Effects of heat pretreatment of wet-milled corn germ on the physicochemical properties of oil

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Abstract Corn germ oil is removed from the milled germ using a conditioning (heating) process, followed by mechanical expelling and/or hexane extraction. In this study, the effect of pretreatment by oven roasting (OR) and microwave (MW) radiation on wet-milled corn germ was investigated. Three OR temperatures (125, 150, and 175 °C) were used with 60 min exposure, and MW pretreatments were established by combining two powers (440 and 800 W) and three pretreatment times (4, 6, and 8 min). The levels of red value, 1,3-diacylglycerol, total diacylglycerol, free fatty acid, and oleic acid increased substantially, while those of triacylglycerol (TAG), linoleic, and linolenic acid decreased significantly following OR. There were no significant differences in TAG compositions following OR and MW treatments. Both heat pretreatments significantly increased the total tocopherol content. δ -Tocopherol showed minimal changes, while β -tocopherol progressively increased after the heat treatments. No sig-

nificant differences in phytosterols levels were observed among most samples. The MW radiation Proper roasting temperatures or MW radiation times could enrich the content of individual tocopherols and phytosterols, and improve the oxidative stability of oil. The MW radiation tends to be more applicable and sustainable for oil industry to improve the quality of corn germ oil.

Keywords Wet-milled corn germ · Oven roasting · Microwave radiation · Physicochemical properties

Introduction

Wet-milled corn germ is a valuable co-product generated during starch production. It contains more than 30% oil, and is the main commercial source of corn oil. In 2014, 3.19 million tons of corn oil was produced worldwide, with the United States (57.01%), the European (8.70%), and China (6.04%) being the top three producers (FAO 2017). Traditionally, corn germ oil is removed from the milled germ using a conditioning (heating) process, followed by mechanical expelling or/and hexane extraction (Le Roux et al. 2011). This heating process is key step for making corn germ oil, since the color, composition, and quality of corn germ oil are all influenced by the conditions of the process.

There are several techniques available to transfer heat to the seeds. The different techniques rely on different mechanisms of heat transfer: conduction, convection, or radiation (Veldsink et al. 1999). Microwave (MW), as a time- and energy-saving treatment based on radiation, has been widely applied in the food industry for several decades. The energy generated by MW is higher than that of several thermal treatments due to its efficient volumetric

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heating production. That is, the whole sample is heated at the same time by MW radiation, with temperature gradient from inside to outside, which is reversed compared to conventional heating (Koubaa et al. 2016). The conventional heating, based on the combined effect of conduction and convection, was classical heating process, and the surface is heated first. Oven heating is one kind of the conventional heating processes, which was pretty prevalent in cooking (conditioning) process in the edible oil extraction factories. This process is one of the most energy and time consuming steps, performed after flaking of oilseeds and just before the extraction or expression. Since the heating mechanisms are different, differences in the quality of resulting oil from heat-pretreated corn germs may be expected.

Heat pretreatments are known to alter the chemical composition and concentration of nutraceuticals in several oil seeds (Hashemi et al. 2017; Azadmard-Damirchi et al. 2010; Anjum et al. 2006; Amaral et al. 2006; Kwon et al. 2004). Thus different oilseeds subjected to the two heat pretreatments have been extensively described by many researchers. Amaral et al. (2006) reported that hazelnut roasting (temperatures: 125–200 °C and times of exposure: 5, 15, and 30 min) modestly decreased phytosterols and individual tocopherol, with minor changes observed in the fatty acid and TAG compositions. Kwon et al. (2004) also reported that phytosterols decreased gradually when rice germ was roasted at 200 °C, and no major differences in fatty acid profile during roasting and MW pretreatment. However, MW pretreatment of rapeseed can enrich nutraceuticals, such as phytosterols (by 15%) and tocopherols (by 55%) in the oil extracted by pressing (Azadmard-Damirchi et al. 2010). Furthermore, the levels of α -tocopherol, α -tocotrienol, and γ -tocotrienol as well as total vitamin E markedly increased when rice bran was treated with MW radiation for up to 30 s, and longer heating times greatly decreased the vitamin E levels (Ko et al. 2003). Similar results were found in the content of α -tocopherol upon roasting and MW pretreatment of rice germ (Kwon et al. 2004). Yang et al. (2013) found that phytosterol and polyphenol contents, as well as the oxidative stability of oils increased proportionally to the MW exposure time (0–7 min) at 800 W. The amount of canolol and oxidative stability of oils after MW pretreatment of rapeseeds dramatically increased compared to control (Wroniak et al. 2016). The fat and fatty acid compositions, molecular species of triacylglycerols (TAGs), and tocopherol distribution of oils obtained from MW-pretreated peanuts were investigated (Yoshida et al. 2003). Pretreatment of corn germ/fiber was mainly examined (Moreau and Hicks 2006; Moreau et al. 1999). The levels of phytosterol components in the corn fiber oil were moderately decreased after roasting at high oven temperatures (e.g., 100, 125, 150, and

175 °C). After MW treatment at 1500 W, fluctuation effects on the levels of ferulate-phytosterol esters (1% increase and 4% decrease for 2.5 and 5.0 min treatments, respectively) were found in corn fiber oil (Moreau et al. 1999). Moreau and Hicks (2006) further found that heat treatment of corn germ or other corn oil-containing fractions at high temperatures (100–175 °C) reduced γ -tocopherol, γ -tocotrienol, and α -tocotrienol levels, thereby decreasing the oil quality by a certain degree.

Studies on the chemical changes, including fatty acid profile, fat composition, TAG composition, oxidative stability, and nutraceutical content (tocopherol and phytosterol) upon pretreatment of corn germ by OR and MW radiation are still lacking. More work need doing to further understand the quality changes resulting from corn germs under the different heating processes.

Therefore we aimed to provide detailed information about the chemical composition such as oxidative stability, fat composition, TAG composition, fatty acid composition, and levels of tocopherols and phytosterols in this study.

Materials and methods

Materials

Corn germ was obtained from commercial wet milling, provided by China National Cereals, Oils, and Foodstuffs Corporation; Standards of 40 fatty acid methyl esters, α -, β -, γ - and δ -tocopherols (purity > 95%), particle acylglycerols (including 2-olein, 1,2-diolein and 1,3-diolein acylglycerols), and 5 α -cholestane were purchased from Sigma-Aldrich Chemical Co. Ltd. (Shanghai, China). Triacylglycerol standards consisting of POP, POS, and SOS (P, palmitic; S, stearic; O, oleic) were obtained from Larodan Fine Chemicals AB (Malmö, Sweden). *n*-Hexane and 2-propanol were of pure chromatography grade, obtained from Fisher Scientific (United States). Other reagents and solvents were provided by Sinopharm Chemical Regent (Shanghai, China). The other chemicals and reagents (diethyl ether, anhydrous ethanol, potassium hydroxide, anhydrous sodium sulfate, ethanol (95%), *n*-hexane, and aqueous methanol) were of analytical grade.

Methods

Heat treatment of corn germ

For each OR pretreatment, 100 g of wet-milled corn germ were arranged in an even layer in Pyrex petri dish (18-cm diameter) in the oven (FED 115, Binder, Germany), and oven-heated at three temperatures (125, 150, and 175 °C)

for 60 min. For each MW radiation pretreatment, 100 g of wet-milled corn germ were arranged in an even layer in Pyrex petri dish (18-cm diameter), then placed on the turntable plate of the MW oven (Model MG823LA3-NR, Midea Group, Guangzhou in China), after which the samples were MW-treated at a frequency of 2450 MHz for two levels of power (440 and 800 W), and three times radiation (4, 6, and 8 min). The real temperatures of the samples, determined with infrared thermometer (AR350, Smart Sensor, China), treated by MW radiation are listed in Table 1. Corn germ sample without heat pretreatment was used as a control one. Later, each sample was performed in duplicates. Following each heating run, seeds were collected and then cooled to ambient temperature before oil extraction. All the extracted oils were stored at -20°C under nitrogen until analysis.

Extraction of corn crude oil

Approximately, 100 g of ground corn germ with an electric grinder were weighed, and then the fat was extracted using a Soxhlet extraction with 500 mL of *n*-hexane at 60°C for 6 h. The solvent was removed at 50°C in a rotary evaporator.

Oxidative stability

The oxidative stability of the oils were detected by a Metrohm Rancimat apparatus model 743 (Herisau, Switzerland) with slight modification (Azadmard-Damirchi et al. 2010). Oil samples (3 g) were heated at 120°C , and at the same time 20 L/h of the cleaned and dried air was bubbled into the hot sample. Effluent air containing small molecular volatile organic acids from the oil sample was collected in a measuring vessel containing 60 mL of distilled water. The conductivity of water was measured automatically as oxidation proceeded. The oxidative stability was expressed in hours (h).

Table 1 Temperatures of corn germ treated by MW radiation

Powers (W)	Time (min)	Temperatures ($^{\circ}\text{C}$)
440	4	82
	6	105
	8	132
800	4	96
	6	146
	8	183

Color determination

Color determination was according to AOCS (Firestone 2003) Method Cc 13b-45. Briefly, oil samples, were placed into 25.4 mm optical path spectrophotometer cell, then tested with AOCS-Tintometer R/Y method with Lovibond PFX 880 Tintometer (Tintometer® Group, UK).

Fatty acid composition

Fatty acid composition was determined according to our former method (Jin et al. 2016). Fatty acids were identified by comparing their retention times with authentic standards, and their levels were reported as the relative percentages.

Fat composition

Triacylglycerol, diacylglycerol, mono-acylglycerol, and free fatty acids were analyzed by Normal Phase HPLC (Waters 1525 Binary HPLC Pump-2414 RI Detector) using a silica column ($5\text{ }\mu\text{m}$, $4.6 \times 250\text{ mm}$, Sepax, USA) according to the reported procedures (Zeng et al. 2015).

Triacylglycerol composition

Triacylglycerol composition was determined based on AOCS (Firestone 2003) method Ce 5-86 using a gas chromatograph system (7890A, Agilent Technologies, USA) equipped with a hydrogen flame ionization detector (FID). Triacylglycerols were separated by a DB-17HT capillary column ($0.15\text{ }\mu\text{m}$, $0.25\text{ mm} \times 30\text{ m}$, Agilent Technologies, USA). Triacylglycerols were identified by carbon numbers and unsaturation degree, and by comparing their retention times of triacylglycerol standards. The content of each triacylglycerol was reported in terms of the relative percentages.

Tocopherol and phytosterol levels

Tocopherols were analyzed by a high-performance liquid chromatographic system (Waters e2695 Separations module) equipped with Multi λ Fluorescence Detector (Waters 2475, Waters, USA) with excitation at 293 nm and emission at 325 nm according to the method described by Moreau and Hicks (2006) and Jin et al. (2017), with small modification. α -, β -, γ - and δ -Tocopherols were identified and quantified by comparing the standards, and their contents were determined by external standard method, and reported in mg/kg.

Sterol analyse was analyzed with a gas chromatograph-mass spectrum system (Thermo Fisher, USA) according to

procedures (Jin et al. 2017), and the contents were reported as mg/kg.

Statistical analysis

The analysis was conducted in triplicate, and the results were expressed as mean values with standard deviations. Variables were compared by analysis of variance (ANOVA), and significant differences between means were determined through Duncan's multiple range tests ($P < 0.05$, significant difference) were performed using the SPSS program version 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results and discussions

Physical and chemical properties of crude corn germ oils after heat pretreatment

Color, oxidative stability and fat composition of oil resulting from raw and heat-treated corn germ are shown in Table 2. The color unit (red value) of crude corn oils greatly increased with increasing roasting temperatures. For example, the red value was 8.4 units at 125 °C, and dramatically increased to 36.0 units at 175 °C. Similar findings were reported by Yen (1990), who found that the color unit (red value) of oils increased with roasting temperature up to 220 °C, but then decreased. Interestingly, different results were observed for samples pretreated by MW radiation. Upon MW pretreatment at 440 W, the red

value initially increased to 9.4 units with a prolonged heating time of up to 6 min, and then greatly decreased by 2.6 units. A gradual decrease was observed in the red value after MW radiation at 800 W. The color increase phenomenon appeared to result from browning substances derived from maillard-type nonenzymatic reactions between reducing sugars or phospholipid and free amino acid or amide or, and further phospholipid degradation, forming polyene substances, was reported to cause browning during heating (Yen 1990), and the red decrease phenomenon due to color substances degradation at higher temperature (175 °C) or longer MV radiation (400 W for 8 min; 800 W for 6 and 8 min). The red color increased with increasing roasting temperatures or MW radiation times (Azadmard-Damirchi et al. 2011; Anjum et al. 2006). Uneven heating of corn germs by OR compared to MW radiation could contributed to the higher red color.

The oxidative stability of oils depends on their fatty acid composition and content of non-triglyceride components. In this study, the stability of oil samples gradually ($P < 0.05$) increased as the roasting temperature increased from 125 to 150 °C, and then decreased markedly ($P < 0.05$) at 175 °C. For example, the oxidative stability of corn germ oils roasted at 125, 150, and 175 °C were 6.54, 8.12, and 6.22 h, respectively. Upon MW radiation at 440 W, significant effects ($P < 0.05$) were observed, with the maximum level (8.05 h) being reached after heating for 8 min. The results were comparable to those of previous study (Azadmard-Damirchi et al., 2010). In contrast, the oxidative stability of oil samples decreased significantly ($P < 0.05$) from 10.40 to 5.94 h upon exposure to MW

Table 2 Color, oxidative stability, and fat compositions of oil extracted from heat-treated corn germs

Temperature/ power	Time (min)	Oxidative stability (h)	Color (R/Y units)	Fat compositions (%)				
				TAG	1,3-DAG	1,2(2,3)-DAG	Total DAG	FFA
125 °C	Unroasted	5.59 ± 0.21 ^c	4.1/70.0	95.27 ± 0.21 ^a	1.30 ± 0.07 ^c	1.79 ± 0.01 ^a	3.09 ± 0.06 ^a	1.64 ± 0.16 ^c
	60	6.54 ± 0.40 ^b	8.4/71.1	94.14 ± 0.42 ^{ab}	1.72 ± 0.40 ^{bc}	1.64 ± 0.01 ^a	3.36 ± 0.38 ^a	2.50 ± 0.04 ^b
	150 °C	8.12 ± 0.21 ^a	13.7/73.6	93.81 ± 0.89 ^{ab}	2.14 ± 0.30 ^{ab}	1.84 ± 0.50 ^a	3.98 ± 0.80 ^a	2.22 ± 0.09 ^b
175 °C	60	6.22 ± 0.04 ^{ab}	36.0/70.1	92.83 ± 0.45 ^b	2.53 ± 0.23 ^a	1.67 ± 0.03 ^a	4.20 ± 0.21 ^a	2.98 ± 0.25 ^a
440 W	Unroasted	5.59 ± 0.21 ^b	4.1/70.0	95.27 ± 0.21 ^a	1.30 ± 0.07 ^a	1.79 ± 0.01 ^a	3.09 ± 0.06 ^a	1.64 ± 0.16 ^b
	4	5.18 ± 0.09 ^b	7.1/72.6	94.90 ± 0.18 ^a	1.35 ± 0.09 ^a	1.95 ± 0.06 ^a	3.30 ± 0.04 ^a	1.80 ± 0.14 ^b
	6	5.34 ± 0.07 ^b	9.4/73.6	94.33 ± 0.60 ^a	1.53 ± 0.40 ^a	1.75 ± 0.10 ^a	3.28 ± 0.49 ^a	2.40 ± 0.11 ^a
	8	8.05 ± 0.17 ^a	6.8/71.5	93.99 ± 0.71 ^a	1.82 ± 0.35 ^a	1.93 ± 0.30 ^a	3.74 ± 0.65 ^a	2.27 ± 0.06 ^a
800 W	Unroasted	5.59 ± 0.21 ^c	4.1/70.0	95.27 ± 0.21 ^a	1.30 ± 0.07 ^b	1.79 ± 0.01 ^a	3.09 ± 0.06 ^{ab}	1.64 ± 0.16 ^b
	4	10.40 ± 0.22 ^a	8.6/70.7	94.92 ± 0.74 ^{ab}	1.44 ± 0.17 ^b	1.76 ± 0.08 ^a	3.20 ± 0.25 ^{ab}	1.88 ± 0.48 ^b
	6	9.16 ± 0.11 ^b	7.9/70.5	95.38 ± 0.28 ^a	1.33 ± 0.01 ^b	1.65 ± 0.13 ^{ab}	2.98 ± 0.14 ^b	1.65 ± 0.15 ^b
	8	5.94 ± 0.06 ^c	7.8/70.0	93.83 ± 0.28 ^b	2.00 ± 0.14 ^a	1.52 ± 0.03 ^b	3.52 ± 0.17 ^a	2.66 ± 0.09 ^a

Values in the same column with different superscript letters are significantly different from those for unroasted seeds within each group ($P < 0.05$)

R, red; Y, yellow; TAG, triacylglycerol; DAG, diacylglycerol; FFA, free fatty acid

radiation (power, 800 W). Generally, heat pretreatments of corn germs enhanced the oxidative stability of oils and consequently extended their shelf life, compared to the untreated corn germs (Wroniak et al. 2016). The heating conditions (150 °C, 60 min; 440 W, 8 min; 800 W, 4 and 6 min) give the resulting oil higher oxidative stability. This also indicates that MW radiation outweigh OR due to its relative high efficiency. Higher temperatures and more powerful MW radiation decreased the oxidative stability of oil samples, probably because of more oxidation reactions under such conditions (Pingret et al. 2013).

The fat composition of corn germ oil in this study mainly included TAG, diacylglycerol (DAG), and free fatty acid (FFA). No trace of mono-acylglycerol was detected in any of the crude corn germ oils. The levels of TAG, 1,3-DAG, 1,2(2,3)-DAG, FFA were 92.83–95.38, 1.30–2.53, 1.52–1.95 and 1.64–2.98%, respectively. The total amount of DAG fluctuated from 2.98 to 4.20%. For both types of heat pretreatments, there was a progressive decrease in TAG content [maximum by 2.56, 1.34, 1.51% for OR and two MW powers (440 and 800 W), respectively]. In contrast, a progressive increase (maximum by 94.62, 40.00 and 53.85%, respectively) in the 1,3-DAG and FFA contents (maximum by 81.71, 38.41 and 62.20%, respectively) in the OR, and 440 and 800 W MW-pretreated samples was found, compared to the control samples. Duncan's test ($P = 0.05$) revealed that a significant difference was observed in levels of TAG, 1,3-DAG, and FFA between the pretreated and control samples. Similar results were previously reported by researchers, who explained that TAGs were randomly and gradually hydrolyzed and/or oxidized by MW radiation or OR to produce FFAs or DAGs (Yoshida et al. 2001, 2003; Yoshida and Takagi 1999; Takagi et al. 1999). In general, regarding the fat compositions, OR caused more pronounced changes in crude corn oil than the two MW radiation powers. Prolonged heating and higher MW powers generally result in much greater levels of deterioration of crude corn germ oils when taking the above-mentioned fat composition changes into consideration. This result is in accordance with previous data (Megahed 2001).

Effect of heat pretreatment on fatty acid composition

The fatty acid composition of MW radiation- and OR-pretreated corn germ samples is recorded in Table 3. The dominant fatty acid components of control corn germ oil were palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and arachic acid at concentrations of 11.61, 2.04, 32.83, 51.95, 1.27 and 0.32%, respectively. When wet-milled corn germ samples were pretreated by OR,

small but significant differences ($P < 0.05$) were detected in the fatty acid profiles of the oils. The levels of linoleic and linolenic acid significantly decreased ($P < 0.05$), while those of oleic acid significantly increased ($P < 0.05$). Linoleic acid was reduced by 2.55%, reaching 50.78%, and the percentages of saturated fatty acids, such as palmitic acid (3.62%) were increased. Similar results were reported by Yoshida and Takagi (1999), who observed greater percentages of palmitic and oleic acids, and lower percentages of linoleic and linolenic acids with longer the roasting times. Thus, the total saturated fatty acids increased and total polyunsaturated and unsaturated ones decreased upon OR. Small but marked changes in the fatty acid profiles of the oil after MW radiation of whole peanuts (Megahed 2001), Chilean hazelnuts (Azadmard-Damirchi et al. 2010; Uquiche et al. 2008), and sesame seeds (Yoshida et al. 2001) were also observed. However, interestingly, a minor significant difference ($P > 0.05$) was found in the fatty acid profiles of oils after the MW pretreatment. This result agrees with previous studies. Yoshida and Kajimoto (1994), and Kim et al. (2002) found no differences among the fatty acid profiles of rice germ and sesame seed oils obtained using various roasting temperatures and times.

Effect of heat pretreatment on TAG compositions

The relative percentages of each TAG for raw and roasted corn germ samples are shown in Table 4. Twelve TAGs were identified and quantified, mainly LLO, LOO, PLL, LLL, POL, and OOO. The levels of these TAGs were in the range of 22.18–23.00, 15.12–16.66, 15.06–16.06, 14.56–17.27, 12.44–13.59 and 5.74–6.76%, respectively. After OR pretreatment, no significant differences ($P > 0.05$) in the levels of most TAGs, such as PPL, POL, PLL, LOO, OOO, LLO, LLLn, and LLL, were observed. A slight increase ($P > 0.05$) in SOO and PPO, and significant decrease ($P < 0.05$) in POS levels appeared were observed. Disappearance of a result that a decrease of TAG containing linoleic acid moieties and an increase of TAG containing oleic, palmitic, and stearic acids might be as a consequence of the small content fluctuations of these fatty acid during OR (Amaral et al. 2006). On the contrary, there were similar contents of most TAGs before and after MW pretreatment at two powers (440 and 800 W), except that PPO and LLLn were significantly decreased at 440 W and LLO and POL were evidently increased ($P < 0.05$) at 440 and 800 W, respectively.

Effect of heat pretreatment on tocopherols

The individual (α -, β -, γ -, and δ -tocopherols) and total tocopherol contents in corn germ oils obtained by OR and

Table 3 Fatty acid compositions of oils extracted from heat treated corn germ (%)

Temperature/power	Time (min)	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidic acid
125 °C	Unroasted	11.61 ± 0.00 ^{ab}	2.04 ± 0.01 ^c	32.83 ± 0.00 ^b	51.95 ± 0.01 ^a	1.27 ± 0.01 ^b	0.32 ± 0.00 ^b
	60	11.60 ± 0.01 ^c	2.05 ± 0.00 ^b	32.87 ± 0.01 ^b	51.88 ± 0.01 ^b	1.30 ± 0.01 ^a	0.32 ± 0.00 ^b
	150 °C	11.63 ± 0.01 ^b	2.03 ± 0.00 ^c	32.86 ± 0.02 ^b	51.90 ± 0.01 ^{ab}	1.27 ± 0.01 ^b	0.32 ± 0.01 ^b
175 °C	60	12.03 ± 0.01 ^a	2.11 ± 0.01 ^a	33.49 ± 0.03 ^a	50.78 ± 0.04 ^c	1.26 ± 0.00 ^b	0.34 ± 0.01 ^a
440 W	Unroasted	11.61 ± 0.00 ^a	2.04 ± 0.01 ^{ab}	32.83 ± 0.00 ^a	51.95 ± 0.01 ^a	1.27 ± 0.01 ^a	0.32 ± 0.00 ^a
	4	11.32 ± 0.37 ^a	2.01 ± 0.03 ^b	32.00 ± 0.95 ^a	50.91 ± 1.73 ^a	1.25 ± 0.04 ^a	0.30 ± 0.04 ^a
	6	11.60 ± 0.01 ^a	2.03 ± 0.01 ^{ab}	32.87 ± 0.03 ^a	51.92 ± 0.04 ^a	1.27 ± 0.00 ^a	0.32 ± 0.00 ^a
	8	11.58 ± 0.00 ^a	2.06 ± 0.01 ^a	32.61 ± 0.00 ^a	52.17 ± 0.01 ^a	1.28 ± 0.00 ^a	0.32 ± 0.00 ^a
800 W	Unroasted	11.61 ± 0.00 ^a	2.04 ± 0.01 ^a	32.83 ± 0.00 ^{ab}	51.95 ± 0.01 ^b	1.27 ± 0.01 ^a	0.32 ± 0.00 ^a
	4	11.59 ± 0.04 ^a	2.04 ± 0.00 ^a	32.77 ± 0.03 ^{bc}	52.02 ± 0.03 ^{ab}	1.28 ± 0.01 ^a	0.31 ± 0.02 ^a
	6	11.55 ± 0.02 ^{ab}	2.04 ± 0.01 ^a	32.76 ± 0.03 ^c	52.07 ± 0.01 ^a	1.28 ± 0.01 ^a	0.32 ± 0.00 ^a
	8	11.46 ± 0.07 ^b	2.04 ± 0.01 ^a	32.88 ± 0.02 ^a	52.05 ± 0.07 ^{ab}	1.27 ± 0.00 ^a	0.31 ± 0.01 ^a

Values in the same column with different superscript letters are significantly different from those for unroasted seeds within each group ($P < 0.05$)

MW pretreatment are shown in Table 5. When corn germ was heated at 125 and 150 °C for 1 h, the α -tocopherol content increased significantly ($P < 0.05$), followed by a dramatic decrease ($P < 0.05$) beyond the both temperatures points, still higher than the content in control sample. Following OR pretreatment, the concentration of γ -tocopherol in the crude corn oils greatly increased to 543.51 mg/kg at 125 °C, and then significantly decreased thereafter. β -Tocopherol and total tocopherols contents increased significantly ($P < 0.05$) with higher OR temperature, reaching maximum levels at 175 °C (317.07 and 894.31 mg/kg, respectively). Upon MW radiation pretreatment at 440 W, α -, β -, γ - and total tocopherols contents considerably increased ($P < 0.05$) with prolonged MW radiation time. Rekas et al. (2015) reported that with increasing roasting temperatures, a modest increase in the α -tocopherol and statistically significant ($P < 0.05$) gradual increase in γ -tocopherol level occurred. Oomah et al. (1998) found that the level of γ -tocotrienol, the major tocopherol of grapeseed oil, increased, while that of δ -tocopherol decreased during heat pretreatment of grapeseed. The heat breaks the bonds between tocopherols and membrane proteins, phosphates or phospholipids (Moreau et al. 1999). Further damage to the oilseed cell membrane by MW pretreatment increase the release of tocopherols and enhances their levels in the extracted oil (Azadmard-Damirchi et al. 2010). On the contrary, a significant ($P < 0.05$) decrease in the amounts of γ - and δ -tocopherol constituents in the MW-treated sunflower oils was found. However, when the corn germs were heated at 800 W, different results were observed, with a slight decline in the levels of α -, γ -, δ - and total tocopherols, which then gradually increased with increasing MW radiation time for up

to 6 min, followed by a moderate decrease up to 8 min. δ -Tocopherol content remained constant during these heating processes. These results are in agreement with previous findings by Ko et al. (2003), who reported that both MW and an electric roaster significantly increased the total vitamin E content, but a longer heating time degraded vitamin E. In general, heat pretreatment of corn germs under this study conditions contributed to enrichment of more tocopherols extracted from oilseeds. Analysis of variance revealed that the time of exposure to MW radiation significantly affected ($P < 0.05$) γ -tocopherol and total tocopherol contents rather than the level of MW power (440 and 800 W), whereas MW power and time both affected α - and β -tocopherol contents ($P < 0.05$), with the power level having a greater effect. No significant effect ($P > 0.05$) on δ -tocopherol content was observed at different power levels.

Effect of heat pretreatment on phytosterol content

Changes in the levels of phytosterols in corn germ oils after the two different heat treatments are shown in Table 6. Three phytosterol derivatives (campesterol, stigmasterol, and sitosterol) were identified and determined, with sitosterol accounting for the major part. For example, sitosterol was predominant (4061.02–5780.39 mg/kg), followed by campesterol (1351.30–1750.20 mg/kg) and stigmasterol (464.92–616.40 mg/kg). At initial heat treatments, the total phytosterol content in oil samples extracted by hexane progressive increased with increasing pretreatment time and then decreased, with the maximum content of total phytosterol at 150 °C (7504.85 mg/kg), 440 W for 6 min (7179.39 mg/kg), and at 800 W for 6 min (8146.98 mg/

Table 4 Changes in the triacylglycerol content of oils extracted from heat treated corn germs (%)

Temperature/ power	Time (min)	Triacylglycerols (%)											
		PPL	PPO	POS	POO	POL	PLL	SOO	OOO	LOO	LLO	LLL	LLLn
125 °C	Unroasted	1.23 ± 0.03 ^a	3.20 ± 0.08 ^a	0.25 ± 0.04 ^{ab}	4.74 ± 0.07 ^b	13.59 ± 0.44 ^a	15.46 ± 0.31 ^a	0.29 ± 0.03 ^b	6.43 ± 0.21 ^a	16.21 ± 0.11 ^a	22.39 ± 0.32 ^a	14.87 ± 0.16 ^a	1.36 ± 0.11 ^a
	60	1.34 ± 0.26 ^a	3.28 ± 0.16 ^a	0.29 ± 0.07 ^{ab}	5.25 ± 0.04 ^a	13.34 ± 0.01 ^a	15.06 ± 0.26 ^a	0.33 ± 0.03 ^{ab}	6.76 ± 0.09 ^a	16.21 ± 0.16 ^a	22.44 ± 0.09 ^a	14.49 ± 0.12 ^a	1.23 ± 0.07 ^a
	60	1.45 ± 0.07 ^a	3.33 ± 0.19 ^a	0.32 ± 0.00 ^a	5.21 ± 0.25 ^a	12.98 ± 0.38 ^a	15.34 ± 0.03 ^a	0.48 ± 0.06 ^a	6.54 ± 0.40 ^a	16.14 ± 0.62 ^a	22.18 ± 0.19 ^a	14.88 ± 0.29 ^a	1.16 ± 0.04 ^a
	60	1.50 ± 0.00 ^a	3.63 ± 0.21 ^a	0.16 ± 0.05 ^b	5.02 ± 0.03 ^{ab}	13.53 ± 0.45 ^a	15.11 ± 0.46 ^a	0.44 ± 0.10 ^{ab}	6.56 ± 0.10 ^a	15.81 ± 0.29 ^a	22.36 ± 0.29 ^a	14.56 ± 0.47 ^a	1.34 ± 0.14 ^a
440 W	Unroasted	1.23 ± 0.03 ^a	3.20 ± 0.08 ^a	0.25 ± 0.04 ^a	4.74 ± 0.07 ^a	13.59 ± 0.44 ^a	15.46 ± 0.31 ^a	0.29 ± 0.03 ^b	6.43 ± 0.21 ^a	16.21 ± 0.11 ^a	22.39 ± 0.32 ^b	14.87 ± 0.16 ^a	1.36 ± 0.11 ^a
	4	1.35 ± 0.01 ^a	3.36 ± 0.05 ^a	0.26 ± 0.09 ^a	5.15 ± 0.01 ^a	13.26 ± 0.20 ^a	15.07 ± 0.19 ^a	0.49 ± 0.11 ^a	6.59 ± 0.03 ^a	16.18 ± 0.22 ^a	22.35 ± 0.03 ^b	14.91 ± 0.05 ^a	1.04 ± 0.04 ^{ab}
	6	1.18 ± 0.13 ^a	3.36 ± 0.22 ^a	0.17 ± 0.05 ^a	4.76 ± 0.30 ^a	13.07 ± 0.31 ^a	15.48 ± 1.02 ^a	0.35 ± 0.03 ^{ab}	6.01 ± 0.85 ^a	16.66 ± 0.51 ^a	22.54 ± 0.25 ^{ab}	15.04 ± 0.90 ^a	1.38 ± 0.24 ^a
	8	1.29 ± 0.10 ^a	3.44 ± 0.18 ^a	0.18 ± 0.0 ^a	4.74 ± 0.15 ^a	13.28 ± 0.23 ^a	15.58 ± 0.00 ^a	0.33 ± 0.03 ^{ab}	6.02 ± 0.03 ^a	16.34 ± 0.21 ^a	23.00 ± 0.00 ^a	14.91 ± 0.08 ^a	0.91 ± 0.07 ^b
800 W	Unroasted	1.23 ± 0.03 ^a	3.20 ± 0.08 ^a	0.25 ± 0.04 ^a	4.74 ± 0.07 ^a	13.59 ± 0.44 ^a	15.46 ± 0.31 ^a	0.29 ± 0.03 ^a	6.43 ± 0.21 ^a	16.21 ± 0.11 ^a	22.39 ± 0.32 ^a	14.87 ± 0.16 ^a	1.36 ± 0.11 ^a
	4	1.16 ± 0.01 ^a	3.03 ± 0.44 ^a	0.34 ± 0.03 ^a	4.71 ± 0.66 ^a	12.44 ± 0.32 ^b	16.06 ± 0.93 ^a	0.32 ± 0.26 ^a	5.74 ± 1.08 ^a	15.12 ± 0.96 ^a	22.67 ± 0.28 ^a	17.27 ± 2.77 ^a	1.15 ± 0.27 ^a
	6	1.29 ± 0.14 ^a	3.50 ± 0.28 ^a	0.30 ± 0.12 ^a	5.00 ± 0.08 ^a	13.09 ± 0.11 ^{ab}	15.33 ± 0.02 ^a	0.37 ± 0.09 ^a	6.32 ± 0.28 ^a	15.74 ± 0.12 ^a	22.85 ± 0.08 ^a	14.93 ± 0.14 ^a	1.29 ± 0.12 ^a
	8	1.18 ± 0.04 ^a	3.27 ± 0.28 ^a	0.27 ± 0.03 ^a	4.84 ± 0.18 ^a	13.18 ± 0.05 ^{ab}	15.46 ± 0.40 ^a	0.41 ± 0.01 ^a	6.51 ± 0.39 ^a	16.20 ± 0.42 ^a	22.67 ± 0.43 ^a	15.09 ± 0.07 ^a	0.92 ± 0.00 ^a

Values in the same column with different superscript letters are significantly different from those for unroasted seeds within each group ($P < 0.05$)
P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid

kg). This result agrees with previously published results by Kwon et al. (2004), who reported that the concentrations of all three phytosterols gradually increased and then decreased. One possible reason for the decrease in the contents of phytosterols in corn germ oil is that phytosterols easily evaporate at higher temperatures (175 °C) and powers for long times (8 min) (Moreau et al. 1999). However, these results do not agree with those of Yang et al. (2013) and Azadmard-Damirchi et al. (2010). Different parameters applied in these studies and different types of oilseeds may be responsible for these discrepancies. A higher MW power resulted in increased total phytosterol content compared to a lower MW power. Similar results were observed (Wang 2011). In general, there were no significant differences ($P > 0.05$) among the contents of phytosterols after each heat treatment. These results show that pretreatment of corn germ by OR or MW radiation under proper conditions can enrich the phytosterol content of extracted oil.

Conclusion

The heat pretreatments with OR and MW radiation showed considerable effects on the physicochemical properties of crude oils from wet-milled corn germ. A constant increase in the FFA, total DAG, and color was observed with increasing roasting temperatures, MW radiation power or time duration. Minor changes in the fatty acid profile and TAG composition were observed after OR and MW radiation pretreatment. Both pretreatments largely increased total tocopherol, and tocopherol isomers performed differently under different heat pretreatments. Phytosterol contents initially increased and then decreased slightly with higher temperature or power with longer heat exposure. Furthermore, both heat pretreatments significantly affected the oxidative stability (5.18–10.40 h) of crude oils, thus contributing to different shelf life of oils. Consequently, seed treated with both heat pretreatment generate oils with an enhanced content of desirable tocopherols compared to the untreated samples. Furthermore, MW radiation at proper conditions (for example, 800 W for 6 min) seemed to be more promising than OR (all three temperatures for 60 min) for commercial use in the near future due to its economical (time- and energy-saving) and quality-improving characteristic (higher oxidative stability and contents of tocopherol and phytosterol). Thus, this study will accelerate further study and application of MW radiation on pilot scale and large scale.

Table 5 Tocopherol contents of oils extracted from heat treated corn germ (mg/kg)

Temperature/power	Time (min)	α -Tocopherol	β -Tocopherol	γ -Tocopherol	δ -Tocopherol	Total tocopherols
125 °C	Unroasted	62.60 $\pm$ 0.24 ^c	79.24 $\pm$ 0.06 ^c	419.87 $\pm$ 0.79 ^d	32.36 $\pm$ 0.10 ^a	594.08 $\pm$ 1.07 ^d
	60	107.27 $\pm$ 1.18 ^b	62.73 $\pm$ 0.70 ^d	543.51 $\pm$ 2.24 ^a	33.63 $\pm$ 0.11 ^a	747.14 $\pm$ 2.61 ^c
	150 °C	116.33 $\pm$ 2.32 ^a	148.22 $\pm$ 3.58 ^b	511.37 $\pm$ 10.09 ^b	33.22 $\pm$ 0.92 ^a	809.13 $\pm$ 16.91 ^b
175 °C	60	108.37 $\pm$ 0.43 ^b	317.07 $\pm$ 2.05 ^a	436.11 $\pm$ 1.94 ^c	32.76 $\pm$ 0.60 ^a	894.31 $\pm$ 5.02 ^a
440 W	Unroasted	62.60 $\pm$ 0.24 ^{bc}	79.24 $\pm$ 0.06 ^b	419.87 $\pm$ 0.79 ^b	32.36 $\pm$ 0.10 ^a	594.08 $\pm$ 1.07 ^b
	4	50.27 $\pm$ 1.13 ^c	81.96 $\pm$ 3.51 ^b	405.13 $\pm$ 16.82 ^b	31.42 $\pm$ 3.44 ^a	568.78 $\pm$ 24.90 ^b
	6	75.72 $\pm$ 9.45 ^b	94.57 $\pm$ 11.90 ^{ab}	472.75 $\pm$ 60.21 ^{ab}	31.79 $\pm$ 4.97 ^a	674.83 $\pm$ 86.53 ^{ab}
	8	93.68 $\pm$ 1.96 ^a	105.30 $\pm$ 0.75 ^a	538.29 $\pm$ 9.94 ^a	35.61 $\pm$ 0.80 ^a	772.89 $\pm$ 13.45 ^a
800 W	Unroasted	62.60 $\pm$ 0.24 ^b	79.24 $\pm$ 0.06 ^c	419.87 $\pm$ 0.79 ^a	32.36 $\pm$ 0.10 ^a	594.08 $\pm$ 1.07 ^a
	4	85.85 $\pm$ 16.05 ^{ab}	115.82 $\pm$ 14.18 ^b	413.00 $\pm$ 76.09 ^a	25.72 $\pm$ 6.10 ^a	640.39 $\pm$ 112.41 ^{ab}
	6	110.93 $\pm$ 14.71 ^a	150.64 $\pm$ 9.92 ^a	535.18 $\pm$ 68.44 ^a	35.14 $\pm$ 3.91 ^a	831.90 $\pm$ 96.98 ^a
	8	109.92 $\pm$ 0.17 ^a	165.47 $\pm$ 0.02 ^a	517.20 $\pm$ 0.43 ^a	34.28 $\pm$ 0.66 ^a	826.87 $\pm$ 0.04 ^a

Values in the same column with different superscript letters are significantly different from those for unroasted seeds within each group ($P < 0.05$)

Table 6 Phytosterols contents of oils extracted from heat treated corn germ (mg/kg)

Temperature/power	Time (min)	Campesterol	Stigmasterol	Sitosterol	Total phytosterols
Control	Unroasted	1558.73 $\pm$ 198.29	533.92 $\pm$ 82.30	5046.45 $\pm$ 788.21	7139.10 $\pm$ 1068.79
125 °C	60	1681.68 $\pm$ 16.09	573.60 $\pm$ 31.38	5241.93 $\pm$ 283.82	7497.21 $\pm$ 331.28
150 °C	60	1662.24 $\pm$ 143.96	558.71 $\pm$ 10.41	5283.91 $\pm$ 181.81	7504.85 $\pm$ 336.18
175 °C	60	1581.36 $\pm$ 97.93	550.13 $\pm$ 9.69	5017.64 $\pm$ 154.23	7149.13 $\pm$ 46.60
440 W	Unroasted	1558.73 $\pm$ 198.29	533.92 $\pm$ 82.30	5046.45 $\pm$ 788.21	7139.10 $\pm$ 1068.79
	4	1351.30 $\pm$ 87.75	464.92 $\pm$ 27.64	4061.02 $\pm$ 239.42	5877.24 $\pm$ 124.03
	6	1551.16 $\pm$ 45.29	563.95 $\pm$ 12.68	5064.28 $\pm$ 22.36	7179.39 $\pm$ 54.96
	8	1548.40 $\pm$ 162.26	548.43 $\pm$ 64.23	4894.50 $\pm$ 623.70	6991.33 $\pm$ 850.2
800 W	Unroasted	1558.73 $\pm$ 198.29	533.92 $\pm$ 82.30	5046.45 $\pm$ 788.21	7139.10 $\pm$ 1068.79
	4	1501.38 $\pm$ 129.81	496.53 $\pm$ 53.17	4950.95 $\pm$ 257.64	6948.86 $\pm$ 440.62
	6	1750.20 $\pm$ 197.60	616.40 $\pm$ 61.07	5780.39 $\pm$ 716.68	8146.98 $\pm$ 975.30
	8	1570.35 $\pm$ 173.36	545.04 $\pm$ 17.77	4964.69 $\pm$ 86.38	7080.07 $\pm$ 277.51

There was no significant differences within each group ($P > 0.05$)

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Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

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