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Research Article

Combined Effects of Isolation and Grinding Technologies on Physico-chemical, Structural and Antioxidant Properties of Dietary Fiber from Doum (*Hyphaene thebaica* L.) Fruit

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Abstract

In current study, dietary fiber (DF) was isolated from Doum fruit by exploiting the alkali and microwave reactor techniques. Grinding technologies were also applied to characterize the chemical composition, particle size, structural, physico-chemical and antioxidant properties of obtained dietary fibers (DFs). Among the DF samples, MEDFSG (DF obtained by microwave extraction with superfine grinding) exhibited the highest contents of soluble dietary fiber (14.33 g/100g), monosaccharides (except for xylose), total polyphenol (72.31 mg GAE/g), ABTS•+ (20.09 mM TEA/100g), DPPH• (88.76%) with a crystallinity of 52.6%. Whereas, the lowest levels of insoluble dietary fiber fractions (excluding cellulose) were found in MEDFSG. Furthermore, superfine grinding led to produce smaller MEDFSG particles with greater number of cracks and holes as observed by scanning electron microscopy, suggesting enhanced functional properties in terms of highest hydration properties and oil adsorption capacity as compared to its counterparts. Notably, the results revealed a novel synergistic approach of microwave reactor extraction and superfine grinding which may prove promising strategy to produce functionally active DFs for their functional applications.

Keywords: Doum fruit; Dietary fiber; Microwave extraction; Superfine grinding; Structural, functional and antioxidant properties

Introduction

Dietary fiber (DF) from the fruit has been reported to contain elevated levels of bioactive compounds [1,2]. *Hyphaene thebaica* is known as Doum or gingerbread palm because its taste and consistency is similar to that of gingerbread. This tree is extensively found along the Nile River in Sudan and Egypt [3]. The high nutritional value of Doum fruit has made it beneficial for the human health, thus leading its applications in food processing industry particularly in baking industry [3]. This fruit has emerged as a novel source of DF, vitamins, essential minerals and antioxidants.

Nowadays, chemical and multistage water extraction using microwave are popular methods for extracting DF from fruit sources. The process conditions affect the structure and composition of DF, which can strongly influence the physico-chemical and functional characteristics [4]. However, Nyman and Svanberg reported that chemical extraction method result in the loss of a large amount of soluble dietary fiber (SDF) due to disruption of glycosidic linkages. In addition, the chemical methods generate large volumes of wastes in form of alkalis and strong acids thus leading to environmental problems. Previously, multistage water extraction using microwave has been applied in the isolation of insoluble date fiber from date flesh [5] and the technique has been found with several advantages such as improved efficiency, yield, water and oil holding capacity. Superfine grinding approach has been reported to improve chemical reactivity, adsorption, dispersion, high solubility and fluidity of reagents during the processing of powders [6]. Previously, Zhu, Huang, Peng, Qian and Zhou have explored that the superfine grinding can improve the antioxidant characteristics of DF. The most common types of grinders are rotor and ball mill which are extensively used in the milling of food materials.

In the present era, as the increasing demand of functional foods has boosted up the market for fiber-enriched food products which may offer a therapeutic measure in addition to providing inherited calories. Thus, it is of utmost need of hour to explore the novel strategies for the production of functionally active DFs. It's a pioneer investigation to use the Doum fruit as a source of DF; which was extracted through alkaline and microwave reactor methods. The grinding technologies were involved to explore the physicochemical, structural and antioxidant properties of obtained dietary fibers (DFs).

Experimental

Materials

Doum fruit was harvested in March, 2016 from its natural habitat in Tayba Garden (Aljazeera Aba, Sudan). Protease and α-amylase were purchased from Sigma Chemical Co., St. (Louis, MO, USA). All other chemicals and solvents used in this study were of analytical grade.

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Dietary fiber isolation

Seeds were removed from Doum fruits and then were ground to pass through 20-mesh screen. DF was isolated by microwave reactor and alkali assisted extraction methods. To prepare DF by microwave extraction (MEDF), a microwave reactor apparatus (XH-200A; Beijing Xianghu Science and Technology Development Co., LTD, China) was employed. Briefly, the Doum fruit powder was dissolved in the distilled water in a powder: water volume ratio of 1:10 (w/v); and poured into a 250 ml round-bottomed flask facilitated with magnetic stirring and microwave heating at 60°C for 60min. The solution was passed through a thin cloth of 0.318 mm pore size. Filtration and extraction processes were repeated 5 times in order to increase the purity. For the preparation of DF by alkali extraction (AEDF), the sample of Doum fruit was assorted with distilled water in a powder: water ratio of 1:50 and the pH was adjusted to 10 with 0.5 N NaOH under continuous homogenization for 3 hours at 60°C in a water bath. The mixture was neutralized with dilute acetic acid 10% (v/v) and the prepared sample was filtrated with a thin cloth of 0.318 mm pore size. The fibers from different extraction methods were washed in distilled water in order to remove dissolved substances. The samples were freeze-dried (Alpha 1-2 LD plus, Christ, Saxony, Germany; Vacuum brand vacuum pump, Germany)and divided into two parts, whereby the first part was ground by using a regular laboratory mill (WK-400B,Jingcheng Pharmaceutical Equipment Manufacturing Co., Ltd, shangdong, China) while the second part was micronized with a planetary ball mill. A flow diagram for the extraction and grinding process is shown in Figure 1.

Micronization of the Doum dietary fibers

Doum DFs obtained after extraction and drying was micronized with a planetary ball mill (PM-100 type micronizer (Retsch GmbH, Haan, Germany)). The ball-milling was done by mixing the Doum DFs and Zirconia balls (10 mm) in a 250 mL grinding jar (5 h, 400 RPM). The obtained powders were sealed in aluminum foil and kept in desiccators for further analysis.

Compositional analysis

Proximate composition analysis of Doum fruit and subsequent obtained DFs were determined by AOAC (AOAC, 2000) official methods: moisture (Protocol No. 925.09), ash (Protocol No.942.05), fat (Protocol No.920.39) and protein (ProtocolNo.955.04). IDF, SDF, and TDF of Doum fruit and obtained DFs were estimated by enzymatic–gravimetric method (AOAC, 1992).

Determination of insoluble dietary fiber fractions

The protocol for neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) determination was adopted from Yaich et al. [7]. Hemicellulose content was calculated by the difference between NDF and ADF, as well as, the difference between ADF and acid ADL contents were used to calculate the amount of cellulose.

Analysis of monosaccharides

The compositions of monosaccharides of Doum DFs were calculated by using a modified protocol reported by Chau and Huang [8]. Individual monosaccharides were determined using a high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (ICS-5000 HPLC system, DIONEX Co., Sunnyvale, USA). Peaks were identified on the chromatogram according to the retention data by standard sugars and peak areas was examined in relation to the standard sugar peak.

Particle size distribution

Particle size of Doum DFs was measured by laser particle size analyzer (S3500, Microtrac, USA) using a dry module. Particle size distributions were expressed as volume diameters at 10% (V_{d10}), 50% (V_{d50}) and 90% (V_{d90}). According to British standards, span is used to measure the width of particle size distributions. Span was calculated according to the following formula:

$$\text{Span} = (V_{d90} - V_{d10})/V_{d50} \quad (1)$$

Physicochemical properties

Hydration properties and oil adsorption capacity (OAC) of Doum DFs: The water swelling (WSC), holding and retention of DFs were determined according to previously described methods [6] while the OAC was estimated using the method reported by Ma and Mu.

Color: Hunter color parameters (L*, a* and b*.) of DFs were measured using a Minolta colorimeter (Lab Scan XE, Hunter Association Laboratory, Reston, VA, USA)after being calibrated using colorimeter standards.

Scanning electron microscopy (SEM): The structure of Doum DFs was studied using a scanning electron microscope (model su1510; Hitachi Co., Japan), and Cambridge 250 MK3 stereo scan operated at 10-20kV. Prior to SEM examination, the samples were coated with gold for 5 min by means of plasma sputtering apparatus.

Fourier transform-infra red spectroscopy (FTIR): The presence of specific functional groups in isolated fiber was recorded on a Nicolet Nexus 470 FT-IR spectrometer (Spectrum One, Perkin Elmer Co., USA) in the range of 4000-400 $cm^{-1}$with 32 scans and a resolution of 4 cm^{-1}. Doum DFs were mixed with KBr (1:250, w/w) followed by pressing the mixture into ultra-thin pellets.

X-ray diffraction (XRD): X-ray diffractometry of the Doum DFs was collected by a Bruker AXS D8 diffractometer (D2 PHASER, Bruker AXS Inc., Germany) using Cu Ka radiation, with a voltage of 40 kV and an incident current of 30 mA. The diffraction intensities were recorded between 5 and 70° (2θ angle range) coupling with a scanning rate of 3°/min and a scanning step length of 0.05°. The degree of crystallinity was calculated from the peak area under the curve with MDI Jade 6.5 software (Materials Data, Inc., California, USA) using the following equation:

$$D_c(\%) = \frac{A_c \times 100}{A_c + A_a} \quad (2)$$

where D_c is a degree of crystallinity; A_c is the crystallized area on the X-ray diffractogram, and A_a is the amorphous area on the X-ray diffractogram.

Extraction of antioxidant from Doum DFs: To determine total phenolics content and antioxidant activity of Doum DFs, 3 g of each sample was extracted with 30 mL of methanol (80%) for 2 h in an ultrasonic cleaner bath at 60 °C. Afterwards, the mixtures were cooled to ambient temperature and then centrifuged at 5000 X g for 15 min. The supernatants were clarified and methanol was eliminated using the rotary evaporator at 50 °C to dryness. The dried extract was re-dissolved in absolute methanol (25 mL) and kept at -4 °C till use.

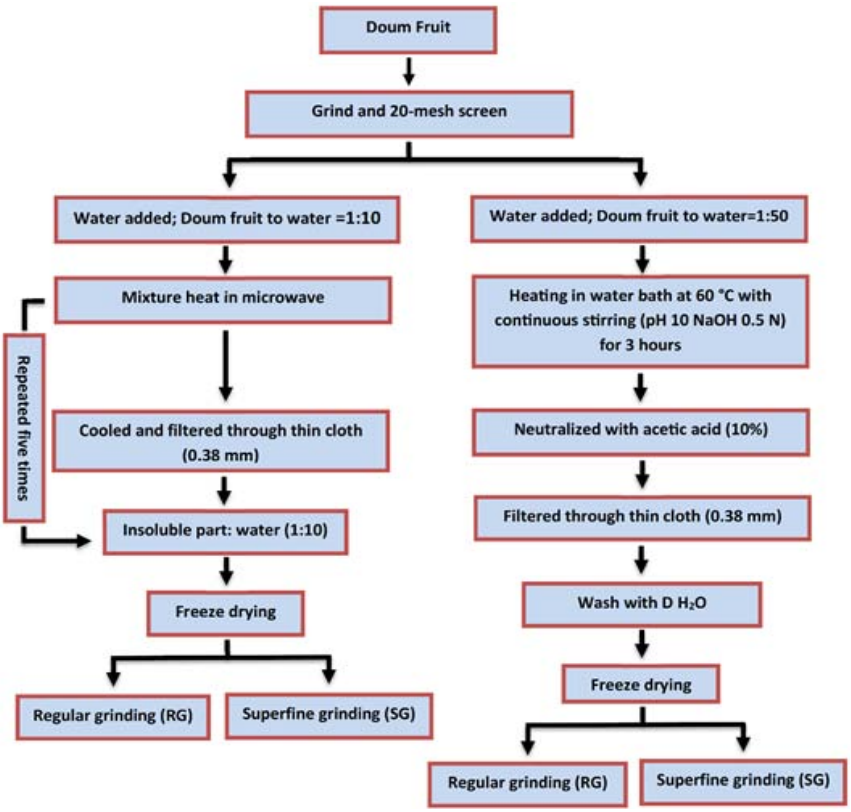

Figure 1: Flow diagram for extraction and grinding of Doum fruit dietary fiber.

Determination of Total phenolic content (TPC): Total polyphenol content of extracts was assessed colorimetrically using Folin-Ciocalteu reagent method. Polyphenol analysis was performed by mixing 0.1 ml of sample extract, 3 ml of distilled water, 0.75 mL of 20% Na_2CO_3 and 0.25 mL of concentrated Folin-Ciocalteu reagent. UV-Vis spectrophotometer at 760 was used to measure the absorbance of the mixture after incubation the in the dark for 30 min at 25°C. Final results of total polyphenol were presented as mg gallic acid equivalents per g dry basis (mg GAE/g db) depending on the linear equation of the gallic acid standard curve at (0.0125-0.125) mg/mL, R^2= 0.998.

Antioxidant activity

Determination of antioxidant activity by the ABTS: *In vitro* radical cation scavenging capacity of Doum DFs against ABTS was determined according to Zhang et al. with some changes. Stock solution of radical cation was prepared by mixing 7 mmol ABTS and 2.45 mmol potassium persulfate in a volume ratio of 1: 0.5; thereafter, the mixture was maintained at ambient temperature in the dark for 16 hours. Working solution of the ABTS was prepared by dilute 7 mmol ABTS radical cation stock solution with PBS (pH 7.4) to an absorbance of 0.68 ± 0.02 at 734 nm. Extract (0.15 mL) of each sample was mixed with 3 ml of ABTS+ working solution. The mixture was kept at 25°C for 10 min and the absorbance was recorded at 734 nm. The antioxidant activity of the extract DDF and the biscuits were presented as mM Trolox equivalent (TE) per 100 g dry basis (TE mM/100 g db) by a linear equation of the standard curve for Trolox. The linearity range of the calibration curve was 0.00 to 0.09 mmol (R^2=0.998).

DPPH free radical scavenging capacity: The DPPH• scavenging effect (DPPH•-SE) of Doum DFs was determined according to [3]. Based on the total phenolic content, 0.15 mL of each extract at different concentrations was treated with a 3 ml of DPPH• methanolic solution (6×10^{-5} mmol/mL). The absorbance after 30 min was measured at 517 nm and the % DPPH•-SE was calculated using the following equation:

$$\%DPPH^{\bullet} - SE = \left(\frac{A_c - A_s}{A_c}\right)\times 100 \qquad (3)$$

where: AC is the absorbance of the control; AS is the absorbance of the sample extract. The half maximal effective concentration (EC50) of DPPH•-SE was calculated using Microsoft Excel (2016).

Data analysis

One-way ANOVA analysis using SPSS software (SPSS, Chicago, Illinois, USA, version 17.0) was conducted to analyze the data. The data were presented as mean ± SD. Duncan's test was performed to determine the significance ($P<0.05$). The EC50 values were calculated using Microsoft Excel 2016.

Results and Discussion

Proximate composition

Proximate composition of Doum fruit and the contents of DFs (AEDFRG, MEDFRG, AEDFSG, and MEDFSG) have been presented in Table 1. The major components of Doum fruit were TDF (48.83 g/100 g), ash (4.6 g/100 g), protein (3.88 g/100 g) and fat (0.95 g/100 g).Furthermore, the TDF of Doum fruit was higher than

Table 1: Proximate composition of Doum fruit and obtained DFs.

	Doum fruit	AEDFRG	MEDFRG	AEDFSG	MEDFSG
Moisture	5.79 ± 0.025^{b}	3.86 ± 0.046^{c}	3.22 ± 0.031^{d}	7.17 ± 0.127^{a}	7.09 ± 0.015^{a}
Ash	4.60 ± 0.111^{a}	1.72 ± 0.060^{c}	1.93 ± 0.090^{c}	1.92 ± 0.046^{c}	2.24 ± 0.075^{b}
Protein	3.88 ± 0.040^{a}	2.39 ± 0.065^{b}	2.45 ± 0.060^{b}	1.88 ± 0.036^{d}	2.21 ± 0.020^{c}
Fat	0.95 ± 0.040^{d}	0.98 ± 0.028^{d}	1.05 ± 0.026^{c}	1.15 ± 0.031^{b}	1.26 ± 0.036^{a}
IDF	42.82 ± 0.415^{d}	88.61 ± 0.625^{a}	88.32 ± 0.491^{a}	75.31 ± 0.591^{b}	72.50 ± 0.732^{c}
SDF	6.01 ± 0.186^{c}	2.42 ± 0.131^{e}	2.91 ± 0.252^{d}	11.72 ± 0.261^{b}	14.33 ± 0.312^{a}
TDF	48.83 ± 0.594^{c}	91.03 ± 0.499^{a}	91.23 ± 0.743^{a}	87.03 ± 0.450^{b}	86.83 ± 0.420^{b}

All the data are expressed as mean ± standard deviation (SD). Data are expressed as ''g/100 g dry basis'' except for moisture content. Means with the different superscript letters within the same line are significantly different (P< 0.05).

Table 2: IDF fractions (%) and Monosaccharides (%)obtained from Doum DFs.

IDF fractions (%)	AEDFRG	MEDFRG	AEDFSG	MEDFSG
NDF	63.39 ± 0.596^{a}	60.07 ± 0.522^{b}	56.72 ± 0.471^{c}	54.41 ± 0.443^{d}
ADF	34.98 ± 0.678^{a}	33.54 ± 0.359^{b}	31.94 ± 0.239^{c}	31.05 ± 0.267^{d}
ADL	15.68 ± 0.632^{a}	15.27 ± 0.397^{a}	11.01 ± 0.587^{b}	10.86 ± 0.346^{b}
Hemicellulose	28.41 ± 0.926^{a}	26.53 ± 0.837^{b}	24.78 ± 0.362^{c}	23.36 ± 0.339^{d}
Cellulose	19.30 ± 0.418^{b}	18.28 ± 0.628^{c}	20.93 ± 0.48^{a}	20.22 ± 0.562^{ab}
Rhamnose	1.69 ± 0.020^{d}	1.96 ± 0.025^{b}	1.84 ± 0.031^{c}	2.42 ± 0.036^{a}
Arabinose	11.18 ± 0.085^{d}	11.54 ± 0.101^{c}	13.37 ± 0.105^{b}	14.55 ± 0.111^{a}
Galactose	3. 17 ± 0.026^{d}	3.51 ± 0.056^{c}	3.64 ± 0.053^{b}	4.61 ± 0.067^{a}
Glucose	4.49 ± 0.066^{c}	2.07 ± 0.036^{d}	10.26 ± 0.092^{a}	7.14 ± 0.075^{b}
Xylose	79.47 ± 0.584^{b}	80.92 ± 0.726^{a}	70.89 ± 0.642^{c}	71.28 ± 0.546^{c}

All the data are expressed as mean ± standard deviation (SD). Means with the different superscript letters within the same line are significantly different (P< 0.05). IDF: Insoluble Dietary Fiber; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ADL: Acid Detergent Lignin.

that of Mango peel (40.60 g/100 g), Apple pomace (30.15 g/100 g), Orange pomace (40.47 g/100 g) and defatted rice bran (31.7 g/100 g) [1,9,10], suggesting that Doum fruit represents an excellent source of DF. Compared to Doum fruit, DFs showed lower levels of protein contents as 2.39, 2.45, 1.88 and 2.21 g/100 g, for AEDFRG, MEDFRG, AEDFSG and MEDFSG respectively; and ash content was found in the range of 1.72 to 2.24 g/100 g. In addition, fat and TDF contents of DFs were higher than Doum fruit and ranged from 0.98 to 1.28 g/100g and 86.83 to 91.23 g/100g respectively. According to results, the TDF contents of the AEDFSG and MEDFSG were more than 86 g/100g, implying that superfine grinding treatment increased the purity. In addition, it was found out that superfine grinding increased the SDF contents from 2.42 g/100g to 11.72 g/100g and from 2.91 g/100g to 14.33 g/100g for alkali and microwave extraction methods respectively. Results revealed that the SDF content of DFs extracted by microwave were significantly (P< 0.05) higher than that extracted by the alkali with both types of grinding. This could be due to the breakdown of glycosidic linkages in dietary fibers as a result of strong alkaline treatment. As shown in Table 1, the SDF content in AEDFSG and MEDFSG were higher than 10%. These findings are in agreement with those reported previously for wheat bran(11.47 g/100 g) and deoiled cumin (7.85-12.26 g/100 g), but lower than buckwheat hull (26 g/100 g) and rice bran dietary fiber (15.1-18.7 g/100 g) [11].

IDF fractions

The amounts ADF, ADL, NDF, hemicellulose and cellulose of Doum DFs are summarized in Table 2. NDF comprised of cellulose, insoluble hemicellulose and lignin while ADF comprised of lignocellulosic fraction. According to results NDF and ADF were predominant fractions in all samples and they were found in the range of 63.39% to 54.41% and 34.98% to 31.05%, respectively. In the case of NDF, fibers from alkali treatments were higher in NDF than those from microwave treatments. It was also found out that dietary fiber processed from regular grinding technique had higher NDF levels than superfine grinding. As shown in Table 2, AEDFRG and MEDFRG had the highest content of ADL and hemicellulose while AEDFSG and MEDFSG contained significantly higher amounts of cellulose. Based on these results, NDF, ADF and hemicellulose contents of DFs were significantly affected by extraction methods and grinding processes, these results are consistent with the findings reported by [11]. Doum DFs had considerable amounts of ADL ranging from 15.68% to 10.86%, with significant variation due to the grinding process. These values were higher than those of alga Ulvalactuca fiber (1.53%-5.57%) [7].

Monosaccharides

The monosaccharide compositions of Doum DF's are presented in Table 3. Among the monosaccharides, xylose and arabinose were in abundance in all samples, followed by glucose, galactose, and rhamnose. As shown in Table 2, DF processed through microwave extraction had higher contents of arabinose, galactose and rhamnose than alkali extraction. Meanwhile, samples processed by superfine grinding had higher content of these monosaccharides than regular grinding due to an increase of SDF in the samples; these results were in conformity with the results listed in Table 1.The high contents of xylose in Doum DFs were related to the hemicellulose (-D-glucan

Table 3: Effect of extraction methods and grinding technologies on particle sizes and physicochemical properties of DFs obtained from Doum fruit.

	AEDFRG	MEDFRG	AEDFSG	MEDFSG
V_{d10} (μm)	79.98 ± 0.226[a]	73.59 ± 0.807[b]	9.08 ± 0.296[c]	7.33 ± 0.365[d]
V_{d50} (μm)	190.49 ± 1.012[a]	187.69 ± 1.169[b]	40.51 ± 0.611[c]	37.93 ± 0.938[d]
V_{d90} (μm)	601.76 ± 1.771[a]	553.79 ± 1.093[b]	88.43 ± 1.014[c]	77.94 ± 0.862[d]
Span	2.74	2.56	1.96	1.86
WHC (g/g)	4.82 ± 0.105[d]	5.03 ± 0.08[c]	5.74 ± 0.101[b]	5.92 ± 0.045[a]
WRC (g/g)	4.05 ± 0.041[d]	4.26 ± 0.087[c]	4.74 ± 0.116[b]	5.13 ± 0.138[a]
WSC (mL/g)	4.97 ± 0.144[c]	6.67 ± 0.255[a]	5.90 ± 0.276[b]	6.88 ± 0.091[a]
OAC (g/g)	1.36 ± 0.031[d]	1.63 ± 0.061[c]	2.37 ± 0.076[b]	2.80 ± 0.031[a]
*L**	69.76 ± 0.225[a]	69.26 ± 0.137[b]	64.68 ± 0.217[c]	60.46 ± 0.192[d]
*a**	8.08 ± 0.137[b]	9.44 ± 0.175[a]	6.60 ± 0.115[c]	9.20 ± 0.185[a]
*b**	15.22 ± 0.177[c]	24.93 ± 0.197[a]	15.87 ± 0.164[b]	13.27 ± 0.131[d]

Values are expressed as mean ± SD (n=3). Means with the different superscript letters within the same line are significantly different (P< 0.05). V_{d10}, V_{d50} and V_{d90} are the equivalent volume diameters at 10%, 50% and 90% cumulative volumes respectively. WHC: Water Holding Capacity; WRC: Water Retention Capacity; WSC: Water Swelling Capacity; OAC: Oil Adsorption Capacity.

Table 4: TPC and antioxidant activity of Doum DFs as determined by the ABTS and % DPPH•-SE (with EC50).

	TPC (mg GAE/g DW)	ABTS (mM TEA/100g DB)	% DPPH•-SE (0.5mg/mL)	EC_{50} (mg/mL)
AEDFRG	68.30 ± 0.271[d]	13.88 ± 0.055[d]	77.73 ± 0.210[d]	0.28
MEDFRG	69.67 ± 0.280[c]	16.39 ± 0.056[c]	82.78 ± 0.368[c]	0.26
AEDFSG	70.58 ± 0.418[b]	16.79 ± 0.074[b]	85.23 ± 0.372[b]	0.23
MEDFSG	72.31 ± 0.417[a]	20.09 ± 0.035[a]	88.76 ± 0.313[d]	0.2

Data are expressed as mean ± standard deviation (n=3) except for EC50 values. Means marked by the same letter within the same column are not significantly different (P< 0.05). TPC: Total Phenolic Contents; EC50: The Extract Concentration Providing 50% Effective Concentration of DPPH•-SE.

containing (1, 4)-linked -d-xylose) content. Moreover, the xylose content of DFs with superfine grinding decreased significantly due to a reduction of cellulose and the amount was significantly lower than samples treated with regular grinding. The glucose content of DFs treated with microwave was lower than those treated with alkaline. This might be so due to the relative decrease of hemicellulose content.

Particle size distributions

The particle size of the Doum DFs measured by the laser particle size analyzer is shown in Table 3. Various reports have studied the effect of particle size on the physico-chemical and functional properties of dietary fiber, and its relation to hydration properties, absorption of glucose, adsorption of bile acid [4]. The average particle sizes of AEDFRG, MEDFRG, AEDFSG and MEDFSG were: 190.49 μm, 187.69 μm, 40.51 μm, and 37.93 μm, respectively. These results have shown that superfine grinding in combination with either alkali or microwave extraction significantly (P< 0.05) decreased the particle size of the dietary fiber powders. Phat et al. reported that the particle size affects the span value and homogeneity of DF powders. According to results, the span values of DFs processed with regular-grind were much higher than those processed with superfine grinding, meanwhile, the alkali extracted fibers had higher span value than microwave extracted fibers possibly due to high IDF contents in alkali fibers.

Physicochemical properties of Doum DFs

Hydration and OAC properties: Hydration properties including water holding capacity (WHC), water retention capacity (WRC), and water swelling capacity (WSC) are shown in Table 3. Previous studies have reported that the particle size of the fiber cannot be the only factor influencing the physico-chemical properties, however the procedure of treatment also has a major impact on the final characteristics [8,11,12]. As shown in Table 3, the hydration properties of Doum DFs increased with decreasing particle size, possibly because of increasing surface area and improved solubilization of SDF by micronization. Thus superfine grinding significantly increased hydration properties of the DFs unlike regular grinding. The results are similar to those reported for carrot insoluble fiber, ginger powder and hull-less barley bran IDF [8].

Oil adsorption capacity (OAC) which determines the ability of DF to adsorb fat was investigated. As shown in Table 4, the OAC values of AEDFRG, MEDFRG, AEDFSG and MEDFSG were 1.36 g/g, 1.63 g/g, 2.37 g/g, and 2.8 g/g, respectively. Thus the OAC values increased with decreasing particle size of Doum DFs. These results are similar to OAC values obtained by both regular and superfine grinding reported previously for carrot insoluble fiber (1.92 g/g and 1.99 g/g), and hull-less barley bran IDF (1.74 g/g and 2.03 g/g) [8].

Color parameters: Results regarding the influence of the extraction methods and grinding technologies on the color of Doum DFs have been summarized in Table 3. As expected, the lightness values decreased from the regular ground to superfine ground with both types of extraction. From Table 3, it is clear that AEDFRG and MEDFRG samples possessed the highest 'L*' values of 69.76 and 69.26, respectively. Meanwhile, the 'b*' values decreased from the samples with regular grinding treatment to superfine grinding treatment, with both kinds of extraction methods. In contrast, the 'a*'values decreased from the treatment AEDFRG to AEDFSG; and the data displayed there was no significant difference (P< 0.05) on

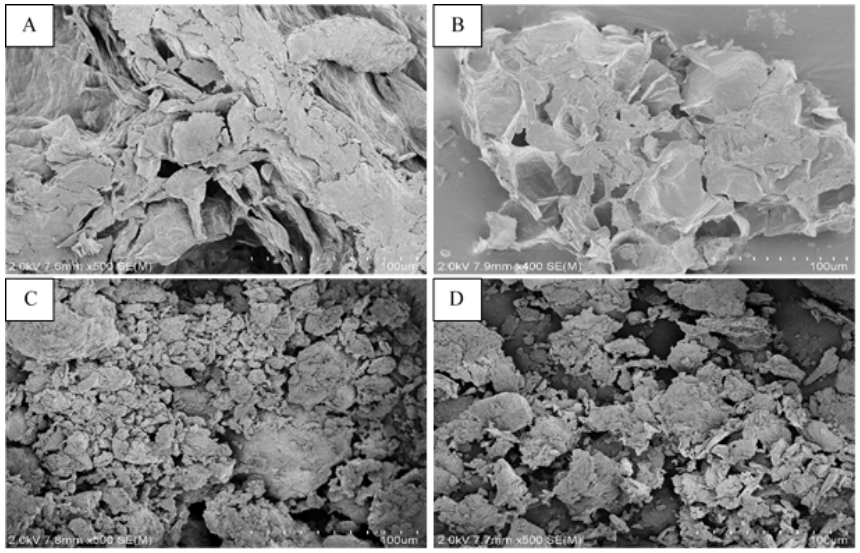

Figure 2: Scanning electron microscopy (SEM) of dietary fiber obtained from Doum fruit by different treatments: (A) AEDFRG; (B) MEDFRG; (C) AEDFSG; (D) MEDFSG.

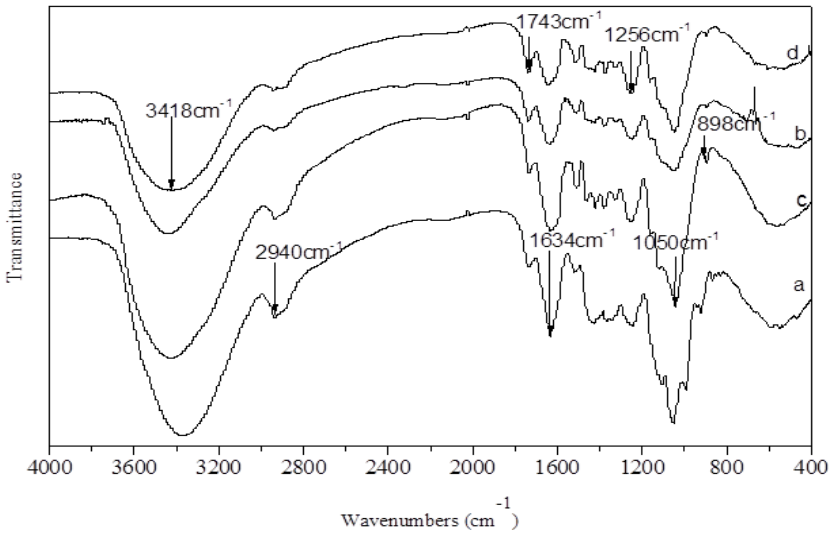

Figure 3: FTIR of dietary fiber obtained from Doum fruit by different treatments: (a) AEDFRG; (b) MEDFRG; (c) AEDFSG; (d) MEDFSG.

'a*'value between MEDFRG and MEDFSG samples. Moreover, MEDFRG were found with higher values of redness and yellowness than the rest of the samples. The variations in color could be due to exposure of samples to heat during extraction and grinding processes. Previously, it was reported by Hu, Chen and Ni that during superfine grinding, samples were subjected to increasing heat, which led to the degradation of chlorophyll and oxidation of catechins.

Scanning Electron Microscopy (SEM), FTIR and XRD analysis

SEM: Figure 2 shows the SEM images of DFs obtained from Doum fruit by different treatments. SEM images of AEDFRG and MEDFRG exposed the existence of a network structure, with some differences among them (Figure 2A and 2B). As shown in the Figure 2A, AEDFRG had larger particle sizes, more irregular shapes with cracks and holes than the rest of the samples. More specifically, a scanning electron micrograph of MEDFRG had a characteristic honeycomb structure, as can be seen in Figure 2B. As shown in Figure 2C and 2D, excessive milling resulted into a number of effects such as break down of the particles into smaller parts, flattening, aggregation and fracture of fibers. These effects led to production of various shapes of particles with a lot of cracks and holes. Furthermore, significant differences between surface morphologies of MEDFSG and AEDFSG were observed possibly due to exposure of samples AEDFSG to alkaline conditions. These findings are consistent with the observations mentioned by Ma and Mu (2016a).

FTIR: Doum DFs were analyzed using Fourier Transform Infrared Raman spectrometer and the FTIR spectrum from 400 to 4000 cm^{-1} has been shown in Figure 3. The FTIR spectra provides

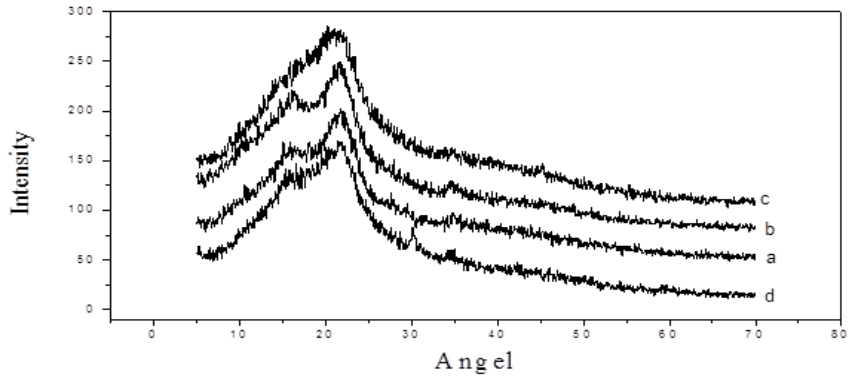

Figure 4: XRD of dietary fiber obtained from Doum fruit by different treatments: (a) AEDFRG; (b) MEDFRG; (c) AEDFSG; (d) MEDFSG.

information about chemical bonding and groups in the structure of organic molecules. The major peaks around 3418, 2940, 1743, 1634 and 1050 cm^{-1} are attributed to the vibrations of O-H, C-H, C-O, C=O and C-O-C. The FTIR spectra of Doum DF powders showed same peaks, because, the chemical composition of the fiber is independent of the particle size as reported by Encalada, Basanta, Fissore, De'Nobili and Rojas. The peak at 3418 cm^{-1} corresponded to characteristic bending or stretching of OH. This hydroxyl group was detected in all samples due to the vibrations of the hydrogen atom involved in the O-H bond found in both cellulose and hemicellulose. Moreover, the characteristic absorption peak of C-H stretching bands at ≈2940 cm^{-1} was also observed in all DF samples due to the presence of methylene group found in polysaccharides. All samples revealed C=O stretching bands at ≈1634 cm^{-1} with strong peak intensity in AEDFRG and AEDFSG samples possibly due to strong alkaline conditions; on the other hand, abroad peak at 1634 cm^{-1} is characteristic of the aromatic benzene associated with lignin. The typical broad band recorded at ≈1743 cm^{-1} is a signal for the carbonyl associated with the ester group present in all samples. All samples had peaks at 1256 and 1050 cm^{-1} which correspond to C-O stretch bond and deformation bands in cellulose and hemicellulose. A weak peak was observed in all samples at 898 cm^{-1} which was suggestive of stretching oscillation of b-glycosidic bonds in polysaccharides.

XRD: XRD analysis can provide valuable information and better understanding about the effects of processing parameters on overall structure, such as crystalline or amorphous natures of the DFs particles. As illustrated in Figure 4, XRD patterns of all tested DFs were found with a prominent 2θ peak at approximately 21.5° and a minor peak at around 16.5°. The peak position and peak width of all the DFs were not significantly different, implying that the crystalline region of Doum DFs wasn't devastated during ultrafine grinding. On the other hand, diffraction peak was observed at 29° (2θ) in MEDFSG sample (Figure 4d) suggesting that the formation of crystallite, which was significantly different from its counterparts. The results revealed that the MEDFSG had the highest scores of crystallinity (52.6%), followed by AEDFSG (51.4%), MEDFRG (47.53%) while AEDFRG showed the lowest value (46.32%). These results show that the superfine grinding had a positive influence on the crystallinity; this effect could be attributed to the decrease of hemicelluloses as well as to increase of celluloses content during grinding of samples [12]. The results are consistent with previous findings in which the amorphous and crystallinity domains of dietary fiber were affected by superfine grinding. On the other hand, alkali extracted fibers had lower crystallinity values than microwave samples with both types of grinding possibly due to denaturation effect of alkali on the cellulose. Previous studies have confirmed that a higher crystallinity of fiber would enhance the thermal stability of fiber and benefit their applications in baking products [12].

Antioxidant properties: The antioxidant activities of the Doum DF extracts were tested by ABTS•+ radical assay, DPPH•-SE assay and total phenolic contents (TPC). The results of TPC of Doum DFs are presented in Table 4. According to results, the MEDFSG were found with the highest contents of TPC (72.31mg GAE/g DB), followed by AEDFSG (70.58mg GAE/g DB) and MEDFRG (69.67mg GAE/g DB) while AEDFRG had the lowest contents (68.30mg GAE/g DB). The differences could be due to the amount of water soluble polysaccharides, non-starch polysaccharides and hemicelluloses. These observations are in accordance with the findings of Ma and Mu.

The ABTS•+, generated by potassium persulfate, is considered as one of the best assay methods for measuring the antioxidant activity of hydrogen-donating antioxidants. Table 4, shows that the antioxidant activity by ABTS•+ radical assay varied considerably, ranging from 13.88 to 20.09 mM TEA/100g DB. These results revealed that the highest antioxidant activity by ABTS•+ were detected in MEDFSG extracts, followed by AEDFSG, MEDFRG, and AEDFRG extracts.

As shown in Table 4, all Doum DF extracts at concentration of 0.5 mg/mL showed strong DPPH•-SE after incubation for 30 min and the values were as follows: MEDFSG (88.76%), AEDFSG (85.23%), MEDFRG (82.78%) and AEDFRG (77.73%). Meanwhile, the EC50 values decreased in the same order thus MEDFSG (0.28 mg/mL), AEDFSG (0.26 mg/mL), MEDFRG (0.23 mg/mL) and AEDFRG (0.20 mg/mL). The extract obtained from AEDFSG and MEDFSG with superfine grinding treatment showed higher antioxidant activities as compared to AEDFRG and MEDFRG with regular grinding. These findings are consistent with those notified in previous studies. It is evident from the results that AEDFSG and MEDFSG had higher TPC and antioxidant activities than the rest of the samples possibly due release and exposure of phenolic compounds as a result of extensive breakdown of the matrix unlike other samples. These results support previous findings of Ma and Mu, in which SDF with high antioxidant activity were also found due to polymers such as pectin, arabinoxylans,

xylans which contain phenol-polysaccharide.

Conclusions

The microwave and alkaline extractions with regular and superfine grinding have proven promising methods for producing DFs from Doum fruit. Superfine grinding decreased particle size of some of Doum DFs and resulted in increasing the physico-chemical properties such as hydration and OAC, and antioxidant potential. The findings of this current study highly favor the application of microwave extraction combined with superfine grinding for the isolation of dietary fiber from other similar agro-food sources. The future research direction may bridge the gap on protective effects of MEDFSG (direct application and/or incorporating into food products) both in *in vitro* and *in vivo* systems as a novel anti-cancer and anti-diabetic therapeutic target.

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