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Evaluation of Extraction Methods of Dietary Fiber from Pomelo Juice Byproducts and Particle Size Distribution on the Physicochemical and Functional Properties

Suwanna Pichaiyongvongdee*, Tita Foophow, Piyawan Yoodee & Nujira Rasamipaiboon

School of Culinary Arts, Suan Dusit University, Bangkok, 10700 Thailand

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Abstract

Pomelo (*Citrus grandis* (L.) Osbeck) is the largest citrus fruit in Thailand, which pomelo juice is commercially produced during the pomelo juice extraction process. After extraction of the juice, the number of by-products that was produced could be a potential source of functional dietary fiber.The objective of this study was to evaluate extraction methods of dietary fiber from pomelo juice byproducts for functional foods and particle size distribution (150, 180, 250 and 425 μm) by pomelo pulp powder (PPP) that was prepared by simply air drying, milling, sieving and grinding. For pomelo pulp dietary fiber powder (PPDFP), the PPP was treated with NaOH and ethanol to remove protein and fat, respectively. After that, analysis of physicochemical and functional properties of PPP and PPDFP found that the PPDFP was richer in total dietary fiber (92.04%), especially insoluble dietary fiber (91.93%). Water holding capacity (WHC) and oil holding capacity (OHC) of PPDFP were higher than those of PPP. Components that can contribute to the bitter flavor of the powders, limonin, naringin and naringenin were considerably reduced in PPDFP, particularly limonin was totally eliminated. Also, the porous structure of PPDFP may facilitate its use in food processing compared to the more sheet-like structure of PPP. The optimum particle size was 150 μm that resulted in powders with increased WHC and OHC. From the above data, it was concluded that the PPDFP was a good source for food dietary fibers that could be used as a functional ingredient in fiber rich food products.

Introduction

The citrus juice industry, for example pomelo, extraction rate for pomelo (*Citrus grandis* (L.) Osbeck) was reported to be only 38.7% juice, 8.3% flavedo, 26.5% albedo, 25.2% pulp and 1.4% seeds (Pichaiyongvongdee & Haruenkit 2009). In Thailand, and some other countries, most of these byproducts are wasted or

* Corresponding Author
e-mail: suwanna_pic@dusit.ac.th

underutilized, but have proven to contain useful compounds including dietary fibers (Pichaiyongvongdee & Rattanapun, 2015). Several studies have demonstrated the physical chemical and functional properties of dietary fiber extracted from residues of the citrus juice industry including orange, lime and lemon albedo, indicating their levels of insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) (Tainara de et al., 2013; Pichaiyongvongdee & Rattanapun, 2015), but not the extraction of dietary fiber from pomelo fruit pulp. In Thailand, the recommended acceptable intake of dietary fiber is 25g/ day for adults (Ministry of public health, 2020). The importance of fiber in the human diet was reviewed by Jinjin et al., 2015) who showed that adequate levels could contribute to the prevention of chronic diseases such as colon cancer, coronary heart disease, hypertension, obesity, diabetes and asthma. Also, an important functional property of dietary fiber is the prevention of loss of water and oil in processed foods and dietary fiber has been used in fruit jellies to reduce water loss (Lilian & Diego, 2019). Dietary fiber from wheat bran was reported to be added to beef burgers to reduce levels of cholesterol and improve their cooking yield, diameter and texture (Mansour & Khalil, 1999). Dietary fiber from apple pomace was used as a partial substituted for wheat flour in cakes and it was found that the volume of the cakes decreased, and their density and hardness increased (Sudha et al., 2007).

Generally, the remaining material from the pomelo, after extraction of juice, is used as animal feed or fertilizer or wasted and can be a source of environmental pollution if disposed of incorrectly. The objective of this paper were to evaluate extraction methods of dietary fiber from pomelo juice byproducts and particle size distribution on the physicochemical and functional properties.

Materials and methods

1. Samples and chemicals

The cultivars pomelo waste pulp (*Citrus grandis* (L.) Osbeck) were Kao Nampheung (KNP), which was collected from a fruit juice processing factory in the Thai province of Nakhon Pathum for the analysis. The chemicals used were gallic acid, trolox, DPPH (2-diphenyl-1-1-picrylhydrazl), naringin, limonin, quercetin, apigenin, luteolin, ferulic acid, hesperedin, naringenin, sinapic acid, p-coumaric acid and caffeic purchased from Sigma-Aldrich, Gillingham, Dorset, UK. Myricetin and kaempferol were purchased from Fluka, Loughborough, Leicestershire, UK. All the chemicals and reagents used were of analytical grade.

2. Preparation of samples

On arrival at the laboratory, the pomelo pulp samples were divided into two parts. One part was processed into pomelo pulp powder (PPP) and the other part was processed into pomelo pulp dietary fiber powder (PPDFP) using the methods described below.

2.1 Preparation of pomelo pulp powder (PPP)

The pomelo pulp was dried in a tray dryer (Memmert 400, Germany) at 70°C until its moisture content was less than 10% and then milled (Muti-function high speed disintegrator, rotation rate 25,000-28,000 RMP at 1 min, made in Taiwan) and sieved (Stainless steel sieves, by Advantech Manufacturing, Inc, U.S.A). The sieved particles were then ground to different particle sizes: 150, 180, 250 and 425 μm in order to obtain PPP and each sample sealed under vacuum in an aluminum packet and stored at 4±2 °C until analyzed.

2.2 Preparation of pomelo pulp dietary fiber powder (PPDFP)

The pomelo pulp was treated to eliminate protein by soaking in 0.01 N NaOH solution (pomelo pulp:NaOH solution, 1:10, w/v) at 37°C for 10 min, followed by washing with distilled water and pressing. Each treated sample was defatted by soaking in ethanol (pomelo pulp:40% ethanol, 1:10, w/v) for 30 min, then again washed with distilled water and pressed. Bitterness reduction was by constant soaking for 60 mins in distilled water pH 7 (adjust the pH of the distilled water from 6.8 to pH 7.0 using 0.1 N NaOH solution) (pomelo pulp: distilled water pH 7, 1:10 w/v) followed by washing twice with distilled water and pressing. Then all the samples were dried in a tray dryer (Memmert 400, Gemmany) at 70°C until the moisture content was less than 10%, followed by milled samples were ground and sieved to different particle sizes: 150, 180, 250 and 425 μm in order to obtain PPDFP and each sample was sealed under vacuum in an aluminum packet and stored at 4±2°C until analyzed.

3. Quality analysis of PPP and PPDFP

3.1 Chemical composition

Dry weight, moisture, fat, protein, ash, and total sugar contents were determined following the methods of AOAC 925.45, 922.06, 981.10, 940.26 and 982.14, respectively (AOAC, 2016).

3.2 Dietary fiber composition

Total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) were determined using the enzyme-gravimetric method 985.29, 985.29 and 985.29, respectively (AOAC, 2016).

3.3 Total phenolic content and antioxidant properties

3.3.1 Total polyphenol content

The total phenolic content was determined using the Folin Ciocalteu colorimetric method described by Yun et al., 2009. The sample (1 g) were extracted with methanol 20 mL (w/v), shaker at 37°C at 6,000 rpm for 30 min and then the supernatant was filtered through Whatman filter paper. The sample 0.4 mL was added to 2 mL of 10% Folin- Ciocalteu reagent and 1.6 mL of 5% Na_2CO_3 solution was added to the mixture. Incubate in darkness at room temperature for 30 min, the absorbance at 765 nm using spectrophotometer (model 1601; Shimadzu Corp; Kyoto, Japan). The sample was expressed as mg of gallic acid equivalent (GAE) per milliliter.

3.3.2 Antioxidant activity using a free radical scavenging assay

The stable DPPH radical was used for determination of free radical-scavenging activity of the pomelo albedo extracts (a modified method from Zigoneanu et al., 2007). The sample 2 mL was added to 2 mL of DPPH reagent (200 μM). After 30 min at room temperature, the absorbance at 515 nm. The antioxidant activity (DPPH) was expressed as micro molar (μM) Trolox equivalent.

3.4 Limonin and naringin

Limonin and naringin give fruit a bitter taste, therefore the dietary fibers were analyzed for both substances because they could affect their use in food. Limonin is a limonoid and is a white, crystalline substance and naringin is a flavonoid. Both occur naturally in citrus fruits and give them a bitter taste.

Extraction sample: 1 g of sample was weighed, 10 mL of methanol was added and shaken for 1 min with Vortex Mixer and then was centrifuged for 30 min at 4,500×g and solution was passed through nylon filter size 0.22 μm, and kept in a vial for analysis of limonin and naringin.

Standards of limonin by diluting the stock solution to 2, 5, 10, 20, 50 and 100 mg/100g. Standard curves were used for the calculation of limonin in the extracts. The linear regression equations for the curves for limonin were y=14726x with a correlation coefficient (r^2) value of 0.9977.

Standards of naringin by diluting the stock solution to 2, 5, 10, 20, 50 and 100 mg/100g. Standard curves were used for the calculation of naringin in the extracts. The linear regression equations for the curves for naringin were y=46918x with a correlation coefficient (r^2) value of 0.9995.

Limonin determination: the analytical method used was modified from Sun et al. (2005) using a HPLC (Water Alliance 2695, USA) system consisted of C_{18} Column (4.5x150 mm, pore size 3 μm), a C_{18} guard column, a Diode array detector (Water Alliance 2996), a computerized recorder/integrator (Waters Empower software). The mobile phase consisted of A: 45% acetonitrile and B: 55% deionized water, a flow rate of 0.7 mL/min. The auto-injection sample 10 μL by a wavelength 210 nm.

Naringin Determination: the method used was modified from Kanaze et al. (2003) using the HPLC system as limonin, but the mobile phase consisted of A: 25% acetonitrile and B: 75% deionized water, a flow rate of 0.7 mL/min and the auto injection sample 10 μL by a wavelength 280 nm.

3.5 Total flavonoid contents

Total flavonoid contents (Apigenin, Ferulic acid, Hesperedin, Kaempferol, Luleotin, Myricetin, Naringenin, Querctin, Sinapic acid, p-Coumaric acid and Caffeic acid) were determined by using a modified method of Hertog et al.1992). The 1-2 g of the sample were weighed and then added BHQ, HO and HCl in a conical flask of 250 mL and swirled. The samples for hydrolyze were put in a hot water-bath of 90°C for 2 hours (swirled occasionally). The sample was cooled for 5 min in a cool water bath. Added to each sample was 1 mL of ascorbic acid solution. Then each sample was put into a volumetric flask with MeOH and diluted to a total volume of 100 mL and sonicate for 5 min. The extract was passed through a 0.22 μm nylon filter seeping into a vial for analysis.

The system consisted of an HPLC system (Agilent 1100 Series), a column: Zorbax Eclipse XDB-C_{18} (4.6x250 mm, pore size 5 μm) were preceded by a guard column Eclipse XDB-C_{18} (4.6x12.5 mm, pore size 5 μm). The column and guard column heater were temperature controlled at 30°C. The mobile phase consisted of A: water: trifluoroacetic acid (TFA) (99.95:0.05), B: acetonitrile: TFA (99.95:0.05) and C: methanol:TFA (99.95:0.05) with a flow rate of 0.6 mL/min (isocratic). By at (1) 0-5 min: 90-85% A, 6-9 % B, 4-6% C; (2) at 5-30 min: 85-71% A, 9-17.4% B, 6-11.6% C; (4) at 30-60 min: 71-0% A, 17.4-85% B, 11.6-15 % C; (5) at 60-61 min: 0-90% A, 85-6% B, 15-4% C; and (5) at

61-66 min: 90% A, 6% B, 4% C. Auto-sample/injector were 20 µL. The detection wavelength was at 210, 280, 325, 338 and 368 nm.

3.6 Color

The color of each sample was measured using a Handy Colorimeter (Minolta Camera Co.; Osaka, Japan) following the system of the *L* a* b.**

3.7 Functional properties

Water holding capacity (WHC) was measured using the method described by Xianliang et al. (2017) 1g of dried powder of sample (W_1) was dissolved in 20 ml distilled water and shaken for 1 min with Vortex mixer and then stood at room temperature for 20 min. After centrifugation at 4,000 rpm for 15 min, the sediment was collected and weighted (W_2). The WHC was calculated as follows: WHC (g/g)=$((W_2–W_1) / W_2)$

Oil holding capacity (OHC) was measured using the method described by Xianliang et al. (2017) 1 g of dried powder of sample (W_1) was dissolved in 20 mL distilled water and shaken for 1 min with Vortex mixer and then stood at room temperature for 18 hr. After centrifugation at 4,000 rpm for 15 min, the sediment was collected and weighted (W_2). The OHC was calculated as follows: OHC (g/g)=$((W_2–W_1)/W_2)$

4. Microstructure analysis

4.1 Scanning electron microscopy (SEM)

PPP and PPDFP samples were sieved through 150 µm size sieves. Samples were coated with a layer of platinum and scanned using a SEM, Model SU3500 Hitachi, Naka Factory, Japan, at voltage of 5kv at 2000x magnification level.

4.2 Fourier-transform infrared spectroscopy (FTIR) spectral analysis

The sample was observed by using Micro-Attenuated Total Reflectance (Micro-ATR) FTIR spectroscopy (Spectrum Spotlight FTIR Imaging System, Perkin-Elmer, Illinois, USA) at a resolution of 4 cm^{-1} and 32 scans per sample in the region range at wave number from 4000-600 cm^{-1}.

5. Statistical analysis

The data were analyzed in triplicate using one-way analysis of variance with SPSS Statistic Version 20.0 (SPSS Inc., Chicago, IL, USA). Data was considered statistically significant at $p<0.05$.

Results and discussion

1. Chemical properties

The chemical properties of PPP and PPDFP is shown in Table 1. It was found that levels of total dietary fibers from PPP were only about a quarter where those from PPDFP were in excess of 90%; more than pomelo albedo dietary fiber powder. The insoluble dietary fiber content in PPDFP (91.93%) was higher than in PPP (22.72%). The levels of insoluble dietary fiber of PPDFP, increased during the extraction process which considerably reduced protein and fat levels. A high proportion of IDF (mainly cellulose, hemicellulose and lignin) in pomelo fruit processing by-products are good source of dietary fiber that are suitable for use in food processing. The proportion of IDF reported in this work were higher than those of orange peel, grapefruit peel and lemon peel (Lei et al., 2015); orange peel fiber (Tainara de et al., 2013) and albedo pomelo fiber (Pichaiyongvongdee & Rattanapun, 2015).

Table 1 Chemical properties level in PPP and PPDFP (Dry matter)

Chemical composition	PPP	PPDFP
Moisture (%)	7.30±0.01	8.98±0.07
Protein (%)	8.47±0.03	3.37±0.03
Fat (%)	2.06±0.01	0.87±0.02
Ash (%)	3.22±0.03	5.74±0.06
Total sugar (%)	47.36±0.06	n.d.
a_w	0.26±0.00	0.27±0.00
Total dietary fiber (TDF)	25.27	92.04
Insoluble dietary fiber (IDF)	22.72±0.02	91.93 ±0.06
Soluble dietary fiber (SDF)	2.55±0.06	0.11±0.01
Limonin (mg/100g)	43.87±0.14	n.d.
Naringin (mg/100g)	263.48±9.61	0.19±0.01
Total phenolic content (mg gallic/g)	4.39±0.03	1.20±0.03
Antioxidant activity (DPPH) (µM trolox/g)	37.54±0.01	25.04±0.01
Naringenin (mg/100g)	25.81±0.77	0.90±0.02
Quercetin, Apigenin, Luteolin, Ferulic Acid, Hesperedin, Sinapic Acid, Kaemferol p-Coumaric acid, Caffeic acid, Myricetin	n.d.	n.d.

Remark: nd=not detected. Means values ±Standard deviation (n=3)

Protein content decreased from 8.47% to 3.37% probably due to the denaturation of protein in contact with sodium hydroxide. The fat reduction from 2.06% to 0.87% was due to ethanol treatment and no sugar was detected in PPDFP due to the preparation period of PPDFP by several of washing, which sugar has soluble properties. (Table 1). In Meng-mei & Tai-hua (2016) study the extracted dietary fiber from cumin using 3 methods (alkali extraction, enzymatic hydrolysis, and shear emulsifying assisted enzymatic hydrolysis) these methods could decrease protein and fat more than 3 times. Clearly, modifying PPP to PPDFP could be used to greatly enhance dietary fiber for its application in food products. These additions could improve the food

products, for example, it has previously been reported that protein in dietary fiber can reduce the water holding capacity of food product and rancidity during long term storage of high fat foods. Removing protein and fats from dietary fiber, as was shown by PPDFP, was previously shown to improve texture and eating quality of coconut cake (Yajun & Yan, 2018). Fats in Chia seeds (*Salvia hispanica*) were reduced by treatment with hexane, and Vazquez-Ovando et al. (2009) found that defatting seeds of Chia improved their physicochemical properties in such a way as to improve their use in functional foods.

The bitterness in pomelo fruit may be unacceptable for use in some functional foods. The two compounds responsible for bitterness, naringin and limonin in PPDFP were almost completely reduced in Table 1. Horowitz & Gentili (1977) reported that naringin is soluble in water, which would account for its reduction. Limonin is more soluble in solvents like chloroform and only slightly in water (Maier et al., 1977), but these results showed that the ethanol plus water treatments effectively controlled levels. Naringenin was decreased from 25.81mg (PPP) to 0.90 mg/100g (PPDFP) while other components were not detected in both PPP and PPDFP. Yoon et al. (1997) reported that flavonoid can be dissolved well in pH 7 but not soluble at pH 6.5 or lower.

2. Effects of particle size on color and functional foods

2.1 Color

The color is an important factor in the applications in food which might mean that in some aspect the color of particles needs to be considered in application to the food industry because it could affect their appearance. The preparation of PPDFP could be used to greatly enhance the color by removal of sugars and another soluble component during the preparation of PPDFP by washing before drying which could be the cause due to Maillard browning reactions that was found such as *L** increased lightness, red to green *(a*)* value and blue to yellow *(b*)* value both decreased (Table 2). Reducing the particle size distribution affected the color of both PPP and PPDFP and it was found that *L** increased lightness whereas *a**and *b** decreased.

2.2 Water and oil holding capacity (WHC and OHC)

The water holding and oil holding capacity of PPDFP was higher than PPP due to PPDFP had IDF higher than PPP. IDFs have the structure of cellulose and hemicellulose that consists of several hundred to many thousands of glucose units connected by a beta acetal linkage, bond of cellulose and hemicellulose had intermolecular hydrogen bond that showed much higher WHC than that of sugar and protein in PPP. In addition, both WHC and OHC increased as particle size decreased. The increase of water holding and oil holding capacity of both PPP and PPDFP were related to particle size. The PPDFP size 150 μm could hold a higher amount of water and oil more than the size of 180, 250 and 425 μm. The PPDFP 150 μm particle exhibited the highest WHC and OHC and had 11.36 g water/g sample and 3.86 g oil/g sample, respectively (Table 3).

Table 2 Effects of particle size on color of PPP and PPDFP that had been sieved to different sizes

Dietary fiber	Sieving size (μm)	*L**	*a**	*b**
PPP	425	69.60±0.31^{h}	7.21±0.21^{a}	27.60±0.31^{a}
	250	72.71±0.24^{g}	7.00±0.15^{b}	26.50±0.26^{b}
	180	75.61±0.37^{f}	6.10±0.28^{c}	24.48±0.27^{c}
	150	77.38±0.31^{e}	3.54±0.12^{d}	21.27±0.32^{d}
PPDFP	425	80.57±0.30^{d}	1.17±0.07^{e}	14.06±0.15^{e}
	250	84.50±0.30^{fc}	0.85±0.05^{f}	13.17±0.15^{f}
	180	86.34±0.25^{b}	0.50±0.08^{g}	12.51±0.24^{g}
	150	88.85±0.31^{a}	0.32±0.05^{h}	11.28±0.15^{h}

Remark: Different letters in the same column indicate that the values have significant differences ($p < 0.05$)

Table 3 Effects of particle size on water holding capacity (WHC) and oil holding capacity (OHC) of PPP and PPDFP that were sieved to different sizes

Dietary fiber	Sieving size (μm)	WHC (g water/g sample)	OHC (g oil/g sample)
PPP	425	3.25±0.10^{h}	0.67±0.07^{g}
	250	3.76±0.15^{g}	0.88±0.02^{f}
	180	4.90±0.09^{f}	0.91±0.05^{ef}
	150	5.32±0.20^{e}	1.08±0.02^{e}
PPDFP	425	8.55±0.14^{d}	2.33±0.08^{d}
	250	9.23±0.26^{c}	2.89±0.16^{c}
	180	10.41±0.29^{b}	3.38±0.17^{b}
	150	11.36±0.18^{a}	3.86±0.05^{a}

Remark: Different letters in the same column indicate that the values have significant differences ($p < 0.05$)

Fengmei et al. (2015) reported that particle size reduction of hull-less barley bran dietary fiber for water retention capacity and oil binding capacity. Similarly, the superfine grinding increased the WHC of oat fiber (Marcin et al. 2016). Zheng & Li (2018) reported that the particle size of coconut cake dietary fiber treated by acid was reduced from 250 to 167 μm and resulted in increased WHC. Marcin et al. (2016) reported that decreasing particle size leads to reduced hydration properties, exposure of hydrophilic groups which is connected with

water absorption capacity. Increasing of surface area and pore volume as well as structural modification contribute to increasing of WHC and OHC. Schneeman (1999) said that the ability of oil holding capacity (OHC) of dietary fiber to adsorb fat or oil can be important in food applications; for example, in preventing fat loss upon cooking and in nutrition where the ability to absorb or bind bile acids and increase their excretion is associated with plasma reduction and the ability to prevent fat loss during food processing and the capacity to reduce serum cholesterol level (Navarro-González et al., 2011) Thus, characteristics of WHC and OHC of different particle sizes are important in food application or can be used for functional ingredient to modify the viscosity and texture of some formulated food and avoid syneresis in food.

2.3 SEM that had been sieved to different sizes

The microstructure of PPP and PPDFP were particle size 150 µm. The differences of their surfaces found that the PPP with sheet-like structures and the surface of the PPDFP was partially disintegrated, indicating that their cell structures were damaged (Fig 1). This effect had previously been reported by Peerajit et al. (2012) who showed that hydrolysing of hemicellulose led to collapse and distortion of the cell wall structure and increased porosity. The SEM examination was consistent with the result of the particle size 150 µm and had an affect on WHC and OHC (Table 3) indicating the increased ability in water/oil binding was mainly related to the porosity of the fibre structure.

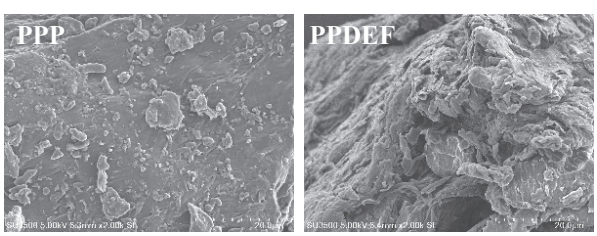

Fig. 1 Scanning electron micrographs of PPP and PPDFP were particle size 150 µm (view under 2000 magnification)

2.4 FTIR spectra of PPP and PPDFP

FTIR spectra of PPP and PPDFP are illustrated in Fig 2. It was found that most characteristic bonds of PPP and PPDFP were similar in FTIR spectra, such as bands at 3348 and 3366 cm^{-1} (O-H in stretching vibration in hemicellulose cellulose), 2932 and 2925 cm^{-1} (C-H in stretching vibration of the methylene group of the saccharide) (Liu et al., 2019; Jin-Shun et al., 2017; Jinjin et al., 2015). A band at 1739 and 1731 cm^{-1} (C=O in stretching vibration of the ester group) (Jin-Shun et al., 2017; Liu et al., 2019), 1622 and 1621 cm^{-1} (C=O in lignin benzene ring) (Liu et al., 2019), 833, 897, 1067, 1064, 1416 and 1424 cm^{-1} (due to the bending of the OH group in C-OH in hemicellulose (Jin-Shun et al., 2017), 1244 cm^{-1} (C-O in hydroxybenzene (Liu et al., 2019). All referred to the characteristic absorption peak of lignin (Jin-Shun et al.. 2017), the beading vibration absorption peak of acetyl groups (Jin-Shun et al., 2017), the beading vibration absorption peak of C-O-C (Jinjin et al., 2015) respectively, proving that the NaOH and ethanol treatment for the PPP reduced the intensity functional group and destroyed the structure of organic molecules.

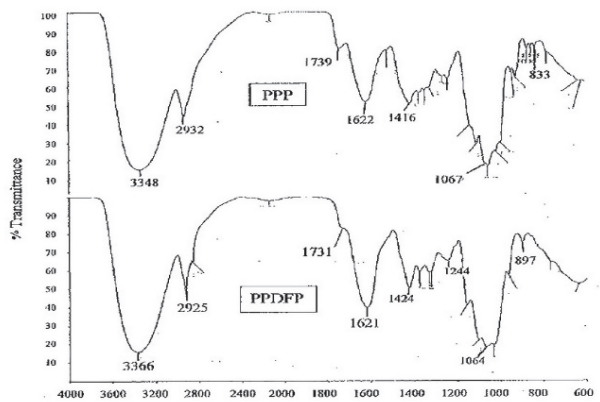

Fig. 2 FT-IR spectra of PPP and PPDFP

Conclusion

This study found that reducing the protein and fat in the pomelo pulp, by using NaOH, ethanol, and washing with distilled water prior to drying, resulted in high yielded pomelo pulp dietary fiber powder with high dry matter and low water activity. Reducing particle size to 150 µm resulted in powders with increased WHC and OHC which are more suitable for inclusion in many food products. However, the color of PPDFP must be considered in its applications to avoid a negative of the foods to which it is added. In addition, the PPDFP was richer in insoluble dietary fiber; higher than 90% which IDF are characterized by their porosity and their low density. Its favorable physical and chemical characteristics offers it to be suitable to use in food formulations such as adding to meat products to improve the texture and stability as well as increase in density and hardness in extruded cereals. In addition it can improve

water absorption and taste in baked products and increase in the water absorption of dough during the process as well as decrease water absorption and swelling of pasta product.

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