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Research Article

Comparative Analysis of Dietary Fibre Extract Isolated from Citrus Juice By-products using Water Extraction, Fermentation and Enzymatic Treatment Methods

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Abstract: Citrus juice by-products are raw materials that have attracted considerable attention as a potential Dietary Fibre (DF) source and a potential ingredient in a healthy diet. In the present study, we evaluate physico-chemical, nutritional and microstructure characteristics of dietary fibres from citrus juice by-products. The effect of three treatments methods (fermentation, enzyme and water bath treatment) on physico-chemical properties was evaluated in order to enhance the value of DF from a functional point of view. Fermentation treatment samples exhibit the highest DF content (77.91 g/100 g) compared with water bath (62.69 g/100 g) and enzyme (64.12 g/100 g) treated samples. Furthermore, when compared to previous reported data, these fermentation samples display some favourable functional characteristics, such as enhanced water retention (13.31 mL water/g powder), increased swelling (8.55 mL/g powder) and oil-holding (8.37 g oil/g powder) capacity, as well as improved heavy metals bounding ability (Cu^{2+}, 27.54 μmol/g; Pb^{2+}, 42.85 μmol/g; Cd^{2+}, 30.92 μmol/g). Together, these results support the use of fermentation treatment samples as potential property modifiers for formulated products.

Keywords: Citrus juice by-products, dietary fibre, fermentation treatment, functional properties, microstructure, physicochemical properties

INTRODUCTION

Citrus is the most abundant crop in the world, with over 122 million tons produced per year since 2008 (Terol *et al*., 2010). Almost half of these fruits are squeezed into juice with the remainder, including peel, segment membranes and insoluble mass, which are considered as Citrus Juice By-Products (CJBPs) (Wilkins *et al*., 2007). An estimated 15 million tons of this solid bulk is generated annually worldwide (Marín *et al*., 2007) and is often destined for the landfill or sold as low-value animal feeds (Tripodo *et al*., 2004). Processes to produce value-added products from CJBPs would enhance the profitability of the citrus industry.

Dietary Fibre (DF) is defined by the Association of Official Analytical Chemists (AOAC) as 'the polysaccharides and remnants of plant materials that are resistant to hydrolysis (digestion) by human digestive enzymes'. More specifically, DF often includes a mixture of plant oligo-and poly-saccharides (e.g., cellulose, hemicelluloses, pectic substances, gums, resistant starch and inulin) that may be associated with lignin and other non-carbohydrate components (e.g., polyphenols, waxes, saponins, cutin, phytates and resistant protein). Generally, DF can be divided into Soluble Dietary Fibre (SDF) and Insoluble Dietary Fibre (IDF) and collectively referred to as Total Dietary Fibre (TDF) (Elleuch *et al*., 2011). The consumption of DF plays an important role in the prevention of diseases, such as obesity, atherosclerosis, coronary heart disease, colorectal cancer and diabetes (Vergara-Valenci *et al*., 2007). Thus, DF is not only desirable for its nutritional value but also for its functional and physicochemical properties.

In recent years, the demand for fruit and vegetable by-products as sources of DF has continued to increase (Fuentes-Alventosa *et al*., 2009). As one of the potential DF sources, CJBPs have gained considerable attention (Fischer and Schieberle, 2009; Juana *et al*., 2009). The main advantage of DF from CJBPs, when compared with other sources lies in its higher proportion of SDF. Moreover, CJBPs are rich in associated bioactive compounds, such as flavonoids and vitamin C. (Marín *et al*., 2007). Chemical and physical properties of citrus DF have been widely investigated. Several studies on CJBPs as a source of functional additive preparation, including chemical treatment techniques, have been reported (Rodríguez *et al*., 2006). However, no exhaustive studies have yet been conducted to investigate the effects of various extraction methods on

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the yield and quality of the resulting functional ingredients. The objective of this study is to evaluate the effects of three different treatment approaches on the composition and functional properties of DF obtained from CJBPs, as well as to assess the influence of these treatments on their microstructure, thus providing practical approaches and valuable insight on the potential use of CJBPs as sources of DF in functional foods or as naturally dietary supplements.

MATERIALS AND METHODS

Experiment methods:

- **Sample preparation:** Samples of Jinchen Sweet Orange Fruit (Citrus sinensis Osbeck) were obtained from an orchard in the Sichuan Province of China. Fresh juice by-products, left over after oil and juice extraction, were dried at 50°C for 48 h in an air tunnel drier (moisture content lower than 10%). The dried samples were ground using a mill to pass a 0.125 mm screen and then the Raw Powder (RP) was stored in a desiccator at room temperature (approximately 25°C) until used (Prior to further analysis, all the samples were treated with this method).

  All other chemicals were of analytical grade.
- **Microorganisms:** The filamentous fungi *T. viride* ATCC 36316, was used in this study. The microorganism was obtained from American Type Culture Collection as ATCC 36316 and was preserved in potato dextrose agar at 4°C.

DF preparation:

- **Water bath treatment:** RP (50.00±0.5 g) was combined with water in a solid-liquid ratio of 1:20 and mixed thoroughly with a glass stirring rod every 5 min in a water bath at 90°C for 4 h. The extract was filtered through a Whatman No. 1 filter paper (Shanghai Mosu Science Equipment Co, ltd, China). The filtrate was concentrated to 30 mL using a rotary evaporator at 50°C under vacuum (using methods from José *et al*. (1997) with adaptation). The concentrate and filter residue were added into a quadruple volume of 95% alcohol and kept standing for 30 min. The precipitate was collected and dried (as described above, 2.1.1).The product is called the water bath extraction powder (WP).
- **Fermentation treatment:** The *T. virde* spores immobilized on potato dextrose agar was activated by washing with sterile water. The mixture containing the spores was poured into an erlenmeryer flask with several small glass balls beads. The flask was agitated for 15 min to disperse the spores. The spore suspension was filter sterilized through 0.45 μm Millipore PVDF membrane filters into a sterile reservoir and adjusted to a concentration of 10^6-10^8 spores/mL. Flasks (250 mL) containing 50.00±0.5 g RP in 150 mL distilled water (a solid-liquid ratio of 1:3) were steam sterilized at 121°C for 20 min. Flasks containing pretreated RP were cooled and the pH was adjusted to 6.5 with 1 mol/L NaOH prior to spore inoculation. A certain volume of spore suspension prepared before (2%, v/v), was added into the flasks. To initiate the fermentation process, flasks were incubated at 30°C in an incubator shaker at 180 rpm for 72 h. All these flasks were sterilized after fermentation and filtered through a Whatman No. 1 filter paper. The filtrate was concentrated to 50 mL using a rotary evaporator at 50°C under vacuum. Then the concentrated and filtered residues were added into a quadruple volume of 95% alcohol and kept standing for 30 min. After removing the alcohol, the deposit was collected and dried (as described above, see 2.1.1). The resulting product was called the fermentation treated powder (FP).
- **Enzymatic treatment:** Flasks (250 mL) containing 50.00±0.5 g RP in 150 mL distilled water (a solid-liquid ratio of 1:3) and well-mixed with a glass stirring rod. A certain concentration of thermostable α-amylase (Activity≥4000 U/gds; concentration is 200 U/mL, 200 μL/g RP) was added into the flasks and incubated in a water bath at 100°C for 30 min. The pH of the mixture was then adjusted to 6.8 with 1 mol/L NaOH and a certain concentration of neutral protease (Activity≥2000 U/gds; concentration is 200 U/mL, 2 mL/g RP) was added into the flasks. The flasks were incubated in a water bath at 45°C for 1 h. Subsequently, the pH was adjusted to 6.0 with 1 mol/L HCl. A certain concentration of cellulase (Activity≥1800 U/gds; concentration is 1800 U/mL, 1800 μL/g RP) was added into the flasks, follow with 4 h incubation in a water bath at 40°C. Then, the treated extract was filtered through a Whatman No. 1 filter paper and the filtrate was concentrated to 50 mL using a rotary evaporator at 50°C under vacuum. The concentrate and filter residue were added into a quadruple volume of 95% alcohol and kept standing for 30 min. After removing the alcohol, the deposit was collected and dried (as described above, see 2.1.1). The product is called the enzymatic treatment powder (EP).

Physicochemical properties:

- **Chemical fibres:** DF composition was determined as previously described by AOAC 991.43 (Lee *et al*., 1992). Triplicate samples were gelatinized with heat stable α-amylase and digested with protease and amyloglucosidase to remove the protein and starch present in the samples. Determination of the contents of undigested protein

and ash was performed for corresponding corrections. TDF is calculated as the sum of SDF and IDF. TDF is expressed as g per 100 g of RP residue on a dry weight basis.

Pectin content was determined by alkaline hydrolysis and colorimetric determination of galactouronic acid derivatized with carbazole (Rangana, 1986).

Cellulose was determined according to the Kurschner method, by acetic-nitric acid digestion of citrus by-products, washed with alcohol, benzene and ether (Lee, 1982).

Hemicellulose content was determined by the Arsenal Royal Woolwich procedure (Powell and Whittaker modification) by titration with thiosulphate (Lee, 1982).

Lignin content was analysed by the Springer method (Lee, 1982), using sequential extractions in a Soxhlet extractor with ether, alcohol and water and subsequent acid hydrolysis.

Crude fat was determined according to method (AOAC, 2000 960.39); Crude protein was assessed using Kjeldahl's procedure (total nitrogen×6.25) (AOAC, 2000 928.08); Ash was determined according to the Muffle-Furnace technique (AOAC, 920.153). Total carbohydrates were determined by the Dubois method (Chaplin, 1986).

- **Colour measurement:** Colour changes of the three samples were analyzed by measuring their reflectance using a colourimeter (Juki, JP7100, Tokyo, Japan). The colour values were expressed using CIE Lab^* values where L^* represents the luminosity (0 = black; 100 = white), a^* the redness ($a^*>0$) or greenness ($a^*<0$) and b^* the blueness ($b^*>0$) or yellowness ($b^*<0$). Each sample was measured ten times and the result was presented as an average.

Functional properties:

- **WRC (Water retention capacity) and OHC (oil-holding capacity):** WRC is expressed as the mL of water/g of dry fibrous residue powder; WRC is determined by centrifugation as described elsewhere with slight modifications. The samples (2.00±0.02 g) were suspended in water (50 mL). After 24 h of equilibration at room temperature (approximately 25°C), the suspension was centrifuged at 4, 200 rpm for 15 min. Subsequently, the supernatant from each sample was discarded and the remaining hydrated content was weighed.
- **SWC (swelling capacity):** A sample (2.00±0.02 g) was added into a calibrated cylinder (2 cm in diameter) and was hydrated with 30 mL of distilled water at room temperature (approximately 25°C) for 24 h. The change in volume was recorded and expressed as the volume/g of the original sample (dry weight).
- **HMBA (heavy metals bonding ability):** The samples (0.1000±0.0005 g) were added into three flasks (250 mL), each containing 100 mL 4μg/mL copper sulphate, 100 mL 4 μg/mL cadmium chloride and 100 mL 16 μg/mL lead nitrate. The three flasks were well-mixed with a glass stirring rod in the water bath at 37°C. After 4 h, the suspension was centrifuged at 4200 rpm for 15 min and the supernatants were measured for the concentration of heavy metals by atomic absorption spectrophotometry (Mehmet *et al*., 2008). Based on the difference in concentrations of the responses before and after the water bath, the heavy metals bonding ability were obtained.
- **Scanning electron microscopy (SEM) analysis:** In order to investigate the influence of fermentation extraction on the structure of the materials and to understand the mechanisms involved in the functional property changes, the RP, WP and FP were analyzed by SEM (Zhang *et al*., 2008). Sample particles were fixed on the silicon wafer and sputtered-coated with gold to a thickness of about 100 nm. The shape and the surface characteristics of the samples were observed and recorded on the SEM (JSM-5510LV, Japan).
- **Statistical analysis:** All experiments were performed in triplicate and randomized. Each data was presented as mean±standard deviation. The data were statistically analyzed using a one-way analysis of variance followed by Duncan's multiple range tests (SPSS 17.0). Difference with p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Chemical fibres and composition of samples: Table 1 shows the content of chemical fibres and composition of the samples (The final product was a powder with 7-9% moisture). As expected, all treatments appreciably removed crude protein and crude fat contents and showed no significant difference. Ash contents of DF ranged from 1.32 g/100 g to 2.87 g/100 g dry weight. The highest amount of ash was observed in raw sample without treatment, whereas the lowest amount (1.32 g/100 g dry weight) was found in water bath treatment group. The other treatments in this study favoured the removal of ash from the RP with no significant difference ($p>0.05$).

Sugars were released from polysaccharides by acid hydrolysis. Sugars in the samples were released using two hydrolytic procedures in order to distinguish the sugars from noncellulosic polysaccharides and cellulose. Sugar contents varied significantly ($p<0.05$) among the samples, ranged from 8.24 g/100 g to 10.29 g/100 g dry weight (Table 1). A higher amount of sugar was obtained when enzymatic treatment was used for extraction of DF. Compared to samples without treatment (RP), samples obtained from different

Table 1: Chemical fibres and composition of samples (g/100 g dry powder)*

Samples	Crude Protein	Ash	Crude Fat	Sugar	Lignin	Cellulose	Hemicellulose	Pectin
RP	4.76±0.04^{a}	2.87±0.06^{a}	7.26±0.03^{a}	8.65±0.81^{ab}	14.88±0.95^{a}	39.51±2.02^{a}	8.79±0.34^{a}	2.47±0.04^{a}
WP	4.24±0.01^{b}	1.32±0.11^{b}	5.39±0.09^{b}	9.51±0.02^{bc}	11.06±0.72^{b}	33.88±1.21^{b}	13.22±1.52^{b}	8.53±0.07^{b}
FP	4.07±0.07^{b}	1.79±0.03^{b}	4.56±0.08^{b}	8.24±0.15^{ab}	7.93±0.11^{c}	26.63±0.69^{c}	11.31±0.45^{c}	23.55±2.23^{c}
EP	4.11±0.02^{ab}	1.59±0.08^{b}	5.56±0.07^{b}	10.29±0.79^{c}	10.57±0.67^{bd}	23.18±0.30^{d}	11.26±0.07^{c}	20.30±0.66^{d}

*: Mean value±standard deviation, n = 3; RP: raw powder; WP: water bath extraction powder; FP: fermentation treatment powder; EP: enzymatic treatment powder; Different letters (a-d) within a column indicate significant differences ($p<0.05$)

Table 2: Compositions of DF (g/100 g dry powder)*

Samples	TDF	IDF	SDF
RP	40.94±2.08^{a}	37.82±1.05^{a}	3.11±0.02^{a}
WP	62.69±3.14^{b}	45.81±2.12^{b}	16.88±0.08^{b}
FP	77.91±3.23^{c}	45.32±1.71^{b}	32.57±0.69^{c}
EP	64.12±1.68^{b}	38.32±0.51^{a}	26.81±0.79^{d}

*: Mean value±standard deviation, n = 3; RP: raw powder; WP: water bath extraction powder; FP: fermentation treated powder; EP: enzyme treated powder; DF: dietary fibre; TDF: total dietary fibre; SDF: soluble dietary fibre; IDF: insoluble dietary fibre; Different letters (a-d) within a column indicate significant differences ($p<0.05$)

treatments (WP, FP and EP) showed lower contents of lignin and cellulose and higher contents of pectin and hemicelluloses. The significant lower recoveries of cellulose in several samples might also suggest some major disruption of the cell wall arrangement during processing, despite cellulose being the most resistant type of cell wall polymer. This may easily be explained by the fact that, in the treatments processing, the polysaccharides that are released into the brines include both soluble compounds such as neutral polysaccharides and pectin; and constituents of IDF such as the portion of hemicelluloses and cellulose (Jiménez *et al*., 1998). Pectic substances are more susceptible to enzyme or heat induced chemical degradation than other polysaccharide components (Levi *et al*., 1988).

Prosky *et al*. (1988) developed an enzymatic-gravimetric method for DF determination. This enzymatic-gravimetric method determines a group of polysaccharides, lignin, some of resistant starch and other associated compounds (waxes, phenolic compounds and Maillard reaction products), whereas oligosaccharide and other types of resistant starch are not quantified by this method (Ohkuma *et al*., 2000). Therefore, the chemical fibres in Table 1 were different from TDF, SDF and IDF described in Table 2.

Mechanism of three treatments: It has been well documented that heating can substantially change the texture of plant tissues, with the modifications being dependent on the composition and structure of the fibre components. Thus, it is crucial to study the effect of thermal treatments on the physicochemical and functional properties of the fibre. Fermentation is a form of CJBPs processing that causes modifications of the composition and structure of DF. These changes occur due to the secretion of enzymes from microorganisms used during the fermentative process. Such enzymes alter the composition and structure mainly by degradation of cell wall polysaccharides. The primary enzymes detected in the fermentative brines are amylase, proteinase, poligalacturonase, cellulase and β-galactosidase that selectively degrade distinct cell wall polysaccharides and as a result, leading to a decrease in cellulosic fibre content, although a portion of the lignin can also be released (Rodríguez *et al*., 2006). Celluases are a multicomponent enzyme system composed of a 1, 4-β-glucan endohydrolase, a cellobiohydrolase and a cellobiase. Plant fibre treated with cellulose could reduce the crystalline structure of cellulose, increases the amorphous region and improve DF considerably (Tang *et al*., 2012). Enzymatic treatment can alter the ratio between soluble and insoluble fibres, e.g., treatment of cell walls with xylanase raises the ratio of SDF (Laurikainen *et al*., 1998).

The processing of CJBPs into DF affects the parameters that known to determine the nutritional and functional value of a product. In the studied samples, increases in TDF, SDF and IDF were observed (Table 2). Previous studies often use water bath to heat the samples over 90°C, however, this process results in the inactivation of virtually all the enzymes that could negatively affect the physicochemical properties of the final products. Moreover, treatment of DF with a high temperature (i.e., drying at 80°C and 90°C) attenuates some useful properties (i.e., WRC, OHC, SWC and antioxidant capacity) (Garau *et al*., 2007). The present study shows that the types of water baths, fermentation and enzymatic treatment could affect the changes in potentially bioactive constituents of DF. Especially, the levels of bioactive compounds can be modified during fermentation by the metabolic activity of microbes. The mechanical treatments (e.g., stirring) in water bath process could eliminate structure of DF by mechanical shear, making free hydroxyl groups from cellulose available to bind with water (Vetter and Kunzek, 2003). In addition, fermentation-induced structural breakdown of raw material cell walls may also occur, leading to the liberation and/or formation of various bioactive compounds.

There appeared to be a widespread deterioration of the sample's innate microstructure. When examining the RP samples, we saw evidence of a particular microstructure. Remarkably, when the CJBPs were treated either by fermentation or enzymatic degradation, the resultant powders appeared less rough (Fig. 1c1 and d1). However, more evident destruction of the microstructure was observed in Fig. 1c2 than that in Fig. 1a2 and b2, potentially due to a loosening process created during fermentation due to

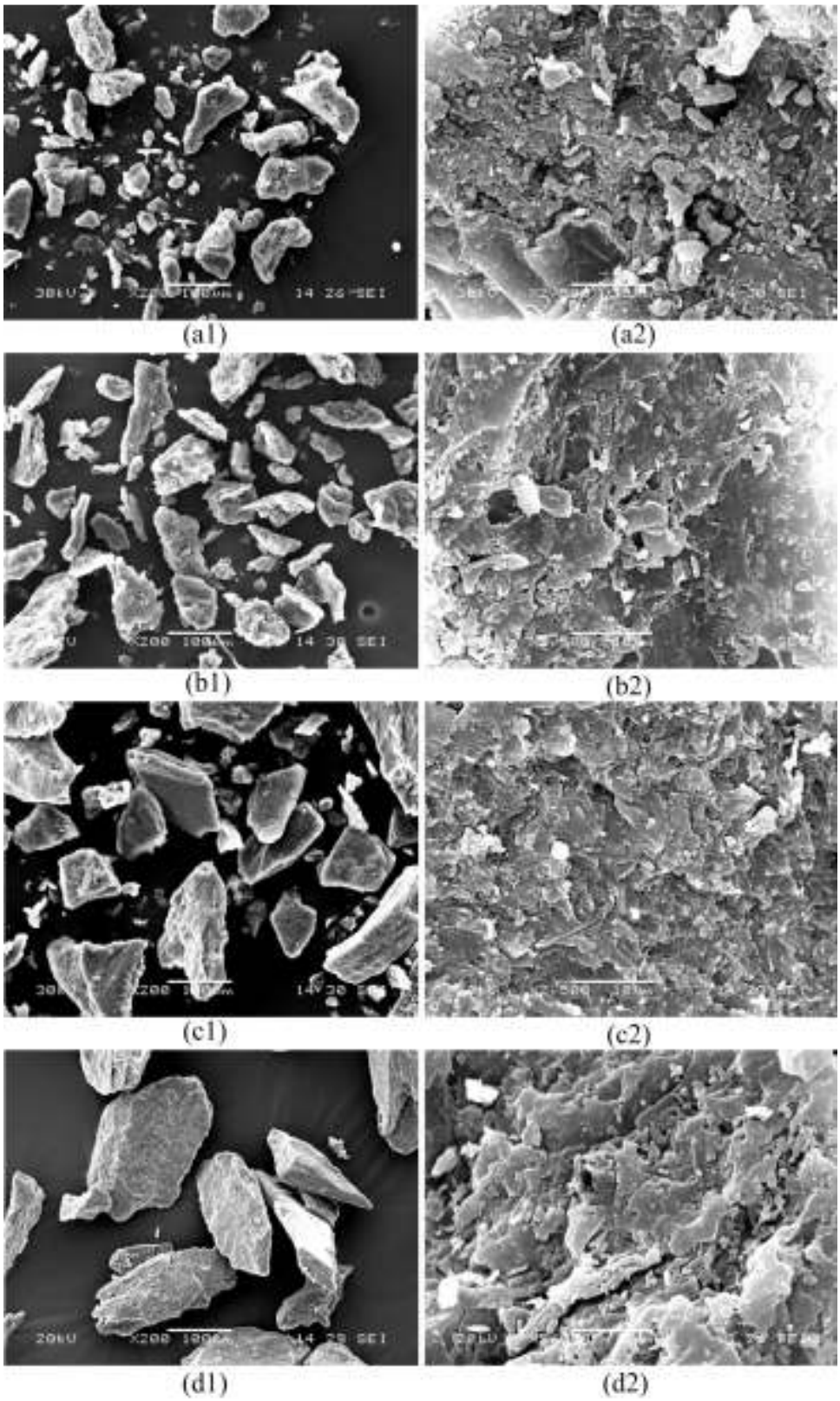

Fig. 1: SEM of DF, (a1) the RP×200 (scale bar 100 μm), (a2) the RP×2,500 (scale bar 10 μm); (b1) the WP×200 (scale bar 100 μm), (b2) the WP×2,500 (scale bar 10 μm); (c1) the FP×200 (scale bar 100 μm), (c2) the FP×2,500 (scale bar 10 μm); (D1) the EP×200 (scale bar 100 μm), (c2) the EP×2,500 (scale bar 10 μm); DF, dietary fibre; RP, raw powder; WP, water bath extraction powder; FP, fermentation treatment powder; EP, enzymatic treatment powder

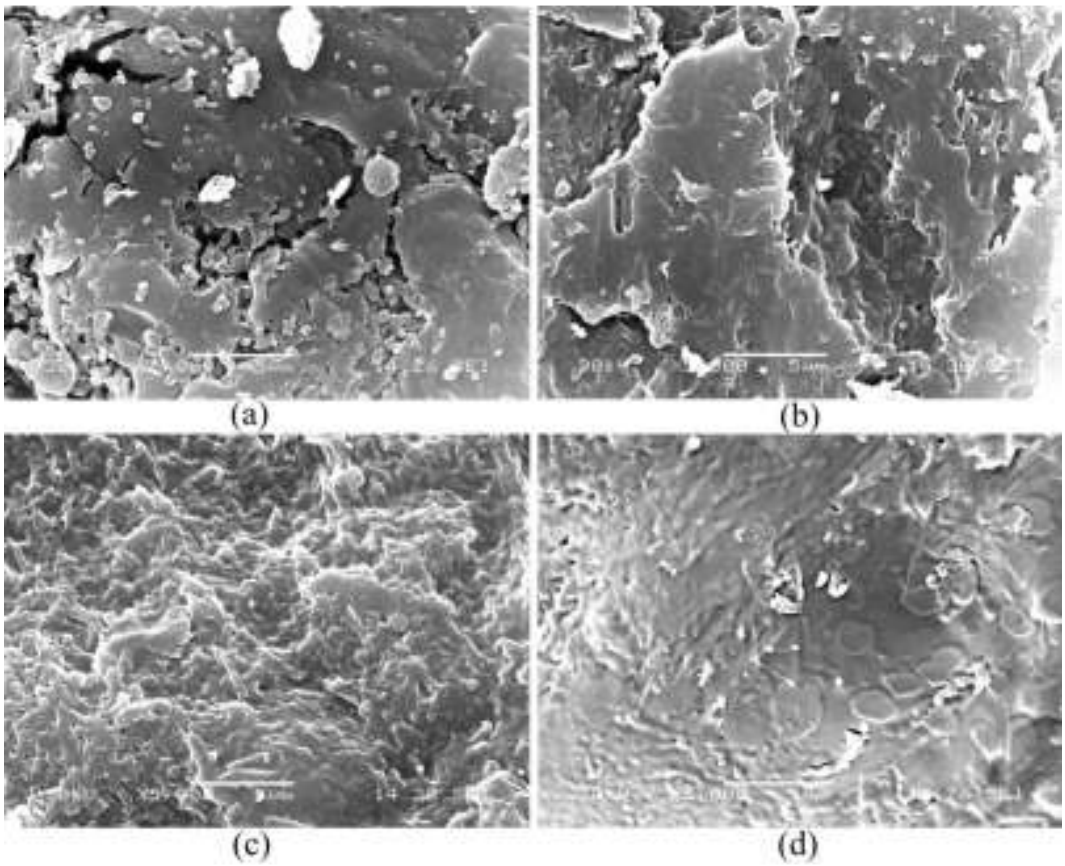

Fig. 2: SEM of SDF, (a) the SDF in RP×2,500 (scale bar 10 μm); (b) the SDF in WP×2, 500 (scale bar 10 μm); (c) the SDF in FP×2, 500 (scale bar 10 μm); (d) the SDF in EP×2,500 (scale bar 10 μm); SEM, scanning electron microscopy; RP, raw powder; WP, water bath extraction powder; FP, fermentation treatment powder; EP, enzymatic treatment powder; SDF, soluble dietary fibre.

gas (This is the cavitation by fermentation). As shown in Fig. 1d2, enzymatic treatment also slightly modified the surface of the microstructure.

Figure 1 shows that a higher SDF ratio corresponded with a more evident crosslinking structure. To further assess the influence of different treatments on the structure of DFs, the microstructure of SDF in the WP, FP and EP samples were examined by SEM (Fig. 2). The surface of SDF after water bath treatment had many noticeable uneven small pits, but it became much smoother and more superficial compared with the surface of the SDF after fermentation treatment. The SDF of EP had the smoothest and neatest surface compared with the WP and FP, indicating that the fermentation treated SDF had the largest surface area and was more likely to show stronger adsorption and dissolving capacity.

Functional properties: Functional properties are related to the chemical structure of the plant polysaccharides (Garau *et al*., 2007). Therefore, in order to evaluate possible modifications affecting the structural of DF samples, hydration-related properties such as WRC, SWC and OHC were measured on RP, WP, FP and EP. The results are presented in Table 3.

Table 3: Functional properties of samples*

Samples	WRC (mL water/g powder)	OHC (g oil/g powder)	SWC (mL/g powder)
RP	7.88±0.05^{a}	3.79±0.12^{a}	4.83±0.02^{a}
WP	10.21±0.12^{b}	5.85±0.06^{b}	7.01±0.12^{b}
FP	13.31±0.20^{c}	8.37±0.12^{c}	8.55±0.14^{c}
EP	13.14±0.11^{c}	7.50±0.05^{d}	7.23±0.09^{bd}

*: Mean value±standard deviation, n = 3; RP: raw powder; WP: water bath extraction powder; FP: fermentation treated powder; EP: enzyme treated powder; TDF: total dietary fibre; SDF: soluble dietary fibre; IDF: insoluble dietary fibre; WRC: Water retention capacity; OHC: oil-holding capacity; SWC: Swelling capacity; Different letters (a-d) within a column indicate significant differences ($p<0.05$)

WRC, quantity of water that remains bound to the hydrated fibres following the application of an external force (pressure or centrifugation), is an important property of DF from both physiological and technological points of view. WRC values measured for FP and EP were significant higher than RP and WP samples ($p<0.05$). Thus, all the treatments could increase the WRC values. As shown in Table 3, RP has a WRC (7.88±0.05 mL water/g) similar to that previously reported (Elleuch *et al*., 2011), whereas WP had a significant higher WRC (10.21±0.12 mL water/g; $p<0.05$) compared with RP. The WRC values of FP and

Table 4: HMBA of samples*

Samples	Cu^{2+} (μmol/g)	Pb^{2+} (μmol/g)	Cd^{2+} (μmol/g)
RP	26.09±0.05[a]	38.30±0.11[a]	28.13±0.18[a]
WP	26.98±0.12[ab]	41.12±0.15[b]	29.61±0.09[b]
FP	27.54±0.11[b]	42.85±0.17[c]	30.92±0.14[c]
EP	28.17±0.06[bc]	40.02±0.08[d]	30.32±0.12[dc]

*: Mean value±standard deviation, n = 3; RP: raw powder; WP: water bath extraction powder; FP: fermentation treated powder; EP: enzyme treated powder; TDF: total dietary fibre; SDF: soluble dietary fibre; IDF: insoluble dietary fibre; Different letters (a-d) within a column indicate significant differences ($p<0.05$)

Table 5: CIE Lab* coordinates of RP, WEP and FEP samples*

Samples	*L**	*a**	*b**	H
RP	69.40±0.16[a]	5.14±0.09[a]	15.78±0.25[a]	71.96±3.21[a]
WP	78.03±0.21[b]	5.83±0.16[a]	28.00±0.48[b]	78.24±4.02[b]
FP	65.66±0.21[c]	5.82±0.08[a]	16.69±0.17[ac]	70.78±1.77[ac]
EP	75.83±0.13[d]	2.54±0.05[b]	17.28±0.07[c]	81.64±3.85[d]

*: Mean value±standard deviation, n = 10; RP: raw powder; WP: water bath extraction powder; FP: fermentation treated powder; EP: enzyme treated powder; TDF: total dietary fibre; SDF: soluble dietary fibre; IDF: insoluble dietary fibre; $H = \tan^{-1}(b^*/a^*)$; Different letters (a-d) within a column indicate significant differences ($p<0.05$)

EP (approximately 13 mL water/g) was in the range of mostly reported for fibres (Lou *et al*., 2009; Larrauri, 1999; Lario *et al*., 2004), e.g., 12.72 mL water/g for fibre-rich burdock root powders, 12.6 mL water/g for peach pulp fibres and approximately 11 mL water/g for lemon fibres (Lou *et al*., 2009). The other by-products had lower values than those mentioned above, e.g., cocoa husks, with a WRC value of approximately 5 mL water/g fibre. Based on these findings, FP and EP could be promoted as a modifier of viscosity and texture for formulated products and they could produce a decrease in calories as well.

The results for OHC of RP (approximately 3.79 g oil/g) are presented in Table 3. All the treated samples exhibited significantly higher ($p<0.05$) OHC values than RP. Water bath slightly enhanced the OHC of WP (approximately 5.85 g oil/g). Fermentation could improve the OHC of samples (approximately 8.37 g oil/g). The OHC value of EP (approximately 7.50 mL oil/g) was slightly lower than FP. Based on the findings of Elleuch *et al*. (2011), the OHC of DF was 0.6-1.8 mL oil/g for apple pomace and citrus peel. The highest reported level was approximately 6 mL oil/g for carrot sarcocarp. Fibre-rich burdock root powder was reported with high values (8.50 mL oil/g) (Lou *et al*., 2009). Garau *et al*. (2007) has reported that Alcohol Insoluble Residues (AIRs) DF from orange by-product were higher than other OHC values reported for the DF concentrates from different orange varieties, from 0.8 to 1.3 g oil/g fibre concentrate. The OHC of FP was higher than AIRs. Thus, FP may be suitable for products for where emulsifying properties are required.

The results for SWC of RP (approximately 4.83 mL/g) are presented in Table 3. All the treated samples exhibited significantly higher ($p<0.05$) SWC values than RP. The value of RP showed a similar order with those of citrus residues. As shown in Table 3, SWC values seemed to be significantly affected by the treatment procedures ($p<0.05$), where high temperature water bath and enzymatic treatment had a considerable effect on this capacity and fermentation treatment exhibited the greatest effect, but in all the values of SWC in all samples were obviously lower than that reported by Garau *et al*. (2007) (approximately 20~30 mL/g). Moreover, FP had the highest TDF content, which suggested the best physicochemical and functional properties. EP had almost the same TDF content as FP, indicating similar physicochemical and functional properties.

Table 4 shows the HMBA of RP, WP, FP and EP. FP had the strongest heavy metal bounding ability among the samples with Pb^{2+} and Cd^{2+} and EP were the strongest one with Cu^{2+} than RP and WP. These results could be explained by the following two reasons. On the one hand, fermentation and enzymatic treatment could increase the surface area of DF, thereby increasing their adsorption capability. On the other hand, different treatment procedures may result in distinct surfactant assemblies, interfering with either electrostatic surfactant-precursor assembly interactions or hydrogen bonding.

Colour of samples: Colour is one of the most important quality parameters in the organoleptic properties of fruits and vegetables. Undoubtedly, dried DF samples would limit their potential applications (Femenia *et al*., 2003). Limitations with the slightly brown hue of apple pectins in very light-coloured foods have been reported (Renard *et al*., 1997). Table 5 shows the Hunter parameters (*L*, *a*, *b*) of CJBPs at various treatments. There were significant changes ($p<0.05$) in all L^*, a^* and b^* values of the samples subjected to water bath treatment, enzymatic treatment and fermentation treatment. The WP samples became redder and with slight loss of yellowness which is likely due to the leaching out of soluble pigments during water bathing (Wolfe and Liu, 2003). Similar results of L^*, a^* and b^* values were found for the FP samples, which is probably attributed to be fermentation treatment with sterilization in a high temperature inhibited enzymatic browning reactions. The influence of fermentation treatment on browning development, taking into account the H parameter, did not equally affect samples from RP. Thus, FP samples underwent a relatively lower colour modification than other samples. Therefore, in order to improve L, an approach that uses 2% H_2O_2 solution for 30 min to bleaching may be required for the samples after fermentation treatment.

CONCLUSION

In this study, DF from CJBPs was treated by water bath, fermentation and enzyme in this study. The effects of different treatment procedures on the functional and physicochemical properties of dietary fibre were

thoroughly evaluated. The total dietary fibre content was significantly increased from 40.94±0.08 g/100 g to 77.91±0.23 g/100 g by fermentation. Compared with the RP, WP, FP and EP, the values of WRC, OHC, SWC and HMBA were significantly improved by the FP. Although FP and EP had similar physicochemical and functional properties properties, the fermentation treatment takes the advantages of highest TDF output, lowest cost and most easily to implement a continuous DF production line for citrus juice by-products. These results indicated the prospect of FP as a functional food ingredient. From the perspective of biomass refining, this study provides an important sequential method for the recovery of valuable compounds from citrus fruit waste using a sustainable development technique.

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