

Improvement of the Flavor and Oxidative Stability of Walnut Oil by Microwave Pretreatment

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Abstract Walnut oil is in great demand due to its high nutritional value. However, it is easily oxidized and often loses its typical flavor. This study focused on the role of microwave pretreatment in improving the flavor and oxidative stability of walnut oil, and also investigated the effects of microwave pretreatment on unsaturated fatty acids (oleic, palmitoleic, linoleic, and linolenic acids) and antioxidant components (tocopherols and phytosterols). The results indicate that microwave pretreatment is effective in generating pyrazine compounds. The typical ‘roasted’ flavor was present when pretreatment for 2 min or more was applied. Meanwhile, compared with the control sample, only the highly treated sample (microwave-pretreated for 4 min) showed higher oxidative stability. Only small changes were found in the composition of the unsaturated fatty acids, while the levels of tocopherols and phytosterols significantly decreased with increasing duration of microwave treatment ($P < 0.05$). The results suggest that the Maillard reaction caused the improvement of oxidative stability, since this reaction can also generate antioxidant products (melanoidins) in addition to pyrazines. Moreover, microwave pretreatment was found to be effective for enhancing the oil yield during pressing. Therefore, despite its adverse effects on tocopherols and phytosterols, microwave pretreatment could be used to improve the flavor and oxidative stability of walnut oil.

Keywords Walnuts · Microwave · Flavor · Oxidative stability

Introduction

Walnuts contain high levels (60 % on average) of oil, which includes substantial amounts of monounsaturated and polyunsaturated fatty acids [1]. It is now established as a good source of edible oil in countries such as France, Spain, Chile, and Argentina [2]. Although China is the largest producer of walnuts globally, producing 1,700,000 tons of shelled walnuts in 2013, it exported only 30,744 tons [3]. Thus, there is great potential for development of the Chinese walnut oil industry.

The optimal balance of n-6:n-3 polyunsaturated fatty acids (at a 4:1 ratio) in walnut oil, along with antioxidant components such as tocopherols and phytosterols, helps this oil to prevent disease and maintain health [4, 5]. However, high levels of unsaturated fatty acids make walnut oil susceptible to oxidation, which results in a short shelf life [6].

Among various newly established techniques for producing high-quality vegetable oil, microwave pretreatment is a simple and effective option [7]. Compared with conventional thermal treatments (roasting and steaming), microwave pretreatment is less time consuming and more energy efficient. It can also improve the oil extraction yield and oxidative stability of some oilseeds [8–11]. Moreover, although microwaves are generally thought not to promote the formation of desired flavors in food [12], research on peanuts has showed that, compared to oven roasting, microwave treatment produced similar odor activity levels but in a shorter time [13].

Pyrazines have been suggested to be the key contributors to the intense nutty/roasted flavor of aromatic peanut oil [14]. Similarly, in cumin seeds, the concentrations of

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pyrazines increased significantly as a result of microwave treatment, leading to a 'roasted' flavor [15]. During our preliminary experiment, an enhanced 'roasted' flavor of walnut kernels was also experienced after microwave treatment for a short period (data not published). Thus, the role of microwave pretreatment in the production of aromatic walnut oil warrants investigation.

The main objective of this research was thus to study the effect of microwave pretreatment on the flavor and oxidative stability of walnut oil. Changes in the composition (unsaturated fatty acids, tocopherols, and phytosterols) and oil yield were also evaluated to obtain a comprehensive understanding of the effects of microwave pretreatment.

Materials and Methods

Plant Materials

Fully mature walnuts (var. Santai) were hand-picked at a commercial plantation in Dayao County, Yunnan Province, China. Immediately after harvest, the green husks were removed and the nuts were dried in an oven at 43 °C to a moisture content of 8 %. The nuts were then shelled manually. Kernels of a similar size and color were selected for microwave pretreatment.

Microwave Pretreatment

The microwave pretreatment was performed with a consumer-model microwave oven at a frequency of 2450 MHz and power output of 600 W. For each microwave pretreatment, approximately 280 g of halved walnut kernels were placed in seven Pyrex Petri dishes (9 cm diameter), arranged along the edge of the glass turntable within the microwave. Samples were processed for 0, 1, 2, and 4 min as control, light, medium, and dark levels of treatment, respectively. Each microwave treatment was performed with three replicates. Three replicates of 280 g of kernels were used for each processing time.

Oil Extraction

Walnut oil was obtained by pressing 280 g of kernels with a laboratory hydraulic press (QYZ-230; Liang Jun Yi You Machinery, Tai'an, China) at 50 MPa for 10 min. The pressed oil was collected and centrifuged at 8000g for 15 min to remove fine particles, and then stored in capped bottles at 4 °C in the dark. Walnut cake was also collected to determine the content of the residual oil. For each microwave pretreatment, extraction was carried out with three 280-g replicates of the kernels.

Pyrazine Quantification and Sensory Assessment

Pyrazine analysis was carried out by headspace solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC–MS) (7890A–5975C; Agilent Technologies, Santa Clara, CA, USA), by a modified version of a previously reported procedure [1]. Briefly, 5 mL of walnut oil was placed into a 20-mL headspace vial fitted with a silicon septa. This vial was then incubated at 50 °C for 10 min. Then, the volatiles were sampled at the same temperature for 30 min with a 1-cm 50/30- μ m SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane. The fiber was then immediately inserted into the injection port (250 °C) in splitless mode. Separation was performed with an HP-INNOWAX capillary column (30 m \times 0.25 mm i.d., 0.25 μ m film thickness; Agilent), using helium (flow rate of 1 mL/min) as the carrier gas. The oven temperature was set as follows: 40 °C for 3 min, decreased to 220 °C at 4 °C/min, and then maintained at that temperature for 5 min. Standards including 2-methylpyrazine, 2,5-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, and 2,3,5-trimethylpyrazine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pyrazines were identified by comparison of the retention time and mass spectral data with those of standards. Quantification was performed by the external standard method, using quantitative ions for each standard. Owing to the lack of standards, 2-ethyl-6-methylpyrazine and 2-ethyl-5-methylpyrazine were tentatively identified by mass spectrum matching with the NIST 2.0 Library. Meanwhile, we found that the detector response was closely related to the molecular weight of pyrazines, which is in accordance with another report [16]. Hence, the levels of both of these pyrazines were calculated by comparing the peak areas with those of 2,3,5-trimethylpyrazine.

Flavor assessment was carried out in our laboratory by 10 trained panelists. In this study, only the flavor corresponding to 'roasted nut' was evaluated, since it could reflect the formation of pyrazines. The panelists were trained for 2 h a day for 3 days. A scale ranging from 1 to 15 points was used to assess the intensity of the 'roasted' flavor. Freshly refined walnut oil (score of 1) and commercially roasted walnut oil (score of 15; purchased from La Tourangelle, Saumur, France) were used as references. During the training, mixtures with different refined oil/roasted oil ratios were assessed by rating on the 1- to 15-point scale, where 1 = no roasted odor (purely refined oil), 5 = lightly roasted odor (refined oil and roasted oil at a ratio of 4:1), 10 = medium roasted odor (refined oil and roasted oil at a ratio of 1:1), and 15 = strongly roasted odor (purely roasted oil). Aliquots (10 mL) of oil samples were assessed in 15-mL amber bottles (i.d., 2.2 cm; surface area,

3.8 cm²) covered with glass sheets. Prior to assessment, the sample was allowed to stand at 25 °C for 2 h in a capped bottle. Distilled water was used for flavor neutralization between samples. The assessment process was performed once a day for 3 days. For each microwave pretreatment, one oil sample was assessed by the panelists per day.

Color development was determined according to GB/T 22460–2008 with a Lovibond tintometer (Model F; The Tintometer, Amesbury, UK) [17]. The results are reported on red and yellow color scales.

Oxidative Stability Evaluation

An oven storage test recommended by the American Oil Chemists' Society was adopted to evaluate the oxidative stability of walnut oil [18]. Six aliquots (15 mL) of each type of pressed walnut oil were stored in the dark at 60 ± 1 °C in 40-mL loosely capped bottles (i.d., 2.6 cm; surface area, 5.3 cm²) for 1, 2, 3, 5, 7, or 9 days. One bottle was taken for analysis at each scheduled time. Three individual batches of samples were used for each pretreatment. To follow the progress of oxidation, peroxide value (POV) and *p*-anisidine value (*p*-AV) were measured, in accordance with GB/T 5538-2005 and GB/T 24304-2009, respectively [19, 20]. The total oxidation value (TOTOX) was used to evaluate the overall oxidation state, calculated as follows: TOTOX = 2POV + *p*-AV [4].

Component Analysis

Fatty Acids

For the preparation of fatty acid methyl esters (FAMES), approximately 20 mg of oil was subjected to esterification by mixing with 2 mL of 0.01 M NaOH in methanol at 60 °C for 30 min under continuous shaking [21]. The tubes were then cooled and supplemented with 2 mL of 10 % sodium hydrogen sulfate (NaHSO₄)/25 % NaCl in water (1:1), 3 mL of water, and 1 mL of hexane. After vigorous shaking, the upper hexane layer containing FAMES was collected for GC analysis. The separations were performed by GC–MS on an Agilent 7890A-5975C with a CP-Sil 88 column (100 m × 0.25 mm i.d., 0.2 μm film thickness). Helium (flow rate of 1 mL/min) was used as a carrier gas at a split ratio of 100:1. The column temperature was set to change from 150 °C (5 min) to 210 °C (10 min) at 3 °C/min; injector and detector temperatures were 270 °C. Reference standards (methyl palmitate, methyl stearate, methyl palmitoleate, methyl oleate, methyl linoleate, and methyl linolenate) from Sigma-Aldrich were used. The FAMES were identified by comparisons of retention times and mass spectral data (NIST 2.0) with those of the standards. Quantification was carried out using an external standard method

with the same standards. The corresponding iodine value was measured according to GB/T 5532–2008 [22].

Tocopherols

Tocopherol levels were determined by high-performance liquid chromatography (HPLC) [23]. Oil samples (100 mg) were dissolved in 10 mL of hexane and filtered through a 0.2-μm nylon syringe filter. The separation was performed using a chromatograph (LC-10AT; Shimadzu, Kyoto, Japan) coupled with a LiChrospher Si-60 column (250 × 4 mm, 5 μm film thickness) and a fluorescence detector (RF-20A; Shimadzu). Hexane/2-propanol (99:1, v/v) was used as the mobile phase with a flow rate of 1 mL/min. The excitation wavelength and emission wavelength were set at 290 and 330 nm, respectively. Tocopherol homologues (α-, β-, γ-, and δ-) purchased from Sigma-Aldrich were used as standards. Each type of tocopherol in oil samples was identified according to the retention times of the standards. The external standard method was used for quantification.

Phytosterols

The levels of phytosterols were determined according to NP EN ISO 12228 [24]. After saponification, separation, and silylation, the phytosterols were analyzed by Agilent 7890A-5975C GC–MS. The separation was performed on an HP-5MS column (30 m × 0.25 mm i.d., 0.25-μm film thickness) at a split ratio of 50:1. The injector and detector temperatures were both 320 °C, and the column temperature was set to change from 250 to 300 °C (12 min) at 2 °C/min. Campesterol, stigmasterol, and β-sitosterol were identified by comparisons of retention times and mass spectral data with the standards purchased from Sigma-Aldrich. Δ⁵-Avenasterol, clerosterol, and sitostanol were tentatively identified by mass spectrum matching with the NIST 2.0 Library and comparisons with a previous report [24]. Owing to the lack of standard compounds, only relative concentrations of individual sterols are given in this paper. The semi-quantification was performed by relating the areas of the internal standard (betulin) to the areas of the sterols of interest.

$$\text{Relative concentration} = \frac{\text{Peak area of particular sterol}}{\text{Peak area of betulin}} \times \text{Concentration of betulin}$$

Residual Oil Content

The residual oil content in walnut cake was determined gravimetrically by extraction from 20 g of finely ground samples with analytical-grade hexane to exhaustion in a Soxhlet apparatus for 4 h at 70 °C [10].

Statistical Analysis

All analyses were performed in triplicate. The results are expressed as means of three replicates \pm standard deviation (SD). One-way ANOVA followed by Duncan's multiple range test was used to assess differences between means. Analyses were performed in SPSS for Windows software (v.19.0; SPSS, Chicago, IL, USA). A difference was considered to be significant at the level of $p < 0.05$.

Results and Discussion

Effect of Microwave Pretreatment on Flavor

Pyrazines impart a nut-like aroma in many different types of food [16]. Figure 1 shows the gas chromatograms of pyrazines in oil samples. Seven pyrazine compounds, namely, 2-methylpyrazine, 2,5-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, and 2,3,5-trimethylpyrazine, were identified and quantified in oils pressed from the microwave-pretreated kernels, as shown in Table 1. These pyrazines were also identified in oils pressed from roasted peanut [13], red pepper seed [16], and soybean [25]. 2,5-dimethylpyrazine was the predominant pyrazine compound in all of the oils pressed from pretreated kernels, followed by 2-methylpyrazine. Trace amounts of 2,5-dimethylpyrazine and 2-methylpyrazine were also found in the control samples, which might have been derived from the extrusion process. The total levels of pyrazines were positively correlated to the intensity of treatment. The clear increase in the level of pyrazines indicated that the Maillard reaction was promoted by the microwave pretreatment, since alkylpyrazines are regarded as products of this reaction through pathways including Amadori rearrangement, Strecker degradation, and heterocyclization [26, 27].

The results of flavor assessment were strongly correlated with the total amount of pyrazines ($R^2 = 0.9462$), as shown in Fig. 2. In fact, almost no pronounced 'roasted' flavor was experienced for the control and light samples. However, starting at the medium sample, a 'roasted' flavor could be easily identified by the panelists. Therefore, microwave pretreatment for 2 min or more can be used to produce walnut oil with a 'roasted' flavor.

The color development with increasing processing time was also determined, the results of which are shown in Fig. 3. Significant ($P < 0.05$) color changes appeared first in the medium sample. Similarly, in a previous study, the color of oil extracted from microwave-treated sunflower seeds changed from light yellow (red unit, 1.60; yellow unit, 16) to brown (red unit, 16.0; yellow unit, 50.4) with increasing process time [11]. It has also been reported

that the Maillard reaction is associated with the development of both aromatic and color components [28]. Hence, in addition to the above pyrazines, the clear color changes also indicated the promotion of the Maillard reaction during microwave pretreatment. Such color changes have also been used to estimate the levels of Maillard reaction products and the related antioxidant attributes in roasted almond oil [29].

Effect of Microwave Pretreatment on Oxidative Stability

The time courses of POV, *p*-AV, and TOTOX of oil during oven storage are shown in Table 2. The data at day 0 indicate that the microwave pretreatment caused small but significant increases in all the indexes ($P < 0.05$). After 3 days of storage, the highest oxidative stability was observed for the high sample, since it possessed the lowest POV and TOTOX. The low sample showed the poorest oxidative stability. However, during storage, the control sample showed a lower *p*-AV than the high sample. *p*-AV was used to measure the secondary oxidation products in oils. Since the formation of pyrazines indicates the promotion of the Maillard reaction, this could be explained by the fact that the higher *p*-AV was caused not only by oil oxidation but also by the Maillard reaction, since both could generate carbonyl compounds [26]. This reaction could also explain the improvement of oxidative stability in the high sample, since it also produced antioxidant products such as melanoidins [30]. Hence, the effect of microwave pretreatment on the oxidative stability depended on the duration and intensity of processing. In our study, 4-min pretreatment could be used to improve the oxidative stability of walnut oil.

Effect of Microwave Pretreatment on Fatty Acids

The changes in fatty acid composition induced by microwave pretreatment are shown in Table 3. According to a worldwide study of walnut oils from unspecified varieties, the levels of fatty acids reported here are almost within the normal ranges for authentic walnut oil, except for a 2 % lower level of linolenic acid [31]. It has been indicated that the genotype is the main source of variability of fatty acid composition in walnut oil [32]. Hence, this discrepancy could be explained by the difference in cultivars.

There were few changes in the fatty acid composition of walnut oil during the microwave pretreatment. This matches a previous paper, which reported that no significant changes were found in fatty acid composition when walnut kernels were treated with a microwave at full power for 3 min [33]. However, small but significant ($P < 0.05$) differences in the unsaturated fatty acids were identified.

Fig. 1 Gas chromatograms of pyrazines in oils pressed from treated and untreated walnut kernels (the numbering of peaks corresponding to pyrazine compounds is in accordance with Table 1)

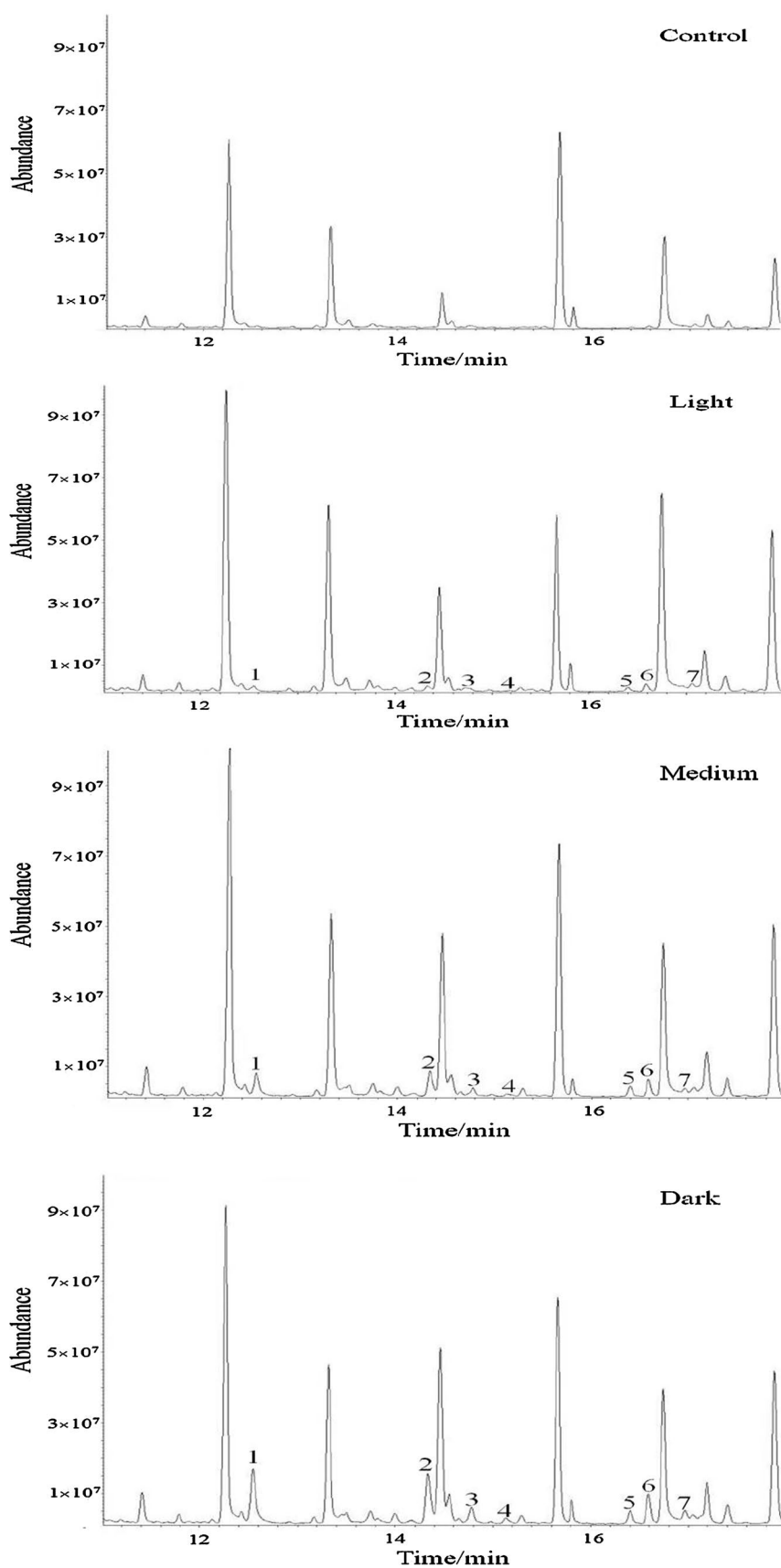
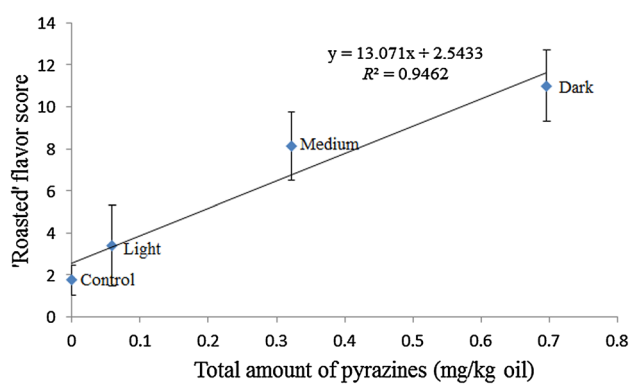
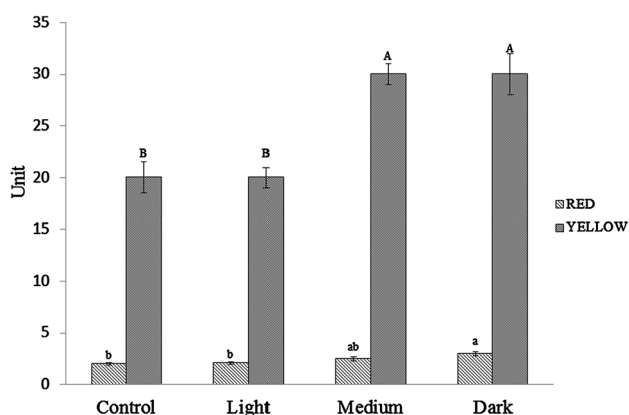


Table 1 Pyrazine compounds identified in the headspace of walnut oils using headspace solid-phase microextraction/gas chromatography–mass spectrometry

Peak no.	Compound	Concentration (mg/kg oil)			
		Control (0 min)	Light (1 min)	Medium (2 min)	Dark (4 min)
1	2-methylpyrazine	Tr	0.010 ± 0.00	0.079 ± 0.002	0.209 ± 0.004
2	2,5-dimethylpyrazine	Tr	0.026 ± 0.00	0.129 ± 0.00	0.261 ± 0.00
3	2-ethylpyrazine	ND	0.008 ± 0.00	0.025 ± 0.001	0.051 ± 0.001
4	2,3-dimethylpyrazine	ND	0.001 ± 0.00	0.011 ± 0.00	0.025 ± 0.00
5	2-ethyl-6-methylpyrazine	ND	0.003 ± 0.00	0.020 ± 0.00	0.038 ± 0.001
6	2-ethyl-5-methylpyrazine	ND	0.007 ± 0.00	0.044 ± 0.001	0.083 ± 0.003
7	2,3,5-trimethylpyrazine	ND	0.004 ± 0.00	0.014 ± 0.00	0.029 ± 0.001
Total		Tr	0.062 ± 0.00	0.325 ± 0.002	0.699 ± 0.008

Values are presented as means of three replicates ± standard deviation

Tr trace, ND not detected

**Fig. 2** Relationship between the ‘roasted’ flavor score and total amount of pyrazines**Fig. 3** Color parameters (red and yellow units) of walnut oils with different degrees of treatment. Uppercase letters represent comparisons of the means of yellow units and lowercase letters are those of red units. Different letters indicate significant differences ($P < 0.05$)

Compared with the control sample, the level of oleic acid in the highly treated sample increased by 5.19 %, while the level of linolenic acid decreased by 3.46 %. Similarly,

other research also indicated that a longer processing time would lead to a higher proportion of oleic acid and a lower proportion of linoleic acid [11]. No significant ($P > 0.05$) changes were observed in the total levels of unsaturated fatty acids, and the proportion of unsaturated fatty acids [expressed as $U/(U+S)$] remained almost the same in all samples. In addition, no significant ($P < 0.05$) changes were found in iodine values, which were used to measure the degree of unsaturation. According to a previous report, the iodine value was too general to allow the correlation of physical and chemical properties with fatty acids, and it depended on both the molecular weight and the amount of unsaturated fatty acids in oils [34].

In other oilseeds such as sesame, the fatty acid composition was essentially the same during the first 8 min of microwave heating [35]. Moreover, few changes in fatty acid composition were found when the sunflower seeds were treated with a microwave for less than 12 min [36]. In addition to the power frequency and processing time, the material characteristics (density, composition, and structure) were also shown to determine the effect of microwave pretreatment [37]. Hence, for walnuts, our results showed that microwave pretreatment lasting for 0–4 min would have no significant effects on the unsaturation of fatty acids. Furthermore, from the perspective of human nutrition, no significant ($P > 0.05$) changes were found in the omega-6/omega-3 ratio after microwave pretreatment. The World Health Organization has recommended a ratio of omega-6/omega-3 of 5:1 to 10:1, and in some countries, this ratio is lower [38]. The consumption of walnut oil could be helpful to obtain an appropriate ratio of omega-6/omega-3 in the diet.

Effect of Microwave Pretreatment on Tocopherols and Phytosterols

Tocopherols and phytosterols are natural antioxidants that can stabilize oils and prevent rancidity [39]. The major

Table 2 The time courses of peroxide value (POV), *p*-anisidine value (AV), and total oxidation value (TOTOX) of walnut oils subjected to different degrees of treatment during oven storage

	Day							
	0	1	2	3	5	7	9	
POV								
Control	0.47 ± 0.01c	2.22 ± 0.04d	6.37 ± 0.08c	12.85 ± 0.26c	21.64 ± 0.64b	32.46 ± 2.06b	41.77 ± 1.41b	
Light	1.03 ± 0.01b	3.83 ± 0.03a	9.47 ± 0.09a	16.28 ± 0.60a	25.82 ± 1.17a	38.50 ± 1.90a	48.14 ± 1.56a	
Medium	1.08 ± 0.01a	3.70 ± 0.03b	9.37 ± 0.08a	14.59 ± 0.49b	22.90 ± 1.26b	32.36 ± 1.20b	44.30 ± 1.58b	
Dark	1.07 ± 0.01ab	3.57 ± 0.05c	8.00 ± 0.21b	12.05 ± 0.29c	18.07 ± 0.37c	27.57 ± 0.86c	37.81 ± 1.44c	
<i>p</i>-AV								
Control	0.33 ± 0.05d	0.86 ± 0.08d	1.44 ± 0.01d	1.65 ± 0.01c	2.92 ± 0.05c	4.51 ± 0.11c	5.97 ± 0.13d	
Light	0.62 ± 0.03c	1.28 ± 0.03c	1.92 ± 0.06c	2.19 ± 0.03b	3.50 ± 0.04b	4.75 ± 0.12c	6.26 ± 0.10c	
Medium	0.85 ± 0.05b	1.44 ± 0.01b	2.12 ± 0.07b	2.58 ± 0.09a	3.94 ± 0.17a	5.57 ± 0.18a	7.37 ± 0.10a	
Dark	1.03 ± 0.06a	1.61 ± 0.02a	2.34 ± 0.06a	2.61 ± 0.02a	3.62 ± 0.14b	5.24 ± 0.13b	7.12 ± 0.08b	
TOTOX								
Control	1.27 ± 0.04d	5.29 ± 0.12c	14.17 ± 0.18b	27.35 ± 0.53c	46.19 ± 1.23c	69.44 ± 4.13b	89.51 ± 2.96c	
Light	2.70 ± 0.01c	8.94 ± 0.10a	22.86 ± 0.22a	34.75 ± 1.20a	55.15 ± 2.32a	81.75 ± 3.71a	102.54 ± 3.22a	
Medium	3.00 ± 0.04b	8.83 ± 0.06ab	22.85 ± 0.21a	31.75 ± 0.89b	49.74 ± 2.37b	70.27 ± 2.44b	95.97 ± 3.12b	
Dark	3.16 ± 0.05a	8.74 ± 0.07b	22.34 ± 0.45a	26.70 ± 0.60c	39.76 ± 0.89d	60.38 ± 1.59c	82.75 ± 2.88d	

Values are presented as means of three replicates ± standard deviation. For each index, different letters in the same column indicate significant differences ($P < 0.05$)

Table 3 Fatty acid compositions of walnut oils with different degrees of treatment

	Degree of treatment			
	Control (0 min)	Light (1 min)	Medium (2 min)	Dark (4 min)
Fatty acid (g/100 g)				
Palmitic (16:0)	7.56 ± 0.09ns	7.57 ± 0.02ns	7.47 ± 0.11ns	7.46 ± 0.10ns
Steric (18:0)	2.23 ± 0.03ns	2.23 ± 0.05ns	2.20 ± 0.01ns	2.20 ± 0.03ns
Palmitoleic (16:1)	0.18 ± 0.02ns	0.18 ± 0.03ns	0.18 ± 0.01ns	0.19 ± 0.02ns
Oleic(18:1)	20.46 ± 0.50b	20.60 ± 0.65b	21.20 ± 0.31ab	21.67 ± 0.57a
Linoleic (18:2)	61.02 ± 0.28a	60.83 ± 0.67ab	60.01 ± 0.45b	60.29 ± 0.16ab
Linolenic (18:3)	8.66 ± 0.32a	8.72 ± 0.14a	8.53 ± 0.25a	8.36 ± 0.10b
ΣPolyunsaturated fatty acids (P)	69.68 ± 0.30a	69.55 ± 0.79ab	68.82 ± 0.69b	68.37 ± 0.14b
ΣUnsaturated fatty acids (U)	90.32 ± 0.38ns	90.33 ± 1.29ns	90.21 ± 0.94ns	90.24 ± 0.63ns
ΣSaturated fatty acids (S)	9.78 ± 0.08ns	9.80 ± 0.05ns	9.68 ± 0.13ns	9.67 ± 0.15ns
U/(U+S)	0.902 ± 0.00ns	0.902 ± 0.00ns	0.903 ± 0.00ns	0.903 ± 0.00ns
Omega-6/Omega-3	7.07 ± 0.29ns	6.97 ± 0.08ns	7.07 ± 0.16ns	7.18 ± 0.11ns
Iodine value (g/100 g)	154.5 ± 2.50ns	155.8 ± 0.65ns	156.4 ± 4.51ns	153.2 ± 2.00ns

Values are presented as means of three replicates ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$)

ns not significant

tocol in walnut oil is γ -tocopherol, while β -sitosterol and campesterol are the predominant sterols [31]. The effects of microwave pretreatment on tocopherols and phytosterols are shown in Table 4. Compared with earlier reports [31], the levels of α -, β -, and δ -tocopherol were generally within the normal ranges for authentic walnut oil; however, the level of γ -tocopherol was outside the normal range (247–525 mg/kg) after microwave pretreatment, probably due to

its low initial level. The phytosterol levels of all samples were generally in agreement with those for authentic walnut oil [31, 40].

During the microwave pretreatment, the tocopherol and phytosterol levels decreased with increasing processing time. For tocopherols, even the low treatment caused significant losses of all components (decreases of 35.1, 17.8, and 9.9 % for α -tocopherol, γ -tocopherol, and

Table 4 Tocopherol and phytosterol compositions of walnut oils with different degrees of treatment

Minor component	Degree of treatment			
	Control (0 min)	Light (1 min)	Medium (2 min)	Dark (4 min)
Tocopherols (mg/kg)				
α -Tocopherol	16.67 \pm 1.74a	10.89 \pm 1.08b	11.30 \pm 0.81b	10.50 \pm 0.68b
β -Tocopherol	Tr	Tr	Tr	Tr
γ -Tocopherol	235.26 \pm 11.23a	193.83 \pm 5.74b	174.73 \pm 9.12c	165.26 \pm 6.66c
δ -Tocopherol	34.26 \pm 2.71a	30.86 \pm 0.55ab	29.37 \pm 2.16b	28.50 \pm 1.11b
Total	286.21 \pm 7.39a	234.59 \pm 5.88b	215.40 \pm 7.59c	204.27 \pm 7.22c
Phytosterols (mg/kg)				
β -Sitosterol	1101.96 \pm 15.01a	1080.70 \pm 2.25b	1077.96 \pm 3.06b	1025.33 \pm 5.95c
Δ 5-Avenasterol	122.53 \pm 8.01a	120.90 \pm 6.17a	118.90 \pm 2.32a	114.26 \pm 2.05a
Campesterol	52.66 \pm 2.45a	43.83 \pm 1.51b	43.86 \pm 1.60b	40.93 \pm 1.97b
Clerosterol	7.72 \pm 0.95a	7.50 \pm 0.24a	6.52 \pm 0.09b	6.31 \pm 0.10b
Stigmasterol	12.47 \pm 0.51a	5.25 \pm 0.35b	5.06 \pm 0.66b	3.83 \pm 0.48c
Sitostanol	40.13 \pm 1.00a	38.80 \pm 1.30ab	36.83 \pm 0.90b	26.76 \pm 1.35c
Total	1337.50 \pm 8.52a	1296.98 \pm 5.51b	1289.15 \pm 4.39b	1217.44 \pm 9.34c

Values in the same row followed by a different letter are significantly different ($P < 0.05$)

Tr trace

δ -tocopherol, respectively). However, when the processing time increased, only γ -tocopherol showed remarkable changes (decreases of 9.3 and 5.7 % at medium and high treatments, respectively). Previously, it was reported that the effectiveness of tocopherols in delaying the oxidation of methyl linoleate was in the following order: $\gamma > \delta > \beta > \alpha$ [41]. Meanwhile, another study showed that, when the concentration of tocopherol mixtures was 750 ppm or less, hydroperoxide formation seemed to be determined by γ -tocopherol [42]. Thus, the decrease in total tocopherol content and the different antioxidant abilities of the individual tocopherols could explain the continuous decrease of γ -tocopherol in this study. Among the phytosterols, small but significant ($P < 0.05$) decreases were observed for all of the individual sterols as the processing time increased. Among the different phytosterols, β -sitosterol was present at the highest concentration, and its level decreased the most during the microwave pretreatment. However, in terms of the percentage loss of each individual sterol, stigmastanol showed greater loss than the other sterols in walnut oil. In the samples subjected to high treatment, 69.2 % of the stigmastanol was destroyed, compared with 6.95 % of the β -sitosterol. Similar results were also reported by other researchers, who explained this by the increase in the unsaturation of the B-ring structure in sterols [43]. Although the microwave pretreatment did influence the levels of tocopherols and phytosterols in walnut oil, the degradation of these components upon normal storage without pretreatment was also significant. It was reported that a decrease in tocopherols (~30 %) was observed after 3 months of refrigerated storage of walnuts

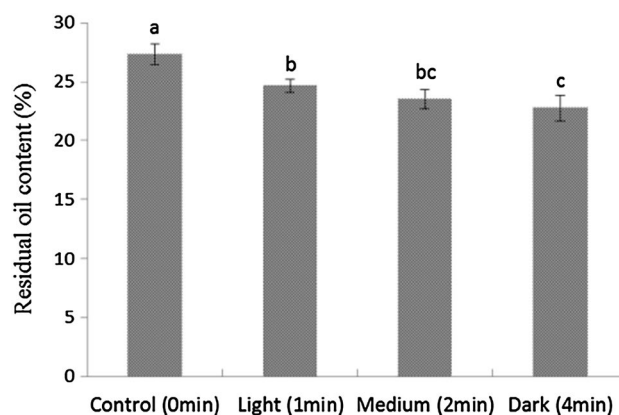


Fig. 4 The effect of microwave pretreatment on residual oil content (%) in walnut cake. Different letters above bars indicate significant differences ($P < 0.05$)

[44]. In our study, the loss of total tocopherols was approximately 28 % when the walnuts were pretreated for 4 min. Thus, compared with the effect of long-term storage, the degree of degradation of tocopherols caused by microwave pretreatment was not severe.

Effect of Microwave Pretreatment on Residual Oil Content

The residual oil content in walnut cake was used to denote the oil yield of walnut during pressing. As shown in Fig. 4, the residual oil content decreased with increasing processing time, and the minimum residual oil content (22.8 %)

was achieved when the processing time was 4 min. The largest difference occurred between the control sample and the low sample, indicating an instant effect of microwave pretreatment on oil yield. The mechanism behind this improvement may be explained by cell damage, as it was reported that microwave treatment facilitated rupture of the cell membrane, generating permanent pores and enabling oil to move through the permeable cell walls [10].

Conclusion

In this study, the effects of microwave pretreatment on flavor and oxidative stability were evaluated, as well as the changes in fatty acids, tocopherols, phytosterols, and oil yield. Microwave pretreatment was found to be effective for generating pyrazine compounds, and a treatment time of 2 min or more resulted in a typical ‘roasted’ flavor of oil. Meanwhile, compared with the control sample, higher oxidative stability was achieved when walnuts were subjected to the 4-min treatment. Moreover, no significant ($P < 0.05$) changes were found in the unsaturation of fatty acids, while the levels of tocopherols and phytosterols decreased significantly ($P < 0.05$) with increased processing time. The degradation of these antioxidant components indicated that the improvement of oxidative stability could be explained by the Maillard reaction and its antioxidant products such as melanoidins. Microwave pretreatment was also found to be effective in enhancing the oil yield during pressing. In general, our research suggests that microwave pretreatment could be applied to walnuts before pressing to improve flavor, oxidative stability, and oil yield.

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