

## Research Article

# Physicochemical properties, volatile compounds, and oxidative stability of cold pressed kernel oils from raw and roasted pistachio (*Pistacia vera* L. Var Kerman)

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Pistachio kernel oil (PKO) was prepared by cold-pressing from raw (RPKO), conventional (CRPKO), and microwave (MRPKO) roasted kernels for Kerman pistachio. Physicochemical properties, bioactive compounds, antioxidant activities, and thermal behaviors of extracted oils were determined in this study. Volatile compounds were tentatively identified and semi-quantified by headspace solid-phase micro-extraction combined with gas chromatography-mass spectrometry technique. The results showed that there was no significant difference between oil samples for some physicochemical parameters and fatty acid profiles. Roasting treatments caused an increase in total phenolics and antioxidant capacity and a decrease in levels of total tocopherols and chlorophylls. The thermal transition temperatures of the RPKO were  $-47.15$  and  $-19.85^{\circ}\text{C}$  for crystallization, and  $-13.11$  and  $3.11^{\circ}\text{C}$  for melting. Among the 43 volatile compounds identified, the major compounds were limonene,  $\alpha$ -pinene,  $\beta$ -myrcene, hexanoic acid, and nonanal in RPKO. The levels of these compounds decreased after roasting. However, concentrations of pyrazines, furans, and pyrroles increased significantly as a result of roasting, especially for CRPKO. Using a PV of 15 meq/kg oil as a quality criterion, PKO stored in transparent glass bottles after exposed to fluorescent light (720 Lux) and room temperature conditions had an acceptable quality for only 30 days of storage.

**Practical applications:** Pistachios are normally consumed as salted and roasted snacks or as an ingredient in bakery and confectionery products, desserts, and ice-creams. With the increasing consumption and demand for novel edible oils, the market is expanding and creating space for the production of the pistachio kernel oil. After analyzing the physicochemical characteristics and volatile compounds, roasting treatments may provide additional volatile compounds in the pistachio kernel oil. The research results suggest that cold-pressed pistachio kernel oil is highly susceptible to photo-oxidative degradation, and must be stored in containers with light-barrier properties together with the addition of some appropriate natural antioxidants.

**Keywords:** Cold press / Oxidative stability / Physicochemical properties / Pistachio kernel oil / Roasting

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**Abbreviations:** AOC, antioxidant capacity; CDV, conjugated diene value; CRPKO, conventional roasted pistachio kernel oil; FFA, free fatty acid; MRPKO, microwave roasted pistachio kernel oil; NPF, non-polar fraction; PF, polar fraction; PKO, pistachio kernel oil; PV, peroxide value; RI, refractive index; RPKO, raw pistachio kernel oil; TO, total oils; TPC, Total polyphenol content

## 1 Introduction

Nuts are nutrient dense foods, generally rich in unsaturated fatty acids, plant protein, and micronutrients, with wide-ranging cardiovascular and metabolic benefits [1]. Among these nuts, the pistachio (*Pistacia vera* L.) is an increasingly important crop, and one of the popular tree nuts of the world. The global pistachio production increases dramatically during the past decade. Based on reports by FAO, as the

production has been raised from 542 037 Mt in 2003 to 916 921 Mt in 2013, which are mainly contributed by three world producers: Iran, USA, and Turkey [2].

Pistachios are normally consumed as salted and roasted snacks or as an ingredient in bakery and confectionery products, desserts, and ice-creams. With the increasing consumption and demand for novel edible oils, the market is expanding and creating for productions of the pistachio kernel oil (PKO). The oil content ranges from 50 to 60% (dry weight, d.w.) in kernels, depending mainly on the cultivar, crop year, and geographic location [3]. Triglycerides constitute the major components of PKO, in which monounsaturated and polyunsaturated fatty acids are present in high levels [4]. The presence of other micronutrients, such as tocopherols, sterols, and plant pigments, has been also documented [5, 6].

PKO is not described by the current Committee on Fats and Oils of the Codex Alimentarius, but it is prized as a specialty oil owing to beneficial effects on human health [7]. Commercial PKO products have been appeared in some Middle East and Europe countries for several purposes, mainly as a salad dressing or gourmet oil, but also used for cosmetic and therapeutic products [8]. Due to high unsaturation and micronutrient levels of nut oils, an extreme care needs to be taken to prevent quality degradation reactions during extraction processes. Mechanical pressing is a simple and safe technique, although more oil remains in the meal than by solvent extraction. For specialty oils with unique in flavor, odor, and special characteristics, such as nut oils, pressing methods could maintain their raw and roasted aroma as compared with solvent extraction, in which subsequent refining may lead to refined oil with a neutral aroma. Especially, cold pressing is the most reliable method for nut oils since heating is not applied to oil seeds during the pressing and can yield very pure, safe, nutritionally rich, and sensorially acceptable virgin oils, which do not require refining and can be consumed directly [9].

Over the last few years, physicochemical properties, aromatic profiles, and oxidative stability of cold pressed oil obtained by either screw press or hydraulic press have been studied for various nuts. Oro et al. [10] studied the physicochemical properties and storage stability of pecan nut oil obtained by hydraulic pressing and found that sensory characteristics of pecan nut oil were unaltered for up to 60 days of storage at room temperature in dark. Bendini et al. [11] using quantitative descriptive analysis evaluated the sensory quality of cold pressed sunflower oil and found that volatile profiles of the sunflower oil were well in agreement with the data provided by the sensory judgment. Uquiche et al. [12] found that microwave pretreatment could be applied to hazelnut oil extraction by hydraulic pressing to improve the oil recovery and quality.

Previous studies concerning PKO have mainly focused on yields of extracted oil, chemical composition, and changes in oxidative stability after roasting [13–15]. In the literature,

however, comprehensive studies on physicochemical or aromatic properties and storage stability of PKO extracted by cold press methods were scarce. The aims of this research were to analyze physicochemical characteristics and volatile compounds of cold-pressed PKO from raw and two roasted kernels prepared by conventional and microwave roasting treatments and to investigate their storage stability during a 4-month storage period with light and dark conditions.

## 2 Materials and methods

### 2.1 Materials

Raw and dried pistachio nuts (in-shell) of the Kerman variety were obtained from Paramount Farming Company (Lost Hills, CA, USA), which is the major cultivar of *Pistacia vera* grown in California, USA. After cleaned manually to remove all foreign matter and broken nuts, they were sealed into polyethylene bags at  $4 \pm 1^\circ\text{C}$  until use. The tested samples were randomly selected from a batch of 10 kg pistachio nuts. All chemicals used in this study were purchased from either Sinopharm Chemical Reagent Co., Ltd (Shanghai, P. R. China) or Sigma-Aldrich (St. Louis, MO, USA).

### 2.2 Kernel preparation and oil extraction

At the beginning of the study, the outer shells of nuts were removed manually. Then the kernels were portioned to be three parts for unroasted raw, conventional and microwave roasting. Conventional roasting was following a traditional method commonly used for industrial roasting treatment [16]. Each batch of 500 g kernels were spread on a stainless tray as a single layer and roasted in a forced convection hot-air oven at  $160^\circ\text{C}$  for 20 min. The microwave roasting intensity for pistachios was selected according to the equivalent thermal energy to the conventional roasting and was thus performed in a 1 kW domestic microwave oven (LGWD700, LG Electronic Appliances, Tianjin, P. R. China) on low power (0.3 kW) with the kernels of 300 g in a Pyrex beaker (500 mL) for 6 min as proposed by Ozel et al. [17]. During microwave heating, the kernels were mixed up at 1 min intervals and the internal temperature was about  $130^\circ\text{C}$  measured by thermocouples at the end of treatment. The roasting process was conducted in triplicate.

After roasting, kernels were cooled to ambient temperature and thoroughly mixed before preparation of unroasted raw PKO (RPKO), conventional roasting PKO (CRPKO), and microwave roasting PKO (MRPKO), respectively. Each batch  $200 \pm 0.5$  g of crushed samples were transferred to the hydraulic press (LefenT50, Dezhou, P. R. China) and then pressed at  $60 \text{ kg/cm}^2$  for 15 min without additional heat treatment. When oil and press cake were collected and weighed after each cold pressing, the fine particles in the extracted oils were separated by centrifugation at  $2862g$  for

10 min (SC-3610, Anhui USTC Zonkia Scientific Instruments Co., Ltd, Hefei, P. R. China). The supernatant oil was then transferred into airtight glass containers and stored at  $-20^{\circ}\text{C}$  in the dark until required. Press cakes were ground in a blender to obtain a homogeneous flour sample and then put into zipped bags and frozen at  $-20^{\circ}\text{C}$  until the analyses.

### 2.3 Proximate analysis of pistachio kernel and press cake

Raw pistachio kernels and press cake flours from different treatments were analyzed for determining their moisture (Vacuum oven,  $95\text{--}100^{\circ}\text{C}$ ,  $68\text{--}85\text{ kPa}$ ), ash (Muffle furnace,  $550^{\circ}\text{C}$ ), fat (Soxhlet, petroleum ether, 16 h), and protein ( $N \times 5.30$ ) contents by the standard methods of AOAC [18]. Total carbohydrate content was estimated by the difference from the total contents. All proximate composition data were reported on a wet basis (w.b.). Sample color was measured using a computer vision system (CVS) described by Kong *et al.* [19] and expressed as CIE ( $L^*$ ,  $a^*$ , and  $b^*$ ) color values.

### 2.4 Physical and chemical analysis of cold pressed oils

#### 2.4.1 General properties and composition

The general properties and composition of cold pressed PKO included: free fatty acid (FFA), peroxide value (PV), and conjugated diene value (CDV), which were determined according to standard methods of AOCS [20]. Refractive index (RI) was obtained using an Abbe refractometer (Model 2WAJ, SHANGHAI CSOIF CO., LTD., Shanghai, P. R. China) at  $25^{\circ}\text{C}$ . CIE ( $L^*$ ,  $a^*$ , and  $b^*$ ) color values were also measured by a computer vision system (CVS) described by Kong *et al.* [19]. The fatty acid (FA) profile was determined by gas chromatography (GC) according to Ling *et al.* [21].

#### 2.4.2 Bioactive compounds and antioxidant activity

Quantification of tocopherols was using a 510 Waters HPLC with a Radial Pak  $C_{18}$  column ( $5\mu\text{m}$ ,  $8\text{ mm} \times 10\text{ cm}$ ) and UV detector according to the GB/T 5009.82-2003 standard method [22], which is the National Standard of the People's Republic of China issued by China State Bureau of Standards. The mobile phase was methanol and  $\text{H}_2\text{O}$  (v/v, 98:2). The flow rate was  $1.7\text{ mL/min}$ . The detection wavelength was  $290\text{ nm}$ .  $\alpha\text{-}V_{\text{E}}$ ,  $\delta\text{-}V_{\text{E}}$ , and  $\gamma\text{-}V_{\text{E}}$  were used as  $V_{\text{E}}$  standard. Chlorophylls were determined spectrophotometrically at  $669\text{ nm}$  in cyclohexane via specific extinction values using the method of Cai *et al.* [23] and the results expressed as pheophytin an equivalent. Total polyphenol content (TPC) and antioxidant capacity (AOC) were determined according to the method of Arranz

*et al.* [24] with minor modifications. Briefly,  $1\text{ g}$  of oil was dissolved in  $2\text{ mL}$  of methanol, and the mixture was vigorously stirred for  $5\text{ min}$ . After centrifuged at  $2862g$  for  $5\text{ min}$ , the methanol phase was taken and the residue was re-extracted with same procedure, the methanol phases were combined as polar fraction (PF), and the remaining oil was used as the non-polar fraction (NPF). TPC was determined in the PF by Folin–Ciocalteu method as described by Cai *et al.* [23]. AOC was measured by DPPH assay as described by Orthofer and Lamuela-Raventos [25] in total oils (TO), PF, and NPF, respectively. AOC was also determined in PF by ABTS assay according to Sánchez-González *et al.* [26] in comparison to DPPH assay.

#### 2.4.3 Thermal behavior

Thermal properties of PKO were determined by a differential scanning calorimeter (DSC Q2000, TA Instruments, New Castle, Delaware, USA) equipped with Refrigerated Cooling System RCS90. The instrument was calibrated using indium and gallium. Nitrogen (99.99% purity) was used as the purge gas at a rate of  $50\text{ mL/min}$ . Oil samples ( $10\text{--}15\text{ mg}$ ) were placed in the hermetically sealed Tzero aluminum pan and the reference pan was an empty aluminum pan with its lid. All samples were subjected to the following temperature programs:  $60^{\circ}\text{C}$  isotherm for  $1\text{ min}$ , cooled at  $3^{\circ}\text{C/min}$  to  $-80^{\circ}\text{C}$ , then held at  $-80^{\circ}\text{C}$  isotherm for  $1\text{ min}$ , and heated at  $3^{\circ}\text{C/min}$  to  $60^{\circ}\text{C}$ . The enthalpy change ( $\Delta H$ ,  $\text{J/g}$ ), onset temperature ( $T_{\text{on}}$ ,  $^{\circ}\text{C}$ ), offset temperature ( $T_{\text{off}}$ ,  $^{\circ}\text{C}$ ) and peak temperature ( $T_{\text{p}}$ ,  $^{\circ}\text{C}$ ) of transition for cooling and heating curves were obtained using Universal Analysis Software (Version 4.5A, TA Instruments).

### 2.5 Volatile compounds

Volatile compounds were analyzed by headspace solid-phase micro-extraction (HS-SPME) coupled to GC–MS as reported earlier with minor modifications [27]. Briefly, Three grams of the oil sample and  $10\mu\text{L}$  of 1,2,3-trichloropropane ( $2.000\text{ mg/mL}$  in methanol) as internal standard (IS) were weighed into a  $15\text{ mL}$  glass vial, sealed with aluminum foil and crimped with aluminum caps, and heated at  $50^{\circ}\text{C}$  for  $10\text{ min}$  to equilibrate the volatiles in the headspace. Then volatiles were sampled for  $30\text{ min}$  from the headspace of the vial, with a pre-conditioned Supelco 57348  $2\text{ cm}$ ,  $50/30\mu\text{m}$  DVB/Carboxen/PDMS Stable-Flex (Supelco, Bellefonte, PA, USA) fiber. After sampling, the fiber was immediately inserted into the injection port ( $250^{\circ}\text{C}$ ) of a Thermo Finnigan Trace GC (Thermo Electron, San Jose, CA, USA) coupled to a Trace DSQ MS, and was thermally desorbed for  $3\text{ min}$  in splitless mode. The tested components were separated on a capillary column DB-WAX ( $30\text{ m} \times 0.25\text{ mm id}$ ,  $0.25\mu\text{m}$  film thickness, J&W Scientific, Folsom, Ca, USA). The GC oven temperature was initially maintained at  $40^{\circ}\text{C}$  for

2.5 min and then programmed at 8°C/min to the final temperature of 230°C, which was maintained for 3 min. Helium was the carrier gas at 0.8 mL/min flow rate. The mass spectrometer in the electron impact mode (MS/EI) at 70 eV was scanned in the range of 33–450 amu. The ion source temperature was 200°C. Volatile compounds were identified by comparison of their mass spectra data with those of the NIST (National Inst of Standards and Technology) mass spectra search library and Kovát indices (KI). Identification was considered as tentative when it was based on only mass spectral data. Volatile compounds were semi-quantification to follow the relative changes in course of the roasting process and to find out the influence of roasting on the flavor of pistachio kernel oil. Therefore, given concentration values are not noted as absolute concentration values but as equivalents to the internal standard. The relative concentrations of the investigated compounds were calculated by relating the areas of the IS to the areas of the compounds of interest. Mean relative concentration (mg/kg) = concentration of IS × peak area of compound/peak area of the IS.

## 2.6 Storage stability test

PKO (100 mL) samples were transferred separately into transparent grinding mouth glass bottle (100 mL). The bottled oils were placed inside a thermostated chamber at 25 ± 1°C. Two sets of bottled oils were prepared: one set was stored under illumination (720 Lux and a photoperiod of 10:14 (L:D) h), and the another one was kept in the dark by wrapping each bottle with an aluminum foil. Each treatment was prepared in duplicate. The progress of lipid oxidation of the oil was assessed by measuring PV and CDV with drawing an aliquot of the sample and testing it every 15 days for a period of 4 months.

## 2.7 Statistical analysis

Data were reported as mean ± standard deviation of triplicate measurements. Significant differences ( $p < 0.05$ ) within means were analyzed using variance and Tukey's honestly significant difference (HSD) test in the statistical software SPSS 16.0 version (SPSS Inc., Chicago, IL, USA).

## 3 Results and discussion

### 3.1 Proximate analysis of pistachio kernel and press cake flour

As shown in Table 1, the pistachio kernels used in this study contained 3.13% moisture, 20.26% crude protein, 23.88% carbohydrate, 2.52% ash, and 50.11% oil, and the results are in agreement with those in the literature [3]. The general compositions of the remaining press cake after aforementioned pressing conditions are also shown in Table 1. No significant differences were observed in different press cake samples in terms of protein, fat and oil yield. However, oil recovery was slightly higher in the treated kernels (33.27 and 32.95%) than those of raw kernel (31.57%). Similar trends were also observed in carbohydrate and ash content, which may be due to the different initial moisture content of kernels caused by roasting. Use of the cold press resulted in the loss of 36% of the oil compared to the Soxhlet method (32 vs. 50% oil on w.b.), which is an unavoidable problem associated with cold pressing. However, with a low-fat and high-protein contents press cake flour seemed to be a promising product, which can be further used as a food ingredient. Compared to full fat kernel flour, defatting significantly improved the  $a^*$  value and reduced the  $b^*$  values (decreased intensity of green and yellow color) as lipid-soluble pigments (e.g., carotenoids

**Table 1.** Proximate composition of the pistachio kernel and press-cake flour

Parameter	Kernel	Press-cake flour		
		Raw	Conventional roasting	Microwave roasting
Moisture (%)	3.13 ± 0.09	4.58 ± 0.04 <sup>a1</sup>	0.91 ± 0.09 <sup>c</sup>	2.06 ± 0.03 <sup>b</sup>
Protein (N% × 5.3)	20.26 ± 0.27	34.10 ± 0.52 <sup>a</sup>	34.75 ± 0.39 <sup>a</sup>	34.93 ± 0.46 <sup>a</sup>
Carbohydrate (%) <sup>2</sup>	23.88 ± 0.97	31.85 ± 0.23 <sup>b</sup>	35.37 ± 0.15 <sup>a</sup>	36.29 ± 0.69 <sup>a</sup>
Ash (%)	2.52 ± 0.02	3.83 ± 0.09 <sup>b</sup>	4.08 ± 0.04 <sup>a</sup>	3.89 ± 0.07 <sup>b</sup>
Fat (%)	50.11 ± 1.34	25.38 ± 0.11 <sup>a</sup>	24.68 ± 0.37 <sup>a</sup>	23.20 ± 0.25 <sup>b</sup>
Oil yield (%)	–	31.57 ± 1.56 <sup>a</sup>	33.27 ± 1.12 <sup>a</sup>	32.95 ± 1.39 <sup>a</sup>
$L^*$	70.21 ± 0.95 <sup>3</sup>	68.27 ± 0.93 <sup>a</sup>	66.63 ± 0.34 <sup>b</sup>	67.69 ± 0.89 <sup>b</sup>
$a^*$	–(5.81 ± 0.29)	–(2.15 ± 0.34) <sup>c</sup>	1.10 ± 0.11 <sup>a</sup>	0.14 ± 0.03 <sup>b</sup>
$b^*$	42.96 ± 0.52	18.59 ± 0.12 <sup>c</sup>	23.65 ± 0.33 <sup>a</sup>	20.58 ± 0.49 <sup>b</sup>

<sup>1</sup>Means followed by different superscript letters represent significant differences in the treatments for measured properties ( $p \leq 0.05$ ).

<sup>2</sup>Carbohydrate content was calculated by subtracting other nutrients from the total weight.

<sup>3</sup>Color values were obtained from kernel flour to reduce the data variability caused by kernel membrane.

and chlorophylls) could be removed with the kernel lipid removal.

## 3.2 Physical and chemical properties of the cold pressed oils

### 3.2.1 General properties and compositions

Table 2 shows the general properties and compositions of cold pressed PKO prepared from raw and roasted kernels. No significant differences were observed in oil samples in terms of RI, FFA, and FA compositions. Values for RI in PKO was 1.471 on average, this index is a specific variable for edible oil related to the degree of unsaturation and presence of unusual components, which can be used to quickly evaluate the level of suspected adulteration in edible oils, Yildiz et al. [28] also reported a similar average RI value of 1.469 for PKO from five variety of Turkey pistachio nut. Both conventional and microwave roasting methods led to a clear decrease in  $L^*$  and  $b^*$  values and increase in  $a^*$  value. In this way, the color of PKO changed gradually from yellow–green before roasting to yellow–brown after roasting, which is most probably due to the appearance of products from the Maillard reaction and the degradation of chlorophylls. Considered for degree of lipid hydrolytic and oxidation, RPKO had very low FFA (0.27), PV (0.40) and CDV (0.72) values. However, roasting treatments caused the PV and CDV of oils increased significantly, but they were still much lower than the maximum values ( $PV < 15$  and  $FFA < 4.0$ ) established by the Codex Alimentarius [29] for non-refined oils. Six major FAs determined in PKO were in agreement with the FA profile in Kerman pistachios reported by Tsantili et al. [3]. However, there was no significant difference in FA profile between different samples. These results are in accordance with those reported by Durmaz and

Gokmen [14], since roasting caused slight variations in fatty acid composition of pistachio kernel oil but without significant difference.

### 3.2.2 Bioactive compounds and antioxidant activity

As shown in Table 3, the total tocopherols were 366.72 mg/kg in RPKO and the major isomers are  $\beta$  and  $\gamma$  tocopherols. It is observed that the contents of  $\alpha$ -tocopherol,  $\beta + \gamma$  tocopherol and total tocopherols decreased clearly after roasting, especially for CRPKO. These results were in accordance with previous studies reported by Durmaz and Gokmen [14], which are generally attributed to the thermal degradation or microstructural changes in kernels during roasting. Chlorophylls are usually associated with color and quality of some plant oils, and RPKO contains relatively high level of chlorophylls (12.21 mg/kg oil), which is similar to the values reported by Saber-Tehrani et al. [30] for cold-pressed pistachio kernel oil from Iran (17.21 mg/kg oil). After roasting chlorophylls levels were reduced slightly (9.95–10.23 mg/kg oil). This degradation may be due to the conversion of chlorophylls to pheophytins and pyropheophytins during thermal treatment. Regarding TPC, PKO showed very low concentrations (38.70–49.46  $\mu\text{g}$  GAE/g oil) compared to those of the dried pistachio kernels (6.3–14.2 mg GAE/g d.w) reported by Tsantili et al. [31]. This might be due to their low oil solubility, and only minor amounts of phenolics could be present in the extracted oil. The AOC of PKO determined by DPPH assay expressed as Trolox equivalent antioxidant capacity (TEAC) values ranging between 136.26 and 171.01  $\mu\text{g}$ /g oil, with roasted samples having higher values. It could be noted that the NPF had always a higher AOC than the PF in three oil samples. Similar results were also observed by previous studies for commercial oilseeds [32], and they generally consider that

**Table 2.** General properties and composition of cold-pressed PKO

Parameter	RPKO	CRPKO	MRPKO
RI at 25°C	1.4712 ± 0.0003 <sup>a1</sup>	1.4710 ± 0.0002 <sup>a</sup>	1.4711 ± 0.0003 <sup>a</sup>
$L^*$	42.37 ± 0.62 <sup>a</sup>	29.22 ± 0.02 <sup>c</sup>	36.89 ± 0.18 <sup>b</sup>
$a^*$	−(3.84 ± 0.10) <sup>a</sup>	−(0.59 ± 0.07) <sup>c</sup>	−(2.22 ± 0.18) <sup>b</sup>
$b^*$	32.12 ± 0.26 <sup>a</sup>	25.41 ± 0.11 <sup>c</sup>	26.30 ± 0.07 <sup>b</sup>
FFA (% oleic acid)	0.27 ± 0.01 <sup>a</sup>	0.28 ± 0.03 <sup>a</sup>	0.27 ± 0.03 <sup>a</sup>
PV (meq/kg oil)	0.40 ± 0.04 <sup>c</sup>	2.65 ± 0.17 <sup>a</sup>	1.16 ± 0.15 <sup>b</sup>
CDV( $K_{232}$ )	0.72 ± 0.03 <sup>b</sup>	0.81 ± 0.02 <sup>a</sup>	0.79 ± 0.02 <sup>a</sup>
Palmitic acid (16:0) <sup>2</sup>	10.95 ± 0.12 <sup>a</sup>	11.03 ± 0.08 <sup>a</sup>	11.11 ± 0.10 <sup>a</sup>
Palmitoleic acid (16:1)	1.05 ± 0.01 <sup>a</sup>	1.08 ± 0.02 <sup>a</sup>	1.01 ± 0.12 <sup>a</sup>
Stearic acid (18:0)	1.05 ± 0.02 <sup>a</sup>	1.06 ± 0.10 <sup>a</sup>	1.11 ± 0.01 <sup>a</sup>
Oleic acid (18:1)	55.37 ± 1.67 <sup>a</sup>	55.89 ± 1.09 <sup>a</sup>	56.05 ± 0.92 <sup>a</sup>
Linoleic acid (18:2)	31.32 ± 1.08 <sup>a</sup>	30.69 ± 0.92 <sup>a</sup>	30.46 ± 0.51 <sup>a</sup>
Linolenic acid (18:3)	0.26 ± 0.04 <sup>a</sup>	0.26 ± 0.01 <sup>a</sup>	0.23 ± 0.05 <sup>a</sup>

<sup>1</sup>Means followed by different superscript letters represent significant differences in the treatments for measured properties ( $p \leq 0.05$ ).

<sup>2</sup>The results were expressed as g of FA per 100 g of total FAs.

**Table 3.** Bioactive compounds and antioxidant capacity of cold-pressed PKO

Parameter	RPKO	CRPKO	MRPKO
$\alpha$ -tocopherols <sup>1</sup>	33.57 ± 1.74 <sup>a2</sup>	24.44 ± 0.08 <sup>b</sup>	34.69 ± 1.32 <sup>a</sup>
$\beta$ + $\gamma$ -tocopherols	308.65 ± 4.31 <sup>a</sup>	280.00 ± 2.26 <sup>c</sup>	296.70 ± 3.54 <sup>b</sup>
$\delta$ -tocopherols	24.50 ± 0.23 <sup>a</sup>	25.47 ± 0.31 <sup>a</sup>	20.25 ± 0.30 <sup>b</sup>
Total tocopherols	366.72 ± 6.29 <sup>a</sup>	329.91 ± 2.04 <sup>c</sup>	351.64 ± 1.92 <sup>b</sup>
Chlorophylls	12.21 ± 0.97 <sup>a</sup>	9.95 ± 0.68 <sup>b</sup>	10.23 ± 0.56 <sup>b</sup>
TPC <sup>3</sup>	38.70 ± 2.94 <sup>b</sup>	49.46 ± 2.49 <sup>a</sup>	46.97 ± 2.00 <sup>a</sup>
AOC-TO <sup>4</sup>	136.26 ± 9.15 <sup>b</sup>	171.01 ± 11.10 <sup>a</sup>	156.93 ± 6.74 <sup>a</sup>
AOC-NPF	95.56 ± 10.99 <sup>b</sup>	118.44 ± 3.05 <sup>a</sup>	109.64 ± 10.37 <sup>ab</sup>
AOC-PF	56.34 ± 6.91 <sup>a</sup>	66.87 ± 3.64 <sup>a</sup>	61.01 ± 5.98 <sup>a</sup>
AOC-PF <sup>5</sup>	75.29 ± 5.65 <sup>b</sup>	90.12 ± 7.07 <sup>a</sup>	84.64 ± 5.92 <sup>ab</sup>

<sup>1</sup>Tocopherols and chlorophylls contents were expressed as mg/kg oil.

<sup>2</sup>Means followed by different superscript letters represent significant differences in the treatments for measured properties ( $p \leq 0.05$ ).

<sup>3</sup>The results were expressed as  $\mu\text{g}$  gallic acid equivalents (GAE)/g oil.

<sup>4</sup>Antioxidant capacity was expressed as  $\mu\text{g}$  trolox equivalent/g oil in TO, NPF, and PF determined by the DPPH assay.

<sup>5</sup>Antioxidant capacity was expressed as  $\mu\text{g}$  trolox equivalent/g oil in PF determined by the ABTS assay.

the higher AOC of NPF might be attributed to the high amount of lipid-soluble compounds, such as tocopherols and pigments, compared to minor amounts of phenolics and water-soluble compounds in oils. Compared to DPPH assay, the TEAC values in PF were significant higher determined by ABTS assay, which was probably due the more sensitivity of ABTS radical for antioxidant compounds. Furthermore, the AOC of PKO determined by ABTS assay for PF showed slight increase after thermal treatment both for conventional and microwave roasting. Similar results were also reported by Wagner et al. [33], and they generally considered that the increase in AOC of polar fraction of food systems with roasting might be due to the formation of Maillard reaction products (MRPs), which was a more efficient antioxidant in hydrophilic media systems especially for ABTS radical scavenging assay.

### 3.2.3 Thermal behavior

DSC is the most widely used thermo-analytical method for oils and fats, and has been used for determination of solid fat content, crystallization and melting profiles, polymorphic forms, and adulteration of edible oils [34, 35]. Table 4 lists the complete thermal transition points derived from DSC curves of three PKO samples. Both crystallization and melting curves of three oils showed two distinct exothermic and endothermic peaks. The major peaks (peak 1 of Fig. 1A and B) were observed at lower temperatures together with the minor (peak 2 of Fig. 1A and B) ones at higher temperatures of DSC curves. Obviously, the cooling curves appeared to be not altered after roasting treatment, and comparisons among these thermal parameters showed no significant differences between three oil samples. The heating

**Table 4.** Thermal transition temperatures of cold-pressed PKO

Thermal behavior	RPKO	CRPKO	MRPKO
<b>Crystallization</b>			
$T_{\text{on}}$	-18.41 ± 0.54 <sup>a1</sup>	-19.24 ± 0.65 <sup>a</sup>	-18.96 ± 0.36 <sup>a</sup>
$T_{\text{off}}$	-51.06 ± 0.27 <sup>a</sup>	-50.32 ± 0.43 <sup>a</sup>	-50.38 ± 0.64 <sup>a</sup>
$T_{\text{p1}}$	-47.15 ± 0.36 <sup>a</sup>	-46.61 ± 0.51 <sup>a</sup>	-46.78 ± 0.43 <sup>a</sup>
$T_{\text{p2}}$	-19.85 ± 0.41 <sup>a</sup>	-20.54 ± 0.43 <sup>a</sup>	-20.08 ± 0.39 <sup>a</sup>
$\Delta H$	54.18 ± 1.16 <sup>a</sup>	53.43 ± 0.35 <sup>a</sup>	52.92 ± 0.21 <sup>a</sup>
<b>Melting</b>			
$T_{\text{on}}$	-20.63 ± 0.46 <sup>a</sup>	-20.50 ± 0.24 <sup>a</sup>	-20.71 ± 0.41 <sup>a</sup>
$T_{\text{off}}$	4.07 ± 0.11 <sup>a</sup>	3.32 ± 0.28 <sup>b</sup>	3.37 ± 0.15 <sup>b</sup>
$T_{\text{p1}}$	-13.11 ± 0.20 <sup>a</sup>	-13.06 ± 0.32 <sup>a</sup>	-13.44 ± 0.21 <sup>a</sup>
$T_{\text{p2}}$	3.11 ± 0.11 <sup>a</sup>	2.57 ± 0.03 <sup>b</sup>	2.51 ± 0.05 <sup>b</sup>
$\Delta H$	55.87 ± 0.91 <sup>a</sup>	52.67 ± 0.64 <sup>b</sup>	53.60 ± 0.41 <sup>b</sup>

<sup>1</sup>Means followed by different superscript letters represent significant differences in the treatments for measured properties ( $p \leq 0.05$ ).

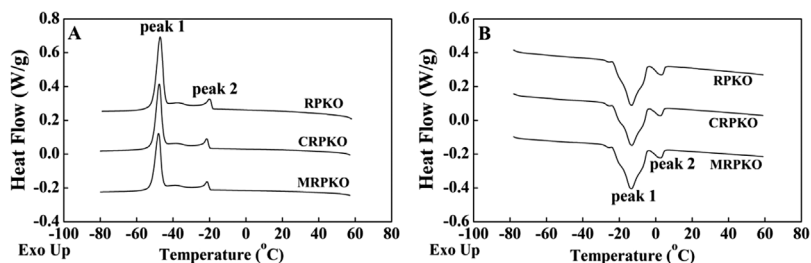


Fig. 1. DSC crystallization (A) and melting (B) curves of cold-pressed PKO.

curves of oils exhibited a more complex than cooling with multiple overlapping transitions. These results illustrate the complex nature of triglyceride in PKO due to the well known phenomena of polymorphism [36]. Although a slight decrease of enthalpy,  $T_{\text{off}}$  and  $T_{\text{p2}}$  were observed after roasting treatment, and the curves were not altered. In a broader comparison, the RPKO displayed a lower transition point than crude olive oil ( $-37.1$  and  $-13.8^{\circ}\text{C}$  for crystallization,  $-5.8$  and  $7.8^{\circ}\text{C}$  for melting) but higher than cold pressed hemp seed oil ( $-61.11^{\circ}\text{C}$  for crystallization,  $-40.10$  and  $-18.12^{\circ}\text{C}$  for melting) as determined by Caponio et al. [37] and Teh and Birch [38], respectively. Taking into account the similarities in total of the percentages of individual unsaturated fatty acids in the above oils, the DSC curves of the two oils are likely influenced more by triglyceride composition and crystal structure than total unsaturation, which requires further research.

### 3.3 Volatile compounds

Forty-three volatiles including 7 aldehydes, 5 alcohols, 2 acids, 13 pyrazines, 4 pyrroles, 5 furans, 5 Terpenes, and 2 additional volatiles were detected in PKO by HS-SPME-GC-MS (Table 5). Sixteen of them were detected in RPKO, and limonene,  $\alpha$ -pinene,  $\beta$ -myrcene, hexanoic acid, and nonanal were the major components, and constitute up to 90% of the total relative concentration of RPKO and the rest were minor constituents. Unfortunately there was no information about volatile compounds from cold pressed PKO to compare with this study. In two studies, however, volatiles of raw pistachio kernels were quantified. In one study, Hojjati et al. [39] characterized 26 volatiles of raw pistachio using a hydrodistillation method and the most abundant compounds were limonene,  $\alpha$ -pinene, nonanal, and terpinolene. Similarly, volatile components of raw pistachio were extracted by HS-SPME and analyzed by GC-MS, 22 compounds were tentatively identified, limonene,  $\alpha$ -Pinene, and 3-carene were found with the highest content [40]. Furthermore, hexanoic acid was found naturally in pistachio kernel and nut oils, such as walnut oil and hazelnut oil [41, 42].

Both conventional and microwave roasting significantly changed the types of volatile compounds and their relative concentrations present in the PKO. New volatile

compounds, such as N-heterocyclic (pyrazines and pyrroles), O-heterocyclic (furans), and some Non-heterocyclic (aldehydes and alcohols), appeared after roasting. But terpenes were still among the predominant volatiles, with the exception of 3-carene and styrene, which disappeared after roasting. Similar results were also observed by Gogus et al. [42] in raw and roasted pistachio kernels. Twelve pyrazines were found in the CRPKO that accounted for 35% total relative concentration and were the highest contributors to the volatile profile of CRPKO. In general, pyrazine compounds contribute significantly to the nutty/roasty aroma with some eliciting earthy or potato-like comments of thermal-treated foods, such as roasted nuts [43], and pretreatment plant oils [44]. Most of the pyrazines identified had high relative concentrations. However, Susan [45] and Mottram [46] reported that replacing one or more of the methyl groups in pyrazines with ethyl can result in a marked decrease in odor threshold value, and some ethyl-substituted pyrazines have sufficiently low threshold values for them to be important in the roast aroma of cooked foods, such as 2-ethyl-5-methyl-pyrazine, 2-ethyl-3-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, and 3,5-diethyl-2-methyl-pyrazine probably play a major role in CRPKO aromas. Four pyrroles were detected in CRPKO. However, pyrrole derivatives have been confirmed as Maillard reaction products, but their importance as dominant aroma constituents is still limited until now. Among these, 2-acetyl-pyrrole has ever been frequently reported to intensify the roast flavor of some plant oils [47]. Furan-containing compounds generated through the thermal degradation of fructose and glucose, and contributed to the caramel-like, sweet and nutty odors of heated carbohydrates. Among four furans identified in CRPKO, the odor threshold values of furfural and furanones are generally at the ppm level [45]. Dihydro-2(3H)-furanone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone have ever been identified in the flavor of roasted peanut oil [27]. Aldehydes were the major non-heterocyclic compounds and generally described as green, tallow, metallic, or rancid flavors. Among these, hexanal is a key volatile compound contributing to rancid flavors and thus considered as an indicator of oil quality, but it did not show remarkable concentration changes after roasting. Furthermore, 2-methyl-propanal, 2-methyl-butanal, and 3-Methyl-butanal were previously reported to be derived from Strecker degradation of leucine

**Table 5.** Volatile compounds of the cold pressed PKO analyzed by HS-SPME-GC-MS

Aromatic compound	K <sub>I,Exp</sub> <sup>2</sup>	K <sub>I,lit</sub> <sup>3</sup>	Aroma descriptions <sup>4</sup>	Relative concentration (mg/kg PO) <sup>1</sup>		
				RPKO	CRPKO	MRPKO
<b>Aldehydes</b>						
2-Methyl-propanal	<1000	819	Pungent, malt, green	n.d <sup>5</sup>	1.16 ± 0.39	n.d
2-Methyl-butanal	<1000	914	Cocoa, almond	n.d	3.55 ± 0.17	0.95 ± 0.11
3-Methyl-butanal	<1000	918	Malt	n.d	5.43 ± 0.54	2.29 ± 0.34
Hexanal	1104	1091	Grass, tallow, fat	0.88 ± 0.16	0.71 ± 0.13	0.60 ± 0.23
Nonanal	1404	1399	Fat, citrus, green	1.21 ± 0.06	n.d	n.d
Benzaldehyde	1546	1534	Almond, burnt sugar	n.d	1.52 ± 0.47	0.56 ± 0.19
(Z)-2-Decenal	1661	1641	Tallow	0.30 ± 0.06	nd	nd
Total aldehydes				2.39 (3.9%) <sup>6</sup>	12.37 (8.9%)	4.4 (6.6%)
<b>Alcohols</b>						
1-Hexanol	1349	1355	Resin, flower, green	0.26 ± 0.06	1.15 ± 0.21	0.59 ± 0.07
1-Octen-3-ol	1442	1443	Mushroom	n.d	0.79 ± 0.05	n.d
1-Octanol	1559	1557	Chemical, metal, burnt	0.61 ± 0.16	n.d	n.d
1-Nonanol	1659	1660	Fat, green	0.68 ± 0.11	3.21 ± 0.35	2.34 ± 0.14
Phenylethyl alcohol	1938	1921	Honey, spice, rose, lilac	n.d	1.17 ± 0.26	0.81 ± 0.06
Total alcohols				1.55 (2.5%)	6.32 (4.5%)	3.74 (5.6%)
<b>Acid</b>						
Pentanoic acid	1738	1733	Sweat	0.87 ± 0.22	n.d	0.69 ± 0.10
Hexanoic acid	1845	1846	Sweat	11.41 ± 0.10	6.19 ± 0.72	5.68 ± 0.38
Total acid				12.28 (20.0%)	6.19 (4.4%)	6.37 (9.5%)
<b>Pyrazines</b>						
2-Methyl-pyrazine	1279	1276	Popcorn	n.d	3.59 ± 0.93	0.44 ± 0.06
2,5-Dimethyl-pyrazine	1330	1331	Cocoa, roasted nut, roast beef, medicine	n.d	8.54 ± 0.01	1.77 ± 0.49
2,6-Dimethyl-pyrazine	1336	1339	Roasted nut, cocoa, roast beef	n.d	1.11 ± 0.22	1.07 ± 0.25
2-Ethyl-pyrazine	1344	1337	Peanut butter, wood	n.d	0.63 ± 0.06	n.d
2,3-Dimethyl-pyrazine	1357	1353	Nut, peanut butter, cocoa, meat	n.d	0.40 ± 0.04	n.d
2-Ethyl-6-methyl-pyrazine	1394	1397	—	n.d	1.90 ± 0.27	0.23 ± 0.02
2-Ethyl-5-methyl-pyrazine	1400	1397	Fruit, sweet	n.d	7.00 ± 0.74	n.d
2-Ethyl-3-methyl-pyrazine	1414	1416	Roast	n.d	6.41 ± 0.28	n.d
2,3,5-Trimethyl-pyrazine	1416	1410	Roast, potato, must	n.d	nd	0.65 ± 0.11
3-Ethyl-2,5-dimethyl-pyrazine	1451	1451	Potato, roast	n.d	8.09 ± 1.07	n.d
2-Ethyl-3,5-dimethyl-pyrazine	1469	1464	Potato	n.d	1.00 ± 0.37	2.50 ± 0.35
3,5-Diethyl-2-methyl-pyrazine	1501	1496	Baked	n.d	2.03 ± 0.30	n.d
Pyrazinamide	1734	1740	—	n.d	8.00 ± 0.67	2.23 ± 0.33
Total pyrazines				0 (0%)	48.7 (35.0%)	8.89 (13.2%)
<b>Pyrroles</b>						
1-Methyl-1H-pyrrole,	1164	1153	—	n.d	7.18 ± 0.00	0.70 ± 0.04
1-Methyl-2-pyrroolidinone	1705	1678	—	n.d	3.03 ± 0.58	n.d
2-Acetyl-pyrrole	1997	1985	Nut, walnut, bread	0.25 ± 0.03	3.18 ± 0.29	1.54 ± 0.15

(Continued)



Table 5. (Continued)

Aromatic compound	K <sub>I,Exp</sub> <sup>2</sup>	K <sub>I,Lit</sub> <sup>3</sup>	Aroma descriptions <sup>4</sup>	Relative concentration (mg/kg PO) <sup>1</sup>			
				RPKO	CRPKO	MRPKO	MRPKO
2-Pyrrolidinone	2078	2032	—	n.d.	1.50 ± 0.24	n.d.	n.d.
Total pyrroles				0.25 (0.4%)	14.89 (10.7%)		2.24 (3.3%)
Furans							
2-Pentyl-furan	1244	1240	Green bean, butter	n.d.	0.40 ± 0.02	n.d.	n.d.
Furfural	1475	1472	Bread, almond, sweet	n.d.	1.41 ± 0.21	n.d.	n.d.
Dihydro-2(3H)-furanone	1662	1647	Caramel, sweet	n.d.	4.73 ± 0.18	n.d.	n.d.
5-Ethylidihydro-2(3H)-furanone	1731	1710	Coumarin, sweet	0.27 ± 0.06	n.d.	n.d.	n.d.
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	2049	2042	Caramel	n.d.	3.12 ± 0.32	n.d.	1.09 ± 0.06
Total furans				0.27 (0.4%)	9.66 (6.9%)		1.09 (1.6%)
Terpenes							
α-Pinene	1031	1028	Pine, turpentine	10.61 ± 1.84	6.07 ± 0.69	9.51 ± 0.02	n.d.
3-Carene	1169	1154	Lemon, resin	0.27 ± 0.04	n.d.	n.d.	n.d.
β-Myrcene	1181	1168	Balsamic, must, spice	1.02 ± 0.06	1.15 ± 0.06	0.75 ± 0.13	n.d.
D-Limonene	1219	1207	Citrus, mint	32.61 ± 1.07	32.82 ± 1.21	30.17 ± 2.42	n.d.
Styrene	1274	1271	Balsamic, gasoline	0.16 ± 0.04	n.d.	n.d.	n.d.
Total terpenes				44.67 (72.8%)	40.04 (28.8%)	40.43 (60.2%)	n.d.
Additional volatiles							
1-Methylene-1H-indene	1777	1763	—	n.d.	0.46 ± 0.06	n.d.	n.d.
Toluene	1057	1053	Paint	0.31 ± 0.08	0.50 ± 0.10	0.27 ± 0.07	n.d.
Total additional volatiles				0.31 (0.5%)	0.96 (0.7%)	0.27 (0.4%)	n.d.
Total aroma compounds				61.32 (100%)	139.13 (100%)	67.16 (100%)	n.d.

<sup>1</sup>Relative concentration to the internal standard (1,2,3-trichloropropane, 6.67 mg/kg PO), average of three replications with the corresponding relative standard deviation.

<sup>2</sup>K<sub>I,Exp</sub>, experimental Kovát index (KI) determined on DB-WAX column using a homologous series of *n*-alkanes (C10–C26) as external references.

<sup>3</sup>K<sub>I,Lit</sub>, KI from NIST mass spectra library (NIST 2014).

<sup>4</sup>Aroma description adapted from flavornet (<http://flavornet.org>; accessed Jan 30, 2015).

<sup>5</sup>n.d., not detected.

<sup>6</sup>Relative percentage of total relative concentration.

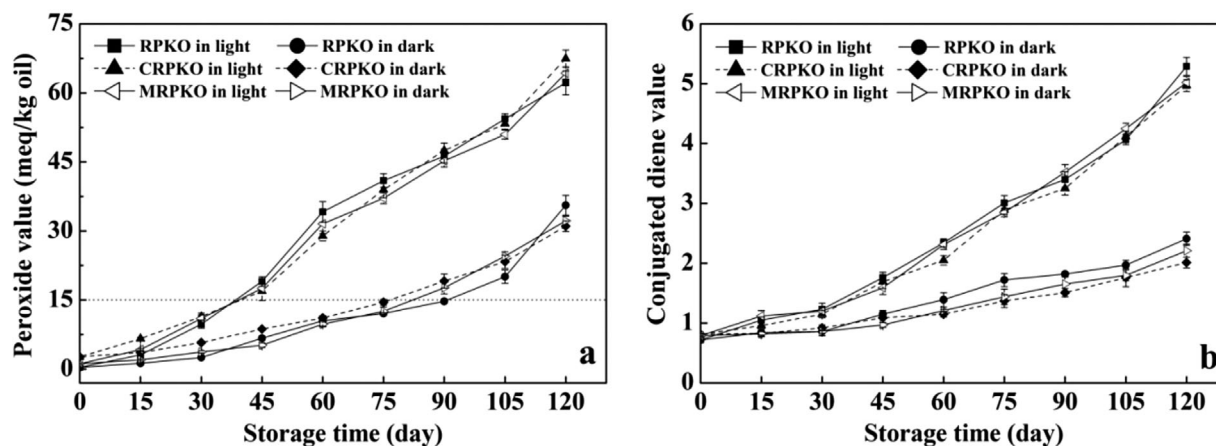


Fig. 2. Peroxide value (a) and conjugated diene value (b) from PKO during storage stability test.

and increased significantly even at low temperatures [48]. As for aliphatic alcohols, some new compounds appeared after roasting. However, it is generally not considered to be important contributors to the flavors of oils and fats due to their relatively high odor threshold values [49].

The MRPKO showed similar groups of volatile compounds as compared to those found in CRPKO. However, their total number and relative concentration of volatiles were significantly lower than those in CRPKO, especially for some key roasted compounds (e.g., pyrazines). These results may be explained by the roasting intensity in microwave roasting condition (final temperature was 130°C after microwave heating for 6 min) was lower than conventional roasting (160°C/20 min). This is agreed that long time roasting under the high temperature is necessary for the formation of the key roasted compounds [39].

The results of aromatic compounds presented in Table 5 are similar to those found in previous studies for raw and roasted pistachios [39, 50]. They found that clear correlations of aroma intensity based on six descriptors (green, sweet, roasted, chocolate/coffee, rancid, and nutty) are observed between measurement technique and sensory evaluation. Generally the nutty and roasted score was highest in the roasted samples, where the pyrazine and furan compounds were in high concentrations. The aroma of the oil obtained from this study with short time and high precision might be also perceived by the consumers. The confirmation study would be next step of the research.

### 3.3.1 Oxidative stability of the oils

Figure 2 shows the changes in the PV and CDV of cold-pressed PKO during storage in the dark and light at room temperature. The PV and CDV of three PKO samples stored in light were significantly greater than those of the oils stored in dark. These results indicated the extremely low stability of PKO against photo-oxidative, and the relative high contents

of chlorophylls present in PKO probably played a major role in the photochemical oxidation. Moreover, it was also showed that CRPKO and MRPKO samples oxidized at similar rate than RPKO under same storage condition, which was different from those previously reported for oils that indicated a better oxidative stability from roasted seeds based on the accelerated quality tests. These differences may be due to different roasting intensity and thermal sensitivity of oil seeds. Furthermore, extreme conditions in the accelerated test may not be identical with real-time storage. Accord to Codex Alimentarius [29], the quality of non-refined oil remains acceptable until the PV reach 15 meq/kg oil. This end point for three oil samples was reached after only 30 and 75 days when stored under light and dark, respectively. Since clear glass and plastic bottles have become popular for storing edible oils due to low cost and direct attractions to consumers, protections against photo-oxidation and addition of appropriate natural antioxidants in these bottles are necessary to keep oil oxidation at the lowest possible level.

## 4 Conclusions

This study showed that good quality virgin oil was extracted using a cold press from raw and roasted pistachio kernels prepared from two roasting methods. Most PKO physicochemical attributes did not change significantly after treated by selected roasting conditions for conventional and microwave roasted pistachios. However, roasted kernels produced slightly oxidized oil and dark color with the decreased contents of tocopherols and chlorophylls but increased TPC and AOC. Three PKO samples exhibited similar transition points of crystallization and melting curves. Differences were observed in volatile compounds among three PKO samples, which were significantly influenced by roasting conditions. Terpenes concentrations of RPKO decreased in roasted samples, but pyrazines, furans, and

pyrroles were present only in roasted samples and their concentrations increased significantly in CRPKO. Highlighted differences between CRPKO and MRPKO were found in some volatile compounds, especially for pyrazines. With high contents of chlorophylls, cold-pressed PKO was highly susceptible to photo-oxidative degradation, and must be stored in containers with light-barrier properties together with the addition of some appropriate natural antioxidants

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