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Research Article

Heating Quality and Stability of Aqueous Enzymatic Extraction of Fatty Acid-Balanced Oil in Comparison with Other Blended Oils

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The heating performance of enzyme-assisted aqueous processing-extracted blended oil (EAEPO), hexane-extracted blended oil (HEBO), and three kinds of blended oils was investigated by varying the heating times. Oil degradation was monitored by analysis of the acid value (AV), peroxide value (PV), *p*-anisidine value (*p*-AV), color, and trans-fatty acid composition. The fatty acid ratios of EAEPO, HEBO, and the three kinds of blended oils were very similar (0.27 : 1.03 : 0.96, 0.27 : 1.08 : 1.16, 0.27 : 0.65 : 0.8, 0.27 : 0.6 : 0.84, and 0.27 : 0.61 : 0.79, resp.). The AV and color increased in proportion to the heating time for all the oils. There was a rapid increase in the PV and *p*-AV of EAEPO and HEBO after heating for only 1 h, whereas the other three blended oils showed a rapid increase after heating for 2 h or 6 h. Despite the highest trans-fatty acid content found for HEBO, this content was relatively low and remained low up to a heating time of 8 h. It was found that after heating, a fatty acid ratio relatively close to its ideal value (0.27 : 0.48 : 0.49) was maintained by EAEPO, which indicates that EAEPO is tolerant to heat treatment and is suitable for maintaining a healthy diet.

1. Introduction

In recent times, the use of oils with a balanced fatty acid ratio has emerged as one of the most important concerns for maintaining a healthy diet. The blending of several oils with different characteristics is one of the simplest procedures for controlling the characteristics of edible oil [1]. The United States Recommended Dietary Allowances (RDA) and the World Health Organization (WHO) recommend the fatty acid ratio of 1 (SFA, saturated fatty acid(s)) : 1 (MUFA, monounsaturated fatty acid(s)) : 1 (PUFA, polyunsaturated fatty acid(s)) as an ideal ratio for health [2]. In addition, a report from the Chinese Nutrition Society (CNS) regarding dietary reference intakes suggested a fatty acid ratio of 0.27 : 1 : 1 (SFA : MUFA : PUFA) after subtracting the daily consumption of fatty acids derived from animal fat [3]. The use of hexane-extracted blended oil (HEBO) is the main method currently used to achieve this aim. However, the hexane derived from petroleum distillates has environmental and safety issues, and thus, it is necessary to develop alternative methods of hexane extraction. Furthermore, the goal of achieving an acid-balanced oil has been realized by extracting oil from a mixture of soybean, rape seed, purple perilla, fructus cannabis, and scabish using an aqueous enzymatic method (EAEP). The oil extraction yield was maximized by applying the simplex-centroid mixture design method. This balanced fatty acid oil may be one of the major nutritional sources in the diet for maintaining human health by providing the body with unsaturated fatty acids, being particularly rich in omega-3, omega-6, and other nutritional components. However, heat treatment of oils is one of the most important methods for preparing food for human consumption worldwide [4] but may affect the shelf life and quality of the oil directly. Heating may simultaneously

induce degradation and oxidation of oil components with the formation of products deleterious to human health. In addition, many reports concluded that the stability of individual vegetable oils against oxidation is unique, being dependent on the fatty acid composition and on the degree of unsaturation in particular [5, 6].

The quality characteristics of many oils, including Deglet Nour, Allig, olive, sunflower, and hydrogenated soybean oil, have been investigated [7, 8]. Microwave heating of soybean oil induces severe quality and composition losses, primarily with more than 3 min of microwave heating [9]. Thus, the reactions that occur during heating of oil are an important concern. During heating, lipids may undergo chemical alterations due to hydrolytic and oxidative rancidity [10]. The influence of heating on the antioxidant activity of certain spice essential oils has been discussed in order to control lipid oxidation during food processing [11]. The influence of these reactions is derived from a number of products generated during heating, including volatile compounds, hydrolysis products, cyclic compounds, trans configuration compounds, polymers, and acrylamide. After the first two frying cycles, the free fatty acid content, peroxide value, and total polar materials increase, whereas the radical scavenging activity decreases [5].

In this paper, the oxidative stability of oils extracted using various techniques, including solvent and aqueous enzymatic methods, is determined. The major reaction conditions and corresponding physical and chemical alterations during heating are also studied by comparative analysis of three blended oils with fatty acid compositions similar to that of EAEPO and HEBO.

2. Materials and Methods

2.1. Materials. Dehulled and full-fat soybean flakes were obtained from a company in Shijiazhuang, and rape seed, purple perilla, fructus cannabis, and scabish were obtained from Anhui, Jiangsu, Guangxi, and Muyang, respectively. Three different types of oils, namely, Golden Arowana blend oil (GABO), Fook Lam Moon Grain blend oil (FGBO), and Fook Lam Moon balanced blend oil (FBBO), were obtained from a local market (RT-Mart, Harbin). Alcalase 2.4 L was sourced from Novo-Nordisk A/S (Bagsvaerd, Denmark). The fatty acid methyl ester (FAME) standards were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Extrusion Process. Water was sprayed onto the cracked soybean flakes to achieve the desired moisture content of 14.5% while tumbling the seeds in a mixer (TMV-100, China). The moisture content of 14.5% was chosen on the basis of previous work on oil extraction using EAEP [12]. The extruder (20 mm screw die diameter) used in this work was manufactured by Engineering College, Northeast Agricultural University, Harbin, China. The rotational speed of the extruder was 105 rpm and the barrel temperature was 90°C. The extruded soybeans were immediately used for the EAEP procedure or stored in polyethylene bags at 4°C in a refrigerator prior to use.

2.2.2. Enzyme-Assisted Aqueous Extraction Process (EAEP). Prior to the extraction procedure, the dehulled forms of rape seed (31.5%), purple perilla (11.8%), fructus cannabis (33.3%), and scabish (21.2%) were individually cracked and then mixed together with the extruded soybean (2.3%). The beaker filled with the seed mixtures and additional water (1 : 6 w/w) was subjected to ultrasonic treatment for 50 min at 55°C at a power of 500 W and then incubated at 55°C in a water bath; the pH of the slurry was adjusted to 9 by the addition of 2 N NaOH [13]. The enzyme dosage of Alcalase 2.4 L was 1.85% (v/w, based on the dry weight of the samples). A continuous stirring device was used to disperse the mixture during the enzymatic hydrolysis process. The reactions were stably maintained at the temperature and pH stated above for 3 h. At the end of the EAEP, the slurry was centrifuged in a 50 mL centrifuge tube at multiples of the force of gravity and expressed at 2,372 g (TGL-16G, China) for 30 min at 20°C. Four distinct layers (oil, cream, skim, and insoluble) were obtained after the centrifugation. The upper oil layer was carefully collected using a Pasteur pipette, and the remaining oil adhering to the cream surface was washed twice with hexane. The hexane was removed by evaporation in a fume hood.

2.2.3. Hexane Extraction (HE) Process. The blending flakes (100 g) (extruded soybean 2.3%, dehulled rape seed 31.5%, dehulled purple perilla 11.8%, dehulled fructus cannabis 33.3%, and dehulled scabish 21.2%) were placed in a Soxhlet extractor equipped with a 0.5 L round-bottom flask and a water condenser. The extraction was carried out on a water bath for 6 h using 3 L of *n*-hexane. After the extraction, hexane was removed under reduced pressure using a vacuum rotary evaporator (R205, China) at 45°C. The recovered oil was stored under refrigeration (4°C) prior to use.

2.2.4. Heat Treatment. To simulate conventional times used in cooking, the heating period was varied as 1, 2, 4, 6, and 8 h. For each oil type and time, three subsamples of 80 mL were, respectively, placed in a domestic electronic heater plate with an intelligent magnetic stirrer (ZNCL, China) and the samples were heated at 200°C. Unheated samples of each oil type were used as controls (corresponding to 0 h). After heating, samples were stored in a brown bottle in a refrigerator (4°C) prior to analysis.

2.3. Oil Quality Analysis

2.3.1. Determination of Fatty Acid and Trans-Fatty Acid Compositions. The trans-fatty acid composition of the oil types was analyzed using a gas chromatograph connected to a mass spectrometer (GC-MS). The fatty acid methyl esters were prepared in two steps: the oils were first saponified with 0.5 M KOH and subsequently methylated with 40% BF_3 in methanol. The separation was performed using an HP-88 capillary column (100 mm × 0.25 mm i.d., 0.2 μm

film thickness; Agilent Technologies, USA) connected to a GC/MS (6890/5973, USA). The operating conditions were as follows: carrier gas pressure, 100 kPa; carrier gas, helium; split ratio, 1 : 30; injection temperature, 250°C; scanning scope, 50–550 amu; and ionization voltage, 70 eV. The oven temperature settings were programmed as follows: the temperature was held at 80°C for 5 min, increased to 150°C at 10°C/min and held at 150°C for 2 min, then increased continuously to 230°C at a rate of 5°C/min, and held for 10 min. The individual fatty acids were identified by comparing their retention times with standards [2].

2.3.2. Determination of Acid Value, p-Anisidine Value, and Color. The acid value was determined according to a literature method (AOAC, Cd-63), where 125 mL of the neutralized solvent mixture and 2 mL phenolphthalein indicator solution were placed into an Erlenmeyer flask and neutralized with alkali to a faint but permanent pink color. The oil sample was then weighed (20 ± 0.05 g) and added to the solution, and the sample was shaken vigorously while titrating with standard alkali to the first permanent pink color of the same intensity as that of the neutralized solvent before the latter was added to the sample.

The *p*-anisidine value of the oxidized oil from fried dough after storage was determined according to the AOCS Recommended Practice Ti la-64 (AOCS, 1990).

An accurately measured amount of oil (100 mg) was dissolved in 25 mL, and the absorbance was measured at 350 nm using a UV-vis spectrophotometer (1600PC, China). This solution (2.5 mL) was combined with 0.5 mL of 0.5% (w/v) *p*-anisidine in acetic acid, and the absorbance was read at 350 nm after 10 min.

The color was determined using a Lovibond Tintometer (WSL-2, China) in the transmittance mode and expressed as red (R) and yellow (Y) values.

2.3.3. Determination of Peroxide Value. An accurately measured amount of oil (300 mg) was dissolved in 9.9 mL of chloroform/methanol (7 : 3, v/v) after which 50.0 μL of 10 mM xylenol orange and 50.0 μL of iron(III) chloride solution were added [14]. The mixture solution was incubated at room temperature for 5 min and then centrifuged at 1000 g for 5 min at 5°C. The supernatant was used for measurement of the absorbance at 560 nm using a UV-vis spectrophotometer (model 1600 PC, China). The iron(III) chloride solution was used to construct a standard curve.

2.3.4. Statistical Analysis. Data from replicate analyses of all samples were subjected to a variance analysis (ANOVA) test using SPSS 18.0 for Windows. The significant difference between the means was determined by Duncan's New Multiple Range Test ($P < 0.05$).

3. Results and Discussion

3.1. Changes in Acid Value. The acid value is considered in the food industry as an indicator of the quality of the oil and the degree of its degradation during heating. An increase in the acid value leads to the development of unpleasant tastes and odors in oils. Although the initial acid values of the oils varied from 0.08 (EAEPO) to 0.16 (HEBO) as shown in Figure 1, at the end of the heating period (8 h), the overall change in the acid value was found to be lowest in the case of EAEPO (0.35), followed by FBBO, FGBO, and GABO. The overall change in the acid value was highest for HEBO (0.48). This variation may be due to differences in the extraction method used. Enzymatic extraction offers the advantages of requiring a lower temperature and not requiring explosive solvents or generating harmful waste relative to solvent extraction. These results are in agreement with the values previously reported for corn oil [15].

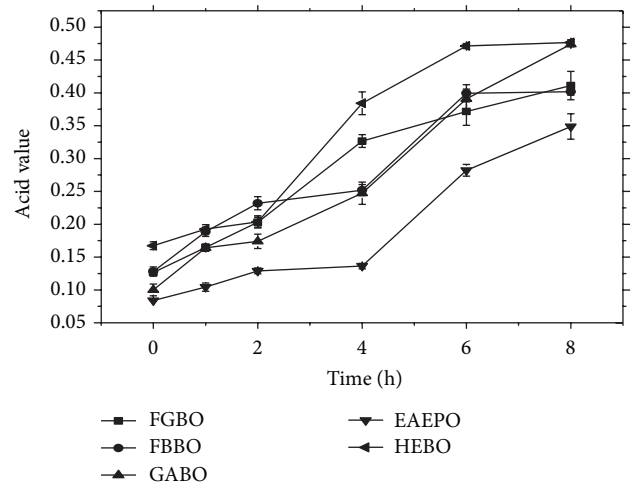

Figure 1: Changes in acid value.

The results also indicated a significant increase in the acid value of the various oil types as heating progressed, which may be attributed to the hydrolysis of TAG and/or cleavage and oxidation of fatty acid double bonds [16]. In addition, the low acid value of the three types of blended oils relative to HEBO might be due to the refining of the former oils. It is recognized that the free fatty acids (FFAs) are removed by saponification; thus, smaller amounts of FFAs are expected for refined oils [17]. However, the lowest acid content of the EAEPO sample might be related to its ideal fatty acid ratio. In a similar study, the stability of soybean and canola oils, with modified fatty acid composition, toward frying was evaluated. The amount of free fatty acids generated during frying was significantly less than that of the corresponding unmodified oils after 5 h of frying [18].

3.2. Changes in Peroxide Value (PV). Figure 2 shows the changes in the PV of the oils with heating from 0 h to 8 h. Prior to the heating process, EAEPO presented the lowest peroxide value (3.18 mEq O_2/kg), followed by HEBO (3.51 mEq O_2/kg), FGBO (3.65 mEq O_2/kg), and FBBO (3.77 mEq O_2/kg). The highest PV was detected for GABO (3.88 mEq O_2/kg). The PV of all the samples was within the acceptable limit of below 10 mEq O_2/kg for vegetable oils [18]. In addition, the lowest PV of EAEPO is plausibly due

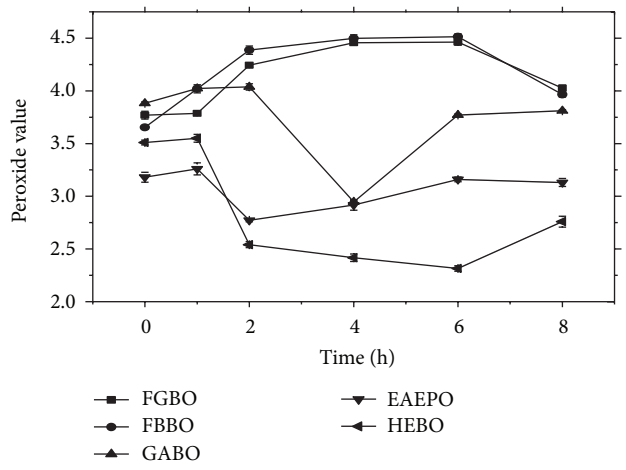

Figure 2: Changes in peroxide value.

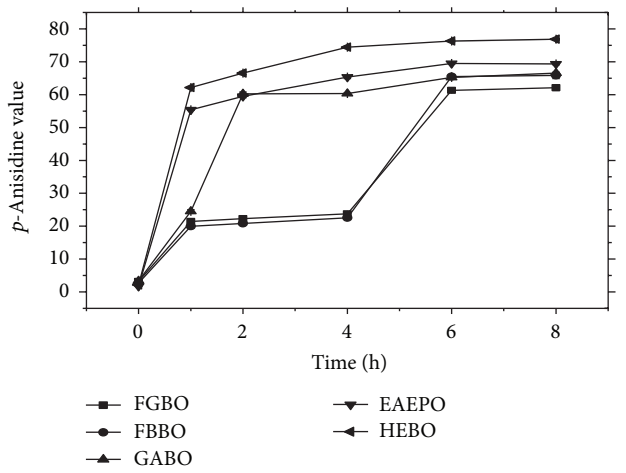

Figure 3: Changes in *p*-anisidine value.

to tocopherol release during the EAEP, which is related to hydrolysis by the enzymes [19].

The peroxide levels of all of the oil types remained similar to the original values during heating with slight variations from the initial amounts (2–4 mEq O_2/kg) due to the volatile nature of peroxides [17]. However, the concentration of peroxides in the various oil types differed significantly.

The PV of the EAEPO and HEBO samples increased during the first 1 h, followed by a decline to the initial levels (3.63 mEq O_2/kg and 2.76 mEq O_2/kg, resp.). In the case of FGBO and FBBO, the PV continued to increase up to 6 h with an eventual return to the initial levels of ca. 4.02 mEq O_2/kg and 3.96 mEq O_2/kg, respectively. The peroxide value of GABO increased to 3.81 mEq O_2/kg after 2 h. The rapid increase in the PV of the EAEPO and HEBO samples indicates that these oils were unstable to oxidative degradation. The EAEPO and HEBO samples had the highest percentage of polyunsaturated fatty acids and demonstrated less stability to oxidation, which is in agreement with the data previously reported for olive oil [17]. The PV decreased after reaching a maximum for all of the oil types, which is attributed to the fact that primary oxidation reactions cause an increase in the concentration of peroxides to a maximum value, beyond which the peroxide concentration decreases due to thermal decomposition thereof into secondary products [20].

3.3. Changes in p-Anisidine Value (p-AV). The *p*-AV is a measure of the content of aldehydes (principally 2-alkenals and 2, 4-alkadienals), which are relatively stable, and is thus a useful tool for monitoring the oxidative state of oil during heating. Therefore, for an accurate estimation of the oxidation status of the oils, both the PV and *p*-AV should be interpreted simultaneously.

Figure 3 shows the changes in the *p*-AV of the oils with heating from 0 h to 8 h. The *p*-AV of all oil types increased with time. Furthermore, the *p*-AV of all the oil types exhibited significant changes at the same heating time. This may be attributed to further decomposition of the less-stable primary oxidative products (hydroperoxides) to form aldehydic compounds, as previously reported [17, 21].

It was also noted that the trend in the variation of the *p*-AV with heating for FGBO was similar to that for FBBO with a maximum rate of change at 6 h of treatment compared to GABO for which the maximum rate of change was achieved at 2 h. However, in the case of EAEPO and HEBO, rapid hydroperoxide formation was detected after 1 h of heat treatment, probably due to the high amounts of PUFA in the two oils. The slightly higher *p*-AV of HEBO may be due to the ability of the solvent to extract other lipid associated substances such as sterols, fat soluble compounds, and hydrocarbons. This increased *p*-AV level was consistent with the PV data presented in Figure 2, which confirms that EAEPO and HEBO present minor resistance to oxidation under heating conditions relative to the other three blended oils.

3.4. Changes in Color. Color is an important food evaluation indicator for rapid monitoring of the quality of heated oil. For the unheated oils, HEBO had the highest R parameter of 34.97 compared to the other blended oils due to the extraction of pigments by the organic solvent. The yellow color of EAEPO was more prominent than that of the other blended oils (55.01), indicative of the presence of more yellow pigments, such as carotenoids, in the EAEPO sample (Tables 1 and 2). However, the lighter color of the other three oils is related to decolorization during oil refining.

Heat treatment induced a considerable increase in the R and Y parameters. In general, the reddish and yellowish coloration intensifies when the pigments developed during oxidation and thermal decomposition of fatty acids diffuse into the oil during heating. In addition, these changes may also be due to traces of carotenoids [22]. The difference in the R parameter of the nontreated and treated oils was slightly higher (34.97 versus 39.72) in the case of HEBO,

Table 1: Variation of yellow color of blended oils.

	Y					
	0 h	1 h	2 h	4 h	6 h	8 h
FGBO	15.08 ± 0.14aB	15.53 ± 0.09bB	15.94 ± 0.05cB	16.09 ± 0.04cB	16.37 ± 0.03215dB	16.65 ± 0.08eB
FBBO	19.70 ± 0.02aC	20.17 ± 0.03bC	20.25 ± 0.03bC	20.39 ± 0.08cC	20.60 ± 0.02082dC	20.87 ± 0.04eC
GABO	29.29 ± 0.05aD	29.49 ± 0.06bD	29.87 ± 0.08cD	30.09 ± 0.01dD	30.26 ± 0.06110dD	30.57 ± 0.09eD
EAEPO	55.01 ± 0.09aE	55.32 ± 0.03bE	55.37 ± 0.07bE	55.80 ± 0.09cE	55.85 ± 0.07000cdE	56.05 ± 0.07eE
HEBO	2.13 ± 0.09aA	2.74 ± 0.03bA	3.07 ± 0.03cA	3.07 ± 0.06dA	3.52 ± 0.02517dA	3.78 ± 0.02eA

Mean values within each row followed by different letters (a, b, c, etc.) are significantly ($P < 0.05$) different. Mean values within each column followed by different letters (A, B, C, etc.) are significantly ($P < 0.05$) different.

Table 2: Variation of red color of blended oils.

	R					
	0 h	1 h	2 h	4 h	6 h	8 h
FGBO	1.21 ± 0.02aA	1.57 ± 0.11bA	2.00 ± 0.06cA	2.56 ± 0.06dB	2.79 ± 0.04eC	3.50 ± 0.09fB
FBBO	1.49 ± 0.02aC	1.87 ± 0.04bC	2.00 ± 0.05bcA	2.07 ± 0.11cA	2.63 ± 0.07cB	2.76 ± 0.04dA
GABO	1.37 ± 0.04aB	1.77 ± 0.04bB	1.97 ± 0.04cA	2.04 ± 0.04cdA	2.15 ± 0.04dA	4.11 ± 0.109eC
EAEPO	2.57 ± 0.05aD	3.60 ± 0.03bD	3.64 ± 0.02bB	4.55 ± 0.05cC	4.67 ± 0.02cD	5.86 ± 0.04dD
HEBO	34.97 ± 0.03aE	38.03 ± 0.09bE	38.27 ± 0.07bC	38.90 ± 0.11cD	39.37 ± 0.05dE	39.72 ± 0.10eE

Mean values within each row followed by different letters (a, b, c, etc.) are significantly ($P < 0.05$) different. Mean values within each column followed by different letters (A, B, C, etc.) are significantly ($P < 0.05$) different.

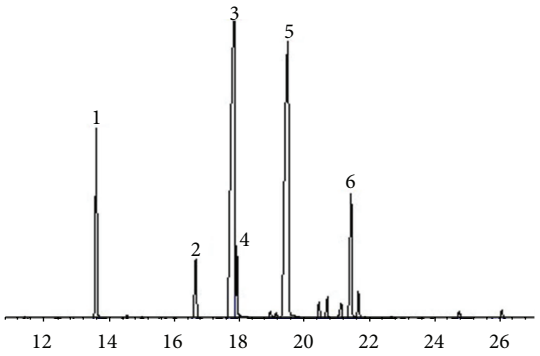

Figure 4: Typical chromatogram of fatty acid methyl ester prepared from EAEPO before heating. Peaks: 1: hexadecanoic acid; 2: octadecanoic acid; 3: 9-octadecenoic acid; 4: 9-octadecenoic acid; 5: 9,12-octadecadienoic acid; 6: 12,15-octadecadienoic acid.

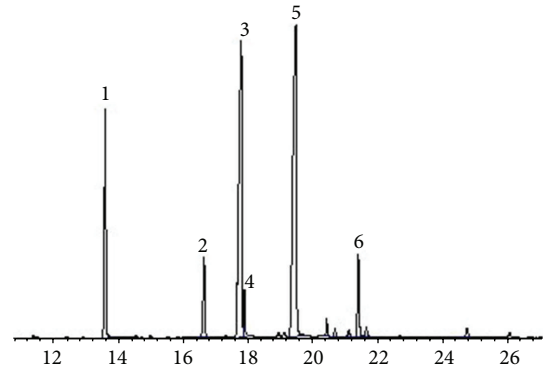

Figure 5: Typical chromatogram of fatty acid methyl ester prepared from EAEPO after heating. Peaks: 1: hexadecanoic acid; 2: octadecanoic acid; 3: 9-octadecenoic acid; 4: 9-octadecenoic acid; 5: 9,12-octadecadienoic acid; 6: 12,15-octadecadienoic acid.

which is more sensitive to oxidation due to its higher relative percentage of PUFA. This also holds for EAEPO (2.57 versus 5.86).

3.5. Changes in Fatty Acids Composition. The fatty acid composition is the most important oil constituent parameter, and the nature of these fatty acids, particularly the degree of unsaturation, determines the oxidative stability of the oils. The unheated oils comprised four major fatty acids: palmitic, stearic, oleic, and linoleic acids (Table 3, Figure 4). The oleic acid content (40.49–47.35%) was found to be the highest, followed by linoleic acid, (35.17–44.63%), palmitic acid (6.61–12.12%), and stearic acid (3.14–9.15%). EAEPO and HEBO, respectively, had the highest linoleic acid content (40.46%, 44.63%) and the lowest palmitic acid content (7.60%, 6.61%). After heating for 4 h, the palmitic acid and stearic acid contents of EAEPO increased significantly, whereas the content of oleic and linoleic acids decreased (Figure 5). Both linoleic acid and palmitic acid are considered as good indicators of the extent of fat deterioration given that linoleic acid is more susceptible to oxidation, whereas palmitic acid is more stable toward oxidation [23].

Table 4 shows the variation of the content of SFA, MUFA, and PUFA of the oils from the initial to the final heating time. Heat treatment induced a decrease in the relative percentages of the unsaturated fatty acids and an increase of the relative percentages of the saturated fatty acids, as has previously been demonstrated in various studies [17]. Progressive heating may

Table 3: Main fatty acids (%) profile.

		FAs					
		0 h	1 h	2 h	4 h	6 h	8 h
$C_{16:0}$	FGBO	9.87 ± 0.03aD	17.25 ± 0.03bB	17.70 ± 0.01bcB	17.48 ± 0.01cB	17.48 ± 0.35cB	18.05 ± 0.02dB
	FBBO	9.15 ± 0.02aC	12.41 ± 0.03bC	13.07 ± 0.02cE	13.14 ± 0.03dD	13.71 ± 0.03eD	16.42 ± 0.04fD
	GABO	12.12 ± 0.02aE	13.12 ± 0.02bD	17.11 ± 0.04cD	17.76 ± 0.04dE	17.76 ± 0.01dE	17.74 ± 0.02dC
	EAEPO	7.60 ± 0.02aB	15.50 ± 0.04bE	16.32 ± 0.01cC	16.71 ± 0.02dA	16.78 ± 0.02eA	16.89 ± 0.02fA
	HEBO	6.61 ± 0.04aA	15.92 ± 0.01bA	16.03 ± 0.02cA	17.22 ± 0.02dC	17.31 ± 0.02eC	18.15 ± 0.01fE
$C_{18:0}$	FGBO	4.10 ± 0.010aC	6.37 ± 0.02bD	7.14 ± 0.03cD	7.60 ± 0.02dD	7.53 ± 0.13dC	9.07 ± 0.02eC
	FBBO	9.15 ± 0.011aE	12.41 ± 0.03bE	13.05 ± 0.02cE	13.13 ± 0.03dE	13.75 ± 0.01eD	16.44 ± 0.00fD
	GABO	4.54 ± 0.047cD	4.15 ± 0.04bC	4.21 ± 0.00bC	4.05 ± 0.01aC	4.07 ± 0.02aB	4.56 ± 0.04cB
	EAEPO	3.60 ± 0.030cB	3.46 ± 0.03aB	3.12 ± 0.00dB	3.48 ± 0.01aB	3.54 ± 0.01bA	3.59 ± 0.00cA
	HEBO	3.14 ± 0.036aA	3.23 ± 0.020bA	3.12 ± 0.01aA	3.36 ± 0.01cA	3.44 ± 0.02dA	3.61 ± 0.02eA
$C_{18:1}$	FGBO	46.74 ± 0.036fD	40.90 ± 0.02dD	40.54 ± 0.04cD	40.09 ± 0.02bC	41.21 ± 0.03eD	40.02 ± 0.02aC
	FBBO	47.35 ± 0.025fE	46.53 ± 0.04eE	45.75 ± 0.01cE	46.13 ± 0.03dE	44.87 ± 0.02bE	42.62 ± 0.04aE
	GABO	45.81 ± 0.032eC	39.79 ± 0.02aC	39.99 ± 0.12bC	40.21 ± 0.03cD	40.55 ± 0.04dC	40.30 ± 0.03cD
	EAEPO	44.49 ± 0.036fB	37.91 ± 0.03eB	35.12 ± 0.02dA	34.67 ± 0.06cA	34.37 ± 0.04bA	34.28 ± 0.02aA
	HEBO	40.49 ± 0.025fA	35.79 ± 0.03eA	35.45 ± 0.01dB	35.23 ± 0.02cB	34.94 ± 0.02bB	34.84 ± 0.03aB
$C_{18:2}$	FGBO	36.34 ± 0.025fB	30.79 ± 0.03eA	30.51 ± 0.04dA	30.18 ± 0.03cA	29.97 ± 0.03bA	27.79 ± 0.03aA
	FBBO	35.17 ± 0.015dA	33.58 ± 0.03cB	32.40 ± 0.02aB	32.37 ± 0.08aB	33.24 ± 0.03bC	32.35 ± 0.02aA
	GABO	35.57 ± 0.05cA	36.30 ± 0.53dC	32.22 ± 0.037abB	32.60 ± 0.07bC	32.40 ± 0.02abB	32.05 ± 0.03aA
	EAEPO	40.46 ± 0.55bC	39.51 ± 0.03aD	39.63 ± 0.03aC	39.72 ± 0.06aE	39.74 ± 0.02aE	39.82 ± 0.04aB
	HEBO	44.63 ± 0.01eD	39.96 ± 0.01cE	40.20 ± 0.01dD	38.97 ± 0.02bD	38.94± 0.02bD	38.68 ± 0.04aB

Mean values within each row followed by different letters (a, b, c, etc.) are significantly ($P < 0.05$) different. Mean values within each column followed by different letters (A, B, C, etc.) are significantly ($P < 0.05$) different.

Table 4: Changes in saturated, monounsaturated, and polyunsaturated fatty acids.

		SFA, MUFA, and PUFA					
		0 h	1 h	2 h	4 h	6 h	8 h
SFA	FGBO	15.28 ± 0.04 aC	26.83 ± 0.04bE	27.75 ± 0.01cE	28.94 ± 0.03dD	27.87 ± 0.04eE	31.45 ± 0.01fE
	FBBO	15.51 ± 0.04aD	18.34 ± 0.02bA	19.92 ± 0.02cA	19.85 ± 0.04dA	20.22 ± 0.02eA	25.32 ± 0.01fC
	GABO	17.93 ± 0.06aE	19.78 ± 0.03bB	24.23 ± 0.01cD	24.74 ± 0.02dC	24.69 ± 0.07dD	25.35 ± 0.01eD
	EAEPO	11.93 ± 0.03aB	20.13 ± 0.02bD	21.11 ± 0.02cC	21.30 ± 0.02dB	21.44 ± 0.00eB	21.65 ± 0.01fA
	HEBO	10.57 ± 0.02aA	20.01 ± 0.02bC	20.03 ± 0.02bB	21.36 ± 0.02cB	21.57 ± 0.02dC	22.54 ± 0.01eB
MUFA	FGBO	47.12 ± 0.02fCD	41.64 ± 0.02dC	41.11 ± 0.01cC	40.70 ± 0.02cB	41.78 ± 0.04eB	40.54 ± 0.02aC
	FBBO	48.15 ± 0.04eD	47.43 ± 0.02dE	46.93 ± 0.05cE	47.65 ± 0.49cD	45.85 ± 0.02bC	41.65 ± 0.03aD
	GABO	45.97 ± 0.01eBC	45.13 ± 0.03dD	41.53 ± 0.01aD	41.55 ± 0.04cC	41.86 ± 0.08cB	41.77 ± 0.01bE
	EAEPO	44.48 ± 0.04cB	40.12 ± 0.02bB	39.28 ± 0.04abA	38.87 ± 0.03abA	38.53 ± 0.02abA	38.46 ± 0.02aA
	HEBO	42.22 ± 0.02dA	39.98 ± 0.02cA	40.18 ± 0.04bB	39.30 ± 0.58cA	38.53 ± 0.65aA	38.74 ± 0.02abB
PUFA	FGBO	37.11 ± 0.01fC	31.46 ± 0.02eA	31.16 ± 0.02cA	30.63 ± 0.02cA	30.31 ± 0.01bA	27.96 ± 0.02aA
	FBBO	36.29 ± 0.03eA	34.24 ± 0.02dB	33.12 ± 0.02bB	33.14 ± 0.03bB	33.91 ± 0.03cC	32.95 ± 0.04aB
	GABO	36.44 ± 0.02fB	35.65 ± 0.01eC	34.23 ± 0.03cC	33.73 ± 0.03cC	33.41 ± 0.02bB	32.95 ± 0.01aB
	EAEPO	42.13 ± 0.03dD	39.54 ± 0.02aD	39.58 ± 0.03aD	39.73 ± 0.05bE	39.74 ± 0.03bE	39.84 ± 0.03cD
	HEBO	45.64 ± 0.02fE	39.97 ± 0.01dE	40.18 ± 0.04dE	38.96 ± 0.02cD	38.89 ± 0.02bD	38.74 ± 0.02aC

Mean values within each row followed by different letters (a, b, c, etc.) are significantly ($P < 0.05$) different. Mean values within each column followed by different letters (A, B, C, etc.) are significantly ($P < 0.05$) different.

induce modification of fatty acids that contain two or three double bonds. Both oxidation and oxidative polymerization occur with progressive heating. Furthermore, the relative oxidation rate of linolenic acid is much higher than that of stearic acid [24].

The fatty acid ratio of EAEPO and HEBO (0.27 : 1.03 : 0.96 and 0.27 : 1.08 : 1.16) approached the ideal fatty acid ratio of 0.27 : 1 : 1 (SFA : MUFA : PUFA), as shown in Table 5; the other three kinds of blended oils also approached this ratio prior to heating (0.27 : 0.65 : 0.8, 0.27 : 0.6 : 0.84, and 0.27 : 0.61 : 0.79

Table 5: Variation of SFA : MUFA : PUFA ratio of blended oils.

	SFA, MUFA, and PUFA					
	0 h	1 h	2 h	4 h	6 h	8 h
FGBO	0.27 : 0.65 : 0.8	0.27 : 0.42 : 0.32	0.27 : 0.40 : 0.30	0.27 : 0.38 : 0.29	0.27 : 0.41 : 0.29	0.27 : 0.35 : 0.24
FBBO	0.27 : 0.6 : 0.84	0.27 : 0.70 : 0.50	0.27 : 0.64 : 0.45	0.27 : 0.64 : 0.45	0.27 : 0.61 : 0.45	0.27 : 0.44 : 0.35
GABO	0.27 : 0.61 : 0.79	0.27 : 0.62 : 0.49	0.27 : 0.46 : 0.38	0.27 : 0.45 : 0.37	0.27 : 0.45 : 0.35	0.27 : 0.45 : 0.35
EAEPO	0.27 : 1.03 : 0.96	0.27 : 0.54 : 0.53	0.27 : 0.49 : 0.50	0.27 : 0.48 : 0.50	0.27 : 0.48 : 0.50	0.27 : 0.48 : 0.49
HEBO	0.27 : 1.08 : 1.16	0.27 : 0.51 : 0.54	0.27 : 0.50 : 0.54	0.27 : 0.47 : 0.49	0.27 : 0.46 : 0.49	0.27 : 0.43 : 0.46

Table 6: Changes in trans-fatty acid profile (mg/mL).

	TFAs					
	0 h	1 h	2 h	4 h	6 h	8 h
FGBO	0.01 ± 0.01aA	4.94 ± 0.01bA	17.18 ± 0.02cA	59.68 ± 0.03dA	157.83 ± 0.08eA	280.16 ± 0.18fA
FBBO	0.01 ± 0.04aC	5.78 ± 0.03bC	21.32 ± 0.01cC	68.79 ± 0.04dC	188.48 ± 0.03eC	257.14 ± 0.08fC
GABO	0.01 ± 0.01aB	5.07 ± 0.06bB	18.54 ± 0.05cB	64.61 ± 0.08dB	162.39 ± 0.03eB	333.44 ± 0.09fB
EAEPO	0.02 ± 0.01aD	9.55 ± 0.03bD	29.88 ± 0.05cD	88.64 ± 0.02dD	232.52 ± 0.07eD	542.39 ± 0.08fD
HEBO	0.02 ± 0.01aC	12.43 ± 0.02bE	30.51 ± 0.03cE	93.09 ± 0.037dE	256.70 ± 0.06eE	558.60 ± 0.14fE

Mean values within each row followed by different letters (a, b, c, etc.) are significantly ($P < 0.05$) different. Mean values within each column followed by different letters (A, B, C, etc.) are significantly ($P < 0.05$) different.

for FGBO, FBBO, and GABO, resp.). At the end of the heating time, the ratio was 0.27 : 0.48 : 0.49 for EAEPO, which indicates that a relatively close to ideal fatty acid ratio for a healthy diet could be maintained for the EAEPO sample as heating progressed.

3.6. Changes in Trans-Fatty Acids. Epidemiological evidence has suggested that the level of trans-fatty acid (TFA) intake is connected to the risk of cardiovascular disease [25]; thus, there have been several studies on TFAs contained in food materials. The amount of total TFAs in the evaluated oil types was in the range of 0.01-0.02 mg/mL, which clearly approximates the requirement for a "zero trans" content. However, a substantial increase in the TFAs of the various oils was observed when the oils were heated for 6 h to 8 h (Table 6). At the final heating time, EAEPO and HEBO presented the highest total TFA content. Several factors, such as the FA composition of each oil and coexisting antioxidants, may contribute to the variation in the thermal TFA accumulation profiles [26].

4. Conclusions

The results of this study demonstrate that the quality of enzyme-assisted aqueous processing extracted blended oil (EAEPO) is superior to that of hexane-extracted blended oil (HEBO) from the initial to the final heating time, which is possibly due to the extraction method of the former that utilizes water as an extraction and separation medium thereby maintaining a higher antioxidant content. However, compared to the other three refined oil samples, the EAEPO and HEBO samples were less stable towards oxidative degradation. Nevertheless, EAEPO was found to have the lowest acid value and offers the additional advantage of containing an ideal fatty acid ratio after heat treatment of the oils (0.27 : 0.48 : 0.49), which has been linked to reduced risk of high cholesterol and heart disease. The advantageous characteristics may be attributed to the superior initial fatty acid composition of EAEPO.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Lianzhou Jiang and Yang Li contributed equally to this paper.

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