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ORIGINAL PAPER

Nutritional Quality, Functional Properties, Bioactivity, and Microstructure of Defatted Pistachio Kernel Flour

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Abstract Defatted pistachio kernel flour (DPKF) is a byproduct of specialty oil industries and a promising product to be further used as a food ingredient. The purpose of this study was to evaluate nutritional value, functional properties, bioactivity, and microstructure of DPKF prepared by cold pressing (partially defatted, ~22 % residue fat d.b.) and pre-press-solvent extraction (totally defatted, <1 % residue fat d.b.) methods both using raw and roasted kernels. DPKF was rich in protein (34.36–46.47/100 g flour, d.b.) and carbohydrate (36.61–48.71/100 g flour, d.b.). Also, DPKF showed low Na contents with high concentrations of K, P, Ca, Mg, Zn, and Se. Excluding the sulfur amino acid, all essential amino acids were significantly higher than the reference values recommended by Food and Agriculture Organization/WHO. Totally defatted flours presented better functional properties than the partially defatted ones with exceptions of foaming stability. DPKF also exhibited appreciably higher total phenolic (552–792 mg gallic acid equivalent/100 g flour, d.b.) and flavonoid (124–280 mg RE/100 g flour, d.b.) contents as well as antioxidant capacities. Therefore, DPKF may have potential applications as functional ingredients and supplements in foods.

Keywords Bioactivity · Defatted pistachio kernel flour · Functional properties · Microstructure · Nutritional value

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Introduction

Pistachio (*Pistacia vera* L.) is one of the most popular and commercially valuable edible nuts in the world because of its high nutritional value, split shell, and unique flavor. Global pistachio production increased dramatically in recent decades. Based on reports by the Food and Agriculture Organization (FAO), the production increased from 542,037 Mt in 2003 to 916,921 Mt in 2013, and the average export prices of raw and dried pistachios exceeded US$5,000 per ton in the international market in 2013 [1].

Pistachio kernels are commonly consumed as salted and roasted snacks or as ingredients in the confectionary industries. With increasing consumption and demand for specialty oils, pistachios have also been used to produce pistachio kernel oil (PKO). Commercial PKO products have appeared in some Middle Eastern and European countries for several purposes, mainly as a salad dressing or gourmet oil, and also used for cosmetic and therapeutic treatments [2].

Traditionally, specialty oils, such as nut oils, are prepared from raw or roasted kernels using cold pressing or pre-press-solvent extraction because nut seeds are high oil-bearing materials [3]. After extracting oil from nut kernels, the residuals are known as partially or totally defatted cake (flour), which is commonly wasted. Although these byproducts create a disposal problem for the industry, they probably retain nutrients and bioactive compounds present in original kernels, which may provide some potential products of various functional ingredients.

During the past few years, the proximate compositions, physicochemical properties, and storage stability of the flour or paste have been studied for different seeds and nuts. Santos et al. [4] evaluated the physicochemical characterization of defatted Brazil nut kernel flour and indicated its nutritional,

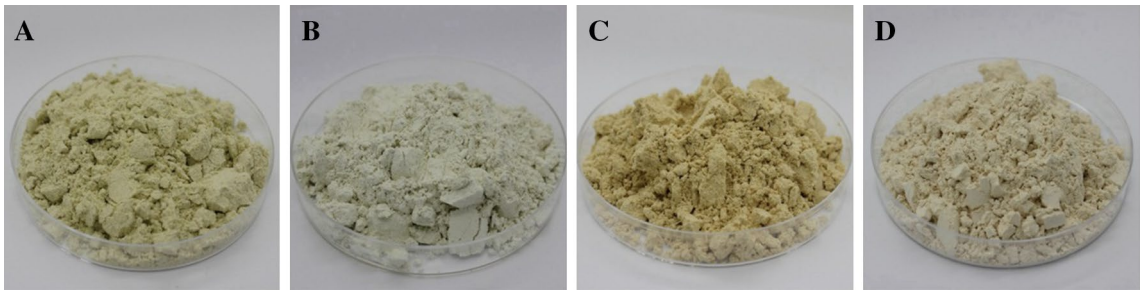

Fig. 1 Photographs of the DPKF obtained by different defatted treatment: **a** PDPKF, **b** TDPKF, **c** PDRPKF, and **d** TDRPKF

functional, and bioactive potentials for use in the application of various food industry sectors. Win et al. [5] reported the effects of roasting treatment at 160 °C for 10, 20, 30, 40, and 50 min on the phenolic composition and antioxidant activity of peanut kernel flour. Using peroxide and fatty acid value as a measure of quality, Ling et al. [6] found that walnut paste could be preserved from oxidation for up to 2 months when stored at room temperature in vacuum or normal packages.

Previous studies concerning pistachios have mainly focused on their kernels and oils, looking at physicochemical characteristics, storage stabilities, changes in color, and volatile compounds after roasting [7–9]. In the literature, however, few comprehensive studies have been conducted on the physicochemical characteristics of defatted pistachio kernel flour (DPKF). The objectives of this research were to evaluate nutritional composition, functional properties, bioactivity, and microstructure of DPKF, and to understand better their possible uses as a functional ingredient in food industry applications.

Materials and Methods

Materials

Raw and dried pistachio nuts (8.0 kg) of the Kerman variety were obtained from Paramount Farm Company (Lost Hills, CA, USA). After removing shells, about 4.0 kg kernels (moisture content 3.77 ± 0.10/100 g in wet basis, w.b.) were sorted to remove any damaged samples and then sealed into polyethylene bags at 4 °C until use. All chemicals used in this study were purchased from either Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) or Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise.

Preparation of DPKF

The kernels were firstly divided into two parts for unroasted raw and dry roasted samples. For dry roasting, to get similar products to those obtained in an industrial roasting treatment, portions of 1 kg of kernels were roasted for 20 min at 160 °C in a laboratory electrical oven [10]. After roasting, both raw and roasted kernels were crushed separately before preparation of partially defatted unroasted (PDPKF) and roasted (PDRPKF) pistachio kernel flour, totally defatted unroasted (TDPKF), and roasted (TDRPKF) pistachio kernel flour, respectively.

To produce partially defatted flours, oil was separated from the crushed kernels using a hydraulic press (Lefen T50, Dezhou, China) at 60 kg/cm^2 for 15 min without additional heat treatment, which obtained a residual fat content of ~22 % (dry basis, d.b.). The resulting press-cake was ground to pass through 50 mesh sieve to obtain partially defatted flour (Fig. 1a, c). To produce totally defatted flour, the resulting partially defatted flour was defatted in a Soxhlet apparatus using petroleum ether (boiling point range of 38.2–54.3 °C) for 24 h to obtain <1 % (d.b.) of residual fat. Then the totally defatted flour was spread into a thin layer (about 3–5 mm) and air-dried for 6 h at ambient temperature in a fume hood, and obtained by passing through a 50 mesh sieve (Fig. 1b, d). All flours were packed in airtight containers and stored in a refrigerator at 4 °C for physiochemical property analyses, which were performed within 1 week.

Determination of Nutritional Composition

Macronutrients and Color Analysis

The proximate compositions including moisture, ash, fat, and protein contents were determined by the AOAC official methods [11]. Total carbohydrate content was estimated by the difference from the total contents. Sample color was measured using a computer vision system (CVS) described by Hou et al. [12] and expressed as CIE (L^*, a^* and b^*) color values.

Micronutrients Analysis

Elemental analysis were according to the method of Çayan et al. [13]. Dried DPKF samples were digested in

a microwave reaction system (MARS 5, CEM Corp., Matthews, NC, USA). The mineral concentrations were determined by inductively coupled plasma mass spectrometer (ICP-MS; Varian 820MS, Varian Pty Ltd, Australia). The operating conditions were: radio frequency applied power of 1.4 kW, sample uptake rate of 300 μL/min, sample depth 7.5 mm, peak hopping scanning mode, dwell time 0.05 s, nebulizer gas flow-rate of 0.86 L/min, plasma gas flow-rate of 18.0 L/min, auxiliary gas flow-rate of 1.8 L/min, and sheath gas flow-rate of 0.13 L/min.

Amino Acid (AA) Profile Analysis

The AA contents (except for tryptophan) in DPKF were determined using an automated amino acid analyzer (L-8900, Hitachi High-Technologies Corp., Tokyo, Japan) according to the standard methods of the instrument. Dried samples of 20–25 mg (d.b.) were hydrolyzed with 6 N hydrochloric acid for 24 h at 110 °C in an evacuated and sealed tube. The hydrolysate was evaporated to dry in a vacuum oven and dissolved in a sodium citrate buffer (pH 2.2) for AA analysis. The AA composition of DPKF was expressed as grams of AA per 100 g protein.

Determination of Functional Properties

Water (WHC) and Oil Holding Capacity (OHC)

WHC and OHC were determined using a modified form of the method described by Kaur and Singh [14]. Approximately 1.0 g (d.b.) samples of each flour (m_1, g) were weighed into a 50 mL centrifuge tube (m_2, g) of known weight when 20 mL of distilled water (or rapeseed oil for OHC) were added. The resulting dispersions were vortexed for 2 min and allowed to stand for 30 min before centrifuging at 2862×g for 20 min (SC-3610, Anhui USTC Zonkia Scientific Instruments Co., Ltd, Hefei, China). The tube with sediment (m_3, g) was reweighed after pouring off the excess water or oil. WHC and OHC were calculated as follows:

$$\text{WHC/OHC (g water or oil/g flour d.b.)} = \frac{m_3 - m_2 - m_1}{m_1}. \quad (1)$$

Emulsifying Capacity (EC) and Stability (ES)

Emulsifying properties were determined using a modified form of the method described by Capitani et al. [15]. Briefly, approximately 2.0 g (d.b.) samples of each flour and 40 mL of distilled water were mixed using a homogenizer (FSH-2A, Chendong Xinrui Instrument Company, Jintan, China) at 16,000 rpm for 1 min. Then, 40 mL of rapeseed oil were added and homogenized at 16,000 rpm for 1 min. A 12.0 mL of the emulsion was centrifuged in a 15 mL graduated centrifuge tube at 716×g for 5 min, and emulsion volume V_1 (mL) was measured. Another 12 mL of the emulsion was heated in 80 °C for 20 min, cooled to room temperature, and centrifuged at 716×g for 5 min. The remaining emulsion volume V_2 (mL) was recorded. EC and ES were calculated as follows:

$$\text{EC (\%, d.b.)} = \frac{V_1}{12.0} \times 100 \quad (2)$$

$$\text{ES (\%, d.b.)} = \frac{V_2}{12.0} \times 100. \quad (3)$$

Foaming Capacity (FC) and Stability (FS)

Foaming properties were determined according to the method described by Cai et al. [16] with some modifications. Approximately 3.0 g (d.b.) samples of each flour were thoroughly mixed with 100 mL of distilled water using an electric blender (JJ-1, Guohua Electric Appliance Co., Ltd, Changzhou, China) at 500×g for 2 min and then immediately poured into a 250 mL graduated cylinder. The total volume V_1 and foam volume V_2 were recorded after 1 min. The FC was expressed as percentage increase in total volume as follows:

$$\text{FC (\%, d.b.)} = \frac{V_1 - 100}{100} \quad (4)$$

The FS was determined by measuring the foam volume V_3 of the mixture after 1 h of standing at ambient temperature and calculated as follows:

$$\text{FS (\%, d.b.)} = \frac{V_3}{V_2} \times 100. \quad (5)$$

Sorption Properties

Water adsorption isotherms were determined using the static gravimetric method standardized by "The European Cooperative Project COST 90" [17]. Completely dehydrated flour samples (~3 g d.b. per plate) were stored in each of three chambers equilibrated with saturated solutions of different salts giving rise to a water activity (a_w) range from 0.113 to 0.843 at 25 °C. Samples were weighed every 2 days until equilibrium was reached. After this period, the final moisture content of each sample was determined according to AOAC official methods [11]. Sorption isotherms were obtained by plotting equilibrium moisture content (EMC, %, d.b.) versus a_w. Experimental data were mathematically modeled with the follow sorption isotherm models: Brunauer Emmett Teller (BET), GAB (Guggenheim Anderson de Boer), Halsey, Oswin, Henderson, and Smith. To evaluate the ability of each model to fit the experimental data, the coefficient of the correlation (R^2), standard error of estimate (SEE), and mean relative deviation (MRD) were determined as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{N} (m_{ep} - m_{pre})^2}{\sum_{i=1}^{N} (m_{ep} - \overline{m_{pre}})^2} \quad (6)$$

$$\text{SEE} = \sqrt{\frac{1}{N - n_c} \sum_{i=1}^{N} (m_{ep} - m_{pre})^2} \quad (7)$$

$$\text{MRD} = \frac{1}{N} \sum_{i=1}^{N} \frac{|m_{ep} - m_{pre}|}{m_{ep}}, \quad (8)$$

where N is the number of experimental observations and n_c the number of constants in each model, m_{ep} represents experimental moisture content values, and m_{pre} denotes value predicted from the model.

Determination of Bioactivity

Extraction of DPKF Bioactives

Extraction of bioactives was carried out according to the method of Tsantili et al. [18] with some modifications. Approximately 3.0 g (d.b.) samples of each flour were weighed into a 50 mL centrifuge tube and homogenized with 30 mL of cold aqueous methanol (80 %, v/v) using a homogenizer at 10,000 rpm for 2 min. The homogenate was sonicated (40 kHz) in the dark for 20 min (KQ5200DE, Kunshan Ultrasonic Instruments, Co., Ltd, Kunshan, China) and was then centrifuged at 3622×*g* for 10 min. The supernatant was collected, and the volume was adjusted to 50 mL with methanol aqueous and stored at −20 °C for further analysis.

Total Phenolic (TPC) and Flavonoid (TFC) Contents

TPC and TFC were measured by modified Folin–Ciocalteu and aluminum chloride colorimetric methods using a spectrophotometer (UV2000, Unico Instrument Co., Ltd, Shanghai, China) as described by Tsantili et al. [18]. The results were expressed as milligrams of gallic acid equivalent (GAE)/100 g flour (d.b.) and milligrams of rutin equivalent (RE)/100 g flour (d.b.) for TPC and TFC, respectively.

Antioxidant Capacity (AC)

Two assays were used for measurement of AC by the radical (2, 2-diphenyl-1-picrylhydrazyl, DPPH·) scavenging capacity test according to Parry et al. [19] and the reducing power (ferric reducing antioxidant power, FRAP) test according to Durmaz and Alpaslan [20]. The results of both DPPH· and FRAP tests were expressed as Trolox equivalent antioxidant capacity values (TEAC, μmol Trolox/g flour, d.b.).

Microscopy Analysis

Characterization of microstructure of DPKF was performed under light microscopy and scanning electron microscopy (SEM) analysis. Briefly, dried samples were suspended in a glycerol/water solution (1:1, v/v) and observed using a light microscope (DMBA400, Motic China Group Co., Ltd, Guangzhou, China) with polarized light. For SEM analysis, samples were mounted on a scanning electron microscope stub with double-sided adhesive tape and coated with gold. Scanning electron micrographs were taken using a scanning electron microscope (JSM-6360LV, JEOL, Ltd, Tokyo, Japan) and viewed at 1000× amplification using an accelerating voltage of 15 kV.

Statistical Analysis

Data were reported as mean ± SD of triplicate measurements. Significant differences ($P < 0.05$) within means were separated by analysis of variance and Tukey's significant difference (HSD) test in the statistical software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Nutritional Compositions

Macronutrient compositions of the DPKF from raw and roasted kernels are listed in Table 1. The protein and carbohydrate were the major components in the DPKF with an average content of about 34–47 and 36–49/100 g, respectively. The protein content of DPKF was approximately 1.7–2.3-fold higher than that of their pristine kernels (~21/100 g, d.b.) and close to that in defatted soybean flour (48/100 g, d.b.) reported by Rosset et al. [21]. A major difference between partially and totally defatted flour was in their fat content, and the residual fat in the press-cake was proposed by considering the composition of the extracted oil using the Soxhlet method. According to the study of oils cold-pressed from the Kerman pistachio kernels, reported by Ling et al. [22], PKOs contained mostly unsaturated fatty acids (UFAs, ~87/100 g oil) and high contents of tocopherols (~367 mg/kg oil). Therefore, it is hypothesized that the residual fat could be considered another nutritional benefit of the partially defatted flour.

Color values (Table 1) confirm the visual perception of the DPKF samples (Fig. 1). No significant differences ($P > 0.05$) were observed in different samples in terms of L^* values except for PDRPKF with slightly lower values. However, the flour obtained from roasted kernels showed higher values in a^* and b^*, causing the flours to be significantly darker than the others, which is most probably due to

Table 1 Macronutrient compositions (g/100 g d.b.) and color values of the DPKF

Composition	PDPKF	TDPKF	PDRPKF	TDRPKF
Moisture	6.01 ± 0.09^{c,C}	8.29 ± 0.08^{a}	3.21 ± 0.05^{d}	6.61 ± 0.05^{b}
Protein^{A}	36.99 ± 0.29^{b}	44.95 ± 0.58^{a}	34.36 ± 0.05^{c}	46.47 ± 1.04^{a}
Lipid	21.90 ± 0.32^{b}	0.59 ± 0.07^{c}	23.55 ± 0.24^{a}	0.67 ± 0.09^{c}
Ash	4.50 ± 0.02^{c}	5.75 ± 0.03^{b}	4.42 ± 0.06^{c}	5.84 ± 0.03^{a}
Carbohydrate^{B}	36.61	48.71	37.67	47.02
*L**	66.20 ± 0.92^{a}	66.74 ± 1.15^{a}	63.62 ± 1.03^{b}	67.71 ± 2.22^{a}
*a**	–2.99 ± 0.23^{c}	–1.98 ± 0.27^{b}	–0.78 ± 0.19^{a}	–0.68 ± 0.33^{a}
*b**	17.20 ± 0.48^{b}	6.36 ± 0.37^{d}	20.45 ± 0.30^{a}	10.25 ± 0.28^{c}

PDPKF partially defatted unroasted flour, *PDRPKF* partially defatted roasted flour, *TDPKF* totally defatted unroasted flour, *TDRPKF* totally defatted roasted flour

[A] Protein: $N \times 5.3$

[B] Carbohydrate content was calculated by subtracting other nutrients from the total weight

[C] Means followed by different superscript letters represent significant differences in the treatments for measured properties ($P \leq 0.05$)

Table 2 Micronutrient compositions (mg/100 g d.b.) of the DPKF

Minerals	PDPKF	TDPKF	PDRPKF	TDRPKF
Macrominerals				
Magnesium (Mg)	136.38 ± 1.27^{d,A}	164.76 ± 2.67^{a}	140.81 ± 0.95^{c}	159.02 ± 1.83^{b}
Potassium (K)	1,356.69 ± 37.64^{b}	1,659.59 ± 26.38^{a}	1,284.35 ± 45.76^{b}	1,711.40 ± 26.91^{a}
Calcium (Ca)	154.01 ± 4.27^{c}	182.13 ± 1.36^{b}	160.69 ± 6.75^{c}	191.16 ± 3.17^{a}
Phosphorus (P)	801.17 ± 13.77^{c}	1,002.20 ± 10.18^{b}	769.80 ± 29.70^{c}	1,103.20 ± 15.70^{a}
Sodium (Na)	2.09 ± 0.04^{c}	3.18 ± 0.10^{a}	2.25 ± 0.06^{b}	3.02 ± 0.15^{a}
Microminerals				
Iron (Fe)	1.13 ± 0.03^{b}	2.54 ± 0.03^{a}	1.16 ± 0.07^{b}	2.63 ± 0.05^{a}
Copper (Cu)	1.62 ± 0.10^{b}	2.06 ± 0.02^{a}	1.73 ± 0.11^{b}	1.98 ± 0.03^{a}
Manganese (Mn)	1.73 ± 0.02^{b}	2.14 ± 0.05^{a}	1.59 ± 0.08^{c}	2.03 ± 0.15^{a}
Zinc (Zn)	3.69 ± 0.08^{b}	4.66 ± 0.06^{a}	3.79 ± 0.19^{b}	4.60 ± 0.10^{a}
Selenium (Se)^{B}	34.00 ± 1.00^{b}	40.67 ± 1.53^{a}	30.33 ± 1.53^{c}	38.67 ± 2.08^{a}

PDPKF partially defatted unroasted flour, *PDRPKF* partially defatted roasted flour, *TDPKF* totally defatted unroasted flour, *TDRPKF* totally defatted roasted flour

[A] Means followed by different superscript letters represent significant differences in the treatments for measured properties ($P \leq 0.05$)

[B] Unit: μg/100 g d.b.

the appearance of Maillard reaction products (MRPs) occurring during roasting treatment. The totally defatted flour was found to have higher *a** and lower *b** values (decreased intensity of green and yellow color) than that corresponding to the partially defatted flour, probably because fat-soluble pigments (e.g. carotenoids and chlorophylls) could be reduced with the seed fat removal. Although DPKF samples revealed unique colors, the different color could be a base for selection in different applications.

The mineral composition analysis in Table 2 showed that K was the most abundant nutrient, followed by P, Ca, and Mg. The patterns of mineral elements in DPKF were similar to those reported for Californian pistachios (variety Kerman) [23]. In turn, there was no clear trend concerning the mineral contents between flours from raw and roasted kernels. An important characteristic of DPKF was in high levels of K, Ca, and Mg contents associated with lower amounts of Na since the lower dietary sodium chloride consumption was the most beneficial for overall good health, and diabetes and coronary heart disease prevention [24]. For microelements, Se was one of the most prominent minerals with average content of 30.33–40.67 μg/100 g (d.b.) in DPKF samples. Zn was another important micromineral essential for various reactions and enzymatic activities with average contents of about 3.7 mg/100 g (d.b.) in the partially defatted flour.

Table 3 presents the AA profile of the partially DPKF compared with those from partially defatted peanut flour

Table 3 AA composition comparison of the partially defatted kernels flour between pistachio and peanut (PDPF)

Amino acid (g/100 g of protein)	Requirement pattern[A]	PDPKF	PDRPKF	PDPF[B]
Essential amino acids (EAAs)				
Histidine (His)	1.6/1.5	2.43 ± 0.12[a,C]	2.17 ± 0.07[b]	2.89 ± 0.29
Threonine (Thr)	2.5/2.3	3.35 ± 0.03[a]	3.10 ± 0.09[b]	2.94 ± 0.05
Lysine (Lys)	4.8/4.5	5.32 ± 0.13[a]	5.43 ± 0.11[a]	3.42 ± 0.17
Methionine + Cystine (Met + Cys)	2.4/2.2	1.69 ± 0.14[a]	1.40 ± 0.07[b]	2.56 ± 0.39
Isoleucine (Ile)	3.1/3.0	4.05 ± 0.15[a]	3.88 ± 0.03[a]	3.18 ± 0.07
Leucine (Leu)	6.1/5.9	7.05 ± 0.05[a]	6.60 ± 0.12[b]	6.53 ± 0.08
Valine (Val)	4.0/3.9	5.51 ± 0.17[a]	5.38 ± 0.02[a]	3.72 ± 0.31
Phenylalanine + Tyrosine (Phe + Tyr)	4.1/3.8	7.20 ± 0.25[a]	7.52 ± 0.07[a]	8.54 ± 0.15
Tryptophan (Try)	0.66/0.6	ND	ND	ND
Total	29.26/27.7	36.60	35.48	33.78
Non-essential amino acids (NEAAs)				
Asparagine (Asp)	–	8.98 ± 0.36[a]	8.42 ± 0.14[b]	11.20 ± 0.43
Glutamine (Glu)	–	23.44 ± 0.36[a]	22.34 ± 0.29[b]	23.05 ± 0.35
Serine (Ser)	–	5.77 ± 0.28[a]	5.26 ± 0.16[b]	5.38 ± 0.20
Glycine (Gly)	–	4.33 ± 0.07[a]	4.12 ± 0.03[b]	5.70 ± 0.14
Argnine (Arg)	–	9.37 ± 0.12[a]	8.96 ± 0.12[b]	11.11 ± 0.29
Alanine (Ala)	–	4.21 ± 0.12[a]	4.12 ± 0.03[a]	4.08 ± 0.25
Proline (Pro)	–	9.71 ± 0.14[a]	8.29 ± 0.10[b]	5.19 ± 0.34
Total	–	65.81	61.52	65.71

PDPKF partially defatted unroasted flour, *PDRPKF* partially defatted roasted flour, *PDPF* partially defatted peanut flour, *ND* not determined

[A] Amino acid requirements for schoolchildren (3–10) and adult over 18 years of age [25]

[B] Source from Zheng et al. [37]

[C] Means followed by different superscript letters represent significant differences in the treatments for measured properties ($P \leq 0.05$)

(PDPF), because peanuts are consumed as a major oil and protein source in food industry. Results indicated that total EAAs of PDPKF formed 36.60 % of the total AA content, and most were at higher levels than those listed in the FAO/WHO [25] with the exception of sulfur amino acid (Met + Cys). Total NEAAs represented 65.81 % of total AA contents while Glu, Pro, Arg, and Asp were found to be in higher contents. Roasting resulted in a significant ($P < 0.05$) decrease in the levels of most AAs, which may be due to the Maillard browning reactions between the AA and reducing sugar the contributed to formation of roasted flavor compounds during roasting. Except for sulfur amino acid (Met + Cys) and aromatic amino acid (Phe + Tyr), all EAAs in PDPKF were similar to or higher than those in PDPF. As for NEAAs, PDPKF even had higher Pro content than PDPF, while Asp, Gly, and Arg contents were lower than those in PDPF. The DPKF samples analyzed are rich sources of Ile (ranging from 3.88 to 4.05/100 g protein), Leu (ranging from 6.60 to 7.05/100 g protein), and Val (ranging from 5.38 to 5.51/100 g protein), equivalent to 1.33–1.88 g Ile, 2.27–3.28 g Leu, and 1.85–2.56 g Val in 100 g (d.b.) of flour samples, respectively. These form a unique and important group among all AAs, act primarily on skeletal muscle, and are known as branched chain AAs. For the NEEAs, Glu presented the highest content with a range of 7.68–10.54/100 g (d.b.) in DPKF, which is an important AA for energy metabolism and the immune system. Also, higher levels of Arg (3.08–4.21/100 g d.b.) with lower Lys (1.87–2.52/100 g d.b.) in the DPKF positively influences in vivo Arg uptake by cells, which may be beneficial for cardiovascular disease [26].

Functional Properties

Water/fat holding capacities of the flour are represented by their ability to bind the structure, reducing moisture and fat loss, and improving the flavor and mouth-feel of food products. The WHC and OHC of DPKF samples ranged from 2.14 to 3.65 and from 1.29 to 1.93 g/g (d.b.), respectively (Table 4). A significant increase ($P < 0.05$) was observed in the WHC and OHC of the totally defatted flour above their counterparts, which were partially defatted both from raw and roasted kernels. This was due to totally removal of oil film (residual fat <1 %, d.b.) from flour particles by permitting exposure of large number of hydrophilic and hydrophobic parts bound to more water and oil units. Moreover,

Table 4 Functional properties of the DPKF at their natural pH

Property	PDPKF	TDPKF	PDRPKF	TDRPKF
WHC (g water/g flour, d.b.)	$2.14 \pm 0.14^{d,A}$	3.32 ± 0.26^{a}	2.41 ± 0.10^{c}	3.65 ± 0.14^{a}
OHC (g oil/g flour, d.b.)	1.29 ± 0.04^{c}	1.64 ± 0.03^{b}	1.30 ± 0.02^{c}	1.93 ± 0.09^{a}
EC (%, d.b.)	57.21 ± 1.90^{b}	62.31 ± 1.50^{a}	58.94 ± 1.07^{b}	60.82 ± 1.77^{ab}
ES (%, d.b.)	58.51 ± 1.01^{c}	62.22 ± 2.15^{ab}	59.73 ± 0.61^{bc}	63.04 ± 1.23^{a}
FC (%, d.b.)	22.17 ± 2.25^{c}	61.00 ± 3.97^{a}	19.00 ± 2.78^{c}	52.33 ± 3.62^{b}
FS (%, d.b.)	67.19 ± 3.58^{a}	60.27 ± 2.25^{b}	62.36 ± 3.73^{ab}	54.92 ± 2.28^{c}

PDPKF partially defatted unroasted flour, *PDRPKF* partially defatted roasted flour, *TDPKF* totally defatted unroasted flour, *TDRPKF* totally defatted roasted flour

[A] Means followed by different superscript letters represent significant differences in the treatments for measured properties ($P \leq 0.05$)

the WHC and OHC of the samples from roasted kernels were slightly larger than those in its counterparts prepared from raw kernels. Similar increases are also reported for processed canola flours, possibly due to the heat dissociation and denaturation of proteins that expose additional binding sites available for water and oil [27].

At the natural pH of the flour, the EC of TDPKF (62.31 % d.b.) and TDRPKF (60.82 % d.b.) was significantly ($P < 0.05$) higher than that of PDRPKF (57.21 % d.b.) and PDRPKF (58.94 % d.b.) ($P < 0.05$, Table 4). However, there were no significant differences ($P > 0.05$) in EC between flours from raw and roasted kernels. Considered for ES of different samples, they followed the same trend noticed for EC. Comparing the emulsifying properties of the totally defatted flour with the data of flour from other sources under similar experimental conditions, TDPKF or TDRPKF showed lower EC and ES than those of chickpea flour, which possessed 65.2–68.8 % (d.b.) and 76.6–77.4 % (d.b.) for EC and ES, respectively [14]. Also, emulsifying properties from TDPKF or TDRPKF were similar to those of chia flour (56.00 and 60.00 % d.b. for EC and ES, respectively) [15]. Although both the chickpea (22.3–24.3/100 g d.b.) and chia (41.36/100 g d.b.) flours had lower protein content than TDPKF or TDRPKF (44.95–47.47/100 g d.b.), the emulsifying properties of the chickpea and chia flours were higher than or similar to those of TDPKF or TDRPKF. This implies that the emulsifying properties of plant flours depends not just on the protein content, but also on protein characteristics, such as the ratios of hydrophilic to hydrophobic amino acids.

Similar to the water/fat holding and emulsifying properties at the natural pH of the samples, the FC of totally defatted flour was significantly higher ($P > 0.05$) than that of partially defatted flour (Table 4). However, partially defatted flour with lower FC [22.17 vs. 61.00 % (d.b.) for PDPKF vs. TDPKF] showed a significantly higher ($P < 0.05$) FS [67.19 vs. 60.27 % (d.b.) for PDPKF vs. TDPKF] compared with their counterparts that were totally defatted. A similar inverse relationship has also been reported for defatted pumpkin seed flours, and it is generally considered that the flours with lower FC can form smaller air bubbles surrounded by thicker and more flexible protein films [28]. As a result, it is more difficult for air bubbles to collapse, and the foams are more stabilized. Furthermore, roasting treatment reduced the FC and FS of the different flours with totally defatted flours having a significant effect ($P < 0.05$). This reduction may be due to high protein–protein interaction, leading to formation of aggregates detrimental to foam formation and diminished nitrogen solubility due to thermal denaturation.

The water sorption phenomenon of the flour as a hygroscopic material exerts a strong influence on its stability and quality during packaging and storage. Figure 2 shows the experimental data of EMC versus a_w at 25 °C for different DPKF. Regardless the flour obtaining procedure, a slow increase in EMC was observed between the a_w of 0.1 and 0.6 followed by a steep increase beyond 0.6, since sorption isotherms can be considered according to the BET classification as type II, this is typically one of the most biological products [29]. Among the flours obtained from raw and roasted kernels, the sorption ability was not significantly different ($P > 0.05$), while the fat content significantly influenced the sorption ability of the flours ($P < 0.05$). With the same EMC, the totally defatted flours had lower a_w than partially defatted ones, indicating that totally defatted flours were more stable than partially defatted ones when they were stored under the same conditions, especially for high relative humidity. This difference in sorption behavior was previously reported and can be ascribed to the differences in the chemical composition of partially and totally defatted flours as shown in Table 1 [30]. Furthermore, this finding was also in agreement with the results observed in WHC (Table 4), with a greater amount of chemical constituents having hydrophilic parts in totally defatted flours, such as proteins and carbohydrates. Thus, the adsorption capacity can be enhanced.

Six known models presented in Table 5 were tested for their goodness-of-fit in describing the isotherms of

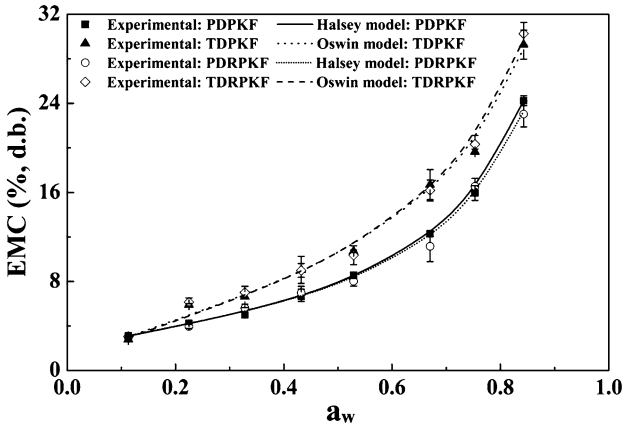

Fig. 2 Experimental and predicted equilibrium moisture (EMC, %, d.b.) as a function of a_w for DPKF at 25 °C

the flours in terms of R^2, SEE and MRD. Among these, the model best fitting the experimental data for partially defatted flours was that proposed by Halsey, while the Oswin model was more adequate to fit data corresponding to totally defatted flours with the highest value of R^2 and lowest value of MRD. The nonlinear regression equations of those models were EMC = $(-8.814/\ln a_w)^{0.808}$ ($R^2 = 0.999$), EMC = $10.49[a_w/(1-a_w)]^{0.603}$ ($R^2 = 0.995$), EMC = $(-9.27/\ln a_w)^{0.788}$ ($R^2 = 0.994$), EMC = $10.504[a_w/(1-a_w)]^{0.619}$ ($R^2 = 0.994$) for PDPKF, TDPKF, PDRPKF, and TDRPKF, respectively.

Bioactivity

TPC and TFC of the DPKF ranged from 552 to 792 mg GAE/100 g and 124 to 280 mg RE/100 g flours (d.b.), respectively (Fig. 3). The results observed in DPKF were higher than those reported for dried whole pistachio kernels from USA (527 mg GAE/100 g d.b. and 143 mg RE/100 g d.b.) [31]. This might be due to the fat extraction results with a relative increase in the proportion of polyphenolic compounds. However, the flours corresponding to the solvent extraction process (TDPKF and TDRPKF) showed significantly ($P < 0.05$) lower TPC and TFC compared with their counterparts obtained by pressing (PDPKF and PDRPKF), which was probably caused by the degradation and solubilisation of non-polar phenolic compounds during solvent extraction.

The ability of antioxidant compounds in DPKF to reduce the DPPH to its non-radical form and reducing Fe^{3+}/ferricyanide complex to Fe^{2+} was compared with that of Trolox, which is a water-soluble analogue of tocopherol (Fig. 4). Flours corresponding to the roasting treatment [67–79 and 103–131 μmol Trolox/g flour (d.b.) for DPPH· and FRAP assays, respectively] showed a great AC than that of flours obtained from raw kernels [50–60 and 75–97 μmol Trolox/g flour (d.b.) for DPPH· and FRAP assays, respectively]. Increase in AC with roasting might be due to the formation of MRPs, which was a more efficient antioxidant in hydrophilic media systems [32]. The partially defatted flours showed significantly ($P < 0.05$) higher AC than totally defatted ones both for flours from raw and roasted kernels, which was probably caused by presence of a high content of polyphenolic compounds in partially defatted flours (Fig. 3). In addition, the higher AC of partially defatted flours can be also expounded by the presence of some plant pigments, which also have higher AC in addition to other naturally occurring polyphenolic substances [33]. On the contrary, those pigments may be removed completely during long solvent extractions, which can be also confirmed by the visual perception of the flours (Fig. 1) due to the reduction of intensity of green and yellow color after totally defatting. According to the similar experimental conditions used in the determination of AC, the DPKF had higher DPPH· scavenging capacity 50–79 μmol Trolox/g flour (d.b.) than that of cold-pressed pumpkin seed flours 2.2 μmol Trolox/g flour (d.b.) [19], as compared to lower DPPH· scavenging capacity than that of cold-pressed grape seed flours 11,800–15,000 μmol Trolox/g flour (d.b.) [34].

Microstructure

The morphological analysis from light microscopy (Fig. 5a) showed that the TDPKF was mainly composed

Table 5 Mathematical expressions, coefficients of correlation (R^2), standard error of estimate (SEE), and mean relative deviation (MRD) of selected sorption models for DPKF at a_w range of 0.113–0.843

Model	Mathematical expression	R^2	SEE	MRD
Smith	$EMC = A - B\ln(1-a_w)$	0.974–0.986	1.129–1.416	0.065–0.125
Oswin	$EMC = A[a_w/(1-a_w)]^B$	0.990–0.995	0.583–0.734	0.054–0.083
Henderson	$EMC = [-\ln(1-a_w)/A]^{1/B}$	0.970–0.982	1.259–1.528	0.110–0.163
Halsey	$EMC = (-A/\ln a_w)^{1/B}$	0.992–0.999	0.177–0.848	0.016–0.096
GAB	$EMC = ABCa_w/[(1-Ba_w)(1-Ba_w+BCa_w)]$	0.833–0.964	0.000–0.000	0.032–0.053
BET	$EMC = ABa_w/[(1-a_w)(1+Ba_w-a_w)]$	0.957–0.991	0.000–0.013	0.034–0.105

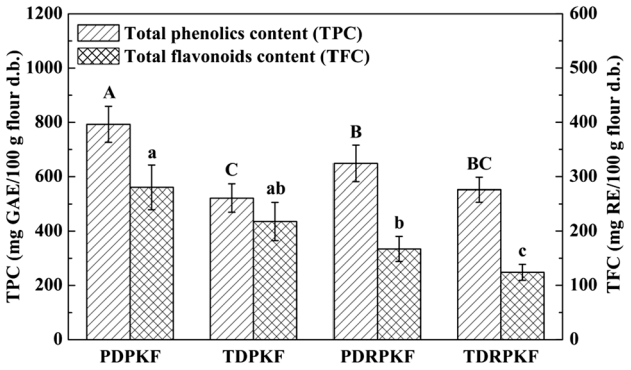

Fig. 3 Total phenolics (TPC) and flavonoids (TFC) contents of the DPKF. Values with *different capital or small letters* are significantly different ($P \leq 0.05$) between the flours for TPC or TFC

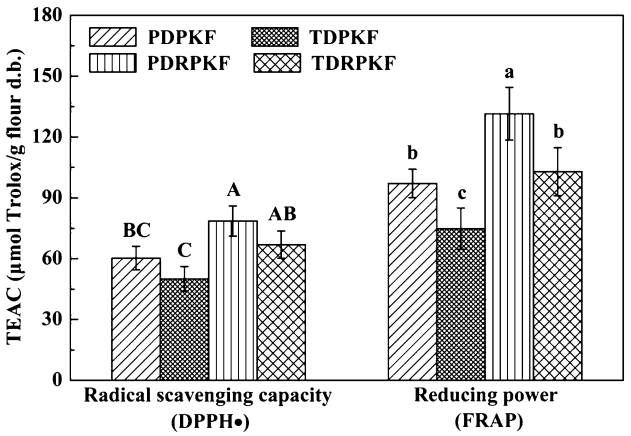

Fig. 4 Trolox equivalent antioxidant capacity (TEAC) values of the DPKF measured by DPPH· and FRAP assay. Values with *different capital or small letters* are significantly different ($P \leq 0.05$) between the flours

of globular structures embedded in flaky kernel tissues. Although the majority of the transparent globular structures found in the light micrographs of nut kernel embryos were normally reported as oil-bearing structures [3], the globules found in the TDPKF were likely to be protein and starch granules rather than liquid bodies, because the flours were already totally defatted (<1 %, d.b.). Also, the occurrence of starch granules located together with protein granules could be found in starchy endosperm of cereals [35] and cotyledon of nut kernels [36]. To distinguish the starch granules from the protein bodies, polarized light was used to observe the TDPKF under the same slide, and the position of starch granules could be identified by noticing the bright particle with birefringence phenomenon (Fig. 5b). The starch granules were distributed throughout the kernel tissue, but with a low level. Generally, the sizes of protein bodies were smaller than those of starch granules, thus the larger globules found in the SEM images (Fig. 6a–d) were starch granules, which were oval in shape with smooth surfaces and approximately 5–15 μm in diameter.

No differences were observed in SEM micrographs between raw and roasted samples. However, PDPKF and PDRPKF (Fig. 6a, c) exhibited large amounts of continuous structure with smooth surfaces surrounding the starch granules and protein clusters compared with a little present in TDPKF or TDRPKF (Fig. 6b, d), which were mainly characterized by the presence of spongy-aspect particles of varied sizes. Taking into account the residual fat content (~22 %, d.b.), the continuous structure in the partially defatted flours may be a lipidic one closely related to the protein and carbohydrate matrix. After totally defatting, the lipid–protein and lipid–starch interaction lead to matrix deformations, which may explain the presence of the large number of spongy-aspect structures after solvent extraction. The microstructure observed here for DPKF could

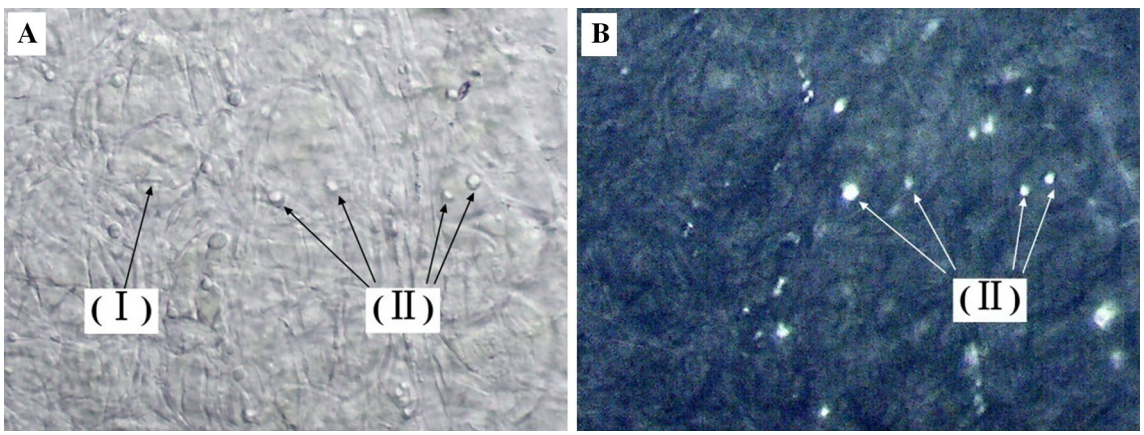

Fig. 5 Normal light micrograph (**a**) and polarized light micrograph (**b**) of TDPKF at the same position under magnification of ×400. Each *arrow* indicates an example of (*I*) kernel tissues and (*II*) starch granules

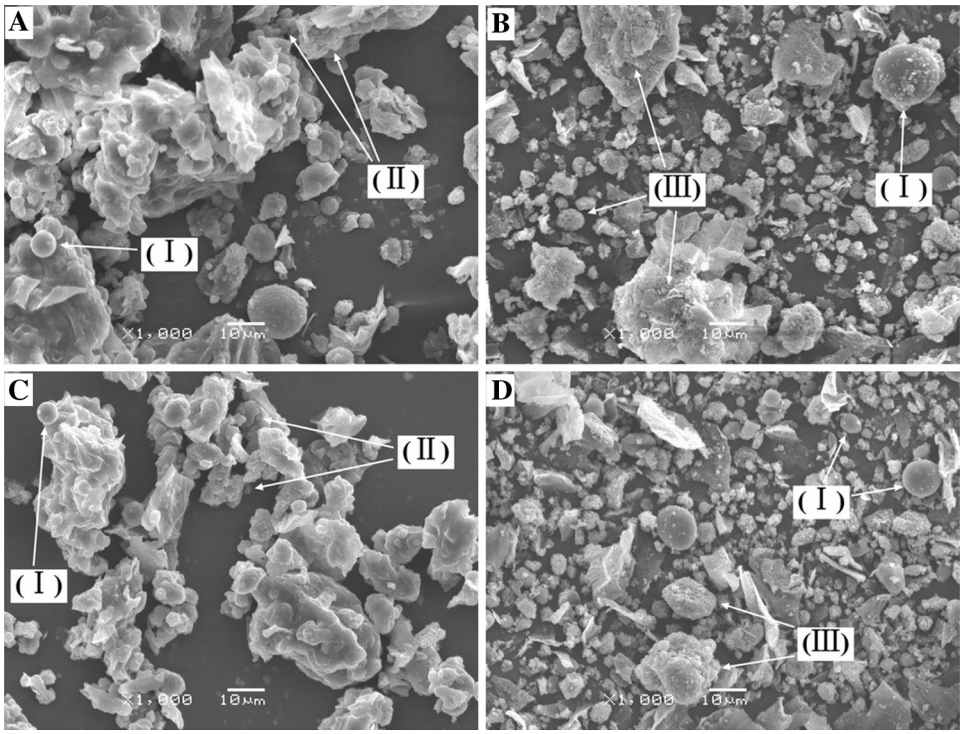

Fig. 6 Scanning electron microstructure of DPKF with **a** PDPKF, **b** TDPKF, **c** PDRPKF, and **d** TDRPKF under magnification of ×1,000. Each *arrow* indicates an example of (*I*) starch granules, (*II*) protein-like structure, and (*III*) sponge-like structure

also provide a better understanding of the analyzed functional properties and how the presence or absence of lipids and the consequently higher concentrations of proteins and carbohydrates affected their functional properties.

Conclusions

DPKF is a good source of proteins and carbohydrates. Presence of abundant EAAs and some minerals plays an important role in regulating various physiological functions in human body. Totally defatted flours presented significantly higher WHC, OHC, EC, ES, and FC than the flours obtained by partially defatting both from raw and roasted kernels. All the flours exhibited a high AC, which is associated with the polyphenolic compounds and MRPs of flours from roasted kernels. Its structural morphology confirmed the differences of functional properties between partially and totally defatted flours. Because of its high protein contents and residual fat associated with UFAs, tocopherols, and bioactives, partially defatted flours can be mainly used as ingredients in bakery and confectionary products to improve nutritional quality. Because they provide not only high protein content, but also better functional properties, the totally defatted flours can be improved both for nutritional and edible qualities of food products.

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