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Characterization and utilization of fruit and vegetable residue flour for the development of functional foods

Caracterização e aproveitamento de farinha de resíduos de frutas e hortaliças para o desenvolvimento de alimentos funcionais

Caracterización y utilización de harinas residuales de frutas y hortalizas para el desarrollo de alimentos funcionales

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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 processing; Flour; Carbohydrates; Microstructure; Candies.

Resumo

A farinha de frutas e resíduos vegetais (FVR) foi obtida a partir do resíduo sólido gerado no processamento total de frutas inteiras (3) e vegetais (8). O objetivo deste estudo foi analisar o perfil de carboidratos da farinha de FVR e propor estruturas de modificação química e enzimática para uso como ingrediente funcional. As propriedades como comportamento de sorção, conteúdo fenólico total e atividade antioxidante também foram avaliadas. Além disso, a farinha FVR foi aplicada na produção de balas. A farinha FVR apresentou apenas 1-kestose (GF2) como oligossacarídeos prebióticos e condição nativa, alteração da conformação de uma estrutura amorfa após diferentes condições de pH, que causou menor estabilidade da farinha FVR quando exposta a variações de aw, suportando apenas até aw = 0,6. O GAB foi o modelo mais adequado para construir isotermas de sorção. O conteúdo fenólico das amostras obtidas pelo processo enzimático foi superior ao encontrado na farinha FVR, amostra 10 (60,29 ± 15,12 mg) e os valores de atividade antioxidante 0,55 ± 0,04g amostra / g DPPH. O conteúdo fenólico da goma e dos doces de cristal, respectivamente, é de 0,289 ± 0,097 mg GAE.g-1 e 0,228 ± 0,011 mg GAE.g-1. Este estudo mostra que é possível promover o processamento viável e sustentável de alimentos sem geração de resíduos.

Palavras-chave: Processamento sustentável; Farinha; Carboidratos; Microestrutura; Doces.

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 processing; Flour; Carbohydrates; Microstructure; Candies.

Resumen

La harina de residuos de frutas y hortalizas (FVR) se obtuvo a partir del residuo sólido generado en todo el procesamiento de frutas y hortalizas enteras (3) y hortalizas (8). El propósito de este estudio fue analizar el perfil de carbohidratos de la harina FVR y proponer una estructura de modificación química y enzimática para usar como ingrediente funcional. También se evaluaron propiedades como comportamiento de sorción, contenido fenólico total y actividad antioxidante. Además, la harina FVR se aplicó para producir dulces. La harina FVR presentó solo 1-kestosa (GF2) como oligosacáridos prebióticos y condición nativa, cambios de conformación a partir de una estructura amorfa luego de diferentes condiciones de pH, que causaron menor estabilidad de la harina FVR cuando fue expuesta a variaciones de aw, solo soportando hasta aw = 0,6. El GAB fue el modelo más adecuado para construir isotermas de sorción. El contenido fenólico de las muestras obtenidas mediante el proceso enzimático fue superior al encontrado en la harina FVR, muestra 10 (60,29 ± 15,12 mg) y los valores de actividad antioxidante 0,55 ± 0,04g de muestra / g DPPH. Los caramelos de goma y cristal con contenido fenólico, respectivamente, es 0,289 ± 0,097 mg GAE.g-1 y 0,228 ± 0,011 mg GAE.g-1. Este estudio muestra que es posible promover un procesamiento de alimentos viable y sostenible sin generación de residuos.

Palabras clave: Procesamiento sostenible; Harina; Carbohidratos; Microestructura; Dulces.

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 et al., 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 et al., 2018), as well as for the production of nutraceuticals from these by-products (Castro-Muñoz et al., 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% (De Laurentiis et al., 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 et al., 2019).

Juices obtained from fruits generate large amounts of waste, such as peels, which are a potential source of dietary fiber (Cypriano et al., 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 (de Sá Mendes, Favre, et al., 2019; Fidelis et al., 2020; Maqsood et al., 2020; Sette et al., 2020; Shadrach et al., 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 et al., 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 et al., 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

2.1 Chemical reagents

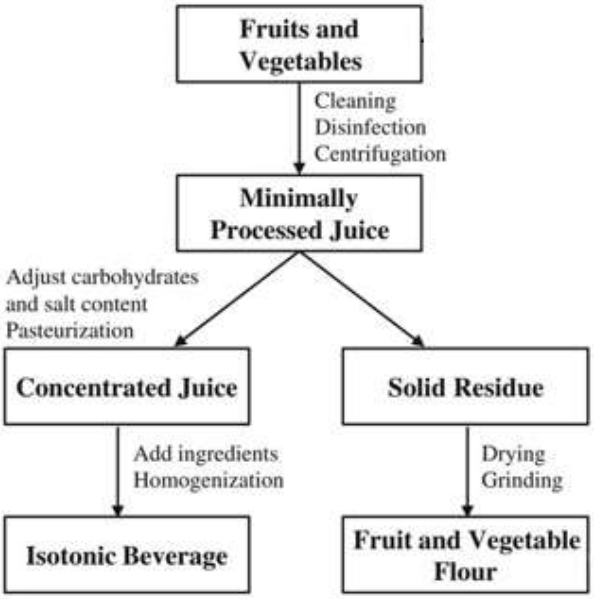
All chemical reagents and solvents applied in this study were of analytical grade (P.A.) obtained from Sigma - Aldrich Brazil.

2.2 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:

2.2.1 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).

2.3 Chemical modification of the structure of FVR flour

2.3.1 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 (R. M. S. Andrade et al., 2016). After filtration, FVR flour was dried in a conventional oven at 105 °C (AOAC, 2012).

2.3.2 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 (R. M. S. Andrade et al., 2016). After filtration, FVR flour was dried in a conventional oven at 105 °C (AOAC, 2012).

2.3.3 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).

2.3.4 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	$X_e = \frac{(X_m C K a_w)}{(1-K a_w)(1-K a_w + C K a_w)}$	(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. Source: Authors.

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

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

Source: Authors.

2.3.5 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 et al., 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).

2.3.6 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 (AOAC, 1990).

2.3.7 Functional Capacity of FVR Flour After Enzymatic Modification

2.3.8 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).

2.3.9 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.).

2.3.10 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

3.1 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 et al., 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.

Source: Authors.

3.2 Chemical Modification in the Structure of FVR Flour

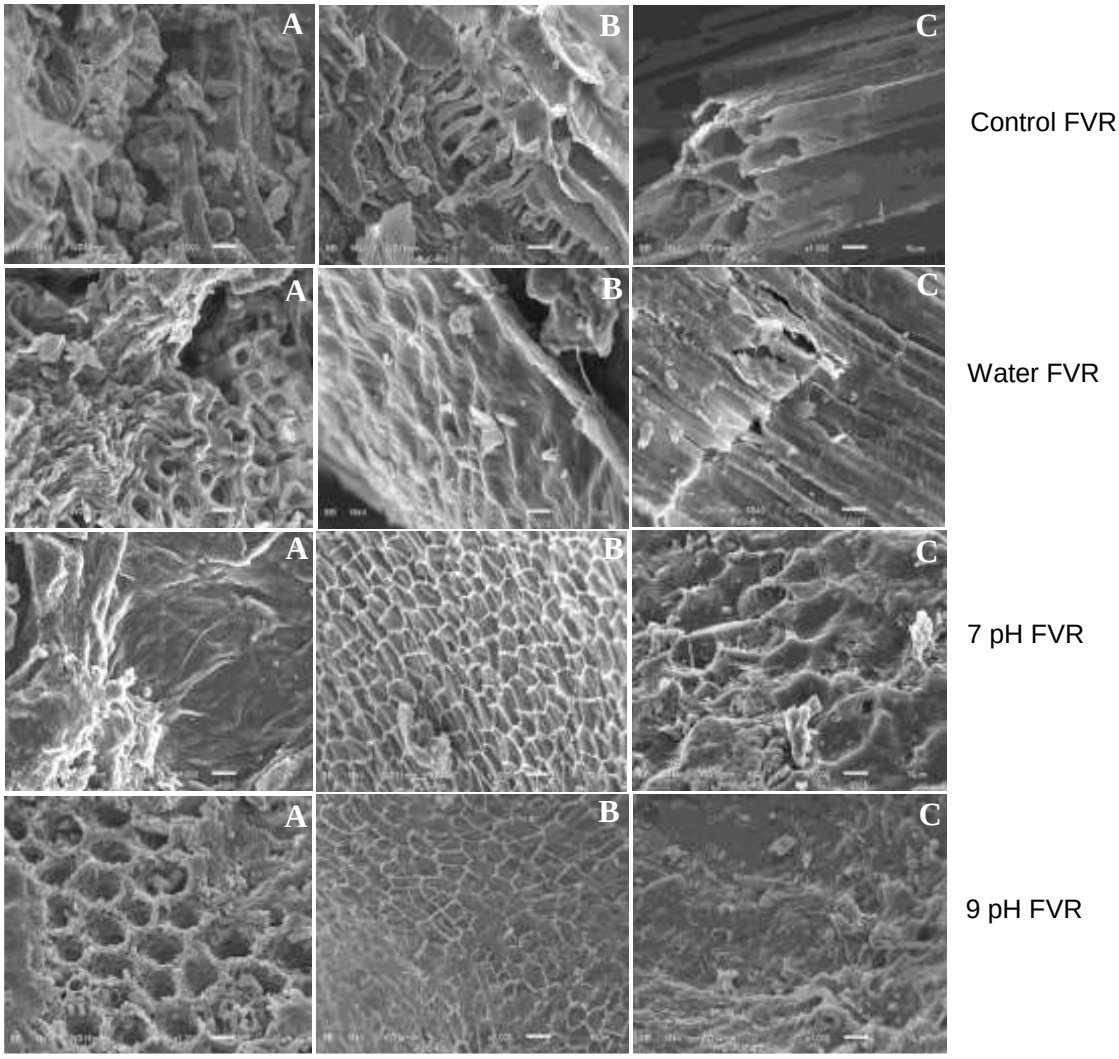
3.2.1 Microstructure

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).

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 et al., 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 (R. M. S. Andrade et al., 2016; Isah et al., 2017).

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.



Source: Authors.

3.3 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

$_m$, *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.

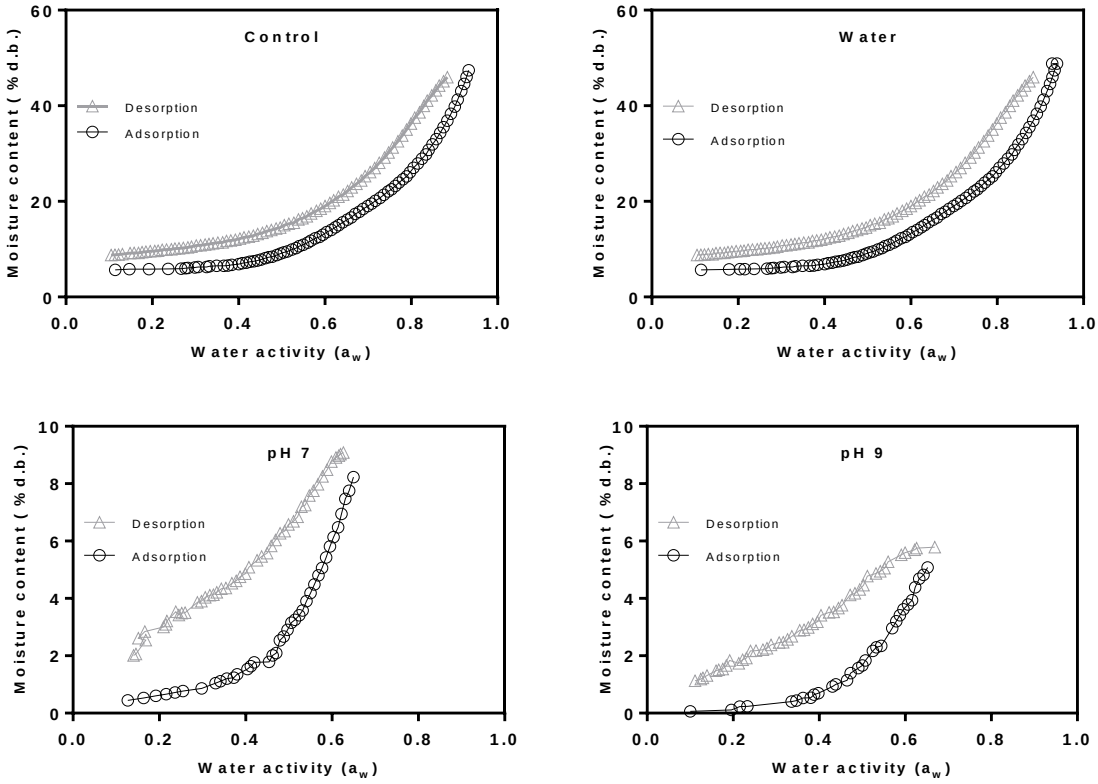
Source: Authors.

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 et al., 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 (R. M. S. Andrade et al., 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.



Source: Authors.

Regarding the hysteresis, according to Caurie (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.

3.4 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 ($p < 0.05$).

Source: Authors.

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. (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 et al., 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).

3.5 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 et al., 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 et al., 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 et al., 2017).

3.6 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 et al., 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.

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

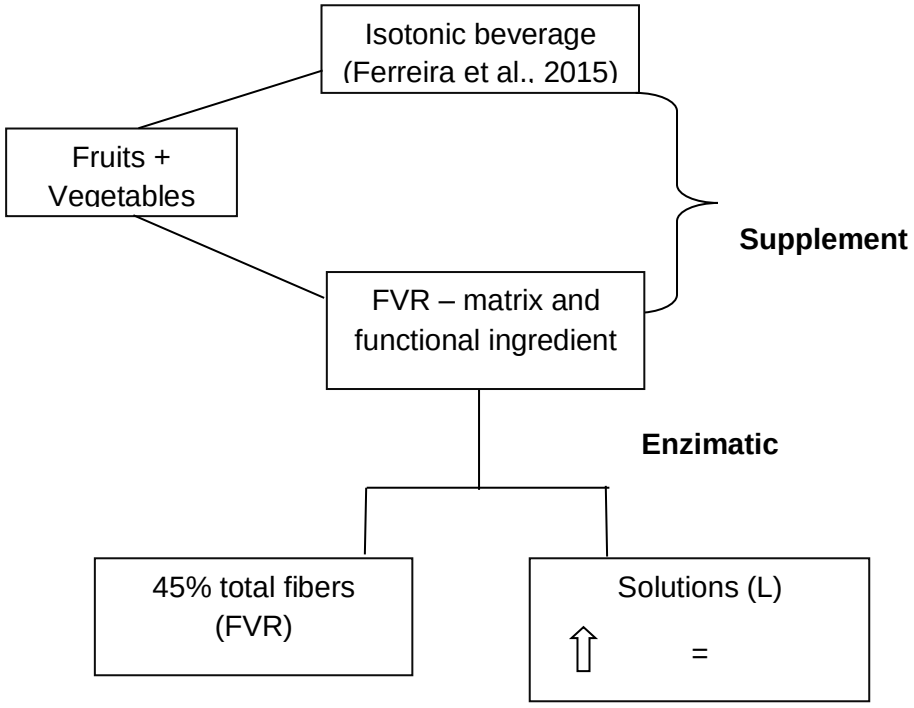

(A)

(B)

Source: Authors.

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.



Source: Authors.

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.

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Conflicts of Interest

The authors declare no conflicts of interest.

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