

Functional capacity of flour obtained from residues of fruit and vegetables

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Article history	<u>Abstract</u>
Received 20 December 2012	Food resid

Received: 30 December 2013 Received in revised form: 12 February 2014 Accepted: 13 February 2014 Kevwords

Fruits and vegetables Residue flour Bowel function Constipation Dietary fibres Antioxidant capacity Food residues represent a rich source of functional compounds such as dietary fibres that may improve bowel function and help to overcome constipation. In this study, fruit and vegetable residue (FVR) flour was evaluated according to the antioxidant activity, dietary fibres and functional capacity through the bowel function improvement. Sixteen female volunteers answered a survey after daily consumption of FVR flour (10 g) for 10 days. The methanolic extract showed a noteworthy antioxidant capacity (61% DPPH inhibition). The insoluble dietary fibres represented 80% total dietary fibres (48.4%). The daily consumption of FVR flour improved constipation symptoms in 87.5% volunteers. After 3 days, 70% volunteers presented significant progress in the bowel function. Body mass index presented a positive relation with constipation symptoms after the consumption of FVR flour. Remarkable dietary fibre content associated with the antioxidant capacity appointed to the strong potential of FVR to overcome constipation and to add nutritional value.

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Introduction

In the last decades, the food industry has been marked by the large volume of waste produced (FAO, 2011). Fruits and vegetables are extensively processed generating a large amount of residue which is frequently discarded. However, agro-industrial residues obtained from fruits and vegetables processing represent a nutritional, inexpensive and environmentally friendly raw material. These residues have called attention to be considered a good source of bioactive compounds, such as polyphenols, carotenoids, vitamins and dietary fibres (Figuerola *et al.*, 2005; Makris *et al.*, 2007; Ajila and Prasada Rao, 2013).

The bioactive compounds have received increasing attention due to their potential role in the prevention and treatment of human diseases through a variety of mechanisms, mainly related to gastrointestinal health and prevention of chronic degenerative diseases (ADA, 2008; Saura-Calixto and Goñi, 2009; Chong et al., 2010; Sun-Waterhouse, 2011). Due to these health benefits, there is a growing demand for functional food and ingredients by consumers (Figuerola et al., 2005). Among the functional compounds, several recent researches have focused on the physiological effects resulting from the human consumption of a wide variety of dietary fibres (Papathanasopoulos and Camilleri, 2010; Montella et al., 2013; Waitzberg et al., 2013). In an exhaustive study, about the relation between fibre intake and constipation, were found that increasing fibre intake may represent an important, inexpensive and feasible therapeutic measure for this widespread digestive complaint (Dukas *et al.*, 2003).

The prevalence of constipation in the worldwide general population can reach 79% being more prevalent and symptomatic in women than men (Mugie *et al.*, 2011). Constipation is a symptom that, although caused by many different disorders, is mostly attributable to functional constipation, which is not associated to organic and anatomic causes or intake of medication. Lack of dietary fibre is known to contribute to constipation, and the primary therapy is based on the increase of fibre intake, along with lifestyle modifications such as improved hydration (Grigelmo-Miguel *et al.*, 1999; Suares and Ford, 2011; Yang *et al.*, 2012).

Dietary fibre can be divided into two categories: the water-soluble and insoluble fractions, presenting different properties. The SDF, such as pectin and gums, dissolves in water forming a gel and is associated with the reduction of cholesterol in blood (Chao *et al.*, 1993). In addition, the SDF can be fermented by bacteria in the colon in a greater extent than IDF, reducing intestinal glucose absorption (Montella *et al.*, 2013). The insoluble part formed by cellulose, hemicelluloses and lignin is related to both water absorption and intestinal regulation.

In terms of health benefits, a balanced ratio of 70-50% insoluble and 30-50% soluble dietary fraction is considered satisfactory. In fruits, the ratio between IDF and SDF fractions is more balanced compared to cereal (Saura-Calixto, 1993; Ajila and Prasada Rao, 2013). Although extensively used as fibre source in food products, the SDF in cereal outer layer fractions attained only about 3% (dry basis) (Griguelmo-Miguel et al., 1999). Thus, plant residues have substantial amounts of total and soluble dietary fibre and may represent a suitable fibre source for use as food ingredient (Nawirska and Kwasniewska, 2005). In fruits and vegetables, the insoluble fraction, mainly represented by cellulose, is resistant to digestion and adsorption in the human small intestine, presenting a complete or partial fermentation in the large intestine, contributing to the acceleration of intestinal transit time (Nawirska and Kwasniewska, 2005; Suares and Ford, 2011; Ajila and Prasada Rao, 2013).

In addition, pomace, peel and seed fractions of some fruits possess higher antioxidant activity than the pulp fractions. Further attention has been paid to these fractions, since natural antioxidants have been associated with reduced risk of chronic diseases and the protection of essential molecules against damage (Guo *et al.*, 2003; Babbar *et al.*, 2011). A recent study conducted in vivo showed that the aqueous extract of passion fruit can reduce oxidative stress (Silva *et al.*, 2013).

Despite the noticeable benefits and recommendations, the intake of plant foods remains low and, consequently, both dietary fibre and antioxidant compounds are usually deficient in most diets around the world (Saura-Calixto and Goñi, 2009). Since plant residues are rich in essential nutrients and available for the population consumption, they have been applied as food ingredients in the development of functional food products (Ayla-Zavala et al., 2011; Sun-Waterhouse, 2011; Ferreira et al., in press). In this way, the aim of this study was to characterize the flour obtained from residues of whole fruit and vegetable processing (FVR flour) in terms of dietary fibre content (SDF and IDF) and antioxidant capacity (DPPH assay) and evaluate its functionality through the improvement of bowel function in adult women with constipation.

Materials and Methods

Fruit and vegetable residue (FVR) flour processing

The FVR flour was formulated based on the solid residue obtained after preparation of isotonic beverage by processing whole fruits and vegetables (Martins *et al.*, 2011). The FVR flour was composed by the following species: Selecta orange (*Citrus sinensis*), passion fruit (*Passiflora edulis*), watermelon (*Citrullus lanatus*), lettuce (*Lactuca*) sativa), courgette (*Cucurbita pepo*), carrot (*Daucus carota*), spinach (*Spinacea oleracea*), mint (*Mentha sp*), taro (*Colocasia esculenta*), cucumber (*Cucumis sativus*) and rocket (*Eruca sativa*). The FVR flour was prepared as previously shown by Ferreira *et al.* (in press).

Determination of DPPH radical scavenging activity

Flour samples (500 mg) were extracted with 40 mL methanol (100%), at room temperature (RT) for 60 minutes. After centrifugation at 1.500 x g for 15 minutes, the supernatant obtained were transferred to 100 mL volumetric flask and completed with distilled water. The antioxidant capacity of the flour was analyzed by DPPH radical scavenging activity, with minor modifications (Brand-Williams et al., 1995). In the dark, aliquots of 1.0 mL of the extract were transferred to tubes containing 2.0 mL of 0.06 mM DPPH in methanol and then homogenized. The standard curve was established from 10 μ M to 60 μ M DPPH in methanol. The absorbance was measured in triplicate at 517 nm after 60 minutes. The radical scavenging activity of FVR flour was expressed in terms of inhibition percentage of DPPH calculated according to the following equation, where control absorbance was the DPPH solution absorbance:

% Inhibition = (control absorbance – sample absorbance) x 100 (control absorbance)

Total, soluble and insoluble dietary fibre contents

Soluble (SDF) and insoluble dietary fibre (IDF) fractions were analyzed in triplicate, according to the AOAC (2000) Method 991.43 based on the enzymatic-gravimetric procedure (Prosky *et al.*, 1984; AOAC, 2000). Total dietary fibre (TDF) was calculated by the sum of soluble and insoluble dietary fibres.

Evaluation of functional capacity of FVR flour

The functional capacity was evaluated by the effect of daily consumption of FVR flour in the improvement of bowel function in adult women with intestinal constipation. Before selecting, female volunteers were submitted to a questionnaire to evaluate inclusion in the group of interest. The evaluation of constipation (dependent variable) was performed according to the Rome II criteria (Drossman *et al.*, 2000). The volunteers must have three or more of the criteria fulfilled in the last three months. The independent variables were the clinical features: age (20 to 57 years) and body mass index (BMI) (20 to 29 kg.m⁻²). Exclusion criteria were personal history of colorectal cancer, ulcerative colitis or Crohn's disease and use of synthetic laxatives.

Evaluation of consumption of FVR flour on bowel function

The study protocol was approved by the Ethics Committee of the Federal University of the Rio de Janeiro State with Certificate of Presentation for Ethics Appreciation number 0009.0.313.000-08. Written informed consent was signed by all participants in accordance with the Ethics Committee of the University. The selected 16 female volunteers consumed on a daily basis for 10 days, 10 g of FVR flour and answered a survey which assessed the frequency of bowel movements, the time that the FVR flour was consumed like with which food it was consumed and changes in lifestyle during the period. It was not request that the volunteers alter their diet in any stage of the research, and the FVR flour was consumed along with any food.

Statistical analyses

All analyzes were performed in triplicate and the results presented as mean followed by standard deviation (SD). Constipation symptoms improvement was expressed as a day score (\leq Day 3 and Day 4 -Day 10) as well as the number of bowel movements (evacuations). Comparisons between two groups were performed by Tukey's test and the significance threshold was set at 5% (P < 0.05). The statistical analysis of the results was performed by the software program XLSTAT 2012, Addinsoft.

Results and Discussion

Fruit and vegetable residues, consisting of stalks, peels, seeds, stems and pomace, although largely wasted, are important sources of nutrients and have been used to develop new products with enhanced functional and nutritional value (Figuerola et al., 2005; Sun-Waterhouse, 2011; Ferreira et al., in press). In a recent study conducted by our research group, the FVR flour obtained was incorporated into cereal bars and biscuits formulations. The chemical, microbiological and sensorial results of the designed products attested the aptness for the use of FVR flour in food products as a new lowcaloric and functional raw material (Ferreira et al., in press). Remarkably, the FVR flour showed high contents of available carbohydrates (53%), crude fibre (21.5%), and significant protein (9.5%) and lipid (5%) contents and were very similar to the proximate composition of fibre concentrates from apple pomace and citrus peel (Figuerola et al., 2005). Altogether, in the present study the FVR flour (Figure 1) was evaluated as potential functional product applied in the improvement of gastrointestinal disorders.

Table 1. Total,	, soluble and	insolubl	e dietary	fibre content
and inhibi	tion percenta	age of D	PPH of F	VR flour

Parameters	Values (%)	
Soluble dietary fiber	9.56 ± 0.88	
Insoluble dietary fiber	38.82 ± 0.55	
Total dietary fiber	48.42 ± 1.43	
% Inhibition of DPPH.	60.98 ± 0.56	
Total, soluble and insoluble die	tary fibers are expressed on d	Irv

matter basis. All data are the mean \pm SD of three replicates.



Figure 1. Fruit and vegetable residue (FVR) flour

Total, soluble and insoluble dietary fibre contents and Inhibition percentage of DPPH

As expected, the TDF content (Table 1) was significantly higher than the crude fibre content of the FVR flour (21.52 \pm 1.61%) (Ferreira *et al.*, in press). Indeed, the crude fibre content gives just a relative idea of the total amount of fibres and may underestimate the true amount from 2 to 16 fold (DeMan, 1999). This is due to the steps performed on the crude fibre determination, where the most part of soluble fraction is lost during the acid digestion to eliminate interfering components such as proteins and carbohydrates. To receive claim of high fibre content, food should have at least 6 g of dietary fibre per 100 g (CODEX, 2009). Thus, FVR flour can be considered a high fibre product, which may help supply the daily requirement of fibre that is 25 g dietary fibre for adult women and 38 g for adult men (IOM, 2002).

The TDF content of FVR flour (48.42% DM) was similar to those reported on grape seed flour (47%) (Özvural and Vural, 2011), raw mango peels (41%) (Ajila and Prasada Rao, 2013) and slight higher than fibre concentrates extracted of peach (30-36% DM) (Grigelmo-Miguel *et al.*, 1999) and grapefruit peel (44.2% DM) (Figuerola *et al.*, 2005). Nonetheless, the TDF of the FVR flour was considerably lower than fruit and vegetable pomace, which ranged between 54-99% DM (Nawirska and Kwaśniewska, 2005).

The content of insoluble fibre (38.82% DM) of FVR flour represented 80% of total dietary fibres. Compared to previous studies, this IDF content was similar to that reported on grapefruit peel (37% DM) (Figuerola *et al.*, 2005) and higher than that of raw mango peels (28%) (Ajila and Prasada Rao, 2013) and peach fibre concentrates (20-23% DM) (Grigelmo-Miguel *et al.*, 1999). Moreover, the insoluble fraction in FVR flour was also higher for

vegetable pomace, which ranged 18% to 35% DM (Nawirska and Uklańska, 2008). Nevertheless, the same authors found superior values (34% to 98% DM) when another method for the determination of dietary fibres fractions were used (Nawirska and Kwaśniewska, 2005), showing the strong influence of the chosen method in these determinations (Li *et al.*, 2002). In this study, the method used was the same adopted for nutrition labelling of dietary fibre by the U.S. Food and Drug Administration (AOAC Method 991.43) (AOAC, 2000).

Fibre derived from fruit (pulp) and vegetables (leaves and flowers) has been described to contain a significant proportion of soluble dietary fibre compared to other foods. However, the proportion of IDF to SDF varies according to the species and the part of the plant in which it was extracted. The vegetable residues (i.e. pomace, stem and seeds) generally have higher proportion of IDF mainly due to the lignin and cellulose contents (Li et al., 2002; Figuerola et al., 2005; Nawirska and Kwaśniewska, 2005). In this study, even if the SDF fraction presented low levels than the previous cited works (Figuerola et al., 2005; Nawirska and Kwaśniewska, 2005; Nawirska and Uklańska, 2008; Ajila and Prasada Rao, 2013) the ratio IDF/SDF was about 4:1 remaining attractive in terms of functionality. It is interesting to note, that despite the quite low level of SDF, the FVR flour presented a high water holding capacity (7.43 g water g⁻¹ FVR flour DM) (Ferreira *et al.*, in press), which can be associated with the high levels of IDF (Figuerola et al., 2005).

Scavenging activity of FVR flour was expressed as inhibition percentage of DPPH and was found in the range of antioxidant activity (43 to 83%) reported in a previous study conducted on several plant residues, such as kinnow seed, kinnow peel, litchi seed, litchi pericarp, grape seed and banana peel (Babbar et al., 2011). In addition, the methanolic extract of the FVR flour presented higher antioxidant activity than fruit residues obtained from pineapple and passion fruit (20-25%) (Oliveira et al., 2009). The FVR flour presented promising results as a natural antioxidant and may even be evaluated as an alternative to synthetic antioxidant. Natural antioxidants compounds increase the food stability not only by preventing lipid peroxidation, but also protecting biomolecules from oxidative damage (Babbar et al., 2011).

Polyphenols, soluble vitamins C and B, vitamins A, B and E and also carotenoids are the most abundant antioxidants in fruits and vegetables. However, by applying a sequential extraction procedure, Pellegrini *et al.* (2007) showed that vitamin C and phenolic

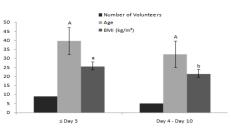


Figure 2. Number of volunteers, mean age and BMI (kg/m²) who showed improvement of constipation in less than 3 days of flour consumption (≤ Day 3) and after 4 days (Day 4 - Day 10). Means followed by different letter means significant difference (p > 0.05).

compounds present in water and acetone extracts were the main contributors to the strong antioxidant activity in some fruits and vegetables. These authors suggested that the total antioxidant capacity of food samples are affected by the solvents used during extraction. Since the FVR flour presented important lipid content, it can be crucial to ensure the complete recovery of antioxidants compounds in a sequential extraction procedure. Indeed, the synergistic effect of these antioxidants in combination with other compounds, such as dietary fibre, may contribute to the protective effects of fruits and vegetables against disease (Saura-Calixto and Goñi, 2009). The FVR flour presented a high antioxidant activity added to significant dietary fibre content, which support its functional properties.

Functional capacity evaluation of RVF flour

In the Figure 2 is represented the number of volunteers who reported constipation symptoms improvement during the daily consumption of the FVR flour. Over the 10 days of the evaluation of bowel function, 87.5% of the volunteers reported a significant improvement in constipation symptoms without changing the habitual diet. Among them, about 70% showed improvement in just 3 days of consumption (\leq 3 Days). These findings clearly suggest that the daily use of the flour may improve symptoms of constipation within a week. The improvement can be directly related to the high total and insoluble dietary fibre content in the FVR flour. As shown before, fruit and vegetable residues (peels, seeds, stems, etc.) are rich sources of insoluble fibre (cellulose and hemicelluloses) and the fibre undergoes minimal change in the digestive tract and shortens colonic transit, causing an increase in the faecal mass and improving the symptoms of constipation (Bijkerk et al., 2004; Ajila and Prasada Rao, 2013).

Low fibre intake is commonly associated with a delay in intestinal transit time. In an exhaustive study conducted with adult women was found that fibre intake was inversely associated with low bowel

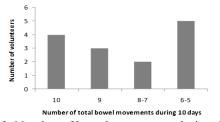


Figure 3. Number of bowel movements during the 10 days of daily consumption of the FVR flour

movement frequency (Dukas *et al.*, 2003). The dough produced mainly from insoluble fibre, or by increasing the microbial mass is required to provide a normal evacuation. Thereby, the increase in the daily intake of insoluble fibre can increase stool weight, promoting normal laxation (Marlett *et al.*, 2002; Slavin, 2005).

In this study, the variation of the age of the volunteers had no influence on the improvement in constipation observed during the FVR flour consumption (Figure 2). However, it was found that the average of body mass index (BMI) showed significant difference between the groups (Figure 2) and the group with higher mean BMI showed faster response (less than 3 days) in constipation symptoms after the consumption of FVR flour. Adults who are overweight have, generally, diets with high energy density and low in dietary fibre (Delgado-Aros et al., 2004). The association between BMI and constipation is not yet clear, however, it can be suggested that the fibre inclusion, such as by the consumption of FVR based flour, in the diet of individuals with reduced consumption would benefit in a faster way than in individuals with adequate fibre intake.

Constipation is also attributed to abnormal consistency of stools, sensation of incomplete evacuation, sensation of obstruction or anorectal blockage and manual manoeuvres to facilitate defecation. Drawn up in 2006, the Rome III criteria includeall these information in diagnosing constipation (Burgers et al., 2012; Waitzberg et al., 2013). When applying the Rome II criteria, the exclusion of such information may be considered too restrictive and the requirement that only two of the six symptoms also very permissive to accurately identify those with constipation (Pare et al., 2001). Usually constipation is defined as less than 3 bowel movements per week. Generally, stool weight is significantly increased by adding sources of insoluble fibre to the diet, and thereby normalizes bowel function in patients with constipation (Chen et al., 1998; ADA, 2008). Most part of volunteers (n = 10) presented between 5 to 9 bowel movements during the 10 days analyzed (Figure 3). This result is consistent with those recent reported in a meta-analysis study (Yang et al., 2012).

These authors related a significant increase in the number of stools per week in the group submitted to the fibre based diet. Even if positive trends in favour of dietary fibre group have been showed, the effect of dietary fibre on different grades of constipation can be different.

Conclusion

Fruit and vegetable residue flour, formulated in order to promote the use of residues from industrial processing, represents a rich source of dietary fibre with a suitable IDF to SDF ratio and can be used in the food products development of added nutritional value. The flour promoted a prompt increase in the number of bowel movements in adult women volunteers. A prominent antioxidant activity with similar to higher values than those reported in several plant residues were found in the FVR flour. Further studies are being conducted to identify the functional compounds involved in the radical scavenging activity and to establish their bioavailability.

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