbiota and can exert effects on the modulation of the intestinal microflora by modifying the number and types of bacteria (Saura-Calixto, 2011), and have been a topic of increasing attention by the scientific community in the last years. Some authors suggest that polyphenols can act as a metabolic prebiotic. Studies have shown the action of polyphenols and their metabolites on the growth of beneficial bacteria (*Lactobacillus* spp.) and in the inhibition of pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*) (Cueva et al., 2010; Dueñas et al., 2015; Hervert-Hernández, Pintado, Rotger, & Goñi, 2009). For example, the antioxidant DF in grapes has demonstrated the potential to improve the gastrointestinal health of the host by stimulating the proliferation of *Lactobacillus* species in in vitro tests and also in the cecum of rats (Pozuelo et al., 2012). Indeed, the prebiotic effect could be enhanced when a substantial amount of polyphenols is associated with DF. Therefore, the regular consumption of a diet rich in plant foods with high polyphenol contents may beneficially balance the gut microbial ecology, helping to prevent gastrointestinal disorders and thus enhancing the health of the host (Hervert-Hernández & Goñi, 2011). However, the effects of dietary polyphenols on the modulation of the intestinal ecology and on the growth of gut microbial species are still poorly understood.

Although there is evidence supporting the efficacy of polyphenols in modulating gut bacteria, the concentrations, bioavailability, and method of delivery varied considerably between studies. Wallace et al. (2015) reported no significant changes in the bacterial population when consuming fruit juice beverages containing fruit fibre, fruit polyphenols, and their combination. The hypothesis for the study was that the combination of polyphenols and fibre would have a greater benefit on gut health than the placebo product or the fibre or polyphenols on their own. According to the authors, the food matrix used to deliver the polyphenols can influence significantly their bioavailability, or in other words, food matrix effects and the interactions between polyphenols and coexisting food components during storage and after ingestion can influence the absorption of polyphenols. Thus, more studies on the metabolism of polyphenols by the intestinal microbiota are therefore crucial for understanding the role of these compounds and their effects on our health.

In addition, researchers also have shown an inverse association between dietary fibre with associated antioxidants consumption and colon cancer (Quirós-Sauceda et al., 2014). Lizarraga et al. (2011) report that consumption of a lyophilized red grape pomace containing proanthocyanidin-rich dietary fibre (grape antioxidant dietary fibre, GADF) by female mice induced alterations in the expression of tumor suppressor genes and proto-oncogenes as well as the modulation of genes from pathways, including lipid biosynthesis, energy metabolism, cell cycle, and apoptosis. This research provides evidence that GADF protects healthy colon tissue against tumor development and reduces the risk of cancer. In particular, phenolic compounds, dietary fibre components and their metabolites come into contact with the gut wall for up to several hours (more than 24). For this reason, the antioxidant environment formed in the colon could modulate the incidence of certain kinds of degenerative diseases, such as colon cancer (Quirós-Sauceda et al., 2014).

Some of the main biological properties associated with the presence of polyphenols linked with DF which have been reported in animal experiments are as follows: increased excretion of lipids, protein, water and feces; positive effects on lipid metabolism in hypercholesterolemic mice, reducing lipid peroxidation, total cholesterol, LDL cholesterol and triglycerides; increased antioxidant activity in the large intestine and the cecum; and inhibition of the proliferation of epithelium of the colon, reducing the total number of crypts in rats (Saura-Calixto, 2011). These results suggested positive effects on gastrointestinal health as well as a reduction in the risk of developing cardiovascular disease and cancer of the colon.

6. Determination of bioactive compounds associated with the fibrous matrix

Several original works and reviews have been published about the antioxidant capacity of plant foods and other materials, however, more attention should be paid to critical steps such as sample preparation or the procedure for extraction of antioxidants such polyphenols. In fact, antioxidant capacity may be a key parameter for both food science and technology and nutritional studies, and for this reason, there is presently a need to develop a correct methodology to measure the content of polyphenols and the total antioxidant capacity in foods.

Polyphenols in foods are classified into two groups: extractable polyphenols (EPPs) and non-extractable polyphenols (NEPPs). According to Saura-Calixto (2012), this classification of dietary polyphenols is a physiological or nutritional concept and not just a concept derived from chemical analysis. EPPs are phenolic compounds of low and medium molecular weight soluble in organic and aqueous solvents (methanol, acetone, ethanol, ethylacetate, etc.). These compounds have a wide range of chemical structures, including flavonoids (flavanols, anthocyanins, flavonols, other subgroups), benzoic and hydroxycinnamic acids, stilbenes, extractable proanthocyanidins (EPA), hydrolysable tannins, and others. EPPs are dissolved in the stomach and small intestine, where they can be absorbed at least partially through the small intestinal mucosa followed by metabolism and systemic effects (Saura-Calixto, 2012). NEPPs are compounds of high molecular weight (condensed tannins and hydrolysable phenolic compounds), or polyphenols that are linked to DF and protein, which can be found in the wastes from aqueous and organic extracts (Pérez-Jiménez & Saura-Calixto, 2005). NEPPs are those dietary polyphenols that, after ingestion, are not significantly released from the food matrix either by mastication, an acidic pH in the stomach or the action of digestive enzymes (Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2013). These compounds are associated with DF by various interactions (hydrogen bonds, hydrophobic interactions and covalent bonds) and are physiologically released from the fibrous matrix by the action of bacterial enzymes in the colon (Saura-Calixto, 2011).

Polyphenols and antioxidant activity are usually measured in food extracts obtained with chemical aqueous-organic solvents. However, there is no solvent that would be entirely satisfactory for extraction of all the antioxidants present in a food, especially those associated with DF. The efficient extraction of these bioactive compounds requires the use of solvents with different polarities and can be improved by the use of acidified solvents (Pérez-Jiménez, Arranz, et al., 2008). Furthermore, studies showed that the extraction is influenced by many other factors such as sample preparation (drying processes and particle size) and the procedure used for the extraction in different food matrices (time, temperature) (Esposito et al., 2005; Saura-Calixto et al., 2010; Sun-Waterhouse et al., 2014).

In this context, there is a considerable amount of antioxidants remaining in the extraction residues, which are ignored in most chemical and biological studies. Most data in the literature regarding polyphenols in foods have been obtained from HPLC analysis of the aqueous or organic extracts of samples (particularly ethanol, ethanol:water or methanol). Thus, these analyses are unable to determine and quantify the antioxidants linked to DF and they underestimate the antioxidant capacity of foods, particularly those that are rich in DF

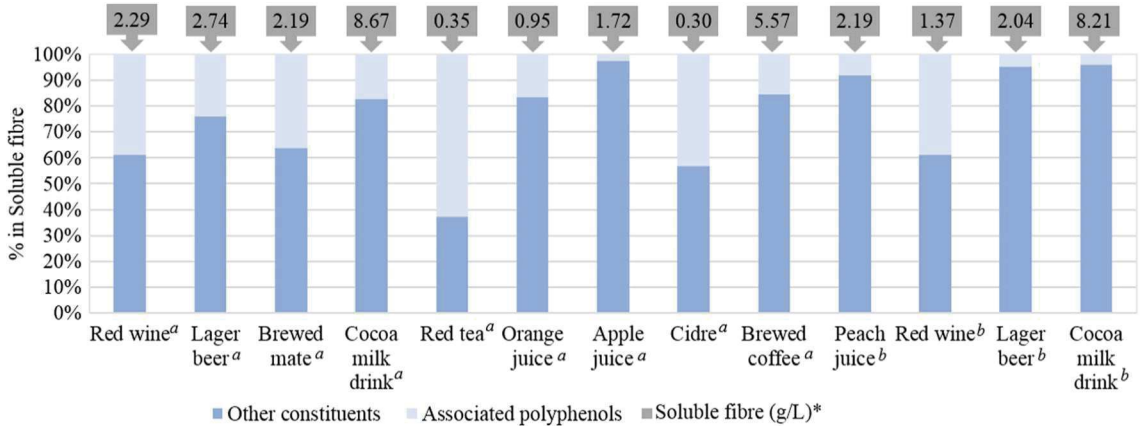

Fig. 3. Soluble fibre and its constituents in beverages. *Dietary fibre content including associated polyphenols. [a] Goñi et al. (2009). [b] Saura-Calixto (2011).

(Saura-Calixto & Díaz-Rubio, 2007). Consequently, biological, nutritional and epidemiological studies generally only investigate extractable polyphenols (bioaccessible and absorbable antioxidants in the small intestine). This means that there are few in vivo scientific studies that address the properties related to health of NEPP associated with DF, compared to hundreds of studies that focus on bioavailable polyphenols in the small intestine.

In fact, if these residues are treated with acids or with enzymes (cellulases, proteases, digestive enzymes, bacterial enzymes), significant amounts of polyphenolic compounds release from the food matrix, which can be analyzed in the corresponding hydrolysates. This shows that a complete determination of PP in foods requires analysis of both extracts and extraction residues (Saura-Calixto, 2012).

The determination of NEPP requires strong acid treatments (HCl, Butanol, H_2SO_4) to release polyphenols from the fibrous matrix. Firstly, acidic conditions (methanol/H_2SO_4; 85 °C/20 h) are required to break the cell wall structure and to hydrolyse the polysaccharides and proteins, allowing the release of hydrolysable polyphenols for evaluation. A further treatment using HCl/butanol (100 °C/60 min) allows the quantification of condensed tannins. The profile and level of NEPP are determined by these two hydrolysates using HPLC–MS and spectrophotometric methods) (Arranz, Silván, & Saura-Calixto, 2010; Pérez-Jiménez & Saura-Calixto, 2005; Pérez-Jiménez et al., 2009; Saura-Calixto, 2011). According to Saura-Calixto (2011), strong acid treatments can degrade some phenolic compounds, especially flavonoids, but acid hydrolysis results in a high recovery rate of polyphenols, and thus far it is the only viable alternative for the analysis of NEPP.

The antioxidant activity of fibre can be enhanced by the presence of polyphenols. Their determination will soon be useful to complement the characterisation of fibre, to estimate its effects on health, and to direct its use as a functional ingredient (Saura-Calixto, 2011). However, this antioxidant activity has been largely overlooked because of the low solubility in water and organic solvents of the associated bioactive compounds. In fact, the correct assessment of this antioxidant capacity requires multiple extraction stages and chemical hydrolysis to release the polyphenols associated with DF, and to enable them to exert antioxidant activity in in vitro assays (Vitaglione et al., 2008).

In recent years, the search for novel sources of DF with antioxidant properties focused widely on plant food by-products (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014). Antioxidant capacity related to DF is measured in its extracts and hydrolysates by common methods (FRAP — ferric reducing antioxidant power, DPPH — 2,2-diphenyl-1-picrylhydrazyl, ABTS — 2,2-azinobis 3-ethyl-benzothiazoline-6-sulfonic acid, ORAC — oxygen radical absorbance capacity) and it represents the quantity of antioxidants units that are carried by it in the human intestine. DF with exceptional antioxidant capacity, which is known as antioxidant dietary fibre, has been found in mango peel, pineapple peel, guava pulp, grape pomace, acerola and some algae (Saura-Calixto, 2011). These materials promise an enhancement in functional properties of foods and at the same time an increase in the antioxidant capacity of the product with exceptional effects on human health (Quirós-Sauceda et al., 2014).

7. Determination of dietary fibre, including oligosaccharides and associated polyphenols, in foods and beverages

The official analytical methods for DF have been developed specifically for solid foods, which has meant that the food composition tables and much of the data contained in the literature consider the DF content in beverages to be zero. However, a certain amount of soluble DF can pass through a beverage during maceration and/or the extraction of solid plant material, which means that some beverages contain appreciable soluble DF content and associated polyphenols that need to be considered (Goñi et al., 2009; Saura-Calixto, 2011). Fig. 3 shows the soluble fibre values in some beverages (from 0.3 to 8.67 g/L) and demonstrates that polyphenols contribute a substantial portion of fibre in beverages such as wine, beer and coffee (38%, 24%, and 15%, respectively). This is biologically significant in that such substances are not bioavailable in the upper part of the human intestine, but they may be susceptible to degradation by colonic microflora.

The presence of polyphenols in the DF obtained by AOAC methods has been reported in studies of plant by-products. However, Goñi et al. (2009) proposed a new methodology for determining the DF content in solid foods and drinks, which agrees with the updated definition of DF, including low molecular weight carbohydrates and polyphenols.

One of the main advantages of the method proposed by Goñi et al. (2009) is that enzymatic treatments are performed at a temperature and pH close to the environment of the human digestive tract, which requires more time for analysis. These analytical conditions, which are almost physiological, prevent changes in the digestibility of starch and protein which may occur when using high temperatures in the classic methods (60–100 °C). Thus, the results obtained for DF using the method of Goñi et al. (2009) are close to the values of substrates that reach the human colon. Another advantage of this method is that the soluble and insoluble fractions are obtained by centrifugation and dialysis, which avoids the loss of indigestible carbohydrates, such as oligosaccharides, which remain solubilised in ethanol. This procedure also allows the isolation of each of these fractions for further chemical–physical analyses, the evaluation of bioactive compounds and antioxidant activity associated with these fractions, and also testing in biological assays.

In short, the substantial amount of antioxidant compounds associated with DF in foods and beverages, along with their relevant physiological properties, supports the inclusion and analysis of these compounds as a constituent of DF and their joint assessment in clinical and biological trials.

8. Conclusion

There has been a great increase in knowledge about DF in recent decades, in both the nutritional and analytical areas. In this context, the concept of DF has evolved significantly and changed from a simple chemical concept for a concept with more physiological focus. It was a long scientific discussion until the current definition was arrived at, suggested by the Codex Alimentarius Commission. However, there are issues that still need to be resolved regarding the current definition of DF, highlighting the importance of including in this definition the low molecular weight carbohydrates (oligosaccharides), because they behave in a similar way to DF in the human body and have numerous beneficial effects on intestinal health. Therefore, the implementation of the new definition of DF and the inclusion of oligosaccharides, require the use of analytical methods that comply with this concept, such as the integrated method (AOAC 2009.01) and its subsequent adaptations and modifications.

The bioactive compounds (such as polyphenols) also are relevant constituents of DF, however they are still omitted from the usual methods of determining. In fact, the transportation of these bioactive compounds through the gastrointestinal tract and production of fermentation metabolites in the colon appear to be important physiological function of DF. This role as a carrier of dietary polyphenols to the colon contributes to intestinal health (modulation of gut microbiota and antioxidant status), nevertheless, should be studied in more detail. In this context, research on association of DF with phenolic compounds offers to be a very promising area.

As seen in this review, the oligosaccharides and phenolic compounds associated with the cell wall of plants potentiate the beneficial effects of DF, because they can impart specific properties to it (as prebiotic and antioxidant effects). Because of this, it is important that they are quantified and addressed in conjunction with the fibre in nutritional studies. This will enable a better interpretation of the results of studies involving the physiological effects of food and the beneficial properties of dietary fibre.

Thus, it is critical to use appropriate methodologies for the analysis of dietary fibre in foods. These should follow the concept of the importance of dietary fibre in the promotion and maintenance of human health.

Conflict of interest

The authors declare no conflict of interest.

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3.2 ARTIGO 2

Artigo submetido à Revista **Food Chemistry**

ACID HIDROLYSIS IMPROVES THE FUNCTIONAL PROPERTIES OF APPLE FIBRE

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Abstract

The possibility of acid hydrolysis altering the physicochemical properties of apple dietary fibre and improving its prebiotic effects was investigated. Apple pomace fibre was submitted to H_2SO_4 treatment and experimental conditions (reaction time and acid concentration) were determined by rotatable central composite design and the results were assessed by the response surface method. Treatment of apple fibre with H_2SO_4 did not increase the soluble fraction, but it improved all the physicochemical properties evaluated, indicating that the acidification process was efficient and produced changes in the structure of the fibrous matrix. In general, more aggressive conditions (lower pH and longer reaction time) enhanced the capacity of the fibre to bind bile acids (55%), to inhibit pancreatic lipase activity (206%), and to retain water (11%) and fat (20%) in its structure. Moreover, the hydrolysed fibre (H_2SO_4 1.5N for 3 h) significantly stimulated the growth of *Bifidobacterium lactis* and *Lactobacillus acidophilus*

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after 48 h of *in vitro* incubation, and the growth was larger ($p < 0.05$) than that detected for the untreated fibre for both probiotic bacteria and similar ($p > 0.05$) to that seen in the inulin-containing broth for *B. Lactis*.

Keywords: physicochemical properties, prebiotic effect, apple pomace, soluble fibre.

1 Introduction

Currently, over 50% of commercially available functional foods have dietary fibre (DF) as their active ingredient.The benefits associated with its consumption include regulation of intestinal transit and prevention or treatment of diabetes, cardiovascular diseases, and colon cancer (Macagnan, da Silva, & Hecktheuer, 2016). The physicochemical properties of DF directly influence the intensity of these beneficial effects and are related to the vegetable source, to the ratio between soluble and insoluble fractions, to particle size, and also to the mode of preparation (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005; Guillon & Champ, 2000).

Apple pomace is an agroindustrial by-product from apple processing and could account for 20 to 40% of the weight of processed apples, depending on the technology used for extraction (Paganini, Nogueira, Silva, & Wosiack, 2005). Apple pomace is utilised for animal feed or discarded inappropriately, causing serious environmental problems. However, apple pomace has a high nutritional potential as alternative source of DF (Macagnan et al., 2015).

Knowledge of the physical and chemical properties of vegetable fibres can be related to their physiological behaviour in the human intestine (Zaragoza, Pérez, & Navarro, 2001). Porosity and the available surface of DF can influence its fermentability (availability for microbial degradation in the colon), while the regiochemistry of the surface layer plays an important role in some physicochemical properties (adsorption or binding of some molecules), and viscosity and ion binding capacity are the major contributing factors for the effects of DF on glucose and lipid metabolism (Guillon & Champ, 2000). In this respect, the physicochemical

properties of DF can be manipulated through chemical, physical, and enzymatic treatments or through a combination thereof, in order to potentiate their functional properties (Caprez, Arrigoni, Amado, & Neukom, 1986; Chau, Wang, & Wen, 2007; Cornfine, Hasenkopf, Eisner, & Schweiggert, 2010; Daou & Zhang, 2012; Esposito et al., 2005; Guillon & Champ, 2000; Hu, Wang, & Xu, 2008; Larrea, Chang, & Martinez-Bustos, 2005; Napolitano et al., 2006; Nyman & Svanberg, 2002; Wennberg & Nyman, 2004).

Recent studies have shown that different chemical changes improve the physicochemical properties of DF (Park, Lee, & Lee, 2013; Pourfarzad, Mahdavian-Mehr, & Sedaghat, 2013; Qi et al., 2016; Wang, Xu, Yuan, Fan, & Gao, 2015; Yang, Xiao, & Wang, 2014). Hydrolysis of cell wall polysaccharides can increase the concentration of soluble fibres. In addition, the swollen volume of insoluble fibres tends to increase, probably due to larger porosity. Chemical hydrolyses are capable of producing structural changes in DF, modifying solubility, fermentability, and functional characteristics (Guillon & Champ, 2000). Chemical treatments, such as acid hydrolysis of polysaccharides, are relatively simple, have low cost, and are easily controlled, as the reaction is halted by neutralisation of the medium (de Moura, Macagnan, & da Silva, 2015; Vitaglione, Napolitano, & Fogliano, 2008). Therefore, the aim of the present study was to assess the effect of acid treatment on the physicochemical and prebiotic properties of apple fibre.

2 Material and methods

2.1 Preparation of apple pomace fibre (APF)

Apple pomace (AP), obtained after pressing the fruit for juice production, was provided frozen by Fischer Juices (Videira, SC, Brazil). In the laboratory, the pomace was defrosted, pre-dried in a forced-air oven (55 °C for 48 h), and ground in a microgrinder (Model MA 630/1, Marconi, Piracicaba, SP, Brazil) to obtain a powder with a particle size of less than 10 mm.

For concentration of the fibre, the apple pomace was defatted with hexane (two washes, 1: 2.5, m/v). After being defatted, the apple pomace was washed twice with ethanol 80% (1: 2.5, m/v) and heated to 80 °C for 5 min to reduce free sugar content in the sample. After that, the APF was dried in a forced-air oven (55 °C for 2 h).

2.2 Acid hydrolysis of APF

An aliquot of 10 g of APF was partially hydrolysed by 250 mL of different concentrations of H_2SO_4 under stirred conditions at 60 °C. Acid concentrations of 0.29, 0.5, 1, 1.5, and 1.7N and reaction times of 0.59, 1, 2, 3, and 3.41 h were used. At the end of the desired reaction time, the mixture was immediately cooled in an ice bath and centrifuged (2400 g for 10 min). The solid sample (precipitate) was washed with deionised water until neutral pH (Cornfine et al., 2010). The supernatant was neutralised with calcium carbonate and the resulting precipitate was removed by centrifugation (Du et al., 2011). The solid sample was combined with the neutralised supernatant, dried in a forced-air oven (55 °C for 24 h) and ground in a microgrinder (Model MA 630/1, Marconi, Piracicaba, SP, Brazil), thus yielding the hydrolysed apple pomace fibre (HAPF) (Figure 1).

2.3 Chemical composition

The AP and APF were analysed for moisture content (105 ºC for 12 h), ash (minerals) (550 ºC for 5 h), and protein (nitrogen determination via the Kjeldahl method, N × 6.25), according to the techniques described by the AOAC (Association of Official Analytical Chemists, 1995). Lipids were quantified using the method of Bligh & Dyer (1959) and sugar concentrations were measured by the chemical method proposed by Lane-Eynon (Ranganna, 1977).

Total dietary fibre content and soluble and insoluble fractions of samples (AP, APF and HAPF) were determined according to the enzymatic-gravimetric method 991.43 of the AOAC (Association of Official Analytical Chemists, 1995). All chemical analyses were performed in triplicate and the results were expressed as g per 100 g of dry matter (DM).

2.4 Water retention capacity (WRC) and absorption capacity of organic molecules (ACOM)

Water retention capacity (WRC) and the absorption capacity of organic molecules (ACOM) were determined by adapting the method proposed by McConnell, Eastwood, & Mitchell (1974) and Zaragoza et al. (2001), respectively. The samples were weighed (1g) in tared centrifuge tubes and added to excess water (20 mL) and canola oil (15 mL), respectively. After 24-h rest at room temperature, the samples were centrifuged (2.000 g for 15 min). Both WRC and ACOM are expressed in grams of water/oil absorbed in a gram of DM, respectively.

2.5 Cation binding capacity (CBC)

Cation binding capacity (CBC) was estimated by measuring copper binding capacity, determined according to McBurney, Van Soest, & Chase (1983). First, a 0.5g sample was homogenised with 50 mL of $CuSO_4$ (1M) in a sintered crucible and incubated for 1 h at room temperature. After vacuum filtration, the sample was washed with distilled water. A solution of propanol/HCl was then added. After filtration by gravity, the whole filtrate was transferred to a 100-mL volumetric flask and brought up to volume with distilled water. An aliquot of this solution was adjusted to a pH between 8 and 9 with NH_4OH (2M) and then transferred to a 50-mL volumetric flask. A cuprizone (Bis-cyclohexanone-oxaldihydrazone, Sigma) reagent solution (0.5%) was added to the flask and completed to volume with distilled water. Absorbance was read at 590 nm after 30 min. $Cu(NO_3)_2$ solutions were used to prepare the standard curve and the results were displayed as mg of copper per g^{-1} of DM.

2.6 Inhibitory activity (%) toward pancreatic lipase (IALip)

The potential of the sample to inhibit pancreatic lipase activity was assessed using the methodology proposed by Chau et al. (2007). 0.5 g of the sample was weighed in the flask, in addition to 10 mL of olive oil and 50 mL of sodium phosphate buffer (0.1 M, pH 7.2), mixed with 10 mL of pancreatic lipase solution. The pancreatic lipase solution was prepared by adding 7.1 mg of pancreatic lipase (L3126, Sigma Chemical) to 10 mL of sodium phosphate buffer. The test tube was incubated in a water bath at 37 ºC for 1 h and then placed in a boiling water bath to cease the reaction. The amount of free fatty acid released was determined by titration with 0.05 N NaOH. Lipase inhibitory activity (%) was defined as the percent decrease in the free fatty acid production rate compared to the control (no fibre).

2.7 Stimulation of growth of probiotic bacteria

Commercially available lactic acid bacteria (DMS Food Specialties), namely *Bifidobacterium lactis* (Lafiti B94) and *Lactobacillus acidophilus* (Lafiti L10), were used to assess the *in vitro* prebiotic effect of the selected sample and of the unhydrolysed fibre (APF).

To reactivate the probiotic microorganisms, the bacteria were inoculated in MRS (Man, Rogosa and Sharpe) broth and incubated at 37 ºC in an anaerobic jar (Anaerobac reagent, Probac do Brasil) for 15 h. Two millilitres of the activated strains (at predefined concentrations) was inoculated in tubes containing 20 mL of MRS broth, whose carbon source (glucose) was replaced with the samples, in order to assess the bifidogenic potential. Thereafter, the tubes were incubated at 37 ºC for 48 h under anaerobic conditions. Glucose-free MRS broth was used as negative control (without any carbon source) and MRS broth containing inulin served as positive control.

The serial dilution method was utilised for quantification of the microbial population. Aliquots of 1 mL were collected and plated onto a specific medium (MRS agar) using the Pour Plate method and incubated at 37 ºC for 48 h under anaerobic conditions. Quantification consisted of the total count of colony-forming units (CFU), between 30 and 300, performed in triplicate, and the values were expressed as $LogCFU.mL^{-1}$.

2.8 Experimental design and data analysis

A rotatable central composite design (RCCD) was used to verify the relationship between the independent variables for chemical hydrolysis (acid concentration and reaction time) and certain chemical and functional parameters of the modified fibre (IF:SF ratio, WRC, CBC, IALip, and ACOM) and to select the condition that provides the best physicochemical properties for assessment of the *in vitro* prebiotic potential. The 2x2 factor design included four cube points, four star points (two star points on the axis of each variable at a distance of 1.41 from the centre) and three centre points, yielding 11 experiments. The results were evaluated by the Response Surface Methodology (RSM). The coded levels and real levels are shown in Table 1. Based on the results obtained from the experimental design, it was possible to determine the regression coefficients (Table 4) for all assessed parameters. For the analysis of microbial growth, the results were submitted to analysis of variance (ANOVA) and compared by Tukey's test at a 5% significance level. A Pearson's correlation analysis was performed. The Statistica 8 (Statistica 8, Statsoft Inc., USA) software program was used for the statistical analysis.

3. Results and discussion

3.1 Chemical composition of apple pomace (AP) and apple pomace fibre (APF)

The procedure used for preparation of apple pomace fibre, increased DF concentration (±11%) by reducing free sugar (±64%) and lipid (±38.85%) levels (Table 2).

3.2 IF:SF ratio

The chemical hydrolysis of APF increased the IF:SF ratio, especially in more aggressive treatments (assays 3, 4, 9, and 11) (Table 3) and had a stronger effect on the soluble fraction (reduction of 18.30% for assay 9 to 23.44% for assay 11), probably because this fraction has a structure that is more susceptible to hydrolysis. Contradictory results were found by Ning, Villota, & Artz (1991), with a significant increase (23%) in soluble fibre content after acid treatment (HCl, pH 2.0, 90 ºC, 4 h) of maize fibre, and this increase was more effective when combined with alkaline treatment (8.7 times higher). According to those authors, chemical hydrolysis led to the partial breakdown of polysaccharide chains of the major components of maize fibre (cellulose and hemicellulose), weakening the interaction between the polysaccharide chains, thereby reducing the chain length. Therefore, the mobility of polysaccharide molecules increased significantly, facilitating their interaction with water molecules and their solubility. In the present study, acid concentration had a significant and positive effect concerning the IF:SF ratio (Table 4 and Figure 2a). Unlike cereal fibres, apple pomace has a higher concentration of soluble fibre, whose main polysaccharide is pectin (Macagnan et al., 2015). The conditions utilised in this experimental study probably caused partial depolymerisation of this soluble polysaccharide, forming compounds with shorter molecular chains and pectic oligosaccharides, the latter of which are not quantified by the method adopted here, but play a crucial role in fermentability. Hence, acid treatments had a

negligible influence on the solubility of the insoluble fraction, but this could have been achieved with higher temperatures and stronger acids, especially when the intention is to break down crystalline structures such as those of cellulose (Ning et al., 1991). However, these operating conditions could be unfeasible at the industry level. Moreover, preliminary tests indicate that temperatures greater than 60 ºC, combined with very acidic conditions, cause pronounced browning of the sample, in addition to allowing for the formation of undesirable compounds such as furfural derivatives (de Moura et al., 2015).

3.3 Physicochemical properties

Effects of the acid hydrolysis of APF on physicochemical properties (WRC, CBC, IALip, and ACOM) are presented in Table 3. The hydration properties of dietary fibre refer to its ability to retain water within its matrix. Fibres with strong hydration properties could increase stool weight and potentially slow the rate of nutrient absorption by the intestine (Figuerola et al., 2005). The fibre's water retention capacity (WRC) increased by 11% after acidification at 1N and a reaction time of 3.41 h (assay 9), followed by assays 4 and 11 (8 and 7% higher). Despite the likely depolymerisation of pectin, hydration was directly related to reaction time and acid concentration (Table 11 and Figure 2b). Ning et al. (1991) described a 57% increase in water holding capacity of maize fibre submitted to acid treatment. Changes in structure and porosity of the insoluble fibre facilitate penetration and retention of water as they allow the hydroxyl groups to bind more easily to cellulose (Daou & Zhang, 2012). This effect contributed in a more substantial manner to water retention within the fibrous matrix of apples and minimised the effects of pectin degradation.

Cation binding capacity is related to the fibre's ability to bind to ions through groups available on its surface (Anninson & Choct, 1994) and it may indicate the capacity of food to bind bile acids, preventing them from being reabsorbed by the intestinal epithelium. This

potential relies on the presence of phytochemicals (e.g., polyphenols), as well as on the physical and chemical structure of the fibre, hydrophobicity, ionic nature, and level of interaction with active binding sites (Kahlon & Smith, 2007). In the present study, the highest CBC was 39.02 $mg.g^{-1}$ of DM, obtained at the concentration of 1.5N and with a reaction time of 3 h (assay 4). The CBC was 55% higher than that observed for the APF before hydrolysis (25.21 $mg.g^{-1}$ of DM), indicating that hydrolysis caused larger degradation of cell wall material, mainly of pectic compounds. This proportionally increased the amount of small fibre fragments and binding to chemically reactive compounds such as copper and, from the physiological standpoint, it potentially increased binding to bile acids. Likewise, acid hydrolysis (H_2SO_4 1M, room temperature) of lupin (*Lupinus angustifolius* L.) dietary fibre contributed to the higher capacity to bind to bile acids (38%) (Cornfine et al., 2010). In this study, there was a close relationship between incubation time (4, 18, 48 h) and cell wall degradation, chiefly of pectic and hemicellulosic compounds. That indicates that the disintegration of cell wall components makes binding sites more accessible and has a considerable impact on the fibre's capacity to bind to bile acids.

When assessing the fibre's potential to inhibit pancreatic lipase activity (IALip), the highest value was that of assay 11 when a larger concentration of acid was used for 2 h, followed by assay 4. These hydrolysis conditions increased the inhibition of the enzyme by 206 and 194%, respectively. Pancreatic lipase is a key enzyme in the hydrolysis and absorption of lipids (Nakai et al., 2005) and dietary fibre could decrease its activity, thus contributing to the prevention of hyperlipidaemia. This inhibitory potential is related to characteristics of the fibrous matrix itself, such as porosity, level of exposure of inhibitory substances found on the surface, fibre's capacity to encapsulate the oil and the enzyme, and reduction in the access of lipase to oil (Chau et al., 2007). Regarding CBC and InibLip, factors such as acid concentration

(L) and reaction time (L) yielded significant and positive regression coefficients, and an increase in any one of these coefficients enhances the response (Table 4 and Figure 2c and 2d).

The fibre's potential to absorb organic molecules could be related to its capacity to bind to molecules such as triglycerides, cholesterol, bile acids, carcinogenic agents, and toxic compounds in the intestinal tract (Zaragoza et al., 2001). The capacity to absorb organic molecules (ACOM) was strongly correlated (r=0.89) with the lipase inhibition rate (IALip), indicating that the larger encapsulation of fat by the fibre contributes significantly to the inhibition of this enzyme. ACOM was also higher in assay 11, followed by assay 4 (20 and 18% higher than that of the untreated fibre), showing a significant effect of acid concentration (L) (Table 4 and Figure 2e). More acidic treatments possibly have a stronger effect on porosity and physical retention of oil within the fibre structure.

3.4 Stimulation of growth of probiotic bacteria

The sample hydrolysed with H_2SO_4 1.5N for 3 h (assay 4) was selected for the assessment of bifidogenic activity as it showed higher CBC and optimised responses to the other physicochemical properties. The results for the untreated fibre (APF) and for the fibre submitted to chemical treatment (HAPF), along with the positive (inulin) and negative (MRS agar without carbon source) controls, are displayed in Table 5. HAPF remarkably stimulated the growth of *B. lactis* and *L. acidophilus* compared to the negative control and to the initial count, and the growth was larger than that observed for the unhydrolysed fibre for both probiotic bacteria. After 48 h of incubation, HAPF showed that the growth of *B. lactis* was similar to that seen in the inulin-containing broth, without any statistical difference.

Dietary fibre is the major source of energy for intestinal bacteria. Its fermentation produces short chain fatty acids, especially acetic, propionic, and butyric acids, which are crucial for maintaining the integrity of the colonic mucosa and function, playing an important

role in lipid and carbohydrate metabolism (Guillon & Champ, 2000). The surface available for the action of bacteria depends on fibre architecture, and accessibility and structural characteristics of polysaccharides (e.g., chain length, branching pattern, nature of monomers, presence and distribution of functional groups) turn out to be limiting factors for fermentation (Guillon & Champ, 2000). The more susceptible the fibre is to the action of colonic bacteria, the higher the level of fermentation and, therefore, the larger the stimulus to the activity and development of caecal microflora.

Accordingly, the present study revealed that acid treatment increased the prebiotic effect of apple fibre through greater stimulation of probiotic bacteria. This finding is also in line with the hypothesis that the hydrolysis of polysaccharides in apple cell walls yielded fragments with a smaller molecular weight, pectins with a lower degree of esterification, and less reticulate structures, facilitating the use of the substrate and consequent stimulation compared to the unhydrolysed fibre. Likewise, acid hydrolysis (HCl 1.0N) of pectin and mucilage extracted from the cladodes of O*puntia ficus-indica* formed oligosaccharides that stimulated the growth of probiotic bacteria, with an increase of 7 log CFU/mL (25%) in the concentration of bifidobacteria by pectic oligosaccharides, whereas treatment with oligosaccharides derived from the mucilage increased mainly the concentration of *Lactobacillus* (23.8%) (Guevara-Arauza et al., 2012).

4 Conclusions

Acid hydrolysis of apple fibre was not efficient in increasing the soluble fibre content, but it improved all the assessed physicochemical properties. By and large, more aggressive experimental conditions (lower pH and longer reaction time) ameliorated the fibre's capacity to bind bile acids (55%), to inhibit the pancreatic lipase activity (206%), and to retain water (11%) and fat (20%) in its structure. It may be inferred that the chemical reaction conditions

used in the present study probably led to the partial breakdown of cell wall polysaccharides, rendering the internal structure of the fibre looser and more porous, which increased the availability of active binding sites to bile acids, water, triglycerides, cholesterol, toxic compounds, and carcinogenic agents in the intestinal tract.

Apple fibre hydrolysed with H_2SO_4 1.5N for 3 h significantly stimulated the growth of *B. lactis* and *L. acidophilus,* which was greater than that of the untreated fibre for both probiotic bacteria. This finding corroborates that acid hydrolysis changed the molecular structure of the fibre and allowed the better use of carbohydrates by beneficial bacteria found in the human colon. In addition, after 48 h of incubation, the hydrolysed fibre revealed that the growth of *B. lactis* was similar to that observed in the inulin-containing broth, without statistical difference.

The results obtained herein indicate that structural changes produced by acid hydrolysis improved the functional properties of apple fibre, turning it into a promising ingredient to be used in human nutrition.

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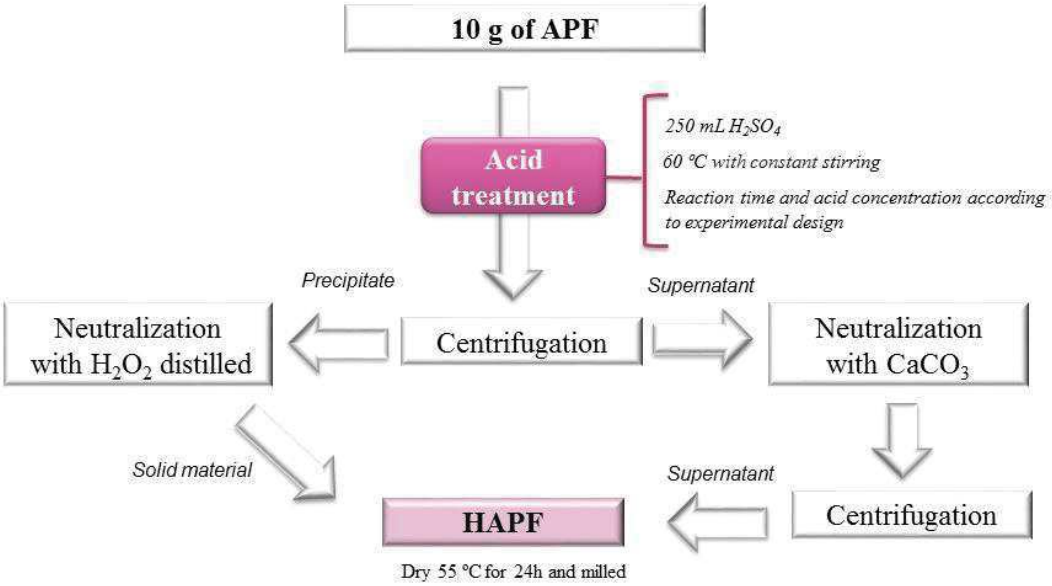

Figure 1. Schematic view of process of acid hydrolysis. APF: apple pomace fibre, HAPF: hydrolysed apple pomace fibre.

Table 1. Coded levels and real levels for independent variables

Independente variables	**Coded levels**				
	-1.41	-1	0	+1	+1.41
	Real levels				
X_1	0.59	1	2	3	3.41
X_2	0.29	0.5	1	1.5	1.7

X_1= Reaction time (hours), X_2 =Acid concentration (N).

Table 2. Chemical composition of the apple pomace (AP) and apple pomace fibre (APF)

Components	**AP**	**APF**
Moisture (%)	4.52	6.75
	% Dry matter	
Ash	1.32	1.34
Protein	7.00	7.24
Lipids	8.03	4.91
Total dietary fibre	72.96	81.14
Insoluble dietary fibre	59.70	66.55
Soluble dietary fibre	13.70	14.59
Sugar	12.75	4.64

Table 3. Effects of acid hydrolysis variables on IF:SF ratio and physico-chemical of apple pomace fibre (APF)

Assays	Independent variables		Responses				
	X_1	X_2	IF:SF ratio	WRC	CBC	IALip	ACOM
				g water.g^{-1} DM	mg Cu.g^{-1} DM	%	g oil.g^{-1} DM
01	1	0.5	4.91	5.81	26.45	13.33	1.88
02	3	0.5	4.68	6.25	27.44	15.43	1.93
03	1	1.5	5.41	6.22	27.83	18.46	1.96
04	3	1.5	5.54	6.42	39.02	26.72	2.07
05	2	1	4.66	6.24	33.98	22.45	1.98
06	2	1	4.67	6.27	35.80	21.49	1.99
07	2	1	4.70	6.25	34.30	23.70	1.97
08	0.59	1	5.13	6.11	25.39	15.28	1.93
09	3.41	1	5.36	6.58	36.20	25.00	1.97
10	2	0.29	4.85	6.18	26.57	10.74	1.90
11	2	1.7	5.60	6.38	30.25	27.78	2.10
APF			4.56	5.95	25.21	9.09	1.75

X_1: Reaction time (hours), X_2: Acid concentration (N), IF: Insoluble dietary fibre, SF: Soluble dietary fibre, WRC: Water retention capacity, CBC: Cation binding capacity, capacity, IALip: Inhibitory activity toward pancreatic lipase, ACOM: Absorption capacity of organic molecules.

Table 4. Regression coefficients for the variables IF:SF ratio, WRC, CBC, IALip and ACOM in the chemical hydrolysis assays

Responses	Factors	Regression coefficients	p-values
IF:SF ratio	X_1 (Q)	0.26	0.0011
	X_2 (L)	0.30	0.0006
	X_2 (Q)	0.25	0.0012
	X_1 X_2	0.09	0.0131
WRC	X_1 (L)	6.25	0.0001
	X_2 (L)	0.16	0.0011
	X_1 X_2	0.11	0.0025
CBC	X_1 (L)	3.44	0.0013
	X_1 (Q)	-1.81	0.0364
	X_2 (L)	2.28	0.0080
	X_2 (Q)	-3.01	0.0052
	X_1 X_2	2.55	0.0195
IALip	X_1 (L)	3.02	0.0039
	X_1 (Q)	-1.51	0.0873
	X_2 (L)	5.07	0.0004
	X_2 (Q)	-1.96	0.0407
	X_1 X_2	1.54	0.1278
ACOM	X_1 (Q)	1.99	0.0001
	X_2 (L)	0.03	0.0166
	X_2 (L)	-0.02	0.0360

X_1: Reaction time (hours), X_2: Acid concentration (N), IF: Insoluble dietary fibre, SF: Soluble dietary fibre, WRC: Water retention capacity, CBC: Cation binding capacity, capacity, IALip: Inhibitory activity toward pancreatic lipase, ACOM: Absorption capacity of organic molecules.

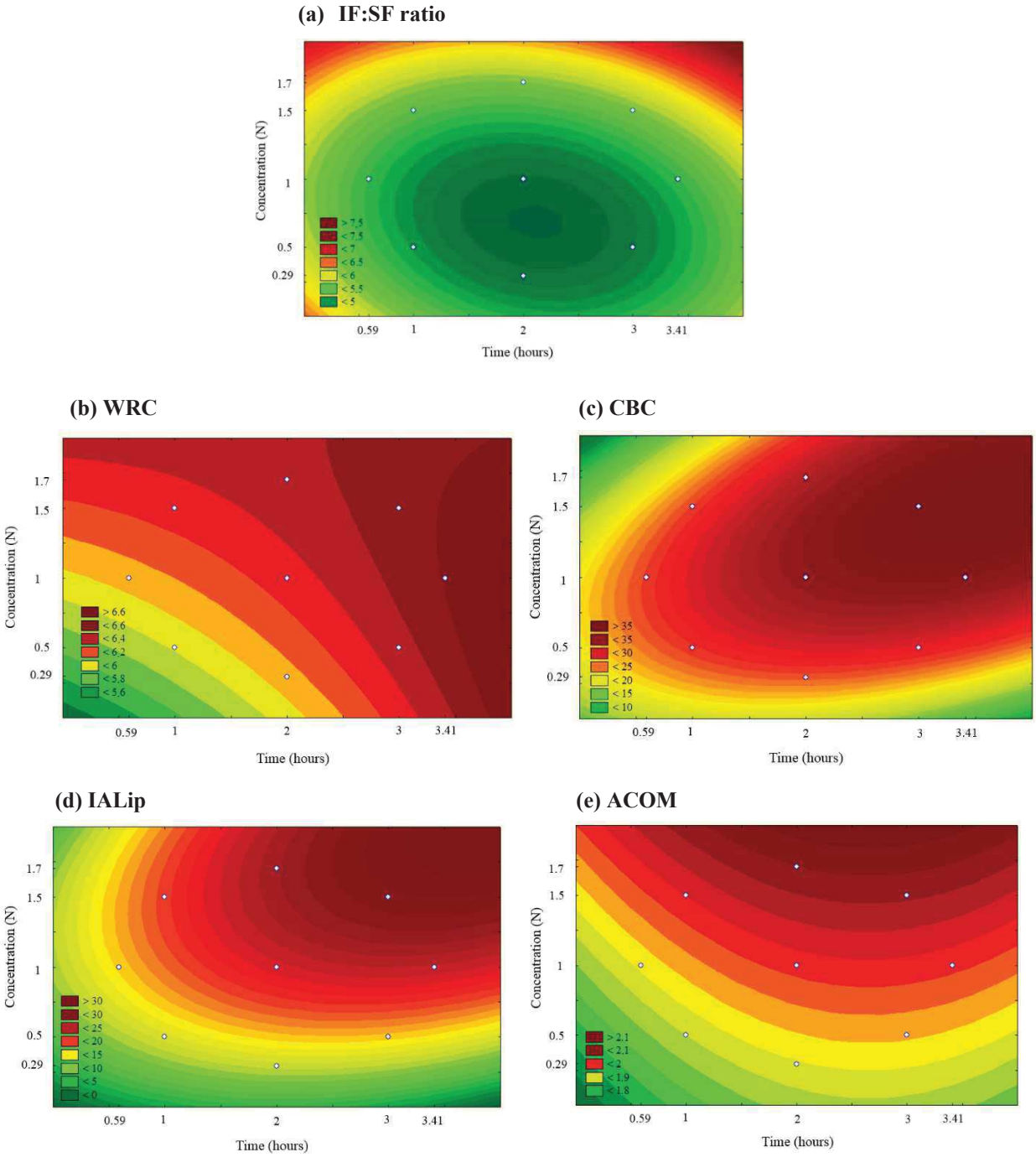

Figure 2. Contour curves of the response surfaces for the variables IF:SF ratio and physico-chemical proprieties as a function of reaction time (hours) and acid concentration (N). (a) Insoluble dietary fibre: soluble dietary fibre ratio (F_c= 32.14 > F_{tab} 0.95, 4.6 = 4.53 R2 = 0.96). (b) Water retention capacity (F_c= 13.91> F_{tab} 0.95, 3.7 = 4.35 R2 = 0.86). (c) Cation binding capacity (F_c= 19.18 > F_{tab} 0.95, 5.5 = 5.05 R2 = 0.86). (d) Inhibitory activity toward pancreatic lipase (F_c= 22.03 > F_{tab} 0.95, 5.5 =5.05 R2 = 0.96). (e) Absorption capacity of organic molecules (F_c= 25.99 > F_{tab} 0.95, 3.7 = 4.35 R2 = 0.92). F_c: Calculated F, F_{tab}: Tabulated F.

Table 5. Growth of *Lactobacillus acidophilus* e *Bifidobacterium lactis* in 48 hours, with values expressed in Log CFU/mL

Time		*L. acidophilus*	*B. lactis*
0 hours	Initial count	7.40 ± 0.06^{e}	7.45 ± 0.07^{c}
48 hours	Negative control	8.44 ± 0.04^{c}	7.35 ± 0.10^{c}
	Inulin	8.96 ± 0.13^{a}	8.36 ± 0.02^{ab}
	APF	8.48 ± 0.05^{c}	8.25 ± 0.05^{b}
	HAPF	8.77 ± 0.05^{b}	8.40 ± 0.03^{a}

Negative control: MRS agar without carbon source, APF: Apple pomace fibre, HAPF: Apple pomace fibre submitted to acid hydrolysis (assay 4).

The results are expressed as the mean ± standard deviation. Mean values followed by a different letter on the same column are significantly different using Tukey`s test ($p<0.05$).

3.3 ARTIGO 3

Artigo submetido à Revista **Food Research International**

MODIFICATION OF APPLE FIBRE THROUGH EXTRUSION

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Abstract

The possibility of extrusion modifying the physicochemical properties of apple dietary fibre and enhancing its biological effects was investigated. Extrusion increased the soluble fibre content by 112.6% and decreased that of insoluble fibre by 22%, without changing total fibre concentration, indicating redistribution among fibre fractions. Cell wall disintegration was larger when experimental conditions were more intense and was confirmed by the reduction in the concentrations of cellulose (8.5%) and hemicellulose (46,8%) and by the increase in soluble pectin content (92%), while lignin content remained unchanged. By and large, more aggressive experimental conditions improved the capacity of apple fibre to bind bile acids (13%), fat (22%), and to inhibit pancreatic lipase activity (by over 70%), but they reduced water retention capacity (8-26%). The energy generated from extrusion led to the partial release of fibre-associated polyphenols, increasing the extractable polyphenol content and reducing that of hydrolysable polyphenols, without changing the concentration of condensed tannins. Only the pomace extruded at 90 ºC and with 33% of moisture showed a significant increase (5%) in its total antioxidant capacity. Extrusion (90 ºC/ 33% of moisture) further stimulated the growth of

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Abbreviations: AC: antioxidant capacity, ACOM: Absorption capacity of organic molecules, AP: Apple pomace, CBC: Cation binding capacity, DF: Dietary fibre, EAP: Extrused apple pomace, IALip: Inhibitory activity toward pancreatic lipase, IF: Insoluble fibre, SF: soluble fibre, TAC: Total antioxidant capacity, TF: Total dietary fibre, WRC: water retention capacity.

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L. acidophilus, but it did not influence the growth of *B. lactis*. The findings obtained in this study demonstrate that structural changes in apple fibre caused by extrusion improved its functional properties and prebiotic effect, turning it into a promising ingredient to be used in functional human nutrition.
Keywords: Apple pomace, soluble fibre, physicochemical properties, polyphenols, prebiotic.

1. Introduction

Dietary fibre (DF) contains several substances with different physical, chemical, and physiological properties. Based on its solubility in water, DF is classified as either soluble (pectin, gums, mucilage, and some hemicelluloses) or insoluble (cellulose, other types of hemicellulose, and lignin) (Elleuch et al., 2011; Mudgil & Barak, 2013). The major effect of insoluble fibre (IF) is the improvement of intestinal peristalsis, which is linked to water retention capacity. The soluble fibre (SF) has multiple functions, being used as substrate for some lactic bacteria and bifidobacteria (prebiotic activity), for control of blood glucose levels, and for reduction of cholesterol plasma levels (Esposito et al., 2005). Pectin, SF found in large amounts in apples, has a high capacity to bind bile acids in the intestine, which is important so that some fibres can contribute to reducing cholesterol serum levels (Cornfine, Hasenkopf, Eisner, & Schweiggert, 2010).

Even though it is important to determine the chemical composition of DF, knowledge about its physicochemical properties is quite useful to understand its physiological effect on the human body, since these properties knowingly influence the gastrointestinal transit of diets, nutrient and bile salt uptake, and lipid and carbohydrate metabolism (Guillon & Champ, 2000; Zaragoza, Pérez, & Navarro, 2001).

Moreover, the presence of antioxidant compounds associated with DF has considerable effects on the physicochemical properties of DF and contributes to its biological effects on humans (Macagnan, da Silva, & Hecktheuer, 2016). SF is less reticulate and can be fermented more easily, which prompts the release of associated polyphenols by the intestinal microflora, increasing their bioavailability to consumers (Vitaglione, Napolitano, & Fogliano, 2008), in addition to producing short-chain fatty acids, which are essential to intestinal health (Tungland & Meyer, 2002).

Apple pomace (AP) is an agroindustrial by-product from the processing of apples, consisting of the heterogeneous mixture of peel, seed, calyx, stalk, and pulp. Apple pomace is utilised for animal feed or disposed of inappropriately, causing serious environmental problems. However, apple pomace is a good source of DF and antioxidant compounds (Macagnan et al.,

2015), both of which are nutritional factors known to help with the prevention of chronic diseases. More than 70% of apple pomace DF is insoluble (Macagnan et al., 2015), which can limit its lipid-lowering and glucose-lowering effects, as well as its prebiotic potential and its antioxidant protection in the body, as this fraction is highly reticulate, hindering the action of the intestinal microflora and the release of polyphenols in the intestine.

Physical treatments such as extrusion can alter the physicochemical and physiological properties of DF. Extrusion cooking consists of heating and homogenisation. In the extruding machine, the material is submitted to intense mechanical shearing and cooking uses high temperature and pressure and low water content. This way, mechanical treatment totally disrupts the original structure of the material (Daou & Zhang, 2012). Furthermore, food processing technologies such as extrusion potentially release phenolic compounds adhered to cell walls (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014) and increase the concentration of bioactive compounds and the antioxidant capacity of the extruded product (Brennan, Brennan, Derbyshire, & Tiwari, 2011).

Depending on the extrusion process conditions, there could be solubilisation of the fibre of some food sources (Vasanthan, Gaosong, Yeung, & Li, 2002; Larrea, Chang, & Martínez Bustos, 2005; Daou & Zhang, 2012; Jing & Chi, 2013; Chen, Ye, Yin, & Zhang, 2014; Arcila, Weier, & Rose, 2015; Yan, Ye, & Chen, 2015; Huang & Ma, 2016). The extruded fibre can also exhibit high viscosity (Daou & Zhang, 2012), higher water and oil retention capacity, and higher swelling capacity (Chen et al., 2014; Jing & Chi, 2013; Yan et al., 2015). Studies also indicate that extrusion can improve the fermentability of the fibre (Arcila et al., 2015) and its lipid-lowering (Chen et al., 2014) and glucose-lowering effects (Céspedes, Martínez Bustos, & chang, 2010).

The referenced studies show that extrusion is a physical process that should be investigated to enhance the functional effects of dietary fibres. In this respect, the aim of the present study is to assess the influence of extrusion on the composition and functional properties of apple fibre.

2. Material and methods

2.1 Preparation of apple pomace (AP)

Apple pomace, obtained after pressing the fruit for juice production, was provided frozen by Fischer Juices (Videira, SC, Brazil). In the laboratory, the pomace was defrosted, pre-

dried in a forced-air oven (55 °C for 48 h), and ground in a microgrinder (Model MA 630/1, Marconi, Piracicaba, SP, Brazil) to obtain a powder with a particle size of less than 10 mm.

2.2 AP extrusion

Extrusion was performed using a single-screw extruder (model Ex-Micro Laboratório, Exteec Máquinas, Ribeirão Preto, SP, Brazil) at different barrel temperature and feed moisture (g/100 g of dry matter) (Table 1). The feeding rate was kept constant at 115 g/min. After processing, the samples were dried in a forced-air oven (55 ºC for 5 h), ground in a microgrinder (Model MA 630/1, Marconi, Piracicaba, SP, Brazil), and stored at -18 ºC in polyethylene bags for further analysis. The extrusion variables were predefined after preliminary tests that allowed obtaining the extrudates.

2.3 Characterisation analysis

2.3.1 Dietary fibre

Total dietary fibre content and soluble and insoluble fractions of all samples were determined according to the enzymatic-gravimetric method 991.43 of the AOAC (Association of Official Analytical Chemists, 1995). Pectin content was obtained according to the method described by Carvalho, Jong, & Bello (2002), based on the neutralisation of the charges of the free galacturonic acid residues by calcium ions, resulting in gelation of pectin and in its precipitation, and calculated as calcium pectate. Neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, and lignin concentrations were determined according to the methodology of Goering & Van Soest (1970). The hemicellulose content was calculated as the difference between NDF and ADF. The results were expressed in g/100 of dry matter.

2.3.2 Concentration of total phenolic compounds and condensed tannins

Phenolic compounds were extracted using the method proposed by Pérez-Jiménez et al. (2008), and the content of phenolic compounds was determined in the aqueous organic extract (extractable polyphenols) and in the residue of this extraction (nonextractable polyphenols: hydrolysable polyphenols and condensed tannins). 0.5 g of the sample was weighed, mixed with 20 mL of methanol/water (50:50,v/v, pH 2) and agitated for 1 h at room temperature. After centrifugation (2500g/ 10 min), the supernatant was allowed to stand. 20 mL of acetone / water (70:30, v/v) was added to the centrifugation residue and the previous step was repeated. The

methanolic and acetonic extracts were mixed and the final extract was combined with extractable polyphenols.

In order to release the polyphenols associated to the cell wall, the residue was treated with 20 mL of methanol and 2 mL of concentrated sulphuric acid. Thereafter, the samples were shaken at 85 °C for 20 h. After centrifugation (2500 g for 10 min), the supernatant was allowed to stand. After the residue was washed twice with distilled water, and the final volume was brought up to 50 mL in a volumetric flask. This extract is associated with hydrolysable polyphenols.

Extractable and hydrolysable polyphenol contents were measured in the corresponding extract by the method of Folin-Ciocalteau and calculated as gallic acid equivalent (GAE) and expressed in mg/g of dry matter (Waterhouse, 2003).

Condensed tannins were quantified according to the method described by Pérez-Jiménez, Arranz, & Saura-Calixto (2009), and the aqueous-organic extract residues were treated with 10 mL of HCl / butanol/ $FeCl_3$ (5:95, v / v) at 100 °C for 60 min. After centrifugation (2500 g for 10 min), the supernatant was allowed to stand. The residue was washed twice with butanol (5 mL), centrifuged, and the supernatants were mixed at a final volume of 25 mL in a volumetric flask. The final extract was used for quantification of condensed tannins by spectrophotometry at 555 nm and the results were expressed in mg/g of dry matter.

2.4 Analysis of functional properties

2.4.1 Physicochemical properties

Water retention capacity (WRC) and the absorption capacity of organic molecules (ACOM) were determined by adapting the method proposed by McConnell, Eastwood, & Mitchell (1974) and Zaragoza et al. (2001), respectively. The samples were weighed (1g) in tared centrifuge tubes and stirred with excess water (20 mL) and canola oil (15 mL), respectively. After 24-h rest at room temperature, the samples were centrifuged (2000 g for 15 min). Both WRC and ACOM are expressed in grams of water/oil absorbed in 1 g of dry matter, respectively.

Cation binding capacity (CBC) was estimated by measuring copper binding capacity, determined according to McBurney, Van Soest, & Chase (1983). First, a 0.5-g sample was homogenised with 50 mL of $CuSO_4$ (1 M) in a sintered crucible and incubated at room temperature for 1 h. After vacuum filtration, the sample was washed with distilled water. A

solution of propanol/HCl was then added. After filtration by gravity, the whole filtrate was transferred to a 100-mL volumetric flask and brought up to volume with distilled water. An aliquot of this solution was adjusted to a pH between 8 and 9 with NH_4OH (2M) and then transferred to a 50-mL volumetric flask. A cuprizone (Bis-cyclohexanone-oxaldihydrazone, Sigma) reagent solution (0.5%) was added to the flask and completed to volume with distilled water. Absorbance was read at 590 nm after 30 min. $Cu(NO_3)_2$ solutions were used to prepare the standard curve and the results were displayed as mg of copper per g of dry matter.

The potential of the sample to inhibit pancreatic lipase activity was assessed using the methodology proposed by Chau, Wang, & Wen (2007). 0.5 g of the sample was weighed in a flask, in addition to 10 mL of olive oil and 50 mL of sodium phosphate buffer (0.1 M, pH 7.2), mixed with 10 mL of pancreatic lipase solution. The pancreatic lipase solution was prepared by adding 7.1 mg of pancreatic lipase (L3126, Sigma Chemical) to 10 mL of sodium phosphate buffer. The test tube was incubated in a water bath at 37 ºC for 1 h and then placed in a boiling water bath to cease the reaction. The amount of free fatty acid released was determined by titration with 0.05 N NaOH. The Inhibitory activity (%) toward pancreatic lipase (IALip) was defined as the percent decrease in the free fatty acid production rate compared to the control (no fibre).

2.4.2 Antioxidant capacity

The antioxidant activity (AA) was measured in the aqueous organic extract and in extrudates by FRAP (Pulido et al., 2000) for extractable and hydrolysable polyphenols and by ABTS (Rufino et al., 2007) for condensed tannins. Analytical reagent Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma) was used for the preparation of the standard curve. Total antioxidant capacity was calculated by adding the AA of each extract, expressed in μM Trolox/ g^{-1} of dry matter.

2.4.3 Stimulating growth of probiotic bacteria

Commercially available lactic acid bacteria (DMS Food Specialties), namely *Bifidobacterium lactis* (Lafiti B94) and *Lactobacillus acidophilus* (Lafiti L10), were used to assess the *in vitro* prebiotic effect of extruded apple fibre (APF).

Probiotic microorganisms were reactivated by inoculation in MRS (Man, Rogosa and Sharpe) broth and incubated at 37 ºC in an anaerobic jar (Anaerobac reagent, Probac do Brasil) for 15 h. Two millilitres of the activated strains (at predefined concentrations) was inoculated in tubes containing 20 mL of MRS broth, whose carbon source (glucose) was replaced with the samples, in order to assess the bifidogenic potential. Thereafter, the tubes were incubated at 37 ºC for 48 h under anaerobic conditions. Glucose-free MRS broth was used as negative control (without any carbon source) and MRS broth containing inulin served as positive control.

The serial dilution method was utilised for quantification of the microbial population. Aliquots of 1 mL were collected and plated onto a specific medium (MRS agar) using the pour plate method and incubated at 37 ºC for 48 h under anaerobic conditions. Quantification consisted of the total count of colony-forming units (CFU), between 30 and 300, performed in triplicate.

2.5 Statistical analysis

The operating conditions of the extrusion process were determined based on the results of preliminary tests, including six assays. AP extrusion was performed in duplicate and each duplicate was analysed twice. The results were submitted to analysis of variance (ANOVA) and compared by Tukey's test at a 5% significance level. A Pearson's correlation analysis was performed. The Statistica 8 (Statistica 8, Statsoft Inc., USA) software program was used for the statistical analysis.

3. Results and discussion

3.1 Dietary fibre

Extrusion can be a powerful tool for the macromolecular disintegration of cell wall (Hwang et al., 1998). Table 2 shows the effects of extrusion assays on total fibre (TF), soluble fibre (SF), and insoluble fibre (IF), as well as the IF:SF ratio. Note that SF content increased whereas IF content decreased in all extruded samples, which led to a decline as high as 63% in the IF:SF ratio, ranging from 1.8 to 2.5. According to Spiller (1986), this ratio should range between 1.2 and 2.3 so as to produce physiological effects associated with both DF fractions. In this respect, AP extrusion allowed obtaining values close to this recommendation. Hwang et al. (1998) demonstrated that the shearing effect also caused the solubilisation of the rigid structure of the AP cell wall.

The increase of the soluble fiber was larger when experimental conditions were more intense and could be interpreted through the breaking of chemical bonds of the fibrous matrix. Moisture acts as a plasticising agent in the extrusion barrel; thus, lower moisture indicates that the sample is subjected to larger abrasion and mechanical rupture (Arcila et al., 2015). This may explain the progressive and significant increase in SF with a decrease in moisture at 90ºC (assays 1, 2 and 3). However, moisture did not have a significant effect when barrel temperature was increased to 115 ºC (assays 4, 5 and 6), indicating that higher temperature strongly contributes to fibre degradation, playing down the effect of moisture. This positive effect of temperature on fibre solubilisation could also be observed when moisture content was set to 41% (assays 3 and 5), where the temperature of 115 ºC increased SF and decreased IF. According to Chen et al. (2014), high temperatures stimulate fibre degradation.

Assay 1, which used pomace with a 33% moisture level and extrusion temperature of 90 ºC, yielded the highest SF concentrations (28.30%), representing a significant increase of 112.6%. Considering that SF is more easily fermented than IF, solubilisation of the apple fibre through extrusion may be important to its availability to colonic bacteria (Björck, Nyman, & Asp, 1984; Guillon & Champ, 2000; Vitaglione et al., 2008).

Unlike SF and IF, TF of apple pomace was not significantly influenced by experimental conditions, tending to decrease as temperature increased. This finding demonstrates redistribution from insoluble to soluble fibre, caused by modification in the cell wall structure, where IF was partially degraded without fully degrading the fibre's polymer structure. This redistribution is corroborated by the strong negative correlation observed in SF and IF contents ($p<0.01$) ($r=-0.98$). Similar results were obtained in recent research carried out by Huang & Ma (2016), in which AP extrusion increased SF content by 75% and reduced IF content by 28%, while TF remained unchanged by the process. Likewise, Jing & Chi (2013) found that extrusion of soybean residue did not alter DF content, but increased SF from 2.05 to 12.65% and decreased IF from 60.82 to 50.39%.

According to Larrea, Chang, & Martínez Bustos (2005), redistribution of DF from insoluble to soluble fractions during orange pulp extrusion could result from the breaking of covalent and non-covalent bonds between carbohydrates and fibre-associated proteins, yielding small molecular fragments, which are more soluble. Moreover, the mechanical breaking of glycosidic bonds during extrusion may increase SF (Vitaglione et al., 2008). As a matter of fact, mechanical stress during AP processing was accountable for the breaking of glycosidic bonds of insoluble polysaccharides, confirmed by the reduction of up to 8.5% in cellulose contents (assay 6) and up to 53% in hemicellulose contents (assay 1), whereas lignin content

remained unchanged (Figure 1). The AP's hemicelluloses showed higher degradation when compared to cellulose, but both were influenced by the intensity of the process. Cellulose (linear polymer with β-1,4-linked glucose units) molecules are closely packed through hydrogen bonding, forming a highly organized structure. Hemicellulose, on the other hand, has a looser (more amorphous) structure or poorly stable glycosidic bonds between pentose and hexose units, which facilitates its degradation (Ning, Villota, & Artz, 1991). Therefore, long and linear molecules such as cellulose are more resistant to shearing forces than shorter and branched molecules such as hemicellulose (Daou & Zhang, 2012).

The AP's soluble pectin content was closely related to the intensity of the process, increasing by 32 to 92% in all extruded samples. The maximum content was observed in assay 1, going from 9.12% to 17.50%. The conditions during the extrusion process were probably sufficient to cause changes in protopectin structure, allowing for its solubilisation and pectin release. Therefore, solubilisation of pectic substances by the mechanical force used in the extrusion process also contributed to the increase in SF content of AP, revealing a strong positive correlation ($p<0.01$) ($r=0.97$) between SF and pectin. A similar result was obtained for orange pulp in the study conducted by Larrea, Chang, & Martínez Bustos (2005), in which all extrusion treatments increased total pectin concentration, but higher soluble pectin concentrations were observed at higher temperatures and lower moisture levels.

Since extrusion facilitates the solubilisation of insoluble pectin, it is an interesting alternative to conventional acid extraction, used to obtain commercial pectin from AP and citrus pulps, and this treatment should be further investigated as to the quality of pectins extracted under different experimental conditions.

3.2 Physicochemical properties

Table 3 shows the effect of different extrusion treatments on the physicochemical characteristics of AP. Assays 1, 4 and 5 demonstrated a significant increase in cation binding capacity (CBC) (13, 11, and 8%, respectively), probably for exposing a larger surface area, uronic acids, or ion binding sites in the apple dietary fibre structure, with a strong positive correlation with soluble pectin content ($p<0.01$) ($r=0.90$). Cation binding capacity is related to the fibre's ability to bind to ions through groups available on its surface (Annison & Choct, 1994) and it may indicate the capacity of food to bind to bile acids, preventing them from being reabsorbed by the intestinal epithelium. It has been suggested that this chemical interaction potential is a powerful mechanism whereby certain dietary fibres, rich in uronic acids and phenolic compounds, have a cholesterol-lowering effect (Guillon & Champ, 2000; Cornfine et

al., 2010). Furthermore, fibres with high CBC can trap, destabilise, and disintegrate lipid emulsion, further reducing the diffusion and absorption of lipids and cholesterol (Furda, 1990). Similarly to what occurred in the present study, extrusion also increased (60%) the cation exchange capacity (CEC) of orange pomace, being associated with a larger amount of uronic acid, with which it had a positive significant correlation (r=0.99) (Huang & Ma, 2016). Daou & Zhang (2012) assessed the CEC of rice bran fibre under different conditions in the digestive tract and noted that extrusion of the bran increased (157%) its CEC only at pH 1.8 (in the stomach).

The fibre's potential to absorb organic molecules could be related to its capacity to bind to molecules such as triglycerides, cholesterol, bile acids, carcinogenic agents, and toxic compounds in the intestinal tract (Zaragoza et al., 2001). AP extrusion changed the surface properties of the fibre and increased access to its hydrophobic binding sites, which is confirmed by the higher ACOM of extruded samples, whose oil uptake capacity was 15 to 22% higher. Jing & Chi (2013) found that the oil retention capacity of soybean residue rose by 25% after extrusion. However, Huang & Ma (2016) demonstrated significant reduction (68%) of this capacity for extruded orange pomace whereas Caprez, Arrigoni, Amado, & Neukom (1986) found no influence on wheat bran, indicating that such differences can be attributed to different conditions during the extrusion process and to the different raw materials used.

Extrusion significantly increased (by more than 70%) the fibre's potential to inhibit pancreatic lipase activity, except under the experimental conditions of assay 3. The higher oil uptake capacity may have contributed to this increase ($p<0.05$) ($r=0.84$). Despite no significant influence from the intensity of the process, there was a positive correlation between the enzyme inhibition potential and fibre solubility ($p<0.01$) ($r=0.92$), as well as between solubility and CBC ($p<0.01$) ($r=0.82$). This indicates disintegration of cell wall components, and formation of smaller soluble fragments has a considerable impact on the fibre's capacity to bind to bile acids and inhibit pancreatic lipase activity. Pancreatic lipase is a key enzyme in the hydrolysis and absorption of lipids (Nakai et al., 2005) and dietary fibre could decrease its activity, thus contributing to the prevention of hyperlipidaemia. This inhibitory potential is related to characteristics of the fibrous matrix itself, such as porosity, level of exposure of inhibitory substances found on the surface, fibre's capacity to encapsulate the oil and the enzyme, and reduction in the access of lipase to oil (Chau et al., 2007).

Hydration properties of DF are concerned with its capacity to hold water within its matrix (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005) and are associated with its structure, particle size, and number of water-binding sites (Daou & Zhang, 2012). Extrusion

increased the water retention capacity in different samples, such as durum wheat by-products (Esposito et al., 2005), soybean residue (Jing & Chi, 2013 Chen et al., 2014), wheat bran (Yan et al., 2015), and orange pomace (Huang & Ma, 2016). Nevertheless, the present study indicated otherwise, as there was a significant reduction in WRC of extruded samples (8-26%). The decrease in WRC of apple pomace after extrusion had already been observed by Hwang et al. (1998) and was associated with disintegration of cell wall by high specific mechanical energy (SME) during the process. Likewise, Ning et al. (1991) described slight ($p=0.05$) reduction in WRC of maize fibre after extrusion. According to those authors, extrusion can cause the fibrous matrix to collapse as a result of large shearing forces and pressure. High disintegration of cell walls triggers the formation of compounds with a lower molecular weight and with a poorer hydration potential, in addition to reducing particle size, which directly affects WRC (Zaragoza et al., 2001; Daou & Zhang, 2012).

Notwithstanding, by analysing extruded samples alone, it is possible to note that WRC is higher the more intense the process is (lower moisture), having a positive correlation ($p<0.05$) ($r=0.82$) with SF content. Quite possibly, higher pectin content and larger changes in the surface structure of insoluble fibre, such as increase in pore size and in groups of free hydroxyls through cellulose, stimulated WRC of extruded samples under more aggressive experimental conditions (Daou & Zhang, 2012).

3.3 Polyphenols and antioxidant capacity

During extrusion, phenolic compounds can undergo decarboxylation due to higher barrel temperature and moisture, which leads to polymerisation of phenols and tannins, consequently reducing extractability and antioxidant activity (Brennan et al., 2011). However, in some cases, the concentration of bioactive compounds in extruded products can increase with thermal and mechanical processes during extrusion (Zielinski, Kozlowska, & Lewczuk, 2001; Morales et al., 2015). The increase in the concentration of certain phenolic acids in extruded products is due mainly to their release from the cell wall matrix (Brennan et al., 2011).

In the present study, AP extrusion did not have a significant impact on total polyphenol levels, except for assay 6 (6% reduction) (Figure 2a). Nonetheless, extrusion significantly reduced hydrolysable polyphenol concentrations in AP under all experimental conditions, yielding higher ($p<0.05$) extractable polyphenol concentrations (Figure 2a). The energy generated by the treatment likely caused the partial release of polyphenols associated with the fibrous matrix, considering that, as pointed out earlier, the extrusion process caused strong cell wall disintegration in AP (Table 2 and Figure 1). The concentration of condensed tannins was

not influenced by the process, being at odds with the findings of Obiang-Obounou & Ryu (2013), which indicates that extrusion effectively reduced (78%) the concentration of tannins in chestnuts.

Antioxidant capacity (Fig. 2b) showed a positive correlation ($p<0.01$) with the concentrations of extractable polyphenols ($r=0.99$), hydrolysable polyphenols ($r=0.95$), and total polyphenols ($r=0.91$). The processing conditions used in assay 1 (90 ºC and moisture of 33%) allowed for a significant increase (5%) in total antioxidant capacity of AP (Fig. 2b), but there was no significant difference in total polyphenol contents (Fig. 2a). The antioxidant activity of extruded products depends not only on the concentration of bioactive compounds, but also on their composition (Brennan et al., 2011). According to Pokorný & Schmidt (2001), although natural antioxidants are partially lost during heating, the total antioxidant properties of heated foods can be maintained or even increased by the development of new antioxidants, such as Maillard reaction products. Accordingly, the formation of compounds generated from Maillard reaction may have contributed, along with polyphenols, to the antioxidant capacity of extruded samples.

One of the major phenolic compounds found in AP is chlorogenic acid, followed by rutin, phloridzin, epicatechin, caffeic acid, and p-coumaric acid (Leyva-Corral et al., 2016). Extrusion from mixtures of oat flour, apple pomace, and potato starch reduced the concentration of total (3-20%) and individual (24-75%) phenolic compounds, with a slight increase in the antioxidant activity of extruded products and full degradation of epicatechin (Leyva-Corral et al., 2016). In another study, extrusion strongly affected the concentration of total phenolic compounds (80 to 64% reduction) and antioxidant activity in two varieties of Kiwicha (*Amaranthus caudatus*) (Repo-Carrasco-Valencia, Peña, Kallio, & Salminen, 2009). According to the authors, phenolic acids can undergo decarboxylation during food processing. Contradictory results were obtained by Stojceska, Ainsworth, Plunkett, & Ibanoğlu (2009), according to whom extrusion cooking increased the concentration of total phenolic compounds and total antioxidant capacity in two different formulations with red cabbage. Hence, it is prudent to conclude that the effect of extrusion on bioactive compounds depends on processing conditions and on the raw materials used.

3.4 Stimulation of growth of probiotic bacteria

Porosity and the surface available for microbial degradation in the colon depend on fibre architecture and have a direct influence on its fermentability (Guillon & Champ, 2000). An extruded sample with 33% of moisture submitted to 90 ºC (assay 1) was used for assessment of

prebiotic potential as this sample had the highest SF content among extrudates and good functional properties. The extruded apple pomace (EAP) showed bifidogenic activity and proved efficient in stimulating the growth of both probiotic bacteria, which turned out to be significant compared to the glucose-free control and to the initial colony-forming unit count (Table 4). Although inulin largely stimulated the growth of *Lactobacillus*, it was not statistically different for *Bifidobacterium*. Extrusion favoured the growth of *L. acidophilus*, but it did not have any impact on the growth of *Bifidobacterium.*

A larger amount of SF may possibly influence the fermentability of AP in the large intestine, as previously reported for extruded wheat flour (Björck et al., 1984) and wheat bran (Arcila et al., 2015). Soluble polysaccharides are more easily fermented than equivalent polysaccharides in an organized network. The porosity and particle size of insoluble fibre determine the available area so that the bacterium can exert control over fermentation (Guillon & Champ, 2000).

Results reveal that the bacteria were capable of growing and fermenting the fibrous substrate of AP, even when it contained carbohydrates with a high degree of polymerisation. It can be inferred that apple fibre solubilisation, combined with changes in particle size, molecular weight, and porosity, contributed to the results obtained here.

4. Conclusion

All experimental conditions in the AP extrusion process caused redistribution among fibre fractions, with a significant increase in SF content. Partial degradation of cell walls was confirmed by the reduction in cellulose and hemicellulose contents and by the increase in soluble pectin levels. The extrusion assay, which utilised a temperature of 90 ºC and a moisture level of 33%, was the most efficient one in fibre solubilisation (112.6% increase), thus increasing its availability to colonic microorganisms and improving its fermentability. The prebiotic effect was improved by the larger stimulation of growth of *Lactobacillus acidophilus* after extrusion.

By and large, more aggressive experimental conditions ameliorated the fibre's capacity to bind bile acids, to retain water and fat in its structure, and to inhibit the pancreatic lipase activity. However, large disintegration of cell wall eventually reduced the WRC. The energy generated from extrusion led to the partial release of fibre-associated polyphenols without changing total polyphenol content. Only the pomace extruded at 90 ºC and with 33% of moisture showed a significant increase (5%) in its total antioxidant capacity. The findings of this study indicate that structural changes in apple fibre caused by extrusion improved its

functional properties and prebiotic effect, turning it into a promising ingredient to be used in human nutrition.

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Table 1. Assays with the respective variables and their levels used for the extrusion test.

Independent variables	**Assays**					
	1	2	3	4	5	6
Temperature (ºC)	90	90	90	115	115	115
Moisture (%)	33	37	41	37	41	45

Table 2. Effect of the extrusion variables on dietary fibre of apple pomace.

Assay	TF	IF	SF	IF:SF ratio
	(% dry matter)			
1	79.51 ± 0.12^{a}	51.22 ± 0.06^{e}	28.30 ± 0.21^{a}	1.81 ± 0.00^{d}
2	79.59 ± 0.49^{a}	55.77 ± 0.29^{c}	23.82 ± 0.20^{b}	2.34 ± 0.01^{cd}
3	79.39 ± 2.03^{a}	60.97 ± 0.53^{b}	18.42 ± 1.51^{c}	3.31 ± 0.24^{b}
4	77.10 ± 0.53^{a}	53.85 ± 0.77^{d}	23.25 ± 0.24^{b}	2.32 ± 0.06^{cd}
5	77.29 ± 0.93^{a}	54.54 ± 0.19^{cd}	22.75 ± 0.93^{b}	2.40 ± 0.09^{c}
6	77.98 ± 0.02^{a}	56.06 ± 1.35^{c}	21.90 ± 1.35^{b}	2.56 ± 0.22^{c}
Unextrused AP	79.03 ± 1.25^{a}	65.72 ± 0.23^{a}	13.31±1.16^{d}	4.94±0.42^{a}

Obs.: TF: Total dietary fibre, IF: Insoluble dietary fibre, SF: Soluble dietary fibre.
The results are expressed as the mean ± standard deviation. Mean values followed by a different letter on the same column are significantly different using Tukey`s test ($p<0.05$).

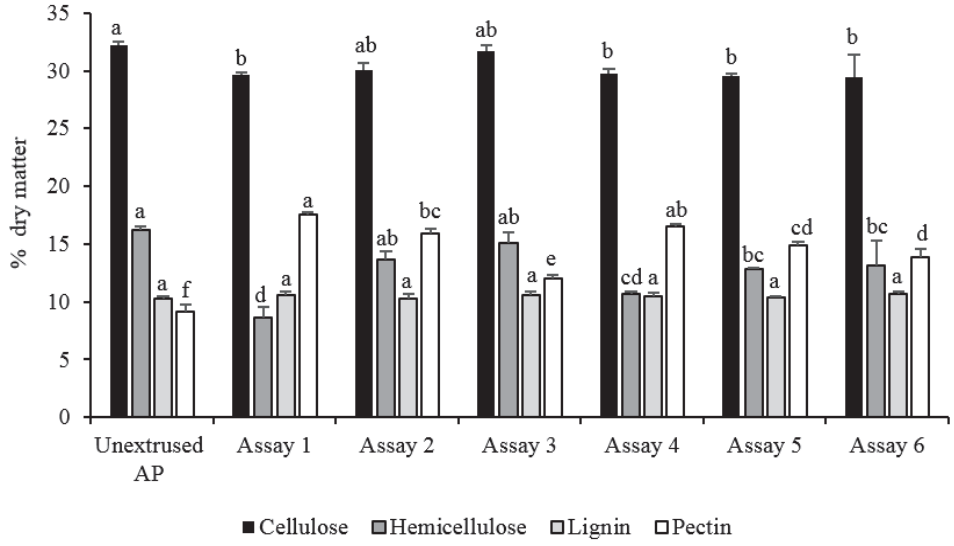


Figure 1. Effect of the extrusion variables on composition of the dietary fibre of apple pomace. Different superscripted letters in columns indicated statistical differences using Tukey`s test ($p<0.05$).

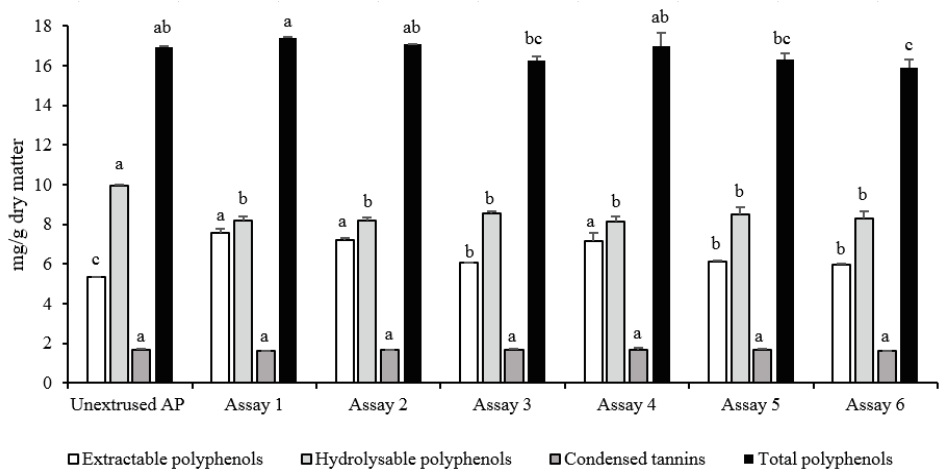
Table 3. Effect of the extrusion variables on physicochemical properties of apple pomace.

Assays	CBC	ACOM	IALip	WRC
	mg Cu/ g DM	g oil/ g DM	%	g water/ g DM
1	29.58 ± 0.49^{ab}	2.06 ± 0.02^{a}	16.80 ± 2.34^{a}	6.92 ± 0.01^{b}
2	28.30 ± 0.05^{bc}	2.06 ± 0.01^{ab}	16.02 ± 1.17^{a}	6.77 ± 0.07^{bc}
3	27.88 ± 0.40^{bc}	2.02 ± 0.02^{b}	13.67 ± 0.78^{ab}	6.06 ± 0.04^{d}
4	30.04 ± 0.08^{a}	1.95 ± 0.02^{c}	17.06 ± 3.25^{a}	6.58 ± 0.04^{c}
5	28.71 ± 0.02^{ab}	1.95 ± 0.0^{c}	16.23 ± 2.71^{a}	6.22 ± 0.04^{d}
6	27.73 ± 1.28^{bc}	1.94 ± 0.02^{c}	15.23 ± 1.56^{a}	5.97 ± 0.06^{d}
Unextrused AP	26.60 ± 0.40^{c}	1.69 ± 0.12^{d}	8.59 ± 1.57^{b}	7.56 ± 0.24^{a}

Obs.: CBC: Cation binding capacity, ACOM: Absorption capacity of organic molecules, IALip: Inhibitory activity toward pancreatic lipase, WRC: Water retention capacity

The results are expressed as the mean ± standard deviation. Mean values followed by a different letter on the same column are significantly different using Tukey`s test ($p<0.05$).

(a)



(b)

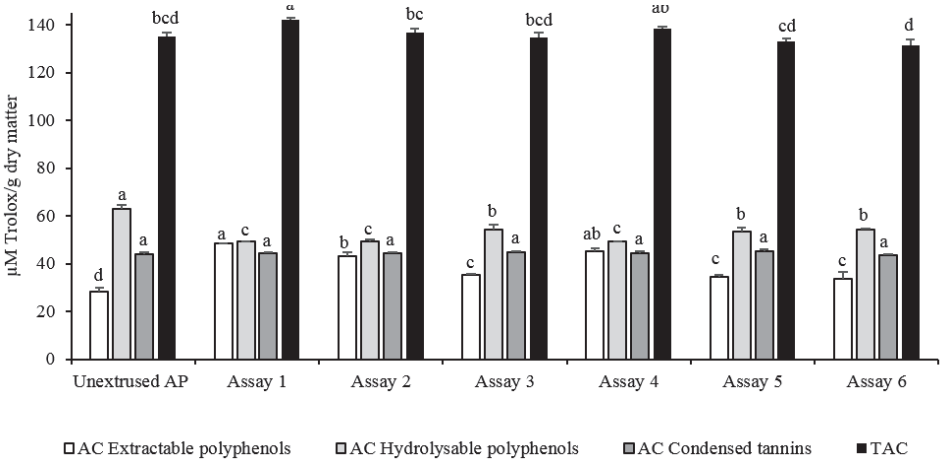


Figure 2. Effect of the extrusion variables on polyphenols *(a)* and antioxidant capacity *(b)* of apple pomace. AC: Antioxidant capacity, TAC: Total antioxidant capacity. Different superscripted letters in columns indicated statistical differences using Tukey`s test ($p<0.05$).

Table 4. Growth of *Lactobacillus acidophillus* e *Bifidobacterium lactis* culture in 48 hours, with values expressed in log CFU/ mL.

Time		*L. acidophillus*	*B. lactis*
0 hours	Initial count	7.40 ± 0,06^{e}	7.45 ± 0.07 ^{b}
48 hours	Negative control	8.44 ± 0.04^{c}	7.35 ± 0.10 ^{b}
	Inulin	8.96 ± 0,13^{a}	8.36 ± 0,02 ^{a}
	AP	8.48 ± 0.03^{c}	8.35 ± 0.03 ^{a}
	EAP	8.66± 0.03^{b}	8.30 ± 0.04 ^{a}

Obs.: Negative control: MRS without carbon source, AP: apple pomace (unextrused), EAP: extrused apple pomace (assay 1).
The results are expressed as the mean ± standard deviation. Mean values followed by a different letter on the same column are significantly different using Tukey`s test ($p<0.05$).

3.4 ARTIGO 4

Artigo em fase de revisão pelos autores para ser submetido à Revista **Food Chemistry**

Influence of chemical and physical modification of apple dietary fibre on metabolic parameters and antioxidant status in rats

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Abstract

The effect of extrusion and acid hydrolysis of apple fibre on metabolic parameters in rats was investigated. Extrusion increased solubility and fermentability of apple dietary fibre, boosting butyric acid production and antioxidant protection of the caecum. The extruded fibre also led to larger excretion of fat and cholesterol in the faeces, reduction of serum triglyceride levels, and decrease of peak postprandial glucose levels. Acid hydrolysis improved the prebiotic effect of apple fibre, reducing faecal pH and increasing the production of short-chain fatty acids, especially of propionic and butyric acids. Consumption of modified and unmodified apple fibres increased the concentration of polyphenols in the serum and in the intestine of

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experimental animals, resulting in better antioxidant protection. This shows that these fibres play an important physiological role as carriers of bioactive compounds through the gastrointestinal tract.

Keywords: extrusion, acid hydrolysis, glucose level, lipids, short-chain fatty acids, antioxidants.

1. Introduction

Dietary fibres (DF) have attracted large interest from the scientific community due to their important role in the promotion and protection of human health. However, the benefits of DF and their effects on the body depend not only upon the consumed amount, but also upon their composition, organizational structure, molecular weight, physicochemical characteristics, and associated bioactive compounds. These characteristics that are inherent to DF are closely related to their vegetable source and to the different preparation methods (Macagnan, da Silva, & Hecktheuer, 2016).

The solubility of DF in water has a decisive influence on their physiological and functional properties. Some types of fibres can help reduce the intestinal absorption of bile acids, glucose, cholesterol, and lipids by way of physical and chemical interactions. Nonetheless, most of the lipid-lowering and glucose-lowering effects of fibres are associated with their soluble fraction (Elleuch et al., 2011; Kendall, Esfahani, & Jenkins, 2010; Mudgil & Barak, 2013). Soluble fibre (SF) is less crosslinked and can be fermented more easily, which prompts the release of associated polyphenols by the intestinal microflora, increasing their bioavailability (Mudgil & Barak, 2013; Vitaglione, Napolitano, & Fogliano, 2008). Fermentation also produces short-chain fatty acids (SCFAs), which play a crucial role in intestinal health (Tungland & Meyer, 2002).

Apple pomace, the major by-product of the industrialisation of apples for juice production, is generated in large amounts and is usually disposed of inappropriately into the environment. However, apple pomace is a good source of DF and antioxidant compounds (Macagnan et al., 2015), both of which dietary factors that act synergistically upon the prevention of chronic diseases. Unfortunately, these bioactive compounds associated with the fibrous matrix have not been given due attention by the scientific community and, consequently, there is a paucity of studies on their composition and biological action in experimental models when compared to hundreds of studies on polyphenols that are bioavailable from the small intestine (Goñi, Díaz-Rubio, Pérez-Jiménez, & Saura-Calixto, 2009; Macagnan, da Silva, & Hecktheuer, 2016).

Physical and chemical treatments can cause structural changes in DF and improve their physicochemical characteristics and fermentability (Guillon & Champ, 2000), altering their digestive and metabolic effects, despite the lack of scientific data. Accordingly, the aim of this study was to assess the influence of extrusion and acid hydrolysis of apple pomace fibre on the biochemical and physiological parameters in rats. In addition, the study assessed the impact of the consumption of modified and unmodified apple fibre on the production of SCFAs and on antioxidant status in the serum and caecum of experimental animals.

2. Material and methods

2.1 Preparation of apple pomace fibre (AP-Fibre)

Apple pomace, obtained after pressing the fruit for juice production, was provided frozen by Fischer Sucos (Videira, SC, Brazil). In the laboratory, the pomace was defrosted, pre-dried in a forced-air oven (55 °C for 48 h), and ground in a microgrinder (Model MA 630/1, Marconi, Piracicaba, SP, Brazil) to obtain a powder with a particle size of less than 10 mm.

For determination of fibre concentration, the apple pomace was defatted with hexane (two washes, 1: 2.5, m/v). After being defatted, the apple pomace was washed twice with ethanol 80% (1: 2.5, m/v) and heated to 80 °C for 5 min to reduce free sugar content in the sample. After that, the AP-Fibre was dried in a forced-air oven (55 °C for 2 h).

2.2 Acid hydrolysis of AP-Fibre

An aliquot of 10 g of AP-Fibre was partially hydrolysed by 250 mL of H_2SO_4 (1.5N) under stirred conditions at 60 °C for 3 h. At the end of the desired reaction time, the mixture was immediately cooled in an ice bath and centrifuged (2400 g for 10 min). The solid sample (precipitate) was washed with distilled water until neutral pH (Cornfine, Hasenkopf, Eisner, & Schweiggert, 2010). The supernatant was neutralised with calcium carbonate ($CaCO_3$) and the resulting precipitate was removed by centrifugation (Du et al., 2011). The solid sample was combined with the neutralised supernatant, thus yielding the hydrolysed apple pomace fibre (HAP-Fibre). The HAP-Fibre was dried in a forced-air oven (55 °C for 24 h), ground in a microgrinder, and stored at -18 ºC.

2.3 AP-Fibre extrusion

Extrusion was performed using a single-screw extruder (model Ex-Micro Laboratório, Exteec Máquinas, Ribeirão Preto, SP, Brazil) at a barrel temperature of 90 ºC and with a feed moisture of 33 g/100 g of dry matter. The feeding rate was kept constant at 100 g/min. After processing, the extruded apple pomace fibre (EAP-Fibre) was dried in a forced-air oven (55 ºC for 5 h), ground in a microgrinder, and stored at -18 ºC.

2.4 Chemical composition

The AP-Fibre, HAP-Fibre and EAP-Fibre were analysed for moisture content (105 ºC for 12 h), ash (minerals) (550 ºC for 5 h), and protein (nitrogen determination via the Kjeldahl

method, N × 6.25), according to the techniques described by the AOAC (Association of Official Analytical Chemists, 1995). Lipids were quantified using the method of Bligh & Dyer (1959). Total dietary fibre content and soluble and insoluble fractions of all samples were determined according to the enzymatic-gravimetric method 991.43 of the AOAC (Association of Official Analytical Chemists, 1995). Pectin content was obtained according to the method described by Carvalho, Jong, & Bello (2002). All chemical analyses were performed in triplicate and the results were expressed as g per 100 g of dry matter.

Phenolic compounds were extracted using the method proposed by Pérez-Jiménez et al. (2008), and the content of phenolic compounds was determined in the aqueous organic extract (extractable polyphenols) and in the residue of this extraction (nonextractable polyphenols: hydrolysable polyphenols and condensed tannins). Extractable and hydrolysable polyphenol contents were measured in the corresponding extract by the method of Folin-Ciocalteau (Waterhouse, 2003), calculated as gallic acid equivalent (GAE) and expressed in mg/g of dry matter. Condensed tannins were quantified according to the method described by Pérez-Jiménez, Arranz & Saura-Calixto (2009) and the results were expressed in mg/g of dry matter. Proximate composition is shown in Table 1.

2.5 Biological assay

2.5.1 Experimental diets and treatments

Four experimental diets were formulated based on the chemical composition of AP-Fibre, HAP-Fibre and EAP-Fibre (Table 1), and in accordance with the recommendations of the American Institute of Nutrition (AIN-93G) (Reeves, Nielsen, & Fahey, 1993), with modifications in DF concentration. The fibre in the control diet (microcrystalline cellulose) was replaced with apple fibres. The treatments consisted of the following four diets: CEL (treatment control with cellulose), AP (test treatment with apple pomace fibre), HAP (test treatment with

hydrolysed apple pomace fibre), and EAP (test treatment with extruded apple pomace fibre). All of the diets were isocaloric and had the same protein, lipid, and DF concentrations (Table 2).

2.5.2 Animals and experiment

The study protocol was approved by the Ethics Committee on Animal Use of Federal University of Santa Maria (UFSM) (protocol 140/2014). Thirty-two male Wistar albino rats (*Rattus norvegicus*) (47.32±5.31 g; age 21 days) were obtained from the "Biotério Central" of UFSM, randomly distributed among the treatments (eight animals per treatment) and individually housed in metabolic cages with free access to feed and water. The temperature was maintained at 21±2 ºC and lighting was controlled with a 12/12 h cycle throughout the experiment. The period of adaptation to the experimental diets and ambient conditions was 5 days. In the experimental period (33 days), feed intake was determined and faeces were collected daily. Body weight was obtained every week. On the 23rd day of the experimental period, all the animals were randomly selected for 4 consecutive days, in groups of eight (two animals per treatment), for analysis of their postprandial blood glucose response. After a 12-hour overnight fast, the animals were fed 2 g of an experimental diet, which was totally consumed within 20 min. Blood samples from the tail vein were taken at fasting (before the consumption of the meal) and at 15, 30, 60, 90, and 180 min after the meal to measure serum glucose levels (Denardin, Walter, Silva, Souto, & Fagundes, 2007).

On the last experimental day, after a 12-hour overnight fast, the animals were weighed, anesthetised with an intraperitoneal injection of ketamine-xylazine (70-10 mg/kg) and blood was collected by cardiac puncture. The blood samples were immediately centrifuged to obtain the serum and were stored at -18 °C for subsequent blood tests. After euthanasia with a lethal dose of anaesthetic, the livers and epididymal fat pads were immediately removed and weighed.

The liver was wrapped in aluminium foil and polyethylene bags and frozen at -18 °C for further analysis of cholesterol (Liver-Chol) and lipids (Liver-Lip). The intestine was carefully removed and the caecal contents were collected in polyethylene bottles and stored at -18 ºC for further analysis of SCFAs, polyphenols, and antioxidant capacity.

2.5.3 Analytical methods

The faeces were partially dried (55 ºC for 48 h), ground in a microgrinder (Model MA 630/1, Marconi, Piracicaba, SP, Brazil), and analysed for moisture (105 °C for 12 h) and total dietary fibre content (method 991.43) according to the methods described by the AOAC (Association of Official Analytical Chemists, 1995). The faecal pH was obtained from a solution of 1 g of partially dried faeces in 10 mL of distilled water. The lipid contents in the faeces and liver were obtained via the method of Bligh & Dyer (1959).

The cholesterol content in the faeces and liver was determined according to the method of Haug & Hostmark (1987) by homogenisation of the organ/faeces in isopropanol (10% w/v). The resulting extract was kept refrigerated for approximately 24 h and centrifuged after quantification of total cholesterol in the supernatant by an enzymatic colorimetric kit from Doles® (Goiânia, Goiás, Brazil). For analysis of fasting plasma glucose levels and postprandial blood glucose response, blood samples were collected from tail veins using a Breeze®2 (Bayer Healthcare, Mishawaka, USA) monitoring kit. Total protein, triglycerides (TG), total cholesterol (Total-C), and HDL cholesterol (HDL-C) in the serum of the animals were determined by colorimetric reactions using specific enzymatic kits from Doles® (Goiânia, Goiás, Brazil). The non-HDL cholesterol (non-HDL-C) was defined as Total-C minus HDL-C.

The feed efficiency ratio (FER) was estimated as the ratio between the increase in body weight and daily consumption (in dry matter). The apparent digestibility of dietary fibre (AD-DF) was determined as the ratio, on a dry basis, of the fibre consumed and not recovered in the

faeces. The weight of the liver and isolated epididymal (Epid-Fat) fat was calculated as g per 100 g of animal weight.

2.5.3.1 Analysis of SCFA production

Intestinal content was assessed as to the production of SCFAs using gas chromatography, as proposed by Bianchi et al. (2011), with some adaptations. SCFAs were extracted by solid phase microextraction (SPME) using Car/PDMS (Supelco, 75µm x 10 mm) fibre at 40 °C under agitation, with a an equilibrium time of 10 min and 30 min of extraction thereafter. The SPME fibre was desorbed in the injector port (250 °C, Split mode; 1:20 ratio) of a gas chromatograph equipped with a flame ionization detector (GC-FID Varian, Star 3400 CX). The volatiles were separated with a ZBWax Plus fused silica capillary column (30 m/0.25 mm, 0.25 µm) using hydrogen as carrier gas (15 psi, 1.3 mL/min). The initial oven temperature was set to 50 ºC for 1 min, followed by a ramp rate of 5 °C per min up to 110 °C, 15 °C per min up to 230 °C, maintaining an isothermal condition for 10 min. The flame ionization detector temperature was 230 °C. SCFAs were identified by comparing hold times of the analyte with authentic standards (Sigma Aldrich). SCFAs were quantified by a calibration curve within 250-3000 mg/L of acetic, propionic, and butyric acid standards.

2.5.3.2 Polyphenol content and antioxidant capacity in serum and caecum

Polyphenols were extracted from the caecal content using the method proposed by Pérez-Jiménez et al. (2008). Extractable polyphenol content was measured in the aqueous organic extract prepared from the caecal content and in serum via the method of Folin-Ciocalteau (Waterhouse, 2003), calculated as gallic acid equivalent (GAE), and expressed in mg/g of dry matter. The antioxidant activity (AA) of caecum and serum was determined by the FRAP (Pulido, Jiménez-Escriga, Orensanzb, Saura-Calixto, & Jiménez-Escrig, 2005) and

ABTS (Rufino et al., 2007) methods. Analytical reagent Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma) was used for the preparation of the standard curve. The antioxidant capacity of the samples was expressed in μmol Trolox/L and μmol Trolox/g of dry matter for serum and caecal content, respectively.

2.6 Statistical analysis

The results obtained were submitted to analysis of variance and the means were compared using Tukey's test at the 5% level of significance. The correlation analyses were performed using Pearson's correlation coefficient. The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 13.0.

3. Results and discussion

3.1 Consumption, weight gain, and feed efficiency ratio

Feed intake was significantly lower for CEL and EAP treatments, which explains the smaller weight gain, but that did not influence the feed efficiency ratio (Table 3). Rats can select food based on their chemical composition and on their palatability, which often influences the amount of food intake. Therefore, the better palatability of experimental diets AP and HAP obtained from the addition of apple fibre can explain their higher intake. On the other hand, the lower intake observed in the EAP diet could be ascribed to the higher concentration of SF (75-113%) and pectin (68-127%) due to solubilisation of the fibre as a result of the extrusion process (Table 1). Soluble fibres, such as pectin, can increase the viscosity of gastric contents, delaying gastric emptying and increasing satiety sensation (Slavin & Green, 2007).

3.2 Faecal analysis

The dry faecal production (DFP) for CEL and EAP treatments can be explained by the lower food intake by animals in these groups. The test treatments had higher faecal moisture content (38-61%) than the control treatment (Table 3), due to the high concentration of SF in apple pomace. This can be applied to the use of processed fibres for the prevention and treatment of constipation and haemorrhoids (Slavin, Savarino, Paredes-Diaz, & Fotopoulos, 2009). However, acid hydrolysis and extrusion of apple fibre did not increase ($p<0.05$) water content in the faeces.

Dietary fibre can increase the excretion of nutrients and also adsorb toxic and carcinogenic substances, excreting them in larger or smaller amounts, depending on the physical and chemical characteristics of fibre components (Zaragoza, Pérez, & Navarro, 2001). Our findings demonstrate that apple fibre has characteristics capable of reducing the absorption and metabolisation of fat and cholesterol. The replacement of cellulose with unmodified apple fibre increased the elimination of lipids in the faeces by 95% (Table 3), a finding that was described in a previous study on apple pomace and other fruit by-products (Macagnan et al., 2015). However, the tested modifications improved these beneficial effects, and extruded fibre increased the faecal excretion of lipids by 18% and that of cholesterol by 36% when compared to unmodified fibre (Table 3). The tested processes probably changed the surface and structural properties of the fibre, causing greater interference in the absorption of these nutrients.

3.3. Final fasting glucose and postprandial glucose levels

Apple fibres did not change fasting glucose levels in normoglycaemic rats. Nevertheless, the postprandial glucose curve revealed significant reduction of the area under the curve (AUC) for AP (6%) and EAP (15%) treatments compared to the control treatment (Table 4). Several studies have reported on the role of DF in the reduction of postprandial

glucose levels, which is mainly associated with larger viscosity provided by SF. Pectin (major component of apple soluble fibre) increases the viscosity of food bolus, hindering glucose diffusion and delaying the absorption and digestion of carbohydrates (Ou, Know, Li, & Fu, 2001; Kendall et al., 2010). Glucose adsorption capacity (avoiding glucose diffusion) and the inhibitory effect on α-amylase activity are other mechanisms whereby soluble and insoluble fibres help reduce blood glucose levels (Ou et al., 2001; Chau & Huang, 2004). Alongside viscosity, such mechanisms may have contributed to better glucose response in animals fed AP and EAP diets.

Modification of the fibre by extrusion was efficient and stood out in the analysis of postprandial glucose levels, as it allowed for a slight increase in glucose concentrations and helped maintain them. EAP had the lowest glycaemic peak ($p<0.05$) and AUC after the meal, which makes extruded apple fibre an interesting ingredient, especially in the diet of diabetic patients, who need to keep blood glucose at normal levels, avoiding hyperinsulinaemia and pancreatic β cell dysfunction or dysregulation (Kopp, 2006). This glucose response can be explained by the larger viscosity of aqueous solutions prepared from extruded apple fibre (data not shown), owing to the presence of a larger amount of water-soluble compounds, mainly pectin (Table 1). The larger viscosity of extruded samples and their positive effect on glucose levels had already been described in *in vitro* studies on other sources of fibre such as orange pulp (Céspedes, Martínez Bustos, & Chang, 2010) and rice bran (Daou & Zhang, 2012).

Unlike extrusion, the chemical modification of fibres proved inefficient in improving glucose response, which was not different from that of the control treatment (Table 4). Acid hydrolysis probably led to partial depolymerisation of pectin, forming compounds with smaller molecular chains, which have little or no influence on viscosity (Tungland & Meyer, 2002).

3.4 Biochemical analysis and relative weight of organs

Modified fibres were better at reducing serum triglyceride (TG) levels, with significantly lower rates in 35% (acid hydrolysis) and 58% (extruded fibre) compared to the control treatment. This reduction can result from the larger faecal excretion of fat by the rats ($p < 0.01$) ($r = - 0.726$). The tested modifications probably changed the surface properties of the fibre and increased access to its hydrophobic binding sites. However, only extrusion was able to significantly increase this lipid-lowering potential, reducing TG levels by 44% (46.69 mg/dL for AP and 26.34 mg/dL for EAP treatments). Viscous fibres form gels that reduce the contact of luminal content with pancreatic lipase and bile due to the formation of DF-lipid aggregates that block the emulsification of fats and micelle formation (Kaczmarczyk, Miller, & Freund, 2012). Hence, the larger viscosity provided by extruded fibre probably interfered with metabolisation and absorption of fat, increasing its faecal excretion.

The capacity to sequester and even to bind chemically to bile acids has been suggested as a powerful mechanism whereby certain dietary fibres, rich in uronic acids (pectin) and phenolic compounds, have a cholesterol-lowering effect (Cornfine et al., 2010; Guillon & Champ, 2000). With fewer circulating bile acids, fat emulsification is not so intense and, therefore, fewer lipids are absorbed by the intestinal epithelium and, consequently, triglyceride levels are lower. It is assumed that the intensification of this mechanism contributed to higher faecal excretion of fat by animals fed modified apple fibres (Table 3) and to the significantly lower levels of cholesterol in the faeces (Table 3) and TG levels in the EAP group (Table 4).

Since cholesterol is the precursor of bile acids, the reduction in enterohepatic circulation stimulates the transformation of hepatic cholesterol into new bile acids, which explains the significantly lower hepatic cholesterol levels (30-34%) in animals fed apple fibre (Table 4). As a consequence, cholesterol flows from the bloodstream into the liver, due to the difference in concentration, reducing its plasma levels (Riegel, 2012). It is inferred that the compensatory

effect in healthy animals might have led to cholesterol depletion only in the liver, revealing lack of significance for total cholesterol (Table 4). Perhaps, the significant positive effect expected for serum cholesterol could be observed with the extension of the experimental period or with the previous induction of hypercholesterolaemia, as reported by Leontowicz, Leontowicz, Gorinstein, Martin-belloso, & Trakhtenberg (2007) and Leontowicz et al. (2001). In those studies, apple fibre had a lipid-lowering effect when added to the diets of cholesterol-fed rats.

Even though cholesterol levels remained unchanged, the findings indicate a positive effect of chemical hydrolysis and, especially, of fibre extrusion, on the capacity of the fibre to influence lipid metabolism. Similarly, extrusion of orange pomace increased the cation exchange capacity by 60%, which is associated with the fibre's capacity to bind to bile acids and to reduce the uptake of lipids and cholesterol (Huang & Ma, 2016). Chen, Ye, Yin, & Zhang (2014) reported that the extrusion of soybean residue also reduced ($p < 0.05$) triglyceride serum levels in rats due to the improvement of the physicochemical properties of the fibre after its processing. Positive results were also found by Cornfine et al. (2010) for the chemical modification of fibres, revealing disintegration of cell wall components (chiefly of pectic and hemicellulosic compounds) by acid hydrolysis of lupin (*Lupinus angustifolius* L.) DF, which made the active binding sites of the fibre more accessible and contributed to a 38% increase in its binding to bile acids.

No effect was observed on serum concentrations of HDL cholesterol across treatments. However, EAP had a significantly lower (37.5%) non-HDL cholesterol (non-HDL-Chol) when compared to the control group (Table 4). This lipid marker measures the cholesterol content in all atherogenic particles. Studies therefore suggest that non-HDL-Chol could be more strongly associated with the risk of developing coronary heart diseases than LDL cholesterol, being more likely to predict cardiovascular events, similarly to dosage of apolipoprotein B (Rana,

Boekholdt, Kastelein, & Shah, 2012). Thus, the result obtained for this parameter reinforces the preventive and lipid-lowering effect of extruded apple fibre.

Despite their strong interference in digestion and use of nutrients, the different experimental diets did not affect total protein levels (Table 4). Also, no significant differences in liver weight and liver fat content were observed across treatments (Table 4). Moreover, the largest weight gain in animals fed AP and HAP and largest faecal excretion of lipids observed in test treatments did not interfere with the stored fat content, which is estimated by the percentage of epididymal fat per body weight (Table 4).

3.5 Faecal pH, apparent digestibility of dietary fibre and SCFA production

Apple fibres added to the test treatments showed higher apparent digestibility (82-153%) than the fibre in the control diet (Table 4). Fibres with higher fermentation rates are consumed in a given volume and excreted in a much lower volume (Riegel, 2012). Thus, both tested modifications contributed to the larger degradation of DF by the intestinal microflora, as shown by the significantly higher AD-DF (28-39%) compared to the unmodified fibre.

Fermentable fibres can bring numerous benefits to health by changing the composition of the intestinal flora (Slavin, 2013). As a fermentative substrate, DF is considered the major source of energy for the bacteria that live in the colon. Its fermentation produces SCFAs, predominantly acetic, propionic, and butyric acids (Guillon & Champ, 2000; Slavin, 2013), which act upon the mucosa and colonic function, influencing lipid and carbohydrate metabolism (Slavin et al., 2009; Slavin, 2013; Riegel, 2012).

Cellulose is a type of poorly fermentable fibre, which is excreted without being adequately broken down by the intestinal flora (Riegel, 2012). It should be noted that the replacement of this insoluble fibre with different apple fibres increased SCFA production (60-128%) in the caecum (Figure 1), leading to acidification of the faeces (Table 4). This reduction

in pH is important to inhibit the growth of pathogenic bacteria and to promote the growth of beneficial ones, contributing to the prevention of gastrointestinal infection and colon cancer (Slavin, 2013; Tungland & Meyer, 2002). The high fermentability of apple fibres is supported by the inverse relationship ($p < 0.01$) between faecal pH and AD-DF ($r = - 0.639$) and SCFA ($r = - 0.749$), as well as by the positive relationship ($p < 0.01$) between SCFA and AD-DF ($r = 0.584$).

Different fibres vary in quantity and ratio of SCFAs, as well as in their rate of production (Slavin, 2013). The modification of fibres by acid hydrolysis increased all SCFAs assessed, especially propionic and butyric acids, whose concentrations increased by more than 65% ($p < 0.05$) (Figure 1), resulting in higher acidification of the faeces ($p < 0.05$) in the HAPF treatment (Table 4). Propionic acid is metabolised in the liver and is the only gluconeogenic substrate, which can influence the hepatic synthesis of cholesterol by inhibition of HMGCoA-reductase. Butyric acid is the main source of energy for colonocytes and has a trophic effect on the intestinal epithelium, stimulating the growth and proliferation of epithelial cells (Slavin, 2013; Riegel, 2012). According to Slavin (2013), the fermentation pattern can be related to the molecular weight of the fibre and chain length and structure – short-chain molecules are more easily fermented. It is therefore inferred that the experimental condition used in the chemical treatment led to the loosening of the fibrous matrix and partial breakdown of cell wall polysaccharides, especially pectin, forming smaller compounds (including pectic oligosaccharides), which contributed to a lower concentration of high molecular weight SF in the HAP-Fibre (Table 1). These changes in the physicochemical and molecular structure of apple fibre helped with its fermentation and increased SCFA production.

During extrusion, the material is submitted to strong mechanical shear and cooking takes place at high temperature and pressure and with a low water content, which leads to total disorganization of the original structure of the material (Daou & Zhang, 2012). It is assumed

that not only did the energy generated during the extrusion of apple fibre lead to solubilisation, but it also caused a large change in fibre structure, rendering it looser and more porous (Daou & Zhang, 2012), which helped with its degradation by the intestinal bacteria, increasing its apparent digestibility (Table 4). The improved fermentability of fibre after its extrusion was also described in an *in vivo* study in which extruded wheat flour had higher solubility and was more intensely degraded in the intestine of rats (Björck, Nyman, & Asp, 1984).

It is commonly known that the surface available for the action of bacteria relies on the cell wall architecture, and that accessibility and structural characteristics of polysaccharides (e.g., chain length, branching pattern, nature of monomers, presence and distribution of functional groups) turn out to be limiting factors for fermentation (Guillon & Champ, 2000). Thus, fermentation patterns and the amount of SCFA produced depends on the type of DF (Kaczmarczyk et al., 2012). In this respect, extrusion, albeit not different from that of the unmodified fibre, changed the SCFA ratio, stimulating the formation of propionic (12%) and butyric (25%) acids, which probably contributed to the significance observed for butyric acid when compared to the control treatment (Figure 1). Likewise, Arcila, Weier, & Rose (2015) reported that the extrusion of wheat bran increased its solubility, which increased *in vitro* fermentation by human faecal microbiota, also increasing the production of SCFA, especially of butyrate. Butyric acid appears to be the SCFA with the strongest topical effect on the health of colonic epithelium and has been given special attention by researchers as it has some beneficial effects regarding colon cancer (Slavin, 2013). According to Riegel (2012), the insufficient production of this SCFA causes injuries to the colon, such as inflammation, polyposis, and cancer.

Therefore, it is possible to say that the tested modifications improved fibre fermentability and increased butyric acid production and health of the intestine. However,

chemical modification was more efficient than extrusion concerning fermentation and the prebiotic potential of apple fibre.

3.6 Soluble polyphenol content and antioxidant status in serum and caecum

Scientific evidence has shown that phenolic compounds associated with the cell wall of plants are important DF components and account for nearly 50% of the total amount of dietary antioxidants. It is widely known that these phenolic compounds are not absorbed in the small intestine and arrive at the colon intact, along with indigestible carbohydrates, where they become fermentable substrates for the bacterial microflora, producing metabolites and an antioxidant environment (Macagnan et al., 2016). In the present study, there was a significantly higher amount of polyphenols in the serum (5.5-6.3%) and in the caecum (128-153%) of animals fed diets supplemented with modified and unmodified apple pomace fibre (Figure 2a and 2b), which can be explained by the considerable amount of nonextractable polyphenols in these alternative sources of fibre (Table 1). The presence of these polyphenols associated with the cell wall of plants is overlooked by most *in vivo* studies, but these compounds protect the colon and other body tissues through their antioxidant action. This enhanced protection was provided by the consumption of apple fibres and supported by a significantly higher antioxidant activity (AA) of serum (15-27% in the ABTS method) and of caecal content (89-171% in the FRAP method and 75-133% in the ABTS method) of animals submitted to the test treatments (Figure 2c and 2d).

It has been known so far that only the intestinal microflora can naturally disrupt the fibrous matrix structure and release associated antioxidant compounds. Intestinal bacteria can hydrolyse, reduce, decarboxylate, demethylate, and dehydroxylate polyphenols, producing several metabolites. Hence, some metabolites of colonic fermentation can be absorbed and have a systemic effect (Macagnan et al., 2016). This supports the hypothesis that polyphenols

associated with apple fibre contributed, alongside polyphenols that are bioavailable in the small intestine, to the higher AA in rat serum. In the same vein, Pérez-Jiménez et al. (2009) measured the antioxidant capacity of plasma and demonstrated that phenolic compounds associated with fibre are partially bioavailable in humans, although the fibre delays their uptake. Vitaglione et al. (2008) argued that the slow and continuous release of polyphenols associated with cereal fibre in the intestinal lumen can exert in vivo antioxidant activity, providing constant protection against diseases whose etiology and progression are governed by a state of redox imbalance. According to the authors, this slow and continuous release can be more advantageous than higher peaks of antioxidants in the bloodstream, which, in some cases, also provides a pro-oxidant environment.

As far as nonabsorbable metabolites and nonfermented polyphenols are concerned, they remain in the colonic lumen, where they can help form an antioxidant environment by the elimination of free radicals and neutralisation of the pro-oxidant effects of the diet (Macagnan et al., 2016). The formation of this antioxidant environment resulting from the presence of nonabsorbable polyphenols in the upper intestinal tract was confirmed in the present study, as modification in apple fibre facilitated the release and/or fermentation of these polyphenols in the intestine, thereby increasing antioxidant activity (21-44%, FRAP method) (9-33%, ABTS method) in the caecum of rats (Figure 2d). This increase was significant for the EAP treatment when the antioxidant activity was measured by the FRAP method (Figure 2d), leading to the assumption that the increase in fibre solubility facilitated its fermentation and release of polyphenols and consequent antioxidant activity in the intestine. Similarly, in the study of Goñi & Serrano (2005), polyphenols from grape seed DF provided remarkable antioxidant status in the large intestine of rats (approximately 80% higher in animals fed grape fibre). This antioxidant environment formed in the colon can modulate the incidence of certain types of degenerative diseases such as colon cancer (Macagnan et al., 2016). Furthermore, as

polyphenols serve as substrate for the colonic microbiota, they can influence the modulation of the intestinal microflora and act as prebiotic metabolites. Studies have shown the action of polyphenols and their metabolites on the growth of beneficial bacteria (*Lactobacillus* spp.) and on the inhibition of pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*) (Macagnan et al., 2016).

In this context, considering that apple fibres have a sizeable amount of associated polyphenols, it may be inferred that they provide important local protection to the colon and have beneficial systemic effects as does DF consumption.

4. Conclusions

Acid hydrolysis and extrusion produced different changes in the structure and physicochemical characteristics of apple fibre, which led to distinct biological responses. The physical modification of apple fibre by extrusion increased its solubility, facilitating its fermentation and intensifying the production of the butyric acid and antioxidant protection of the caecum, factors that are crucial for maintaining the integrity of the intestinal mucosa. The extrusion of the fibre also interfered positively and significantly in lipid and glucose metabolism, reducing serum triglyceride and postprandial glucose levels. Acid hydrolysis improved the prebiotic potential of the fibre, increasing its fermentability and the production of SCFA, especially of propionic and butyric acids. It is inferred that acid hydrolysis changed the physicochemical and molecular structure of the fibre, leading to the loosening of the fibrous matrix and partial breakdown of long-chain pectin polymers into smaller and more easily fermentable compounds.

The consumption of modified and unmodified apple fibres eventually increased the concentration of polyphenols in the serum and intestine of rats, providing higher antioxidant

protection. This demonstrates that these fibres also play an important physiological role as carriers of bioactive compounds through the gastrointestinal tract.

Briefly, both extrusion and acid hydrolysis of apple fibre enhanced some of its beneficial properties and improved its protective effect on intestinal health, proving efficient and capable of producing active ingredients that can be explored in human nutrition.

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Table 1. Chemical composition of the dietary fibres used in the formulation of the experimental diets.

Components	**AP-Fibre**	**HAP-Fibre**	**EAP-Fibre**
Moisture (%)	7.99 ± 0.07	8.18 ± 0.24	7.34 ± 0.50
	% dry matter		
Ash	1.37 ± 0.03	5.96 ± 0.02	1.26 ± 0.05
Protein	7.99 ± 0.94	6.87 ± 0.56	7.64 ± 0.25
Lipids	4.59 ± 0.03	4.45 ± 0.11	4.48 ± 0.01
Total dietary fibre	79.06 ± 0.24	72.10 ± 1.23	75.79 ± 0.81
Insoluble dietary fibre	65.83 ± 0.22	61.23 ± 0.63	52.68 ± 0.84
Soluble dietary fibre	13.24± 0.42	10.86 ± 1.21	23.12 ± 1.01
Non-fibrous carbohydrates	6.99 ± 0.67	10.62 ± 1.44	10.84± 0.60
Soluble pectin	9.74 ± 0.09	7.21 ± 0.10	16.34 ± 0.20
	mg /100g dry matter		
Extractable polyphenols	331.02 ± 18.41	175.49 ± 6.03	473.36 ± 26.33
Non-extractable polyphenols			
Tannins	182.40 ± 22.40	177.74 ± 20.30	175.84 ± 21.59
Hydrolysable polyphenols	955.38 ± 44.44	894.64 ± 30.78	802.52 ± 37.33
Total polyphenols	1468.80 ± 42.17	1247.87 ± 39,81	1451.72 ± 40.65

AP-Fibre: apple pomace fibre; HAP-Fibre: hydrolysed apple pomace fibre; EAP-Fibre: extruded apple pomace fibre

Table 2. Composition of the experimental diets fed to the rats according to the treatments.

	CEL	**AP**	**HAP**	**EAP**
		g/Kg		
Casein	200.00	193.48	193.77	193.47
Sucrose	100.00	96.25	93.67	95.77
Soybean oil	70.00	66.49	66.29	66.45
Starch	519.36	510.67	504.99	508.23
Cellulose	*60.00*	-	-	-
AP Fibre †	-	*82.47*	-	-
HAP Fibre †	-	-	*90.63*	-
EAP Fibre †	-	-	-	*85.43*
Mineral Mix *	35.00	35.00	35.00	35.00
Vitamin mix **	10.00	10.00	10.00	10.00
L-Cystine	3.00	3.00	3.00	3.00
Choline bitartrate	2.50	2.50	2.50	2.50
		%		
Dietary fibre	6.00	6.00	6.00	6.00
Protein	20.00	20.00	20.00	20.00
Lipids	7.00	7.00	7.00	7.00
NFC§	61.94	61.07	60.05	60.82
Crude energy (Kcal)	359.00	358.87	357.11	357.55

CEL: Control treatment with cellulose, AP: test treatment with apple pomace fibre, HAP: test treatment with hydrolysed apple pomace fibre, EAP- test treatment with extruded apple pomace fibre.

† AP Fibre: apple pomace fibre, HAP Fibre: hydrolysed apple pomace fibre, EAP Fibre: extruded apple pomace fibre

* Mineral Mix (g or mg/kg mix): Ca 142.94 g; P 44.61 g; K 102.81 g; Na 29.11 g; Cl 44.89 g; S 8.57 g; Mg 14.48 g; Fe 1.00 g; Zn 0.86 g; Si 0.14 g; Mn 0.30 g; Cu 0.17 g; Cr 0.03 g; B 14.26 mg; F 28.73 mg; Ni 14.31 mg; Li 2.85 mg; Se 4.28 mg; I 5.93 mg; Mo 4.32 mg; V 2.87 mg.

** Vitamin mix (g or mg/kg mix): nicotinic acid 3.00 g; Ca pantothenate 1.60 g; pyridoxine–HCl 0.70 g; thiamin–HCl 0.60 g; riboflavin 0.60 g; folic acid 0.20 g; biotin 0.02 g; vitamin B12 2.50 mg; vitamin E 15.00 g; vitamin A 0.80 g; vitamin D3 0.25 g; vitamin K1 0.075 g.

§ NFC = Non-fibrous carbohydrates

Table 3. Consumption, weight gain, feed efficiency ratio (FER), dry faecal production (DFP), faecal moisture, faecal lipids and faecal cholesterol of treatments.

	CEL	AP	HAP	EAP
Consumption (g/day)	10.57 ± 1.43^{b}	13.04 ± 1.18^{a}	13.60 ± 0.85^{a}	10.56 ± 0.72^{b}
Weight gain (g)	$107.45 \pm 11,41^{b}$	144.83 ± 19.06^{a}	147.72 ± 9.88^{a}	113.51 ± 13.28^{b}
FER	0.31 ± 0.26^{a}	0.34 ± 0.18^{a}	0.33 ± 0.11^{a}	0.33 ± 0.24^{a}
DFP (g/day)	0.77 ± 0.11^{b}	1.00 ± 0.13^{a}	1.00 ± 0.11^{a}	0.77 ± 0.06^{b}
Faecal moisture (%)	26.40 ± 4.20^{b}	36.48 ± 6.91^{a}	40.61 ± 3.90^{a}	42.44 ± 6.4^{a}
Faecal lipids (%)	3.81 ± 0.45^{d}	7.45 ± 0.44^{c}	7.81 ± 0.77^{b}	8.77 ± 1.50^{a}
Faecal cholesterol (μmol/g)	18.58 ± 1.60^{b}	18.90 ± 2.35^{b}	20.33 ± 4.31^{b}	25.76 ± 3.87^{a}

CEL: Control treatment with cellulose, AP: test treatment with apple pomace, HAP: test treatment with hydrolysed apple pomace fibre, EAP- test treatment with extruded apple pomace fibre.

The results are expressed as the mean ± standard deviation. Mean values followed by a different letter on the same line are significantly different using Tukey`s test ($p<0.05$). N=8

Table 4. Final fasting glucose (Glucose), Glycaemic peak, total area under the curve for glucose (AUC), serum triglycerides (TG), serum total cholesterol (Total-C), serum HDL cholesterol (HDL-C), non-HDL cholesterol (Non-HDL-C), serum total protein (Total protein), liver lipids content (Liver-Lip), liver total cholesterol (Liver-Chol), faecal pH, and apparent digestibility of dietary fibre (AD-DF), weight liver (Liver), weight epididymal fat (Epid-Fat) of treatments.

Parameters	CEL	AP	HAP	EAP
Glucose (mg/dL)	85.37 ± 10.51^{a}	83.14 ± 8.07^{a}	80.43 ± 10.23^{a}	87.71 ± 5.06^{a}
Glycaemic peak (mg/dL)*	169.88 ± 25,40^{a}	152.38 ± 8.26^{a}	161.29 ± 10.55^{a}	135.00 ±10.38^{b}
AUC (mg/dL.min)	139.70 ± 4.94^{a}	130.86 ± 5.68^{b}	139.92 ± 6.64^{a}	118.92 ± 6.41^{c}
TG (mg/dL)	62.73 ± 8.26^{a}	46.69 ± 14.04^{ab}	40.97 ± 13.34^{bc}	26.34 ± 8.55^{c}
Total-C (mg/dL)	134.12 ± 16.34^{a}	115.88 ± 22.96^{a}	116.78 ± 19.94^{a}	114.65 ± 18.83^{a}
HDL-C (mg/dL)	92.21 ± 13.43^{a}	83.14 ± 15.21^{a}	84.73 ± 14.79^{a}	88.46 ± 19.66^{a}
Non-HDL-C (mg/dL)	41.91 ± 7.88^{a}	32.74 ± 8,98^{ab}	32.05 ± 8.71^{ab}	26.19 ± 6.99^{b}
Total protein (mg/dL)	5.24 ± 0,17^{a}	5.54 ± 0,20^{a}	5.54 ± 0,30^{a}	5.38 ± 0,27^{a}
Liver-Chol (µmol/g)	16.66 ± 1.82^{a}	11.06 ± 2.40^{b}	11.63 ± 2.38^{b}	11.50 ± 2.58^{b}
Liver-Lip (%)	4.71 ± 0.20^{a}	4.55. ± 0.38^{a}	4.57 ± 0.12^{a}	4.53 ± 0.23^{a}
Liver (% body weight)	4.32 ± 0.29^{a}	4.18. ± 0.31^{a}	3.97 ± 0.30^{a}	3.89 ± 1.18^{a}
Epid-Fat (% body weight)	0.93 ± 0.16^{a}	1.18. ± 0.30^{a}	1.08 ± 0.38^{a}	1.26 ± 0.31^{a}
AD-DF (%)	16.83 ± 1.03^{c}	30.56 ± 3.49^{b}	39.20 ± 4.80^{a}	42.52 ± 5.46^{a}
Faecal pH	5.79 ± 0.18^{a}	5.47 ± 0.17^{b}	5.24 ± 0.13^{c}	5.51 ± 0.12^{cb}

CEL: Control treatment with cellulose, AP: test treatment with apple pomace, HAP: test treatment with hydrolysed apple pomace fibre, EAP- test treatment with extruded apple pomace fibre.

* Glycaemic peak = 30 min after the meal

The results are expressed as the mean ± standard deviation. Mean values followed by different letters on the same line are significantly different using Tukey`s test ($p<0.05$). N=8

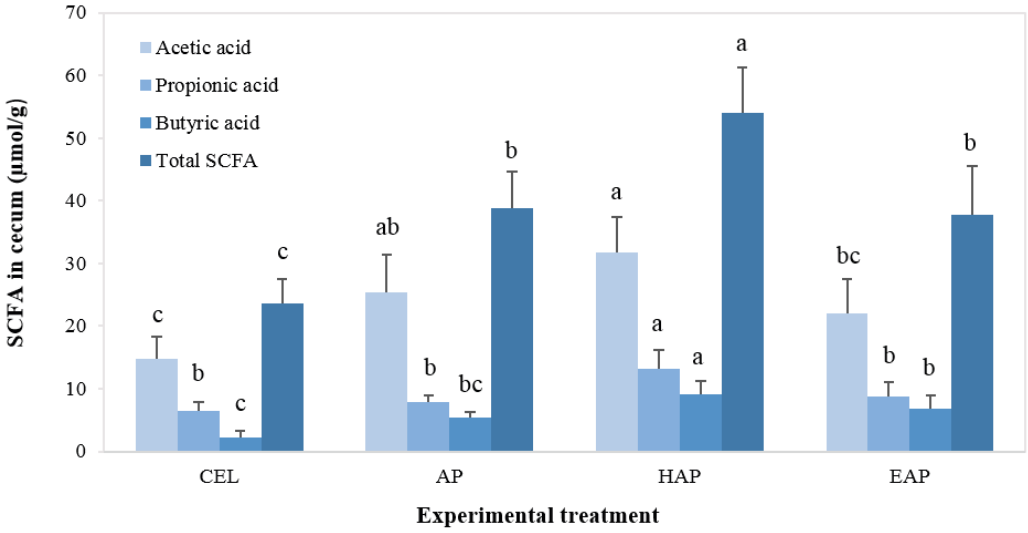

Figure 1. Short-chain fatty acid (SCFA) concentration of acetic acid, propionic acid, butyric acid and total SCFA in caecum of rats. CEL – Control treatment with cellulose, AP – test treatment with apple pomace, HAP – test treatment with hydrolysed apple pomace fibre, EAP – test treatment with extruded apple pomace fibre. The values represent the means ± standard deviation. Different superscripted letters in columns indicated statistical differences using Tukey`s test ($p<0.05$). N=7

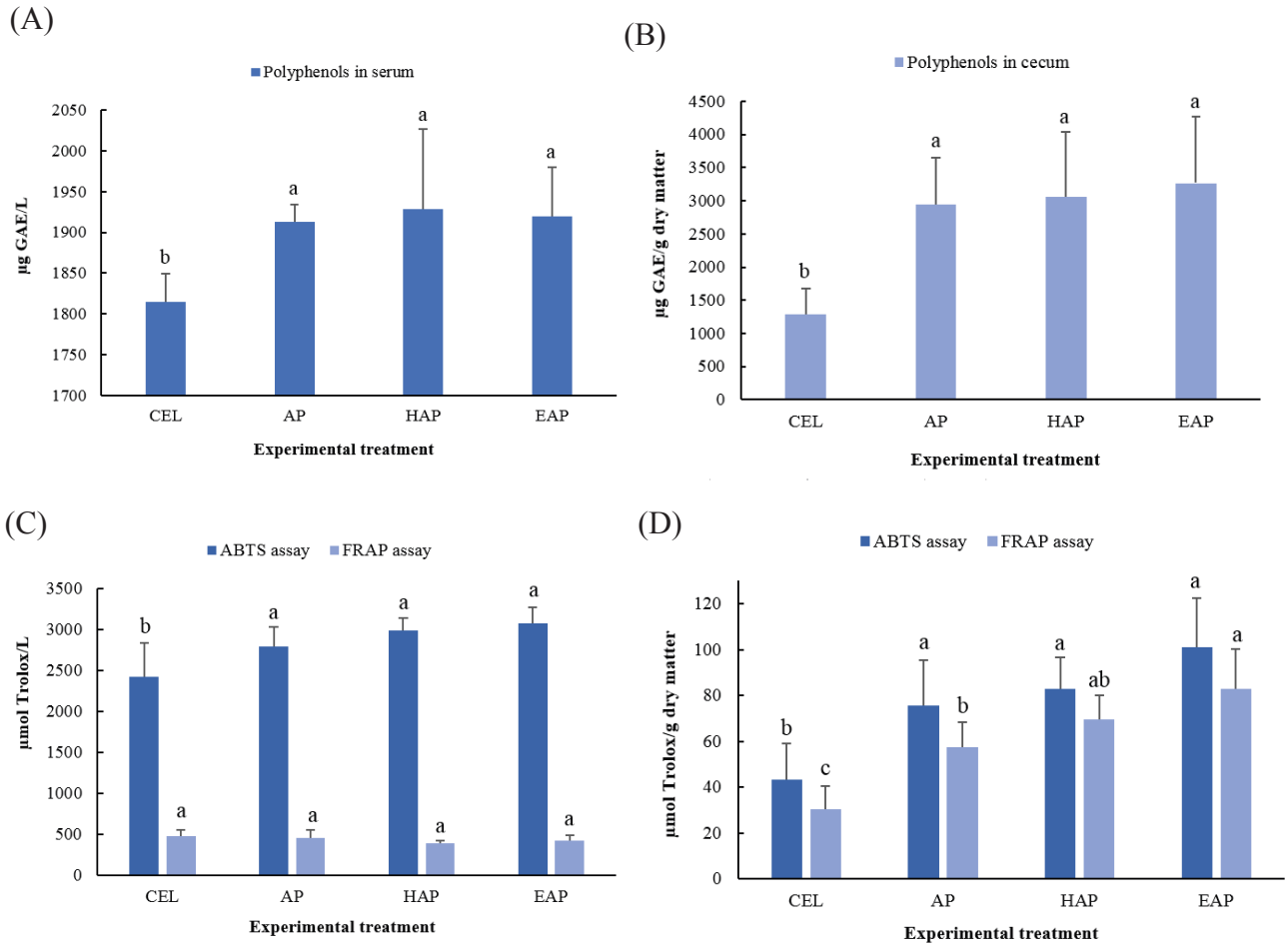

Figure 2. Soluble polyphenols and antioxidant status in serum and caecal contents of rats. (A) Content of polyphenols in serum, (B) Content of polyphenols in caecum, (C) Antioxidant capacity in serum, (D) Antioxidant capacity in caecum. Different superscripted letters in columns indicated statistical differences using Tukey`s test ($p<0.05$). N=8.

4 DISCUSSÃO

Nas últimas décadas, inúmeros conhecimentos novos acerca do componente dietético fibra alimentar foram descobertos (MACAGNAN et al., 2016). Assim, a fibra deixou de ser vista apenas como um regulador do trânsito intestinal e passou a ser considerada uma potente aliada no tratamento e prevenção de patologias como a aterosclerose, dislipidemias, diabetes e câncer de cólon (KACZMARCZYK et al.; 2012; KENDALL et al., 2010). Atualmente, ainda há muito o que se explorar nessa área, tanto em relação a importância biológica da presença de compostos bioativos associados à fibra, como em relação a utilização de métodos diferenciados de modificação que visem aprimorar suas qualidades funcionais e direcionar sua aplicação de forma eficiente na nutrição humana.

O Brasil apresenta produção expressiva de maçã e a sua industrialização gera grandes quantidades de bagaço como subproduto, o qual é descartado incorretamente ou utilizado para nutrição animal. Entretanto, o bagaço de maçã é um subproduto valioso para ser utilizado na alimentação humana, pois é boa fonte de compostos antioxidantes e de fibra alimentar (MACAGNAN et al., 2015), ambos fatores dietéticos reconhecidos na prevenção de doenças crônicas. A pesquisa no campo da ciência e tecnologia dos alimentos é fundamental na produção de conhecimentos científicos que ofereçam subsídios para o melhor aproveitamento de subprodutos agroindustriais e que visem, além da proteção do meio ambiente, a promoção e a manutenção da saúde humana. Nesse sentido, este trabalho estudou a influência da hidrólise ácida e da extrusão sobre as características físico-químicas e biológicas da fibra do bagaço de maçã, buscando a potencialização de seus efeitos benéficos e a sua valorização industrial e científica. Aliado a isso, investigou-se também os compostos associados à parede celular vegetal e a sua relação com a maior proteção antioxidante do organismo.

Os resultados obtidos nesse estudo demonstram que a fibra oriunda do bagaço de maçã apresenta elevada fermentabilidade e boas propriedades biológicas. Entre os resultados positivos encontrados para o consumo da fibra de maçã não modificada pelos animais experimentais em relação ao consumo da fibra controle celulose, foram: melhor fermentabilidade, aumento da umidade fecal, redução do pH das fezes, maior excreção fecal de gordura, melhor resposta glicêmica pós-prandial, menor nível de colesterol no fígado e maior produção de AGCCs, principalmente do acético, seguido do propiônico e do butírico. O perfil de fermentação encontrado para a fibra de maçã foi semelhante ao relatado para a pectina de maçã em outros estudos (CAMBRODÓN & MARTÍN-CARRÓN, 2001).

Contudo, as modificações testadas da fibra de maçã mostraram-se eficazes, pois intensificaram algumas de suas propriedades benéficas e melhoraram seu efeito protetor da saúde intestinal. Tais tratamentos provocaram diferentes alterações na estrutura e nas características físico-químicas da fibra, o que resultou em respostas biológicas diferenciadas.

Os resultados obtidos com a hidrólise ácida da fibra de maçã demostraram que esse tratamento melhorou todas as características físico-químicas da fibra, devido provavelmente a alterações na sua estrutura física e molecular. De forma geral, condições experimentais mais agressivas (pH menor e tempo de reação maior) proporcionaram à fibra maior capacidade em ligar ácidos biliares (55%), inibir a atividade da enzima lipase pancreática (206%) e reter água (11%) e gordura (20%) na sua estrutura. Entretanto, observou-se redução expressiva da proporção de fibra solúvel (FS). Resultados contrários foram obtidos no estudo de Ning et al. (1991), o qual demonstrou aumento significativo (23%) no teor de fibra solúvel após o tratamento ácido (HCl, pH 2,0, 90ºC, 4 horas) da fibra de milho. Diferente da maioria das fibras de cereais, o bagaço de maçã apresenta teor relevante de fibra solúvel tipo pectina, a qual é facilmente hidrolisada por aquecimento, em meio ácido (CANTERI et al., 2012). Nesse sentido, infere-se que as condições utilizadas no planejamento experimental não foram agressivas o suficiente para solubilizar os polissacarídeos insolúveis. Contudo, provocaram despolimerização parcial da pectina, formando compostos de cadeias moleculares menores e inclusive, oligossacarídeos pécticos, os quais não são quantificados pelo método enzimático-gravimétrico utilizado na determinação da fibra, mas podem exercer efeito positivo sobre a sua fermentabilidade (MACAGNAN et al., 2016). Tal hipótese é apoiada pelos resultados favoráveis obtidos no estudo *in vitro* da atividade bifidogênica da fibra. Nessa avaliação, a fibra de maçã hidrolisada (H_2SO_4 1.5N por 3 horas) estimulou significativamente o crescimento das culturas de *Bifidobacterium lactis* e *Lactobacillus acidophilus,* sendo que o crescimento de ambas as culturas probióticas mostrou-se superior ao encontrado para a fibra não tratada. Além disso, em 48 horas de incubação, a fibra hidrolisada apresentou para a cultura de *B.lactis* crescimento similar a inulina, não diferindo estatisticamente desse prebiótico comercial.

A potencialização do efeito prebiótico da fibra de maçã pela hidrólise ácida foi constatada também no estudo *in vivo* com ratos Wistar. Essa modificação química destacou-se por melhorar ($p<0,05$) a digestibilidade aparente da fibra, reduzir o pH fecal e a aumentar de forma significativa a produção de AGCCs (39%), em particular propiônico (67%) e butírico (66%). Dessa forma, pressupõem-se que a hidrólise ácida alterou a estrutura da fibra e possibilitou seu melhor aproveitamento pelas bactérias benéficas presentes no cólon humano. Apesar da efetiva melhora das propriedades físico-químicas na avaliação *in vitro*, a fibra de

maçã modificada quimicamente não interferiu de forma significativa nos parâmetros bioquímicos dos ratos quando comparada a fibra não modificada e não se mostrou eficaz na melhora da resposta glicêmica pós-prandial. Observou-se, contudo, intensificação da excreção fecal de gordura (4,8%) ($p < 0,05$) e colesterol (7,6%) ($p > 0,05$), bem como da redução (35%) ($p > 0,05$) do nível de triglicerídeos sérico, devido provavelmente a sua maior capacidade de ligar ácidos biliares e aprisionar gordura na sua estrutura e de influenciar a metabolização dos lipídeos pela lipase pancreática.

Todas a condições experimentais testadas no processo de extrusão do bagaço de maçã levaram à redistribuição entre as frações da fibra, aumentando de forma significativa o teor de FS. A degradação parcial da parede celular foi confirmada pela redução nos seus teores de celulose e hemicelulose e aumento no seu teor de pectina solúvel, sendo o conteúdo de lignina inalterado pelo processo. O ensaio de extrusão, que utilizou temperatura de 90ºC e teor de umidade de 33%, foi o mais eficiente na solubilização da fibra, aumentando os valores de FS de 13,31 para 28,30%, e o teor de pectina de 9,12 para 17,50%. A solubilização da fibra é um fator importante para aumentar sua disponibilidade aos microrganismos do cólon e melhorar a sua fermentabilidade, fato evidenciado pelo maior estímulo ao crescimento da cultura probiótica de *Lactobacillus acidophillus* do bagaço de maçã após o processo de extrusão.

O efeito positivo da extrusão sobre a fermentabilidade da fibra também foi confirmado no ensaio biológico. Durante o processo de extrusão, o material é submetido a intenso cisalhamento mecânico e o cozimento ocorre com temperatura e pressão elevada e com baixo teor de água. Dessa forma, esse tratamento físico é capaz de desorganizar completamente a estrutura original do material (DAOU & ZHANG, 2012). Pressupõe-se que energia gerada durante a extrusão da fibra de maçã, além de provocar a sua solubilização, causou forte alteração na estrutura da fibra, tornando-a mais aberta e porosa (DAOU e ZHANG, 2012), o que facilitou a sua degradação pelas bactérias intestinais, aumentando de forma significativa em 39% a sua digestibilidade aparente. A melhora da fermentabilidade da fibra após o processo de extrusão também foi relatada em estudo *in vivo*, no qual a farinha de trigo extrusada apresentou maior solubilidade e foi mais extensivamente degradada no intestino de ratos (BJÖRCK et al., 1984).

Os padrões de fermentação e a proporção de AGCCs produzida dependerá do tipo de fibra alimentar (KACZMARCZYK et al., 2012). Nesse sentido, embora não tenha diferido estatisticamente da fibra não modificada, a extrusão alterou a proporção entre os AGCCs, intensificando a formação do propiônico (12%) e, principalmente, do butírico (25%). De forma semelhante, Arcila et al. (2015) relataram que a extrusão do farelo de trigo aumentou sua solubilidade, o que permitiu maior poder de fermentação *in vitro* pela microbiota fecal humana,

com incremento da produção de AGCCs, especialmente do butirato. O ácido butírico parece ser o AGCC de mais acentuado efeito tópico sobre a saúde do epitélio colônico e vem recebendo atenção especial dos pesquisadores por apresentar efeitos benéficos em relação ao câncer de cólon (RIEGEL, 2012; PACHECO & SGARBIERI, 2001). Segundo Riegel (2012), a insuficiente produção desse AGCC ocasiona lesões no cólon, tais como inflamações, polipose e câncer. Nesse sentido, pode-se afirmar que ambas as modificações testadas melhoraram a fermentabilidade da fibra, intensificaram a produção do ácido butírico, e aumentaram a proteção e a saúde do intestino. Contudo, a modificação química mostrou-se mais eficaz que a extrusão em relação a melhora do perfil de fermentação e do potencial prebiótico da fibra de maçã.

O consumo da fibra extrusada proporcionou maior ($p<0.05$) excreção fecal de gordura (18%) e colesterol (36%), bem como redução expressiva ($p<0.05$) no nível sanguíneo de triglicerídeos (44%) dos animais experimentais. Esses resultados positivos são subsidiados pela melhora das propriedades físico-químicas observadas na avaliação *in vitro* após o processo de extrusão (maior capacidade em ligar ácidos biliares e reter gordura em sua estrutura, e maior potencial de inibição da enzima lipase pancreática).

A modificação da fibra pelo processo de extrusão destacou-se também na análise da glicemia pós-prandial, pois permitiu aumento tênue da glicose sanguínea e contribuiu na manutenção da sua estabilidade. (Apêndice C). O tratamento com a fibra extrusada apresentou o menor pico glicêmico ($p<0.05$) e AUC após o desjejum, o que torna a fibra de maçã extrusada ingrediente interessante de ser explorado, especialmente, na dieta de pacientes diabéticos, que necessitam manter regulares os níveis de glicose no sangue, evitando hiperinsulinemia e disfunção ou desregulação das células β-pancreáticas (KOPP, 2006). Essa boa resposta glicêmica pode ser justificada, principalmente, pelo aumento da viscosidade do conteúdo intestinal devido à presença de quantidade superior de compostos solúveis em água, em particular da pectina (Apêndices B e C). A maior viscosidade das amostras extrusadas e sua influência positiva sobre a glicemia, já haviam sido relatadas em estudos *in vitro* para outras fontes alimentares de fibra como a polpa de laranja (CÉSPEDES et al., 2010) e o farelo de arroz (DAOU e ZHANG, 2012). Fibras viscosas formam géis que diminuem o contato do conteúdo luminal com a lipase pancreática e a bile, devido a formação de agregados FA-lipídeo, que bloqueiam a emulsificação da gordura e a formação de micelas (KACZMARCZYK et al., 2012). Nesse sentido, supõe-se que a maior viscosidade proporcionada pela fibra extrusada interferiu também na metabolização e absorção da gordura, intensificando a excreção fecal de lipídeos e, consequentemente, o potencial hipolipidêmico da fibra de maçã.

Sabe-se que os compostos fenólicos associados à parede celular vegetal não são absorvidos no intestino delgado, e assim, chegam intactos ao cólon juntamente com os carboidratos indigeríveis, onde se tornam substratos fermentáveis para a microbiota bacteriana, produzindo metabólitos e um ambiente antioxidante (GOÑI et al., 2009; MANACH et al., 2005; SAURA-CALIXTO, 2011; SAURA-CALIXTO & DÍAZ-RUBIO, 2007). No presente estudo, encontrou-se quantidade significativamente superior de polifenois no soro (5.5 -6.3%) e no ceco (128 – 153%) dos animais alimentados com as dietas contendo as fibras do bagaço de maçã (modificada e não modificada). Isso pode ser justificado pelo teor considerável de polifenois não extraíveis (polímeros de polifenois e polifenois de baixo peso molecular associados à fibra) nessas fontes alternativas de fibra. A presença de polifenois associados à fibra é ignorada na maioria dos estudos científicos *in vivo*, provavelmente, por se acreditar que eles são constituintes irrelevantes do ponto de vista quantitativo e fisiológico (MACAGNAN et al., 2016). Entretanto, exercem importante proteção antioxidante ao cólon e demais tecidos corporais. Esse fato é subsidiado pela atividade antioxidante significativamente superior do soro (15-27% pelo ensaio ABTS) e do conteúdo cecal (89-171% pelo ensaio FRAP; 75 – 133% pelo ensaio ABTS) dos animais submetidos aos tratamentos teste. De acordo com a análise do teor de polifenois totais, a fibra de maçã apresentou quantidade relevante de polifenois não extraíveis (hidrolisáveis e taninos), os quais exercem considerável atividade antioxidante. Dessa forma, os resultados positivos encontrados no ensaio biológico indicam que a fibra de maçã atua como importante carreadora desses compostos bioativos através do trato gastrointestinal e proporciona maior proteção antioxidante ao organismo.

As modificações da fibra da maçã aumentaram a sua digestibilidade, o que, provavelmente, contribuiu para facilitar a liberação e/ou fermentação dos polifenois no intestino, resultando no incremento da atividade antioxidante (21-44%, ensaio FRAP; 9-33%, ensaio ABTS) no ceco dos ratos. Esse aumento se mostrou significativo para o tratamento adicionado de fibra extrusada pelo método de FRAP. Os resultados obtidos no estudo do processo de extrusão do bagaço de maçã sob diferentes condições de temperatura e umidade, mostraram que a energia gerada pelo processamento possibilitou a liberação parcial dos polifenois associados à fibra. Contudo, no estudo *in vivo*, não se observou diferença no teor de polifenois e atividade antioxidante do soro. Infere-se assim, que mesmo com menor quantidade de polifenois associados a fibra, a maior fermentabilidade da fibra extrusada facilitou a liberação daqueles polifenois associados para exercerem ação antioxidante no intestino dos ratos.

Esse ambiente antioxidante formado no cólon pode modular a incidência de certos tipos de doenças degenerativas, como o câncer do cólon. Além disso, pelo fato dos polifenois servirem de substrato para microbiota colônica, eles podem exercer efeitos na modulação da mesma e atuar como metabólitos prebióticos. Estudos tem mostrado a ação dos polifenois e seus metabólitos no crescimento de bactérias benéficas (*Lactobacillus* spp.) e na inibição de bactérias patogênicas (*Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*) (MACAGNAN et al., 2016).

Nesse sentido, percebe-se que as propriedades físico-químicas da fibra alimentar podem ser manipuladas por meio de métodos químicos e físicos, como a extrusão e a hidrólise ácida, com o intuito de melhorar as suas características biológicas. Ressalta-se também, que tanto a análise química quanto a avaliação *in vitro* das propriedades físico-químicas da fibra, são determinações importantes, pois podem prever seu comportamento fisiológico e direcionar as melhores condições de processamento. Contudo, o estudo *in vivo* é essencial para avaliar a real ação que a fibra desempenha em um meio biológico complexo como o organismo e, comprovar seus benefícios. Nesse estudo, a hidrólise ácida e a extrusão da fibra de maçã mostraram-se tratamentos eficazes, produzindo ingredientes ativos diferenciados para serem explorados na nutrição funcional humana.

5 CONCLUSÃO

Os métodos de modificação da fibra alimentar da maçã avaliados no presente trabalho foram eficazes, intensificando algumas de suas propriedades benéficas e melhorando seu efeito protetor da saúde intestinal. Tais métodos provocaram diferentes alterações na estrutura e nas características físico-químicas da fibra, o que resultou em respostas biológicas diferenciadas.

A fibra de maçã submetida a hidrólise ácida apresentou melhora em todas as suas propriedades físico-químicas no estudo *in vitro*. Contudo, contribuiu de maneira menos eficaz no metabolismo lipídico e glicídico dos animais experimentais, proporcionando aumento da excreção fecal de gordura e intensificando a redução dos níveis séricos de triglicerídeos. A hidrólise química se destacou por melhorar as propriedades prebióticas da fibra, tanto em relação ao estímulo do crescimento das bactérias benéficas do cólon, quanto a maior produção de AGCC. Dessa forma, sua inclusão na nutrição humana poderia contribuir de forma mais eficaz na proteção, promoção e manutenção da saúde intestinal.

A fibra extrusada apresentou propriedades hipolipidêmicas e hipoglicêmicas que fortalecem seu uso em dietas que visam a redução e/ou manutenção dos níveis de lipídeos sanguíneos, e também, o maior controle da glicemia pós-prandial. Além disso, o processo de extrusão aumentou o teor de fibra solúvel, a fermentabilidade e a capacidade da fibra de maçã estimular o crescimento da cultura probiótica de *Lactobacillus acidophillus*, fornecendo assim, maiores benefícios a integridade intestinal.

O trabalho revelou também que, grande parte dos compostos fenólicos do bagaço de maçã encontra-se associada à fibra ou não são solúveis em solventes orgânicos e aquosos, sendo que as condições experimentais utilizadas no processo de extrusão foram capazes de provocar a liberação parcial desses compostos a partir da matriz da parede celular e aumentar a atividade antioxidante relativa aos polifenois extraíveis. Com os resultados obtidos no ensaio biológico, pode-se inferir que a presença de compostos bioativos associados à fibra da maçã, desempenham importante papel na saúde intestinal, proporcionando maior proteção antioxidante ao intestino.

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APÊNDICES

APÊNDICE A – Bagaço de maçã *in natura* (A), após a secagem em estufa de circulação (B) e a após a moagem em micromoinho (C).

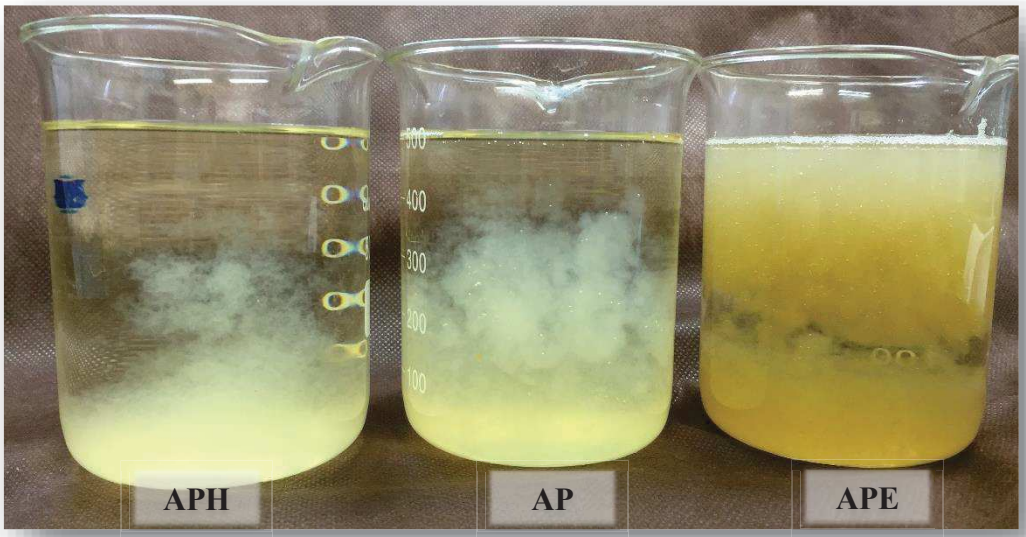
(A)

(B)

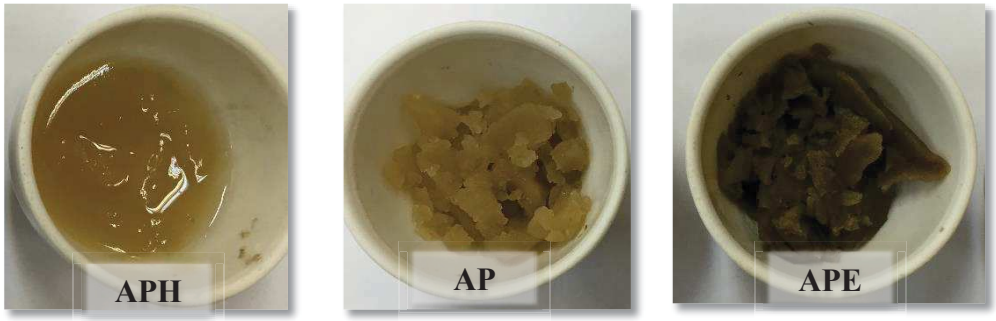
(C)

APÊNDICE B – Análise do teor de pectina nas amostras. (A) Precipitação da pectina, (B) Pectina filtrada após a precipitação. APH: Fibra do bagaço de maçã. AP: Fibra do bagaço de maçã submetida a hidrólise ácida (H_2SO_4, 1,5N, 3 h). APE: Fibra do bagaço de maçã submetida ao processo de extrusão (33% de umidade, 90 ºC).

(A)

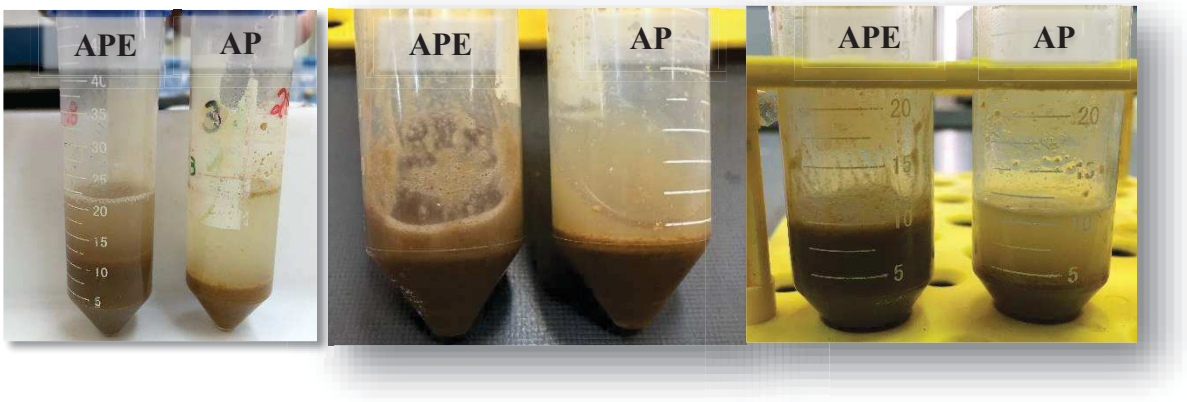


(B)

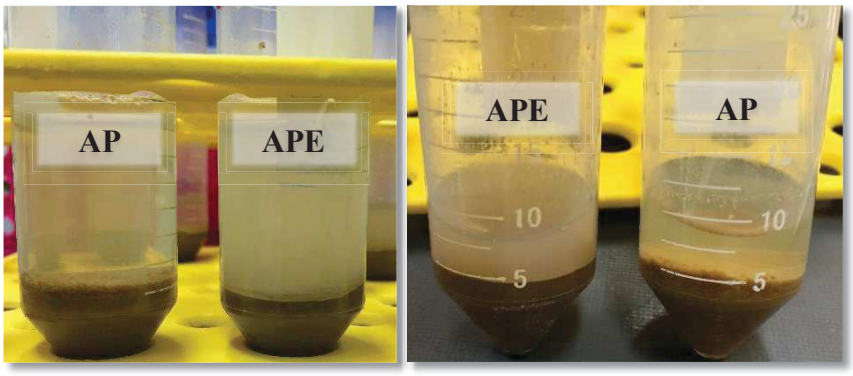


APÊNDICE C- Soluções aquosas de fibra de maçã (1g/20mL e 1g/10mL) antes e após o processo de extrusão (A) Soluções aquosas de fibra antes da centrifugação, (B) Soluções aquosas de fibra após a centrifugação. AP: fibra do bagaço de maçã, APE: fibra do bagaço de maçã submetida ao processo de extrusão (33% de umidade, 90 ºC).

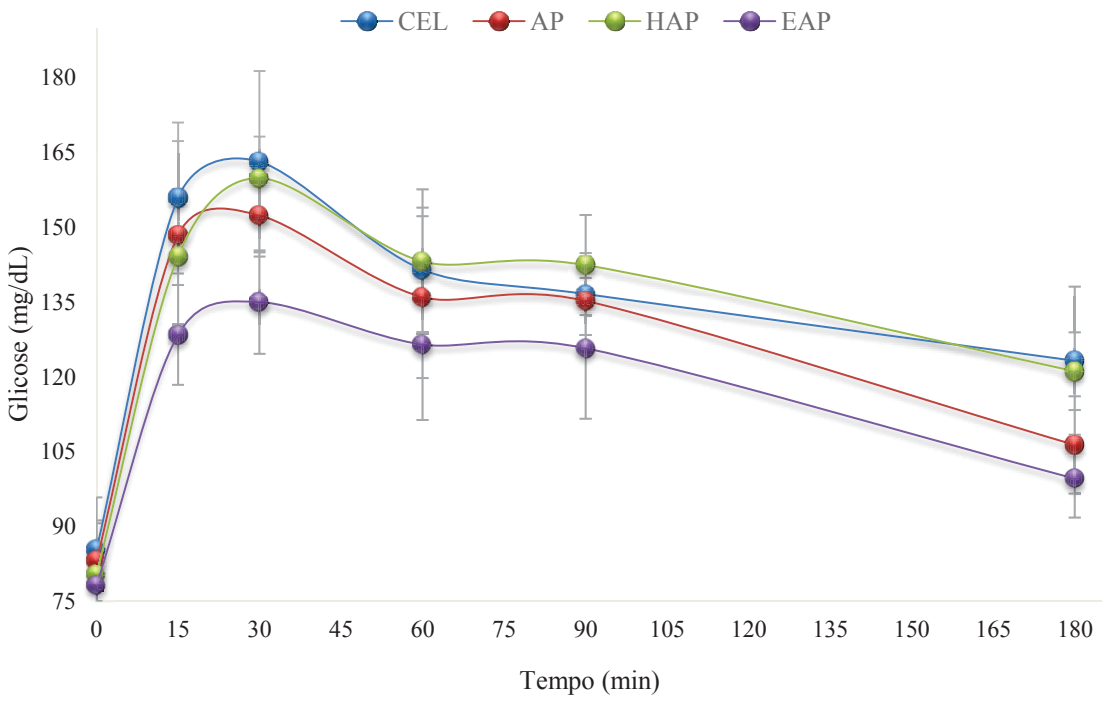
(A)



(B)



APÊNDICE D – Concentração plasmática pós-prandial de glicose em resposta ao consumo de dietas com diferentes fibras alimentares. CEL: tratamento controle com celulose, AP: tratamento teste com fibra de maçã, HAP: tratamento teste com fibra de maçã submetida a hidrólise ácida, EAP: tratamento teste com fibra de maçã submetida ao processo de extrusão.



APÊNDICE E – Cromatogramas dos AGCCs (ácido acético, ácido propiônico e ácido butírico) presentes no conteúdo cecal dos ratos. (A) Padrões, (B) tratamento controle com celulose, (C) tratamento teste com fibra de maçã, (D) tratamento teste com fibra de maçã submetida a hidrólise ácida, (E) tratamento teste com fibra de maçã submetida ao processo de extrusão.