PROGRAMA DE PÓS-GRADUAÇÃO EM ALIMENTOS E NUTRIÇÃO
CENTRO DE CIÊNCAS BIOLÓGICAS E DA SAÚDE
UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO

Nathânia de Sá Mendes

Capsicum (*baccatum* e *pubescens*): UM POTENCIAL INGREDIENTE FUNCIONAL

Capsicum (*baccatum* and *pubescens*): A POTENTIAL FUNCTIONAL INGREDIENT

de Sá Mendes, Nathânia
d538 Capsicum (baccatum e pubescens): UM POTENCIAL INGREDIENTE FUNCIONAL / Nathânia de Sá Mendes. -- Rio de Janeiro, 2020.
144 p.

Orientadora: Édira Castello Branco de Andrade Gonçalves.
Coorientadora: María del Pilar Buera.
Tese (Doutorado) - Universidade Federal do Estado do Rio de Janeiro, Programa de Pós-Graduação em Alimentos e Nutrição, 2020.

1. Pimentas. 2. Compostos fenólicos. 3. Isotermas . 4. SEM - EDS. 5. Ingrediente funcional. I. Castello Branco de Andrade Gonçalves, Édira , orient. II. del Pilar Buera, María , coorient. III. Título.

NATHÂNIA DE SÁ MENDES

Capsicum (*baccatum* e *pubescens*): UM POTENCIAL INGREDIENTE FUNCIONAL

Tese de Doutorado – Programa de Pós-Graduação em Alimentos e Nutrição da Universidade Federal do Estado do Rio de Janeiro, como requisito parcial para obtenção do título de Doutor em Alimentos e Nutrição.

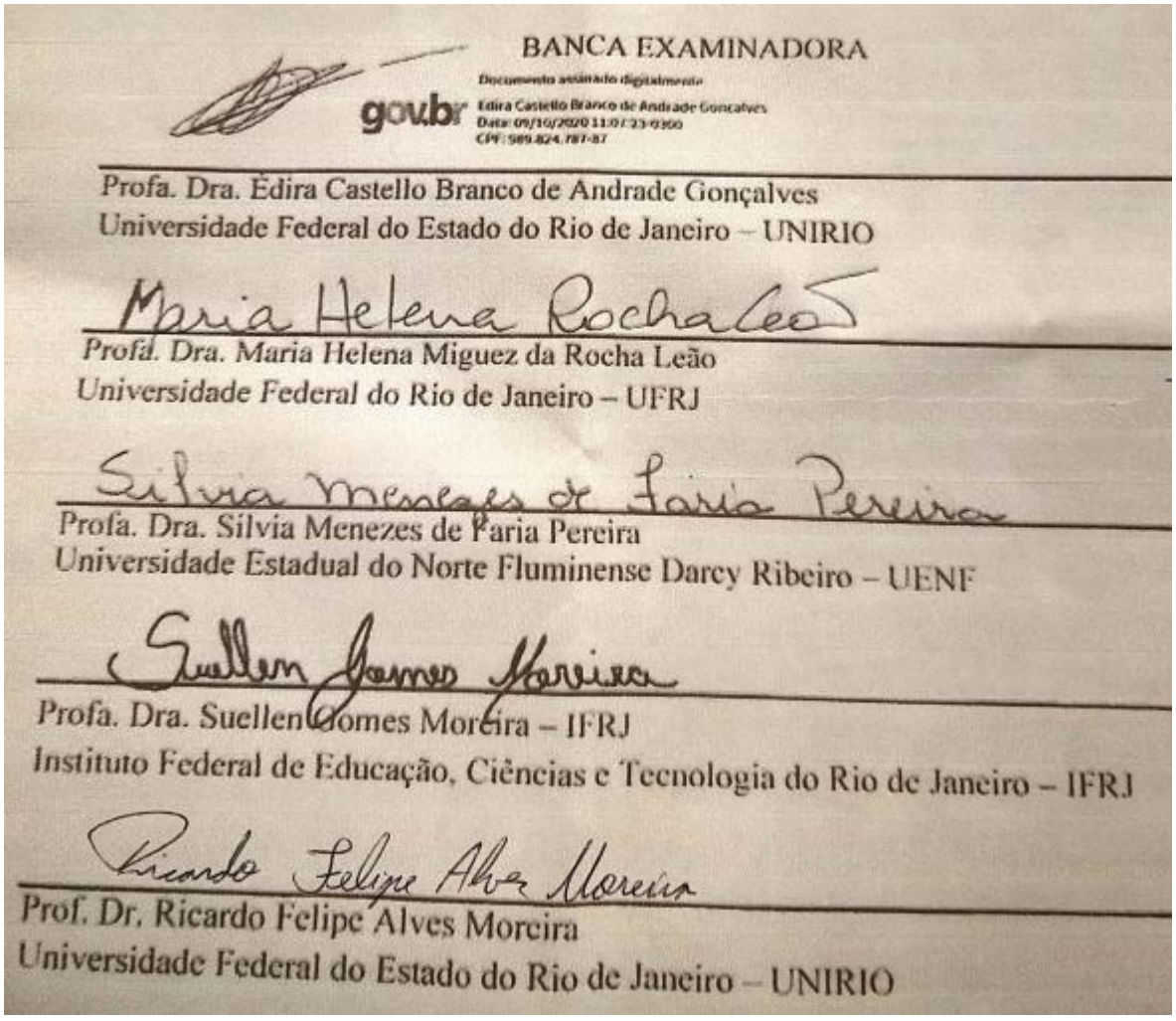
Orientadora: Profa. Dra. Édira Castello Branco de Andrade Gonçalves
Coorientadora: Profa. Dra. María del Pilar Buera

Nathânia de Sá Mendes

Capsicum (*baccatum* e *pubescens*): UM POTENCIAL INGREDIENTE FUNCIONAL

Tese de doutorado apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição na Universidade Federal do Estado do Rio de Janeiro.

Aprovada em: 11/09/2020



RIO DE JANEIRO
2020

Dedico este trabalho aos meus queridos pais,
irmãos e sobrinha Tayla

AGRADECIMENTOS

À Deus por nunca ter me abandonado, por sempre guiar e iluminar meus caminhos, e por ter a oportunidade de concluir mais uma importante etapa na minha vida;

Aos meus pais, Hélio e Suzete, que apostaram em mim mais do que ninguém. Quero agradecer vocês por tudo;

Aos meus irmãos Tauana e Helisson, pelo apoio incondicional, orações e por acreditar na minha força de trabalho;

Minha sobrinha querida Tayla, uma bênção sem igual na minha vida;

À UNIRIO pela oportunidade do curso e por disponibilizar a infraestrutura para a realização do trabalho;

À FAPERJ, pelo suporte financeiro e ao programa Santander pela oportunidade de fazer uma parte do meu doutorado na Argentina;

À orientadora Édira, por abraçar a idéia deste trabalho, aceitando me orientar, por toda a ajuda, ensinamento e dedicação, sempre se mostrando acessível e disposta a qualquer momento, e por acreditar que sou capaz;

À coorientadora Pilar, minha gratidão pela oportunidade da parceria e valiosa contribuição a este trabalho;

Aos professores Maria Helena, Ricardo, Silvia e Suellen por aceitarem fazer parte da banca examinadora deste trabalho;

À todos os professores e funcionários do PPGAN com os quais tive a oportunidade de conviver e pelo conhecimento que comigo compartilharam;

Aos amigos “de bancada” Tamara, Pedro, Monica e Roberta pelo incentivo, amizade e por sempre ter acreditado e participado de momentos importantes da minha história;

À amiga Tamara pela amizade sincera, pelos conselhos, ensinamentos e companheirismo;

À Marilene, que me recebeu com tanto carinho que me senti em casa, além de incentivo, preocupação e dedicação. Você tem lugar especial no meu coração;

À todos aqueles que, direta ou indiretamente, contribuíram para a realização deste trabalho o meu mais profundo agradecimento;

Mais uma etapa foi vencida!

“Suba o primeiro degrau com fé
não é necessário que você
veja toda a escada
apenas dê o primeiro passo”

Damião Maximino

RESUMO

Devido ao seu conteúdo em compostos bioativos, é necessária atenção especial na caracterização e exploração de pimentas do gênero *Capsicum* com usos múltiplos, atualmente pouco estudadas. O objetivo deste trabalho foi estudar as propriedades metabolômicas, morfológicas e químicas de duas pimentas: *C. baccatum* e *C. pubescens*. Para tal, os objetivos específicos foram caracterizar o perfil de compostos fenólicos por cromatografia líquida de ultra eficiência acoplada a espectrometria de massas (UPLC-MS^E), caracterizar fisicamente aplicando microscopia eletrônica de varredura (MEV) acoplada à espectroscopia por energia dispersiva (EDS) e isotermas de sorção, além de avaliar as propriedades físico-químicas de farinha de pimenta (PF). Um total de 42 e 61 compostos fenólicos foram identificados em *C. baccatum* e *C. pubescens*, respectivamente. Dez compostos em comum foram encontrados nessas espécies, indicando grande variação no perfil destas. O modelo que melhor se ajustou às isotermas de sorção foi o GAB e as análises de microestrutura e composição elementar mostraram superfície rugosa composta principalmente por polissacarídeos, sendo o potássio e o magnésio os elementos mais abundantes. A adição de PF (*C. baccatum)* à farinha de frutas e hortaliças (FVR) melhorou sua estabilidade e propriedades funcionais, e a relação entre o conteúdo fenólico total e a atividade antioxidante foi positiva para MIX (PF / FVR). Para *C. pubescens*, o uso de extratores, água e etanol (50%), não influenciou o conteúdo total de compostos fenólicos (ensaio de Folin-Ciocalteu) e atividade antioxidante (ensaios ABTS, FRAP e ORAC). Todas as amostras estudadas têm potencial como ingrediente alimentar para usos funcionais e tecnológicos.

Palavras-chave: *Capsicum*; compostos fenólicos; isotermas; SEM - EDS; ingrediente funcional.

ABSTRACT

Due to its content in bioactive compounds, special attention is needed in the characterization and exploration of *Capsicum* peppers with multiple uses, currently little studied. The objective of this work was to study the metabolomic, morphological and chemical properties of two peppers: *C. baccatum* and *C. pubescens*. To this end, the specific objectives were to characterize the profile of phenolic compounds by ultra-efficient liquid chromatography coupled with mass spectrometry (UPLC-MS^E), to characterize physically using scanning electron microscopy (SEM) coupled with dispersive energy spectroscopy (EDS) and sorption isotherms, in addition to assessing the physico-chemical properties of pepper flour (PF). A total of 42 and 61 phenolic compounds were identified in *C. baccatum* and *C. pubescens*, respectively. Ten common compounds were found in these species, indicating great variation in their profile. The model that best adjusted to the sorption isotherms was the GAB and the microstructure and elemental composition analyzes showed a rough surface composed mainly of polysaccharides, with potassium and magnesium being the most abundant elements. The addition of PF (*C. baccatum*) to fruit and vegetable flour (FVR) improved its stability and functional properties, and the relationship between total phenolic content and antioxidant activity was positive for MIX (PF / FVR). For *C. pubescens*, the use of extractors, water and ethanol (50%), did not influence the total phenol content (Folin-Ciocalteu test) and antioxidant activity (ABTS, FRAP and ORAC tests). All samples studied have potential as a food ingredient for functional and technological uses.

Keywords: *Capsicum*; phenolic compounds; isotherms; SEM - EDS; functional ingredient.

SUMÁRIO

1. INTRODUÇÃO

A demanda dos consumidores por melhor qualidade dos alimentos cresceu nos últimos dez anos, o que é facilmente explicado por novos estilos de vida. Esse fato impulsiona a busca por novos ingredientes saudáveis e sustentáveis (Harich et al. 2018; Takwa et al. 2018). Os frutos de pimenta que são uma especiaria do gênero *Capsicum*, comercializada mundialmente, contém micro e macronutrientes e uma série de compostos bioativos com propriedades funcionais e tecnológicas de relevante interesse industrial (Mendes et al., 2019a,b; 2020).

Estudos mostraram usos tradicionais da pimenta na indústria, principalmente como corantes e condimentos em molhos, sopas e carnes processadas, doces, bebidas alcoólicas, trazendo suas características em termos de cor, pungência e sabor, além de outras propriedades atualmente consideradas no setor de processamento de alimentos, como atividades antimicrobianas e antioxidantes (Baenas et al. 2019; Téllez-Pérez et al. 2015). Esses frutos também podem ser consumidos diariamente como único ingrediente na minha dieta ou como suplemento dietético (Sricharoen et al. 2017) e são adequados para aplicações de produtos alimentícios prontos para consumo (Guadarrama-Lezama et al. 2014). No entanto, a aplicação de pimentas como ingredientes funcionais ainda é limitada (Mendiratta, Shinde, and Mane 2013; Nath et al. 2018), uma alternativa seria complementar com a farinha de frutas e hortaliças (FVR), para obtenção de ingredientes alimentares sustentáveis e funcionais (Mendes et al., 2019b).

A FVR, por exemplo, foi produzida a partir de resíduos de bebidas isotônicas com base na exploração completa de várias espécies regionais de frutas e vegetais: laranja, maracujá, melancia, alface, abobrinha, cenoura, espinafre, hortelã, inhame, pepino e rúcula, aplicada com sucesso na reformulação de barras e biscoitos de cereais (Ferreira et al. 2015). Nesta farinha, um total de 88 compostos fenólicos foram identificados: ácidos fenólicos (28), flavonoides (32) e outros polifenois (28), sendo a hesperidina o composto principal (Gonçalves et al. 2018).

Diante do exposto, a proposta deste trabalho é estudar as propriedades morfológicas, quimicas e metabolômicas de duas espécies de pimentas *Capsicum*. A partir destas caracterizações, incorporar a FVR, visando a ação funcional desses ingredientes. A escolha desta proposta justifica-se pela possibilidade de inclusão destas farinhas, como farinha de pimenta (FP) e FVR, com a finalidade de aumentar o consumo de bioativos antioxidantes na dieta e a vida de prateleira dos produtos alimentícios.

A presente tese de doutorado é apresentada na forma de artigos científicos, conforme descrito nos capítulos seguintes.

No primeiro, é apresentado um artigo de revisão bibliográfica, publicado pela revista *Trends in Food Science & Technology*. O artigo intitula-se “The role of bioactive components found in peppers”. Neste capitulo, é relatado os resultados mais recentes de uma investigação sobre compostos bioativos (capsaicinoides, compostos fenólicos, carotenoides e vitaminas) em cinco principais espécies de pimentas do gênero *Capsicum* da família Solanaceae, utilizadas tanto como especiaria quanto como hortaliça, do ponto de vista da saúde humana e/ou preservação de alimentos. Este estudo permitiu concluir que poucas espécies foram minuciosamente estudadas em relação aos seus bioativos de modo a contribuir com futuros estudos sobre o assunto.

No capítulo 2, é apresentado o artigo original publicado na revista *LWT - Food Science and Technology*. O artigo é intitulado “Characterization of pepper (*Capsicum baccatum*) - A potential functional ingredient”. Nele, são apresentados os resultados focados na caracterização desta espécie de pimenta em termos de abordagens morfológicas, químicas e metabolômicas. A partir deste estudo, foi possível considerar a farinha de pimenta como um potencial ingrediente funcional.

No capítulo 3, é apresentado o artigo intitulado "*Capsicum pubescens* as a functional ingredient: microencapsulation and phenolic profilling by UPLC-MS^E" publicado na revista *Food Research International*. Os resultados expostos neste estudo relacionam-se à morfologia, química e metabolomica da farinha de pimenta (*Capsicum pubescens*). A partir desta farinha foi feita a microencapsulação. Foram realizados neste pó, atividade antioxidante (ensaios ABTS, FRAP e ORAC) e micrografias MEV.

O 4° e ultimo capitulo compreende o artigo intitulado "Flour from 'fruits and vegetables' waste with addition of a South-American pepper (*Capsicum baccatum*) proposed as food ingredient" publicado na revista *International Journal of Food Science and Technology*. Os resultados apresentados neste artigo indicam que a farinha de pimenta e a farinha de frutas e hortaliças representam uma boa combinação de matérias-primas, com qualidades tecnológicas interessantes para a produção de ingredientes funcionais, bem como uma solução viável para a valorização de subprodutos do processamento de alimentos, resíduos agroindustriais e recursos regionais.

2. CAPÍTULO I – THE ROLE OF BIOACTIVE COMPONENTS FOUND IN PEPPERS

Nathânia de Sá Mendes, Édira Castello Branco de Andrade Gonçalves
Artigo publicado na revista "*Trends in Food Science & Tecnology"*, 99 (2020), 229–243.
https://doi.org/10.1016/j.tifs.2020.02.032

Abstract

Background: To this day, many species of peppers, genus *Capsicum*, have been identified and are the subject of several researches. However, only 5 are commonly used: *C. annuum*; *C. baccatum*; *C. chinense*; *C. frutescens* and *C. pubescens*. When associated with a healthy diet, the frequent intake of peppers has been positively correlated to improvements in human health. Most effects are due to the presence of a wide range of bioactive compounds, responsible for their functional properties as well as their technological potential as a food additive. Among the bioactive compounds present in these fruits are capsaicinoids, phenolic compounds, carotenoids, vitamins C and E.

Scope and Approach: The goal of this review is to summarize the main findings regarding the bioactive compounds found in peppers and their technological and functional applications.

Key Findings and Conclusions: Results show that despite all health claims and technological potential reported, not all species were thoroughly studied and their potential is still unclear.

Keywords: *Capsicum*; capsaicinoids; phenolic compounds; carotenoids; vitamins

1. Introduction

Spices such as pepper (*Capsicum* spp.) originally grown in tropical and humid regions of South and Central America, belong to the Solanaceae family and hold a prominent position among the oldest, most highly commercialized plants in the world (da Silveira Agostini-Costa, da Silva Gomes, de Melo, Reifschneider, & da Costa Ribeiro, 2017; Giuffrida et al., 2013; Silva et al., 2014). The genus *Capsicum* includes many species, of which only five are used fresh or as culinary spices: *C. annuum*; *C. baccatum*; *C. chinense*; *C. frutescens* and *C. pubescens* (Mendes et al., 2019b). However, according to the author, only two are highly used: *C. annuum* and *C. frutescens*.

Bioactive compounds from pepper species are known for their analgesic, anti-obesity, cardioprotective, pharmacological, neurological and dietic properties. These substances display a significant antibiotic activity and the capacity to reduce serum cholesterol levels when consumed in small quantities as part of a normal diet (Conforti, Statti, and Menichini 2007; Gurnani et al. 2016; Lu, Ho, and Huang 2017). Several studies, both in vitro and in vivo, have associated *Capsicum annuum* species to some useful protective effects, mainly antioxidant activity and anticancer (Ghasemnezhad, Sherafati, and Payvast 2011; Jeong et al. 2011). Also, *C. annuum* role reducing or preventing chronic diseases (Kim et al. 2016a) and dietary lipid accumulation have been reported (J.-S. Kim et al. 2017). *Capsicum frutescens* has been described as a source of new antimicrobial compounds and antioxidants, as a flavoring and coloring agent. It also has ethnomedicinal prestige and is used in the treatment of several human diseases (Gurnani et al. 2016; Nascimento et al. 2014).

In addition to the micro and macronutrients, all peppers contain a wide range of bioactive compounds with functional and technological properties with relevant industrial interest (N. de S. Mendes, Favre, et al. 2019; N. de S. Mendes, Santos, et al. 2019). Among these compounds, capsaicinoids, phenolic compounds, carotenoids (provitamin A) and vitamins (C and E) stand out. However, their concentration can vary according to the amount of sunlight, soil, season, crop region, temperature changes, variety of fruit and maturity level (Bae et al., 2014; Dias et al., 2016; Menichini et al., 2009). Pepper fruits exposed to temperatures below 15 °C, during development and cultivation, did not show oxidative stress (Mateos et al. 2013).

In addition, chemical composition and bioactive compounds diversity, can be explained not only by species differences and condition of crops, but also by different extraction

procedures (Gurnani et al. 2016; Schweiggert, Carle, and Schieber 2006). The stability of this fruit during storage was also evaluated, in the period of 12 months, where it was observed that the production methods (conventional and organic) and the harvest period affected, in great part, the bioactive content (Koncsek et al. 2016). This review aims to summarize studies regarding bioactive constituents of peppers, their health benefits and potential as functional ingredients and / or food products.

2. Bioactive compounds in peppers from the genus *Capsicum* used as fresh fruit and spices

2.1. Capsaicinoids

The pungent compounds of the *Capsicum* fruit known as capsaicinoids are bioactive vanillylamides containing 9-11 carbons. It is estimated that capsaicin and dihydrocapsaicin occur in quantities above 80% of the total capsaicinoids and their quantities are largely determined by the level of pungency (Lu, Ho, and Huang 2017; Saha et al. 2015; Urbina et al. 2017). In addition to these most abundant compounds, there is evidence of other less common (in lower abundancy) capsaicinoids. It is worth highlighting that the natural pattern and content of individual capsaicinoids in *Capsicum* fruit changes according to species, varieties and environmental factors (Gurnani et al. 2016; Nagy et al. 2017; Schweiggert, Carle, and Schieber 2006). Table 1 presents the capsaicinoids found in the five domesticated species of *Capsicum* largely cultivated by man.

Of the two main capsaicinoids previously quantified and presented above, capsaicin was the most highly abundant in several *Capsicum* species analyzed. Another capsaicinoid, nordihydrocapsaicin, was also found in these peppers, but in small amounts (Table 1). According to Menichini et al. (2009), *C. chinense* specie is considered one of the most spicy in the world, e.g. Habanero pepper, which can be measured by its pungency. This hypothesis was supported by Sarpras et al. (2016), who compared the high pungency of *C. chinense* with other two types of peppers: *C. annuum* and *C. frutescens*. In comparison, *C. chinense* presented greater pungency (Table 1). Another pungent spice is ginger (*Zingiber officinale*), but capsaicin is not the main component responsible for this property. This fact is justified both by its small amount and by the high pungency of gingerols (Ali et al. 2008; Oyedemi et al. 2019; Sajeev et al. 2011).

Daily consumption of pungent peppers plays a significant role associated with multiple biological benefits (Nagy et al. 2017). Studies have demonstrated the capsaicinoids

antioxidant, anticarcinogenic, anti-inflammatory and thermogenic properties (Giuffrida et al. 2013; Lu, Ho, and Huang 2017). In particular, the thermogenic effect of capsaicin can both promote weight loss and help maintain body mass following weight loss and, therefore, it can be an important addition in treating the current global obesity epidemic (Kantar et al. 2016; Urbina et al. 2017). Also, capsaicin has been found to induce apoptosis in different cancerous cells, as well as inhibiting carcinogenesis in the prostate, skin, breast, colon, lung and human urinary-bladder. However, it must be highlighted that excessively exposure to capsaicin can be toxic, causing local irritation, respiratory problems, as well as an increased potential to certain types of cancer, as a result of the consumption of large amounts of capsaicin (Fernández-Bedmar and Alonso-Moraga 2016; Lu, Ho, and Huang 2017).

Among all capsaicinoids, capsaicin is the most thoroughly studied in experimental and clinical investigations. Especially regarding the development of new drugs due to its beneficial properties (Santos et al. 2015; Fernández-Bedmar and Alonso-Moraga 2016). Furthermore, capsaicin has the potential to treat nervous system disorders, including arthritis, cystitis and the human immunodeficiency virus (HIV), in addition to its noteworthy antimicrobial properties, which can be applied as a natural inhibitor to pathogenic microorganisms in food (Nascimento et al. 2014; Topuz et al. 2011).

More recently, in a study carried out by Gurnani et al. (2016), the in vitro antimicrobial activity of *C. frutescens* extracts was particularly notable against a number of pathogenic microorganisms (*Pesudomaonas aeruginosa, Klebsilla pneumonae, Staphylococcus aureus and Candida albicans*). In literature, there is little information on capsaicinoid bioavailability. However, some studies show that it has a dose-dependent response. Given that the level of capsaicinoids range a great deal among pepper species from 1.2 to 6580 µg/g of fresh pepper, a possible interference on the digestibility of the fruit, the release of capsaicinoids and, as a consequence, the bioavailability of these compounds is expected (Victoria-Campos et al. 2015).

Table 1. Capsaicinoids present in the five species used as both fresh fruit and spices belonging to the genus *Capsicum.*

CAPSAICINOIDS	SPECIES	MAXIMUM QUANTIFICATION (mg kg^{-1} dry weight)	EXTRACTION SOLVENT	REFERENCE
Capsaicin	*C. annuum*	2495	Methanol / Acetonitrile/ Hexane/ Acetone	(Kozukue *et al*. 2005; Ziino *et al*. 2009; Ornelas-Paz *et al*. 2010; Bae *et al*. 2012; Giuffrida *et al*. 2013)
	C. baccatum	1770	Ethyl acetate	(Dias *et al*. 2017)
	C. chinense	8175	Methanol / Acetone	(Ornelas-Paz *et al*. 2010; Giuffrida *et al*. 2013)
	C. frutescens	917	Acetone / Dichloromethane / Methanol	(Schweiggert *et al*. 2006; Giuffrida *et al*. 2013; Santos *et al*. 2015; Lu *et al*. 2017)
	C. pubescens	158.4	Methanol	(Ornelas-Paz *et al*. 2010; Meckelmann *et al*. 2015)
	Blend (*C. annuum* + *C. Frutescens*)	0.164	Acetone	(Nagy et al., 2017)
Dihydrocapsaicin	*C. annuum*	1016	Acetonitrile / Methanol / Hexane / Acetone	(Kozukue *et al*. 2005; Ziino *et al*. 2009; Ornelas-Paz *et al*. 2010; Bae *et al*. 2012; Giuffrida *et al*. 2013)
	C. baccatum	730	Ethyl acetate	(Dias *et al*. 2017)
	C. chinense	4273	Methanol/ Acetone	(Ornelas-Paz *et al*. 2010; Giuffrida *et al*. 2013)
	C. frutescens	351	Acetone / Dichloromethane / Methanol	(Schweiggert *et al*. 2006; Giuffrida *et al*. 2013; Santos *et al*. 2015; Lu *et al*. 2017)
	C. pubescens	514.4	Methanol	(Ornelas-Paz *et al*. 2010; Meckelmann *et al*. 2015)
	Blend (*C. annuum* + *C. Frutescens*)	0.095	Acetone	(Nagy et al., 2017)
Nordihydrocapsaicin	*C. annuum*	180	Acetonitrile / Methanol / Acetone	(Ziino *et al*. 2009; Ornelas-Paz *et al*. 2010; Giuffrida *et al*. 2013)
	C. baccatum	110	Ethyl acetate	(Dias *et al*. 2017)
	C. chinense	340	Methanol/ Acetone	(Ornelas-Paz *et al*. 2010; Giuffrida *et al*. 2013)
	C. frutescens	66	Acetone / Dichloromethane / Methanol	(Schweiggert *et al*. 2006; Giuffrida *et al*. 2013; Santos *et al*. 2015; Lu *et al*. 2017)
	C. pubescens	68.2	Methanol	(Ornelas-Paz *et al*. 2010; Meckelmann *et al*. 2015)
	Blend (*C. annuum* + *C. Frutescens*)	0.007	Acetone	(Nagy et al., 2017)
Homocapsaicin-I	*C. annuum*	--	Methanol/Acetone	(Kozukue *et al*. 2005; Giuffrida *et al*. 2013)
	C. baccatum	--	Ethyl acetate	(Dias *et al*. 2017)
	C. chinense	--	Acetone	(Giuffrida et al. 2013)

	C. frutescens	--	Acetone/ Methanol	(Giuffrida *et al*. 2013; Santos *et al*. 2015)
Homodihydrocapsaicin-I	*C. annuum*	--	Methanol	(Kozukue *et al*. 2005)
	C. baccatum	--	Ethyl acetate	(Dias *et al*. 2017)
	C. frutescens	--	Dichloromethane	(Lu *et al*. 2017)
N-Vanillyl nonanamide	*C. annuum*	--	Methanol	(Thompson *et al*. 2005)
	C. chinense	--	Methanol	(Thompson *et al*. 2005)
	C. frutescens	--	Acetone	(Schweiggert *et al*. 2006)
5-ene-7-methyl norcapsaicin	*C. annuum*	--	Methanol	(Thompson *et al*. 2005)
	C. chinense	--	Methanol	(Thompson *et al*. 2005)
6-ene-8-methyl capsaicin	*C. annuum*	--	Methanol	(Thompson *et al*. 2005)
	C. chinense	--	Methanol	(Thompson *et al*. 2005)
6-ene-8-methyl homocapsaicin	*C. annuum*	--	Methanol	(Thompson *et al*. 2005)
	C. chinense	--	Methanol	(Thompson *et al*. 2005)
6-ene-9-methyl homocapsaicin	*C. annuum*	--	Methanol	(Thompson *et al*. 2005)
	C. chinense	--	Methanol	(Thompson *et al*. 2005)
7-methyl nordihydrocapsaicin	*C. annuum*	--	Methanol	(Thompson *et al*. 2005)
	C. chinense	--	Methanol	(Thompson *et al*. 2005)
8-methyl dihydrocapsaicin	*C. annuum*	--	Methanol	(Thompson *et al*. 2005)
	C. chinense	--	Methanol	(Thompson *et al*. 2005)
8-methyl homodihydrocapsaicin	*C. annuum*	--	Methanol	(Thompson *et al*. 2005)
	C. chinense	--	Methanol	(Thompson *et al*. 2005)
9-methyl homodihydrocapsaicin	*C. annuum*	--	Methanol	(Thompson *et al*. 2005)
	C. chinense	--	Methanol	(Thompson *et al*. 2005)
Homocapsacicin-II	*C. annuum*	--	Methanol	(Kozukue *et al*. 2005)
Homodihydrocapsaicin-II	*C. annuum*	--	Methanol	(Kozukue *et al*. 2005)
Nonivamide	*C. annuum*	--	Methanol	(Kozukue *et al*. 2005)
N- Vanillyl decanamide	*C. frutescens*	--	Acetone	(Schweiggert *et al*. 2006)
N- Vanillyl octanamide	*C. frutescens*	--	Acetone	(Schweiggert *et al*. 2006)
Norcapsaicin	*C. frutescens*	--	Acetone	(Schweiggert *et al*. 2006)
Nornorcapsaicin	*C. frutescens*	--	Acetone	(Schweiggert *et al*. 2006)
Nornordihydrocapsaicin	*C. frutescens*	--	Acetone	(Schweiggert *et al*. 2006)

-- Not quantified.

2.2. Phenolic compounds

All known species of pepper from the genus *Capsicum* are rich in phenolic compounds, secondary metabolites that are readily found in plants as a result of their adaptation to biotic and abiotic stress (Dias et al. 2016; Hallmann and Rembialkowska 2012; Mokhtar et al. 2015; Silva et al. 2014). The term "phenolic" (or "polyphenol") can be defined as compounds that contain at least one aromatic ring attached to one or more hydroxyl groups. They comprise more than 8000 substances with highly diversified structures and a variation in molecular mass ranging from small molecules (<100 Da), such as phenolic acids, to large molecules (>30,000 Da) of highly polymerized compounds (Juániz, Ludwig, Bresciani, et al. 2016; Lucci, Saurina, and Núñez 2017) as shown in Figure 1.

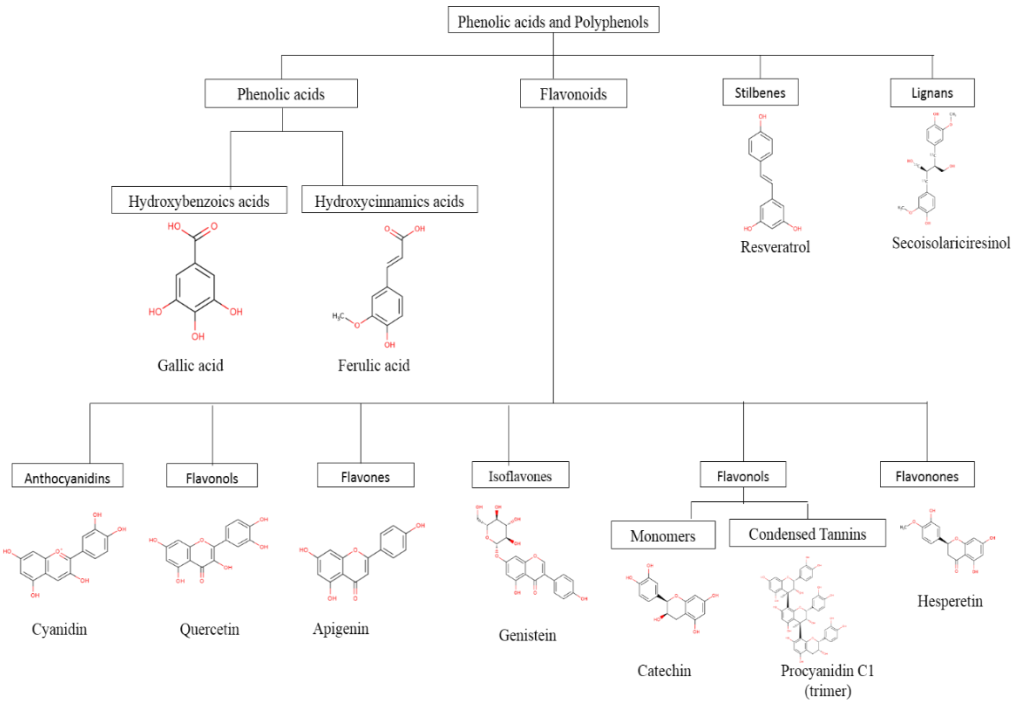


Fig 1. Classification and chemical structures of some phenolic acids and polyphenols. Source: (LUCCI *et al*. 2017)

Considering the bioactive compound profile of peppers, the diversity of their phenolic compounds has been extensively investigated, in terms of their medicinal properties, such as the prevention of cancer, atherosclerosis and anti-inflammatory activity (Dias et al. 2016; Jeong et al. 2011). It is worth highlighting that in these species of peppers there is a sound correlation between the antioxidant activity and the phenolic compounds, suggesting that these compounds are primarily responsible for the antioxidant capacity of peppers. However, it has been found that not only an isolated compound, but in fact a synergy of compounds present in peppers is

responsible for their antioxidant property (Carvalho et al. 2015; Ghasemnezhad, Sherafati, and Payvast 2011; Gurnani et al. 2016). In an *in vitro* study carried out by Oboh & Rocha (2008), samples of the *C. pubescens* species, both green and ripe, inhibited the lipid peroxidation on rat brains. Ripe pepper was however the most potent inhibitor of lipid peroxidation, possibly due to the greater content of phenolics and stronger reducing power.

As previously reported in the literature, flavonoids are the main classes of phenolic compounds found in pepper (Table 2) (Mudric et al., 2017). Flavonoid biosynthesis follows the phenylpropanoid pathway, and consequently, the environment is expected to heavily impact on production. Nutrient deficiency, UV radiation or an increase in stress levels caused by pathogens can largely influence the production of flavonoids in various types of peppers (Meckelmann, Riegel, et al. 2015; Rao and Ravishankar 2000). Nascimento et al. (2014), mentioned a botanical classification scale, considering foods as low (0.1-39.9 mg kg-1), moderate (40-99.9 mg kg-1) and high (> 100 mg kg-1), based on the concentration of flavonoids. They also demonstrated the quantitative variation of flavonoids in peppers, with concentrations ranging from a few mg/kg to hundreds of mg/kg. Another study reported that pungent peppers contain a moderate level of polyphenol content when compared to wild mint and grapes generally present in high concentrations (Nagy et al. 2015).

It is well known that the main phenolic compounds found in peppers are vanillic, caffeic, ferulic, *p*-coumaric, and p-hydroxybenzoic acids. Ferulic acid has strong antiradical properties and vanillic acid is primarily used as a flavor intensifier (Mudric et al. 2017). Studies show that the majority of flavonoids found in peppers are glycosides and aglycones of myricetin, quercetin, luteolin, apigenin and kaempferol (Nascimento et al. 2014; Juániz et al. 2016a).

Jeong et al. (2011), verified that the derivatives of quercetin from *Capsicum* fruit effectively inhibited the viability and proliferation of several human cancer cells both in vitro and *in vivo*. Therefore, this flavonoid has been associated to the pepper's ability to prevent diseases such as cancer. In addition, a high concentration of quercetin in green fruits have been related to the protective function of the photosynthetic device. It has been reported that the flavonoids which absorbed higher levels of UVB radiation, in the range of 280-315 nm, can act as filters to UV radiation and, thus, protect the photosynthesized cells (Ghasemnezhad, Sherafati, and Payvast 2011; Meckelmann, Riegel, et al. 2015).

Juániz et al. (2016a) highlighted how thermal treatment influences the level of phenolic compounds in peppers, particularly for the chlorogenic acids, which suggests that the thermal destruction of the cell walls and sub-cellular compartments during the cooking process tends to cause an increasing bioavailability of these compounds. Juániz et al. (2016b) also verified that

the bioaccessibility of the phenolic compounds after gastrointestinal digestion was greater in cooked samples when compared to raw samples. This was particularly evident in green pepper, which presented a larger quantity of phenolic compounds after the digestion process and, as a consequence, increased health benefits.

Ghasemnezhad et al. (2011) found that the phenolic compounds accumulated in pepper fruits can be affected by storage conditions. The fruit stored at 8 °C accumulated derivatives of hydroxycinnamic acid, while at 4 °C, the accumulation of phenolics appeared to be partially inhibited. Antimicrobial properties of polyphenol extracts of pepper are of extreme interest as natural additives, both to the food industry, and human healthcare, as they can negatively impact micro-organisms, such as intestinal bacterias (Mokhtar et al. 2015; Nascimento et al. 2014).

According to Carvalho et al. (2015), these compounds have an important role acting directly as free radicals scavengers, as well all modulating the activity of detoxification enzymes, oxidation and reduction processes. Also, strengthening the immune system, regulating gene expression, cell signaling, and hormone metabolism. In addition, the relevance of the content of polyphenols, including phenolic acids, catechins and some flavonoids in food products play an important role in food quality, as it has a strong influence on color and taste properties (Lucci, Saurina, and Núñez 2017). As an example, anthocyanins, phenolic compounds characterized by the basic core, the flavylium ion, are responsible for the characteristic red color found in species of *Capsicum* (Carvalho et al. 2015).

Table 2. Phenolic compounds present in the five species used as both fresh fruit and spices belonging to the genus *Capsicum.*

PHENOLIC COMPOUNDS	SPECIES	MAXIMUM QUANTIFICATION (mg kg^{-1} dry weight)	EXTRACTION SOLVENT	REFERENCE
Hydroxybenzoic acids				
Protocatechuic acid	*C. annuum*	0.83	Methanol	(Mudric *et al*. 2017)
	C. frutescens	2.35	Ethyl acetate	(Rao & Ravishankar 2000)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Vanillic acid	*C. annuum*	13.29	Methanol	(Li *et al*. 2015; Mudric *et al*. 2017)
	C. frutescens	--	Ethyl acetate	(Rao & Ravishankar 2000)
Gallic acid	*C. annuum*	865.9	Methanol	(Hallmann & Rembialkowska 2012; Mudric *et al*. 2017)
Benzoic acid	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
P-Hydroxybenzoic acid	*C. annuum*	6.42	Methanol	(Li *et al*. 2015; Lin *et al*. 2016; Mudric *et al*. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
2,6-dihydroxybenzoic acid	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
3-hydroxybenzoic acid	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Syringic acid	*C. annuum*	5	Methanol	(Lin *et al*. 2016; Mudric *et al*. 2017)
Vanillic acid glucoside	*C. annuum*	4020	Hydrochloric acid	(Mokhtar *et al*. 2015)
Hydroxycinnamic acids				
Caffeic acid	*C. annuum*	53.7	Water; Methanol	(Silva *et al*. 2014; Juániz *et al*. 2016a; Mudric *et al*. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Ethyl trans-caffeate (Caffeic acid ethyl ester)	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Caffeic acid glucoside I	*C. annuum*	83	Methanol	(Juániz *et al.* 2016a)
Caffeic acid glucoside II	*C. annuum*	31.4	Methanol	(Juániz *et al.* 2016a)
Caffeic acid 4-O-hexoside	*C. annuum*	--	Methanol	(Mudric *et al*. 2017)
Cinnamic acid	*C. annuum*	0.24	Methanol	(Mudric et al. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Chlorogenic acid	*C. annuum*	877	Methanol	(Hallmann & Rembialkowska 2012)
3-hydroxycinnamic acid	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)

CQA	*C. annuum*	10	Ethanol	(Juániz *et al*. 2016b)
5-CQA	*C. annuum*	342.62	Methanol	(Juániz *et al*. 2016a)
4-CQA	*C. annuum*	540	Methanol	(Juániz *et al*. 2016a)
P-Coumaric acid	*C. annuum*	75	Hydrochloric acid; Methanol	(Mokhtar *et al*. 2015; Juániz *et al*. 2016a; Mudric *et al*. 2017)
P-Coumaric acid 4-O-hexoside	*C. annuum*	--	Methanol	(Mudric *et al*. 2017)
P-Coumaroyl glycolic acid	*C. annuum*	6470	Hydrochloric acid	(Mokhtar *et al*. 2015)
Ferulic acid	*C. annuum*	12.45	Methanol	(Lin *et al*. 2016; Mudric *et al*. 2017)
Ferulic acid 4-O-hexoside	*C. annuum*	3.72	Methanol	(Mudric *et al*. 2017)
Feruloyl hexoside	*C. annuum*	53.4	Methanol	(Jeong *et al*. 2011)
Isoferulic acid	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
P-Coumaryl tyrosine	*C. annuum*	6810	Hydrochloric acid	(Mokhtar *et al*. 2015)
5-O-*p*-Coumaroylquinic acid	*C. annuum*	2.88	Methanol	(Mudric *et al*. 2017)
5-O-Caffeoylquinic acid	*C. annuum*	23.33	Water; Methanol	(Mikulic-Petkovsek *et al*. 2013; Silva *et al*. 2014; Mudric *et al*. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Sinapic acid	*C. annuum*	132.2	Water; Methanol	(Silva *et al*. 2014; Mudric *et al*. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Sinapic acid 4-O-hexoside	*C. annuum*	2.31	Methanol	(Mudric *et al*. 2017)
Sinapoyl hexoside	*C. annuum*	72.4	Methanol	(Jeong *et al*. 2011)
Trans-*p*-sinapoyl-β-D-glucopyranoside	*C. annuum*	419	Ethanol	(Materska & Perucka 2005)
Trans-*p*-feruloyl-β-D-glucopyranoside	*C. annuum*	359	Ethanol	(Materska & Perucka 2005)
Flavonols				
Kaempferol	*C. annuum*	42	Ethyl acetate; Methanol	(Bae *et al*. 2012; Hallmann & Rembialkowska 2012)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
	C. pubescens	--	Methanol	(Meckelmann *et al*. 2015a)
Quercetin	*C. annuum*	10810	Methanol; Hydrochloric acid	(Bae *et al*. 2012; Hallmann & Rembialkowska 2012; Mokhtar *et al.* 2015; Mudric *et al*. 2017)
	C. pubescens	1500	Methanol	(Meckelmann *et al*. 2015a)
Kaempferol diglucoside	*C. annuum*	17170	Hydrochloric acid	(Mokhtar *et al*. 2015)
Kaempferol pentosyldihexoside	*C. annuum*	42.1	Methanol	(Jeong *et al*. 2011)
Kaempferol 3-O-sophoroside (Sophoraflavonoloside)	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)

Quercetin glucoside	*C. annuum*	19860	Methanol; Hydrochloric acid; Ethanol	(Hallmann & Rembialkowska 2012; Mokhtar *et al*. 2015; Juániz *et al*. 2016a, b)
Quercetin rhamnoside	*C. annuum*	925	Hydrochloric acid; Methanol; Ethanol	(Mokhtar *et al*. 2015; Juániz *et al*. 2016a, b)
Quercetin 3-O-galactoside (Hyperoside)	*C. annuum*	2.16	Methanol	(Mudric *et al*. 2017)
Quercetin 3-O-rhamnoside	*C. annuum*	1502	Methanol; Water	(Jeong *et al*. 2011; Silva *et al*. 2014; Mudric *et al*. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Quercetin-3-O-rutinoside	*C. annuum*	100.1	Methanol; Water	(Hallmann & Rembialkowska 2012; Mikulic-Petkovsek *et al*. 2013; Silva *et al.* 2014)
Quercetin 3-O-α-L rhamnoside	*C. annuum*	5400	Ethanol	(Materska 2014)
Quercetin 3-O-α-L-rhamnoside-7-O-β-D-glucoside	*C. annuum*	12300	Ethanol	(Materska 2014)
Quercetin 3-glucosyl(1-3) rhamnosyl(1-6)galactoside	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Quercetin O-rhamnosyl-O-hexoside	*C. annuum*	23.1	Methanol	(Jeong *et al*. 2011)
Quercetin 3-O-(6’’-O-rhamnosyl) glucoside (Rutin)	*C. annuum*	2.30	Methanol	(Mudric *et al*. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Quercetin 3-O-rhamnoside-7-O-hexoside	*C. annuum*	3.69	Methanol	(Mikulic-Petkovsek *et al*. 2013; Mudric *et al*. 2017)
Quercetin 3-O-(2”-O-hexosyl) rhamnoside	*C. annuum*	--	Methanol	(Mudric *et al*. 2017)
Quercetin 3-glucoside-7-rhamnoside	*C. annuum*	26.2	Methanol	(Juániz *et al*. 2016a)
Quercetin 3-sambubioside-7-rhamnoside	*C. annuum*	10	Methanol; Ethanol	(Juániz *et al.* 2016a,b)
Quercetin 3-O-α-L-rhamnopyranoside-7-O- β -D-glucopyranoside + trans-p-ferulyl-alcohol-4-O-[6-(2-methyl-3- hydroxypropionyl)] glucopyranoside	*C. annuum*	365	Ethanol	(Materska & Perucka 2005)
Quercetin 3-O-neohesperidoside	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Quercetin 3-O-α-L-rhamnopyranoside	*C. annuum*	993	Ethanol	(Materska & Perucka 2005)
Quercetin 3-O-hexoside	*C. annuum*	13.5	Methanol	(Jeong *et al*. 2011)
Isorhamnetin	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Isorhamnetin 3-O-[6”-O-(5-hydroxyferuloyl) hexoside]-7-O-rhamnoside	*C. annuum*	1.32	Methanol	(Mudric *et al*. 2017)
Myricetin	*C. annuum*	261.7	Methanol	(Hallmann & Rembialkowska 2012)

Myricetin-3-O-rhamnoside	*C. annuum*	672.2	Water	(Silva *et al*. 2014)
Flavones				
Apigenin	*C. annuum*	18.3	Methanol	(Bae *et al*. 2012; Mudric *et al*. 2017)
	C. pubescens	--	Methanol	(Meckelmann *et al*. 2015a)
Luteolin	*C. annuum*	880	Methanol; Hydrochloric acid	(Bae *et al*. 2012; Hallmann & Rembialkowska 2012; Mokhtar *et al*. 2015; Mudric *et al*. 2017)
	C. pubescens	--	Methanol	(Meckelmann *et al*. 2015a)
Apigenin C-pentosyl-C-hexoside	*C. annuum*	7.4	Methanol	(Jeong *et al*. 2011)
Apigenin 6-C-hexoside-8-C-pentoside	*C. annuum*	3.04	Methanol	(Mikulic-Petkovsek *et al*. 2013)
Apigenin 6-C-hexoside-8-C-pentoside 2[b]	*C. annuum*	--	Methanol	(Mikulic-Petkovsek *et al*. 2013)
Apigenin 6,8-di-C-hexoside	*C. annuum*	2.58	Methanol	(Mudric *et al*. 2017)
Apigenin 6-C-pentoside-8-C-hexoside	*C. annuum*	2.82	Methanol	(Mikulic-Petkovsek *et al*. 2013; Mudric *et al*. 2017)
Apigenin 6-C-β-D-glucoside-8-C-α-L-arabinoside	*C. annuum*	900	Ethanol	(Materska 2014)
Apigenin 6-C-β-D-glucopyranoside-8-C-R-L-arabinopyranoside	*C. annuum*	109	Ethanol	(Materska & Perucka 2005)
Apigenin 7-O-(2”-O-apiosyl) glucoside (Apiin)	*C. annuum*	3.16	Methanol	(Mudric *et al*. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Chrysoeriol 7-O-(2-apiosyl-6-acetyl) glucoside[b]	*C. annuum*	64.2	Methanol	(Mikulic-Petkovsek *et al*. 2013)
Apigenin 8-C-glucoside (Vitexin)	*C. annuum*	2.02	Methanol	(Mudric *et al*. 2017)
Hispidulin	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Isoscoparin	*C. annuum*	23.4	Methanol	(Jeong *et al*. 2011)
Luteolin acetylglucoside I	*C. annuum*	--	Methanol	(Juániz *et al.* 2016a)
Luteolin C-pentosyl-C-hexoside	*C. annuum*	17.4	Methanol	(Jeong *et al*. 2011)
Luteolin O-(apiosyl)hexoside	*C. annuum*	40.1	Methanol	(Jeong *et al*. 2011)
Luteolin O-(apiosylacetyl)glucoside	*C. annuum*	19.2	Methanol	(Jeong *et al*. 2011)
Luteolin O-malonylpentosyldihexoside	*C. annuum*	105.3	Methanol	(Jeong *et al*. 2011)
Luteolin O-(apiosylmalonyl)glucoside	*C. annuum*	206.6	Methanol	(Jeong *et al*. 2011)
Luteolin glucoside	*C. annuum*	5090	Hydrochloric acid	(Mokhtar *et al*. 2015)
Luteolin-6-C-glucoside (Isoorientin)	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Luteolin 6-C-hexoside-8-C-pentoside 2	*C. annuum*	--	Methanol	(Mikulic-Petkovsek *et al*. 2013)

Luteolin 6-C-pentoside-8-C-hexoside 2	*C. annuum*	--	Methanol	(Mikulic-Petkovsek *et al*. 2013)
Luteolin-6-C-(6-malonyl) hexoside-8-C-pentoside[b]	*C. annuum*	84.4	Methanol	(Mikulic-Petkovsek *et al*. 2013)
Luteolin 6-C-β-D-glucoside -8-C-α-L-arabinoside	*C. annuum*	--	Ethanol	(Materska 2014)
Luteolin 6,8-di-C-hexoside	*C. annuum*	74.8	Methanol	(Jeong *et al*. 2011; Mikulic-Petkovsek *et al*. 2013; Mudric *et al*. 2017)
Luteolin 6-C-hexoside-8-C-pentoside	*C. annuum*	2.61	Methanol	(Mikulic-Petkovsek *et al*. 2013; Mudric *et al*. 2017)
Luteolin 6-C-pentoside-8-C-hexoside	*C. annuum*	1.86	Methanol	(Mikulic-Petkovsek *et al*. 2013; Mudric *et al*. 2017)
Luteolin 6-C-hexoside	*C. annuum*	250.6	Methanol	(Jeong *et al*. 2011; Mikulic-Petkovsek *et al*. 2013; Mudric *et al*. 2017)
Luteolin 6,8-di-C-glucoside	*C. annuum*	24.2	Methanol	(Juániz *et al*. 2016a)
Luteolin 6-C-hexoside-8-C-pentoside	*C. annuum*	48.7	Methanol	(Juániz *et al*. 2016a)
Luteolin 6-C-pentoside-8-C-hexoside	*C. annuum*	--	Methanol	(Juániz *et al*. 2016a)
Luteolin 6-C-β-D-glucopyranoside-8-C-α-L-arabinopyranoside	*C. annuum*	92	Ethanol	(Materska & Perucka 2005)
Luteolin-7-O-(2-apiosyl-6-malonyl) glucoside	*C. annuum*	468.4	Water; Ethanol	(Silva *et al*. 2014; Juániz *et al*. 2016b)
Luteolin-7-O-(2-apiosyl-6-acetyl)hexoside	*C. annuum*	--	Methanol	(Mikulic-Petkovsek *et al*. 2013)
Luteolin-7-O-(2-apiosyl-6-malonyl)hexoside	*C. annuum*	--	Methanol	(Mikulic-Petkovsek *et al*. 2013)
Luteolin-7-O-(2-apiosyl)-hexoside[b]	*C. annuum*	--	Methanol	(Mikulic-Petkovsek *et al*. 2013)
Luteolin 7-O-[2''-O-(5''''-O-sinapoyl) pentosyl] hexoside	*C. annuum*	1.90	Methanol	(Mudric *et al*. 2017)
Luteolin 7-O-glucoside (Cynaroside)	*C. annuum*	13.5	Water; Methanol	(Silva *et al*. 2014; Mudric *et al*. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Luteolin diglucoside	*C. annuum*	5660	Hydrochloric acid	(Mokhtar *et al*. 2015)
Luteolin 7-O-[2-(β-D-apiosyl)-β-D-glucoside]	*C. annuum*	--	Ethanol	(Materska 2014)
Luteolin 7- O- [2- (β-D-apiosyl)-4- (β-D-glucosyl)- 6-malonyl]- β-D- glucoside	*C. annuum*	--	Ethanol	(Materska 2014)
Luteolin 7-O-(2''-O-pentosyl-4''-O-hexosyl) hexoside	*C. annuum*	1.85	Methanol	(Mudric *et al*. 2017)
Luteolin 7-O-(2''-O-pentosyl) hexoside	*C. annuum*	2.81	Methanol	(Mudric *et al*. 2017)

Luteolin 7-O-[2”-O-(5””-O-sinapoyl) pentosyl-hexoside	*C. annuum*	1.90	Methanol	(Mudric *et al*. 2017)
Luteolin 7-O-(2”-O-pentosyl-4”-O-hexosyl-6”-O-malonyl) hexoside	*C. annuum*	3.47	Methanol	(Mudric *et al*. 2017)
Luteolin 7-O-(2”-O-pentosyl-6”-O-malonyl) Hexoside	*C. annuum*	2.59	Methanol	(Mudric *et al*. 2017)
Luteolin7-O-(2-apiosyl) glucoside	*C. annuum*	332	Methanol	(Juániz *et al*. 2016a)
Luteolin 7-O-(2-apiosyl-6-malonyl) glucoside I	*C. annuum*	238	Methanol	(Juániz *et al*. 2016a)
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside II	*C. annuum*	--	Methanol	(Juániz *et al*. 2016a)
Lutoeolin 7-O-[2-(β-D-apiofuranosyl)-β-D-glucopyranoside]	*C. annuum*	231	Ethanol	(Materska & Perucka 2005)
Luteolin 7-O-[2-(β-D-apiofuranosyl)-4-(β-D-glucopyranosyl)-6- malonyl]-β-D-glucopyranoside	*C. annuum*	136	Ethanol	(Materska & Perucka 2005)
Luteolin 8-C-hexoside	*C. annuum*	267	Methanol	(Jeong *et al*. 2011; Mikulic-Petkovsek *et al*. 2013; Juániz *et al*. 2016a; Mudric *et al*. 2017)
Orientin	*C. annuum*	12.7	Methanol	(Jeong *et al*. 2011)
Vicenin-2	*C. annuum*	12.7	Methanol	(Jeong *et al*. 2011)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Alkylphenols				
4-vinylphenol	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Anthocyanins				
Caffeoyl glucoside	*C. annuum*	2590	Hydrochloric acid	(Mokhtar *et al*. 2015)
Trans-*p*-feruloyl-β-D-glucoside	*C. annuum*	6700	Ethanol	(Materska 2014)
Trans-*p*-sinapoyl-β-D-glucoside	*C. annuum*	5600	Ethanol	(Materska 2014)
Hydroxycoumarins				
Aesculin	*C. annuum*	0.20	Methanol	(Mudric *et al*. 2017)
Esculetin	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Hydroxycoumarin	*C. annuum*	2420	Hydrochloric acid	(Mokhtar *et al*. 2015)
Umbeliferone	*C. annuum*	14.61	Methanol	(Mudric *et al*. 2017)
4-hydroxycoumarin	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)

Hydroxybenzaldehydes				
Vanillin	*C. annuum*	5	Methanol	(Lin *et al*. 2016; Mudric *et al*. 2017)
	C. frutescens	5.63	Ethyl acetate	(Rao & Ravishankar 2000)
p-hydroxybenzaldehyde	*C. annuum*	3	Methanol	(Lin *et al*. 2016)
4-hydroxybenzaldehyde	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
p-anisaldehyde	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Flavanones				
Naringenin	*C. annuum*	4.83	Methanol	(Mudric *et al*. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Naringenin 7-O-hexoside	*C. annuum*	1.66	Methanol	(Mudric *et al*. 2017)
Naringenin 7-O-glucoside (Prunin)	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Hydroxycinnamaldehydes				
Coniferyl aldehyde	*C. annuum*	2.99	Methanol	(Mudric *et al*. 2017)
Hydroxyphenylacetic acids				
p-hydroxyphenylacetic acid	*C. annuum*	1.98	Methanol	(Mudric *et al*. 2017)
	C. baccatum	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
2-methoxy-2-phenylacetic acid	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Homovanillic acid	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Hydroxyphenylpropanoic acids				
Hydrocaffeic acid	*C. annuum*	3030	Hydrochloric acid	(Mokhtar *et al*. 2015)
Methoxyphenols				
Guaiacol	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Other phenolic compounds				
Daphnetine	*C. annuum*	16290	Hydrochloric acid	(Mokhtar *et al*. 2015)
Hydroxybenzoylhexose	*C. annuum*	3290	Hydrochloric acid	(Mokhtar *et al*. 2015)
Isovanillin	*C. annuum*	1	Methanol	(Lin *et al*. 2016)
Isovanillic acid	*C. annuum*	3	Methanol	(Li *et al*. 2015)
Methylparaben	*C. annuum*	3	Methanol	(Lin *et al*. 2016)
Paeonol	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)
Pyrogallol	*C. baccatum*	--	Butanol; Ethanol	(Mendes *et al.* 2019b)

-- Not quantified.

2.3. Carotenoids

Carotenoids are bioactive compounds widely found in plants and they are responsible for coloring *Capsicum* peppers (Rodríguez-Burruezo et al. 2010; da Silveira Agostini-Costa et al. 2017*). Capsicum* peppers are one of the richest sources of carotenoids and their different colors are due to the different carotenoid profiles (Carvalho et al. 2015). They can influence the flavor of the peppers: yellow, orange and red peppers, are sweeter than green peppers, they can also be related to the development of a higher glucose content with the advance of ripening (Thuphairo, Sornchan, and Suttisansanee 2019).

Taking into account its chemical structure, these compounds are isoprenoids that are characterized by a $C_{40}H_{56}$ with polyene chains and different terminal groups (β, ε, κ) that can be classified as oxygen deprived carotenes, or xanthophylls, when they contain oxygen as a result of enzymatic oxidation or addition and, when ripe, they contain esterified bonds with fatty acids (Bernstein et al. 2016; Nagy et al. 2017; Williams et al. 2013). The profile and concentration of *Capsicum* fruit carotenoids are different among species (Table 3), considering aspects such as growing conditions, part of the plant, stage of maturity at harvest and post-harvest management practice. In addition, the selection, processing and extraction of samples in adequate conditions are essential to maintaining maximum levels of carotenoids in vegetable materials (Bernstein et al. 2016; Carvalho et al. 2015; Olivares-Tenorio et al. 2016).

Interest surrounding the health benefits of peppers is attributed, in part, to their carotenoid content. These fat-soluble compounds have been found to gather important beneficial aspects, especially those related to the prevention of certain types of cancer, gastric ulcers, cardiovascular disease, age-related macular degeneration (AMD), cataracts, strengthening the immune system and other degenerative diseases (Fernández-Bedmar and Alonso-Moraga 2016; Pugliese et al. 2013; Sricharoen et al. 2016).

Carotenoid compounds can act as antioxidants, protecting cells from free-radical damage by reactive oxygen species (ROS) and delaying the aging processes (Campos et al. 2013). The antioxidant activity of carotenoids is due to the presence of a system of conjugated double bonds, which enable the free radicals inactivation (Carvalho et al. 2015). Thus, these compounds are highly valuable to the food industry and consumers who can benefit from their health claims (Sricharoen et al. 2016). Carotenoids from different species of *Capsicum* have been studied for decades and attracted attention due to their diverse functional effects on the human body (J. S. Kim et al. 2016; Pugliese et al. 2013).

Recent studies show that capsanthin in *Capsicum* species can prevent or reduce dietetic lipid

accumulation. In this context, the ingestion of peppers can be beneficial due to their role in inhibiting inflammation and improving plasma lipid profiles in the human body (Kim et al. 2016, 2017). The carotenoid zeaxanthin is found in fruits and vegetables, including kale, peppers (*C. annuum*), corn and spinach, which is believed to contain some of the highest concentrations of xanthophyll (Nwachukwu et al. 2016; Kim et al. 2017).

Kim et al. (2016) compared the profiles of carotenoids and concentrations of different colored peppers. The authors suggested the ingestion of red pepper, which contains capsanthin and capsorubin, since it was considered a source of carotenoids and is the most consumed kind of pepper. However, in this study, the orange-colored pepper was identified as an important source of zeaxanthin, the carotenoid which provides the greatest benefits to ocular health. Lutein, however, was the most abundant carotenoid in yellow peppers, noted for its role in preventing AMD and cancer. In addition, the β-cryptoxanthin was found to improve bone health of ovariectomized rats and humans.

Food intake of carotenoids, such as β-carotene and α-carotene, can reduce the risk of type 2 diabetes in generally healthy men and women due to their antioxidant properties (Sluijs et al. 2015). Among the studied spices and condiments, red peppers (1310 mg / 100 g) and smilax (2136 mg / 100 g) are good sources of β-carotene, while turmeric (60 μg / 100 g) and cloves (70 μg / 100 g) have low amounts of β-carotene (Kandlakunta, Rajendran, and Thingnganing 2008). The coriander, widely used as a culinary spice, had levels of β-carotene of 12 mg / 100g (Divya, Puthusseri, and Neelwarne 2012).

It is also important to mention that only α and β-caroten and β-criptoxanthin present vitamin A activity (Carvalho et al. 2015; Topuz et al. 2011). According to O'Sullivan et al. (2010) the bioaccessibility of pepper carotenoids range from 6.2% to 100%. To Pugliese et al. (2013), little is known about the role of the bioaccessability of capsanthin, violaxanthin or neoxanthin in peppers. However, the xanthophylls, mainly capsanthin and capsorubin, which are characteristic of pepper, allow their application in several areas, such as the production of natural dyes (e.g oleoresins), widely used in food and cosmetics industries (Meckelmann et al. 2013; Wahyuni et al. 2011).

Table 3. Carotenoids present in the five species used as both fresh fruit and spices belonging to the genus *Capsicum.*

CAROTENOIDES	SPECIES	MAXIMUM QUANTIFICATION (mg kg^{-1} dry weight)	EXTRACTION SOLVENT	REFERENCE
CAROTENES				
ß –Carotene	*C. annuum*	108000	Acetone	(Giuffrida *et al*. 2013; Pugliese *et al*. 2013; Carvalho *et al*. 2015; Kim *et al*. 2016a, 2017; da Silveira Agostini-Costa *et al*. 2017)
	C. baccatum	4541		(Pugliese *et al*. 2013; Carvalho *et al*. 2015)
	C. chinense	191000		(Giuffrida *et al*. 2013; Pugliese *et al*. 2013; Carvalho *et al*. 2015; da Silveira Agostini-Costa *et al*. 2017)
	Blend (*C. annuum* + *C. Frutescens*)	0.049		(Nagy et al., 2017)
α –Carotene	*C. annuum*	516.64		(Hallmann & Rembialkowska 2012; Carvalho *et al*. 2015; Kim *et al*. 2016b)
	C. baccatum	391.17		(Carvalho *et al*. 2015)
	C. chinense	98000		(Giuffrida *et al*. 2013)
Phytoene	*C. annuum*	1000		(Giuffrida *et al*. 2013)
	C. chinense	1000		(Giuffrida *et al*. 2013)
	C. frutescens	1000		(Giuffrida *et al*. 2013)
(13Z)-cis- ß-Carotene	*C. chinense*	13000		(Giuffrida *et al*. 2013)
	Blend (*C. annuum* + *C. Frutescens*)	0.004		(Nagy et al., 2017)
cis- ß-Carotene	*C. annuum*	43		(Hallmann & Rembialkowska, 2012)
Phytofluene	*C. annuum*	1000		(Giuffrida *et al*. 2013)
(9Z)-cis-α-Carotene	*C. chinense*	6000		(Giuffrida *et al*. 2013)
XANTHOPHYLLS				
Zeaxanthin	*C. annuum*	460.03	Acetone	(Giuffrida *et al*. 2013; Pugliese *et al*. 2013; Carvalho *et al*. 2015; Kim *et al*. 2016b, 2017; da Silveira Agostini-Costa *et al*. 2017)
	C. baccatum	1291		(Pugliese *et al*. 2013; Carvalho *et al*. 2015)
	C. chinense	108000		(Giuffrida *et al*. 2013; Carvalho *et al*. 2015; da Silveira Agostini-Costa *et al*. 2017)
	C. frutescens	2000		(Giuffrida *et al*. 2013)
Zeaxanthin DE 1 – DE 3	Blend (*C. annuum* + *C. Frutescens*)	0.006 - 0.012		(Nagy et al., 2017)
(13Z)-cis-ß-Cryptoxanthin	*C. annuum*	2000		(Giuffrida *et al*. 2013)
	C. chinense	73000		(Giuffrida *et al*. 2013)
All-trans-lutein	*C. annuum*	312.79		(Carvalho *et al*. 2015)
	C. baccatum	139.85		(Carvalho *et al*. 2015)
	C. chinense	687.71		(Carvalho *et al*. 2015)

Antheraxanthin	*C. annuum*	5000		(Hallmann & Rembialkowska 2012; Giuffrida *et al*. 2013; Pugliese *et al*. 2013; da Silveira Agostini-Costa *et al*. 2017)
	C. baccatum	283		(Pugliese *et al*. 2013)
	C. chinense	99000		(Giuffrida *et al*. 2013; Pugliese *et al*. 2013; da Silveira Agostini-Costa *et al*. 2017)
	C. frutescens	2000		(Giuffrida *et al*. 2013)
	Blend (*C. annuum* + *C. Frutescens*)	0.001	Methanol	(Nagy et al., 2017)
Capsanthin	*C. annuum*	125000		(Giuffrida *et al*. 2013; Pugliese *et al*. 2013; Kim *et al*. 2016b, 2017; da Silveira Agostini-Costa *et al*. 2017)
	C. baccatum	592		(Pugliese *et al*. 2013)
	C. chinense	86000		(Giuffrida *et al*. 2013; Pugliese *et al*. 2013; da Silveira Agostini-Costa *et al*. 2017)
	C. frutescens	33000		(Giuffrida *et al*. 2013)
	Blend (*C. annuum* + *C. Frutescens*)	0.016		(Nagy et al., 2017)
Capsanthin-C12:0	*C. annuum*	66000		(Giuffrida *et al*. 2013)
	C. frutescens	51000		(Giuffrida *et al*. 2013)
Capsanthin-C12:0, C14:0	*C. annuum*	148000		(Giuffrida *et al*. 2013)
	C. chinense	113000		(Giuffrida *et al*. 2013)
	C. frutescens	152000		(Giuffrida *et al*. 2013)
Capsanthin-C12:0, C16:0	*C. annuum*	19000		(Giuffrida *et al*. 2013)
	C. chinense	5000		(Giuffrida *et al*. 2013)
	C. frutescens	13000		(Giuffrida *et al*. 2013)
Capsanthin-C14:0	*C. annuum*	204000		(Giuffrida *et al*. 2013)
	C. chinense	167000		(Giuffrida *et al*. 2013)
	C. frutescens	115000		(Giuffrida *et al*. 2013)
Capsanthin-C14:0, C14:0	*C. annuum*	103000		(Giuffrida *et al*. 2013)
	C. chinense	114000		(Giuffrida *et al*. 2013)
	C. frutescens	95000		(Giuffrida *et al*. 2013)
Capsanthin-C14:0, C16:0	*C. annuum*	52000		(Giuffrida *et al*. 2013)
	C. chinense	63000		(Giuffrida *et al*. 2013)
	C. frutescens	12000		(Giuffrida *et al*. 2013)
Capsanthin-C16:0	*C. annuum*	22000		(Giuffrida *et al*. 2013)
	C. chinense	35000		(Giuffrida *et al*. 2013)
	C. frutescens	14000		(Giuffrida *et al*. 2013)
Capsanthin-C16:0, C16:0	*C. annuum*	28000		(Giuffrida *et al*. 2013)
	C. chinense	92000		(Giuffrida *et al*. 2013)

Capsanthin DE 1	Rubin BE-blend 1*	0.006	Methanol	(Nagy et al., 2017)
Capsanthin DE 2 – DE 8	Blend (*C. annuum* + *C. Frutescens*)	0.001 - 0.113	Acetone	(Nagy et al., 2017)
Capsolutein	*C. annuum*	278200		(Topuz & Ozdemir 2007)
Cis-Capsanthin	*C. annuum*	34000		(Giuffrida *et al*. 2013; da Silveira Agostini-Costa *et al*. 2017)
	C. chinense	21000		(Giuffrida *et al*. 2013; da Silveira Agostini-Costa *et al*. 2017)
	C. frutescens	8000		(Giuffrida *et al*. 2013)
Cis-Capsanthin-C14:0	*C. annuum*	14000		(Giuffrida *et al*. 2013)
	C. chinense	5000		(Giuffrida *et al*. 2013)
	C. frutescens	23000		(Giuffrida *et al*. 2013)
13-cis-capsanthin	Blend (*C. annuum* + *C. Frutescens*)	0.002		(Nagy et al., 2017)
13-cis-capsanthin DE 1 - DE 3	Blend (*C. annuum* + *C. Frutescens*)	0.006 - 0.033		(Nagy et al., 2017)
Cis-capsanthin ME 2	Blend (*C. annuum* + *C. Frutescens*)	0.026		(Nagy et al., 2017)
Capsorubin	*C. annuum*	460000		Topuz & Ozdemir 2007; Kim et al. 2016b, 2017
Capsorubin DE 1 - DE 2	Blend (*C. annuum* + *C. Frutescens*)	0.001 - 0.020		(Nagy et al., 2017)
Cis-capsorubin DE	Blend (*C. annuum* + *C. Frutescens*)	0.013		(Nagy et al., 2017)
Cryptocapsin-C14:0	*C. annuum*	11000	Acetone	(Giuffrida *et al*. 2013)
	C. chinense	17000		(Giuffrida *et al*. 2013)
	C. frutescens	22000		(Giuffrida *et al*. 2013)
Cryptoxanthin-C16:0	*C. chinense*	21000		(Giuffrida *et al*. 2013)
Cryptoxanthin-5,6-epoxide	*C. annuum*	4000		(Giuffrida *et al*. 2013)
	C. chinense	2000		(Giuffrida *et al*. 2013)
	C. frutescens	2000		(Giuffrida *et al*. 2013)

Cucurbitaxanthin-B	Blend (*C. annuum* + *C. Frutescens*)	0.005		(Nagy et al., 2017)
Lutein	*C. annuum*	11800		(Hallmann & Rembialkowska 2012; Giuffrida *et al*. 2013; Pugliese *et al*. 2013; Kim *et al*. 2017)
	C. baccatum	59.2		(Pugliese *et al*. 2013)
	C. chinense	483000		(Giuffrida *et al*. 2013)
Lutein-C14:0	*C. chinense*	38000		(Giuffrida *et al*. 2013)
ß –Cryptoxanthin	*C. annuum*	620		(Hallmann & Rembialkowska 2012; Pugliese *et al*. 2013; Carvalho *et al*. 2015; Kim *et al*. 2016b, 2017; da Silveira Agostini-Costa *et al*. 2017)
	C. baccatum	1456		(Pugliese *et al*. 2013; Carvalho *et al*. 2015)
	C. chinense	21000		(Giuffrida *et al*. 2013; Pugliese *et al*. 2013; da Silveira Agostini-Costa *et al*. 2017)
ß -Cryptoxanthin-C12:0	*C. annuum*	7000		(Giuffrida *et al*. 2013)
	C. chinense	2000		(Giuffrida *et al*. 2013)
	C. frutescens	2000		(Giuffrida *et al*. 2013)
ß -Cryptoxanthin-C14:0	*C. annuum*	21000		(Giuffrida *et al*. 2013)
	C. chinense	12000		(Giuffrida *et al*. 2013)
ß -Cryptoxanthin-C16:0	*C. chinense*	19000		(Giuffrida *et al*. 2013)
ß-Carotene-5,6-epoxide	*C. annuum*	21000		(Giuffrida *et al*. 2013)
	C. chinense	16000		(Giuffrida *et al*. 2013)
	C. frutescens	13000		(Giuffrida *et al*. 2013)
Violaxanthin	*C. annuum*	1119	Acetone	(Topuz & Ozdemir 2007; Pugliese *et al*. 2013; Kim *et al*. 2017)
	C. baccatum	214		(Pugliese *et al*. 2013)
	C. chinense	9.4		(Pugliese *et al*. 2013)
	Blend (*C. annuum* + *C. Frutescens*)	0.006	Methanol	(Nagy et al., 2017)
Zeaxanthin-C12:0	*C. annuum*	9000	Acetone	(Giuffrida *et al*. 2013)
	C. chinense	6000		(Giuffrida *et al*. 2013)
	C. frutescens	15000		(Giuffrida *et al*. 2013)
Zeaxanthin-C12:0, C12:0	*C. annuum*	16000		(Giuffrida *et al*. 2013)
	C. chinense	62000		(Giuffrida *et al*. 2013)
	C. frutescens	17000		(Giuffrida *et al*. 2013)
Zeaxanthin-C14:0	*C. annuum*	16000		(Giuffrida *et al*. 2013)
	C. chinense	19000		(Giuffrida *et al*. 2013)
	C. frutescens	9000		(Giuffrida *et al*. 2013)
Zeaxanthin-C14:0, C14:0	*C. annuum*	16000		(Giuffrida *et al*. 2013)
	C. chinense	33000		(Giuffrida *et al*. 2013)
	C. frutescens	14000		(Giuffrida *et al*. 2013)
Zeaxanthin-C14:0, C16:0	*C. annuum*	8000		(Giuffrida *et al*. 2013)
	C. chinense	32000		(Giuffrida *et al*. 2013)
	C. frutescens	3000		(Giuffrida *et al*. 2013)

Zeaxanthin ME 1	Blend (*C. annuum* + *C. Frutescens*)	0.007		(Nagy et al., 2017)
Zeaxanthin ME 2	Blend (*C. annuum* + *C. Frutescens*)	0.008	Methanol	(Nagy et al., 2017)
α-Cryptoxanthin	*C. annuum*	1000	Acetone	(Giuffrida *et al*. 2013)
	C. chinense	3000		(Giuffrida *et al*. 2013)
Antheraxanthin-C12:0	*C. annuum*	23000		(Giuffrida *et al*. 2013)
	C. frutescens	2000		(Giuffrida *et al*. 2013)
Antheraxanthin-C14:0	*C. annuum*	11000		(Giuffrida *et al*. 2013)
Capsanthin-5,6-epoxy-C14:0	*C. annuum*	32000		(Giuffrida *et al*. 2013; da Silveira Agostini-Costa *et al*. 2017)
Cis-Capsanthin-C12:0	*C. annuum*	7000		(Giuffrida *et al*. 2013)
	C. frutescens	14000		(Giuffrida *et al*. 2013)
Cryptocapsin	*C. chinense*	15000		(Giuffrida *et al*. 2013)
Mutatoxanthin	*C. annuum*	--		(da Silveira Agostini-Costa *et al*. 2017)
	C. chinense	--		(da Silveira Agostini-Costa *et al*. 2017)
Neoxanthin	*C. annuum*	54.4		(Pugliese *et al*. 2013)
	C. baccatum	35.1		(Pugliese *et al*. 2013)
(13Z)-cis-Cryptocapsin	*C. chinense*	103000		(Giuffrida *et al*. 2013)
Cis-Zeaxanthin	*C. annuum*	1.2		(Hallmann & Rembialkowska, 2012)
Cis-zeaxanthin ME	Blend (*C. annuum* + *C. Frutescens*)	0.010	Methanol	(Nagy et al., 2017)
Cryptoflavin	*C. annuum*	21.3	Acetone	(Hallmann & Rembialkowska, 2012)
Cryptoxanthin	*C. annuum*	1.7		(Hallmann & Rembialkowska, 2012)
Luteoxanthin	*C. chinense*	7000		(Giuffrida *et al*. 2013)
Pheophytin a	*C. chinense*	22000	Acetone	(Giuffrida *et al*. 2013)
ß-Carotene-5,8-epoxide	*C. chinense*	19000		(Giuffrida *et al*. 2013)
Zeaxanthin-C16:0	*C. chinense*	22000		(Giuffrida *et al*. 2013)
Zeaxanthin-C16:0, C16:0	*C. chinense*	11000		(Giuffrida *et al*. 2013)
15-cis-capsanthin + cis-zeaxanthin	Blend (*C. annuum* + *C. Frutescens*)	0.002		(Nagy et al., 2017)
Capsanthin-epoxide ME	Blend (*C. annuum* + *C. Frutescens*)	0.015		(Nagy et al., 2017)

Capsanthin ME 1	Blend (*C. annuum* + *C. Frutescens*)	0.004	Methanol	(Nagy et al., 2017)
Capsanthin ME 2 - ME 4	Blend (*C. annuum* + *C. Frutescens*)	0.015 - 0.030	Acetone	(Nagy et al., 2017)
β-cryptocapsin + cis-capsanthin ME	Blend (*C. annuum* + *C. Frutescens*)	0.016	Methanol	(Nagy et al., 2017)
Antheroxanthin ME - ME 2	Blend (*C. annuum* + *C. Frutescens*)	0.020 - 0.023	Acetone	(Nagy et al., 2017)
Cryptokapszin ME	Blend (*C. annuum* + *C. Frutescens*)	0.010		(Nagy et al., 2017)
Cis-capsanthin DE 1 - DE 4	Blend (*C. annuum* + *C. Frutescens*)	0.003 - 0.014		(Nagy et al., 2017)

-- Not quantified.

2.4. Vitamin C

Ascorbic acid is another important bioactive in the *Capsicum* species, which are well known sources of this vitamin with antioxidant activity (Meckelmann, Riegel, et al. 2015; Rodríguez-Ruiz et al. 2017; da Silveira Agostini-Costa et al. 2017). Studies show that 50g of fresh pepper provide 50% or more of the recommended daily intake (RDI) of vitamin C for humans (Palma et al. 2015; Perla et al. 2016). Some varieties of pepper also contain about twice as much vitamin C as orange, apple or tomato per gram of fruit weight, for example (Kantar et al. 2016; Zhuang et al. 2012).

In data verified by Wahyuni et al. (2011) on the *Capsicum* pepper, levels of vitamin C were shown to be 10 times higher than those found in tomatoes, and thus was considered a good source of vitamin C. Perla et al. (2016), when analyzing the level of this same compound, it was also observed that the highest level of ascorbic acid (> 2 mg/g FW) was registered in red peppers (*C. annuum*) considering 66 foods tested. Peppers (120 mg/100g) and gourds (180 mg/100g) were considered sources of vitamin C (Davey et al. 2000), as well as coriander (160 mg/100g) (Divya, Puthusseri, and Neelwarne 2012). Vitamin C has also been identified in food spices produced in Ghana, such as kapok seed (1,029 mg/100 g) and *Tetrapleura tetraptera* (0.88 to 1.20 mg/100g) (Adadi, Barakova, and Krivoshapkina 2019).

The levels of ascorbic acid also depends on the species, environmental conditions, the harvest season, production practices and stage of maturity and storage. During ripening, peppers store more reducing sugars, which are the precursors of L-ascorbic acid, and this confirms the fact that they increase the vitamin C content with ripening (Nagy et al. 2015). The different methods of extraction can also influence the stability and content of vitamin C in peppers (Ornelas-Paz et al. 2013; Palma et al. 2015; da Silveira Agostini-Costa et al. 2017; Teodoro et al. 2013). Although we are talking about the same genus (*Capsicum*), different species have different genetic characteristics, thus resulting in different chemical compositions (Carvalho et al. 2015), as shown in Table 4.

Table 4. Vitamin C present in the five species used as both fresh fruit and spices belonging to the genus *Capsicum.*

SPECIES	EXTRACTION SOLVENT	RANGE QUANTIFICATION (mg g^{-1})	REFERENCE
C. annuum	Methanol; Metaphosphoric acid; Oxalic acid; TCEP-HCI	2.81 – 327.29	(Bae *et al*. 2014; Carvalho *et al*. 2015; da Silveira Agostini-Costa *et al.* 2017; Dubey *et al*. 2015; Nagy *et al*. 2015; Tilahun *et al*. 2013)
C. baccatum	Oxalic acid; Water distilled; Metaphosphoric acid	11.3 – 264.13	(Carvalho *et al*. 2015; Perla *et al*. 2016; Rodríguez-Burruezo *et al*. 2009; Wahyuni *et al*. 2011)
C. chinense	Metaphosphoric acid; TCEP-HCI; Oxalic acid	1.51 – 315.04	(Bae *et al*. 2014; Campos *et al*. 2013; Carvalho *et al*. 2015; da Silveira Agostini-Costa *et al*. 2017; Dubey e*t al*. 2015; Teodoro *et al*. 2013)
C. frutescens	Metaphosphoric acid; DCFI; Oxalic acid	2.08 – 249.79	(Dubey *et al*. 2015; Nagy *et al*. 2015; Tilahun *et al*. 2013; Zhuang *et al*. 2012)
C. pubescens	Water distilled; Oxalic acid; Metaphosphoric acid	0.21 – 221.25	(Dubey *et al*. 2015; Oboh & Rocha, 2008; Ornelas-Paz *et al*. 2013; Rodríguez-Burruezo *et al*. 2009)

TCEP-HCI (tris 2-carboxyethyl-phosphine hydrochloride); DCFI (2-6-diclorofenol-indofenol)

The maximum amount of vitamin C in peppers reported in literature varied considerably between 221.25 and 327.29 mg of ascorbic acid/100 g (dry basis) and these contents are related to the species *C. pubescens* and *C. annuum*, respectively (Table 4). However, all five species of *Capsicum* present vitamin C levels above those currently recommend for the adult population - 75mg for women and 90mg for men – and these amounts can be achieved by diet, from pepper in natura or in hot sauces with a higher content of antioxidants (Cerqueira, De Medeiros, and Augusto 2007; Perla et al. 2016; Teodoro et al. 2013).

Ascorbic acid plays important protective roles in human health, such as preventing scurvy, DNA mutations induced by oxidative stress and chronic human diseases, including certain types of cancer, coronary artery disease, arteriosclerosis, cataracts and kidney disease (Campos et al. 2013; Rodríguez-Burruezo et al. 2009; Rodríguez-Ruiz et al. 2017; Teodoro et al. 2013), it also stimulates the immune system, inhibits the formation of nitrosamines and blocks the metabolic activation of carcinogens (Campos et al. 2013; Carvalho et al. 2015; Cerqueira, De Medeiros, and Augusto 2007).

Capsicum peppers play a significant role combating the oxidization of food lipids due to the relevance of ascorbic acid (da Silveira Agostini-Costa et al. 2017). Ascorbic acid present in peppers, participate in various antioxidant processes in plants. The accumulation of this compound slows down important metabolic changes that take place in the maturation process of peppers, acting in their preservation and prolonging their commercial value, as well as

improving post-harvest quality (Bae et al. 2014; Rodríguez-Ruiz et al. 2017).

As an antioxidant, vitamin C is the most sensitive to thermal drying in peppers, so temperatures above 80 °C should be avoided in order to keep it at a desired level in the final products, also preserving other valuable compounds, such as carotenoids (Daood et al. 2014; Meckelmann et al. 2013). The levels of vitamins A and C have recently been associated with the levels of capsaicin and, with the advances in genetic engineering, crops with higher levels of nutrients could be developed and, therefore, contribute to a healthy diet and combat vitamin deficiency (Kantar et al. 2016).

2.5. Vitamin E

Vitamin E is a generic term that refers to the tocopherols and tocotrienols, i.e. α-, β-, δ-, γ-tocopherol and α-, β-, δ-, γ-tocotrienol. Structurally, these compounds consist of a chromanol nucleus attached to an aliphatic side chain (Cerqueira, De Medeiros, and Augusto 2007; Grebenstein and Frank 2012). Both α and γ-tocoferol are found in large quantities in pepper, with α-tocopherol located in the tissue of the pericarp and γ-tocopherol in seeds (Nagy et al. 2017). It has been acknowledged in the last decade that the consumption of certain foods and spices, such as peppers from the genus *Capsicum*, could promote health benefits due to high levels of vitamin E. However, some aspects can influence their nutritional value, including climate conditions, growing techniques, ripeness, duration of storage, extraction and species (Table 5) (Daood et al. 2014; Kantar et al. 2016; Menichini et al. 2009).

This compound plays a key role in protecting approximately 80 diseases, by scavenging free radicals, preventing cancer, anemia, diabetes and cardiovascular diseases, inhibition of oxidation of low-density lipoproteins, disorders of the skin, eye, lungs and other constituents of the lipid-rich body (Ching and Mohamed 2001; Meckelmann et al. 2013). According to Daood et al. (2014), because of their content in antioxidant vitamins, *Capsicum* species are excellent raw-materials for producing high quality products that can be easily commercialized, as well as being a natural source of the daily recommended intake of vitamin E. These peppers contain significant quantities of fat-soluble antioxidants, such as tocopherols (mainly vitamin E) (Conforti, Statti, and Menichini 2007; Meckelmann et al. 2013).

Table 5. Vitamin E present in five species used as both fresh fruit and spices belonging to the genus *Capsicum.*

SPECIES	EXTRACTION SOLVENT	RANGE QUANTIFICATION (mg g^{-1})	REFERENCE
C. annuum	n-hexane-Ethyl acetate; Methanol; Hexane	1.74 – 89.49	(Ching & Mohamed, 2001; Conforti *et al*. 2007; Daood *et al*. 2014; Le Grandois *et al*. 2017; Meckelmann *et al*. 2015)
C. baccatum	2-propanol	303.66	(Meckelmann *et al*. 2015)
C. chinense	Ethanol; Methanol: chloroform	5.90 – 16.32	(Menichini *et al*. 2009; Wahyuni *et al*. 2011)
C. frutescens	n-hexane-Ethyl acetate	95.4	(Ching and Mohamed 2001)
C. pubescens	2-propanol	18.4	(Meckelmann *et al*. 2015)

Tocopherols, with antioxidant properties, are synthesized only by photosynthetic organisms and play positive roles in human health, inactivating reactive oxygen species (ROS). In animals, deficiency of this vitamin, causes neurological weakness and dysfunction (Tavva et al. 2007). In a study carried out by Ching & Mohamed (2001) on the content of vitamin E in 62 edible tropical plants, it was possible to observe that the red pepper *C. annuum* (155.4mg/kg), stood out with one of the highest levels among these foods, such as, for example, garlic (*Allium sativum*) (1.23 mg / 100 g). Ghanaian spices, such as kapok seed (2.9916 mg / 100 g) and *Tetrapleura tetraptera* (2.66-3.69mg / 100g), also had low vitamin E content (Adadi, Barakova, and Krivoshapkina 2019).

Tocopherols, components of vitamin E, are about 250 times more effective than BHT (Koncsek, Helyes, and Daood 2017). According to the author, α-tocopherol has been associated with the antioxidant action of peppers and that the γ-tocopherol content of pepper seed oils provides oxidative stability of the auto-oxidation processes and can be used in the cosmetic and pharmaceutical industries, further improving the bioefficiency of many products. Menichini et al. (2009) reported a positive result of *C. annuum* against neurodegenerative diseases. According to the authors, several studies highlight the association between carotenoids, nutrient deficiency in vitamins E and C, memory deficiencies and learning disabilities.

2.6. Food application

Spice consumption when compared to the consumption of food products from other food groups has been lower and is still frequently ignored in research related to food intake (Gajewska, Katarzyna, and Szkop 2019). *Capsicum* pepper is the second most popular spice,

following Piper peppers (N. D. S. Mendes et al. 2019). Between 2006 and 2016, total pepper production increased by 25%, being of great agricultural and economic importance (Baenas et al. 2019).

Evaluating the functional action of the bioactive components present in this matrix, it is possible to apply it to the food industry, improving functional and sensory quality, but considering the current behavior of consumers and industries, there are still few works related to the characterization and exploration of this raw material, plant or plant extract, with potential uses (Baenas et al. 2019). For example, the application of peppers in food preparation with functional appeal is restricted and is currently restricted to the addition of nuggets (Mendiratta, Shinde, and Mane 2013), spaghetti (Padalino et al. 2013) and bakery products (Danza et al. 2014).

As the matrix for the production of ingredients, the most extracted bioactive components are capsaicinoids and carotenoids, usually paprika oleoresin, used as a natural dye in sauces, soups, processed meats, sweets and alcoholic beverages (Baenas et al. 2019; Téllez-Pérez et al. 2015). The industrial production of pepper seed oil indicated high levels of bioactives, such as linoleic acid and polyunsaturated fatty acids, carotenoids and tocopherols, considered a product with high nutritional value and application in nutrition and food processing (Koncsek, Helyes, and Daood 2017).

Powdered pepper is an ingredient that improves the color retention of dehydrated foods, and supplementation with rosemary extract was advantageous in retaining antioxidant bioactive (Koncsek et al. 2019). It is important to note that bleaching for 5 minutes at 90 and 100 °C, was appropriate for the production of pepper powders with low microbial load and high content of carotenoids and capsaicinoids (Schweiggert et al. 2007).

It is known that encapsulation promotes efficient retention of bioactive substances, positively impacting chemical and functional stability (Ozkan et al. 2019). To explore the possibilities of using the peppers, the encapsulation process was proposed; the obtained ingredient developed an improved stability, favoring storage and becoming suitable for future applications in hydrophilic media. The results presented in this study indicate that the sample studied has the potential for industrial uses, such as baking ingredients or spices (N. de S. Mendes, Favre, et al. 2019).

In recent years, a high consumption of meat products has been reported three to four times a week, associated with the consumption of "ready-to-eat" products, directly related to changes in consumers' lifestyle and time savings (Solomando, Antequera, and Perez-palacios 2020). However, the addition of antioxidant ingredients is common in order to increase physical,

chemical, enzymatic and microbial stability in meat derivatives (Zehiroglu, Beyza, and Sarikaya 2019). For this reason, it is believed that *Capsicum* peppers among the possible functional and technological applications can be used as a natural antioxidant ingredient due to important barrier properties (Mendes et al., 2019b) for this branch of the food industry.

3. Conclusions

Capsicum peppers present various bioactive compounds with functional properties of relevant industrial interest, such as capsaicinoids, phenolic compounds, carotenoids and vitamins. All these important constituents are reported mainly in *Capsicum annuum* (Baenas et al. 2019). Thus, it is evident that there is a promising field of study for all pepper species of the genus *Capsicum*, considering the benefits for human health and, food industry, with interesting technological qualities for the production of functional ingredients.

Funding acknowledgement statement

Supported by hte Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ, Brasil, Project No. E-26/202.086/2016.

Declaration of competing interest

The authors declare no conflicts of interest. All authors read and approved the final version of the manuscript and agree to its submission to TIFS.

Acnowledgement

We thank to Mônica Cristine Pereira dos Santos for her valuable help for translating and revising the manuscript.

3. CAPÍTULO II – CHARACTERIZATION OF PEPPER (*CAPSICUM BACCATUM*) – A POTENTIAL FUNCTIONAL INGREDIENT

Nathânia de Sá Mendes, Mônica Cristine Pereira dos Santos, Millena Cristina Barros Santos, Luiz Claudio Cameron, Mariana Simões Larraz Ferreira, Édira Castello Branco de Andrade Gonçalves
Artigo publicado na revista "*LWT - Food Science and Technology",* 112(2019),108–209.
https://doi.org/10.1016/j.lwt.2019.05.107

Abstract

This study aimed to characterize *Capsicum baccatum* fruits by morphological, chemical and metabolomics approaches. Fruits were obtained, processed into flour, and stored for a period not exceeding 1 month at 25 °C and 80% HR until analysis. The pepper flour (PF) was scanned by an electron microscope coupled with an energy-dispersive spectrometer (EDS) and, sorption isotherms were assessed. Also, an extraction procedure was performed with butanol or ethanol, and phenolic compounds were identified by UPLC-ESI-Q-TOF-MS/MS. SEM images showed a granular matrix with particles of different shapes and sizes. The most abundant element observed were carbon, oxygen and, potassium. The GAB model was the most suitable, and the reduced hysteresis area indicated good stability. 42 phenolic compounds were identified, and quercetin 3-*O*-rhamnoside, luteolin 7-*O*-glycoside, and naringenin were the most abundant. The pepper flour was considered a potential food ingredient with functional and technological properties.

Keywords: *Capsicum baccatum*; microstructure; sorption isotherms; phenolics; functional ingredient

1. Introduction

Several herbs and spices have been reported as potential sources of bioactive compounds with antioxidant activity. However, to be considered a functional ingredient it needs to be described by epidemiological and clinical studies associating the fruit and vegetable intake with a lower risk of developing chronic diseases. Also, it needs to increase the public belief that phytochemicals present in the diet are better and safer than synthetic chemicals (Uribe et al. 2016). Used worldwide, since ancient times, peppers have become a symbol of cooking, and, among the different genus, *Capsicum* peppers are the second most popular spice, succeeding *Piper* peppers (Calixto et al., 2016). The use of *Capsicum* peppers as functional ingredients in food formulations and nutritional supplements has been explained on the basis of their rich nutritional value and antioxidant properties, due to their high contents of polyphenols and ascorbic acid (Kantar et al. 2016; Mudric et al. 2017). They are used in the food industry as additives and dyes because of their characteristic flavor and color, and are suitable for ready-to-eat food product applications (Guadarrama-Lezama et al., 2014).

The *Capsicum* peppers have a great importance due to the presence of capsaicinoids, responsible for pungency, carotenoids, phenolic compounds, vitamins C and E and other natural antioxidants that are found in these fruits with potential activity on human health and food preservation (Calixto et al. 2016; Carvalho et al. 2015; Mokhtar et al. 2015). Although many peppers from the *Capsicum* genus of the Solanaceae family are known, only five are cited in literature as fresh or culinary spices: *C. annuum, C. baccatum, C. chinense, C. frutescens* and *C. pubescens* (Kantar et al. 2016; Meckelmann et al. 2013; Rigon et al. 2012) and only two are most commonly used: *C. annuum* and *C. frutescens* (Fernández-Bedmar and Alonso-Moraga 2016; Gurnani et al. 2016). Liquid chromatography coupled with mass spectrometry (LC-MS) analysis have been used to identify, analyze and quantify polyphenols in *Capsicum annuum* (Lucci, Saurina, and Núñez 2017).

Considering *Capsicum baccatum* is a valuable source of disease resistance (Cremona et al. 2018) and that it may play an important role in the research of functional food in the future (Perla et al. 2016), there are few reports in literature, especially on its phenolic compounds (Rigon et al. 2012). The metabolomics approach has been a potential tool for identification of phenolics in plants and foods (J. P. S. Oliveira et al. 2018). Metabolomics allows the use of a multiplatform that combine different techniques such as

ultraperformance liquid chromatography (UPLC) coupled with mass spectrometry (MS). It allows an extensive coverage of polar and nonpolar compounds, and a faster analysis (Vorkas et al. 2015). Moreover, the use of electrospray ionization (ESI) techniques and independent data acquisition (EID) methods with simultaneous low and high energy fragmentation (MSE), allow accurate measurements of the mass of the precursor and fragments, generating high resolution and reliable results (Ramirez-Ambrosi et al. 2013). Therefore, considering that there are several reports for functional and technological characterization of some species of *Capsicum*, the importance of this work focused on the characterization of *Capsicum baccatum* peppers by morphological, chemical and metabolomic approaches is justified.

2. Materials and methods

2.1. Preparation of pepper flour

Full ripeness fruits from *Capsicum baccatum* L. var. *Pendulum* (red "dedo de moça" peppers) was obtained from a local supermarket (Rio de Janeiro / Brazil) in May/2016. All fruits were washed thoroughly under running water. They were sanitized for 30 min in a bath containing 200 ppm of sodium hypochlorite (NaClO) before rinsing in standard distilled water, and dried in an air circulation drying oven (Marconi, model MA035, Brazil) at 65 °C for 6 hours. Following that, they were dried at 90 °C for 1 hour, crushed, homogenized and stored for a period not exceeding 1 month (with an average temperature of 25 °C and average relative humidity of 80%) in metalized sachets, obtaining the pepper flour (PF) (Ferreira et al. 2015).

2.2. Pepper flour (PF) microstructure and elemental composition

PF was analyzed using a scanning electron microscope (SEM, Oxford Industries, England) coupled with an X-ray energy dispersive spectrometer (EDS; Oxford Industries) for structure (shape and size) and elemental composition according to the method described by Andrade, Ferreira, & Gonçalves (2016).

2.3. Determination of sorption isotherms

AquaLab VSA (Decagon Devices, Inc., Pullman, Washington, USA) was used to construct the moisture sorption isotherms at 25 ± 1 °C of the pepper flour. The Aquasorp

was set to create isotherms utilizing the water activity and gravimetric analysis method called Dynamic Dewpoint Isotherm (DDI). Adsorption curves (DDI) were generated with settings of minimum water activity of 0.03 a_w, a maximum water activity setting of 0.90 a_w, a flow rate of 80 ml/min, resolution setting of 0.01 a_w, and starting sorption direction adsorption. The software for data analysis was SorpTracTM Version 1.14 for AquaSorp Isotherm Generator.

Moisture was determined by gravimetrical analysis of the moisture contents of samples immediately before measuring the sorption isotherms at 105 °C (Association of Official Analytical Chemists (AOAC) 1984). The average values of the moisture contents, which were calculated in % dry basis, were used to assess the sorption isotherms.

2.3.1. Mathematical modeling of sorption data

Table 1 shows that adsorption and desorption isotherms were adjusted with five mathematical models: Guggenheim, Anderson and Boer (GAB), D'Arcy and Watt (GDW), Halsey, Henderson and Oswin, through non-linear regression analysis, a statistical procedure, using GraphPad Prism 6 software. The coefficient of determination (R^2), mean relative percentage deviation (E) (Equation (6)) and root mean square (RMSE) (Equation (7)) were the criteria to verify the degree of adjustment of the models (Téllez-Pérez et al. 2014). The area comprised between the desorption and adsorption curves of the PF was utilized to calculate the hysteresis. The integration method was used in order to calculate the area between the desorption and adsorption curves.

Table 1. Selected isotherm models.

Model	Equation	
GAB	$X_e = \frac{(X_m C K a_w)}{(1-Ka_w)(1-Ka_w+CKa_w)}$	(1)
GDW	$X_e = \frac{k_1 k_2 a_w}{(1+k_1 a_w)} + K_5 a_w + \frac{k_3 k_4 a_w}{1-k_3 a_w}$	(2)
Halsey	$X_e = a\left[T \ln\left(\frac{1}{a_w}\right)\right]^{-1/b}$	(3)
Henderson	$X_e = \left[\frac{\ln\left(\frac{1}{1-a_w}\right)}{a(T+b)}\right]^{1/c}$	(4)
Oswin	$X_e = a\left(\frac{a_w}{1-a_w}\right)^b$	(5)

T - temperature °C; X_e - equilibrium moisture, b.s .; a_w - Water activity, dimensionless; X_m - moisture content in the molecular monolayer, kg kg^{-1}; a, b, C, K, k_1, k_2, k_3, k_4, k_5 - model fit constants; n - number of molecular layers.

$$E\% = \frac{1}{N}\sum_{i=1}^{N}\frac{|m_i - m_{pi}|}{m_i} \quad (6) \qquad RMSE\% = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\frac{m_i - m_{pi}}{m_i}\right)^2} \quad (7)$$

Where m_i and m_{pi} are the actual and predicted moisture content values respectively, and N is the
number of observations. The best model was selected as one with the highest R^2 and least error values (E and RMSE).

2.4. Sample Preparation for UPLC ESI-Q-TOF-MS/MS metabolomics analysis of phenolic compounds

Each sample was prepared by extracting 2g of pepper flour in Falcon tubes (50 mL) with 30 mL of ethanol: water solution (50:50, v/v) or 30 mL of butanol: water solution (50:50, v/v) (Gurnani et al. 2016; M. C. P. Santos and Gonçalves 2016). All extracts were shaken for 10 hours at 30 °C and centrifuged at 2000 x g for 15 min. Then, all samples were filtrated in a paper filter, and only the supernatant was recovered and stored at -20°C until analysis (M. C. P. Santos and Gonçalves 2016).

2.4.1. UPLC ESI-Q-TOF-MS/MS analysis

For UPLC-MS analysis, 4 uL of extracts and standards were injected in triplicate onto a UPLC Q-TOF-MS/MS system equipped with an electrospray ionization source (ESI) (Xevo G2-S QTOF, Waters Corporation, UK) operating in negative ion mode ESI (-). Chromatographic separation was carried out on an ACQUITY UPLC® HSS T3 C18 column (100 mm x 2.1 mm, 1.8 µm particle size). The column and autosampler were maintained at 30 °C and 8 °C, respectively.

During each sample running, the flow rate was 0.6 mL.min-1, and the mobile phase gradient elution was conducted with two mobile phases consisting of acidified water (0.3% formic acid v/v) (pump A) and acetonitrile containing 0.3% formic acid and 5 mM ammonium formate (pump B). The gradient was 97% A and 3% B at 0 min, 50% A and 50% B at 6.78 min, 15% A and 85% B at 7.36 - 8.51 min, followed by an additional equilibration step 97% A and 3% B until 9.09 min.

Data were collected from m/z 50 to 1000 operating in negative ion mode. The capillary and cone voltages were set at 2.0 kV and 30 V, respectively. The desolvation gas (high

purity nitrogen, N2) was set at 600 L.h-1 at a temperature of 450 °C, the cone gas was set at 50 L.h-1, and the source temperature was set at 120 °C. Data were acquired using a multiplexed MS/MS acquisition with alternating low and high energy acquisition (MSE) on centroid mode. MSE experiments were performed with a collision energy range from 30 to 55 eV using ultra-high pure argon (Ar) as the collision gas. Data acquisition was performed using MassLynx 4.1 (Waters Corporation, UK).

All acquisitions were performed by infusing lock mass calibration with leucine-enkephaline (Waters Corporation, USA) (m/z 554.2615) at a concentration of 1,0 ng. L-1 in acetonitrile: H_2O (50:50, v/v) with 0.1% (v/v) formic acid at a flow rate of 10 μL.min-1, to ensure accuracy and reproducibility. Scan time for the lock mass was set to 0.3 s, at intervals of 15 s and 3 scans to average with a mass window of ±0.3 Da.

2.4.2. Data processing

The raw data of all replicates obtained from UPLC Q-TOF-MS/MS analysis were processed with Progenesis QI v2.1 (Nonlinear Dynamics, Waters Corporation, UK) with the following conditions: all runs, automatic limits, centroid data, resolution full-width at half maximum (FWHM) of 30.000, ionization negative ion mode, deprotonated molecule [M - H]-. The identification of phenolics compounds was performed by searching for polyphenols with MetaScope, a fully integrated search tool that allowed the use of the customized database PolyphenolsPubChem ID by using the following parameters: precursor mass error ≤ 5 μg/g, fragment tolerance ≤ 10 μg/g and retention time limits 0.3−11.0 min. Target analysis was also applied for identification of the phenolic compounds by comparing the run parameters of 19 phenolic standards such as the retention time, exact mass, mass error and the MS-MS spectrum, besides the other above mentioned parameters. In addition, the database Phenol Explorer was used for confirmation and classification of the phenolics identified. Only the compounds present in the three technical replicates (3/3) were tentatively identified, presenting coefficient of variation (CV) < 20%.

2.5. Statistical analysis

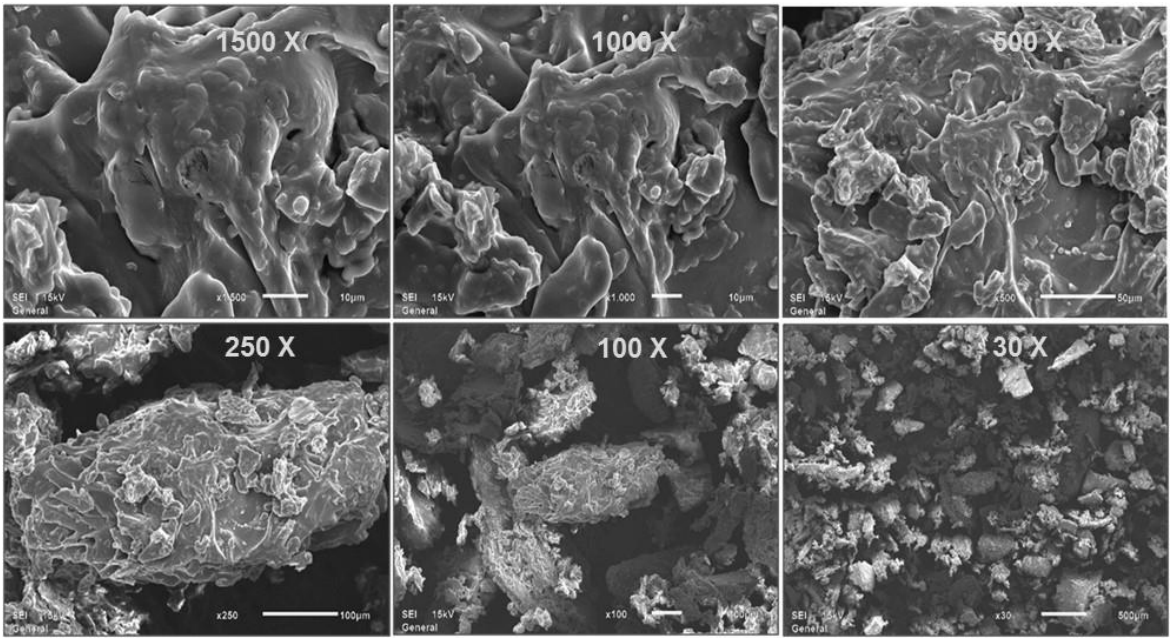
One-way analysis of variance (ANOVA) and Tukey tests for comparison of the average between relative ion abundance of phenolic class ($P < 0.05$) were performed using the XLSTAT software (Addinsoft, version 2018.2.50452).

3. Results and discussion

3.1. Pepper flour (PF) microstructure and elemental composition

Peppers are known to contain essential nutrients that include proteins, carbohydrates, vitamins, minerals, dietary fiber and other health-promoting substances (Mudric et al. 2017; Olatunji and Afolayan 2018). The microstructure of PF, using SEM analysis (Figure 1) showed amorphous spheres immersed in a rugged surface formed mainly by polysaccharide (Roman-Gutierrez, Guilbert, and Cuq 2002; Romdhane et al. 2017) with minimum cell wall rupture (Baby and Ranganathan 2016). The plant-based food materials that are subjected to drying processes can be treated as hygroscopic, porous and amorphous media which undergo multiphase transport of heat and mass (Khan et al. 2017). Amorphous solids can either be found in their brittle, ‘‘glassy” state or a less viscous, ‘‘rubbery” or ‘‘sticky” state. In order to obtain PF, thermic and grinding process was applied, justifying the rubbery state (Mitchell et al. 2017).

Through the elemental composition of the PF analyzed by EDS, the peaks, mainly carbon, oxygen and potassium were identified (Figure 1). The most relevant element in pepper flour, according to the peak-intensity obtained by EDS, was potassium. These results agree with those reported by other researchers, where potassium was also the most abundant mineral in other types of *Capsicum* peppers (Baenas et al. 2019; Embaby and Mokhtar 2011; Mamedov et al. 2015).



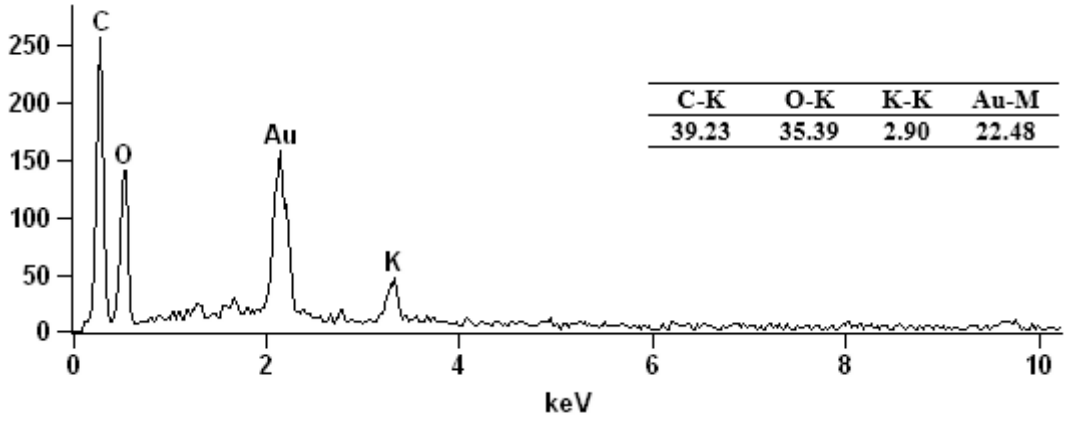


Figure 1. Scanning electron microscopy (SEM) and X-ray microanalysis spectroscopy characteristic of EDS with discrimination table of analyzed elements of pepper flour (*Capsicum baccatum*).

3.2. Modeling of Sorption Isotherm of pepper flour

The results of the non-linear regression analysis were used to fit the experimental data to the five equations presented on Table 2. All the models presented values of determination coefficients (R^2) higher than 0.99 for PF except the Oswin model, which presented 0.98 for the desorption isotherm, and the Henderson's model for adsorption and desorption. However, to evaluate the best mathematical model, the lowest error values (E and RMSE) were also considered. Therefore, the GAB equation was the most suitable followed by Halsey, for the PF sample studied. The worst results were obtained from the Henderson model. Similar results were found by Seid & Hensel (2012) in studies with *C. annuum*. Also, Phomkong & Singthongla (2009) found the GAB model as the most suitable to describe the desorption isotherms data and recommended the Oswin model to represent the adsorption isotherms for this same species.

Table 2. Parameters of the proposed models for moisture sorption isotherms for pepper flour *Capsicum baccatum*).

Models	Parameters	Adsorption	Desorption
GAB	X_m	8.237	8.894
	C	12.43	19.70
	K	0.9340	0.9578
	R^2	0.9979	0.9914
	%E	4.130	5.189
	%RMSE	43.318	46.124
GDW	M	5.260	5.773
	K	9.562	3.400
	k	0.9034	0.8806
	w	1.963	2.293
	R^2	0.9992	0.9939
	%E	6.741	12.240
	%RMSE	70.387	112.184
Halsey	A	31.84	30.00
	B	1.443	1.353
	R^2	0.9969	0.9911
	%E	4.419	5.431
	%RMSE	46.350	47.969
Henderson	A	0.048	0.033
	B	0.997	1.068
	R^2	0.9830	0.9705
	%E	11.316	15.174
	%RMSE	118.146	139.904
Oswin	A	14.39	16.81
	B	0.574	0.5614
	R^2	0.9966	0.9851
	%E	5.209	9.072
	%RMSE	54.392	82.156

X_m, M is the water hydration limit ("monolayer value", % dry basis); C, K, k, w, A, B are constants of the models; R^2 is the coefficient of determinant; %E is the mean relative percentage deviation and %RMSE is the root mean square.

The GAB is considered a model that allows a good fit between several varieties of peppers, in a wide range of a_w (0.1 to 0.9) (Téllez-Pérez et al. 2014; Vega-Gálvez et al. 2007). The parameters C and K of the GAB model determined for PF are within the range defined by Lewicki (1997) ($0{,}24 < K \leq 1$ and $5{,}67 \leq C \leq \infty$) to have a good description of the isotherm. According to the author, maintaining the constants C and K within this range, ensure that the calculated values do not differ from ± 15.5% of the real capacity of the monolayer.

The hydration limits (X_m, called "monolayer value") obtained through the GAB equation are 8.237 g H_2O/g dry basis and 8.894 g H_2O/g dry basis for adsorption and desorption isotherms respectively (Table 2), according to the values obtained are according to the values reported for fruits and pepper dried products by Pérez-Alonso et al., (2009) and Oliveira et al., (2014). The value of X_m found for PF indicates good stability, except for lipid oxidation (Fonteles et al., 2016; Oliveira et al., 2014). Polysaccharides are the main fraction in this kind of matrix,

as showed by SEM (Olatunji and Afolayan 2018) and the three major components that constitute the cell wall of plant parts are cellulose, hemicellulose and pectin (Baby and Ranganathan 2016). Phenolic compounds are able to form covalent bound to cellulose, hemicellulose, lignin and pectin (Gonçalves et al. 2018). Interactions between polyphenols and carbohydrates were mostly based on different non-covalent hydrophobic interactions, and this can protect polyphenols from oxidation (Jakobek 2015). In order to avoid or minimize these oxidative processes that can affect the antioxidant capacity of the PF's matrix, airtight and waterproof packaging should be used (Ballesteros et al. 2017).

The parameter *C* of GAB model indicates the energy of sorption of the adsorbed monolayer water molecules at the primary binding sites. The higher *C* value obtained indicates the greater water binding force of monolayer (Téllez-Pérez et al., 2014). Also, the GAB constants *C* and *K* are indicative of the isotherm type (Brunauer et al., 1938). Observing the parameters, it is possible to note that $K < 1$ and $C > 2$ were obtained in the PF (Table 2). According to the classification of Brunauer et al. (1938), these values correspond to type II, sigmoidal (Figure 2) characteristic of carbohydrates (Chisté et al. 2012).

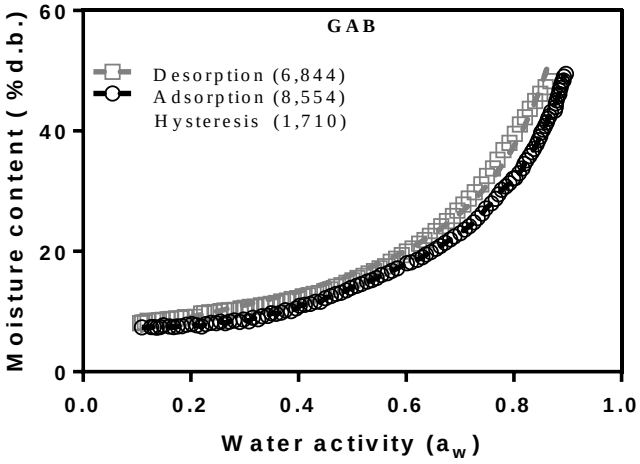


Figure 2. Hysteresis of the GAB model of pepper flour *(Capsicum baccatum*).

The type II isotherm takes into account the existence of multilayers on the inner surface of the material (Fonteles et al. 2016). It is characterized by a relatively slow increase in adsorption capacity at low a_w and a marked increase in higher a_w, as observed in pepper dried products (Vega-Galvéz et al., 2007; Pérez-Alonso et al., 2009), in banana flour (Aguirre-Cruz et al. 2010), pinyon flour (Cladera-Olivera et al. 2011) and tapioca flour (Chisté et al. 2012). It can be observed, still in Figure 2, that the adsorption curve is below the desorption curve in the

whole range of a_w at 25 ± 1 °C, characterizing the hysteresis effect, indicating good stability of PF (Caurie 2007).

3.3. UPLC-MS metabolomics profile of phenolic compounds

A total of 42 phenolic compounds, among flavonoids, phenolic acids, and other phenolics were tentatively identified in PF and were presented in Table 3. The number of identified phenolics in 50% aqueous butanol and 50% aqueous ethanol solutions were 35 and 41, respectively. It was also verified that the average abundance of the relative ions presented significant difference with greater efficiency of butanol as an extractor for the classes of flavonoids and other phenolics. Furthermore, there was no significant difference in the phenolic acids class. Several studies point out a better efficiency in the extraction of polyphenols in plants when organic solvents are added to the solvent extraction system (Khoddami, Wilkes, and Roberts 2013; Turkmen, Sari, and Velioglu 2006). Besides, it has been reported that ethanol may be a suitable solvent for the extraction of low molecular weight polyphenols because the chemical nature of these compounds ranges from the simplest to highly polarized (Ksibi et al. 2015).

As noted on Table 3, the use of ethanol is highlighted by its greater variety of extracted compounds, and it is also a better choice from an industrial point of view, since it is nontoxic and may be reused and generate less waste at the end of the process (Chuichulcherm et al. 2013; Dias et al. 2017). Despite the high abundance of extraction obtained with butanol, this solvent is not suitable for obtaining extracts for application in food industries. Flavonoids were the main phenolic class found in this study for butanol (83.7% flavonoids, 9.7% phenolic acids and 6.6% other polyphenols) and, ethanol (77% flavonoids, 18% phenolic acids and 5% of other polyphenols). These findings are in agreement with other studies in which the main phenolics observed in pepper flour were also, flavonoids (Ksibi et al. 2015; Mudric et al. 2017). Most phenolic compounds identified in this study were free phenolics, esterified with sugars or others compounds that have a low molecular mass, like the quercetin 3-*O*-rhamnoside, luteolin 7-*O*-glucoside and naringenin, the most abundant phenolic compounds detected in all extracts (Figure 3).

The extracted-ion chromatograms (XIC) (Figure 3) were obtained with the mass of these compounds, as described by (Katajamaa and Orešič 2005). It was noted that, in comparison with the other phenolic compounds, quercetin 3-*O*-rhamnoside showed the higher ion-intensity in both extracts. Although some compounds are common for both solvents, they also have a particularity, observed as a variation in the ion-intensity of specific compounds. For

example, the rutin, 3-hydroxycinnamic acid, 5-caffeoylquinic acid and ethyl trans-caffeate, are present among the most abundant in ethanol, but not in butanol extracts.

UPLC-ESI-Q-TOF MS/MS chromatographic techniques were efficient metabolomics tools to characterize and identify the phenolic compounds in *Capsicum* peppers (Abu-Reidah et al. 2015; Cádiz-Gurrea, Fernández-Arroyo, and Segura-Carretero 2014). Furthermore, it is important to note the advantage of this technique is that although it is not quantitative, we can quantify relatively the compounds, even the isomeric forms and that do not have chemical standard.

The chromatogram in BPI (base peak intense) (Figure 3) also shows a few different peaks more intense than those identified as phenolic compounds. These peaks are related to the compounds presented in *Capsicum* species like vitamins, carotenoids, capsaicinoids and other secondary metabolites (Wahyuni et al. 2013). The flavonoids extracted in the aqueous butanol solution consisted of quercetin derivatives as dominant components (65.9% of total flavonoids identified), luteolin 6-*C*-glycoside (13.9%), naringenin derivatives (12.6%), kaempferol derivatives (3.2%), apigenin derivatives (2.4%), rutin (1.3%) and phlorizine (0.6%). Moreover, the flavonoids extracted in the aqueous ethanol solution were quercetin (63.8%), luteolin 6-*C*-glucoside and naringenin derivatives (12.3%), kaempferol derivatives (4.3%) and, apigenin derivatives (3.7%), rutin (2.8%) and amounts of phlorizine (0.7%). These results show that the polyphenol profile of *C. baccatum* is similar to those reported in previous studies for *C. annuum* (Mokhtar et al. 2015; Neacsu et al. 2015).

Nowadays, important phenolic compounds comparable to those found in pepper flour have been reported as bioactive compounds with significant biological activity. As seen in Figure 4, quercetin 3-*O*-rhamnoside was the most abundant flavonoid in extracts, as mentioned by other authors (Juániz, Ludwig, Bresciani, et al. 2016; Materska et al. 2015; Mokhtar et al. 2015). Their function as antioxidant and anticarcinogenic (Jeong et al. 2011), its greater radioprotective effect on human lymphocytes in response to X-ray induced oxidative damage (Materska et al. 2015) and pancreatic lipase inhibitory activity (Zhang et al. 2018) have been described. Within this context, there are many benefits associated with PF consumption, which makes it an alternative for the extraction of this compound.

In contrast, the main compounds, such as luteolin 7-*O*-glycoside (called cynaroside) and naringenin (Figure 4), found in both extracts, though in a smaller quantity, also have potential antioxidant and cytotoxic action (Kil et al. 2017; Song and Park 2014), as well as antidepressant function (J. H. Kim et al. 2013). Additionaly, they play a role in the combat and prevention of type 2 diabetes (Priscilla, Jayakumar, and Thirumurugan 2015). Therefore,

the results exposed in this study related to the morphological, chemical and metabolomics analysis evaluated in *Capsicum* peppers were able to characterize PF samples as a valuable source of functional ingredients to be included in food and nutraceutical formulations.

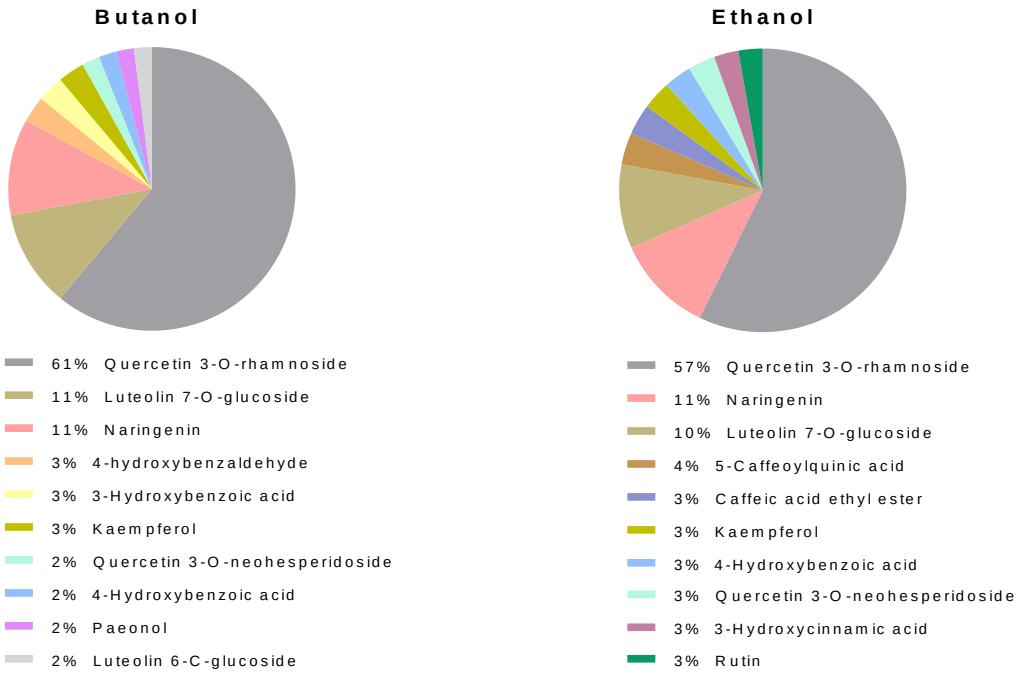


Figure 4. Most abundant phenolic compounds in pepper flour (*Capsicum baccatum*).

Table 3. Phenolic compounds identified in pepper flour (*Capsicum baccatum*) by UPLC-MS^{E}.

N°	Identification	*m/z*	tR_1	Formula	Score	FS_2	EM_3	SI_4	Relative ion abundance	
									Butanol	Ethanol
				Flavonoids						
C1	Quercetin 3-*O*-rhamnoside	447.0926	3.87	$C_{21}H_{20}O_{11}$	55.6	81.6	-1.57	98.32	1410841	654362
C2	Naringenin	271.0606	5.53	$C_{15}H_{12}O_5$	39.2	0.86	-2.01	97.74	266098	126864
C3	Luteolin 7-*O*-glucoside (Cynaroside)	447.0925	3.50	$C_{21}H_{20}O_{11}$	51.6	68.5	-1.71	91.36	267257	109845
C4	Kaempferol	285.0398	4.94	$C_{15}H_{10}O_6$	55.3	82.8	-2.38	96.74	62138	37049
C5	Quercetin 3-*O*-neohesperidoside	609.1453	3.72	$C_{27}H_{30}O_{16}$	52.5	74.5	-1.27	89.37	56322	35057
C6	Rutin	609.1454	2.56	$C_{27}H_{30}O_{16}$	52.6	71	-1.19	93.31	28191	31299
C7	Luteolin 6-*C*-glucoside (Isoorientin)	447.0926	2.99	$C_{21}H_{20}O_{11}$	57.4	90.4	-1.51	98.40	43314	26413
C8	Apigenin 6-*C*-glucoside (Isovitexin)	431.0975	3.38	$C_{21}H_{20}O_{10}$	52.6	76.8	-1.93	88.62	26837	14089
C9	Apigenin 6,8-di-*C*-glucoside (Vicenin 2)	593.1504	2.67	$C_{27}H_{30}O_{15}$	37.8	0	-1.26	90.48	2563	12116
C10	Apigenin-7-(2-*O*-apiosylglucoside) (Apiin)	563.1399	3.11	$C_{26}H_{28}O_{14}$	37.6	0	-1.37	89.71	7051	11641
C11	Kaempferol 3-*O*-sophoroside (Sophoraflavonoloside)	609.1453	3.34	$C_{27}H_{30}O_{16}$	37.7	0	-1.35	90.14	9760	10840
C12	Quercetin 3-glucosyl(1-3) rhamnosyl(1-6)galactoside	771.1983	3.65	$C_{33}H_{40}O_{21}$	36.7	0	-0.86	84.39	nc	10820
C13	Naringenin 7-*O*-glucoside (Prunin)	433.1132	4.35	$C_{21}H_{22}O_{10}$	38.7	8.12	-1.80	87.36	14913	8903
C14	Phlorizine	435.1288	3.71	$C_{21}H_{24}O_{10}$	36.1	0	-1.99	82.77	14378	7973
C15	Isorhamnetin	315.0514	2.12	$C_{16}H_{12}O_7$	37.9	4.5	1.11	86.15	nc	5229
C16	Hispidulin	299.0556	7.13	$C_{16}H_{12}O_6$	36.7	0	-1.82	85.47	16941	3241
				TOTAL					2226604[a]	1105742[b]
				Phenolic acids						
C17	5-caffeoylquinic acid	353.0872	2.30	$C_{16}H_{18}O_9$	55.5	85.1	-1.81	94.64	32735	41626
C18	Ethyl trans-caffeate (Caffeic acid ethyl ester)	207.0657	5.10	$C_{11}H_{12}O_4$	56.6	88.5	-2.93	98.11	nc	39790
C19	4-hydroxybenzoic acid	137.0238	4.10	$C_7H_6O_3$	38.6	0	-4.36	98.21	45756	35804
C20	3-hydroxycinnamic acid	163.0395	2.33	$C_9H_8O_3$	56.5	88.9	-3.49	97.62	19714	31384
C21	2-methoxy-2-phenylacetic acid	165.0550	3.99	$C_9H_{10}O_3$	57.9	96.2	-4.22	98.14	9276	27101
C22	3-hydroxybenzoic acid	137.0238	2.25	$C_7H_6O_3$	38.7	0	-4.27	98.31	63390	22291
C23	3,4-dihydroxybenzoic acid (Protocatechuic acid)	153.0186	1.78	$C_7H_6O_4$	38.4	0	-4.56	97.16	40632	12980
C24	*p*-hydroxyphenylacetic acid	151.0395	2.55	$C_8H_8O_3$	38.9	0	-3.90	99.04	9756	8274
C25	Cinnamic acid	147.0444	1.37	$C_9H_8O_2$	39.5	13	-4.91	90.29	nc	8212
C26	Isoferulic acid	193.0501	2.57	$C_{10}H_{10}O_4$	54.9	85.9	-2.99	92.05	6114	6468

C27	2,6-dihydroxybenzoic acid	153.0187	2.80	$C_7H_6O_4$	37.4	0	-4.22	91.84	5199	5283
C28	Trans-*p*-coumaric acid 4-glucoside	325.0924	2.52	$C_{15}H_{18}O_8$	46.2	40	-1.44	92.64	14400	4958
C29	Benzoic acid	121.0289	2.46	$C_7H_6O_2$	37.4	0	-4.93	92.85	3913	3954
C30	Caffeic acid	179.0343	1.98	$C_9H_8O_4$	56.5	95.2	-3.66	91.49	3142	3924
C31	Homovanillic acid	181.0499	2.71	$C_9H_{10}O_4$	37.1	0	-3.82	89.87	nc	3416
C32	N- phenylacetylglycine	192.0658	3.19	$C_{10}H_{11}NO_3$	36.7	0	-4.45	88.78	nc	3297
C33	Sinapic acid	223.0604	2.03	$C_{11}H_{12}O_5$	40.9	21	-3.46	87.65	4074	3108
C34	3-hydroxybenzeneacetic acid	151.0394	1.97	$C_8H_8O_3$	37.2	0	-4.50	91.05	nc	2964
			TOTAL						258102[c]	264834[c]
				Other polyphenols						
C35	4-hydroxybenzaldehyde	121.0289	2.86	$C_7H_6O_2$	38.4	0	-4.79	97.62	68611	24072
C36	Paeonol	165.0551	3.28	$C_9H_{10}O_3$	38.4	0	-3.94	96.75	43744	23462
C37	Esculetin	177.0186	2.60	$C_9H_6O_4$	38.5	0	-4.07	97.05	31884	10470
C38	2-hydroxychromen-4-one (4-hydroxycoumarin)	161.0238	2.00	$C_9H_6O_3$	55	85.6	-3.89	93.74	9193	7602
C39	Pyrogallol	125.0239	1.31	$C_6H_6O_3$	37.7	0	-4.05	93.04	11042	3537
C40	*p*-anisaldehyde	135.0448	2.82	$C_8H_8O_2$	37.7	0	-2.49	91.26	2898	1843
C41	Guaiacol	123.0445	1.59	$C_7H_8O_2$	37.3	0	-4.95	92.24	4699	1673
C42	4-vinylphenol	119.0496	3.44	C_8H_8O	37.2	0	-4.94	91.46	3768	nc
			TOTAL						175840[c]	72659[d]

Relative ion abundance adjusted for 0.1g of pepper flour. [1]Retention time; [2]Fragmentation Score; [3]Mass Error (ppm); [4]Similarity Isotopic. nc: the relative ion abundance with CV (%) > 20% was not considered. Different letters in the same line differ significantly, using the Tukey test (P <0.05).

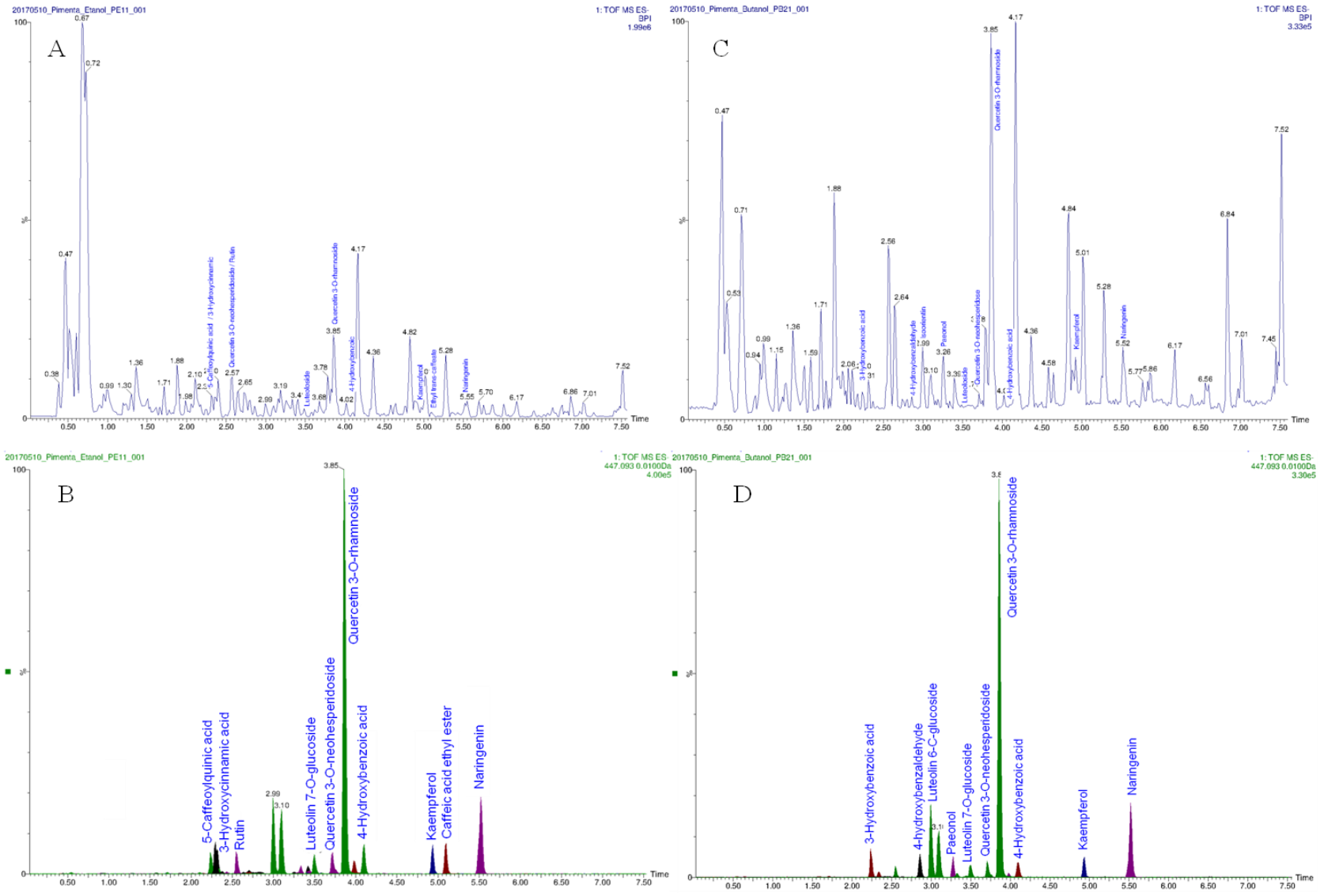


Figure 3. UPLC-ESI-Q-TOF-MS/MS chromatograms of pepper flour with BPI (A – ethanol; C – butanol) and XIC (B – ethanol; D – butanol).

4. Conclusions

According to the SEM analysis, the pepper flour structure displayed amorphous spheres formed mainly by polysaccharide. The hydration limits obtained by the GAB equation indicates good stability, except for lipid oxidation, but interactions between polyphenols and carbohydrates can protect polyphenols from oxidation. Furthermore, in this study all extracts of pepper flour from *Capsicum baccatum* species were characterized as rich in different phenolic compounds, with functional activity already described in the literature. Thus, the pepper flour can also be considered an excellent source of bioactive compounds, especially flavonoids such as quercetin 3-*O*-rhamnoside, luteolin 7-*O*-glucoside and naringenin with potential uses as nutraceuticals or food ingredient. This characteristic can be useful for the food industry, once this type of matrix may possess significant barrier properties and therefore it can be applied in processed foods, enhancing the nutritional potential and stability during storage. Ultimately, the addition of PF in different food matrix can add desirable value to the sensorial properties of the food.

Conflicts of interest

None.

Acknowledgments

This work was supported by the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (grant no. E-26/202.086/2016, FAPERJ, Brazil).

4. CAPÍTULO III – *CAPSICUM PUBESCENS* AS A FUNCTIONAL INGREDIENT: MICROENCAPSULATION AND PHENOLIC PROFILLING BY UPLC-MS^{E}

Nathânia de Sá Mendes, Pedro Paulo Saldanha Coimbra, Millena Cristina Barros Santos, Luiz Claudio Cameron, Mariana Simões Larraz Ferreira, María del Pilar Buera, Édira Castello Branco de Andrade
Artigo publicado na revista "Food Research International". (2020).
https://doi.org/10.1016/j.foodres.2020.109292

Abstract

This study aimed to characterize the profile of phenolic compounds (PC) of *Capsicum pubescens* by metabolomics approach, with further microencapsulation as a means of identifying its functional properties. The metabolomic analyses from the pepper flour (PF) of *C. pubescens* extracted with butanol and ethanol tentatively identified 61 PC. The most abundant was 3-feruloylquinic acid. Physical properties indicated PF could be used as a stable ingredient and its color may suggest applications as a natural food coloring in different types of foods or cosmetics. Experimental water adsorption data was well adjusted to the GAB model. Hydration limits obtained by the GAB equation indicate good stability except for lipid oxidation, but interactions between polyphenols and carbohydrates may protect polyphenols from oxidation. SEM micrographs showed a rough surface composed mainly of polysaccharides, while microencapsulated samples exhibited spherical particles with a smooth surface, some irregularities and good antioxidant capacity. Both PF and microcapsules are indicated as potential functional ingredients to be included in food or nutraceutical products.

Keywords: SEM - EDS; adsorption isotherm; UPLC-MS^{E}; polyphenols; food powders

1. Introduction

There has been growing interest in discovering the functional and technological properties of bioactive compounds present in fruits and vegetables and their extracts, due to their health benefits, related to the reduced risk of cancer, cardiovascular and neurodegenerative diseases, which have been attributed, mainly, to the antioxidant activity of phenolic compounds (PC) in these matrices (Juániz, Ludwig, Bresciani, et al. 2016; Sormoli and Langrish 2016). In parallel, there is a growing concern about synthetic additives and a greater than ever pressure to replace synthetic food colorings by natural antioxidants. These facts, coupled with advances in analytical instrumentation, has promoted studies of pepper fruits as a potential source of bioactives (Baenas et al., 2019).

Capsicum peppers are the second most important spice traded worldwide, succeeding *Piper* peppers and it is considered one of the healthiest fruits in the world, due to the significant amount and diversity of bioactive compounds, such as PC, vitamins C and E, as well as capsaicinoids and carotenoids (Mendes et al., 2019a). They are also reported to contain essential nutrients including carbohydrates, proteins, lipids, minerals and dietary fibers (Olatunji & Afolayan, 2018). Among the many known peppers of the genus *Capsicum* of the Solanaceae family, five main species are used as both spices and vegetables: *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens* (Kantar et al. 2016).

In order to preserve the many functional properties of bioactive compounds such as PC, spray drying technology offers a practical and economical alternative, making this technique the most widely used in the food industry (Chew, Tan, & Nyam, 2018; Guadarrama-Lezama et al., 2012). Kaderides & Goula (2019) mentioned the incorporation of pure PC in food products that are affected by rapid release, low bioavailability, low solubility, low permeation and easy destruction against environmental stresses. Tsali & Goula (2018) also reported that the instability of PC in food processing, distribution or storage, as well as in the gastrointestinal tract, limits their activity and other beneficial health effects.

In this sense, plant and fruit extracts can be spray dried with the addition of various matrices to obtain physically stable and non-adherent powders, with PC stability and improved storage for various food applications (Rezende, Nogueira, and Narain 2018). Several studies have been performed using spray drying as the microencapsulation technology for *C. annuum* (Guadarrama-Lezama et al., 2012; Romo-Hualde et al., 2012) and pepper products (Pérez-Alonso et al., 2009). Maltodextrin is the most traditional wall material used in microencapsulation due to its low cost, mild aroma and taste, low viscosity at high solids concentrations and protection against oxidation (Chew, Tan, and Nyam 2018).

In addition, when mixtures of components are prepared, PC may interact with other constituents, such as proteins, through hydrophobic or hydrophilic interactions, among others, so that they play an important role in antioxidant activity (Gonçalves et al. 2018; Ren et al. 2019). Ksibi et al. (2015) highlighted that the PC present in *Capsicum* extracts interact with biomolecules, such as carbohydrates, proteins and other food components and, therefore, a better solvent should be found for their extraction. Many studies showed the use of the mixtures of organic solvents such as butanol, methanol or ethanol, in different proportions with water can improve the extraction of different PC due to their variety of chemical structures. However, ethanol is more attractive because it has a low cost compared to other solvents and also a better choice in the manufacturing process of food products from a safety and sustainability point of view (Alcântara et al., 2018).

Metabolomic approaches such as ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-Q-TOF-MS/MS) have been reported as essential tools for effective characterization and identification of *Capsicum* pepper - PC, having the advantage to identify and relatively quantify compounds, even the isomeric forms that do not have chemical standards, as well as extensive coverage of polar and non-polar compounds and a much faster analysis (Mendes et al., 2019a). Another advantage is that the use of ESI techniques and independent data acquisition (EID) methods with simultaneous low and high energy fragmentation (MS^E), is based on the most accurate measurements of the mass of the precursor and fragments, to obtain high resolution and reliability of results (Alves et al. 2019). Thus, the non-directed UPLC-MS analysis method has been the most used in different plant matrices, based on the acquisition of MS-MS multiplexed with MS^E to measure the largest possible number of secondary metabolites, establishing a detailed characterization of the metabolomic profile of the sample (M. C. B. Santos et al. 2019).

A lot of studies show antioxidant activity by PC in *Capsicum*, especially *C. annuum* (N. de S. Mendes and Gonçalves 2020; Ribes-Moya et al. 2018; Rodrigues et al. 2019) and *C. chinense* (Aguiar et al. 2019; Bogusz Jr et al. 2018; Pérez-Ambrocio et al. 2018). A recent study was also carried out tracing the phenolic profile of the species *C. baccatum* and indicating the high potential for application of the flour of this pepper as a functional ingredient (Mendes et al., 2019a, b). Although, the *C. pubescens* species is less exploited, especially regarding to its chemical composition (Meckelmann, Jansen, et al. 2015), it is noteworthy that it is a source of health-promoting compounds (Rodríguez-Burruezo, Gonzalez-Mas, and Nuez 2010) acting as antihemorrhoidal, antirheumatic, antiseptic, diaphoretic, digestive, irritant, rubefacients, sialagogue and tonic when taken in relatively small amounts. Externally, it is used in several treatments, such as neuralgia, pleurisy, sprains and unbroken chilblains (Oboh and Rocha 2008). In this context, the

objectives of this study were: (i) to characterize the profile of PC comparing two organic solvents (butanol and ethanol) in the pepper flour (PF) of *Capsicum pubescens* by modern metabolomics approach (UPLC-ESI-Q-TOF-MS^E); (ii) to obtain a functional ingredient in the form of microcapsules with aqueous extract.

2. Materials and methods

2.1. Preparation of Samples

2.1.1. Pepper Flour (PF) - Full ripeness fruits from *Capsicum pubescens* were obtained from a local supermarket (Buenos Aires / Argentina) in September/2017. The PF was processed according to our previous work (Mendes et al., 2019a).

2.1.2. Microencapsulated PF (MPF) – in order to obtain a food ingredient, PC extraction from PF was carrried out with ethanol, the solvent regulated for food safety, thus justifying its use (Alcântara et al. 2018). The microencapsulated extract was obtained according to the procedure described by Mendes et al. (2019b) with modifications. Briefly, PC were extracted from 7% (w/v) of PF in ethanol: water solution (50:50, v/v), incubated in a shaker (TE-420, Tecnal, Brazil) at 200 rpm for 10 hours at 30 °C. After centrifugation at 20°C and 2000 ×g for 15 min, the supernatant was filtered and polyphenol extract from pepper flour (18.3 °Brix) was adjusted to 32 °Brix, with maltodextrin (29.3%) and was spray dried (GEA, AS0340D Niro Atomizer, Germany) under the following operating conditions: flow rate (8 mL/min), air pressure (3.2 kPa), nozzle diameter (1.5 mm), inlet temperature (190 °C) and outlet temperature (90 °C).

2.2. Physicochemical and metabolomics characterization – PF

2.2.1. Bulk and tapped density

Bulk (δ_B) and tapped (δ_T) density were determined in triplicate according to Santhalakshmy et al. (2015).

2.2.2. Flowability and cohesiveness

Carr index (C_I) (Equation (1)) was estimated from the relation of the Bulk (δ_B) and tapped (δ_T) density and the cohesiveness was analyzed in terms of Hausner ratio (H_R) (Equation (2)) (Jinapong, Suphantharika, and Jamnong 2008). All analyses were carried out in triplicate.

$$C_I = \frac{(\delta_T - \delta_B)x100}{\delta_T} \qquad (1) \qquad\qquad H_R = \frac{\delta_T}{\delta_B} \qquad (2)$$

2.2.3. Water activity (a_w)

The water activity of the samples was measured in triplicate using a water activity meter (AquaLab VSA, Decagon Devices, Inc., Pullman, Washington, USA) at 25 ± 1 °C.

2.2.4. Hygroscopicity

Hygroscopicity was performed in triplicate as described by Santhalakshmy et al. (2015).

2.2.5. Solubility

Solubility was determined in triplicate according to the procedure described by Mendes et al. (2019b).

2.2.6. Colorimetric determinations

The color was determined in triplicate using a colorimeter (Konica Minolta CM-5 digital colorimeter, Japan), using the parameters L* (lightness), a* (red/green intensity) and b* (yellow/blue intensity) of the CIE-Lab color space (International Commission on Illumination). Dark and white plates were used as standards to calibrate (0% and 100%, respectively) the sample color measurements.

2.2.7. Water adsorption isotherms

In order to determine the adsorption isotherms, the same approach was used as described in our previous study (Mendes et al., 2019a). Regarding the adjustment of the mathematical models (Table 1), a nonlinear analysis was performed, using the GraphPad Prism 6 software. The coefficient of determination (R^2), mean relative percentage deviation (E) (Equation (5)) and root mean square (RMSE) (Equation (6)) were used in order to compare the adjustment precision of the adsorption models.

Table 1. Selected isotherm models to fit the experimental data.

Model	Equation	
GAB	$X_e = \frac{(X_m C K a_w)}{(1-Ka_w)(1-Ka_w+CKa_w)}$	(1)
Halsey	$X_e = a\left[T \ln\left(\frac{1}{a_w}\right)\right]^{-1/b}$	(2)

Henderson	$X_e = \left[\frac{\ln\left(\frac{1}{1 - a_w}\right)}{a(T+b)}\right]^{1/c}$	(3)
Oswin	$X_e = a\left(\frac{a_w}{1-a_w}\right)^{b}$	(4)

T: temperature °C; X_e: equilibrium moisture, d.b.; a_w - water activity; X_m: moisture content in the molecular monolayer; *a*, *b*, *C*, *K*: model fit constants; n: number of molecular layers.

$$E\% = \frac{1}{N}\sum_{i=1}^{N}\frac{|m_i - m_{pi}|}{m_i} \quad (5) \qquad RMSE\% = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\frac{m_i - m_{pi}}{m_i}\right)^2} \quad (6)$$

Where m_i and m_{pi} are the actual and predicted moisture content values respectively, and N is the number of observations. The goodness of fit of the different models was evaluated with the highest R^2 and least error values (E and RMSE).

2.2.8. Determination of PC by UPLC ESI-Q-TOF-MS^E

A sample was prepared by extracting 0.5 g of pepper flour in Falcon tubes (50 mL) with 7.5 mL of ethanol: water solution (50:50, v/v) or 7.5 mL of butanol: water solution (50:50, v/v) (Gurnani et al. 2016; M. C. P. Santos and Gonçalves 2016). All extracts were shaken for 10 h at 30 °C and centrifuged at 2000 *x*g (centrifuge ST 16R Thermo Scientific) for 15 min. The supernatant was collected and the solvent was evaporated in a vacuum concentrator (Savant Speedvac, Thermo-Scientific). Then, all samples were filtered through a 0.22 µm syringe filter and stored at -20 °C until analysis.

The UPLC-MS^E analysis was carried out according to our previous study (Mendes et al., 2019a) using *Capsicum baccatum* species. Four µL of extracts and standards were injected in triplicate onto a UPLC Q-TOF-MS/MS system equipped with an electrospray ionization source (ESI) (Xevo G2-S QTOF, Waters Corporation, UK) operating in negative ion mode ESI (−). Chromatographic separation was carried out on an ACQUITY UPLC® HSS T3 C18 column (100mm×2.1 mm, 1.8 µm particle size). The column and autosampler were maintained at 30 °C and 8 °C, respectively.

During each sample running, the flow rate was 0.6 mL.min-1, and the mobile phase gradient elution was conducted with two mobile phases consisting of acidified water (0.3% formic acid v/v) (pump A) and acetonitrile containing 0.3% formic acid and 5mM ammonium formate (pump B). The gradient was 97% A and 3% B at 0 min, 50% A and 50% B at 6.78 min, 15% A and 85% B at 7.36–8.51 min, followed by an additional equilibration step 97% A and 3% B until 9.09 min. Data were collected from m/z 50 to 1000 operating in negative ion mode. The capillary and cone voltages were

set at 2.0 kV and 30 V, respectively. The desolvation gas (high purity nitrogen, N_2) was set at 600 L.h-1 at a temperature of 450 °C, the cone gas was set at 50 L.h-1, and the source temperature was set at 120 °C.

Data were acquired using a multiplexed MS/MS acquisition with alternating low and high energy acquisition (MS^E) on centroid mode. MS^E experiments were performed with a collision energy range from 30 to 55 eV using ultra-high pure argon (Ar) as the collision gas. Data acquisition was performed using MassLynx 4.1 (Waters Corporation, UK). All acquisitions were performed by infusing lock mass calibration with leucine-enkephaline (Waters Corporation, USA) (m/z 554.2615) at a concentration of 1,0 ng. L-1 in acetonitrile: H2O (50:50, v/v) with 0.1% (v/v) formic acid at a flow rate of 10 μL.min-1, to ensure accuracy and reproducibility. Scan time for the lock mass was set to 0.3 s, at intervals of 15s and 3 scans to average with a mass window of ± 0.3 Da.

The raw data of all replicates obtained from UPLC Q-TOF-MS/MS analysis were processed with Progenesis QI v2.1 (Nonlinear Dynamics, Waters Corporation, UK) with the following conditions: all runs, automatic limits, centroid data, resolution full-width at half maximum (FWHM) of 30.000, ionization negative ion mode, deprotonated molecule [M - H]-. The identification of phenolics compounds was performed by searching for polyphenols with MetaScope, a fully integrated search tool that allowed the use of the customized database Polyphenols PubChem ID by using the following parameters: precursor mass error ≤5μg/g, fragment tolerance ≤10 μg/g and retention time limits 0.3-11.0 min. Target analysis was also applied for identification of the PC by comparing the run parameters of 19 phenolic standards such as the retention time, exact mass, mass error and the MS-MS spectrum, besides the other above mentioned parameters. In addition, the database Phenol Explorer was used for confirmation and classification of the phenolics identified. Only the compounds present in the three technical replicates (3/3) were tentatively identified, presenting coefficient of variation (CV) < 30%.

2.3. Microstructure and elemental composition

The analysis of PF and MPF was performed in a scanning electron microscope (SEM, Oxford Industries, England) coupled with an X-ray energy dispersive spectrometer (EDS; Oxford Industries) for structure and elemental composition, according to Andrade, Ferreira, & Gonçalves (2016).

2.4. Antioxidant activity (AA) determination - MPF

2.4.1. Sample preparation

PC was extracted from 7% (w/v) of MPF in ethanol: water solution (50:50, v/v), and incubated in a shaker (TE-420, Tecnal, Brazil) at 200 rpm for 10 h at 30 °C. After centrifugation at 20 °C and 2000 *x*g for 15 min, the supernatant was filtered and applied to prepare water solution 10% (v/v) (Mendes et al., 2019a; Santos & Gonçalves, 2016). This solution was prepared and applied to all antioxidant activity tests on the same day.

2.4.1.1. Total PC by Folin-Ciocalteu method

The total PC content was determined in triplicate using the Folin-Ciocalteu method (Singleton, Orthofer, and Lamuela-Raventós 1999). The analyses were made on a Victor Nivo Microplate Reader (Perkin Elmer, German). The results were expressed in mg of galic acid equivalent per gram of sample (mg GAE.g^{-1}).

2.4.1.2. ABTS method

The AA by the ABTS radical was determined in triplicate as described by Re et al. (1999). The analyses were made on a Victor Nivo Microplate Reader (Perkin Elmer, German). The results were expressed in mmol of Trolox equivalent per gram of sample (mmol TE.g^{-1}).

2.4.1.3. FRAP method

The AA by the reduction of iron (FRAP) was determined in triplicate as described by Benzie & Strain (1996). The analyses were made on a Victor Nivo Microplate Reader (Perkin Elmer, German). The results were expressed in mmol of reduced iron per gram of sample (mmol Fe^{2+}.g^{-1}).

2.4.1.4. ORAC method

This assay was determined in triplicate as described by Zulueta et al. (2009), and performed on a Victor Nivo Microplate Reader (Perkin Elmer, German) with the results expressed in mmol of Trolox equivalent per gram of sample (mmol TE.g^{-1}).

2.5. Statistical analysis

The statistical analysis used in this study was one-way ANOVA (Tukey, $P < 0.05$) with the aid

XLSTAT software (Addinsoft, version 2018.2.50452).

3. Results and discussion

3.1. Physicochemical characterization – PF

The parameters obtained from the characterization of the powder are shown in Table 2. Bulk and tapped density values were lower (0.30 and 0.43 respectively) than those reported by Mendes et al. (2019b) for pepper, *C. baccatum*. These results are relevant for packaging and material handling purposes in the food industry (Suriya et al., 2017) and are useful in formulating complementary foods (Awolu 2017). As can be seen, cohesiveness in terms of H_R was intermediate according to Jinapong et al. (2008) and the flowability expressed as C_I was fair, as described by Swaminathan et al. (2015).

Table 2. Physicochemical characterization of pepper flour (PF).

	PF
Bulk Density (δ_B) (g/mL)	0.30±0.03
Tapped Density (δ_T) (g/mL)	0.43±0.07
Cohesiveness (HR)	1.42±0.07
Flowability (CI)	29.63±3.21
a_w	0.53±0.00
Hygroscopicity (g.a.w/100g)	12.39±0.12
Solubility (%)	52.20±0.54
L*	31.76±1.25
a*	11.33±0.19
b*	21.00±0.81

g.a.w: g absorbed water. Values are means ± standard deviation of triplicate analysis.

PF a_w was less than 0.6 indicating that it is microbiologically stable, with no possibility of microbial growth, unless deterioration occurs due to chemical reactions (Álvarez-Henao et al. 2018). The sample presented hygroscopicity value of 12.39% (dry basis). According to Tontul & Topuz (2017), powders with less than 20% hygroscopicity are considered good products, since high hygroscopicity means a greater tendency to absorb water from the environment. Solubility was higher than *C. baccatum* peppers (43%) (Mendes et al., 2019b) with potential to be a functional ingredient, considering that they must have good solubility to be useful and functional (Nunes et al., 2015; Vardanega et al., 2019). The color measurements, CIELAB coordinates (L^*, a^* and b^*) for pepper flour were 31.76, 11.33 and 21.00, respectively (Table 2), indicating dark, red and yellow powder, associated with the presence of pigments carotenoids (Nath et al. 2018).

The fit of the mathematical models of GAB, Halsey, Henderson and Oswin to the experimental data of PF are presented in Table 3. All models showed R^2 values above 0.99 for PF, except Henderson's model, which presented R^2 of 0.98. However, in order to evaluate the fit of the proposed models, besides the highest value of R^2, the lowest error value was considered (E and RMSE). As a result, the GAB equation was the most suitable for the sample analyzed. In contrast, the model proposed by Henderson was the one that presented the least adjustment of experimental data. Similar results were found for peppers *C. annuum* (Seid and Hensel 2012) and *C. baccatum* (Mendes et al., 2019a).

Table 3. Parameters of the proposed models for moisture adsorption isotherms for pepper flour (C*apsicum pubescens*).

Models	Parameters	Adsorption
GAB	X_m	4.473
	C	17.98
	K	0.9596
	R^2	0.9969
	%E	2.558
	%RMSE	35.727
Halsey	A	21.88
	B	1.539
	R^2	0.9925
	%E	3.652
	%RMSE	49.137
Henderson	A	0.2245
	B	0.6638
	R^2	0.9883
	%E	5.573
	%RMSE	82.286
Oswin	A	8.396
	B	0.6122
	R^2	0.9938
	%E	3.303
	%RMSE	44.438

X_m, is the water hydration limit (monolayer value, % dry basis); C, K, A, B are constants of the models; R^2 is the coefficient of determinant; %E is the mean relative percentage deviation and %RMSE is the root mean square.

The GAB model has been reported to be useful in describing the sorption isotherm of food products such as pepper varieties due to its adaptability and better fit over a wide range of a_w (Téllez-Pérez et al., 2014; Vega-Gálvez et al. 2007). Through this model, it is possible to evaluate the moisture content of the monolayer (X_m) of the food, allowing a physical understanding of the adsorption theory. According to Fonteles et al. (2016), X_m indicates the amount of water that is

strongly adsorbed in specific places of the food, considered as the optimal value to ensure its stability. The X_m value of 4.473 g H_2O/g dry basis (Table 3) is within the reported values for *C. baccatum* peppers (Mendes et al., 2019a) and pepper dried products (Pérez-Alonso et al., 2009). The X_m value for PF has low moisture content in the monolayer, thus indicating good stability, except for lipid oxidation that may occur at the storage temperature (25 °C) studied here. As a result of this, it suggests that the packaging should impervious to air and light to avoid and minimize these possible oxidative processes (Fonteles et al. 2016; D. M. Oliveira, Clemente, and da Costa 2014).

Parameter *C* of the GAB model indicates the sorption energy of monolayer water molecules adsorbed at the primary binding sites. The highest *C* value obtained indicates the highest water binding strength of the monolayer (Téllez-Pérez et al. 2014). *K* is the parameter related to interactions between molecules of liquid water and molecules adsorbed in the multilayer (Kaderides and Goula 2017). Timmermann et al. (2001) state that *K* values greater than 1.0 would be physically inadequate indicating infinite adsorption. In this study the value of this constant was less than 1 (Table 3). In addition, the values '*C*' and '*K*' are in accordance with the range determined by Lewicki (1997) ($0.24<K\leq1$ and $5.67\leq C\leq\infty$) to have a good description of the isotherm, so that the calculated values do not differ by ± 15.5% of the actual capacity of the monolayer.

According to the classification of Brunauer et al. (1938), these values correspond to the sigmoid form which is known as type II isotherm, illustrated in Fig. 1 and is characteristic of carbohydrates (Chisté et al. 2012). Fonteles et al. (2016) explained that this isotherm represents the existence of multilayers on the inner surface of the material. Sample data showed a relatively slow increase in adsorption capacity at low a_w and a sharp increase at higher a_w, as reported for *Capsicum* peppers (Mendes et al., 2019a; Vega-Gálvez et al., 2007) and pepper dried products (Pérez-Alonso et al., 2009).

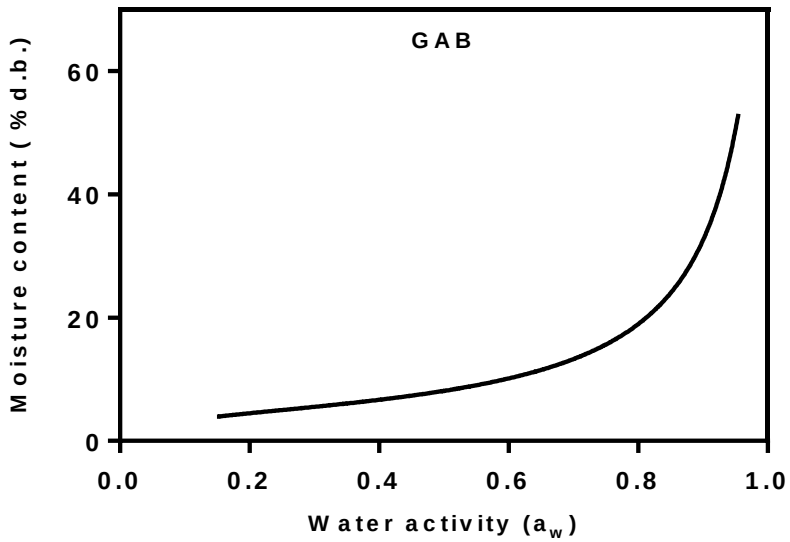


Figure 1. Adsorption isotherm for pepper flour (*Capsicum pubescens*) - GAB.

3.2. Metabolomic profile of PC

Functional properties of PC from *Capsicum* peppers were extensively reported mainly by *C. annuum*, but identified PC in this fruits applying metabolomic is a promissing field of study and recent reported (Mendes et al. 2019a; Mendes & Gonçalves, 2020). The complete list of tentatively identified PC with the respective parameters found is presented in Table 4. A number of 61 PC were tentatively identified from *C. pubescens* pepper, among them, 48 and 56 compounds were identified in 50% aqueous butanol and 50% aqueous ethanol solutions, respectively. Recently, 42 PC were identified in another specie (*C. baccatum)*, applying the same methodology used in this work (Mendes et al., 2019a). According to literature data, the phenolic profiling of *C. annuum* species using liquid chromatography coupled with mass spectrometry (LC-MS) ranged from 18 to 49 identified compounds (Jeong et al. 2011; Lucci, Saurina, and Núñez 2017; Mokhtar et al. 2015; Mudric et al. 2017).

Phenolic acids were the major class showing the highest number of PC identified in the both extracts, but in terms of relatively ion abundance, was the main class found only in butanol (68% phenolic acids, 19% flavonoids, 12% other polyphenols and 1% lignin) while with ethanol the other polyphenols (48% of other polyphenols, 38% phenolic acids, 13% flavonoids, and 1% lignin) (Figure 2). Butanol and ethanol extracts have different profile of PC, as well as different relative abundance of identified compounds. This fact can be observed in figures 3A and 3B, analyzing the chromatographic profile in BPI (base peak intense), where there are the same peaks but with

different intensities such as compound feruloylquinic acid RT: 0.40, *m/z* 367.1051, the most abundant compound in butanolic extract but not in the ethanolic extract. In figure 3C and 3D, are showed the most abundant compounds in each extract were extracted by XIC (extracted ion chromatogram). And, once again, it can be said that different organic solvents allow extracting a greater or lesser variety of PC, as well as extracting the same compound but quantitatively in different proportion. The phenolic profile found in studies by Mudric et al. (2017) and Mendes et al. (2019a) who worked with *C. annum* and *C. baccatum* peppers, respectively, presented phenolic acids and flavonoids as an abundant class.

Interestingly, only 10 common compounds were found in the *Capsicum annuum, baccatum and pubesce*ns species of the genus *Capsicum* (Jeong et al., 2011; Mendes et al., 2019a; Mudric et al., 2017): quercetin 3-*O*-rhamnoside, apigenin 7-*O*-apiosyl-glucoside, apigenin 6-*C*-glucoside, kaempferol, naringenin, 5-caffeoylquinic acid, cinnamic acid, caffeic acid, 4-hydroxyphenylacetic acid, 4-hydroxybenzoic acid, indicating wide variation of compounds in profile of these. It is emphasized that of the five most abundant compounds, 3-feruloylquinic acid (53%) was not identified in the *Capsicum annuum* and *baccatum* species (Jeong et al., 2011; Mendes et al., 2019a; Mudric et al., 2017). This compound is associated with important biological and pharmacological effects, such as the improvement of human hypertension (Matsui et al. 2007).

Table 4. Phenolic compounds tentatively identified in pepper flour (*Capsicum pubescens*) by UPLC-MS^{E}.

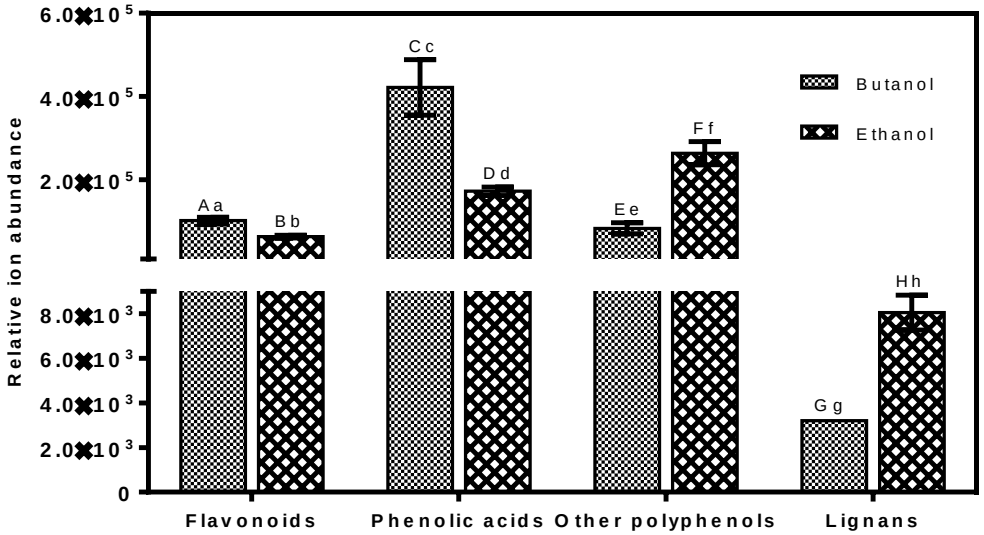
Tentative identification	*m/z*	TR_1	Formula	Score	FS_2	ME_3	SI_4	Isotope Distribution	Relative ion abundance	
									Butanol	Ethanol
FLAVONOIDS										
Quercetin 3-*O*-rhamnoside	447.0909	2.98	$C_{21}H_{20}O_{11}$	37	-	-5.42	92	100 - 18.8	6333.54	5725.22
Apigenin 7-*O*-apiosyl-glucoside	563.1416	3.21	$C_{26}H_{28}O_{14}$	36	-	1.81	84	100 - 15.8	8288.18	4445.82
Apigenin 6-*C*-glucoside	431.0976	3.25	$C_{21}H_{20}O_{10}$	38	-	-1.67	92	100 - 18.8	12180.01	5993.72
6-geranylnaringenin	407.1833	3.80	$C_{25}H_{28}O_5$	35	-	-7.56	85	100 - 12.3	4938.08	8001.71
Irilone	297.0394	3.81	$C_{16}H_{10}O_6$	36	-	-3.60	85	100 - 2.01	6368.91	5940.81
Kaempferol	285.0392	4.78	$C_{15}H_{10}O_6$	38	-	-4.26	94	100 - 12	2690.89	1819.81
Naringenin	271.0598	5.35	$C_{15}H_{12}O_5$	38	-	-5.01	94	100 - 11.5	6664.20	4774.34
Hesperetin / Homoeriodictyol	301.0707	5.47	$C_{16}H_{14}O_6$	38	-	-3.56	93	100 - 12.4	5980.82	3843.51
Hispidulin	299.0555	5.58	$C_{16}H_{12}O_6$	37	-	-1.93	89	100 - 7.94	18200.82	9413.61
3.7-dimethylquercetin / Jaceosidin	329.0659	5.92	$C_{17}H_{14}O_7$	37	-	-2.39	88	100 - 7.46	29919.95	13158.59
PHENOLIC ACIDS										
5-caffeoylquinic acid	353.0899	0.40	$C_{16}H_{18}O_9$	45	40.1	5.85	91	100 - 10.9	16347.61	nc
3-feruloylquinic acid	367.1051	0.40	$C_{17}H_{20}O_9$	40	8.0	4.46	96	100 - 14.7 - 2.79	273622.04	10959.41
Phenylacetylglycine isomer	192.0660	0.67	$C_{10}H_{11}NO_3$	38	-	-3.04	94	100 - 5.39	nc	3184.27
Cinnamic acid	147.0447	1.34	$C_9H_8O_2$	38	-	-3.07	91	100 - 0.773	3233.74	5816.27
4-hydroxymandelic acid / 3.4-dihydroxyphenylacetic acid / Vanillic acid	167.0350	1.48	$C_8H_8O_4$	39	-	0.17	94	100 - 3.84	5954.59	8150.50
Phenylacetylglycine isomer	192.0655	1.62	$C_{10}H_{11}NO_3$	37	-	-5.62	89	100	nc	4651.81
3-hydroxybenzeneacetic acid	151.0390	1.90	$C_8H_8O_3$	37	-	-6.87	94	100 - 3.76	3655.68	2039.81
3-hydroxybenzoic acid	137.0239	2.12	$C_7H_6O_3$	38	-	-3.48	96	100 - 4.57	32973.19	14996.26
Caffeic acid	179.0348	2.17	$C_9H_8O_4$	57	96.3	-1.21	90	100	465.63	2640.86
m-Coumaric acid	163.0400	2.22	$C_9H_8O_3$	57	96.2	-0.49	91	100 - 19.1	13610.39	21452.81
Isoferulic acid	193.0500	2.39	$C_{10}H_{10}O_4$	38	-	-3.12	95	100 - 7.08	1852.45	2099.46
4-hydroxyphenylacetic acid	151.0389	2.42	$C_8H_8O_3$	43	29.3	-7.60	95	100 - 4.57	9522.39	4039.80
Feruloyl glucose	355.1021	2.48	$C_{16}H_{20}O_9$	38	-	-3.67	95	100 - 15.3	8880.56	6505.57

Caffeic acid ethyl ester	207.0649	2.64	$C_{11}H_{12}O_4$	48	50.9	-6.51	95	100 - 16.7	2586.26	42958.73
4.5-dicaffeoylquinic acid 3.5-dicaffeoylquinic acid 3.4-dicaffeoylquinic acid	515.1198	2.74	$C_{25}H_{24}O_{12}$	38	-	0.51	88	100 - 19.4	nc	8338.69
4-hydroxymandelic acid 3.4-dihydroxyphenylacetic acid Vanillic acid	167.0343	2.82	$C_8H_8O_4$	38	-	-3.90	96	100 - 5.58	8675.44	7003.36
Phenylacetylglycine isomer	192.0658	3.06	$C_{10}H_{11}NO_3$	38	-	-4.31	95	100 - 7.26	nc	2709.87
Ferulic acid Isoferulic acid	193.0498	3.37	$C_{10}H_{10}O_4$	37	-	-4.22	92	100 - 3.62	31575.18	18675.63
4-hydroxybenzoic acid	137.0238	3.96	$C_7H_6O_3$	38	-	-4.62	97	100 - 5.86	7812.20	5945.25
Benzoic acid	121.0285	7.40	$C_7H_6O_2$	37	-	-7.83	92	100	1037.40	nc
OTHER POLYPHENOLS										
Bergapten Xanthotoxin	215.0329	0.40	$C_{12}H_8O_4$	39	16.8	-9.73	87	100	2842.14	3585.42
Pyrogallol isomer	125.0243	0.47	$C_6H_6O_3$	39	-	-1.01	94	100 - 13 - 0.552	2208.57	18757.89
Pyrogallol isomer	125.0243	0.85	$C_6H_6O_3$	39	-	-1.94	99	100 - 6.15 - 0.312	10123.03	94828.09
Pyrogallol isomer	125.0243	1.37	$C_6H_6O_3$	39	-	-1.10	98	100 - 5.28	7309.78	62498.19
3-methoxyacetophenone 4-vinylguaiacol isomer	149.0607	2.1903	$C_9H_{10}O_2$	40	-	-0.45	98	100 - 8.5	nc	2015.44
Phenol	93.0339	2.21	C_6H_6O	37	-	-6.90	93	100	1140.34	466.41
Pyrogallol isomer	125.0247	2.22	$C_6H_6O_3$	39	-	2.16	95	100 - 2.55	82.04	14205.07
Mellein Ferulaldehyde	177.0548	2.40	$C_{10}H_{10}O_3$	39	-	-5.39	99	100 - 10.7 - 0.308	406.39	33875.33
Esculetin	177.0186	2.46	$C_9H_6O_4$	37	-	-4.15	90	100	4564.41	2781.37
4-hydroxybenzaldehyde	121.0287	2.71	$C_7H_6O_2$	38	-	-6.75	98	100 - 9.16	45921.64	19922.67
Pyrogallol isomer	125.0235	2.98	$C_6H_6O_3$	37	-	-6.95	93	100	nc	1316.75
p-anisaldehyde	135.0442	3.03	$C_8H_8O_2$	37	-	-7.00	91	100	1797.33	nc
4-vinylphenol isomer	119.0492	3.10	C_8H_8O	37	-	-8.55	96	100 - 4.67	nc	1565.12
Coumarin	145.0288	3.85	$C_9H_6O_2$	38	-	-4.82	93	100 - 2.81	6540.35	5183.88
4-ethylguaiacol	151.0758	4.60	$C_9H_{12}O_2$	37	-	-4.13	90	100	nc	2428.19
LIGNANS										
7-hydroxyenterolactone	313.1103	0.42	$C_{18}H_{18}O_5$	35	-	6.93	81	100	nc	3475.67
Schisandrol B	415.1723	1.01	$C_{23}H_{28}O_7$	35	-	-9.43	86	100 - 11.9	nc	4580.10

Isolariciresinol Lariciresinol	359.1479	3.17	$C_{20}H_{24}O_6$	37	-	-5.99	91	100 - 14.3	3201.94	nc

Relative ion abundance adjusted for 0.1g of pepper flour. [1]Retention time; [2]Fragmentation Score; [3]Mass Error (ppm); [4]Similarity Isotopic. nc: the relative ion abundance with CV (%) > 30% was not considered. Different letters in the same line differ significantly, using the Tukey test (P <0.05).

A



B

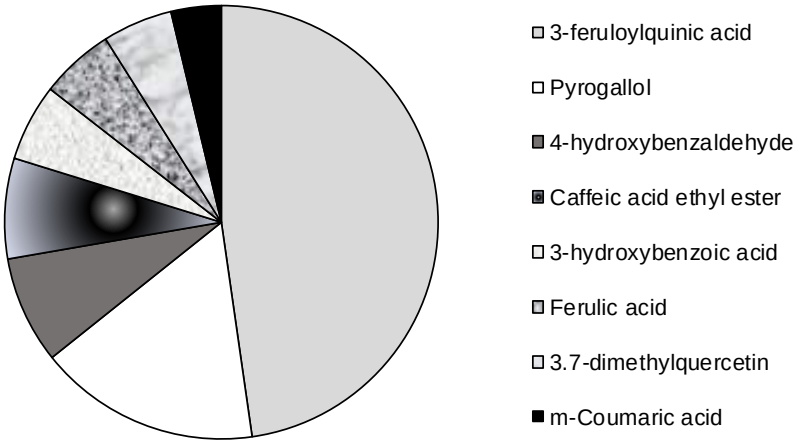


Figure 2. Phenolic compounds in *Capsicum pubescens:* A - normalized abundance by UPLC-ESI-Q-TOF-MS by classes of flavonoids, phenolic acids, other polyphenols and lignans; B - Most abundant phenolic compounds. Smaller letters indicate difference between extractors in the same class; larger letters indicate difference between classes of same solvent.

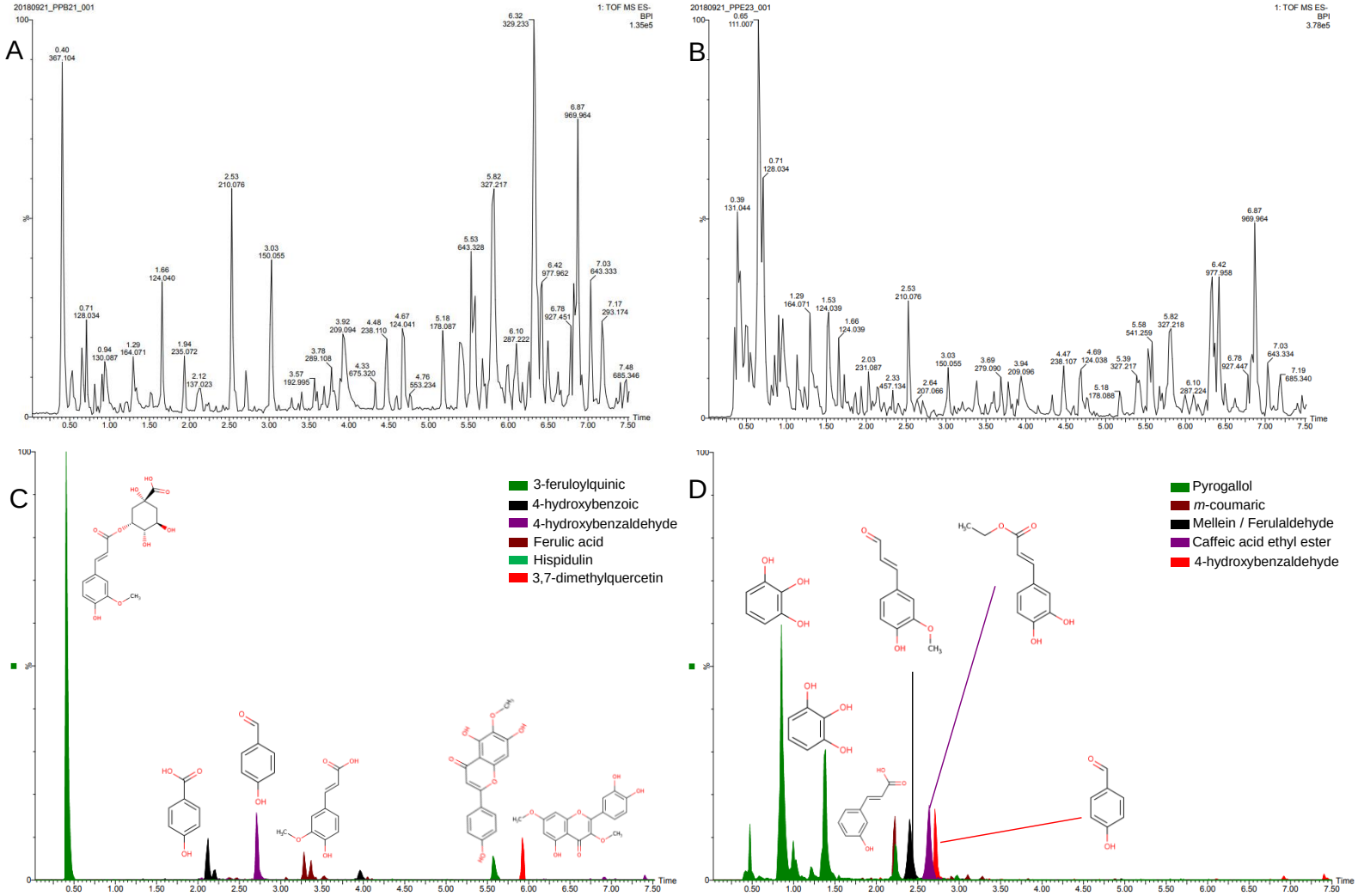


Figure 3. UPLC-ESI-Q-TOF-MS chromatograms of pepper (*Capsicum pubescens)*: BPI (base peak intense) of butanol extract (A) and ethanol extract (B); XIC (extracted ion chromatogram) of butanol extract (C) and ethanol extract (D).

3.3. Microencapsulated flour pepper (MPF)

SEM micrographs of PF and MPF are shown in Figure 4 with different magnifications. The PF sample showed rough surface composed mainly of polysaccharides (Roman-Gutierrez, Guilbert, and Cuq 2002; Romdhane et al. 2017), presenting mainly potassium and magnesium peaks (Fig. 4a). This result coincides with the work of Mendes et al. (2019a) where potassium was the most abundant mineral in *Capsicum baccatum* peppers.

Regarding MPF, there was no variation of the main minerals and the results showed the presence of spherical microcapsules, presenting smooth surface with different irregularities and sizes and absence of fissures (Fig. 4b), which indicates better protection of the active material, as well as good matrix encapsulation barrier (Busch et al., 2017), greater stability (Díaz et al. 2019), and is more favorable in terms of higher dispersibility and rehydration of powders (Guadarrama-Lezama et al. 2012). Similar behavior was reported by Rezende et al. (2018) for the extract of the pulp and residue of acerola (*Malphigia emarginata* DC) encapsulated by spray drying, which presented spherical conformation, irregular shapes and few fissures, although some had a smooth surface.

Also, MPF showed good antioxidant activity (Table 5). It can be observed, still in Table 5, that there was no difference between the different extractors (ethanol and water), indicating that they can be applied as nutraceuticals when compared to other functional products (Batista et al. 2018).

Table 5. Total phenolics contents and antioxidant activities of microencapsulated PF (MPF).

	ETHANOL 50%	**H_2O**
Total phenolics contents (mg GAE/g)	105.42 ± 12.8 [a]	91.36 ± 18.2 [a]
ABTS (mM trolox/g)	2.16 ± 0.08 [a]	2.20 ± 0.06 [a]
FRAP (mmol Fe/g)	4.45 ± 0.52 [a]	4.22 ± 0.46 [a]
ORAC (mmol TE/g)	2.63 ±. 0.03 [a]	2.57 ± 0.01 [a]

Values are means ± standard deviation of triplicate analysis. Different letters in the same line differ significantly, using the Tukey test ($P < 0.05$).

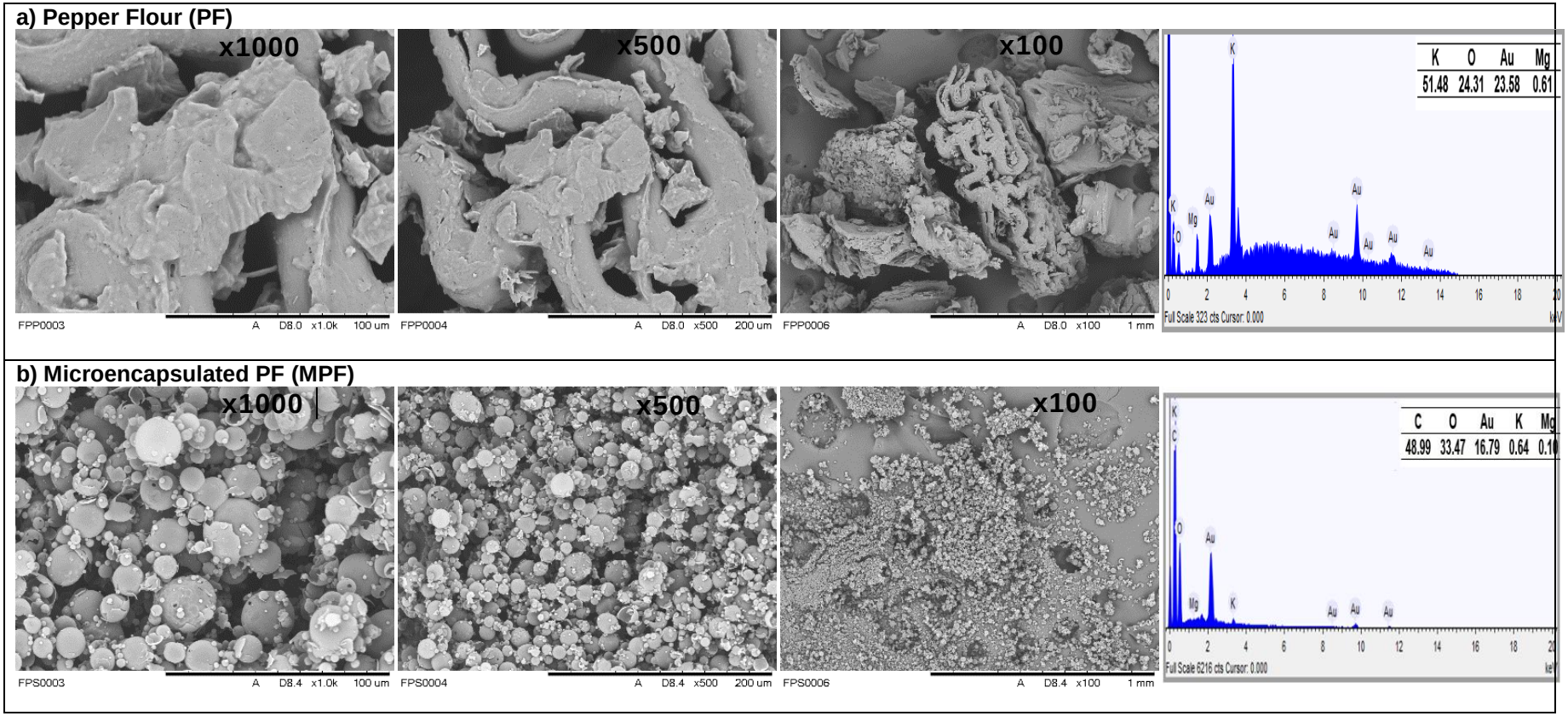


Figure 4. Scanning electron microscopy (SEM) and X-ray microanalysis spectroscopy characteristic of EDS with discrimination table of analyzed elements of pepper flour (*Capsicum pubescens*).

4. Conclusions

The results presented in this study indicated the PF with good physical properties, in terms of bulk and tapped density, water activity, hygroscopicity and solubility. The GAB equation showed the best fit and hydration limits indicated good stability. 61 phenolic compounds were identified, divided into 4 classes: flavonoids (12), phenolic acids (27), other polyphenols (18) and lignans (4), and 3-feruloylquinic acid was the most abundant compound. Additionally, micrographs of PF showed a rough surface composed mainly of polysaccharides, while MPF exhibited spherical particles with a smooth surface, some irregularities as well as good antioxidant capacity. All the studied samples presented potential as food ingredients for functional and technological uses. Parallel studies considering the standardization of these food ingredients in terms of color, pungency, taste and biological activity are needed to expand the applications of these compounds in the market.

Acknowledgments

This work was supported by the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (grant no. E-26/202.086/2016, FAPERJ, Brazil).

5. CAPÍTULO IV- FLOUR FROM FRUITS AND VEGETABLES WASTE WITH ADDITION OF A SOUTH-AMERICAN PEPPER (*CAPSICUM BACCATUM*) PROPOSED AS FOOD INGREDIENT

Nathânia de Sá Mendes, Leonardo Cristian Favre, Guido Rolandelli, Cristina dos Santos Ferreira, Édira Castello Branco de Andrade Gonçalves, María del Pilar Buera
Artigo publicado na revista "International Journal of Food Science and Technology". (2019). https://doi.org/10.1111/ijfs.14358

Abstract

The objective of this work was to evaluate the physicochemical properties of previously characterized flours obtained by milling the solid waste from the manufacture of an isotonic drink produced with various fruits and vegetables (FVR) to which powered pepper fruits (PF) were added. Aqueous extracts were also prepared and encapsulated for protecting their functional compounds and improving their solubility. The encapsulation yields of the spray-drying processes were 90% and 64% for PF and FVR-PF, respectively. The addition of PF to FVR improved antioxidant capacity, stability and appearance, providing reddish color. FT-IR spectra reflected the addition of PF by changes in the absorbances at wave-numbers typical of carotenoids, acylglycerols, chlorophylls and those related to antioxidant capacity. The encapsulated extracts could be applied when solubility is needed in hydrophilic media. The obtained flours with PF addition are suitably cheap, stable functional food ingredients for industrial uses, such as breading or seasoning ingredients.
Keywords: Pepper; beverage waste; encapsulation; isotherms; powder stability; antioxidant activity

1. Introduction

Agroindustrial activity generates a dramatic amount of waste and their disposal (landfilling, incineration) was defined as the worst environmental option. Agri-food waste prevention is a better option and its utilization to yield value-added products is considered an interesting waste minimization strategy (Galanakis et al., 2018).

Recently, the residues from an isotonic beverage composed of fruits and vegetables (FVR), processed as flour, have been successfully used in the formulation of cereal bars and biscuits to increase microbiological stability, water retention capacity, mineral and fiber content (Ferreira et al. 2015; Neacsu et al. 2015). These novel ingredients demonstrated the ability to overcome constipation and can be used for the development of functional foods (Gonçalves et al. 2018; Roberta, Mariana, and Édira 2014).

Pepper fruits (from *Capsicum* genus), commercialized worldwide, may complement the flour from fruits and vegetables, providing flavor and color characteristics, improved nutritional value and antioxidant properties (Palma et al. 2015; Perla et al. 2016)). A typical South-American pepper (*Capsicum baccatum*) is the most consumed in Brazil, and highly relevant in regional gastronomy as flavoring and colorant agent. Besides antioxidant properties, *C. baccatum* extracts display anti-inflammatory activities, may combat antibiotic-resistant bacteria, prevent bacterial adhesion and biofilm formation (von Borowski et al., 2019).

Although FVR composition, antioxidant capacity, colorimetric and rheological properties related to the film forming capacity was reported (Brito et al., 2019), the characterization of the product obtained by its combination with pepper has not been yet performed. Thus, the objective of this work was to evaluate the applicability of combined flours from fruits and vegetables waste (FVR) with pepper flour (PF), or of their spray-dried aqueous extracts, for the development of functional food ingredients.

2. Materials and methods

2.1. Preparation of samples

2.1.1. Pepper Flour (PF) was obtained from fully ripe pepper fruits "dedo-de-moça" (*Capsicum baccatum L. var. Pendulum*) purchased at Hortifrutti, a local market in Rio de Janeiro, Brazil, in May 2016. The peppers were authenticated by a Food Agricultural Engineers staff member, and processed according to the methodology applied by Ferreira et al. (2015), consisting in convective drying at 75 ºC for 5 hours, then at 90 ºC for 1 hour, milled, homogenized and stored at 25 ºC. One

lot of 1000g fresh pepper was processed, from which 141g of PF were obtained.

2.1.2. Fruits aand vegetables flour (FVR) was prepared with residues from the manufacture of an isotonic beverage, as previously described by Ferreira et al. (2015). The beverage has been formulated with a stablished compostion and proposed as a potential functional product applied in the improvement of gastrointestinal disorders (Andrade et al., 2014).

The beverage was composed of the following species: 11% of sweet orange (*Citrus sinensis*), 19% of passion fruit (*Passiflora edulis*), 22% of watermelon (*Citrullus lanatus*), 8.5% of cucumber (*Cucumis sativus*) and courgette (*Cucurbita pepo*), 2% of rocket (*Lactuca sativa*), spinach (*Spinacea oleracea*) and taro (*Colocasia esculenta*), entirely processed for the drink preparation, including non-convencional edible parts such as pulp, stalks, peels, seeds and stems (Ferreira et al., 2015). The remaining solid residues were processed as flour and previously characterized, containing dietary fiber (48%, 80% of which was insoluble), carbohydrates (26%), proteins (9.5%) and lipids (5%). Analysis of different lots in different years allows standardization for assuring the composition constancy of the waste (Brito et al., 2019).

2.1.3. Mix of PF and FVR (MIX): PF and FVR flours were mixed in the proportion of 1:1 (w/w) and homogenized manually in a mortar, using liquid nitrogen to avoid the material stickiness due to exposure to ambient humidity.

2.1.4. Microencapsulated extracts: PF or FVR were suspended in aqueous solutions of 30% (w/w) maltodextrin (MD, DE 15) from Saporiti S.A. (Buenos Aires, Argentina) to obtain a final concentration of 6.4%. For the microencapsulated mix (MPVR), PF and FVR were added in order to obtain 3.2% of each one. The suspensions were homogenized at 500rpm for 10min with Ultra Turrax T18 (IKA, Konigswinter, Germany) and 15,000 rpm for 2min. Subsequently the systems were submitted to the Ultrasonic Processor UP 100H (Ultrasound Technology) for 5min. After centrifugation at 10,000 rpm for 15min at 10 °C, the supernatant was collected and filtered twice in a Buchner system using paper filters (Whatman1.20-µ pore). The filtrate was spray dried (in a Buchi B290, Flawil, Switzerland drier) at a flow rate 8 mL/min, air pressure 3.2 kPa, nozzle diameter 1.5 mm, inlet temperature 174 °C and outlet temperature 95 °C. The product yields of samples after spray drying were calculated according to the following formula:

$$\%\,Yield = \frac{Mass\ of\ powder\ obtained\ after\ the\ spray-drying\ process}{Mass\ of\ initial\ soluble\ solids\ (form\ flour + maltodextrin)} \times 100$$

2.2. Physicochemical characterization

2.2.1. Bulk density

Bulk density (g/mL) was determined according to Santhalakshmy et al. (2015) by measuring the volume of 1.00 g of powder gently introduced into a 10.00 mL graduated cylinder, at 25 °C.

2.2.2. Water activity (a_w)

a_w values were measured using an electronic a_w-meter Aqualab Series 3 (Decagon Devices, Pullman, WA, USA), based ~~in~~on the dew point determination by water condensation on a mirror as temperature decreased.

2.2.3. Hygroscopicity

Hygroscopicity evaluation was performed as described by Santhalakshmy et al. (2015) with modifications. One gram of the sample was placed in a container at 25 °C with a saturated NaCl solution (75% RH). Samples were weighed every 30 min for 285 min and during 2 days until constant weight. Hygroscopicity was expressed in grams of water adsorbed per 100 grams of dry matter (g/100 g d.b.).

2.2.4. Solubility

Solubility was determined according to the procedure described by Cano-Chauca et al. (2005)with modifications. Briefly, 1g of dry powder was carefully added to 50 mL of distilled water into a plastic tube, and stirred at high velocity for 5min. The solution was centrifuged at 3000g during 5 min. An aliquot of 20 mL of the supernatant was transferred to pre-weighed Petri dishes and immediately oven-dried at 105 ºC for 5h. Then the solubility (%) was calculated by weight difference.

2.2.5. Colorimetric determinations

A Minolta CM-508-d tristimulus photocolorimeter (Minolta Corp., Ramsey, NJ, USA), with integrating sphere was employed to analyze the color attributes of the samples. Transparent recipients of 2 cm diameter and 0.5 cm height were employed. The chromatic coordinates in the CIELAB space were obtained, which represent the color attributes: L^* *(*lightness, representing the psychophysical quality of clarity with values 0 for black up to 100 for white), a^*(red-green axis) and b^*(yellow-blue axis). The color coordinates were calculated for the CIE D65 illuminant and 2° observer angle.

2.2.6. Water adsorption isotherms

The isopiestic method was employed for obtaining adsorption isotherms, by exposing the samples at saturated salt solutions at water activities (a_w) values 0.22, 0.43, 0.53, 0.75 and 0.84 at 25 ± 1 °C (Greenspan,(1977). The adsorption isotherms were adjusted with BET, GAB and GDW (D'Arcy and Watt 1970) models, using GraphPad Prism 6 software. The coefficient of determination (R^2), relative mean deviation (%E), equation (1) and mean square error (RMS), equation (2), were calculated to verify the degree of fit of the studied models (Téllez-Pérez et al. 2014; Vega-Gálvez et al. 2007).

$$\%E = \frac{1}{N}\sum_{i=1}^{N}\frac{|m_i - m_{pi}|}{m_i} \quad (1) \qquad \%RMS = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\frac{m_i - m_{pi}}{m_i}\right)^2} \quad (2)$$

where m_i and m_{pi} are the actual and predicted moisture content values, respectively, and N is the number of observations.

2.2.7. FT-IR spectroscopy

The analysis of compositional aspects and component interactions in the samples was performed by FT-IR spectra obtained with a Spectrum 400 spectrometer (Perkin Elmer, Inc., Shelton, CT, USA) with an attenuated total reflection (ATR) device, by averaging 96 scans over the spectral range of 600 to 4000 cm^{-1}. Data analysis of each sample was performed with OriginPro 2017 program (OriginLab, Northampton, MA, U.S.A.). The average of triplicates for each system was reported. Baseline was corrected and the spectra were normalized.

2.3. Antioxidant activity

The extracts were obtained from 5% of dry solids in water or in 1:1 ethanol: water solution, vortexed for 30 min and centrifuged during 10 minutes at 10.000 rpm. The supernatant was recovered for analysis of total phenolic compounds and antioxidant activity.

2.3.1. Total polyphenolscontents by Folin-Ciocalteu method

Total phenolic contents (TPC) of the extracts were determined by the Folin–Ciocalteu method, with some modifications (Busch et al., 2017). Briefly, 125mL of a solution of Na_2CO_3 (20% w/w), 800mL of distilled water and 50 µL of sample were added to 125 µL of the Folin-Ciocalteau reagent (Biopack®, Zarate, Buenos Aires, Argentina). The absorbance at 765 nm was measured in a UV-Vis spectrophotometer (JASCO Inc., Maryland, USA) after 30 min at 25 ºC in the dark. Total polyphenols (TP) were expressed as mg gallic acid per 100 g of dry matter (mg GAE/100 g of d.b.), through a calibration curve.

2.3.2. Free radical scavenging by DPPH•

The radical scavenging activity (RSA) was calculated as a percentage of the free radical DPPH• (2,2-diphenyl-1-picryl-hydrazyl) discoloration in 30 minutes, using Equation (3):

$$\%RSA = \frac{(A_{DPPH\bullet} - A_{EXT})}{A_{DPPH\bullet}} x\,100 \qquad (3)$$

where $A_{DPPH\bullet}$ is the absorbance value of the DPPH• test solution and A_{EXT} is the difference between the absorbance values of the test solution with the extract and of its blank at 30 min (Busch et al., 2017).

3. Results and discussion

3.1. Physicochemical characterization

The parameters color coordinates, bulk density, hygroscopicity and solubility, as so as the a_w values of the samples, are shown in Table 1.

Table 1. Physicochemical characterization of pepper flour (PF), fruits and vegetables flour (FVR),mix of PF and FVR (MIX) and dry powders obtained by spray drying: PF Microcapsules (MPF); PF and FVR Microcapsules (MPVR).

	PF	FVR	MIX	MPF	MPVR
Bulk Density (g/mL)	0.54 ± 0.04	0.42 ± 0.01	0.55 ± 0.02	0.49 ± 0.01	0.50 ± 0.01
a_w	0.37 ± 0.02	0.34 ± 0.04	0.39 ± 0.02	0.09 ± 0.01	0.09 ± 0.01
Hygroscopicity (g.a.w/100 g)	13.0 ± 0.1	16 ± 2	14.8 ± 0.3	13.3 ± 0.8	15.72 ± 0.01
Solubility (%)	43 ± 2	42 ± 1	38.01 ± 0.03	99 ± 1	100.00 ± 0.01
L*	52.8± 0.5	55.8± 0.3	50.7± 0.4	84.7± 0.6	92.1± 0.7
a*	21.4 ± 0.2	2.6± 0.1	13.7± 0.2	16.1± 0.1	7.3± 0.2
b*	37.8 ± 0.2	20.0± 0.3	31.9± 0.3	18.1± 0.3	14.9± 0.1

g.a.w: g of absorbed water. All results are the means ± SD (n = 3).

All the samples were of intermediate lightness, since L^* values (representing luminosity) were close to 50. The visual appearance of FVR was of a greenish-brown coloration, and reflected in the color coordinates, since the a^* value was positive but close to 0 (slightly in the red region) and b^* was >0, well in the yellow zone. The PF sample was visually reddish-yellow, with higher a^* and b^* values. As a consequence, the color coordinates of MIX had intermediate chroma values, providing

a reddish-brown color, with positive and intermediate *a** and *b** values. In the spray-dried powders the visual appearance was governed by the presence of maltodextrin, the samples were almost achromatic, with very high luminosity (*L** value close to 85), being MPF slightly pink.

The encapsulation efficiencies for PF and MIX were 90% and 64% w/w, respectively. The different yields can be related to the nature of the raw material, since the spray-drying conditions were maintained as a constant (Tontul and Topuz, 2017).

PF and MIX powders presented higher bulk density than FVR. As higher is the bulk density, less air is occluded within the powder particles. Considering that the heavier material can be more easily accommodated in the spaces between particles (Santhalakshmy et al. (2015), there is less possibility of product oxidation and thus storage stability is increased due to less contact with atmospheric oxygen. High bulk density is also favorable for transportation and packaging (Tontul and Topuz 2017). Consequently, the addition of PF to FVR potentially favors the functional components stability. No differences in bulk density were observed among the microencapsulated samples, since it was predominantly governed by the maltodextrin matrix.

a_w and hygroscopicity play important roles for storage stability, while solubility is related to the powders reconstitution (Rezende et al.,(2018). The a_w of the samples were between 0.09 and 0.3, indicating stability against chemical or enzymatic reactions. Spray-drying with maltodextrin as wall materials resulted in the lowest a_w values, important for packaging specifications. The samples presented hygroscopicity values from 13.26 to 16.63% (d.b.), which are considered adequate, since values lower than 20% indicate a low tendency to absorb water (Tontul and Topuz 2017). In agreement with other researchers, encapsulation by spray-drying with maltodextrin as wall material, which decreased degradation of bioactive compounds (Busch et al., 2017; Rezende et al., 2018), increased the solubility and water absorption of the powders in aqueous media.

3.2. Water adsorption isotherms

Water sorption isotherms at 25 °C presented sigmoidal shape, characteristic of type II isotherms (Fig. S1), indicating the existence of multilayers in the inner surface of the material (Fonteles et al. 2016).

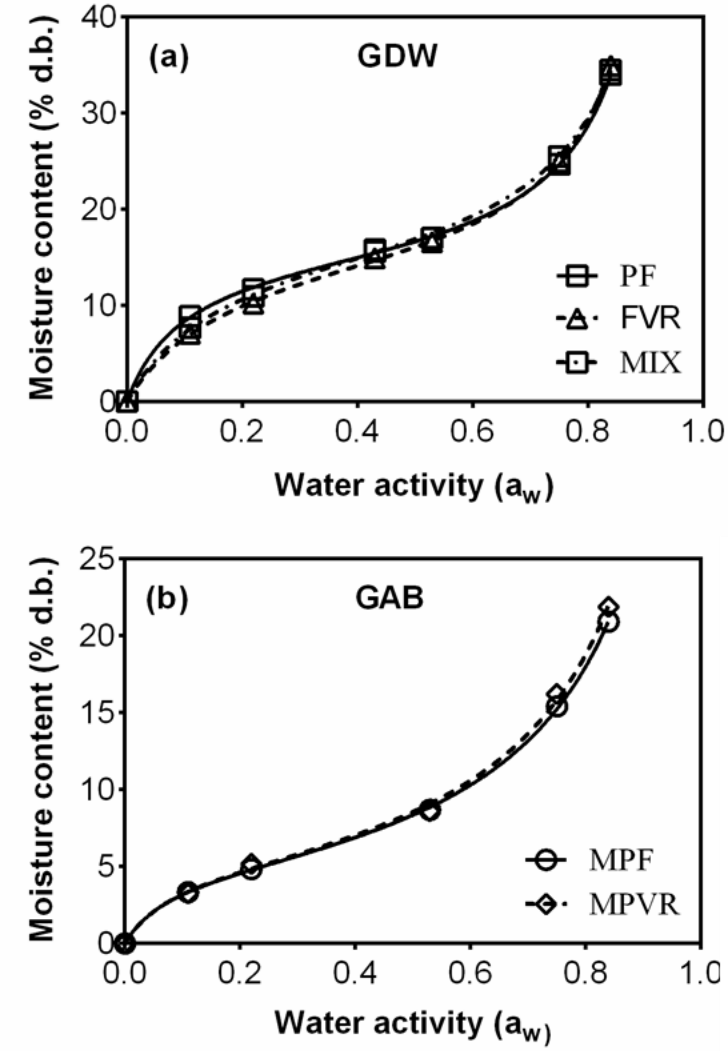


Figure S1. Adsorption isotherms at 25 °C for unencapsulated samples, with the curves obtained by applying the generalized D'Arcy and Watt –GDW- model **(a)** and for encapsulated samples, with the curves obtained by applying the GAB model **(b)**.

Symbols represent the experimental points. PF: pepper flour; FVR: Fruit and vegetable residues; MIX: PF mixed with FVR (1:1); MPF: spray-dried extract of pepper flour; MPVR: spray-dried extract of fruit and vegetable residues. The mean relative percentage deviation was below 5% and error bars lay below the symbols.

The BET, GAB and GDW models employed provided adequate description of the experimental data (Table 2), with determination coefficients (R^2) higher than 0.99, and %E less than 10% (Téllez-Pérez et al.,(2014), being the BET model limited to a_w values lower than 0.5 (Kaderides and Goula 2017).

Table 2. Parameters of the proposed models for moisture sorption isotherms at 25 °C.

Model	Constants	PF	FVR	MIX	MPF	MPVR
BET	X_m	9.359	9.175	9.516	4.674	5.118
	C	51.69	19.45	23.08	14.45	11.39
	R^2	0.997	0.998	0.998	1.000	0.998
	%E	1.886	1.498	1.768	0.306	1.909
	%RMS	3.772	2.997	3.536	0.613	3.819
GAB	X_m	10.13	9.916	10.02	4.895	4.985
	C	44.38	19.36	25.91	14.15	13.64
	K	0.839	0.859	0.849	0.918	0.926
	R^2	0.998	0.999	0.997	0.999	0.998
	%E	2.345	1.985	3.172	0.799	3.069
	%RMS	5.744	4.863	7.771	1.959	7.519
GDW	M	16.29	17.50	18.03	5.042	5.606
	K	10.06	5.798	6.300	13.49	10.97
	k	1.025	1.027	1.006	0.906	0.919
	w	0.215	0.2248	0.2337	1.102	0.985
	R^2	0.999	0.999	0.999	0.999	0.998
	%E	0.922	0.855	0.744	1.070	3.338
	%RMS	2.438	2.262	1.798	2.823	8.831

X_m, M: water hydration limit ("monolayer value", % dry basis); C, K, k, w, A, B: model parameters; R^2: determinant coefficient; %E:mean relative percentage deviation; %RMS: root mean square.

The hydration limits (X_m, or "monolayer values") obtained by the GAB equation for PF was in the range of those obtained for different pepper varieties (Seid and Hense, (2012)). As higher is the GAB constant C, greater is the water binding force at the monolayer (Téllez-Pérez et al., (2014). For the analyzed systems GAB constants values, $k < 1$ and $C > 2$ were obtained for all studied samples (Table 2), which is also typical of type II isotherms.

The GDW model, previously used to describe water sorption isotherms of different food products (Furmaniak et al., 2009), maintains all the considerations for the GAB model, but assumes that only a proportion of water molecules bound to primary adsorption centers can act as secondary centers

and *w* is lower than a value of 1. When each one of the water molecules adsorbed in primary sites is converted to a secondary sorption site, the parameter *w* equals 1 and the GDW model is reduced to GAB model. In some cases, one primary center can adsorb more than one water molecule (Furmaniak et al., 2009), and in this case $w > 1$. As shown in Table 2, *w* was quite lower than 1 for the un-encapsulated systems and quite close to 1 for the encapsulated systems. This indicates that the raw milled samples had a denser or tortuous microstructure while the spray-dried samples presented a more open and less compact structure, which allowed the full conversion of primary sites into secondary sites for water adsorption. This fact explains why the spray-dried samples were well represented by the GAB equation while GDW provided a better description for the water sorption in raw powders.

As previously observed (Furmaniak et al., 2009), M_e values of the GDW model were higher than those obtained for X_m of the GAB model. Sorption kinetic constants for the primary sites (*K*) presented values higher than one, corresponding to type II isotherms. The *K* values indicate that the FVR and MIX milled systems have slower water sorption than the FP and encapsulated extracts. The sorption kinetic constants for the secondary sites (*k*) were slightly higher than 1 for the milled systems and slightly lower than 1 for the encapsulated extracts.

3.3. Antioxidant activity assay

The total phenolic contents were higher for the aqueous extracts than for the ethanolic extracts (Table 3). The addition of PF to the FVR increased the phenolic content. Recently, 42 phenolic compounds were identified by UPLC-ESI-Q-TOF-MS/MS in PF, of which quercetin 3-O-rhamnoside, luteolin 7-O-glycoside and naringenin were the most abundant (Mendes et al., 2019). On the other hand, 88 compounds were tentatively identified in the FVR: phenolic acids (28), flavonoids (32) and other polyphenols (28), being hesperidin the main compound extracted (Gonçalves et al. 2018).

As shown in Table 3, the ethanol extract of PF showed higher free radical scavenging activity than FVR and the MIX. Non-spray-encapsulated samples, showed similar anti-radical capacity in aqueous and ethanol media. The lowest antioxidant activity of the samples was observed for the spray dried samples (MPF followed by the MPVR, Table 3), due to their dilution in the maltodextrin matrix. The antiradical capacity was higher for the samples extracted with water, in parallel with their higher total polyphenols content.

Table 3. Total phenolic contents and antioxidant activity of PF, FVR, MIX, MPF and MPVR.

Samples	Total phenolic contents (mg GAE/g extract)		DPPH• (%of DPPH• discoloration, 30')	
	H_2O	ETHANOL 50 %	H_2O	ETHANOL 50 %
PF	387 ± 2 $^{a, A}$	300 ± 22 $^{a, B}$	73.7 ± 0.5 $^{a, A}$	84 ± 1 $^{a, A}$
FVR	314 ± 15 $^{b, A}$	271 ± 13 $^{a, A}$	70 ± 2 $^{a. A}$	69 ± 3 $^{b, A}$
MIX	361 ± 13 $^{a, A}$	308 ± 1 $^{a, A}$	74 ± 6 $^{a, A}$	70 ± 2 $^{b, A}$
MPF	151 ± 9 $^{c, A}$	118 ± 13 $^{b, A}$	13 ± 3 $^{b, A}$	2.9 ± 0.5 $^{c, B}$
MPVR	159 ± 3 $^{c, A}$	82 ± 4 $^{b, B}$	19 ± 6 $^{b, A}$	3 ± 0.2 $^{c, B}$

All results are the means ± SD (n = 3). Different lower case letters in the same column indicate significant differences between samples using Tukey's multiple range test (p *<0.05*). Different uppercase letters in the same line indicate significant differences between samples using Tukey's multiple range test (p *<0.05).*

3.4. FT-IR spectroscopy

The main differences in the FT-IR spectra of PF, FVR and MIX are indicated in Figure 1 a, b and c, respectively. The normalized absorbance values of those signals are presented in Figure 2 as a function of the proportion of PF (Fig. 2a), or of total polyphenols content (Fig. 2b). The absorbance values at frequencies typical of the hydrocarbonated skeleton of carotenoids (which are those at 2922 cm^{-1} and 2853 cm^{-1} related to CH_3 and CH_2 vibrations, around 1450 cm^{-1}, due to the bending vibration of methylene $–CH_2$, and those around 1367 cm^{-1}, caused by scissoring and bending bonds of alkanes (Kushwaha et al., 2014), followed the order FVR < MIX < PF (Figure 2a). The absorbances of the band at 1743 cm^{-1}, attributed to the ester carbonyl group of acylglycerols, were in the same order (Figure 2a), due to the higher proportion of lipids in PF.

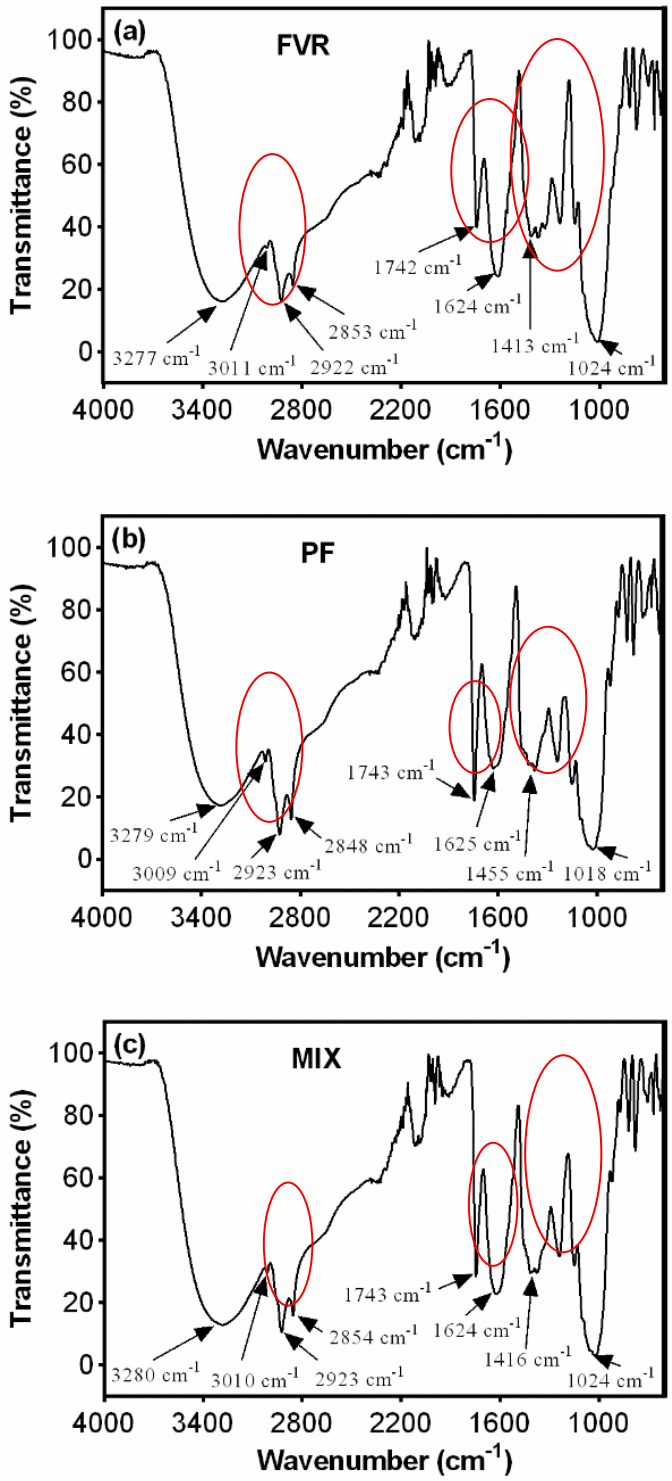


Figure 1. Fourier transform infrared (FT-IR) spectra in the range 4000-700 cm^{-1} for fruit and vegetable flour (FVR), pepper flour (PF) and MIX (PF/FVR)**.** The circles indicate the main differences of the spectral bands. Bands located in the ranges 3270-3320 cm^{-1} and 1743-1663 cm^{-1} are typical of polyphenols. Peaks in the region around 1625 cm^{-1} are attributed to chlorophylls and proteins, contributions of carotenoids are located at 1450 and 1250 cm^{-1}.

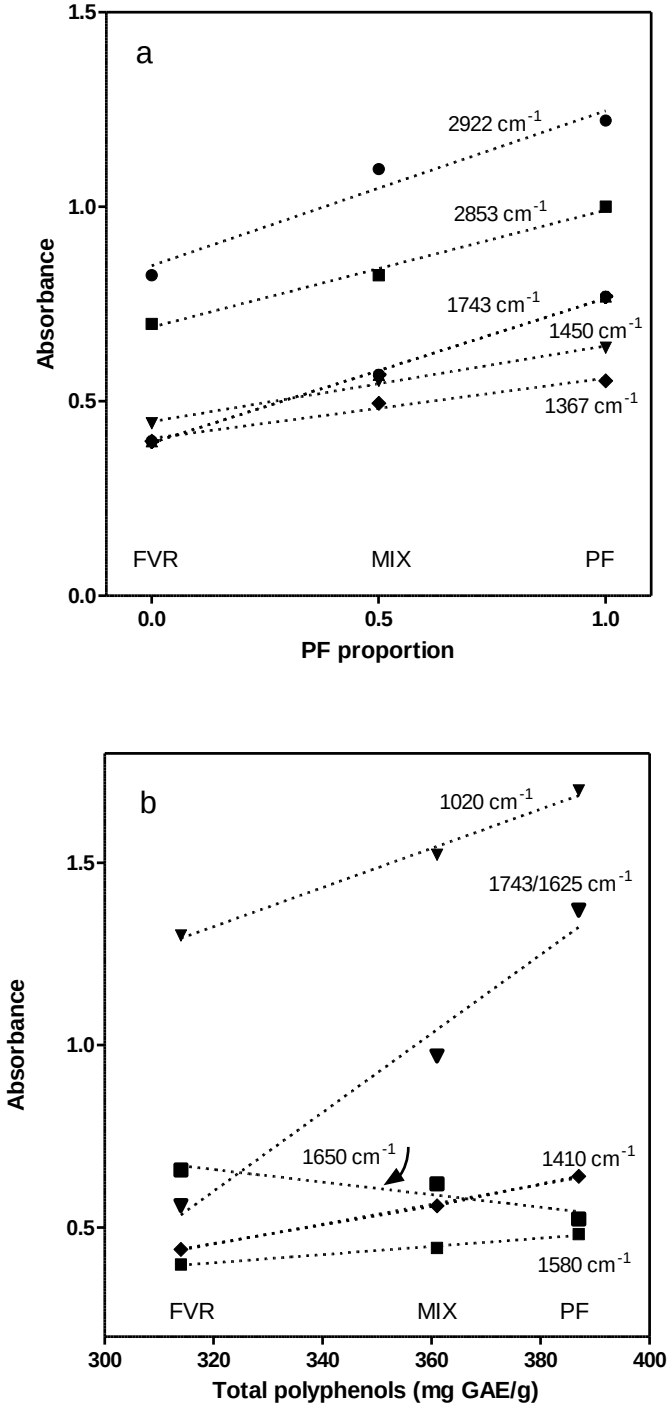


Figure 2. Absorbances in the IR regions at which differences were observed when changing the proportion pepper flour (PF) and fruit and vegetable flour (FVR).
a) Absorbance values at 1450, 1367, 2922 and 2853 cm^{-1} (attributed to carotenoids), at 1743 cm^{-1} (mainly attributed to lipids), as a function of the mass fraction of PF.
b) Absorbance values at 1020, 1410, 1580 and 1650 $cm^{-1}$and the absorbances ratio between 1743 and 1625cm^{-1}as a function of total polyphenols in the aqueous extracts.

As shown in Figure 2b, the absorbance values at 1580, 1410 and at 1020 cm^{-1}, which have been associated with the antioxidant activity of fruit extracts (Lu and Rasco, 2012), and the absorbances at 1650 cm^{-1}, caused by chlorophylls and proteins (Kushwaha et al., 2014) increased with higher total polyphenolic contents and with the PF content.

The ratio of absorbances at 1625 cm^{-1} (related to chlorophylls) and 1743 cm^{-1} (lipids + chlorophylls) was very sensitive to the compositional changes (Figure 2b).

The FT-IR bands in the ranges 3270-3320 cm^{-1}, 1629-1663 cm^{-1} and 1014-1019 cm^{-1} have been associated to polyphenol contents of tea extracts (Senthilkumar et al., 2017). However, for the analyzed samples, only the absorbances at 1020 cm^{-1} were related to increasing PF proportion and with the antioxidant capacity (Figure 2b).

No frequency displacements in the range 3470 to 3230 cm^{-1}(which corresponds to –OH interactions) were detected by PF addition, reflecting that potential molecular interactions of polyphenols with other components (Fig. 1, a-c), would not affect the antioxidant capacity, in agreement with the data shown in Table 3.

4. Conclusions

Dried fruits and vegetables by-products combined with pepper flour represent an interesting alternative for the production of functional ingredients. The addition of pepper flour to the fruits and vegetables flour increased the red coloration, modified the bulk density, improving its stability, and functional properties, also increasing polyphenols content and antioxidant capacity. The absorbance of selected FT-IR bands, mainly those related to carotenoids, phenolics and chlorophylls, reflected the addition of PF to the fruit and vegetable extract.

FVR, PF and MIX could be used after a very easy drying and milling procedure when there are no solubility requirements, as in the case of snacks and seasonings for breaded preparations. On the other side, flours extracts encapsulation by spray-drying may be the choice when the water solubility of the powders is needed. Spray dried powders are characterized by their reduced water content, without a significant change in hygroscopicity. By the encapsulation process, the ingredients obtained developed an improved stability and are suitable for applications in hydrophilic media. The proposed ingredients represent an attractive alternative for the development of innovative products, as well as a viable solution for the valorization of food processing by-products, agroindustrial waste and regional resources, adding value to unappreciated materials.

Conflict of interest

All authors declare that there is no conflict of interest.

Ethical Guidelines

Ethics approve was not required for this research.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Acknowledgments

The authors acknowledge financial support from Agencia Nacional de Promoción Científica y Tecnológica (PICT 2013-1331), Universidad de Buenos Aires (UBACYT 20020170100459BA) and PDTS CIN-CONICET 0196. N.S.M. acknowledges Universidad de Buenos Aires and to Programa de Movilidad en el Posgrado de la Red de Macrouniversidades Públicas de América Latina y el Caribe, for the financial support.

CONSIDERAÇÕES FINAIS

Primeiramente, destaco a pandemia instalada pelo Covid-19 que surpreendeu a todos no mundo. Tendo em vista a oportunidade de desenvolvimento de pesquisa associada à aplicação tecnológica dos ingredientes funcionais caracterizados durante a tese, para empanado de peixe tilápia, mas devido a uma crise como essa, as atividades que seriam realizadas nos meses de março e abril foram adiadas. Neste trabalho, foi aplicado um teste prévio, nas proporções de PF + FVR de 3%; 4.5% e 6%. Seriam realizadas analises de oxidação de lipídios e proteínas, perfil de textura e avaliação instrumental de cores, permitindo avaliar a viabilidade desses ingredientes funcionais. Além disso, foi possível aprimorar ainda mais meus conhecimentos e desenvolver sempre um trabalho de qualidade com a participação do artigo intitulado "Utilization of Fruit and Vegetable Residue Flour for the Development of Functional Foods" (Anexo 1), tema de pesquisa de vários estudiosos, que visa contribuir para o desenvolvimento das indústrias alimentícias e também no âmbito da pesquisa cientifica.

CONCLUSÃO GERAL

Os resultados obtidos neste estudo enfatizam a importância de *C. baccatum* e *C. pubescens*, como fonte potencial de polifenóis para serem usados como "ingredientes funcionais", com aplicação nas indústrias alimentícia e nutracêutica. Os polifenóis foram extraídos com etanol e butanol, sendo identificados por UPLC-MS^E, um total de 42 e 61 compostos fenólicos em *C. baccatum* e *C. pubescens*, respectivamente. Quercetin 3-*O*-rhamnoside, luteolin 7-*O*-glycoside e naringenin foram os mais abundantes em *C. baccatum* e o 3-feruloylquinic acid para *C. pubescens*.

O modelo baseado em GAB resultou nos melhores ajustes para os dados experimentais dessas espécies de pimenta, e os limites obtidos por essa equação indicam boa estabilidade, exceto pela oxidação lipídica, mas as interações entre polifenóis e carboidratos podem proteger os polifenóis da oxidação. As imagens SEM evidenciaram aspecto granular com partículas de diferentes formas e tamanhos, compostas principalmente por polissacarídeos. O elemento mais abundante nessas pimentas em função da intensidade relativa ao pico obtido pela EDS depois do carbono e oxigênio foi o potássio. Além disso, microencapsulação adicional foi realizada em *C. pubescens* como forma de identificar suas propriedades funcionais, mostrando partículas esféricas com superfície lisa, algumas irregularidades e boa capacidade antioxidante.

É importante destacar que a PF (*C. baccatum*) e a FVR representam uma boa combinação de matérias primas, com qualidades tecnológicas interessantes para a produção de ingredientes funcionais. Desta forma, dentre as possibilidades de utilização, os ingredientes propostos podem ser utilizados após um procedimento de secagem e moagem muito fácil, quando não há requisitos de solubilidade como no caso de lanches e temperos para preparações à milanesa. Por outro lado, o encapsulamento dos extratos de farinha na matriz de maltodextrina por secagem por pulverização são indicados para futuras aplicações em meios hidrofílicos.

Esses resultados promissores sugerem o uso de PF como ingredientes funcionais para enriquecer produtos à base de carne, pois abrem novas possibilidades interessantes e promissosas para aplicação na indústria de alimentos. Entre os vários alimentos usados com antioxidantes estão os produtos à base de carne e um alto consumo de produtos cárneos foi relatado, associado à ingestão de produtos "prontos para o consumo". Assim, sugere-se que a adição de pimentas em alimentos processados, por exemplo, tenha potencial para a indústria alimentícia, devido a importantes propriedades de barreira, aumentando o potencial nutricional bem como a estabilidade durante o armazenamento, além da qualidade sensorial do produto enriquecido. Obviamente, aspectos reológicoss e tecnológicos devem ser realizados em estudos mais detalhados, a fim de melhorar a caracterização dos pós e identificar possíveis aplicações industriais. Estudos futuros serão

necessários para beneficiar a saúde humana e atender às expectativas dos consumidores.

REFERÊNCIAS

Abu-Reidah, Ibrahim M. et al. 2015. "HPLC-DAD-ESI-MS/MS Screening of Bioactive Components from Rhus Coriaria L. (Sumac) Fruits." *Food Chemistry* 166: 179–91.

Adadi, Parise, Nadezhda V Barakova, and Elena F Krivoshapkina. 2019. "Scientific Approaches to Improving Artisan Methods of Producing Local Food Condiments in Ghana." *Food Control* 106(May): 106682. https://doi.org/10.1016/j.foodcont.2019.06.008.

Aguiar, Ana Carolina de et al. 2019. "Sequential High-Pressure Extraction to Obtain Capsinoids and Phenolic Compounds from Biquinho Pepper (Capsicum Chinense)." *The Journal of Supercritical Fluids* 150: 112–21. https://doi.org/10.1016/j.supflu.2019.04.016.

Aguirre-Cruz, Andrés, Alberto Alvarez-Castillo, Roselis Castrejón-Rosales, Teodoro Carmona-García, and Luis A Bello-Pérez. 2010. "Moisture Adsorption Behavior of Banana Flours (Musa Paradisiaca) Unmodified and Modified by Acid-Treatment." *Starch* 62: 658–66.

Alcântara, Maristela Alves et al. 2018. "Effect of the Solvent Composition on the Profile of Phenolic Compounds Extracted from Chia Seeds." *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2018.09.133.

Ali, Badreldin H, Gerald Blunden, Musbah O Tanira, and Abderrahim Nemmar. 2008. "Some Phytochemical, Pharmacological and Toxicological Properties of Ginger (Zingiber Officinale Roscoe): A Review of Recent Research." *Food and Chemical Toxicology* 46: 409–20.

Álvarez-Henao, María Victoria et al. 2018. "Microencapsulation of Lutein by Spray-Drying: Characterization and Stability Analyses to Promote Its Use as a Functional Ingredient." *Food Chemistry* 256(118): 181–87. https://doi.org/10.1016/j.foodchem.2018.02.059.

Alves, Thais O, Carolina T S D Almeida, Katharina A Scherf, and Mariana S L Ferreira. 2019. "Modern Approaches in the Identification and Quantification of Immunogenic Peptides in Cereals by LC-MS / MS." *Frontiers in Plant Science* 10(November): 1–13.

Andrade, Roberta M. S., Mariana S. L. Ferreira, and Édira C. B. A. Gonçalves. 2016. "Development and Characterization of Edible Films Based on Fruit and Vegetable Residues." *Journal of Food Science* 81(2): E412–18. http://doi.wiley.com/10.1111/1750-3841.13192.

Association of Official Analytical Chemists (AOAC). 1984. "Official Methods of Analysis of the Association of Official Analytical Chemists." In *Official Methods of Analysis of the Association of Official Analytical Chemists*, Arlington, VA, 1141.

Awolu, Olugbenga Olufemi. 2017. "Optimization of the Functional Characteristics, Pasting and Rheological Properties of Pearl Millet-Based Composite Flour." *Heliyon* (December 2016): e00240. http://dx.doi.org/10.1016/j.heliyon.2017.e00240.

Baby, Kumaranthara Chacko, and Thottiam Vasudevan Ranganathan. 2016. "Effect of Enzyme Pretreatment on Yield and Quality of Fresh Green Chilli (Capsicum Annuum L) Oleoresin and Its Major Capsaicinoids." *Biocatalysis and Agricultural Biotechnology* 7: 95–101.

Bae, Haejin et al. 2014. "Ascorbic Acid, Capsaicinoid, and Flavonoid Aglycone Concentrations as a Function of Fruit Maturity Stage in Greenhouse-Grown Peppers." *Journal of Food Composition and Analysis* 33(2): 195–202. http://dx.doi.org/10.1016/j.jfca.2013.11.009.

Baenas, N et al. 2019. "Industrial Use of Pepper (Capsicum Annum L.) Derived Products: Technological Benefits and Biological Advantages." *Food Chemistry* 274(April 2018): 872–85.

Ballesteros, Lina F et al. 2017. "Encapsulation of Antioxidant Phenolic Compounds Extracted from Spent Coffee Grounds by Freeze-Drying and Spray-Drying Using Different Coating Materials." *Food Chemistry* 237: 623–31. http://dx.doi.org/10.1016/j.foodchem.2017.05.142.

Batista, Patrício Ferreira, Maria Auxiliadora Coêlho de Lima, Ricardo Elesbão Alves, and Rafaela Vieira Façanha. 2018. "Bioactive Compounds and Antioxidant Activity in Tropical Fruits Grown in the Lower-Middle São Francisco Valley." *Revista Ciência Agronômica* 49(4): 616–23.

Benzie, Iris F F, and J J Strain. 1996. "The Ferric Reducing Ability of Plasma (FRAP) as a Measure of '." *The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "' Antioxidant Power*

’”: The FRAP Assay 76: 70–76.
Bernstein, Paul S. et al. 2016. “Lutein, Zeaxanthin, and Meso-Zeaxanthin: The Basic and Clinical Science Underlying Carotenoid-Based Nutritional Interventions against Ocular Disease.” *Progress in Retinal and Eye Research* 50: 34–66. http://dx.doi.org/10.1016/j.preteyeres.2015.10.003.
Bogusz Jr, Stanislau et al. 2018. “Brazilian Capsicum Peppers: Capsaicinoid Content and Antioxidant Activity.” *J Sci Food Agric* 98(June): 217–24.
Brunauer, S, P H Emmett, and E Teller. 1938. “Adsorption of Gases in Multimolecular Layers.” *Journal of American Chemistry Society* 60: 309–12.
Busch, V M et al. 2017. “Propolis Encapsulation by Spray Drying : Characterization and Stability.” *LWT - Food Science and Technology* 75: 227–35.
Cádiz-Gurrea, María de la Luz, Salvador Fernández-Arroyo, and Antonio Segura-Carretero. 2014. “Pine Bark and Green Tea Concentrated Extracts: Antioxidant Activity and Comprehensive Characterization of Bioactive Compounds by HPLC-ESI-QTOF-MS.” *International Journal of Molecular Sciences* 15(11): 20382–402.
Calixto, Rafael et al. 2016. “Chronic Ozone Exposure Alters the Secondary Metabolite Profile, Antioxidant Potential, Anti-in Flammatory Property, and Quality of Red Pepper Fruit from Capsicum Baccatum.” *Ecotoxicology and Environmental Safety* 129: 16–24. http://dx.doi.org/10.1016/j.ecoenv.2016.03.004.
Campos, Maria Rubi Segura, Karen Ramírez Gómez, Yolanda Moguel Ordoñez, and David Betancur Ancona. 2013. “Polyphenols, Ascorbic Acid and Carotenoids Contents and Antioxidant Properties of Habanero Pepper (Capsicum Chinense) Fruit.” *Food and Nutrition Sciences* 4(August): 47–54. http://dx.doi.org/10.4236/fns.2013.48A006%5Cnhttp://www.scirp.org/journal/fns.
Cano-Chauca, Milton, P. C. Stringheta, A. M. Ramos, and J. Cal-Vidal. 2005. “Effect of the Carriers on the Microstructure of Mango Powder Obtained by Spray Drying and Its Functional Characterization.” *Innovative Food Science and Emerging Technologies* 6(4): 420–28.
Carvalho, Ana Vânia et al. 2015. “Bioactive Compounds and Antioxidant Activity of Pepper (Capsicum Sp.) Genotypes.” *Journal of Food Science and Technology* 52(11): 7457–64.
Caurie, Matthew. 2007. “Hysteresis Phenomenon in Foods.” *International Journal of Food Science and Technology* 42(1): 45–49.
Cerqueira, Fernanda Menezes, Marisa Helena Gennari De Medeiros, and Ohara Augusto. 2007. “Antioxidantes Dietéticos: Controvérsias E Perspectivas.” *Quimica Nova* 30(2): 441–49.
Chew, Sook Chin, Chin Ping Tan, and Kar Lin Nyam. 2018. “Microencapsulation of Refined Kenaf (Hibiscus Cannabinus L.) Seed Oil by Spray Drying Using β-Cyclodextrin/gum Arabic/sodium Caseinate.” *Journal of Food Engineering* 237(May): 78–85. https://linkinghub.elsevier.com/retrieve/pii/S026087741830219X.
Ching, L S, and S Mohamed. 2001. “Alpha-Tocopherol Content in 62 Edible Tropical Plants.” *Journal of agricultural and food chemistry* 49(6): 3101–5.
Chinn, Mari S., Ratna R. Sharma-Shivappa, and Jacqueline L. Cotter. 2011. “Solvent Extraction and Quantification of Capsaicinoids from Capsicum Chinense.” *Food and Bioproducts Processing* 89(4): 340–45. http://dx.doi.org/10.1016/j.fbp.2010.08.003.
Chisté, Renan C, Priscila A Silva, Alessandra S Lopes, and Rosinelsonn da Silva Pena. 2012. “Sorption Isotherms of Tapioca Flour.” *International Journal of Food Science and Technology* 47: 870–74.
Chuichulcherm, Sinsupha, Somprattana Prommakort, Penjit Srinophakun, and Anusith Thanapimmetha. 2013. “Optimization of Capsaicin Purification from Capsicum Frutescens Linn. with Column Chromatography Using Taguchi Design.” *Industrial Crops and Products* 44: 473–79. http://dx.doi.org/10.1016/j.indcrop.2012.10.007.
Cladera-Olivera, Florencia, Ligia Damasceno Ferreira Marczak, Caciano Pelayo Zapata Norena, and

Ana Carolina Pettermann. 2011. “Modeling Water Adsorption Isotherms of Pinhão (Araucarua Angustifolia Seeds) Flour and Thermodynamic Analysis of the Adsorption Process.” *Journal of Food Process Engineering* 34: 826–43.

Conforti, Filomena, Giancarlo A. Statti, and Francesco Menichini. 2007. “Chemical and Biological

Variability of Hot Pepper Fruits (Capsicum Annuum Var. Acuminatum L.) in Relation to Maturity Stage.” *Food Chemistry* 102(4): 1096–1104.

Cremona, Gaetana et al. 2018. “Production of Embryo Rescued Hybrids between the Landrace ‘“Friariello”’ (Capsicum Annuum Var. Annuum) and C. Baccatum Var. Pendulum: Phenotypic and Cytological Characterization.” *Euphytica* 214(8): 1–11. https://doi.org/10.1007/s10681-018-2211-6.

D’Arcy, R. L., and I. C. Watt. 1970. “Analysis of Sorption Isotherms of Non-Homogeneous Sorbents.” *Transactions of the Faraday Societyt* 66: 1236–45.

Danza, Alessandra et al. 2014. “Processing and Characterization of Durum Wheat Bread Enriched with Antioxidant from Yellow Pepper Flour.” *LWT - Food Science and Technology* 59(1): 479–85. http://dx.doi.org/10.1016/j.lwt.2014.06.001.

Daood, Hussein G. et al. 2014. “Carotenoid and Antioxidant Content of Ground Paprika from Indoor-Cultivated Traditional Varieties and New Hybrids of Spice Red Peppers.” *Food Research International* 65(PB): 231–37. http://dx.doi.org/10.1016/j.foodres.2014.04.048.

Davey, Mark W et al. 2000. “Plant L-Ascorbic Acid: Chemistry, Function, Metabolism, Bioavailability and Effects of Processing.” *Journal of the Science of Food and Agriculture* 860(December 1999): 825–60.

Dias, Arthur Luiz Baião et al. 2016. “Effect of Ultrasound on the Supercritical CO2 Extraction of Bioactive Compounds from Dedo de Moça Pepper (Capsicum Baccatum L. Var. Pendulum).” *Ultrasonics Sonochemistry* 31: 284–94.

———. 2017. “Ultrasound-Assisted Extraction of Bioactive Compounds from Dedo de Moça Pepper (Capsicum Baccatum L.): Effects on the Vegetable Matrix and Mathematical Modeling.” *Journal of Food Engineering* 198: 36–44.

Díaz, D I, E Lugo, L A Pascual-Pineda, and M Jiménez-Fernández. 2019. “Encapsulation of Carotenoid-Rich Paprika Oleoresin through Traditional and Nano Spray Drying.” *Ital. J. Food Sci.* 31: 125–39.

Divya, Peethambaran, Bijesh Puthusseri, and Bhagyalakshmi Neelwarne. 2012. “Carotenoid Content, Its Stability during Drying and the Antioxidant Activity of Commercial Coriander (Coriandrum Sativum L.) Varieties.” *Food Research International* 45(1): 342–50. http://dx.doi.org/10.1016/j.foodres.2011.09.021.

Embaby, Hassan El-Sayed, and Sayed Mohamed Mokhtar. 2011. “Chemical Composition and Nutritive Value of Lantana and Sweet Pepper Seeds and Nabak Seed Kernels.” *Journal of Food Science* 76: 736–41.

Fernández-Bedmar, Zahira, and Angeles Alonso-Moraga. 2016. “In Vivo and in Vitro Evaluation for Nutraceutical Purposes of Capsaicin,capsanthin, Lutein and Four Pepper Varieties.” *Food and Chemical Toxicology* 98: 89–99.

Ferreira, Mariana S.L. et al. 2015. “Formulation and Characterization of Functional Foods Based on Fruit and Vegetable Residue Flour.” *Journal of Food Science and Technology* 52(2): 822–30.

Fonteles, Thatyane Vidal et al. 2016. “Ultrasound Processing to Enhance Drying of Cashew Apple Bagasse Puree: Influence on Antioxidant Properties and in Vitro Bioaccessibility of Bioactive Compounds.” *Ultrasonics Sonochemistry* 31: 237–49. http://dx.doi.org/10.1016/j.ultsonch.2016.01.003.

Gajewska, Danuta, Paulina Kęszycka Katarzyna, and Michał Szkop. 2019. “Dietary Salicylates in Herbs and Spices.” *Food & Function* 10: 7037–41.

Ghasemnezhad, Mahmood, Mohamad Sherafati, and Gholam Ali Payvast. 2011. “Variation in Phenolic Compounds, Ascorbic Acid and Antioxidant Activity of Five Coloured Bell Pepper

(Capsicum Annum) Fruits at Two Different Harvest Times." *Journal of Functional Foods* 3(1): 44–49. http://dx.doi.org/10.1016/j.jff.2011.02.002.

Giuffrida, Daniele et al. 2013. "Characterization of 12 Capsicum Varieties by Evaluation of Their Carotenoid Profile and Pungency Determination." *Food Chemistry* 140(4): 794–802. http://dx.doi.org/10.1016/j.foodchem.2012.09.060.

Gonçalves, E.C.B.A. et al. 2018. "Byproduct Generated During the Elaboration Process of Isotonic Beverage as a Natural Source of Bioactive Compounds." *Journal of Food Science*. http://doi.wiley.com/10.1111/1750-3841.14336.

Grebenstein, Nadine, and Jan Frank. 2012. "Rapid Baseline-Separation of All Eight Tocopherols and Tocotrienols by Reversed-Phase Liquid-Chromatography with a Solid-Core Pentafluorophenyl Column and Their Sensitive Quantification in Plasma and Liver." *Journal of Chromatography A* 1243: 39–46. http://dx.doi.org/10.1016/j.chroma.2012.04.042.

Greenspan, Lewis. 1977. "Humidity Fixed Points of Binary Saturated Aqueous Solutions." 81(1).

Guadarrama-Lezama, Andrea Yazmin et al. 2012. "Preparation and Characterization of Non-Aqueous Extracts from Chilli (Capsicum Annuum L.) and Their Microencapsulates Obtained by Spray-Drying." *Journal of Food Engineering* 112(1–2): 29–37. http://dx.doi.org/10.1016/j.jfoodeng.2012.03.032.

———. 2014. "Effects of Storage Temperature and Water Activity on the Degradation of Carotenoids Contained in Microencapsulated Chili Extract." *Drying Technology* 32(12): 1435–47.

Gurnani, Neelam, Madhu Gupta, Darshana Mehta, and Bhupendra Kumar Mehta. 2016. "Chemical Composition, Total Phenolic and Flavonoid Contents, and in Vitro Antimicrobial and Antioxidant Activities of Crude Extracts from Red Chilli Seeds (Capsicum Frutescens L.)." *Journal of Taibah University for Science* 10: 462–70. http://linkinghub.elsevier.com/retrieve/pii/S165836551500120X.

Hallmann, Ewelina, and Ewa Rembialkowska. 2012. "Characterisation of Antioxidant Compounds in Sweet Bell Pepper (Capsicum Annuum L.) under Organic and Conventional Growing Systems." *Journal of the Science of Food and Agriculture* 92(12): 2409–15.

Harich, Mehdi, Behnoush Maherani, Stephane Salmieri, and Monique Lacroix. 2018. "Evaluation of Antibacterial Activity of Two Natural Bio-Preservatives Formulations on Freshness and Sensory Quality of Ready to Eat (RTE) Foods." *Food Control* 85: 29–41. https://doi.org/10.1016/j.foodcont.2017.09.018.

Jakobek, Lidija. 2015. "Interactions of Polyphenols with Carbohydrates, Lipids and Proteins." *Food Chemistry* 175: 556–67.

Jeong, Won Y. et al. 2011. "Determination of Polyphenols in Three Capsicum Annuum L. (Bell Pepper) Varieties Using High-Performance Liquid Chromatography-Tandem Mass Spectrometry : Their Contribution to Overall Antioxidant and Anticancer Activity." *J. Sep. Sci.* 34: 2967–74.

Jinapong, Nakarin, Manop Suphantharika, and Pimon Jamnong. 2008. "Production of Instant Soymilk Powders by Ultrafiltration, Spray Drying and Fluidized Bed Agglomeration." *Journal of Food Engineering* 84(2): 194–205.

Juániz, Isabel, Iziar Amaia Ludwig, Letizia Bresciani, et al. 2016. "Catabolism of Raw and Cooked Green Pepper (Capsicum Annuum) (Poly)phenolic Compounds after Simulated Gastrointestinal Digestion and Faecal Fermentation." *Journal of Functional Foods* 27: 201–13. http://dx.doi.org/10.1016/j.jff.2016.09.006.

Juániz, Isabel, Iziar A. Ludwig, Estibaliz Huarte, et al. 2016. "Influence of Heat Treatment on Antioxidant Capacity and (Poly)phenolic Compounds of Selected Vegetables." *Food Chemistry* 197: 466–73.

Kaderides, Kyriakos, and Athanasia M Goula. 2019. "Encapsulation of Pomegranate Peel Extract with a New Carrier Material from Orange Juice by-Products." *Journal of Food Engineering*

253(November 2018): 1–13. https://doi.org/10.1016/j.jfoodeng.2019.02.019.
Kaderides, Kyriakos, and Athanasia M. Goula. 2017. "Development and Characterization of a New Encapsulating Agent from Orange Juice by-Products." *Food Research International* 100(June): 612–22. http://dx.doi.org/10.1016/j.foodres.2017.07.057.
Kandlakunta, Bhaskarachary, Ananthan Rajendran, and Longvah Thingnganing. 2008. "Carotene Content of Some Common (Cereals, Pulses, Vegetables, Spices and Condiments) and Unconventional Sources of Plant Origin." *Food Chemistry* 106: 85–89.
Kantar, Michael B. et al. 2016. "Vitamin Variation in Capsicum Spp. Provides Opportunities to Improve Nutritional Value of Human Diets." *PLoS ONE* 11(8): 1–12.
Katajamaa, Mikko, and Matej Orešič. 2005. "Processing Methods for Differential Analysis of LC/MS Profile Data." *BMC Bioinformatics* 6.
Khan, Md Imran H et al. 2017. "Experimental Investigation of Bound and Free Water Transport Process during Drying of Hygroscopic Food Material." *International Journal of Thermal Sciences* 117: 266–73.
Khoddami, Ali, Meredith A. Wilkes, and Thomas H. Roberts. 2013. "Techniques for Analysis of Plant Phenolic Compounds." *Molecules* 18(2): 2328–75.
Kil, Yun Seo, Sally T. Pham, Eun Kyoung Seo, and Mahtab Jafari. 2017. "Angelica Keiskei, an Emerging Medicinal Herb with Various Bioactive Constituents and Biological Activities." *Archives of Pharmacal Research* 40(6): 655–75.
Kim, Hong Gi et al. 2016. "Binding, Antioxidant and Anti-Proliferative Properties of Bioactive Compounds of Sweet Paprika (Capsicum Annuum L.)." *Plant Foods for Human Nutrition* 71(2): 129–36. http://dx.doi.org/10.1007/s11130-016-0550-9.
Kim, J.-S. et al. 2017. "Red Paprika (Capsicum Annuum L.) and Its Main Carotenoid Capsanthin Ameliorate Impaired Lipid Metabolism in the Liver and Adipose Tissue of High-Fat Diet-Induced Obese Mice." *Journal of Functional Foods* 31: 131–40. http://dx.doi.org/10.1016/j.jff.2017.01.044.
Kim, Ji Ho, Yeon Kyung Son, Gun Hee Kim, and Keum Hee Hwang. 2013. "Xanthoangelol and 4-Hydroxyderricin Are the Major Active Principles of the Inhibitory Activities against Monoamine Oxidases on Angelica Keiskei K." *Biomolecules and Therapeutics* 21(3): 234–40.
Kim, Ji Sun et al. 2016. "Carotenoid Profiling from 27 Types of Paprika (Capsicum Annuum L.) with Different Colors, Shapes, and Cultivation Methods." *Food Chemistry* 201: 64–71. http://dx.doi.org/10.1016/j.foodchem.2016.01.041.
Koncsek, Arnold et al. 2016. "STORAGE STABILITY OF CAROTENOIDS IN PAPRIKA FROM CONVENTIONAL, ORGANIC AND FROST-DAMAGED SPICE RED PEPPERS AS INFLUENCED BY ILLUMINATION AND ANTIOXIDANT SUPPLEMENTATION." *Journal of Food Processing and Preservation* 40: 453–462.
———. 2019. "Improvement of Antioxidant Content and Color Stability in Spice Paprika Powder by Rosemary Extract Supplementation." *Journal of Food Processing and Preservation* (December 2018): 1–10.
Koncsek, Arnold, Lajos Helyes, and Hussein G. Daood. 2017. "Bioactive Compounds of Cold Pressed Spice Paprika Seeds Oils." *Journal of Food Processing and Preservation* (May): 1–9.
Ksibi, Imen El et al. 2015. "Mixture Approach for Optimizing the Recovery of Colored Phenolics from Red Pepper (Capsicum Annum L.) by-Products as Potential Source of Natural Dye and Assessment of Its Antimicrobial Activity." *Industrial Crops and Products* 70: 34–40. http://dx.doi.org/10.1016/j.indcrop.2015.03.017.
Lewicki, Piotr P. 1997. "The Applicability of the GAB Model to Food Water Sorption Isotherms." *International Journal of Food Science and Technology* 32(6): 553–57.
Lu, Muwen, Chi-tang Ho, and Qingrong Huang. 2017. "Extraction, Bioavailability, and Bioefficacy of Capsaicinoids." *Journal Food and Drug Analysis* 25: 27–36.
Lucci, Paolo, Javier Saurina, and Oscar Núñez. 2017. "Trends in LC-MS and LC-HRMS Analysis

and Characterization of Polyphenols in Food." *TrAC Trends in Analytical Chemistry* 88: 1–24. http://linkinghub.elsevier.com/retrieve/pii/S0165993615302053.

Mamedov, M I et al. 2015. "Quality Characteristics of Paprika Pepper Varieties (Capsicum Annum L.) under Moscow Oblast Conditions." *Russian Agricultural Sciences* 41(5): 326–30.

Mateos, Rosa M et al. 2013. "Antioxidant Systems from Pepper (Capsicum Annuum L.): Involvement in the Response to Temperature Changes in Ripe Fruits." : 9556–80.

Materska, Małgorzata. 2014. "Bioactive Phenolics of Fresh and Freeze-Dried Sweet and Semi-Spicy Pepper Fruits (Capsicum Annuum L.)." *Journal of Functional Foods* 7: 269–77. http://linkinghub.elsevier.com/retrieve/pii/S1756464614000528.

Materska, Małgorzata, Maria Konopacka, Jacek Rogoliński, and Krzysztof Ślosarek. 2015. "Antioxidant Activity and Protective Effects against Oxidative Damage of Human Cells Induced by X-Radiation of Phenolic Glycosides Isolated from Pepper Fruits Capsicum Annuum L." *Food Chemistry* 168: 546–53.

Matsui, Yuji et al. 2007. "Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry for Simultaneous Analysis of Chlorogenic Acids and Their Metabolites in Human Plasma." *Journal of Chromatography B* 858: 96–105.

Meckelmann, Sven W. et al. 2013. "Compositional Characterization of Native Peruvian Chili Peppers (Capsicum Spp.)." *Journal of Agricultural and Food Chemistry* 61(10): 2530–37.

Meckelmann, Sven W., Dieter W. Riegel, et al. 2015. "Capsaicinoids, Flavonoids, Tocopherols, Antioxidant Capacity and Color Attributes in 23 Native Peruvian Chili Peppers (Capsicum Spp.) Grown in Three Different Locations." *European Food Research and Technology* 240(2): 273–83.

Meckelmann, Sven W., Christian Jansen, et al. 2015. "Phytochemicals in Native Peruvian Capsicum Pubescens (Rocoto)." *European Food Research and Technology* 241(6): 817–25.

Mendes, Nathânia de Sá, Mônica C P Santos, et al. 2019. "Characterization of Pepper (Capsicum Baccatum) - A Potential Functional Ingredient." *LWT - Food Science and Technology* 112(May): 108209. https://doi.org/10.1016/j.lwt.2019.05.107.

Mendes, Nathânia de Sá, Leonardo Cristian Favre, et al. 2019. "Flour from 'Fruits and Vegetables' Waste with Addition of a South-American Pepper (Capsicum Baccatum) Proposed as Food Ingredient." *International Journal of Food Science + Technology*.

Mendes, Nathânia De Sá et al. 2019. "Characterization of Pepper (Capsicum Baccatum) - A Potential Functional Ingredient." *LWT - Food Science and Technology* 112(January): 108–209. https://doi.org/10.1016/j.lwt.2019.05.107.

Mendes, Nathânia de Sá, and Édira Castello Branco de Andrade Gonçalves. 2020. "The Role of Bioactive Components Found in Peppers." *Trends in Food Science & Technology* 99(May): 229–43.

Mendiratta, S K, A T Shinde, and B G Mane. 2013. "Effect of Added Vegetable (Carrot, Radish and Capsicum) as Functional Ingredients in Mutton Nuggets." *JOURNAL OF MEAT SCIENCE AND TECHNOLOGY* 1(2): 71–76.

Menichini, Federica et al. 2009. "The Influence of Fruit Ripening on the Phytochemical Content and Biological Activity of Capsicum Chinense Jacq. Cv Habanero." *Food Chemistry* 114(2): 553–60. http://dx.doi.org/10.1016/j.foodchem.2008.09.086.

Mitchell, W. Robert et al. 2017. "Compaction of Food Powders: The Influence of Material Properties and Process Parameters on Product Structure, Strength, and Dissolution." *Chemical Engineering Science* 167: 29–41.

Mokhtar, Meriem et al. 2015. "Determination of the Polyphenolic Content of a Capsicum Annuum L. Extract by Liquid Chromatography Coupled to Photodiode Array and Mass Spectrometry Detection and Evaluation of Its Biological Activity." *Journal of Separation Science* 38(2): 171–78.

Mudric, Sanja Z. et al. 2017. "The Polyphenolics and Carbohydrates as Indicators of Botanical and

Geographical Origin of Serbian Autochthonous Clones of Red Spice Paprika." *Food Chemistry* 217: 705–15.

Nagy, Zsuzsa et al. 2017. "The Simultaneous Determination of Capsaicinoids, Tocopherols and Carotenoids in Pungent Pepper Powder." *Journal of Liquid Chromatography & Related Technologies* 6076(February).

Nagy, Zsuzsa, Hussein Daood, Zsuzsanna Ambrózy, and Lajos Helyes. 2015. "Determination of Polyphenols, Capsaicinoids, and Vitamin C in New Hybrids of Chili Peppers." *Journal of Analytical Methods in Chemistry* 2015: 10.

Nascimento, Patrícia L A et al. 2014. "Quantification, Antioxidant and Antimicrobial Activity of Phenolics Isolated from Different Extracts of Capsicum Frutescens (Pimenta Malagueta)." *Molecules* 19(4): 5434–47.

Nath, Prerna, S. J. Kale, Charanjit Kaur, and O. P. Chauhan. 2018. "Phytonutrient Composition, Antioxidant Activity and Acceptability of Muffins Incorporated with Red Capsicum Pomace Powder." *Journal of Food Science and Technology* 55(6): 2208–19. http://link.springer.com/10.1007/s13197-018-3138-6.

Neacsu, M. et al. 2015. "Phytochemical Profile of Commercially Available Food Plant Powders: Their Potential Role in Healthier Food Reformulations." *Food Chemistry* 179: 159–69. http://dx.doi.org/10.1016/j.foodchem.2015.01.128.

Nunes, Graciele Lorenzoni et al. 2015. "Microencapsulation of Freeze Concentrated Ilex Paraguariensis Extract by Spray Drying." *Journal of Food Engineering* 151: 60–68. http://dx.doi.org/10.1016/j.jfoodeng.2014.10.031.

Nwachukwu, Ifeanyi D., Chibuike C. Udenigwe, and Rotimi E. Aluko. 2016. "Lutein and Zeaxanthin: Production Technology, Bioavailability, Mechanisms of Action, Visual Function, and Health Claim Status." *Trends in Food Science & Technology* 49: 74–84. http://www.sciencedirect.com/science/article/pii/S0924224415301369.

Oboh, G., and J. B T Rocha. 2008. "Water Extractable Phytochemicals from Capsicum Pubescens (Tree Pepper) Inhibit Lipid Peroxidation Induced by Different pro-Oxidant Agents in Brain: In Vitro." *European Food Research and Technology* 226(4): 707–13.

Olatunji, Tomi L, and Anthony J Afolayan. 2018. "The Suitability of Chili Pepper (Capsicum Annuum L.) for Alleviating Human Micronutrient Dietary Deficiencies : A Review." *Food Science & Nutrition* 6(January): 2239–51.

Olivares-Tenorio, Mary Luz, Matthijs Dekker, Ruud Verkerk, and Martinus A J S van Boekel. 2016. "Health-Promoting Compounds in Cape Gooseberry (Physalis Peruviana L.): Review from a Supply Chain Perspective." *Trends in Food Science and Technology* 57: 83–92. http://dx.doi.org/10.1016/j.tifs.2016.09.009.

Oliveira, Dalany Menezes, Edmar Clemente, and José Maria Correia da Costa. 2014. "Hygroscopic Behavior and Degree of Caking of Grugru Palm (Acrocomia Aculeata) Powder." *Journal of Food Science and Technology* 51(10): 2783–89.

Oliveira, J. P. S. et al. 2018. "Tissue Culture and Metabolome Investigation of a Wild Endangered Medicinal Plant Using High Definition Mass Spectrometry." *Plant Cell, Tissue and Organ Culture (PCTOC)* 134(1): 153–62. http://link.springer.com/10.1007/s11240-018-1408-7.

Ornelas-Paz, José de Jesús et al. 2013. "Effect of Heat Treatment on the Content of Some Bioactive Compounds and Free Radical-Scavenging Activity in Pungent and Non-Pungent Peppers." *Food Research International* 50(2): 519–25. http://dx.doi.org/10.1016/j.foodres.2011.01.006.

Oyedemi, Blessing OM., E.M. Kotsia, Paul D. Stapleton, and Simon Gibbons. 2019. "Capsaicin and Gingerol Analogues Inhibit the Growth of Efflux-Multidrug Resistant Bacteria and R-Plasmids Conjugal Transfer." *Journal of Ethnopharmacology* 245.

Ozkan, Gulay et al. 2019. "A Review of Microencapsulation Methods for Food Antioxidants: Principles, Advantages, Drawbacks and Applications." *Food Chemistry* 272(July 2018): 494–506. https://doi.org/10.1016/j.foodchem.2018.07.205.

Padalino, Lucia et al. 2013. "Manufacture and Characterization of Gluten-Free Spaghetti Enriched with Vegetable Flour." *Journal of Cereal Science* 57: 333–42. http://dx.doi.org/10.1016/j.jcs.2012.12.010.

Palma, José M. et al. 2015. "Physiology of Pepper Fruit and the Metabolism of Antioxidants: Chloroplasts, Mitochondria and Peroxisomes." *Annals of Botany* 116(4): 627–36.

Papillo, Valentina A et al. 2019. "Cocoa Hulls Polyphenols Stabilized by Microencapsulation as Functional Ingredient for Bakery Applications." *Food Research International* 115(September 2018): 511–18. https://doi.org/10.1016/j.foodres.2018.10.004.

Pérez-Alonso, C., and M. E. Fabela-Morón, M. F., Guadarrama-Lezama, A. Y., Barrera-Pichardo, J.F., Alamilla-Beltrán, L., & Rodríguez-Huezo. 2009. "Interrelationship between the Structural Features and Rehydration Properties of Spray Dried Manzano Chilli Sauce Microcapsules." *Revista Mexicana de Ingeniería Química* 8(2): 187–96.

Pérez-Ambrocio, A et al. 2018. "Effect of Blue and Ultraviolet-C Light Irradiation on Bioactive Compounds and Antioxidant Capacity of Habanero Pepper (Capsicum Chinense) during Refrigeration Storage." *Postharvest Biology and Technology* 135(June 2017): 19–26. http://dx.doi.org/10.1016/j.postharvbio.2017.08.023.

Perla, Venu et al. 2016. "Vitamin C and Reducing Sugars in the World Collection of Capsicum Baccatum L. Genotypes." *Food Chemistry* 202: 189–98. http://dx.doi.org/10.1016/j.foodchem.2016.01.135.

Phomkong, W., and N. Singthongla. 2009. "Inactivation of Enzymatic Browning Reaction Affects Sorption Isotherms of Chilli." *Drying Technology* 27(6): 754–60. http://www.tandfonline.com/doi/abs/10.1080/07373930902828096.

Priscilla, David Hansi, Murugesan Jayakumar, and Kavitha Thirumurugan. 2015. "Flavanone Naringenin: An Effective Antihyperglycemic and Antihyperlipidemic Nutraceutical Agent on High Fat Diet Fed Streptozotocin Induced Type 2 Diabetic Rats." *Journal of Functional Foods* 14: 363–73. http://dx.doi.org/10.1016/j.jff.2015.02.005.

Pugliese, Alessandro et al. 2013. "The Effect of Domestic Processing on the Content and Bioaccessibility of Carotenoids from Chili Peppers (Capsicum Species)." *Food Chemistry* 141(3): 2606–13. http://dx.doi.org/10.1016/j.foodchem.2013.05.046.

Ramirez-Ambrosi, M. et al. 2013. "A New Ultrahigh Performance Liquid Chromatography with Diode Array Detection Coupled to Electrospray Ionization and Quadrupole Time-of-Flight Mass Spectrometry Analytical Strategy for Fast Analysis and Improved Characterization of Phenolic Compounds in Ap." *Journal of Chromatography A* 1316: 78–91. http://dx.doi.org/10.1016/j.chroma.2013.09.075.

Rao, Sathuluri Ramachandra, and Gokare Aswathanarayana Ravishankar. 2000. "Biotransformation of Protocatechuic Aldehyde and Caffeic Acid to Vanillin and Capsaicin in Freely Suspended and Immobilized Cell Cultures of Capsicum Frutescens." *Journal of Biotechnology* 76(2–3): 137–46.

Re, Roberta et al. 1999. "Antioxidant Activity Applying an Improved ABTS Radical." *Free Radical Biology & Medicine* 26: 1231–37.

Ren, Cong, Wenfei Xiong, Jing Li, and Bin Li. 2019. "Comparison of Binding Interactions of Cyanidin-3-O-Glucoside to β- Conglycinin and Glycinin Using Multi-Spectroscopic and Thermodynamic Methods." *Food Hydrocolloids* 92(January): 155–62. https://doi.org/10.1016/j.foodhyd.2019.01.053.

Rezende, Yara Rafaella Ribeiro Santos, Juliete Pedreira Nogueira, and Narendra Narain. 2018. "Microencapsulation of Extracts of Bioactive Compounds Obtained from Acerola (Malpighia Emarginata DC) Pulp and Residue by Spray and Freeze Drying: Chemical, Morphological and Chemometric Characterization." *Food Chemistry* 254(February): 281–91. https://doi.org/10.1016/j.foodchem.2018.02.026.

Ribes-Moya, Ana Maria et al. 2018. "Effect of the Genotype and Growing Conditions on the Main

Phenolic Compounds in Capsicum Peppers."
Rigon, Aline et al. 2012. "Antioxidant and Anti-Inflammatory Properties of Capsicum Baccatum : From Traditional Use to Scientific Approach." *Journal of Ethnopharmacology* 139(1): 228–33. http://dx.doi.org/10.1016/j.jep.2011.11.005.
Roberta, M.S.A., S. L.F. Mariana, and C. B.A.G. Édira. 2014. "Functional Capacity of Flour Obtained from Residues of Fruit and Vegetables." *International Food Research Journal* 21(4): 1675–81.
Rodrigues, Carina A et al. 2019. "Determination of Phenolic Compounds in Red Sweet Pepper (Capsicum Annuum L.) Using a Modified QuEChERS Method and UHPLC-MS/MS Analysis and Its Relation to Antioxidant Activity Carina." *J. Braz. Chem. Soc.* 30(6): 1229–40.
Rodríguez-Burruezo, A., J. Prohens, M. D. Raigón, and F. Nuez. 2009. "Variation for Bioactive Compounds in Ají (Capsicum Baccatum L.) and Rocoto (C. Pubescens R. & P.) and Implications for Breeding." *Euphytica* 170(1): 169–81.
Rodríguez-Burruezo, A, M. del C Gonzalez-Mas, and F Nuez. 2010. "Carotenoid Composition and Vitamin A Value in Aji (Capsicum Baccatum L.) and Rocoto (C. Pubescens R. & P.), 2 Pepper Species from the Andean Region." *J Food Sci* 75(8): S446-53. http://www.ncbi.nlm.nih.gov/pubmed/21535519.
Rodríguez-Ruiz, Marta et al. 2017. "Characterization of the Galactono-1,4-Lactone Dehydrogenase from Pepper Fruits and Its Modulation in the Ascorbate Biosynthesis. Role of Nitric Oxide." *Redox Biology* 12(January): 171–81. http://www.sciencedirect.com/science/article/pii/S2213231716303962.
Roman-Gutierrez, Alma Delia, Stéphane Guilbert, and Bernard Cuq. 2002. "Description of Microstructural Changes in Wheat Flour and Flour Components during Hydration by Using Environmental Scanning Electron Microscopy." *Lebensmittel Wissenschaft & Technologie* 35: 730–40.
Romdhane, Molka Ben et al. 2017. "Optimization of Polysaccharides Extraction from Watermelon Rinds: Structure, Functional and Biological Activities." *Food Chemistry* 216: 355–64.
Romo-Hualde, A et al. 2012. "Supercritical Fluid Extraction and Microencapsulation of Bioactive Compounds from Red Pepper (Capsicum Annum L.) by-Products." *Food Chemistry* 133(3): 1045–49. http://dx.doi.org/10.1016/j.foodchem.2012.01.062.
Saha, Supradip et al. 2015. "Capsaicinoids, Tocopherol, and Sterols Content in Chili (Capsicum Sp.) by Gas Chromatographic-Mass Spectrometric Determination." *International Journal of Food Properties* 18(7): 1535–45. http://www.tandfonline.com/doi/abs/10.1080/10942912.2013.833222.
Sajeev, S. et al. 2011. "Genetic Diversity Analysis in the Traditional and Improved Ginger (Zingiber Officinale Rosc.) Clones Cultivated in North-East India." *Scientia Horticulturae* 128: 182–88.
Santhalakshmy, Swaminathan et al. 2015. "Effect of Inlet Temperature on Physicochemical Properties of Spray-Dried Jamun Fruit Juice Powder." *Powder Technology* 274: 37–43. http://dx.doi.org/10.1016/j.powtec.2015.01.016.
Santos, Millena Cristina Barros et al. 2019. "Metabolomic Approach for Characterization of Phenolic Compounds in Different Wheat Genotypes during Grain Development." *Food Research International* 124(July 2018): 118–28. https://doi.org/10.1016/j.foodres.2018.08.034.
Santos, Mônica C P, and Édira C B A Gonçalves. 2016. "Effect of Different Extracting Solvents on Antioxidant Activity and Phenolic Compounds of a Fruit and Vegetable Residue Flour." *Scientia Agropecuaria* 7(1): 7–14.
Santos, Philipe et al. 2015. "Supercritical Carbon Dioxide Extraction of Capsaicinoids from Malagueta Pepper (Capsicum Frutescens L.) Assisted by Ultrasound." *Ultrasonics Sonochemistry* 22: 78–88. http://dx.doi.org/10.1016/j.ultsonch.2014.05.001.
Sarpras, M et al. 2016. "Comparative Analysis of Fruit Metabolites and Pungency Candidate Genes Expression between Bhut Jolokia and Other Capsicum Species." *PLOS ONE* 9: 1–19.

Schweiggert, Ute, Reinhold Carle, and Andreas Schieber. 2006. "Characterization of Major and Minor Capsaicinoids and Related Compounds in Chili Pods (Capsicum Frutescens L.) by High-Performance Liquid Chromatography/atmospheric Pressure Chemical Ionization Mass Spectrometry." *Analytica Chimica Acta* 557(1–2): 236–44.

Schweiggert, Ute, Christina Kurz, Andreas Schieber, and Reinhold Carle. 2007. "Effects of Processing and Storage on the Stability of Free and Esterified Carotenoids of Red Peppers (Capsicum Annuum L.) and Hot Chilli Peppers (Capsicum Frutescens L.)." *Eur Food Res Technol* 225: 261–70.

Seid, R. M., and O. Hensel. 2012. "Experimental Evaluation of Sorption Isotherms of Chili Pepper: An Ethiopian Variety, Mareko Fana (Capsicum Annum L.)." *Agricultural Engineering International: CIGR Journal* 14(4): 163–72.

Sharma, Rakesh, V. K. Joshi, and M. Kaushal. 2015. "Effect of Pre-Treatments and Drying Methods on Quality Attributes of Sweet Bell-Pepper (Capsicum Annum) Powder." *Journal of Food Science and Technology* 52(6): 3433–39.

Silva, Luís R. et al. 2014. "Inoculation of the Nonlegume Capsicum Annuum (L .) with Rhizobium Strains. 1. Effect on Bioactive Compounds, Antioxidant Activity, and Fruit Ripeness." *Journal of Agricultural and Food Chemistry* 62: 557–64.

da Silveira Agostini-Costa, Tânia et al. 2017. "Carotenoid and Total Vitamin C Content of Peppers from Selected Brazilian Cultivars." *Journal of Food Composition and Analysis* 57: 73–79. http://linkinghub.elsevier.com/retrieve/pii/S088915751630237X.

Singleton, Vernon L., Rudolf Orthofer, and Rosa M. Lamuela-Raventós. 1999. "Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent." *Polyphenols and Flavonoids* 299(1974): 152–78.

Sluijs, I et al. 2015. "Dietary Intake of Carotenoids and Risk of Type 2 Diabetes." *Nutrition, Metabolism and Cardiovascular Diseases*: 8–13. http://dx.doi.org/10.1016/j.numecd.2014.12.008.

Solomando, Juan Carlos, Teresa Antequera, and Trinidad Perez-palacios. 2020. "Evaluating the Use of Fish Oil Microcapsules as Omega-3 Vehicle in Cooked and Dry-Cured Sausages as Affected by Their Processing, Storage and Cooking." *Meat Science* 162(October 2019): 108031. https://doi.org/10.1016/j.meatsci.2019.108031.

Song, Young Sun, and Chung Mu Park. 2014. "Luteolin and Luteolin-7-O-Glucoside Strengthen Antioxidative Potential through the Modulation of Nrf2/MAPK Mediated HO-1 Signaling Cascade in RAW 264.7 Cells." *Food and Chemical Toxicology* 65: 70–75. http://dx.doi.org/10.1016/j.fct.2013.12.017.

Sormoli, Mona Edrisi, and Timothy A G Langrish. 2016. "Spray Drying Bioactive Orange-Peel Extracts Produced by Soxhlet Extraction: Use of WPI , Antioxidant Activity and Moisture Sorption Isotherms." *LWT - Food Science and Technology* 72: 1–8. http://dx.doi.org/10.1016/j.lwt.2016.04.033.

Sricharoen, Phitchan et al. 2016. "Phytochemicals in Capsicum Oleoresin from Different Varieties of Hot Chilli Peppers with Their Antidiabetic and Antioxidant Activities due to Some Phenolic Compounds." *Ultrasonics Sonochemistry*. http://dx.doi.org/10.1016/j.ultsonch.2016.08.018.

Sricharoen, Phitchan, Nattida Lamaiphan, Pongpisoot Patthawaro, and Nunticha Limchoowong. 2017. "Phytochemicals in Capsicum Oleoresin from Different Varieties of Hot Chilli Peppers with Their Antidiabetic and Antioxidant Activities due to Some Phenolic Compounds." *Ultrasonics - Sonochemistry* 38: 629–39. http://dx.doi.org/10.1016/j.ultsonch.2016.08.018.

Suriya, M et al. 2017. "Functional and Physicochemical Characteristics of Cookies Prepared from Amorphophallus Paeoniifolius Flour." *Journal of Food Science and Technology* 54(7): 2156–65.

Takwa, Sallawi et al. 2018. "Arbutus Unedo L. and Ocimum Basilicum L. as Sources of Natural Preservatives for Food Industry: A Case Study Using Loaf Bread." *LWT - Food Science and*

Technology 88: 47–55. https://doi.org/10.1016/j.lwt.2017.09.041.

Tavva, Venkata S, Yul-ho Kim Isabelle, Randy D Dinkins Kyung-hwan Kim, and Glenn B Collins. 2007. "Increased α -Tocopherol Content in Soybean Seed Overexpressing the Perilla Frutescens γ -Tocopherol Methyltransferase Gene." *PLANT CELL REP* 26: 61–70.

Téllez-Pérez, Carmen et al. 2014. "Impact of Swell-Drying Process on Water Activity and Drying Kinetics of Moroccan Pepper (Capsicum Annum)." *Drying Technology*: 1–12.

———. 2015. "Impact of Swell-Drying Process on Water Activity and Drying Kinetics of Moroccan Pepper (Capsicum Annum)." *Drying Technology* 33(2): 131–42.

Teodoro, A. F. P. et al. 2013. "Vitamin C Content in Habanero Pepper Accessions (Capsicum Chinense)." *Horticultura Brasileira* 31(1): 59–62. http://dx.doi.org/10.1590/S0102-05362013000100009.

Thuphairo, Kantamanee, Puttacha Sornchan, and Uthaiwan Suttisansanee. 2019. "Bioactive Compounds, Antioxidant Activity and Inhibition of Key Enzymes Relevant to Alzheimer's Disease from Sweet Pepper (Capsicum Annuum) Extracts." *Prev. Nutr. Food Sci.* 24(February): 327–37.

Timmermann, E O, J Chirife, and H A Iglesias. 2001. "Water Sorption Isotherms of Foods and Foodstuffs: BET or GAB Parameters?" *Journal of Food Engineering* 48: 19–31.

Tontul, Ismail, and Ayhan Topuz. 2017. "Spray-Drying of Fruit and Vegetable Juices: Effect of Drying Conditions on the Product Yield and Physical Properties." *Trends in Food Science and Technology* 63: 91–102. http://dx.doi.org/10.1016/j.tifs.2017.03.009.

Topuz, Ayhan et al. 2011. "Influence of Different Drying Methods on Carotenoids and Capsaicinoids of Paprika (Cv.; Jalapeno)." *Food Chemistry* 129(3): 860–65. http://dx.doi.org/10.1016/j.foodchem.2011.05.035.

Tsali, Alexandra, and Athanasia M Goula. 2018. "Valorization of Grape Pomace: Encapsulation and Storage Stability of Its Phenolic Extract." *Powder Technology* 340: 194–207. https://doi.org/10.1016/j.powtec.2018.09.011.

Turkmen, Nihal, Ferda Sari, and Y. Sedat Velioglu. 2006. "Effects of Extraction Solvents on Concentration and Antioxidant Activity of Black and Black Mate Tea Polyphenols Determined by Ferrous Tartrate and Folin-Ciocalteu Methods." *Food Chemistry* 99(4): 835–41.

Urbina, Stacie L. et al. 2017. "Effects of Twelve Weeks of Capsaicinoid Supplementation on Body Composition, Appetite and Self-Reported Caloric Intake in Overweight Individuals." *Appetite* 113: 264–73. http://linkinghub.elsevier.com/retrieve/pii/S0195666317302623.

Uribe, Elsa et al. 2016. "Assessment of Vacuum-Dried Peppermint (Mentha Piperita L.) as a Source of Natural Antioxidants." *Food Chemistry* 190: 559–65. http://dx.doi.org/10.1016/j.foodchem.2015.05.108.

Vardanega, Renata et al. 2019. "Obtaining Functional Powder Tea from Brazilian Ginseng Roots: Effects of Freeze and Spray Drying Processes on Chemical and Nutritional Quality, Morphological and Redispersion Properties." *Food Research International* 116(September 2018): 932–41. https://doi.org/10.1016/j.foodres.2018.09.030.

Vega-Gálvez, A., R. Lemus-Mondaca, P. Fito, and A. Andrés. 2007. "Note: Moisture Sorption Isotherms and Isosteric Heat of Red Bell Pepper (Var. Lamuyo)." *Food Science and Technology International* 13(4): 309–16.

Victoria-Campos, Claudia I. et al. 2015. "The Effect of Ripening, Heat Processing and Frozen Storage on the in Vitro Bioaccessibility of Capsaicin and Dihydrocapsaicin from Jalapeno Peppers in Absence and Presence of Two Dietary Fat Types." *Food Chemistry* 181: 325–32. http://dx.doi.org/10.1016/j.foodchem.2015.02.119.

Vorkas, Panagiotis A. et al. 2015. "Untargeted UPLC-MS Profiling Pipeline to Expand Tissue Metabolome Coverage: Application to Cardiovascular Disease." *Analytical Chemistry* 87(8): 4184–93.

Wahyuni, Yuni et al. 2011. "Metabolite Biodiversity in Pepper (Capsicum) Fruits of Thirty-Two

Diverse Accessions: Variation in Health-Related Compounds and Implications for Breeding." *Phytochemistry* 72(11–12): 1358–70. http://dx.doi.org/10.1016/j.phytochem.2011.03.016.

———. 2013. "Metabolomics and Molecular Marker Analysis to Explore Pepper (Capsicum Sp.) Biodiversity." *Metabolomics* 9(1): 130–44.

Williams, David J. et al. 2013. "Vegetables Containing Phytochemicals with Potential Anti-Obesity Properties: A Review." *Food Research International* 52(1): 323–33. http://dx.doi.org/10.1016/j.foodres.2013.03.015.

Zehiroglu, Cuma, Sevim Beyza, and Ozturk Sarikaya. 2019. "The Importance of Antioxidants and Place in Today's Scientific and Technological Studies." *Journal of Food Science and Technology* 56(11): 4757–74. https://doi.org/10.1007/s13197-019-03952-x.

Zhang, Chengting et al. 2018. "Phenolic Composition, Antioxidant and Pancreatic Lipase Inhibitory Activities of Chinese Sumac (Rhus Chinensis Mill.) Fruits Extracted by Different Solvents and Interaction between Myricetin-3-O-Rhamnoside and Quercetin-3-O-Rhamnoside." *International Journal of Food Science and Technology* 53(4): 1045–53.

Zhuang, Yongliang, Long Chen, Liping Sun, and Jianxin Cao. 2012. "Bioactive Characteristics and Antioxidant Activities of Nine Peppers." *Journal of Functional Foods* 4(1): 331–38. http://dx.doi.org/10.1016/j.jff.2012.01.001.

Zulueta, Ana, Maria J Esteve, and Ana Frígola. 2009. "ORAC and TEAC Assays Comparison to Measure the Antioxidant Capacity of Food Products." *Food Chemistry* 114(1): 310–16. http://dx.doi.org/10.1016/j.foodchem.2008.09.033.

ANEXO 1

Utilization of Fruit and Vegetable Residue Flour for the Development of Functional Foods

N.S. Mendes[a], M.C.P. Santos[a], M.S. Pumar[b], F.C. Silva[c], P.P.S. Coimbra[a], A.E.C. Faia[d], J.D.R.P. Souza[e], H.Y. Kawaguti[a], S.G. Moreira[f], E.C.B.A. Gonçalves [a,b*]

[a] Laboratory of Bioactives – LabBio - Food and Nutrition Postgraduate Program (PPGAN) – Federal University of Rio de Janeiro State (UNIRIO), Av. Pasteur, 296, 22290-240 Rio de Janeiro, Brazil.

[b] Nutrition School, Federal University of Rio de Janeiro State (UNIRIO), Av. Pasteur, 296, 22290-240 Rio de Janeiro, Brazil.

[c] Biosciences Institute, Federal University of Rio de Janeiro State (UNIRIO), Av. Pasteur, 296, 22290-240 Rio de Janeiro, Brazil.

[d] Departament of Basic and Experimental Nutrition, Institute of Nutrition, Rio de Janeiro State University (UERJ), Rio de Janeiro/RJ, Brazil.

[e] Federal University of Sergipe, Agroindustry Nucleus, Nossa Senhora da Glória/SE, Brazil.

[f] Federal Institute of Rio de Janeiro (IFRJ), Rua Pereira de Almeida, 88, Praça da Bandeira, 20260-100 Rio de Janeiro, Brazil.

*Corresponding author at: Laboratory of Bioactive, Federal University of State of Rio de Janeiro, UNIRIO. Av. Pasteur, 296, 22290-240 Rio de Janeiro, Brazil.
E-mail address: ediracba@analisedealimentos.com (E. Gonçalves).

"6,362 words"

ABSTRACT: Fruits and vegetable residues (FVR) flour were obtained from the solid residue generated from the whole processing of whole fruits (3) and vegetables (8). The purpose of this study was to analyze the FVR flour carbohydrate profile, and to propose chemical and enzymatic modification structure to use as functional ingredient. The properties such as sorption behavior, total phenolic content and antioxidant activity also were evaluated. Besides, the FVR flour was applied to produce candies. The FVR flour presented only 1-kestose (GF2) as prebiotic oligosaccharides and native condition, conformation changes from an amorphous structure after different pH conditions, that caused lower stability of the FVR flour when it was exposed to variations of a_w, only supporting up to $a_w = 0.6$. The GAB was the most suitable model to construct sorption isotherms. The phenolic content of the samples obtained through the enzymatic process was higher than that found in FVR flour, sample 10 (60.29 ± 15.12 mg) and the antioxidant activity values 0.55 ± 0.04g of sample/g DPPH. Phenolic content gum and crystal candies, respectively, is 0,289 ± 0,097 mg $GAE.g^{-1}$ and 0,228 ± 0,011 mg $GAE.g^{-1}$. This study shows that it is possible to promote viable and sustainable food processing without waste generation.

Keywords: Sustainable food processing; Flour; Carbohydrates; Microstructure; Chemical analysis; Candies.

1 Introduction

Today, the new tendencies respect to economic development model in the process of agro-industrial materials are oriented to circular economy in which the treatment and reuse of wastes and by-product play a crucial role. The valorization of agro-food by-products and wastes are a current scope of research. In addition to this, different valorization concepts of agro-food residues have been developed (e.g. Universal Recovery Process) (Castro-Muñoz, Boczkaj, Gontarek, Cassano, & Fíla, 2019; Castro-Muñoz & Fíla, 2018). Some pressure-driven membrane-based technologies to reduce environmental pollution from various agri-food by-products have been reported in the literature, using mainly microfiltration, ultrafiltration and nanofiltration membranes to recover phenolic compounds from various types of food by-products (Cassano, Conidi, Ruby-Figueroa, & Castro-Muñoz, 2018), as well as for the production of nutraceuticals from these by-products (Castro-Muñoz, Vlastimil, & Durán-Páramo, 2017).

It is well know that the management of waste is a great trouble in the world, approximately one third of the food produced for human consumption is lost (FAO, 2016), fruits and vegetables are responsible for 63% (Laurentiis, Corrado, & Sala, 2018). Furthermore, losses and wastes in the supply chain alters according to the economic level of the country (Kowalska et al., 2017). In accord with Mirabella et al. (2014), 39% food loss in the EU occur in the food manufacturing industry and this promotes an great environmental problem, that involves all food supply chain, such as agriculture, food manufacturing and final consumers. Fruits and vegetable wastes, for instance, are responsible for 47% and 40% of the total food waste in South Africa and United States, respectively (Gonçalves et al., 2018). Latin America is among the main regions in the world that loses and wastes more fruits and vegetables, being responsible for 55% of total production (Shirzad, Panahi, Dashti, Rajaeifar, Mohammad Ali Aghbashlo, & Tabatabaei, 2019).

Juices obtained from fruits generate large amounts of waste, such as peels, which are a potential source of dietary fiber (Cypriano, da Silva, & Tasic, 2018; Kosseva, 2009). Citrus residues have total solids content from 8 to 18%, in which the organic fraction is composed of 75% sugars and hemicellulose, 9%

cellulose and 5% lignin, with a moisture content of 80 to 90% (Kosseva, 2009). Sucrose, glucose and fructose are principle component of pineapple juice waste that is applied to produce one of the most important organic acid for the industry, lactic acid (Mochamad Busairi, 2008).

The polyphenols, essentially secondary metabolites of plants, that are present in the residues of fruits and vegetables process being recovered for application in conventional and new products (Fidelis et al., 2020; Maqsood, Adiamo, Ahmad, & Mudgil, 2020; N. de S. Mendes et al., 2019; Sette et al., 2020; Shadrach, Banji, & Adebayo, 2020). Polyphenols (10-11%) were identified in the waste of grape juice production and can be used as food colors, antioxidants and anti-cancer agents (Varadharajan, Shanmugam, & Ramaswamy, 2017). Also, the presence of bioactive compounds such as flavonoids and carotenoids with their antioxidant properties associated with the physiological effects of fiber can result in antioxidant dietary fibers (ADF) for food applications (Amaya-Cruz et al., 2015; Shea, Arendt, & Gallagher, 2012).

Studies have highlighted fruits and vegetables residues (FVR) obtained through their complete exploration, including peel, pulp, stalks, seeds and pits (Ferreira et al., 2015). As a consequence of that, these parts, often discarded, transform the flour with a large amount of fibers, minerals, vitamins, in addition to antioxidant compounds present in them (Brito et al., 2019; Mendes et al., 2019a). According to the authors, proximate composition of FVR flour indicated dietary fibers (48.4%, with 80% insoluble), available carbohydrates (26.5%), proteins (9.5%), moisture (5.9%), lipids (5%) and ashes (4.9%). Recently, 88 phenolic compounds were identified by UPLC-ESI-Q-TOF-MS/MS in FVR: phenolic acids (28), flavonoids (32) and other polyphenols (28) showing that it can potentially be used in the development of food products with added nutritional value (Gonçalves et al., 2018).

For instance, FVR flour was applied in the reformulation of cereal products and their microbiological stability, water retention capacity and mineral and fibrous content were better. (Ferreira et al., 2015), and good functional as prebiotic (Andrade, Ferreira, & Gonçalves, 2014). Considering the functional capacity and the rich composition of bioactive compounds in FVR flour, the purpose of this study was

to analyze the flour's carbohydrate profile, and to propose chemical and enzymatic modification structure to use as a functional ingredient in a processing line without residues generation.

2 Materials and Methods

Chemical reagents

All chemical reagents and solvents applied in this study were of analytical grade (P.A.) obtained from Sigma - Aldrich Brazil.

Sample

In this study, the following species were used: 11% of sweet orange (*Citrus sinensis*), 19% of passion fruit (*Passiflora edulis*), 22% of watermelon (*Citrullus lanatus*), 8.5% of cucumber (*Cucumis sativus*) and courgette (*Cucurbita pepo*), 2% of rocket (*Eruca sativa*) and mint (*Mentha* sp), 13% of carrot (*Daucus carota*) and 5.5% of lettuce (*Lactuca sativa*), spinach (*Spinacea oleracea*) and taro (*Colocasia esculenta*). All species were purchased in a supermarket located in Rio de Janeiro (Brazil), taken to the laboratory for immediate use. Fruits and vegetables were properly washed in flowing water, after they were sanitized for 30min in a bath containing 200 ppm of sodium hypochlorite (NaClO) before rinsing in flowing destilated water. After the concentrated juice was obtained, the solid residue generated was immediately dried in a Marconi ventilated oven model MA-035/5 at 65 °C. After drying the material was ground in a Walita model food processor, returning to the oven for another 60 minutes at 90 °C. Finally, the whole batch was homogenized to obtain the fruit and vegetable flour (FVR) and stored in metalized plastic sachets at room temperature (20 °C - 30 °C) until the date of analysis (Ferreira et al., 2015). The FVR flour were characterized containing dietary fiber (48%, 80% of which was insoluble), carbohydrates (26%), proteins (9.5%) and lipids (5%). Analysis of different lots in different years allows standardization for assuring the composition constancy of the waste (Brito et al., 2019). The flow diagram of the FVR flour production is showed in the Figure 1.

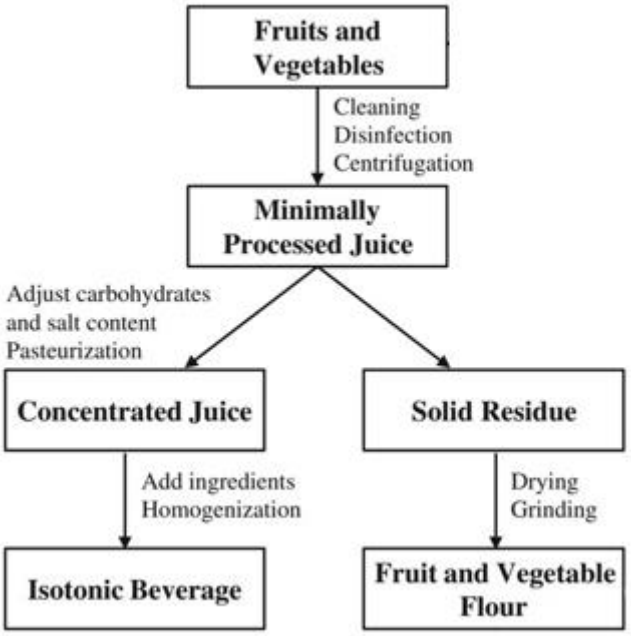


Figure 1. Flow diagram for the isotonic beverage and, fruit and vegetable residue flour.
Source: Ferreira et al., 2015

The FVR flour was applied to determination carbohydrates profile and chemical (pH; water content), and enzymatic modification as described below:

Carbohydrates profiles by high-performance anion-exchange chromatography with pulsed amperometry detection (HPAE-PAD)

Carbohydrate profile was performed according to Sancho et al. (2017) and L'homme et al. (2001) with modifications. A high-performance anion-exchange chromatography system coupled with pulsed amperometry detection (HPAEC-PAD) with Chromeleon 7.0 Chromatographic CHM-1, automation software, Dionex (USA) was employed. All analyses were performed in triplicate. A flour (25 mg/mL) sample was homogenized with deionized water and in ultraturrax during 2 minutes. The sample was centrifuged (5 °C, 15 min, 10,000 RPM). The supernatant was removed, diluted in deionized water and filtered through a 0.20 mm regenerated cellulose membrane filter before analysis.

For fructooligosaccharides and maltoligosaccharides a CarboPac PA-100 (4 x 250 mm) column equipped with a CarboPac PA 100 (4 x 50 mm) guard column was used. The following solutions were

used for gradient elution: A (100 mM sodium hydroxide) and B (500 mM sodium acetate and 100 mM sodium hydroxide). The running was started with 97% (A) and 3% (B) for 2 min, followed by 18 min with a linear gradient from 3 to 40% of B, followed by cleaning with 100% of A for 5 min and stabilization for 5 min at the same initial status, totaling 28 min at a flow rate of 1.0 mL/min at 30 °C. Compounds were quantified using a linear calibration curve of the following carbohydrate standards 1-kestose (GF2), nystose (GF3), and 1-fructofuranosylnystose (GF4) (Wako Pure Chemical Industries, Osaka, Japan), and maltotriose (G3), maltotetraose (G4), maltopentaose (G5), maltohexaose (G6), and maltoheptaose (G7), (Supelco, Bellefont, PA, USA). The results are expressed in mg/100 g of sample (wet matter).

Glucose, fructose and sucrose were quantified using CarboPac PA-1 (4 x 250 mm) column equipped with a CarboPac PA 100 (4 x 50 mm) guard column. The following solutions were used for gradient elution: A (200 mM sodium hydroxide) and B (water). The running was isocratic with 80% (A) and 20% (B) for 10 min, followed by cleaning with 100% of A for 5 min and stabilization for 5 min at the same initial status, totaling 20 min at a flow rate of 1.0 mL/min at 30 °C. Compounds were quantified using a linear calibration curve of the carbohydrate standards. The results are expressed in g/100 g of sample (wet matter).

Chemical modification of the structure of FVR flour

Dehydrated FVR flour

Water solution of FVR flour (8%) was heated at 70 °C under constant agitation (200 rpm) in a water bath (Dubnoff type, M.S. Mistura, Rio de Janeiro, RJ, Brazil) for 45 min (Andrade, Ferreira, & Gonçalves, 2016). After filtration, FVR flour was dried in a conventional oven at 105 °C (AOAC, 2012).

Dehydrated FVR flour (pH 7 and pH 9)

Buffer solution of FVR flour (8%), prepared in ammonium hydroxide and metaphosphoric acid (pH 7); and ammonium hydroxide and phosphoric acid (pH 9), was heated at 70 °C under constant agitation (200 rpm) in a water bath (Dubnoff type, M.S. Mistura, Rio de Janeiro, RJ, Brazil) for 45 min (Andrade et al., 2016). After filtration, FVR flour was dried in a conventional oven at 105 °C (AOAC, 2012).

Microstructure of FVR after chemical modification

Samples of FVR flour after chemical modification was analyzed using a scanning electron microscope (SEM, Oxford Industries, England) coupled with X-ray energy dispersive spectrometer (EDS; Oxford Industries) according to the method described by Andrade, Ferreira, & Gonçalves (2016).

Moisture sorption isotherm of FVR after chemical modification

Moisture sorption isotherm of samples of FVR after chemical modification to construct adsorption and desorption moisture isotherms at 25 °C (Mendes et al., 2019a). The curves were adjusted with four mathematical models: Guggenheim, Anderson and Boer (GAB), Halsey, Henderson and Oswin (Table 1), through non-linear regression analysis, using GraphPad Prism 6 software. The coefficient of determination (R^2), relative percentage deviation (E) (Equation (5)) and root mean square (RMSE) (Equation (6)) were used to evaluate the adjustment of the models.

Table 1. Selected isotherm models.

Model	Equation	
GAB	$X_e = \frac{(X_m C K a_w)}{(1-Ka_w)(1-Ka_w+CKa_w)}$	(1)
Halsey	$X_e = a\left[T \ln\left(\frac{1}{a_w}\right)\right]^{-1/b}$	(2)
Henderson	$X_e = \left[\frac{\ln\left(\frac{1}{1-a_w}\right)}{a(T+b)}\right]^{1/c}$	(3)
Oswin	$X_e = a\left(\frac{a_w}{1-a_w}\right)^{b}$	(4)

X_m, M is the water hydration limit ("monolayer value", % dry basis); C, K, A, B are constants of the models; R^2 is the coefficient of determinant; %E is the mean relative percentage deviation and %RMS is the root mean square.

$$E\% = \frac{1}{N}\sum_{i=1}^{N}\frac{|m_i - m_{pi}|}{m_i} \qquad (5)$$

$$RMSE\% = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\frac{m_i - m_{pi}}{m_i}\right)^2} \qquad (6)$$

in which m_i and m_{pi} are the actual and predicted moisture content values respectively, and N is the number of observations.

Enzymatic modification of the structure of FVR flour

The FVR flour was submitted to the process of enzymatic treatment with commercial enzyme (viscozyme®), in the conditions following (enzyme/temperature): 1 (125 µL/30 °C); 2 (125 µL/60 °C); 3 (375 µL/30 °C); 4 (375 µL/60 °C); 5 (250 µL/45 °C); 6 (75 µL/45 °C); 7 (425 µL/45 °C); 8 (250 µL/24 °C); 9 (250 µL/66 °C); 10 (250 µL/45 °C) (Meyer, Dam, & Lærke, 2009), in aqueous solution in water-bath with shaking (200 rpm) for 30 min (Fai et al., 2016). After enzymatic treatment, the samples were treated as follows:

A - filtration in polyester filters and the residue (RF) was dried in a drying oven with air renewal and circulation (Marconi, model MA035, Brazil) at 105 °C, and liquid (L), was applied to obtain sweets.

B - dried in a drying oven with air renewal and circulation (Marconi, model MA035, Brazil) at 105 °C (RD).

Total dietetic fiber, soluble and insoluble FVR after enzymatic modification

The levels of total dietary fiber (TDF), dietetic soluble (FDS) and insoluble (FDI) were analyzed in triplicate, according to the enzymatic-gravimetric method described by AOAC Method 991.43 (1990).

Functional capacity of FVR flour after enzymatic modification

Antioxidant activity assay

The extracts of RF and RD were obtained from ethanol 75% in a shaker (Incubator shaker NT 715) at

40 °C after 24 hours at 200 rpm (Naspolini et al., 2016). The supernatant was recovered for analysis of total phenolic compounds and antioxidant activity. Total phenolics compounds (TPC) in the extracts were determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965). The results were expressed as gallic acid equivalents in milligrams per 100 g of dry matter (mg GAE=100 g of d.m.).

Free radical scavenging activity of FVR was measured regarding radical scavenging ability, using DPPH [di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium] as described by Brand-Williams, Cuvelier, & Berset, (1995) with few modifications. A 60 µM solution of DPPH was prepared, and 2.0 mL of this solution was added to 1 mL of aqueous extract of FVR. The mixture was shaken vigorously and kept at room temperature, in the dark for 60 min, to ensure the development of the reaction, then the absorbance was read at 517 nm, using spectrophotometer (Shimadzu, UV-2700, Japan). Blank samples were prepared to replace DPPH with methanol. The antioxidant activity was expressed as EC50 (concentration required to obtain a 50% antioxidant effect).

FVR flour after enzymatic modification as a functional ingredient in a processing line without residues generation

Fibers supplement and candy production by sustainable exploitation were proposed using an enzymatic process with FVR in the best conditions (2, 6 and 10). The resulting solution (L) from enzymatic treatment was used for the production of two candies. The first one (gum candy) was prepared with gelatin as follows: 15 grams of unflavored gelatin were diluted in 20 mL and heated underwater vapor until total dissolution and sequentially were taken to refrigeration for 10 minutes. The second, crystal candy was prepared with sugar as follows: 50 grams of sugar were dissolved in 15 mL over medium heat for 10 minutes until caramelization. Total phenolics compounds (TPC) in candies were determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965). The results were expressed as gallic acid equivalents in milligrams per 100 g of dry matter (mg GAE=100 g of d.m.).

Statistical analysis

The data were subjected to analysis of variance (one-way ANOVA) and the means were compared through the Tukey test (95% confidence level) in the XLSTAT statistical software (Addinsoft, version2018.2.50452). A triplicate was performed for each analysis.

3 Results and Discussion

Carbohydrate profile in FVR flour

Oligosaccharides with prebiotic function have significant impacts on gut microbiota and are associated with various health beneficial effects. It is already pointed out that vegetables are a natural source of these components and the combinations of different oligosaccharides are potentially more effective as prebiotics than the consumption of only one type. In other words, prebiotic activity is consequent of a synergy between the chemical nature of the oligosaccharides and metabolic machinery of the gut microbiota (Ose et al., 2018; Pereira et al., 2018; Rajendran et al. 2017; Sancho et al., 2017).

Table 2 shows the carbohydrate profile (mono-, di, malto- and fructooligosaccharides) observed in FVR flour. Carbohydrates were composed mostly of simple sugars, from which fructose was the most abundant. GF2 was the only prebiotic oligosaccharides observed in FVR flour (Table 2). The GF2 is the most common oligosaccharide found in various fruits and vegetables (Jovanovic-Malinovska, Kuzmanova, & Winkelhausen, 2014; L'homme et al., 2001; L'homme, Puigserver, & Biagini, 2003; Pereira et al., 2017). The other oligosaccharides assayed in this sample were not identified. It is important to note that vegetable foods enclose a complex mixture of carbohydrates with a degree of polymerization varying from 2 to 60 units. As a result, identification and quantification of sugar and oligosaccharides in those matrices represent a challenging area of study (Arruda, Pereira, & Pastore, 2017)**.** This study demonstrates that vegetable by-products, such as FVR flour, could contribute to the daily intake of natural sugars and fructooligosaccharides consumption.

Table 2. Carbohydrate profile in FVR flour.

Sugars	Glucose (g/100g)	7.77 ± 0.310
	Fructose (g/100g)	10.86 ± 0.065
	Sucrose (g/100g)	1.76 ± 0.005
Oligosaccharides	GF2 (mg/100g)	11.48 ± 0.220
	G5 (mg/100g)	125.54 ± 2.27
	G6 (mg/100g)	27.25 ± 0.340

Values are means ± standard deviation of triplicate analysis.

Chemical modification in the structure of FVR flour

Microstructure

The microstructures of FVR after chemical modifications, using SEM analysis, presented in Figure 2. Based on previous studies, FVR showed granular and lentil-shaped structures, indicating polysaccharides and proteins in the matrix (Andrade, Ferreira, & Gonçalves, 2016; Reis & Gonçalves, 2014). The microstructures of dehydrated FVR flour without pH modification (water FVR) are not affected as dehydrated FVR flour with pH modification (7pH FVR; 9pH FVR). It is also well known that pH changes can modify the polymer structures of various polymers, such as carbohydrate and protein polymers, changing the charge of polar sites (Carneiro-da-Cunha et al., 2011).

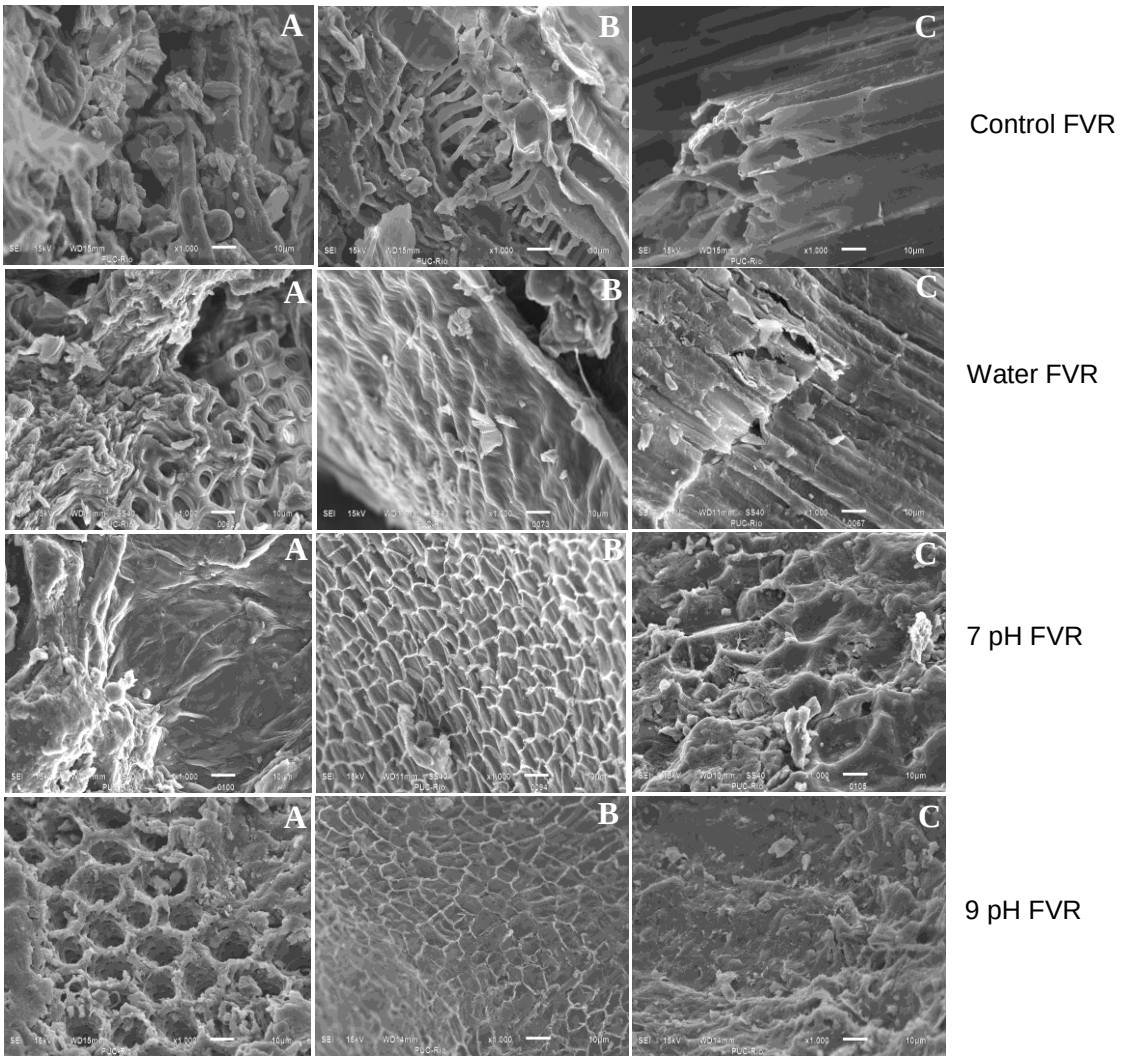


Figure 2. Scanning electron micrographs of the fruit and vegetal residue flour (FVR flour) after extraction with following conditions (magnifications 1000x): control FVR, water FVR, 7 pH FVR and 9 pH FVR.

Considering that FVR flour has native acidic pH (Brito et al., 2019), the stable polymers in this pH condition will be affected by neutralizing the pH or rendering it more basic (pH> 7). The way FVR flour responds to different a_w is directly correlated with the stability of the polymeric structure of its dietary fiber, since water can infiltrate the vacuoles of this polymeric structure, especially in the hydrophilic sites (Mudgil, Barak, & Khatkar, 2014).

Since FVR flour was exposed to pH conditions different from its native condition, its polymers underwent three-dimensional conformation changes, from a three-dimensional polymer structure (Control, SEM C) to an amorphous structure (pH 7, SEM C and pH 9, SEM C). The alteration of the

polymeric structure to amorphous caused a lower stability of the FVR flour when it was exposed to variations of a_w, only supporting up to $a_w = 0.6$, being this value lower than the control and the aqueous extraction FVR flour (Mendes et al., 2019a). It is noteworthy that the greatest change occurs when the pH becomes neutral, since the initial pH changes immediately act on the polar sites charge of the polymer, modifying them and, once modified, the increase of the pH only maintains the post-change condition, causing no major changes (Andrade et al., 2016; Isah, Oshodi, & Atasie, 2017).

Mathematical modeling of sorption data

According to Mendes et al. (2019a,b), a model presents a good fit when the R^2 value is close to the unit and minimum error values (E and RMSE). Therefore, the GAB equation was the most suitable model for all the samples studied (Table 3). These results agree with those reported by other researchers, highlighting that the GAB model was the best model to describe the water sorption isotherms for food systems (Brito et al., 2019).

Table 3. Parameters of the proposed models for moisture sorption isotherms at 25 °C.

Adsorption Isotherm					
Models	**Parameters**	**FVR flour**			
		Control	**Water**	**7 pH**	**9 pH**
GAB	X_m	9.332	8.987	10.937	10.230
	C	2.053	2.123	0.112	0.068
	K	0.881	0.888	1.100	1.101
	R^2	0.997	0.997	0.997	0.993
	%E	7.490	7.863	8.252	18.073
	%RMSE	65.729	69.001	50.870	98.990
Halsey	A	14.48	26.59	1.121	0.866
	B	1.296	1.510	0.447	0.418
	R^2	0.980	0.974	0.994	0.991
	%E	9.750	14.374	7.980	16.226
	%RMSE	82.156	128.566	52.330	91.789
Henderson	A	0.092	0.092	0.440	0.559
	B	0.874	0.872	0.411	0.381
	R^2	0.992	0.992	0.990	0.995
	%E	8.200	8.406	16.279	10.865
	%RMSE	71.960	75.187	100.35	63.355
Oswin	A	10.87	10.96	2.983	1.721
	B	0.589	0.580	1.673	1.805
	R^2	0.984	0.985	0.994	0.995
	%E	10.662	10.823	12.044	10.778
	%RMSE	93.565	96.805	74.246	60.969

Desorption Isotherm					
Models	**Parameters**	**FVR flour**			
		Control	**Water**	**7 pH**	**9 pH**
GAB	X_m	11.246	10.652	4.294	4.831
	C	7.895	9.913	5.064	2.657
	K	0.854	0.869	0.931	0.695
	R^2	0.989	0.989	0.994	0.994
	%E	5.426	5.417	2.573	4.634
	%RMSE	47.929	48.151	17.828	31.086
Halsey	A	39.05	39.34	5.061	2.948
	B	1.458	1.460	1.062	1.004
	R^2	0.989	0.989	0.995	0.985
	%E	5.626	5.731	2.648	4.883
	%RMSE	49.369	50.616	18.348	32.291
Henderson	A	0.027	0.026	0.067	0.120
	B	1.149	1.156	1.224	1.184
	R^2	0.955	0.953	0.986	0.992
	%E	14.817	15.223	4.757	4.085
	%RMSE	130.862	135.310	32.961	27.097
Oswin	A	16.38	16.42	6.628	4.314
	B	0.537	0.535	0.628	0.655
	R^2	0.980	0.980	0.993	0.993
	%E	9.349	9.618	3.378	2.623
	%RMSE	82.573	85.489	23.403	17.404

$_n$, *M* is the water hydration limit ("monolayer value", % dry basis); *C, K, A, B* are constants of the models; ^{2} is the coefficient of determinant; %E is the mean relative percentage deviation and %RMSE is the root ean square.

As stated by Fonteles et al. (2016) and Goula & Adamopoulos (2008), the molecular monolayer (X_m) is the primary food layer, and its water content interferes with the hygroscopicity or water affinity of the molecules, so that the amount of moisture in the monolayer provides maximum stability of food with minimal loss of food quality; below this value, rates of deterioration reactions, except oxidation of unsaturated fats, are minimal, especially in dehydrated foods. The X_m obtained through the GAB equation is 8.987 to 10.937 g H2O/g dry basis and 4.294 to 11.246 g H2O/g dry basis for adsorption and desorption isotherms respectively (Table 3) (Oliveira, Clemente, & da Costa, 2014). In addition, the sample of FVR flour treated with water presented similar values of monolayer (X_m) in comparison to the control. The value of X_m found for samples indicates good stability, with the exception of lipid oxidation that may occur during storage (Mendes et al., 2019a), but previous studies using FVR as raw material demonstrated promising results such its antioxidant capacity and phenolic compounds after 180 days (Santos & Gonçalves, 2016).

It is possible to note that the values of the *C* constant in the GAB model increased for the water FRV flour, which favors the interaction force between adsorbate adsorbent causing an increase in the values of the constant *C*. The value of the control constant K in the GAB model, increased in flour treated with pH 7.0 and pH 9.0. Timmermann et al. (2001) state that the constant *K* of the GAB model increases with the interaction force between adsorbate adsorbent and values greater than 1.0 would be physically unsuitable indicating infinite sorption.

Figure 3 compares the FRV flour sorption isotherms with different conditions (water, defatted, pH 7 and pH 9) at 25 °C. The comparison shows how the pH increase significantly reduces the sorption capacity of FRV, with a_w = 0.6, which is smaller than the others analyzed. This can be attributed to FVR flour which has an acid character in which they were affected by changes in pH different from their native form, thus reducing the sorption degree of water with increasing pH. The pH is an important factor affecting sorption due to the ionization of surface functional groups and solution composition (Hernández-Hernández, Solache-Ríos and Díaz-Nava, 2013). Figure 3 also shows that the

curve is of type J, its first part is flatter, indicating presence soluble components, such as sugars, which describes the water sorption by hydrophilic polymers (Al-Muhtaseb, McMinn and Magee, 2002). According to Andrade, Ferreira, & Gonçalves (2016) products with high carbohydrate content, such as the green banana flour and a dried sample of fully ripe pineapple, show isotherms in this way.

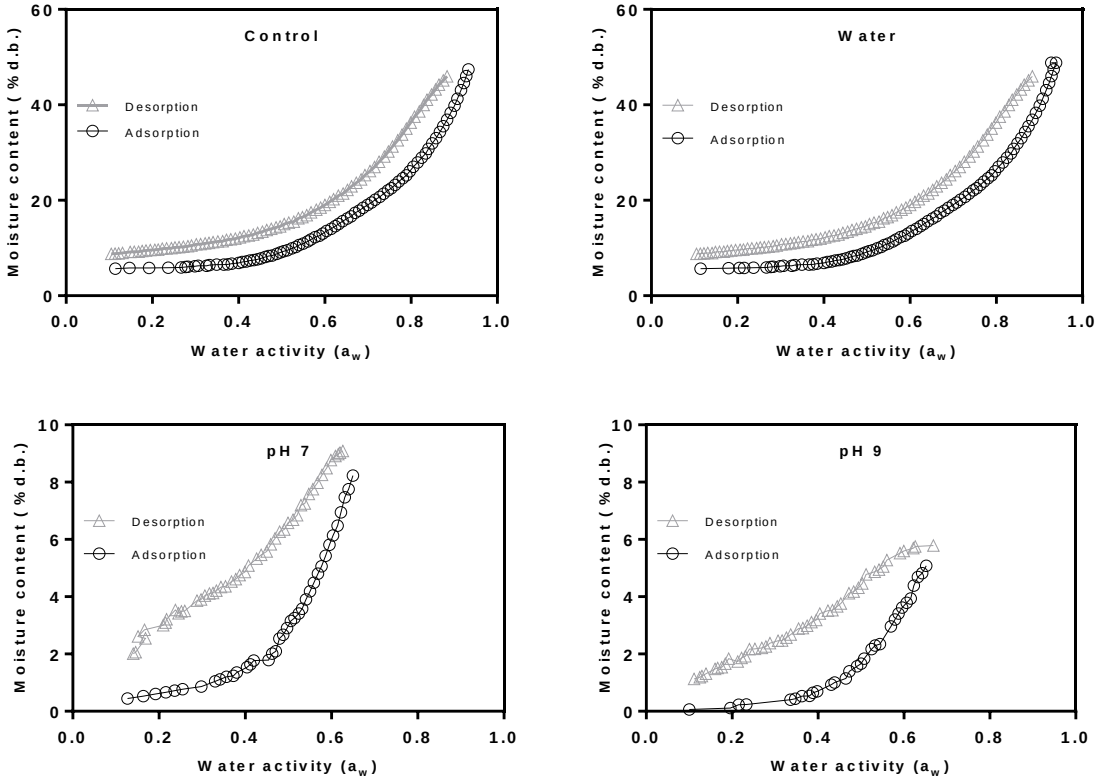


Figure 3. Hysteresis of the GAB model of fruit and vegetable residue flour (FVR flour) of the control, extraction with water, pH 7 and pH 9 solutions.

Regarding the hysteresis, according to Caurie (2007) and Mendes et al. (2019a), is a good indication of the quality of food, because the lower the effect of hysteresis the greater the stability of the product. For all flour fractions (Figure 3), the hysteresis extended from a lower to a higher a_w, and the behavior of the hysteresis was practically the same for the control FVR flour and water. However, it was observed that for the treatment of the FVR flour with pH 7 and 9 there was an increase in the hysteresis effect.

Enzymatic modification of the structure of FVR flour

Table 4 shows the values of total dietetic fiber, insoluble and soluble in different concentrations of

Viscozyme® and temperatures to verify the behavior from fibers according to changes of both variables and influence from the substrate in each sample. The data treatment with the ANOVA and Tukey test showed that the variables were influenced by the variation of the enzyme concentration, but the temperature did not interfere in the process. Regarding the total fiber, the results were higher than those found by Andrade, Ferreira, & Gonçalves (2014), 48,42% in fruit and vegetable flour without treatment. Besides, the results obtained from soluble fiber were mostly equal to zero, which may be lost during the acid digestion from the fibers. The values found by Laufenberg, Kunz, & Nystroem (2003) of the total dietetic fiber of apple pomace (62,5%) and barley pomace (65,3%) were next to the ones found in this work, indicating that the FVR flour after enzymatic treatment has high fiber content, taking into account that to be considered a food with a high content of these components it is necessary to contain 6 g of total fibers per 100 g of sample (Codex, 2001).

Table 4. Contents of total fiber and fractions in FVR flour after enzymatic treatment.

Treatments (enzyme µL/temperature °C)	Insoluble fiber (mg)	Soluble fiber (mg)	Total fiber (mg)
1 (125/30)	67,62 ± 4,61	0	67.62 ± 4.61[a]
2 (125/60)	69.17 ± 2.88	0	69.17 ± 2.88[a]
3 (375.5/30)	71.12 ± 0.20	0	71.12 ± 0.20[b]
4 (375.5/60)	67.94 ± 2.82	0	67.94 ± 2.82[a]
5 (249/45)	66.37 ± 2.32	0	66.37 ± 2.32[a,c]
6 (73/45)	66.84 ± 1.32	0	66.84 ± 1.32[a,c]
7 (425/45)	66.67 ± 0.75	0	66.67 ± 0.75[a,c]
8 (249/23.8)	65.34 ± 2.91	0	65.34 ± 2.91[c]
9 (249/66)	66.42 ± 0.50	0	66.42 ± 0.50[c]
10 (30/45)	67.49 ± 6.55	0,29 ± 0,02	68.43 ± 6.55[a]

Values are means ± standard deviation of triplicate analysis. Different letters on each column mean statistical difference, using the Tukey test ($p < 0.05$).

Enzymatic complexes, which contain cellulases, arabinases, hemicellulases, glucanases and xylanases,

promote modification in the vegetable issues, favoring the extraction of compounds (Meyer et al., 2009). Besides that, the optimum temperature of Viscozyme® activity was 55 °C in a study done by Rosset et al. (2012).

Glucanases and xylanases are added to hydrolyze glucans (likely cellulose, but with β-1,3 and β-1,4 connections) already xylans contain xylose polymers, the main hemicellulosic component. The α and β-amylases are used to achieve starch degradation (Damodaran, Parkin, & Fennema, 2010). Therefore, the hydrolysis of the carbohydrate molecules allows the breaking of specific bonds, reducing the units and separating them (Rosset et al., 2012), which explains the increase of insoluble fibers in the FVR flour. In addition, the results of soluble fibers can also be explained in this way, since their broken molecules can be transformed into oligosaccharides and monosaccharides (Park & Yoon, 2015).

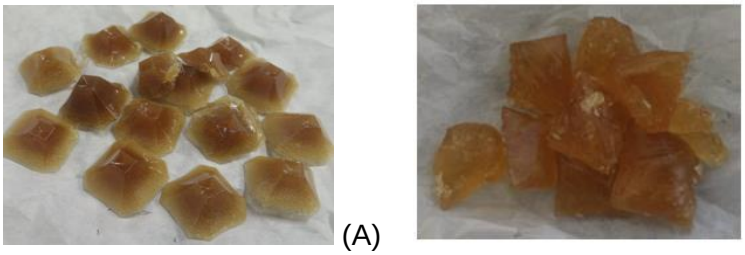
Chemical analysis of FVR flour after enzymatic modification

The phenolic content of the samples that passed through the enzymatic process was higher than that found in FVR flour, sample 2 (59.42 ± 12.52), 6 (51.63 ± 11.45) and 10 (60.29 ± 15.12), and there was no significant difference between the three treatments (p<0.05). Regarding the analysis of phenolic compounds by Folin-Ciocalteau, it is known that carbohydrates, lipids and proteins can interfere in this method (Otemuyiwa, Williams, & Adewusi, 2017). For this reason, it is important to note that the DP value is so high (20%), perhaps as a result of the contribution to sugar in this determination.

EC50 DPPH values were: 0.56 ± 0.05g of sample/g DPPH for sample 2; 0.57 ± 0.06g of sample/g DPPH for 6; and 0.55 ± 0.04g of sample/g DPPH for 10, presenting no significant difference between them. As mentioned, viscozyme is an enzyme complex that includes cellulases, hemicellulases, pectinases (de Figueiredo, Yamashita, Vanzela, Ida, & Kurozawa, 2018); and FVR flour has cellulose, hemicellulose, soluble lignin, insoluble lignin and resistant starch (Brito et al., 2019), the enzymatic process promotes release interaction bound polyphenols and biopolymers increase extraction capacity (Rajha et al., 2018; Waterhouse, Sun-Waterhouse, Su, Zhao, & Zhao, 2017).

FVR flour after enzymatic modification as a functional ingredient in a processing line without residues generation

Solutions (L) were applied to produce candies, Figure 4. Phenolics compounds are one of various phytochemicals classes in fruits and vegetables, generally as free or soluble conjugated (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014), FVR flour presents 88 phenolics compounds, tentatively identified, as previously cited (Gonçalves et al., 2018) and enzymatic treatment does not promote a significant difference in antioxidant capacity of the matrix, as mentioned. Phenolic content gum and crystal candies, respectively, are 0,289 ± 0,097 mg GAE.g^{-1} and 0,228 ± 0,011 mg GAE.g^{-1}, no significant difference.



(A) (B)

Figure 4. Gum (A) and crystal (B) candies produced with solutions residues of FVR flour enzymatic treatment.

In order to propose an industrial process without residues generations and use all potential food matrix, Figure 5 shows a summary and perspectives to use FVR flour as matrix and functional ingredient. It is possible to mention that FVR flour is a multifunctional food ingredient, and enzymatic treatment produces new matrix application as a sustainable food processing (Kowalska et al., 2017). High total fibers value can be considered to apply FVR flour in other value-added products, as bioconversion via solid-state fermentation and biosorbents (Laufenberg et al., 2003).

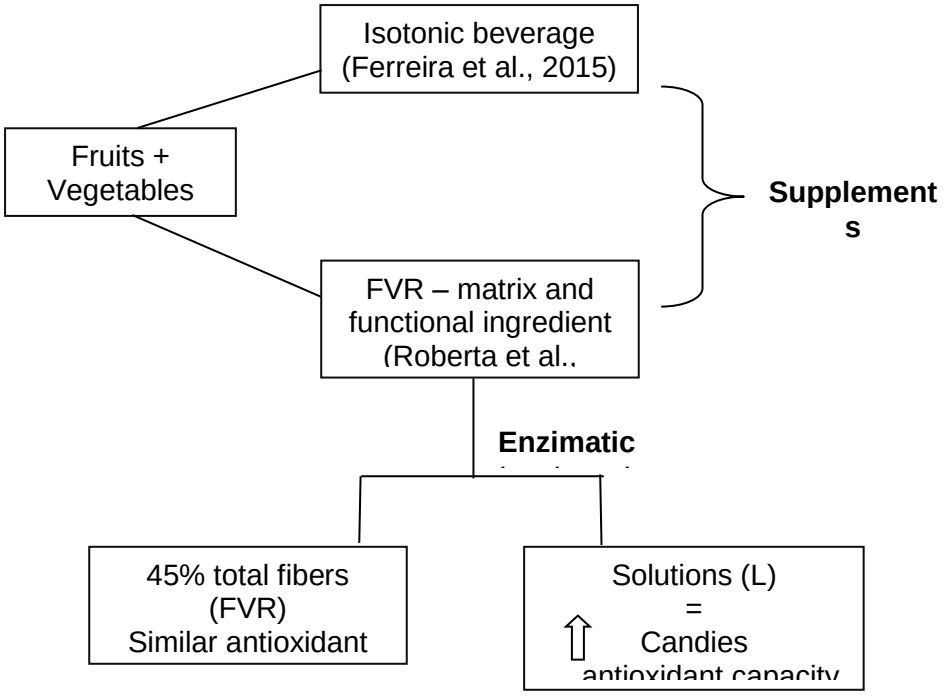


Figure 5. Processing line, isotonic beverage and functional ingredients of fruits and vegetables without residues generation.

4 Conclusion

The high fiber content of FVR flour and the presence of fructooligosaccharides indicate the functional potential of this matrix. Chemical and enzymatic modifications of FVR, respectively, promotes increasing hysteresis and increase fiber. Since FVR flour was exposed to pH conditions different from its native condition, its polymers underwent three-dimensional conformation changes from a three-dimensional polymer structure to an amorphous structure. The alteration from the polymeric structure to amorphous caused a lower stability of the FVR flour when it was exposed to variations of aw, only supporting up to aw = 0.6. The monolayer values were higher in the FRV control samples, defatted and treated with water, when compared to the samples treated with solutions pH 7.0 and 9.0. The result confers on these samples a lower hygroscopicity, which explains the lower affinity for water. The functional capacity of FVR flour after enzymatic treatment was observed. The phenolic content of the samples obtained through the enzymatic process was higher than that found in FVR flour, sample 2 (59.42 ± 12.52), 6 (51.63 ± 11.45) and 10 (60.29 ± 15.12); and the EC50 DPPH values were obtained from sample 2 (0.56 ± 0.05g of sample/g DPPH), sample 6 (0.57 ± 0.06g of sample/g DPPH), and sample 10 (0.55 ± 0.04g of sample/g DPPH).

Processing line, isotonic beverage and functional ingredients of fruits and vegetables without residues

generation indicate four news products, two supplements (isotonic beverage, FVR flour), candies that presented good antioxidant capacity and functional ingredient with good antioxidant capacity and high fiber amount (FVR flour after the enzymatic process) characterizing sustainable process. Ultimately, the wastes of fruit and vegetable processing are a promising source for the recovery of bioactive compounds such as natural antioxidants, as sources of health benefits and functional properties. Recovery of the high-added value compounds has the potential for their use as food additives.

Acknowledgments

The authors are grateful to the Coordination for Improvement of Personnel with Higher Education (CAPES) (23038007379/2011-18), Foundation for Research Support of State of Rio de Janeiro (FAPERJ 26/111.684/2012; 26/010.001651/2014; 26/202.086/2016); Federal University of State of Rio de Janeiro (UNIRIO); Nutritionist Tamara Righetti Tupini Cavalheiro.

Author Contributions

N.S. Mendes, M.C.P. Santos, M.S. Pumar, F.C. Silva, P.P.S. Coimbra assisted in preparing the samples and in the final correction of the work. A.E.C. Faia, J.D.R.P. Souza, H.Y. Kawaguti, S.G. Moreira the supervisors of the article and contributed with an interpretation of data. E.C.B.A. Gonçalves, principal counselor and supervisor of the research.

Conflicts of Interest

The authors declare no conflicts of interest.

References

Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A., & Serna-Saldívar, S. O. (2014). Bound phenolics in foods, a review. *Food Chemistry*, *152*, 46–55. https://doi.org/10.1016/j.foodchem.2013.11.093

Al-Muhtaseb, A. H., McMinn, W. A. M., & Magee, T. R. A. (2002). Moisture Sorption Isotherm Characteristics of Food Products: A Review. *Food and Bioproducts Processing*, *80*(2), 118–128.

https://doi.org/10.1205/09603080252938753

Amaya-Cruz, D. M., Rodríguez-González, S., Pérez-Ramírez, I. F., Loarca-Piña, G., Amaya-Llano, S., Gallegos-Corona, M. A., & Reynoso-Camacho, R. (2015). Juice by-products as a source of dietary fibre and antioxidants and their effect on hepatic steatosis. *Journal of Functional Foods*, *17*, 93–102. https://doi.org/10.1016/j.jff.2015.04.051

Andrade, R. M. S., Ferreira, M. S. L., & Gonçalves, É. C. B. A. (2016). Development and Characterization of Edible Films Based on Fruit and Vegetable Residues. *Journal of Food Science*, *81*(2), E412–E418. https://doi.org/10.1111/1750-3841.13192

AOAC. (1990). Official Methods of Analysis. *Association of Official Analytical Chemist*, *15th*(Volume 2).

AOAC. (2012). Official Methods of Analysis of AOAC International. *Association of Official Analysis Chemists International*, Method ce 2-66. https://doi.org/10.3109/15563657608988149

Arruda, H. S., Pereira, G. A., & Pastore, G. M. (2017). Oligosaccharide profile in Brazilian Cerrado fruit araticum (*Annona crassiflora* Mart.). *LWT - Food Science and Technology*, *76*, 278–283.

Brito, T. B., Carrajola, J. F., Gonçalves, E. C. B. A., Martelli-Tosi, M., & Ferreira, M. S. L. (2019). Fruit and vegetable residues flours with different granulometry range as raw material for pectin-enriched biodegradable film preparation. *Food Research International*, *121*(January), 412–421. https://doi.org/10.1016/j.foodres.2019.03.058

Cassano, A., Conidi, C., Ruby-Figueroa, R., & Castro-Muñoz, R. (2018). Nanofiltration and Tight Ultrafiltration Membranes for the Recovery of Polyphenols from Agro-Food By-Products. *International Journal of Molecular Sciences*, *19*(351), 1–21. https://doi.org/10.3390/ijms19020351

Castro-Muñoz, R., Boczkaj, G., Gontarek, E., Cassano, A., & Fíla, V. (2019). Membrane technologies assisting plant-based and agro-food by-products processing: A comprehensive review. *Trends in Food Science & Technology*. https://doi.org/10.1016/j.tifs.2019.12.003

Castro-Muñoz, R., & Fíla, V. (2018). Membrane-Based technologies as an emerging tool for separating

high-added-value compounds from natural products. *Trends in Food Science & Technology*. https://doi.org/10.1016/j.tifs.2018.09.017

Castro-Muñoz, R., Vlastimil, F., & Durán-Páramo, E. (2017). A Review of the Primary By-product (Nejayote) of the Nixtamalization During Maize Processing : Potential Reuses. *Waste and Biomass Valorization*, *0*(0), 0. https://doi.org/10.1007/s12649-017-0029-4

Caurie, M. (2007). Hysteresis phenomenon in foods. *International Journal of Food Science and Technology*, *42*(1), 45–49. https://doi.org/10.1111/j.1365-2621.2006.01203.x

Codex. (2001). Codex Alimentarius Commission Standards. *CODEX STANDARD FOR HONEY*. https://doi.org/10.1007/978-3-540-88242-8

Cypriano, D. Z., da Silva, L. L., & Tasic, L. (2018). High value-added products from the orange juice industry waste. *Waste Management*, *79*, 71–78. https://doi.org/https://doi.org/10.1016/j.wasman.2018.07.028

Damodaran, S., Parkin, K. L., & Fennema, O. R. (2010). *Quimica de Alimentos de Fennema*. (Artmed, Ed.), *Quimica de Alimentos de Fennema* (4th ed.). Porto Alegre.

de Figueiredo, V. R. G., Yamashita, F., Vanzela, A. L. L., Ida, E. I., & Kurozawa, L. E. (2018). Action of multi-enzyme complex on protein extraction to obtain a protein concentrate from okara. *Journal of Food Science and Technology*, *55*(4), 1508–1517. https://doi.org/10.1007/s13197-018-3067-4

Fai, A. E. C., Souza, M. R. A. de, Barros, S. T. de, Bruno, N. V., Ferreira, M. S. L., & Gonçalves, É. C. B. de A. (2016). Development and evaluation of biodegradable films and coatings obtained from fruit and vegetable residues applied to fresh-cut carrot (*Daucus carota* L.). *Postharvest Biology and Technology*, *112*, 194–204. https://doi.org/10.1016/j.postharvbio.2015.09.021

FAO. (2016). *El Estado Mundial de la Agricultura y la Alimentacion Cambio Climático*.

Ferreira, M. S. L., Santos, M. C. P., Moro, T. M. A., Basto, G. J., Andrade, R. M. S., & Gonçalves, É. C. B. A. (2015). Formulation and characterization of functional foods based on fruit and vegetable residue flour. *Journal of Food Science and Technology*, *52*(2), 822–830.

https://doi.org/10.1007/s13197-013-1061-4

Fidelis, M., do Carmo, M. A. V., da Cruz, T. M., Azevedo, L., Myoda, T., Miranda Furtado, M., … Granato, D. (2020). Camu-camu seed (*Myrciaria dubia*) – From side stream to anantioxidant, antihyperglycemic, antiproliferative, antimicrobial, antihemolytic, anti-inflammatory, and antihypertensive ingredient. *Food Chemistry*, *310*, 125909. https://doi.org/https://doi.org/10.1016/j.foodchem.2019.125909

Gonçalves, E. C. B. A., Lozano-Sanchez, J., Gomes, S., Ferreira, M. S. L., Cameron, L. C., & Segura-Carretero, A. (2018). Byproduct Generated During the Elaboration Process of Isotonic Beverage as a Natural Source of Bioactive Compounds. *Journal of Food Science*. https://doi.org/10.1111/1750-3841.14336

Hernández-Hernández, K. A., Solache-Ríos, M., & Díaz-Nava, M. C. (2013). Removal of brilliant blue FCF from aqueous solutions using an unmodified and iron-modified bentonite and the thermodynamic parameters of the process. *Water, Air, and Soil Pollution*, *224*(5). https://doi.org/10.1007/s11270-013-1562-9

Isah, S., Oshodi, A. A., & Atasie, V. N. (2017). Physicochemical properties of cross linked acha (digitaria exilis) starch with citric acid. *Chemistry International*, *3*(2), 150–157. https://doi.org/10.31221/OSF.IO/USYRC

Kosseva, M. R. (2009). *Chapter 3 - Processing of Food Wastes*. *Advances in Food and Nutrition Research* (1st ed., Vol. 58). Elsevier Inc. https://doi.org/10.1016/S1043-4526(09)58003-5

Kowalska, H., Czajkowska, K., Cichowska, J., & Lenart, A. (2017). What's new in biopotential of fruit and vegetable by-products applied in the food processing industry. *Trends in Food Science & Technology*, *67*, 150–159. https://doi.org/10.1016/j.tifs.2017.06.016

L'homme, C., Peschet, J. L., Puigserver, A., & Biagini, A. (2001). Evaluation of fructans in various fresh and stewed fruits by high-performance anion-exchange chromatography with pulsed amperometric detection. *Journal of Chromatography A*, *920*, 291–297.

Laufenberg, G., Kunz, B., & Nystroem, M. (2003). Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations. *Bioresource Technology*, *87*(2), 167–98.

Laurentiis, V. De, Corrado, S., & Sala, S. (2018). Quantifying household waste of fresh fruit and vegetables in the EU. *Waste Management*, *77*, 238–251. https://doi.org/10.1016/j.wasman.2018.04.001

Maqsood, S., Adiamo, O., Ahmad, M., & Mudgil, P. (2020). Bioactive compounds from date fruit and seed as potential nutraceutical and functional food ingredients. *Food Chemistry*, *308*, 125522. https://doi.org/https://doi.org/10.1016/j.foodchem.2019.125522

Mendes, N. D. S., Santos, M. C. P., Santos, M. C. B., Cameron, L. C., Ferreira, M. S. L., & Gonçalves, É. C. B. A. (2019). Characterization of pepper (*Capsicum baccatum*) - A potential functional ingredient. *LWT - Food Science and Technology*, *112*(January), 108–209. https://doi.org/10.1016/j.lwt.2019.05.107

Mendes, N. de S., Favre, L. C., Rolandelli, G., Ferreira, C. dos S., Gonçalves, É. C. B. de A., & Buera, M. del P. (2019). Flour from "fruits and vegetables" waste with addition of a South-American pepper (*Capsicum baccatum*) proposed as food ingredient. *International Journal of Food Science + Technology*. https://doi.org/10.1111/ijfs.14358

Meyer, A. S., Dam, B. P., & Lærke, H. N. (2009). Enzymatic solubilization of a pectinaceous dietary fiber fraction from potato pulp: Optimization of the fiber extraction process. *Biochemical Engineering Journal*, *43*(1), 106–112. https://doi.org/10.1016/j.bej.2008.09.006

Mirabella, N., Castellani, V., & Sala, S. (2014). Current options for the valorization of food manufacturing waste: a review. *Journal of Cleaner Production*, *65*, 28–41. https://doi.org/10.1016/j.jclepro.2013.10.051

Mochamad Busairi, A. (2008). Conversion of pineapple juice waste into lactic acid in batch and fed-batch fermentation systems. *Reaktor*, *12*(2), 98. https://doi.org/10.14710/reaktor.12.2.98-101

Mudgil, D., Barak, S., & Khatkar, B. S. (2014). Guar gum: processing, properties and food applications—A Review. *Journal of Food Science and Technology*, *51*(3), 409–418. https://doi.org/10.1007/s13197-011-0522-x

Oliveira, D. M., Clemente, E., & da Costa, J. M. C. (2014). Hygroscopic behavior and degree of caking of grugru palm (*Acrocomia aculeata*) powder. *Journal of Food Science and Technology*, *51*(10), 2783–2789. https://doi.org/10.1007/s13197-012-0814-9

Otemuyiwa, I. O., Williams, M. F., & Adewusi, S. A. (2017). Antioxidant activity of health tea infusions and effect of sugar and milk on in-vitro availability of phenolics in tea, coffee and cocoa drinks. *Nutrition & Food Science*, *47*(4), 2017. https://doi.org/10.1108/NFS-08-2016-0134

Park, S. Y., & Yoon, K. Y. (2015). Enzymatic Production of Soluble Dietary Fiber from the Cellulose Fraction of Chinese Cabbage Waste and Potential Use as a Functional Food Source. *Food Sci. Biotechnol.*, *24*(2), 529–535. https://doi.org/10.1007/s10068-015-0069-0

Rajha, H. N., El Kantar, S., Afif, C., Boussetta, N., Louka, N., Maroun, R. G., & Vorobiev, E. (2018). Selective multistage extraction process of biomolecules from vine shoots by a combination of biological, chemical, and physical treatments. *Comptes Rendus Chimie*, *21*(6), 581–589. https://doi.org/10.1016/J.CRCI.2018.02.013

Roberta, M. S. A., Mariana, S. L. F., Édira, C. B. A. G., Andrade, R. M. S. de., Ferreira, M. S. L., & Gonçalves, E. C. B. A. (2014). Functional capacity of flour obtained from residues of fruit and vegetables. *International Food Research Journal*, *21*(4), 1675–1681.

Rosset, M., Prudencio, S. H., & Beléia, A. D. P. (2012). Viscozyme L action on soy slurry affects carbohydrates and antioxidant properties of silken tofu. *Food Science and Technology International*, *18*(6). https://doi.org/10.1177/1082013211433076

Sancho, R. A. S., Souza, J. D. R. P., Lima, F. A. De, & Pastore, G. M. (2017). Evaluation of oligosaccharide profiles in selected cooked tubers and roots subjected to in vitro digestion. *LWT - Food Science and Technology*, *76*, 270–277. https://doi.org/10.1016/j.lwt.2016.07.046

Santos, M. C. P., & Gonçalves, É. C. B. A. (2016). Effect of different extracting solvents on antioxidant activity and phenolic compounds of a fruit and vegetable residue flour. *Scientia Agropecuaria*, *7*(1), 7–14. https://doi.org/10.17268/sci.agropecu.2016.01.01

Sette, P., Fernandez, A., Soria, J., Rodriguez, R., Salvatori, D., & Mazza, G. (2020). Integral valorization of fruit waste from wine and cider industries. *Journal of Cleaner Production*, *242*, 118486. https://doi.org/https://doi.org/10.1016/j.jclepro.2019.118486

Shadrach, I., Banji, A., & Adebayo, O. (2020). Nutraceutical potential of ripe and unripe plantain peels: A comparative study. *Chemistry International*, *6*(2), 83–90. https://doi.org/10.5281/ZENODO.3364199

Shea, N. O., Arendt, E. K., & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science and Emerging Technologies*, *16*, 1–10. https://doi.org/10.1016/j.ifset.2012.06.002

Shirzad, M., Panahi, H. K. S., Dashti, B. B., Rajaeifar, Mohammad Ali Aghbashlo, M., & Tabatabaei, M. (2019). A comprehensive review on electricity generation and GHG emission reduction potentials through anaerobic digestion of agricultural and livestock/slaughterhouse wastes in Iran. *Renewable and Sustainable Energy Reviews*, *111*(November 2018), 571–594. https://doi.org/10.1016/j.rser.2019.05.011

Singleton, V. L., & Rossi, S. A. (1965). Colorimetric of total phenolics with phosphomolibicphosphotungstic acid reagents. *J. Enol. Vitic.*, *16*(3), 144–158. https://doi.org/10.12691/ijebb-2-1-5

Timmermann, E. O., Chirife, J., & Iglesias, H. A. (2001). Water sorption isotherms of foods and foodstuffs: BET or GAB parameters? *Journal of Food Engineering*, *48*, 19–31.

Varadharajan, V., Shanmugam, S., & Ramaswamy, A. (2017). Model generation and process optimization of microwave-assisted aqueous extraction of anthocyanins from grape juice waste.

Journal of Food Process Engineering, *40*(3), e12486. https://doi.org/10.1111/jfpe.12486

Waterhouse, G. I. N., Sun-Waterhouse, D., Su, G., Zhao, H., & Zhao, M. (2017). Spray-Drying of Antioxidant-Rich Blueberry Waste Extracts; Interplay Between Waste Pretreatments and Spray-Drying Process. *Food and Bioprocess Technology*, *10*(6), 1074–1092. https://doi.org/10.1007/s11947-017-1880-9