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Comparison of wheat germ and oil characteristics and stability by different stabilization techniques

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ABSTRACT

In this study, the effects of four stabilization techniques (vibrated fluidized bed, oven, microwave, and extrusion) on the characteristics and stability of wheat germ and its oil were evaluated. The results showed that all methods were highly effective in inactivating lipoxygenase activity, although significant differences were observed in their ability to inactivate lipase activity. Among the techniques, extrusion was found to be the most effective, reducing residual lipase and lipoxygenase activity to less than 5% of that of fresh wheat germ. Acid value remained stable in the stabilized samples after 6 weeks of storage at 37 °C. However, the extrusion, oven, and microwave-treated samples showed rapid oxidation and/or significant losses of tocopherols (84.9%, 99.6%, and 33.9%, respectively). In contrast, wheat germ treated with the vibrated fluidized bed technique exhibited the best lipid stability, attributed to the suitable a_w and few structural changes. Consequently, vibrated fluidized bed technique is recommended as the most suitable method for stabilizing wheat germ and producing high-quality wheat germ oil.

1. Introduction

Wheat germ (WG) is the byproduct of the wheat industry and constitutes 2–3% of the whole wheat grain (Yu et al., 2015). The world production is estimated to be 25 million tons annually (Song et al., 2019). It is rich in nutrients and bioactive compounds, including proteins, lipids, sugars, minerals, B vitamins, carotenoids, flavonoids, tocopherols, sterols, octacosanol, and other micronutrients (Dunford, 2005). However, most wheat germs are still used as low-value feed, and human consumption remains restricted. Hence, urgent attention should be paid to wheat germ processing in order to gain its nutritional and economic advantages.

Furthermore, WG is a potential source of high-value, healthy edible oil as it contains as high as 8–14% of oil (Brandolini & Hidalgo, 2012). Wheat germ oil (WGO) is rich in PUFAs and is additionally the most abundant source of tocopherols and sterols among vegetable oils (Ghafoor et al., 2017). Accordingly, WGO is well-known for its medicinal and nutritional value and has been widely used in cosmetics, pharmaceuticals, health foods, and dietary supplements, however not yet fully utilized in the food industry as a cooking oil.

After immediately separating from the kernel, fresh WG undergoes rapid rancidity caused by lipolytic enzymes, mainly lipase (LA) and lipoxygenase (LOX), resulting in accelerated lipid hydrolysis and oxidation, which restricts its wide utilization (Megahed, 2011). Firstly, unsaturated lipids in WG can be rapidly hydrolyzed to generate a large amount of free fatty acids by LA, and then these free fatty acids will undergo enzymatic oxidation in the presence of LOX. The hydroperoxides produced by oxidation are unstable and then degrade into a wide variety of volatile compounds as well as nonvolatile products, resulting in rancid flavors and reduced nutritional quality. The free fatty acid content of WGO can gradually increase and reach up to 25% without stabilizing, which would also increase the difficulty of oil refining and result in significant losses in oil (Wang & Johnson, 2001).

Consequently, wheat germ should be immediately stabilized after separation to improve its stability and extend shelf life through deactivating lipolytic enzymes. So far, many attempts have been made, including physical, chemical, and biological methods (Boukid, Folloni, Ranieri, & Vittadini, 2018). Physical stabilization is commonly considered for WG, including thermal treatments like steaming (Srivastava, Sudha, Baskaran, & Leelavathi, 2007), fluidization (Giner & Calvelo, 1987), and roasting (Zou, Gao, He, & Yang, 2018), radiation-mediated treatments like microwave (MW) (Xu et al., 2013), infrared radiation (Li et al., 2016), gamma radiation (Jha, Kudachikar, & Kumar, 2013), and hot-air-assisted radio frequency (Liao et al., 2020), as well as

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thermal/mechanical treatments like extrusion (Gu, Qian, & Zheng, 2002). Most of these methods have been verified to effectively deactivate lipase, thus inhibiting the rapid increase in FFA. However, these methods are likely to degrade the nutritional value of WG because of high temperature and prolonged processing (Arslan, Demir, Acar, & Arslan, 2020). In addition, these methods have their own advantages and disadvantages. For example, extrusion has been widely used in the industrial stabilization of rice bran, as the formed porous structure can significantly improve the efficiency of oil extraction (Kim, Byun, Cheigh, & Kwon, 1987). Steaming leads to high moisture content of wheat germ and requires an additional drying process (Srivastava et al., 2007). Fluidization provides intense heat and mass transfer between materials and the hot air, which implies a uniform treatment, but huge investments and energy consumption need to be considered (Chan & Kuo, 2018). Roasting is usually used for the preparation of roasted wheat germ for food use, with a particular flavor and golden color generated from the maillard reactions (Zou et al., 2018). Microwave technology has high energy efficiency and can reduce energy costs; however, few industrial applications on WG stabilization have been reported and the same as other radiation treatments (Boukid et al., 2018).

Yet, few studies have systematically compared these different methods, especially little attention was paid to compare stability parameters after stabilization. Consequently, the purpose of the present research was to compare the effects of four common stabilization treatments (fluidized bed drying, oven drying, microwave, and extrusion) on wheat germ and oil characteristics, and to evaluate the storage stability as well.

2. Materials and methods

2.1. Materials and chemicals

Fresh WG was obtained from Yihai Kerry (Kunshan) Foodstuffs Industries Co. Ltd. After the wheat milling process, the fresh WG was placed into polyethylene bags and stored at −20 °C for further stabilization. Refined soybean oil was purchased from a local supermarket. Standards such as Boron trifluoride-methanol solution (BF_3 in 14% methanol), a 37-component fatty acid methyl ester (FAME) mix ranging from C4 to C24, α-, β-, γ-, δ-tocopherol, sterols (campesterol, campestanol, stigmasterol, δ-7-campesterol, δ-5, 23-stigmastadienol, clerosterol, β-sitosterol, sitostanol, δ-5-avenasterol, δ-5, 24-stigmastadienol, δ-7-stigmastenol, and δ-7-avenasterol), and dehydrocholesterol were supplied by Sigma-Aldrich (St. Louis, MO, USA). Isopropyl alcohol and n-hexane of HPLC grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The purchase of other chemicals of analytical grade was completed with the help of Anpel laboratory technologies (Shanghai, China).

2.2. Stabilization methods

Four stabilization methods (fluidized bed drying, oven drying, microwave and extrusion) were optimized in advance with the goal of maximizing enzymes (mainly LA & LOX) inactivation in the WG as well as suppressing over-heating.

2.2.1. Fluidized bed drying

Fresh WG was dried continuously in a plant-scale vibrated fluidized bed (VFB, OTW-300, BUHLER, Germany). The air temperature in the VFB was set up to 140 °C, and the wheat germ was exposed to hot air for 10 min.

2.2.2. Oven drying

Approximately 180 g of fresh wheat germ was transferred to open shallow trays (33 cm length, 23 cm width) in uniform layers. These trays were then placed in a hot-air circulated oven (FD531, Binder, Germany) maintained at 160 °C and dried for 25 min.

2.2.3. Microwave

About 30 g of fresh wheat germ was put in a pyrex dish (20 cm in diameter) and then placed in the center of a microwave oven (EMA34GTQ-SS, Midea, China). The wheat germ was treated for 2.5 min at 900 Watts.

2.2.4. Extrusion

The extrusion of WG was performed using a co-rotating twin-screw extruder (SYSLG30-IN, Saibainuo Technology Development Co. Ltd., China). The length/diameter ratio of the extruder was 22, and the diameter of the circular die was 6 mm. Once the individual temperature of each barrel section reached a constant level (60 °C, 90 °C, 120 °C, 150 °C), the feeding rate of WG and the screw speed were set to 5.4 Hz and 10 Hz, respectively. The residence time of WG inside the barrel was approximately 2–3 min.

2.3. Oil extraction

Differently stabilized wheat germ was firstly ground into powder, then mixed and extracted with n-hexane in a ratio of 1:3 (m/V) at ambient temperature for 2 h. The supernatant was separated by vacuum filtration to remove the WG meal and the solvent was evaporated by a rotatory evaporator (R206, SENCO Technology Co. Ltd., China) to recover the oil. The obtained crude oil was stored at 4 °C for further investigation.

2.4. Accelerated stability test

Approximately 330 g of differently stabilized wheat germ was placed in a food-grade polyethylene terephthalate bag (24 cm length, 16 cm width) and heat-sealed. The samples were then stored at 37 °C in a hot-air circulated oven (FP720, Binder, Germany) for 6 weeks. Stability analysis was conducted at 2-week intervals, starting from the first day. Wheat germ oil was extracted, and the physicochemical properties and bioactive compounds (tocopherols and phytosterols) were measured periodically.

2.5. Analysis

2.5.1. Moisture content and water activity

The moisture content (MC) of WG was determined by drying it in a hot-air circulating oven (FD531, Binder, Germany) at 105 °C for 2h according to the Chinese National Standards GB 5009.3–2016. The water activity (aw) of WG was measured using a water activity meter (AQUALAB 4 TE, METER Group, Inc. USA) at 25 °C, in accordance with the Chinese National Standards GB 5009.238–2016.

2.5.2. Lipase activity

The lipase (LA) activity of WG was determined in accordance with the Chinese National Standard GB/T 5523-2008, with slight modifications. Initially, 2 g of wheat germ with an accuracy of 0.01 g were weighed. The wheat germ was then ground for 3 min with one spoonful of quartz sand, 1 mL of refined soybean oil (AV < 0.2 mg KOH/g), and 5 mL of pH 7.4 phosphate buffer in a mortar. The mixture was gradually added with 5 mL of distilled water in three separate additions and transferred into a sealable tube. After incubation at 30 °C for approximately 24 h, the mixture was dispersed in 35 mL of 95% ethanol and vortexed for 1 min. The suspension was then centrifuged at 4000 r/min for 8 min, and 10 mL of the supernatant was collected for titration. The lipase activity was expressed as milligrams of potassium hydroxide per gram of wheat germ.

2.5.3. Lipoxygenase activity

The lipoxygenase (LOX) activity was carried out according to the reported method (Cato, Halmos, & Small, 2006) with some modifications. The results were determined by measuring the increase in

absorbance at 234 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). In brief, 0.5 g (with an accuracy of 0.01 g) of ground (sifted by a 20 mesh sieve) WG was transferred into 2.5 mL of phosphate buffer (0.1 mol/L, pH 7.5) and stirred for 30 min at 4 °C. The slurry was then centrifuged at 8000 r/min for 10 min at 4 °C, and the supernatant was the raw lipoxygenase enzyme solution. The substrate solution consisted of tween-20, linoleic acid, and boric buffer mixture. The pH of the substrate solution was adjusted to 7.0 using HCl. The entire reaction system was composed of 2.375 mL of sodium acetate buffer solution (0.05 mol/L, pH 5.6), 75 μL of the substrate solution, and 50 μL of the raw enzyme solution. The reaction mixture was stirred for 10 s before measurement. The blank consisted of a mixture of substrate solution and sodium acetate buffer (2.5 mL). Finally, the absorbance was recorded at 30 s intervals until 210 s. One unit of lipoxygenase activity was defined as an increase in the absorbance of 0.01 at 234 nm per minute per milligram of wheat germ.

2.5.4. Physicochemical properties

The acid value (AV) was measured by using the potassium hydroxide (KOH) standard solution to neutralize the free fatty acids in accordance with the AOCS Official Methods Cd 3d-63. The peroxide value (PV) was determined by adding an acetic acid/chloroform (3:2, v/v) solvent to dissolve the oil and measuring the iodine produced from potassium iodide and peroxides present, according to the AOCS Official Methods Cd 8–53.

2.5.5. Fatty acid composition

The fatty acid composition (FAC) was determined in accordance with the Chinese National Standard GB 5009.168–2016 with minor modifications. Approximately 60 mg of oil were weighed in a sealable tube, and 8 mL of sodium hydroxide methanol solution (2 g/100 g) was added. The mixture was heated to 80 °C in a water bath for 5–7 min. Then, 7 mL of BF3 in methanol was transferred into the tube for a 2 min reaction at 80 °C. The cooled mixture was vortexed for 2 min after adding 10 mL of heptane and 2 mL of saturated sodium chloride solution, and left to clarify. 5 mL of the supernatant was taken, and approximately 3 g of anhydrous sodium sulfate were added and vortexed for 1 min. After the salt precipitated out, the upper FAME solution was transferred to a sample bottle for gas chromatography (GC) analysis. The FAMEs were identified by retention time comparison with the corresponding standards.

Chromatographic peak area normalization method was used for calculating their relative content of compositions.

2.5.6. Tocopherol content

Tocopherol analysis was determined in accordance with the AOCS Official Method Ce 8–89 with minor modifications. Approximately two grams of oil were weighed in a 25 mL volumetric flask and dissolved with n-hexane. The mixture was then filtered through a 0.45 μm PTFE membrane filter for HPLC analysis (Agilent 1200 system, Palo Alto, CA, USA). The injection volume was 20 μL for each sample, and the column equipped was Agilent ZORBAX RX-SIL (250 mm, 4.6 mm i.d., 5 μm). The mobile phase composed of n-hexane/isopropyl alcohol (99.5/0.5, v/v) was used under isocratic elution mode, and UV absorption at 292 nm was monitored. The flow rate was set at 1.0 mL/min, and the column oven temperature was set at 30 °C. Standard solutions of α, β, γ, and δ-tocopherols, formulated using 0–500 mg/L standard concentration, were used for quantification purposes.

2.5.7. Sterol content

Sterol analysis was determined according to the Chinese National Standard GB/T 25223-2010, with minor modifications. One hundred milligrams of the oil sample were carefully weighed in a 50 mL sealable tube. Saponification was carried out using 5 mL of a potassium hydroxide ethanol solution (2 mol/L) at 60 °C for 1 h with 4 mg of dehydrocholesterol used as an internal standard. Once the tube was cooled to room temperature, 4 mL of distilled water and 10 mL of n-hexane were added for extraction. The hexane phase was then dried over anhydrous sodium sulfate, followed by drying under nitrogen. The sample was subsequently silylated using 200 μL of BSTFA-TMCS (99:1) at 105 °C for 20 min. The individual sterol fractions were analyzed by gas chromatography (GC), identified using standards, and quantified using dehydrocholesterol.

2.5.8. Scanning electron microscopy

The stabilized WG flakes were placed on the sample holder using double-sided scotch tape and then coated with gold in a sputter coater (1.5 min, 2 mbar). The morphology of samples was observed using a scanning electron microscopy (SEM, S–3400N, Hitachi Ltd, Tokyo, Japan) at an accelerating voltage of 15 kV.

2.5.9. Differential scanning calorimetry

The gelatinization degree of differently stabilized wheat germ was analyzed using a Q2000 differential scanning calorimeter (DSC, TA Instruments, USA). Approximately 5 mg of ground wheat germ was weighed into an aluminum pan with double distilled water in a 1:2 ratio, and an empty pan was used as a reference. After sealing and being equilibrated at 25 °C for 24 h, the temperature was held at 15 °C for 10 min, and then increased from 15 °C to 140 °C at a rate of 5 °C/min. Gelatinization characteristics, i.e. transition enthalpy (△H), the onset (To), peak (Tp) and completion (Tc) temperatures were obtained from the DSC thermogram.

2.6. Statistical analysis

All analyses obtained from the above experiments were performed in triplicate, and the results were expressed as mean and standard deviation (SD) values. Statistical analysis was carried out using SPSS program version 19.0. Significant differences among the data were analyzed by One-way Variance (ANOVA) combined with Tukey's adjustment test ($P \leq 0.05$). All the line charts and histograms were generated using Origin 8.5 software.

3. Results and discussion

3.1. Effect of stabilization methods on enzyme activities

Enzyme activities are typically used as one of the most important indexes to describe the stabilization effect of different methods. LA and LOX are the most important enzymes in this regard. Therefore, we applied various methods to stabilize WG and then measured the LA and LOX activities of both fresh and differently stabilized WG. As shown in Table 1, after using different stabilization methods (VFB, oven drying, microwave or extrusion), both enzyme activities of WG significantly decreased ($p < 0.05$) compared to fresh, unstabilized WG. The LOX activity after stabilization using VFB, oven drying, microwave or extrusion were reduced to 17.50%, 5.86%, 10.27% and 2.40% of fresh WG (464.90 U), respectively. However, significant differences appeared in the inactivation of LA. Relative to fresh WG (34.54 ± 0.25 mg KOH/g), the residual LA activity post-extrusion was merely 3.10%, while other methods led to much higher residual LA activity to varying degrees (56.80–86.31%). Enzyme inactivation is primarily attributed to thermal treatment, and past research has demonstrated that LOX is more heat-sensitive than LA in WG (Xu et al., 2016). In our experiments, extrusion proved to be the most effective way to inactivate both LA and LOX, and it was shown before that this is mainly due to the high temperature and high humidity which induced protein denaturation (Singh, Gamlath, & Wakeling, 2007). The enzyme deactivation ability of extrusion was also verified previously, for example, the inactivation of fathydrolyzing enzymes in rice bran (Sharma, Chauhan, & Agrawal, 2004). In our experiments, VFB was the least effective method to lower both LA and LOX enzyme activities. The microwave and oven methods were also

Table 1
Enzyme activity, MC & a_w of WG treated by different stabilization and tocopherol & phytosterol contents of WGO obtained from related WG.

	Fresh	VFB	Oven	MW	Extrusion
Lipase activity (mg KOH/g)	34.54 ± 0.25[a]	29.81 ± 3.10[b]	19.62 ± 0.68[c]	21.15 ± 1.90[c]	1.07 ± 1.02[d]
Lipoxygenase activity (U)	464.90 ± 24.40[a]	81.35 ± 1.91[b]	27.25 ± 2.33[cd]	47.75 ± 1.20[c]	11.15 ± 5.55[d]
MC (%)	12.31 ± 0.01[a]	1.63 ± 0.01[d]	0.10 ± 0.06[e]	2.85 ± 0.19[c]	7.14 ± 0.04[b]
a_w	0.67 ± 0.00[a]	0.25 ± 0.08[cd]	0.13 ± 0.05[d]	0.27 ± 0.08[c]	0.53 ± 0.01[b]
α-tocopherol (mg/kg)	2222±5[a]	2105 ± 44[bc]	1964 ± 65[d]	2138 ± 19[b]	2032 ± 43[cd]
β-tocopherol (mg/kg)	684±0[b]	721±6[a]	650 ± 19[c]	706 ±3[ab]	726 ± 23[a]
γ-tocopherol (mg/kg)	36±2[c]	54±1[a]	39±4[c]	36±1[c]	48±0[b]
Total tocopherols (mg/kg)	2939±7[a]	2880 ± 51[ab]	2653 ± 89[c]	2880 ± 23[ab]	2806 ± 67[b]
Campesterol (mg/kg)	9798 ± 245[b]	10032 ± 46[a]	9107 ± 10[d]	9578 ± 28[c]	8785 ± 57[e]
Campestanol (mg/kg)	426±3[d]	648±1[a]	531±4[b]	492±4[c]	540±7[b]
Stigmasterol (mg/kg)	239±9[a]	229±6[a]	208±7[b]	230±2[a]	203±0[b]
δ-7-Campesterol (mg/kg)	405±8[b]	451±0[a]	391±1[c]	413±1[b]	386±0[c]
δ-5,23-Stigmastadienol (mg/kg)	462 ± 13[c]	540±1[a]	503±6[b]	496±0[b]	495±4[b]
Clerosterol (mg/kg)	222±6[a]	225±5[a]	204±3[c]	214±1[b]	200±3[c]
β-Sitosterol (mg/kg)	25327 ± 759[a]	25386 ± 353[a]	23330 ± 253[c]	24423 ±6[b]	22466 ± 187[d]
Sitostanol (mg/kg)	479±2[d]	770 ± 22[a]	626±2[b]	555±1[c]	642±2[b]
δ-5-Avenasterol (mg/kg)	1999 ± 72[b]	2249 ± 119[a]	2044 ± 17[b]	2059 ± 14[b]	1980 ± 28[b]
δ-5,24-Stigmastadienol (mg/kg)	508 ± 20[b]	559 ± 37[a]	519±1[b]	510±2[b]	513 ± 12[b]
δ-7-Stigmastenol (mg/kg)	1011 ± 34[b]	1101 ±8[a]	1040 ±5[b]	1042 ±1[b]	1039 ± 22[b]
δ-7-Avenasterol (mg/kg)	1302 ± 38[c]	1471 ±0[a]	1350 ± 14[b]	1358 ±1[b]	1346 ± 17[b]
Total phytosterols (mg/kg)	42179 ± 1205[b]	43687 ± 356[a]	39876 ± 268[c]	41371 ± 54[b]	38594 ± 324[d]

Values are expressed as means ± SD (n = 3). Values within each row with different superscripts (a, b, c and d) are significantly ($P < 0.05$) different.

not as effective as the extrusion method. The explanation may be that these three methods primarily lowered the a_w of wheat germ to varying levels, resulting in the suppression of only lipase activity rather than more efficient protein denaturation observed after extrusion (Xu et al., 2013).

3.2. Effect of stabilization methods on MC and a_w

A_w is a key factor affecting WG quality and stability because the catalytic activity of LA and the resulting lipid hydrolysis rate of wheat germ is positively correlated with aw (Rose & Pike, 2006). As listed in Table 1, the original MC and aw of WG were 12.31% and 0.67. After stabilization, MC and aw both significantly decreased due to water evaporation during these treatments ($p < 0.05$), which destroyed the beneficial environment for enzyme activities and microbial growth (Labuza & Dugan, 1971). Oven-dried WG had the lowest MC and aw (0.10% and 0.13), which may make it very susceptible to lipid auto-oxidation. The MC and aw of VFB and MW stabilized WG were 1.63% and 2.85% and 0.25 and 0.27, respectively, which may be safer for the inhibition of both oxidation and enzyme activities. However, extrusion was not very effective in drying WG and the MC and aw were therefore as high as 7.14% and 0.53, which were much higher than the safe MC (≤4%) for storage of WG mentioned in the Chinese industrial standard LS/T 3210-1993. Lipid fluidity and hydrolysis may both be enhanced with increased moisture because it is a good carrier and reactant (AI-Muhtaseb, McMinn, & Magee, 2002).

3.3. Effect of stabilization methods on lipid stability

3.3.1. Physicochemical properties

The parameters AV and PV indicate lipid degradation caused by hydrolysis and oxidation, respectively, leading to oil rancidity. As shown in Fig. 1, AV (0 weeks) all significantly decreased, while no significant changes were found in PV (0 weeks) after different treatments. The AV decrease was in accordance with the studies on rice bran stabilized by extrusion cooking (Randall, et al., 1985) and wheat germ stabilized by roasting (Zou et al., 2018). During the accelerated storage, AV of fresh WG increased rapidly to 29.7 mg KOH/g after 2 weeks and reached as high as 47.2 mg KOH/g after 6 weeks. However, AV only increased slightly after stabilization, no matter which method was used, and was therefore significantly lower than that of fresh WG throughout the storage experiment. It was concluded that all these stabilization methods were effective to maintain acceptable oil quality (AV<10 mg KOH/g) after 6 weeks of storage, which is probably due to the limitation of the reaction rates causing rancidity due to a low aw value or the effective inactivation of LA activity. Interestingly, significant differences in PV changes during the storage experiment were observed among these stabilization methods. PV of oven roasting and extrusion increased sharply at 4 weeks and 2 weeks of storage, respectively, and reached 198 and 103 mmol/kg, respectively, at the end of storage, while PV of fresh, VFB, and MW only increased slightly and were still lower than 5 mmol/kg after 6 weeks of storage. As previously mentioned, the LOX activity had been effectively inactivated to lower than 20%; however, oven roasting and extrusion still showed a high oxidation rate. Accelerated lipid oxidation could be found both at relatively low or high aw, as appropriate water in foods could protect unsaturated fat against the

Table 2
Fatty acid composition (% of total fatty acids) of oil obtained from different stabilized WG before and after 6 weeks of storage at 37 °C.

	Fresh		VFB		Oven		MW		Extrusion	
	0 w	6 w	0 w	6 w	0 w	6 w	0 w	6 w	0 w	6 w
C16:0	17.13 ± 0.23[a]	17.35 ± 0.03[a]	17.07 ± 0.01[a]	17.37 ± 0.01[b]	16.78 ± 0.03[a]	18.62 ± 0.06[b]	16.80 ± 0.02[a]	17.68 ± 0.12[b]	16.72 ± 0.01[a]	18.45 ± 0.08[b]
C18:0	0.64 ± 0.03[a]	0.60 ± 0.01[a]	0.64 ± 0.03[a]	0.59 ± 0.00[b]	0.62 ± 0.01[a]	0.64 ± 0.00[b]	0.61 ± 0.00[a]	0.65 ± 0.03[b]	0.61 ± 0.00[a]	0.64 ± 0.00[b]
C18:1	15.88 ± 0.08[a]	16.22 ± 0.06[b]	14.74 ± 0.07[a]	15.09 ± 0.13[b]	14.92 ± 0.20[a]	17.36 ± 0.17[b]	15.29 ± 0.01[a]	16.40 ± 0.10[b]	14.82 ± 0.00[a]	16.05 ± 0.12[b]
C18:2	56.75 ± 0.40[a]	56.99 ± 0.19[a]	58.36 ± 0.12[a]	58.34 ± 0.07[a]	58.56 ± 0.10[a]	54.70 ± 0.25[b]	57.94 ± 0.00[a]	56.41 ± 0.32[b]	58.78 ± 0.03[a]	56.98 ± 0.14[b]
C18:3n3	7.07 ± 0.05[a]	7.22 ± 0.08[a]	6.49 ± 0.01[a]	6.97 ± 0.04[b]	6.55 ± 0.07[a]	6.58 ± 0.10[a]	6.95 ± 0.01[a]	6.83 ± 0.21[a]	6.56 ± 0.02[a]	6.04 ± 0.04[b]
C20:1	1.51 ± 0.02[a]	1.02 ± 0.06[b]	1.37 ± 0.01[a]	0.93 ± 0.07[b]	1.37 ± 0.02[a]	1.18 ± 0.03[b]	1.44 ± 0.00[a]	1.09 ± 0.10[b]	1.38 ± 0.01[a]	1.10 ± 0.01[b]
Others	1.03 ± 0.13[a]	0.59 ± 0.11[b]	1.33 ± 0.00[a]	0.72 ± 0.03[b]	1.20 ± 0.00[a]	0.93 ± 0.11[b]	0.97 ± 0.00[a]	0.93 ± 0.03[b]	1.13 ± 0.00[a]	0.75 ± 0.03[b]

Values are expressed as means ± SD (n = 3). Values within each row with different superscripts (a & b) are significantly ($P < 0.05$) different.

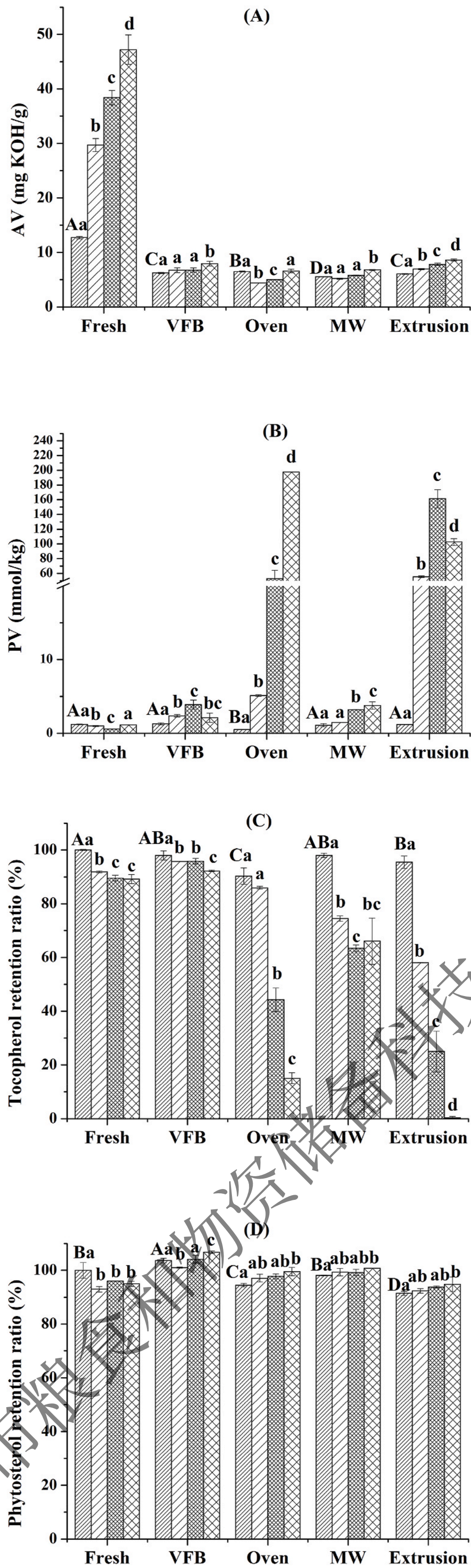

Fig. 1. AV (A), PV (B), total tocopherol (C) and phytosterols (D) retention ratio of fresh and stabilized WG during 6 weeks of storage at 37 °C (Columns are storage weeks: 0, 2, 4, 6; error bars represent SD of each column mean values (n = 3); different capital letters indicate significant differences at $p < 0.05$ among fresh and stabilized WG, while different lowercase letters indicate significant differences at $p < 0.05$ among storage weeks.).

direct contact of oxygen molecules, and the minimum oxidation rate usually happened at or near the aw of the monolayer (Kim, Kim, & Lee, 2014). The monolayer may impede the access of the oxygen molecules to unsaturated fat and/or change the mobility of transition metals in lipids, thus retarding the rate of lipid oxidation. If aw was below the monolayer level, the oxidative rate was rapidly increased and this may be highly attributed to the direct contact of oxygen molecules and lipids (Bell, 2020). As listed in Table 1, aw of oven-roasted WG was only 0.13 and the low aw may accelerate the autoxidation of lipids during storage. On the other hand, aw of 0.25 and 0.28 obtained by VFB and MW, respectively, were shown to be safe for inhibiting high autoxidation of lipids. These aw numbers are near the aw of a monolayer where the optimal oxidative stability is acquired (Labuza, Heidelbaugh, Silver, & Karel, 1971). However, the high aw obtained by extrusion is probably not the main factor for the high oxidation rate seen in this method. It was likely that lipids were released from cells due to the physical breaking of cytoderm, and meanwhile, the large surface area of porous structure was generated by the air cells during extrusion, thus favoring lipid oxidation (Singh et al., 2007).

3.3.2. *Nutritional quality*

The main fatty acid composition of fresh and differently treated WG before and after storage is listed in Table 2. Wheat germ oils are mainly composed of unsaturated fatty acids, accounting for over 80% of the total fatty acids. The content of linoleic acid (C18:2) is the highest (approximately 58%), followed by oleic acid (C18:1, around 15%) and α-linolenic acid (C18:3n3, around 7%). Palmitic acid (C16:0) is the main saturated fatty acid with a content of approximately 17% in wheat germ oils. Other fatty acids such as stearic acid (C18:0, around 0.6%) and eicosenoic acid (C20:1, around 1.4%) are comparatively low in content. The fatty acid composition of oils from fresh and treated WG was similar, indicating no significant difference in the fatty acid composition of WG oil. The results are in agreement with previous research (Li et al., 2016; Ling, Lyng, & Wang, 2018a; Zou et al., 2018; Liao et al., 2020). However, at the end of storage, significant decreases in C18:2 were observed to varying extents in oven roasting, extrusion, and MW-treated samples, while there was no change in C18:2 of VFB-treated samples. In addition, a significant decrease in C18:3n3 was observed in extrusion-treated WG. It is known that polyunsaturated fatty acids (PUFA) are firstly oxidized during storage, leading to a decrease in PUFA. Wheat germ oil with higher C18:2 and reasonable C18:3n3 is prone to higher primary oxidation (hydroperoxide formation) during storage, thus PV increases significantly and reaches higher values at the end of the storage period (Megahed, 2011).

The tocopherol and phytosterol contents of WGO obtained from different stabilization methods are summarized in Table 1. In WGO, α-tocopherol is the primary tocopherol accounting for more than 70% of total tocopherols, followed by approximately 25% of β-tocopherol and 1–2% of γ-tocopherol. The total tocopherol content of oil from fresh WG was 2939 mg/kg and only decreased slightly after VFB and MW treatments, while extrusion and oven roasting led to a loss of 4.5% and 9.7%, respectively. The loss of tocopherol may be because of its sensitivity and low stability at high temperatures (Kose, 2022). Similar results were found for wheat germ roasted at 180 °C for 20 min, with an 8.8% decrease compared to fresh WGO (Zou et al., 2018). Furthermore, it was reported that extrusion had a significant reduction effect on vitamin E, leading to a 30% decrease in rice bran (Rafe & Sadeghian, 2017). Thus, it can be seen that mild treatment has little effect on tocopherol, while severe conditions may lead to a large loss of tocopherol.

It has been confirmed that intake of phytosterol could lower cardiovascular disease risk and low-density lipoprotein cholesterol levels in the blood (Woyengo, Ramprasath, & Jones, 2009). Phytosterols are abundant in wheat germ, and the total phytosterol content of oil from fresh WG was 42179 ± 1205 mg/kg. Twelve sterols were qualified and quantified in wheat germ oil, among which β-sitosterol is dominant (about 60%), followed by campesterol (23%), δ-5-avenasterol (4.7%),

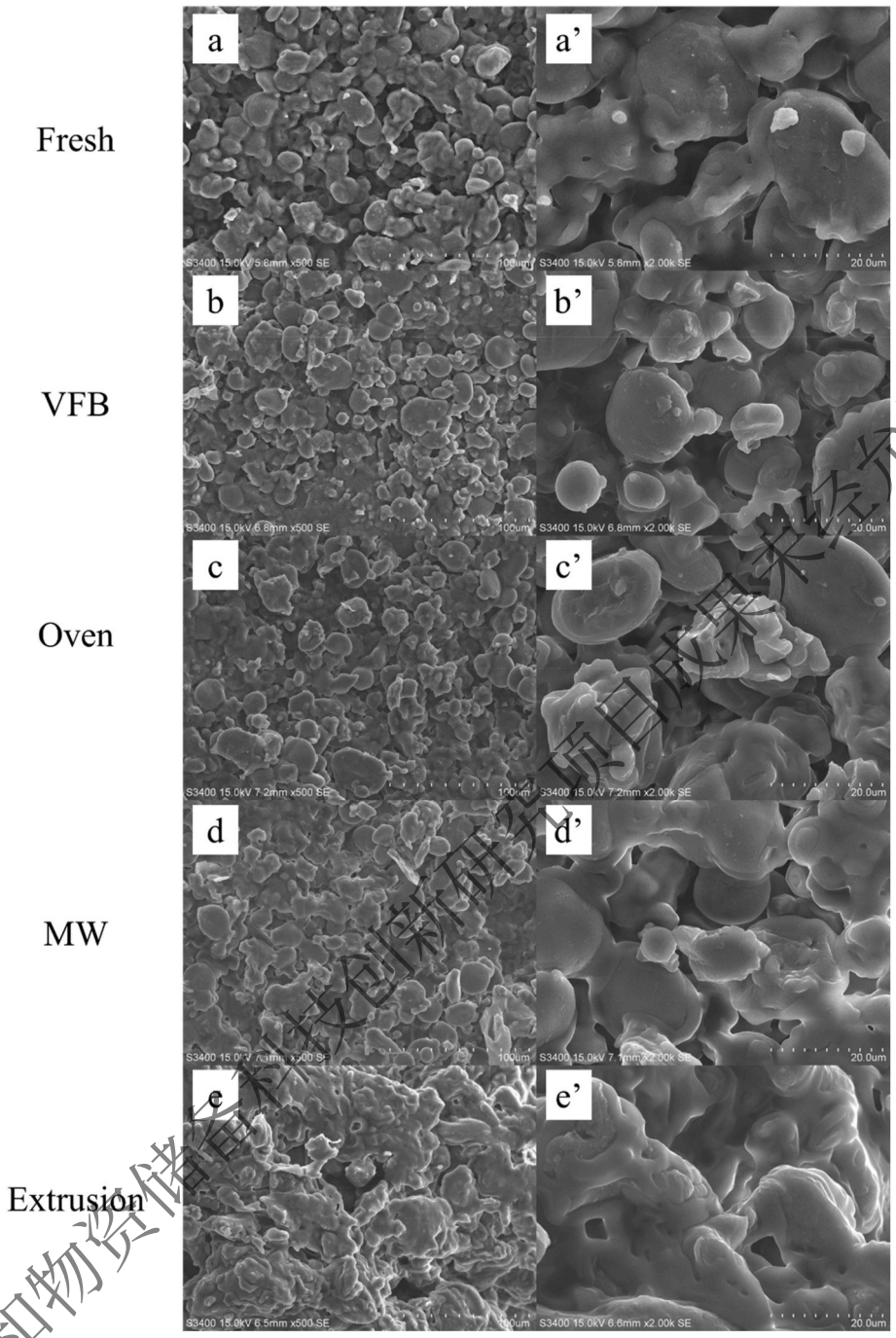

Fig. 2. SEM images of fresh (a, a'), VFB (b, b'), oven (c, c'), MW (d, d') and extrusion (e, e') treated wheat germ (a–e: magnification = × 500, a'-e': magnification = × 2000).

δ-7-avenasterol (3.1%), δ-7-stigmastenol (2.4%), δ-5,24-stigmastadienol (1.2%), sitostanol (1.1%), δ-5,23-stigmastadienol (1.1%), campestanol (1.0%), δ-7-campesterol (1.0%), stigmasterol (0.6%), and clerosterol (0.5%). A similar loss trend seen in total tocopherol was found in phytosterols. Phytosterols were stable in VFB and MW-treated samples, while extrusion and oven roasting resulted in a small but significant loss of 8.5% and 5.5% of total phytosterols, respectively.

The contents of total tocopherol and phytosterols during the 6 weeks of storage at 37 °C were also compared in Fig. 1. Sharp decreases in tocopherol content were observed in oven-roasted and extrusion treated samples, with nearly 85% and 100% of tocopherols being lost after 6 weeks of storage. This may be due to the oxidation and the protective effect of tocopherols against the high lipid oxidation of these two samples. It has been speculated that a low aw obtained by oven roasting led to the loss of protection of the monolayer of water and thus accelerated the oxidation of tocopherol located on the cell membrane. Similar phenomena were observed in extruded rice bran at different temperatures ranging from 110 °C to 140 °C, with a significant loss (46%–60%) of tocopherols during storage after 105 days (Shin, Godber, Martin, & Wells, 1997). Possible factors affecting tocopherol content in extrusion might be trace minerals originating from the extruder and the physical force of the extruder damaging the cell membrane where the tocopherol was present. Fresh and VFB treated samples remained mainly stable and had only a 10% loss of tocopherols during the entire storage. However, a significant decrease was observed in MW treated samples, where the content decreased from 2880 mg/kg to 2191 mg/kg after 2 weeks and

continued to decrease to 1943 mg/kg after 6 weeks. It was speculated that MW extensively destroys the oil body membrane and distorts the structure of cells, causing oil to coalesce and pool. This increased contact with oxygen, resulting in more oxidation (Dickey et al., 2007).

In contrast, the phytosterols in all samples showed high stability during the entire storage period. The loss of phytosterols in all samples was less than 5% after 6 weeks of storage. Other researchers have also found that phytosterols were stable during storage. Oxidation products of β-sitosterol in potato chips did not appear until 95 days of storage at 40 °C, while higher peroxide values were seen after 49 days and a rancid taste and musty odor began after 24 days (Lee et al., 1985). In addition, no significant differences in sterol oxidation products were observed in instant baby foods stored at room temperature for 9 months (García-Llatas et al., 2008). The antioxidants such as tocopherols existing in foods and oils were thought to be protective against the oxidation of sterols. Additionally, unsaturated fatty acids were more easily oxidized and consumed more oxygen, which may decrease the oxidation of sterols during thermal treatments (Xu, Guan, Sun, & Chen, 2009).

3.4. *Effect of stabilization methods on microstructure*

3.4.1. *Morphological characteristics*

The SEM images of WG samples were observed at two magnifications (× 500 and × 2000), which were presented in Fig. 2. The surface of WG was mainly covered with starch granules. Microstructures of fresh WG showed intact starch granules, which had distinct boundaries and uniform distribution. Micrographs of VFB-dried WG were similar to fresh WG with little gelatinization. Oven and MW roasted wheat germs presented a large number of elongated and nonspherical granules as a result of partial gelatinization. Micrographs of extrusion-treated WG clearly showed there were few intact starch granules, which indicated that they had been largely gelatinized, thus the granule boundaries disappeared and turned into continuous sheeting. From the microstructure, it can be seen that the degree of gelatinization varies among different stabilized WG samples. Among these stabilization methods, VFB exhibited the lowest degree of gelatinization, while extrusion exhibited the highest extent of gelatinization and porosity. The results are in accordance with other studies (Sudha, Srivastava & Leelavathi, 2007; Liu et al., 2020).

3.4.2. *Pasting characteristics*

The pasting properties of differently treated WG samples were then analyzed and are shown in Fig. 3 and Table 3. The gelatinization temperature of starch generally started at about 60 °C. The initiation temperature of gelatinization increased with varying degrees depending on the different stabilization treatments, of which extrusion was the most obvious. This indicated that the starch had already gelatinized to different degrees, especially the extruded starch, which was almost completely gelatinized. A very small amount of ungelatinized starch is more difficult to gelatinize, so the temperature required for gelatinization was much higher. Meanwhile, there were two endothermic peaks in the fresh and differently treated WG samples, except for extrusion. The first endothermic peak was at around 68 °C, which is the characteristic peak of starch gelatinization. Another peak appeared near 86 °C, which is presumed to be the starch-lipid complex peak (Singh, Singh, Isono, & Noda, 2010). The transition enthalpy (△H) was used to express the energy required to break the intermolecular hydrogen bonds of the starch particles, reflecting the energy required for starch phase change (Ratnayake & Jackson, 2008). △H significantly decreased to varying degrees according to different stabilization treatments, with extrusion showing the lowest decrease. All of these results were consistent with the results observed by SEM, indicating that the starches had already undergone different degrees of gelatinization. Extrusion showed the highest degree, followed by MW and oven roasting, and VFB drying resulted in the least gelatinization.

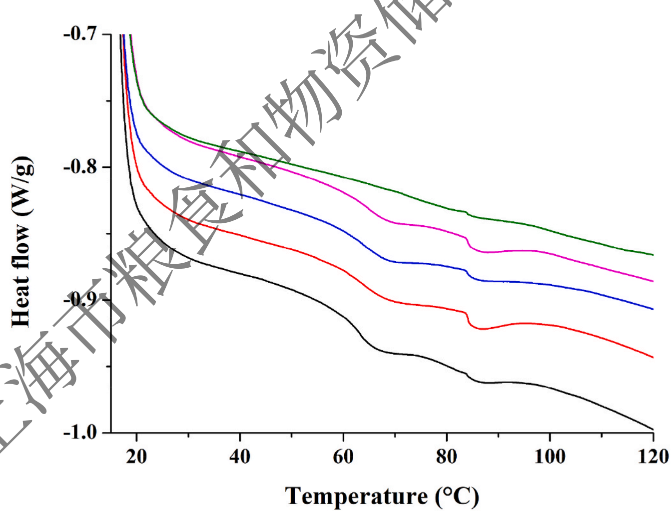

Fig. 3. Pasting properties of fresh and stabilized WG (Lines with different colors means: —fresh, —VFB, — oven, —MW, — extrusion). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Conclusion

Wheat germ has high nutritional value; however, its application is greatly limited by the presence of lipolytic enzymes. In the present study, four commonly used stabilization methods in the food industry (fluidized bed drying, oven roasting, microwave, and extrusion) were adopted to stabilize wheat germ. The effects of these methods on the structural and biochemical indexes and lipid stability were then systematically compared and evaluated. Interestingly, all methods showed a significant difference in inactivating lipase activity, but AV of oils extracted from WG stabilized by these different treatments remained stable after 6 weeks of storage at 37 °C, which was due to the inhibition of lipase at low a_w. Noteworthy, all methods had a remarkable effect on inactivating most of the lipoxygenase activity. However, oven-roasted, extrusion, and MW treated samples showed high oxidation rates and/or high losses of tocopherols, which had an adverse effect on WG oil quality. This indicates that these lipolytic enzymes are not the sole factors contributing to the instability of wheat germ. Furthermore, AV can be easily controlled after stabilization, but if over-processed, oxidation may become a major problem during storage. We found that WG with suitable aw and few structural changes had the best lipid stability, while rapid oxidation during storage was attributed to lipid exposure to air due to suboptimal aw or structural damage. Based on the different characteristics of these methods, VFB with efficient heat and mass transfer, and uniform heating can better control aw and reduce

Table 3
Thermodynamic parameters of fresh and stabilized WG.

	To (°C)	Tp1 (°C)	Tp2 (°C)	Tc (°C)	△H (J/g)
Fresh	59.86 ± 0.39^{bc}	68.52 ± 2.06^{a}	86.99 ± 1.56^{a}	96.18 ± 0.61^{b}	6.79 ± 0.67^{a}
VFB	59.37 ± 1.10^{c}	68.45 ± 0.64^{a}	86.86 ± 1.20^{a}	97.67 ± 0.14^{a}	6.01 ± 0.02^{b}
Oven	60.85 ± 0.62^{bc}	68.51 ± 0.88^{a}	86.52 ± 0.95^{a}	97.26 ± 0.49^{a}	5.43 ± 0.09^{b}
MW	61.50 ± 0.28^{b}	68.92 ± 0.42^{a}	86.19 ± 0.08^{a}	97.90 ± 0.30^{a}	4.22 ± 0.10^{c}
Extrusion	70.52 ± 1.42^{a}	N.D.^{b}	85.70 ± 0.24^{a}	94.93 ± 0.76^{c}	0.77 ± 0.25^{d}

To: the onset temperature, Tp1: the first endothermic peak temperature, Tp2: the second endothermic peak temperature, Tc: the completion temperature, △H: transition enthalpy. Values are expressed as means ± SD (n = 3). Values within each column with different superscripts (a, b, c and d) are significantly ($P < 0.05$) different.

structural damage, making it the most potential and appropriate method for stabilizing wheat germ for high-quality wheat germ oil production. In addition, investigating mild processing conditions for other methods to obtain a suitable range of aw and minimal structural damage is also worth further investigation.

CRediT authorship contribution statement

Xiaojun Liu: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Chengye Chi:** Investigation, Formal analysis, Data curation. **Shengmin Zhou:** Supervision, Project administration, Funding acquisition, Conceptualization. **Yuanrong Jiang:** Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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