Oil processing

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Preheating detoxification of flaxseed and its impact on the quality of flaxseed

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Abstract: Aiming to provide a theoretical basis for possible uses of flaxseed as a food supplement and functional ingredient, the heat treatment of flaxseed was carried out using steaming, roasting, and microwave methods to investigate the detoxification effects of these three pretreatment methods on flaxseed, as well as the impact of the three methods on the quality of flaxseed. The results showed that all three pretreatment methods had better detoxification effects on flaxseed, in which, microwave treatment was the most effective method. After 5 min of microwave treatment, the hydrogen cyanide (HCN) content in flaxseed decreased from (94.65 ± 1.68) mg/kg to (7.80 ± 0.57) mg/kg. All three pretreatment methods significantly reduced the water content in flaxseed but had a weaker effect on protein, fat, and ash contents. After pretreatment by the three methods, the polyphenol content, peroxide value (POV), and a^* value of flaxseed increased significantly, thiobarbituric acid reactive substances (TBARS) increased, while polyunsaturated fatty acids (PUFA) content, amino acid content, and L^*, W^*, and b^* values decreased, with varying degrees of wrinkles and cracks appearing on the surface of flaxseed, and the overall signal pattern of FTIR spectra did not change much. During the 40 ℃ accelerated storage process, the quality of flaxseed treated by all three preheating methods generally declined, and correlation analysis revealed that color change was a good indicator of quality changes in flaxseed. Notably, all three pretreatment methods extended the shelf - life of flaxseed. Compared with steaming (120 ℃ for 20 min) and roasting (100 ℃ for 40 min), microwave (560 W for 4 min) is recommended to remove cyanogenic glycosides and improve the stability and quality characteristics of flaxseed.

Key words: flaxseed; detoxification; polyphenol; color; stability; accelerated storage

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Flax (*Linum usitatissimum*) is an annual or biennial plant that has been grown commercially in more than thirty nations worldwide. It produces seeds containing an average of 41% fat, 20% protein, 28% total dietary fibre, 7.7% moisture, and 3.4% ash[1]. Flaxseed oil has gained popularity recently due to its high α - linolenic acid (ALA, C18:3, *n* - 3) content. Flaxseed can be added to flour products, dairy products and meat products to improve the nutritional

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quality, making it a popular nutritional supplement in the food industry.

Except for nutrients, flaxseed also has high content of antinutritive substances such as cyanogenic glycosides, which are glycosides of the α - hydroxy nitriles. For every 100 g, flaxseed typically contains 264 - 354 mg of cyanogenic glycosides [2]. When cyanogenic glycosides are consumed, they may break down into hazardous hydrogen cyanide (HCN). In addition to being extremely harmful to mammals' neurological, endocrine and respiratory systems, HCN may also affect how well nutrients are absorbed from meals. Adults may die from cyanogenic glycosides poisoning if they consume 100 mg [3]. As a result, before using flaxseed powder to food, its cyanide component must be removed by appropriate processing.

In order to ensure the safety of flaxseed for consumption and to remove potential toxins, heat - based detoxification treatments such as boiling, steaming, roasting, and microwave are commonly used in the industrial production of flaxseed products. Zhao et al[4] detoxified flaxseed using microwaves at a power of 640 W for 2 min, resulting in a cyanogenic glycosides content of 6.987 mg/kg. Tian et al[5] recommended roasting flaxseed at 100 ℃ for 30 min, which reduced the cyanogenic glycosides content to 15.174 mg/kg. Due to the high content of unsaturated oil, flaxseed is prone to oxidation during heat treatment, which may affect the edible qualities such as nutritional composition, odor, and color. Currently, many studies are being conducted to investigate the effects of storage factors (storage temperature, moisture content, and storage time) on the degradation parameters (apparent mold, germination, moisture content, protein content, and free fatty acid value) of flaxseed in order to produce safe storage standards. However, there are currently few reports on the impact of different heat detoxification methods on the quality of flaxseed powder. A thorough analysis of the effects of different heat detoxification treatment methods on the oxidative stability of flaxseed during subsequent storage has not been fully carried out.

Therefore, three different heat treatment methods, namely microwave, roasting, and steaming, were used in this study to detoxify flaxseed. By monitoring the cyanogenic glycosides content, basic nutritional composition, microstructure, peroxide value (POV), thiobarbituric acid reactive substances (TBARS), fatty acid composition, amino acid composition, polyphenol content, color, and Fourier transform infrared spectroscopy (FTIR) of flaxseed before (corresponding to the accelerated storage test at 0 d) and after accelerated storage that have been detoxified under different conditions, the impacts of different preheating treatments (steaming, roasting and microwave) on the quality of flaxseed were revealed. The correlation of quality indexes of different preheated flaxseed was analyzed. Moreover, the shelf - life was predicted combined with the Arrhenius model to reveal the impact of different heat detoxification treatments on the storage stability of flaxseed powder. The results will offer a theoretical foundation for the possible use of flaxseed as a food supplement and functional ingredient.

1 Materials and methods

1.1 *Materials*

Flaxseed (Longya 14), Gansuhuining Jianwei Edible Oil Co., Ltd.; standard for analysis of cyanide in water, Macklin Chemical Reagent Co., Ltd.; gallic acid (HPLC grade, ≥99%), 37 fatty acid methyl ester mixed standards (FAME, ≥98%) and methanol (HPLC grade, ≥99.9%), Sigma Group Co., Ltd.; hydrogen peroxide isopropylbenzene (HPLC grade, ≥95%) and *n* - hexane (HPLC grade, ≥97%), Shanghai Aladdin Biochemical Technology Co., Ltd.; amino acid standards (≥98%), Shanghai Hepu Biotechnology Co., Ltd.; all other related reagents (analytical grade), Tianjin Kemio Chemical Reagent Co., Ltd.

Hattiecs 919E Household low temperature mill, Zhongshan Tuozhen E - commerce Co., Ltd.; P70F20EN3P - ZSB Microwave oven, Guangdong Galanz Microwave Household Electrical Appliance Manufacturing Company; HWS - 24 Electrothermal constant - temperature drying box, Shanghai Yiheng Scientific Instrument Co., Ltd.; SCC - WE101 Universal steam oven, German Rational Company; JSM - 7800F Scanning electron microscope (SEM), JEOL Ltd., Tokyo, Japan; TECAN infinite M200 Multifunctional microplate reader, Tican, USA;

UltraScan Pro Colorimeter, HunterLab, USA; 7890B GC – 5977A Gas chromatograph, Agilent, USA; LA8080 Automatic amino acid analyzer, Hitachi, Japan; Frontier Fourier transform infrared spectrometer (FTIR), Perkin Elmer, USA.

1.2 *Methods*

1.2.1 Preheating detoxification treatment of flaxseed

(A) Microwave treatment: 40 g of flaxseed were weighed and placed in a glass culture dish with a diameter of 20 cm × 20 cm. Microwave treatment was carried out in a microwave oven for 1, 2, 3, 4 min, and 5 min at a microwave power of 560 W.

(B) Roasting treatment: 40 g of flaxseed were weighed and placed in an aluminum foil tray (25 cm × 20 cm). Roasting treatment was carried out in an electrothermal constant – temperature drying box for 10, 20, 30, 40 min, and 50 min at a roasting temperature of 100 ℃.

(C) Steaming treatment: 40 g of flaxseed were weighed and placed in an aluminum foil tray (25 cm × 20 cm). Steaming treatment was carried out in a steam oven for 5, 10, 15, 20 min, and 25 min at a steam temperature of 120 ℃.

After cooling to room temperature, the above – mentioned flaxseed were crushed using a household low temperature mill. Subsequently, these obtained powder were sieved through a 0.25 mm(60 mesh) sieve.

1.2.2 Accelerated storage test of flaxseed

Each preheated flaxseed powder was divided into three portions (40 g each) in zip – lock bags, which were subsequently put in an oven at 40 ℃ for 42 d. One bag of flaxseed powder was taken from the oven at each time point (0, 21 d and 42 d) to be tested.

1.2.3 Extraction of total lipids

Total lipids were extracted from flaxseed powder by using a modified method reported by Folch et al [6]. Briefly, 30 g of flaxseed powder was mixed with 30 mL of deionized water, and then 100 mL of mixed solvent composed of chloroform and methanol (volume ratio 2∶1) was added. After thorough mixing, 37.5 mL of chloroform was added and kept stirring at 40 ℃ for 2 h, then centrifuged at 7 800 × *g* and 4 ℃ for 10 min to collect the organic phase layer. The combination of vacuum rotary evaporation and nitrogen blowing was used to remove organic solvents. The extracted lipids were sealed in centrifuge tubes and stored at –80 ℃ for later analysis.

1.2.4 Cyanide content analysis

The cyanide content was determined by a spectrophotometric method according to GB 5009.36 – 2023, and the result was calculated by HCN content in sample.

1.2.5 Analysis of basic indexes of flaxseed

GB 5009.3 – 2016 (Direct drying method) served as the basis for the determination of water; GB 5009.6 – 2016 (Soxhlet extraction method) for the determination of fat; GB 5009.5 – 2016 (Kjeldahl method) for the determination of protein; GB 5009.4 – 2016 (High temperature calcination method) for the determination of ash.

1.2.6 Analysis of polyphenol content

Polyphenol content was analyzed by a modified method reported by Sarkis et al [7]. In short, 25 mL of 80% (volume fraction) ethanol was combined with 5 g of flaxseed powder and swirled for 1 h at room temperature. To obtain the supernatant, the mixture was centrifuged for 30 min at 4 ℃ and 4 800 × *g*. This supernatant was diluted at a ratio of 1∶9 (volume ratio) using 80% (volume fraction) ethanol. A mixture of 1 mL of Folin – Ciocalteu reagent and 2 mL of sodium carbonate solution (mass concentration 15 g/mL) was added to 0.5 mL of diluted liquid. The mixture was then diluted to 10 mL with deionized water. After a 2 h period at room temperature, the reaction solution was collected, and the absorbance at 760 nm was measured. the polyphenol content was determined using the gallic acid standard curve (y = 0.826 4x + 0.042 7, R^2 = 0.999 3).

1.2.7 SEM observation

Freeze – dried flaxseed powder was prepared using a vacuum freeze dryer, and then dispersed on conductive carbon tape and coated with a thin layer of gold. The microscopic morphology was observed using a SEM at 5 000 × magnification. The observations were performed at two time points of 0 d and 42 d, corresponding to the storage conditions during the accelerated storage test.

1.2.8 Color analysis

The color of flaxseed powder with different preheating treatments was measured using a colorimeter

combined with a three – point detection method reported by Skipnes et al [8]. Prior to determination, the colorimeter was calibrated using a standard white board. As a result, L^* represents brightness, a^* represents redness/greenness, ranging from green (negative) to red (positive), and b^* represents yellowness/blueness, ranging from blue (negative) to yellow (positive). The whiteness (W^*) was then calculated according to formula(1).

$$W^* = 100 - \{(100 - L^*)^2 + a^{*2} + b^{*2}\}^{0.5} \quad (1)$$

1.2.9 Analysis of amino acid composition

The amino acid composition was determined according to GB 5009.124 – 2016 using an automatic amino acid analyzer. The detection conditions were column temperature 57 ℃, detector temperature 135 ℃, wavelengths 440 nm for proline and 570 nm for other amino acids, and injection volume 20 μL. Quantification was performed using the external standard method.

1.2.10 FTIR analysis

FTIR analysis was performed and the spectra were analyzed based on the method reported by Suri et al[9], with slight modifications. Briefly, the flaxseed powder obtained from different preheating treatments were mixed with dried potassium bromide powder. FTIR analysis was performed to obtain the spectra. Especially, the measurements were conducted with 32 scans at a resolution of 8 cm^{-1}, and the infrared absorption spectrum was scanned in the wavenumber range of 400 cm^{-1} to 4 000 cm^{-1}.

1.2.11 Analysis of TBARS

With flaxseed total lipids as material, the TBARS was determined by the improved colorimetric method by John et al [10].

1.2.12 Analysis of POV

With flaxseed total lipids as material, the POV was measured by spectrophotometric analysis at 510 nm, as described by Pei et al [11].

1.2.13 Analysis of fatty acid composition

Fatty acid composition was determined by converting total lipids to fatty acid methyl esters (FAME) and then analyzed by gas chromatography, as described by Xie et al[12].

1.2.14 Shelf – life prediction

The shelf – life of flaxseed powder from different preheating treatments was predicted by the modified method reported by Li et al [13].

Predicted shelf – life: for each type of flaxseed powder, eight portions of flaxseed powder (40 g each) were packaged in zip – lock bags, which were subsequently put in ovens at 40, 50 ℃ and 60 ℃. One bag of flaxseed powder was taken from the oven at each time point (0, 7, 14, 21, 28, 35, 42 d and 49 d), which was subsequently subjected to the extraction of total lipids as 1.2.3. The POVs of these lipid samples were determined. The measured POVs was used to construct the kinetic model of oxidation with zero – order kinetics model and first order kinetics model, respectively. Zero – order kinetics model, first – order kinetics model, Arrhenius equation and shelf – life (L) calculation are given by formula (2), (3), (4) and (5).

$$P = k_0 t + P_0 \quad (2)$$

$$\ln P = kt + \ln P_0 \quad (3)$$

$$\ln k = -E_a/RT + \ln A \quad (4)$$

$$L = (\ln P_{lim} - \ln P_0)/(A \times e^{-E_a/RT}) \quad (5)$$

Where: k_0 and k are reaction rate constants; t is reaction time; P, P_0 and P_{lim} are the POV before and after storage, and national limited; A is the pre – exponential factor; E_a is the activation energy, J/mol; T is the temperature, K; R is the molar constant of the gas, 8.314 4 J/(mol · K).

Actual shelf – life: six portions of flaxseed powder (40 g each) were packaged in zip – lock bags, which were subsequently put in an oven at 25 ℃. One bag of flaxseed powder was taken from the oven at each time point (0, 14, 28, 42, 56 d and 70 d), which was subsequently subjected to the extraction of total lipids as described in 1.2.3. Based on the determined POVs of these samples, the POV – t curve was plotted. With the national limit for POV as standard, the shelf – life of flaxseed powder treated by different preheating methods at 25 ℃ was calculated.

1.2.15 Statistical Analysis

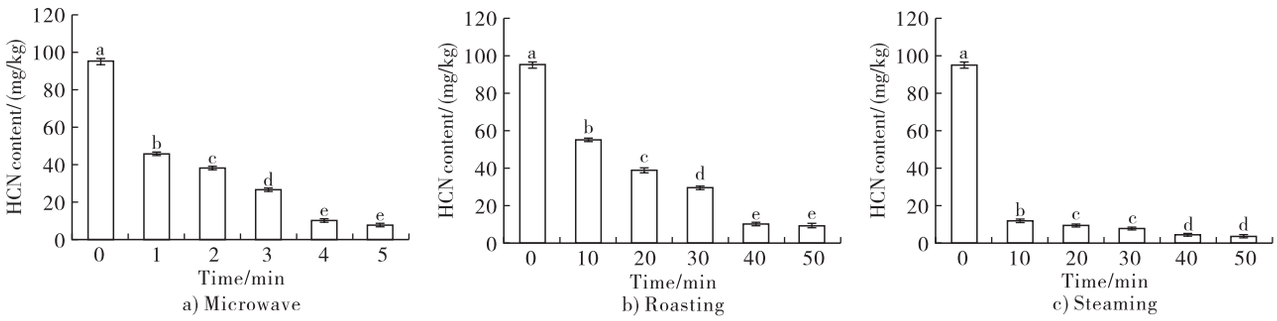
All samples were prepared in triplicate. The means ± standard deviations (calculated from five parallel measurements) were displayed as the results. The statistical analysis was carried out using SPSS version 20.0 software (SPSS Inc., Chicago, Illinois, USA), and the Duncan multi – range test was

employed to determine significance. A Pearson correlation analysis was performed to determine the correlation between each index using Metabo Analyst 4.0. Significant changes ($p < 0.05$) were denoted by different letters.

2 Results and discussion

2.1 *Effect of preheating treatment methods on cyanide content of flaxseed*

The effect of preheating treatment methods on cyanide content of flaxseed is shown in Fig. 1.



Note: Different letters indicate significant differences ($p < 0.05$). The same below

Fig. 1 Effect of preheating treatment methods on cyanide content of flaxseed

As shown in Fig. 1, all three preheating treatment methods could effectively reduce the cyanide content, and the cyanide content began to sharply decrease in the early stage, and then the reduction rate gradually slowed down until the cyanide content tended to be stable.

Two processes are necessary for the effective removal of cyanide: the breakdown of cyanogenic glycosides to HCN and the volatilization of HCN that is produced during this process. It was obvious that compared with the microwave and steaming treatment, the roasting treatment was less effective in removing cyanide. This is mainly due to the fact that compared with the steaming treatment, the roasting treatment is carried out under dry conditions, and there is no (or very little) water, the activity of the enzyme is not as suppressed as in steaming treatment, leading to a higher conversion of cyanogenic glycosides into HCN[14]. Regarding the microwave treatment, it allows the treated material to interact with microwaves, the heat generated by microwaves accelerates the breakdown of cyanogenic glycosides and enhances the volatilization of HCN, leading to a significant reduction in cyanide content. By contrast, microwave treatment was the most effective detoxification method among the three preheating treatment methods mentioned in this study for removing cyanide, and most of the cyanogenic glycosides could be removed in a short period of time. As shown in Fig. 1, after 5 min of microwave treatment, the HCN content in flaxseed was reduced from (94.65 ± 1.68) mg/kg to (7.80 ± 0.57) mg/kg.

The content of HCN in flaxseed decreased with the extension of microwave time, roasting time, and steaming time. Moreover, there was little change after 4 min of microwave treatment, 40 min of roasting treatment, and 20 min of steaming treatment, respectively. Therefore, roasting (100 ℃ for 40 min), steaming (120 ℃ for 20 min) and microwave (560 W for 4 min) were selected as the detoxification processes for subsequent experiments.

2.2 *Effect of preheating treatment methods on the changes of basic indexes of flaxseed during accelerated storage*

The effect of preheating treatment methods on the changes of basic indexes of flaxseed during accelerated storage is shown in Fig. 2.

As shown in Fig. 2, the contents of water, fat, protein and ash in fresh flaxseed were (5.61 ± 0.15)%, (46.40 ± 0.68)%, (23.80 ± 0.03)% and (3.99 ± 0.03)%, which was consistent with the results reported by Tuncel et al [15]. Three preheating treatment methods can significantly reduce the water content of flaxseed, but had an overall weaker effect on protein, fat, and ash contents. The samples that underwent with microwave treatment were observed the greatest decrease in water content, followed by the roasting method, and steaming method was the least effective method for water removal. This difference can be attributed to the fact that microwave treatment directly heats water molecules inside the flaxseed, facilitating faster water evaporation compared with the other methods relying on heat transfer through

external sources. The changes in water content were the most pronounced during the accelerated storage process.

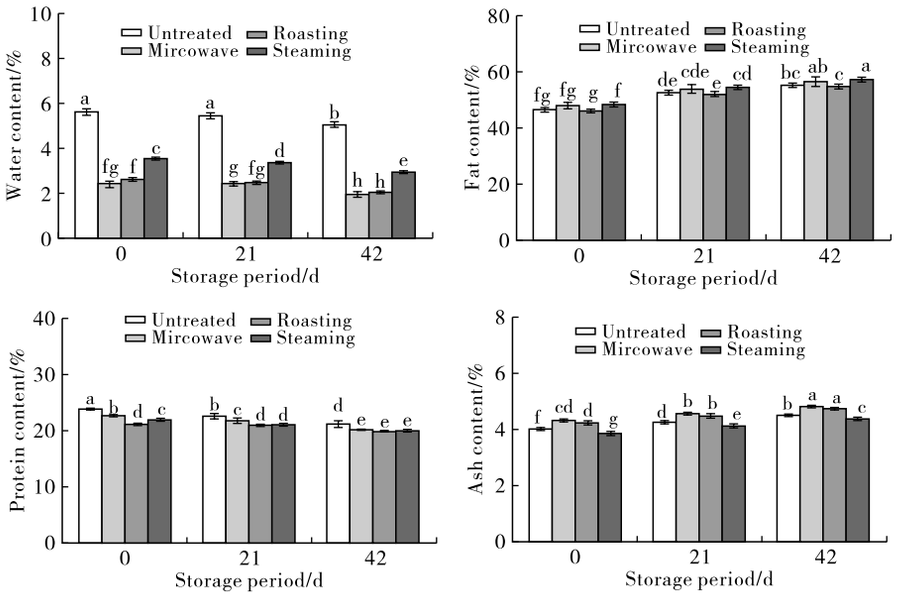


Fig. 2 Effect of preheating treatment methods on the changes of basic indexes of flaxseed during accelerated storage

Meanwhile, it was worth noting that the protein content showed a decreasing trend during accelerated storage, suggesting that the Maillard reaction might occur in flaxseed during accelerated storage. This reduction in protein content was most significant in the samples treated by microwave. Fat and ash contents remained relatively stable throughout storage process, with minor fluctuations being observed. These findings suggest that water content is the most sensitive to preheating treatments and storage conditions, while the fat, protein, and ash contents are less affected but still contribute to the overall quality of the flaxseed.

2.3 *Effect of preheating treatment methods on the polyphenol content of flaxseed during accelerated storage*

The effect of preheating treatment methods on polyphenol content of flaxseed during accelerated storage is shown in Fig. 3.

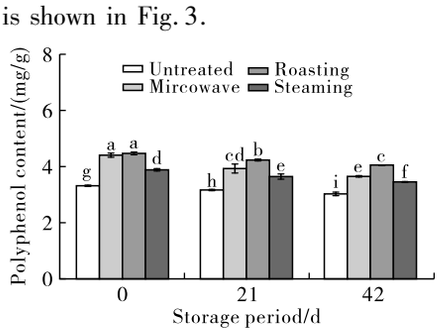


Fig. 3 Effect of preheating treatment methods on polyphenol content of flaxseed during accelerated storage

As shown in Fig. 3, the content of polyphenol in fresh flaxseed was (3.32 ± 0.02) mg/g. After microwave, roasting and steaming treatments, the corresponding values increased to (4.41 ± 0.07) mg/g, (4.47 ± 0.05) mg/g and (3.88 ± 0.04) mg/g, respectively. Owing to the nature of their chemical structures, phenolic compounds can be found in food both in their free form and attached to other dietary components[16]. Phenolic compounds in flaxseed have been found in four distinct forms: free, etherified, esterified and insoluble bound phenolic acids[17]. In summary, flaxseed is a source of free - form phenylpropanoids, including *o* - and *p* - coumaric, ferulic, *p* - hydroxybenzoic, vanillic, and sinapic acids[18]. Nonetheless, bound forms of ferulic acid glucoside, *p* - coumaric acid glycoside, and caffeic acid glucoside are present[19]. Usually, free forms of polyphenol will be released from their bound forms after preheating treatment[20]. Therefore, the polyphenol contents in the heat - treated groups were significantly higher than that in the untreated group.

As shown in Fig. 3, the polyphenol content of all flaxseed samples gradually decreased with the extension of storage time at 40℃. During food storage, polyphenol can provide hydrogen atoms to combine with and inactivate free radicals, thus effectively interrupting the free radical chain reaction[21] and further inhibiting lipid oxidation in food. However, the antioxidant process itself leads to the oxidation of polyphenol molecules, resulting in the loss of polyphenol.

2.4　*Effect of preheating treatment methods on the SEM observation of flaxseed during accelerated storage*

The effect of preheating treatment methods on the SEM observation of flaxseed during accelerated storage is shown in Fig. 4. The black arrows in the microscope images were lipid droplets.

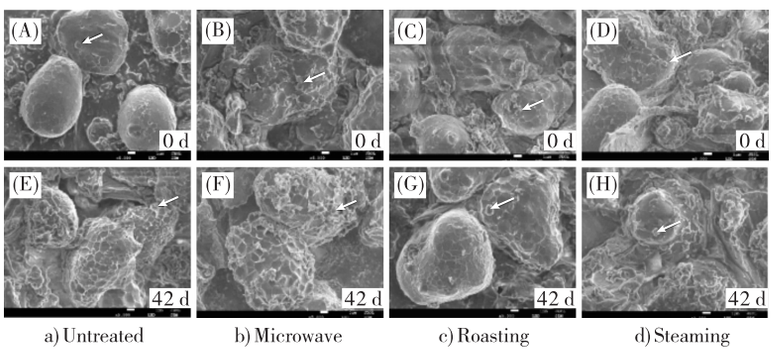


Fig. 4　SEM photographs (5 000 ×) of flaxseed powder treated by different preheating treatment methods

As shown in Fig. 4A, the fresh flaxseed powder exhibited a relatively dense and smooth surface morphology. After microwave, roasting and steaming treatments, different degrees of wrinkles and cracks appeared on the surface (Fig. 4B, 4C and 4D), because of the fact that under high temperature conditions of heat – treatment, the liquid water inside the flaxseed vaporized, thus increasing the pressure inside the flaxseed and further causing changes in the surface structure. Especially, microwave treatment had the strongest destructive effect on the structure, mainly because of the fact that the water molecules inside the flaxseed can effectively absorb microwaves, generating heat inside the cells, which is more conducive to the destruction of the cell wall [14].

As shown in Fig. 4, after 42 d of accelerated storage at 40 ℃, the structure of the untreated flaxseed was more severely disrupted compared with the heat – treated flaxseed. Because of that preheating treatments could also affect the lipid droplet structure in flaxseed. After preheating treatments, the surface of the lipid droplets was covered with a large amount of polyphenols and other accompanying substances, thereby reducing the oxidation of the oil in accelerated storage[22], which could possibly improve the accelerated storage stability of flaxseed powder.

2.5　*Effect of preheating treatment methods on the color of flaxseed during accelerated storage*

Color is one of the most important indicators for evaluating the quality of flaxseed powder. The effect of preheating treatment methods on the color of flaxseed during accelerated storage is shown in Fig. 5.

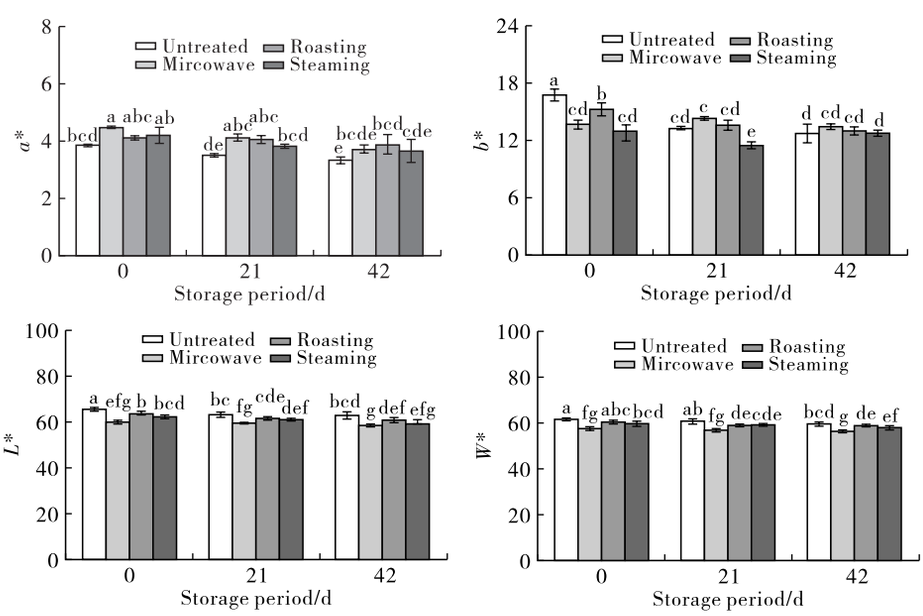


Fig. 5　Effect of preheating treatment methods on the color of flaxseed during accelerated storage

As shown in Fig. 5, the L^*, W^* and b^* values of flaxseed powder decreased after preheating treatments. High temperature caused wrinkles and cracks on the surface of flaxseed particles (Fig. 4), which reduced the sample's reflectivity, thereby leading to a decrease in L^* value[23]. As for the decrease of W^* and b^* values, they are closely related to the oxidative degradation of some carotenoids and flavonoid pigments in flaxseed [9].

The a^* value of flaxseed powder increased after preheating treatments. For example, after microwave, roasting and steaming treatments, the a^* value increased from 3.85 ± 0.03 to 4.47 ± 0.03, 4.11 ± 0.04 and 4.19 ± 0.28, respectively. Such increase may be caused by the Maillard reaction and the oxidation of polyphenol. Under high temperature conditions, the reducing sugar in flaxseed undergoes a Maillard reaction with free amino acids (or amides) to produce melanoidins [23]. Melanoidins are considered as a mixture of some complex, exhibit brown color and possess high molecular weights. Consistent with the findings in the above sections, microwave was found to have the greatest impact on the color of flaxseed among the three preheating treatment methods. Similarly, Suri et al. [9] reported that microwave treatment (540 W, 10 min) significantly increased a^* value, browning index, while decreased the L^* and b^* values of flaxseed oil. Especially, Maillard reaction products were only detected in oil after microwave treatment at 540 W for 10 min.

In accelerated storage process, L^*, W^*, a^* and b^* values of all flaxseed samples generally showed a gradual decrease with the extension of storage time at 40 ℃. This may be related to the oxidative degradation of polyphenol and flavonoid pigments during accelerated storage, leading to the production of browning products in flaxseed. Research findings show that polyphenol oxidase catalyzes the hydroxylation and oxidation of polyphenol to *o* – quinones, leading to browning in flaxseed during preheating treatments [24].

2.6 *Effect of preheating treatment methods on amino acid composition of flaxseed during accelerated storage*

The amino acid compositions of different preheated flaxseed during accelerated storage is shown in Table 1.

Table 1 Amino acid composition of different preheated flaxseed during accelerated storage g/100 g

Amino acid	Content in 0 d				Content in 42 d			
	Untreated	Mircowave	Roasting	Steaming	Untreated	Mircowave	Roasting	Steaming
Asp	2.41 ± 0.13a	2.20 ± 0.00b	2.14 ± 0.00b	2.22 ± 0.00b	1.89 ± 0.01c	1.70 ± 0.00de	1.62 ± 0.01e	1.79 ± 0.01cd
Thr*	0.87 ± 0.00a	0.83 ± 0.00b	0.80 ± 0.00c	0.83 ± 0.00b	0.73 ± 0.01d	0.66 ± 0.01f	0.62 ± 0.00g	0.68 ± 0.00e
Ser	1.01 ± 0.02a	0.95 ± 0.00b	0.95 ± 0.00c	0.96 ± 0.00b	0.91 ± 0.00d	0.80 ± 0.00f	0.78 ± 0.00g	0.85 ± 0.00e
Glu	4.28 ± 0.04a	4.09 ± 0.00b	3.94 ± 0.01c	4.09 ± 0.00b	3.62 ± 0.01d	3.27 ± 0.01f	3.09 ± 0.02g	3.38 ± 0.01e
Pro	0.54 ± 0.03ab	0.49 ± 0.01ab	0.46 ± 0.01ab	0.48 ± 0.01ab	0.64 ± 0.11a	0.53 ± 0.04ab	0.37 ± 0.04b	0.46 ± 0.06ab
Gly	1.02 ± 0.00a	0.98 ± 0.00b	0.94 ± 0.00c	0.97 ± 0.01b	0.84 ± 0.01d	0.75 ± 0.00f	0.76 ± 0.00f	0.79 ± 0.00e
Ala	1.38 ± 0.05a	1.35 ± 0.01ab	1.31 ± 0.00b	1.32 ± 0.03b	1.20 ± 0.01c	1.08 ± 0.00e	1.09 ± 0.00e	1.15 ± 0.00d
Val*	0.81 ± 0.48a	1.17 ± 0.01a	1.11 ± 0.01a	1.15 ± 0.03a	1.00 ± 0.01a	0.89 ± 0.01a	0.88 ± 0.00a	0.91 ± 0.00a
Met*	0.15 ± 0.01a	0.07 ± 0.00g	0.14 ± 0.01b	0.09 ± 0.00e	0.10 ± 0.01d	0.06 ± 0.00h	0.11 ± 0.01c	0.08 ± 0.00f
Ile*	0.59 ± 0.02a	0.56 ± 0.00b	0.55 ± 0.02b	0.57 ± 0.00b	0.46 ± 0.02c	0.39 ± 0.00d	0.39 ± 0.00d	0.41 ± 0.00d
Leu*	1.18 ± 0.04a	1.12 ± 0.00ab	1.11 ± 0.04ab	1.13 ± 0.00ab	1.07 ± 0.03b	0.93 ± 0.01c	0.91 ± 0.00c	0.97 ± 0.01c
Tyr	1.03 ± 0.17a	0.78 ± 0.01a	0.91 ± 0.18a	0.76 ± 0.00a	0.82 ± 0.12a	0.65 ± 0.01a	0.68 ± 0.00a	0.73 ± 0.01a
Phe*	1.54 ± 0.45a	1.10 ± 0.03a	1.42 ± 0.43a	1.08 ± 0.01a	1.32 ± 0.04a	1.18 ± 0.02a	1.19 ± 0.00a	1.24 ± 0.01a
Lys*	0.92 ± 0.26a	0.57 ± 0.02a	0.85 ± 0.28a	0.62 ± 0.01a	0.73 ± 0.19a	0.45 ± 0.00a	0.53 ± 0.00a	0.54 ± 0.00a
His	0.63 ± 0.06a	0.53 ± 0.03a	0.60 ± 0.07a	0.54 ± 0.03a	0.48 ± 0.04a	0.40 ± 0.00a	0.40 ± 0.00a	0.42 ± 0.00a
Arg	2.06 ± 0.03a	1.92 ± 0.00b	1.87 ± 0.01c	1.94 ± 0.00b	1.78 ± 0.00d	1.54 ± 0.00g	1.54 ± 0.01f	1.68 ± 0.01e
Total	20.40 ± 1.16a	18.71 ± 0.10ab	19.08 ± 1.02ab	18.75 ± 0.05ab	17.57 ± 0.34b	15.29 ± 0.06c	14.98 ± 0.05c	16.04 ± 0.06c

Note: * represents essential amino acid; different letters in the same row indicate significant differences ($p < 0.05$). The same below

As shown in Table 1, the fresh flaxseed mainly contained 16 kinds of amino acids, including 7 kinds of essential amino acids, which was consistent with the amino acid composition reported by Sharma et al [25].

After preheating treatments including microwave, roasting and steaming, the contents of amino acids decreased. Taking total amino acids (TAA) as an example, their contents decreased from (20.40 ± 1.16) g/100 g (fresh) to (18.71 ± 0.10) g/100 g (microwave), (19.08 ± 1.02) g/100 g (roasting) and (18.75 ± 0.05) g/100 g (steaming), respectively. The possible reason was that during the preheating treatment process, the amino acids in flaxseed participated in the Maillard reaction, producing volatile flavor compounds and brown macromolecules. It was also possible that heat treatment caused damage to the amino acids[15].

In this study, it was also obvious that after 42 d of accelerated storage, the TAA contents of all flaxseed samples showed a decreasing trend, and the content of TAA in preheated flaxseed was lower than that in untreated flaxseed. Protein oxidation during storage could contribute to the release of free amino acids, and during prolonged storage, these amino acids could undergo further oxidation or degradation, leading to an overall decrease in their levels.

2.7 *Effect of preheating treatment methods on FTIR spectra of flaxseed during accelerated storage*

Infrared spectroscopy can be used to analyze the structure and functional groups of oil and its composition. FTIR spectra of different preheated flaxseed during accelerated storage is shown in Fig. 6.

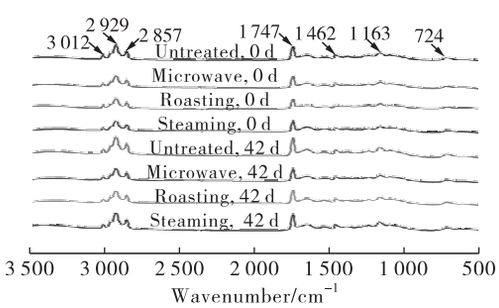


Fig. 6　FTIR spectra of different preheated flaxseed during accelerated storage

As shown in Fig. 6: the peak of the IR spectrum was found at 724 cm^{-1}, which was attributed to the overlap of the CH_2 rocking vibrations and the out – of – plane vibration of the *cis* – disubstituted olefins; 1 163 cm^{-1}, which was linked to the stretching vibration of the C—O group in esters; 1 462 cm^{-1}, which was designated to the bending vibrations of the CH bonds of the aliphatic CH_2 and CH_3 groups; 1 747 cm^{-1}, which corresponded to the stretching vibrations of the carbonyl (C═O) group; 2 929 cm^{-1} and 2 857 cm^{-1}, which were attributed to the asymmetric and symmetric stretching vibrations of the CH bonds of the aliphatic CH_2 groups, respectively; 3 012 cm^{-1}, which was attributed to the symmetric stretching vibration of the CH bonds of the *cis* – olefinic groups ═CH [9].

The overall signal pattern of FTIR spectra of flaxseed before and after preheating treatment and after storage for 42 d was similar, but there were slight differences in intensity of some peaks. This may be related to the oxidation of flaxseed during heat treatment and storage[26].

2.8 *Effect of preheating treatment methods on the POV and TBARS of flaxseed during accelerated storage*

The POV and TBARS were detected to reflect the lipid oxidation in flaxseed treated with different preheating treatment methods. The effect of preheating treatment methods on POV and TBARS of flaxseed is shown in Fig. 7.

As shown in Fig. 7, the three preheating treatment methods could significantly improve the oxidative stability of flaxseed during accelerated storage. Under accelerated storage conditions (40 ℃), lipoxygenase can increase the oxidation of lipid components in flaxseed. It has been widely accepted that preheating treatments can reduce or eliminate the activity of lipoxygenase by disrupting the three – dimensional structure of the enzyme [27]. It could also be observed that during the accelerated storage process, the POV and TBARS values of flaxseed treated by roasting were the lowest, which might be because roasting more effectively increased the content of free polyphenol compared with microwave and steaming (Fig. 3). The polyphenol is an excellent antioxidants and can significantly enhance the storage stability of flaxseed.

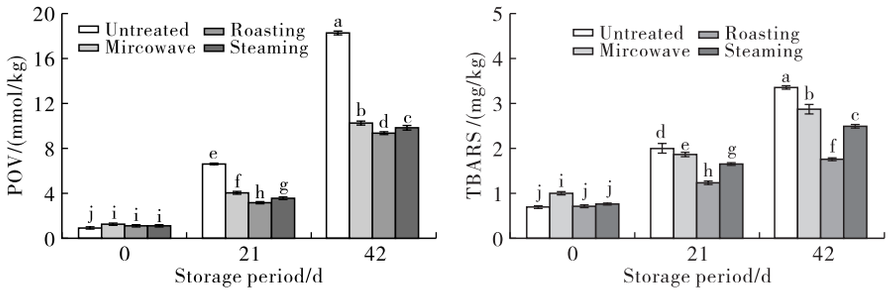


Fig. 7 Effect of preheating treatment methods on POV and TBARS of flaxseed

2.9 *Effect of preheating treatment methods on the fatty acid composition of flaxseed during accelerated storage*

The fatty acid composition and relative content of different preheated flaxseed during accelerated storage is shown in Table 2.

Table 2 Fatty acid composition and relative content of different preheated flaxseed during accelerated storage **g/100 g**

Fatty acid	Content in 0 d				Content in 42 d			
	Untreated	Mircowave	Roasting	Steaming	Untreated	Mircowave	Roasting	Steaming
C16:0	6.02 ± 0.08bc	6.94 ± 0.15a	5.60 ± 0.10c	6.43 ± 0.44abc	6.31 ± 0.06abc	6.93 ± 0.05a	5.72 ± 0.04bc	6.56 ± 0.08ab
C18:0	4.49 ± 0.01b	5.47 ± 0.17a	5.34 ± 0.76a	4.84 ± 0.20ab	4.65 ± 0.06ab	5.47 ± 0.11a	5.53 ± 0.14a	4.95 ± 0.07ab
C18:1	15.43 ± 0.06c	16.34 ± 1.01ab	15.69 ± 0.50bc	16.11 ± 0.36ab	16.51 ± 0.30a	16.10 ± 0.11bc	15.86 ± 0.12bc	16.32 ± 0.01ab
C18:2	15.35 ± 0.06b	16.47 ± 1.01a	16.22 ± 0.50a	15.88 ± 0.36a	14.66 ± 0.30b	16.38 ± 0.11a	16.10 ± 0.12a	15.81 ± 0.01a
C18:3	58.71 ± 1.11a	54.78 ± 0.88d	57.14 ± 1.81b	56.74 ± 0.72b	57.87 ± 0.09a	55.12 ± 0.23cd	56.80 ± 0.05b	56.37 ± 0.26bc
SFA	10.51 ± 0.09c	12.41 ± 0.32a	10.94 ± 0.72bc	11.27 ± 0.54abc	10.96 ± 0.12bc	12.40 ± 0.15a	11.25 ± 0.09bc	11.51 ± 0.07ab
MUFA	15.43 ± 0.06c	16.34 ± 1.01ab	15.69 ± 0.50bc	16.11 ± 0.36ab	16.51 ± 0.30ab	16.10 ± 0.11bc	15.86 ± 0.12bc	16.32 ± 0.01ab
PUFA	74.06 ± 1.18a	71.25 ± 1.86b	73.36 ± 2.10ab	72.62 ± 0.36ab	72.53 ± 0.38ab	71.50 ± 0.31b	72.90 ± 0.17ab	72.18 ± 0.25ab

Note: The data in the table are calculated by the peak area normalization method

As shown in Table 2, the fresh flaxseed mainly contained five kinds of fatty acids, namely linolenic acid (C18:3), oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0) and stearic acid (C18:0). The fatty acid composition was consistent with the research results reported by Tuncel et al [15]. They found flaxseed contained (51.37 ± 0.48)% of linolenic acid, (21.48 ± 0.09)% of oleic acid, (15.18 ± 0.33)% of linoleic acid, (7.84 ± 0.31)% of palmitic acid and (4.11 ± 0.07)% of stearic acid.

Obviously, after preheating treatments including microwave, roasting and steaming, the content of polyunsaturated fatty acids (PUFA) in flaxseed slightly decreased from (74.06 ± 1.18)% to (71.25 ± 1.86)%, (73.36 ± 2.10)% and (72.62 ± 0.36)%, respectively, which might be caused by the slight oxidation of the lipid components in flaxseed during preheating treatment. Similarly, Huang et al [23] found that preheating treatment methods including explosion - puffing, microwave and roasting could significantly decrease the content of PUFA in sesame seeds. In this study, the overall content of PUFA in flaxseed before and after preheating treatment decreased after accelerated storage, while the SFA content increased.

2.10 *Correlation analysis*

The correlation of quality indexes of different preheated flaxseed during accelerated storage is shown in Fig. 8.

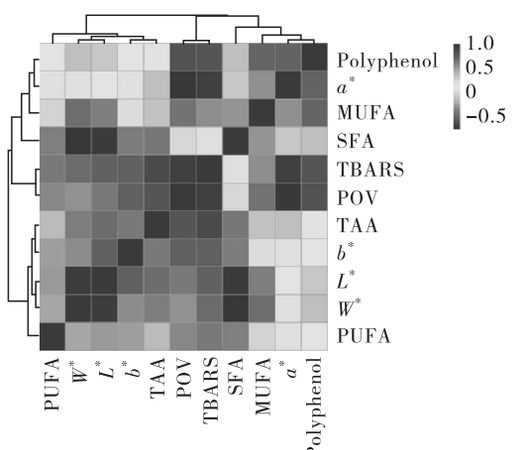


Fig. 8 Correlation of quality indexes of different preheated flaxseed during accelerated storage

As shown in Fig. 8: b^*, L^* and W^* showed a moderately positive correlation with total amino acids content; a^*, b^*, L^* showed a moderately negative correlation with POV and TBARS; L^* and W^* showed a highly negative correlation with SFA content; a^* showed a highly positive correlation with polyphenol content. Color is an important indicator to be considerea during food storage. Especially, nuts, oilseeds, and fruit juices often undergo browning during storage. Browning is well known to be associated with polyphenol oxidation, and therefore it may be accompanied by changes in the degree of oxidation [28]. Polyphenol oxidation belongs to non – enzymatic browning, and it is worth noting that the products of polyphenol oxidation also participate in the Maillard reaction [25], thereby affecting the degree and rate of color change in non – enzymatic browning. In this study, during accelerated storage, the polyphenol content of flaxseed decreased (Fig. 3) and the color became lighter, indicating that the quality of flaxseed had deteriorated due to oxidation. Given this, the color change is a good indicator of the quality change of flaxseed.

2.11 *Shelf – life prediction*

The oxidation kinetics of different preheated flaxseed and shelf – life is shown in Table 3.

Table 3　Oxidation kinetics of different preheated flaxseed and shelf – life

Methods	Temperature/℃	R^2		Arrhenius model	R^2	Shelf – life at 25 ℃/d	
		Zero – order model	First – order model			Predicted value	Tested value
Untreated	40	0.920 9	0.977 7	$\ln k = -289.42/T - 1.733\ 8$	0.999 9	42.79	40.71
	50	0.921 3	0.967 7				
	60	0.937 5	0.954 7				
Mircowave	40	0.947 2	0.989 0	$\ln k = -731.33/T - 0.668\ 4$	0.913 2	62.98	63.83
	50	0.970 6	0.972 6				
	60	0.925 7	0.990 4				
Roasting	40	0.901 4	0.998 9	$\ln k = -597.92/T - 1.084\ 1$	0.883 4	63.42	66.57
	50	0.917 1	0.995 2				
	60	0.939 6	0.979 3				
Steaming	40	0.910 5	0.999 1	$\ln k = -588.41/T - 1.099\ 7$	0.992 5	61.70	64.35
	50	0.886 8	0.993 4				
	60	0.944 5	0.983 7				

As shown in Table 3, the first – order model for all flaxseed samples fitted the changes in POV better than the zero – order model based on the value of R^2. Arrhenius equations for the samples of untreated, microwave – treated, roasting – treated and steaming – treated samples were $\ln k = -289.42/T - 1.733\ 8$, $\ln k = -731.33/T - 0.668\ 4$, $\ln k = -597.92/T - 1.084\ 1$ and $\ln k = -588.41/T - 1.099\ 7$, respectively. The corresponding predicted shelf – life for untreated, microwave – treated, roasting – treated and steaming – treated samples stored at 25 ℃ were 42.79, 62.98, 63.42 d and 61.70 d, while the tested values were 40.71, 63.83, 66.57 d and 64.35 d, respectively. For untreated flaxseed, the relative error (RE) of the shelf life at 25 ℃ between the tested and predicted values was 5.10%; for microwave – heated flaxseed, it was 1.34%; for roasting – treated flaxseed, it was 4.73%; and for steaming – treated flaxseed, it was 4.12%. Results indicated that preheating treatment can effectively improve the storage stability of flaxseed, thereby extending the shelf – life.

3　Conclusion

In this study, the effects of microwave, roasting and steaming treatment on the removal of cyanogenic glycosides and quality of flaxseed were investigated. On the one hand, microwave, roasting and steaming were effective in reducing the content of toxic cyanogenic glycosides in flaxseed. On the other hand, these three preheating treatments significantly improved the shelf – life of flaxseed. In contrast, microwave treatment is recommended to remove cyanogenic glycosides and improve the stability and quality charateristics of flaxseed. In addition, the color change is a good indicator of the change in quality of flaxseed.

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是影响植物甾醇等活性物质损失的关键因素[13]。

3 结 论

压榨菜籽原油中生育酚和植物甾醇含量分别为662～706 mg/kg和7 731～8 568 mg/kg,浸出菜籽原油中二者含量分别为774～885 mg/kg和9 177～10 136 mg/kg,两种菜籽原油中生育酚以γ-生育酚和α-生育酚为主。浸出菜籽原油中生育酚和植物甾醇含量明显高于压榨菜籽原油。菜籽油中的生育酚在精炼各工段均有损失,损失大小顺序为脱臭>中和>脱色,总损失率为11.37%～18.84%。中和工段α-生育酚占比降低,(β+γ)-生育酚占比升高,脱臭工段与之相反,中和工段和脱臭工段4种生育酚组分变幅大小顺序为α-生育酚>(β+γ)-生育酚>δ-生育酚。菜籽油中的植物甾醇在精炼过程中损失率为3.27%～11.89%。脱臭馏出物酸值及生育酚含量受中和油酸值、生育酚含量及脱臭条件等因素的影响,通过在中和工段适当地降低中和油的酸值,可以降低脱臭馏出物的酸值,同时提高其生育酚的含量,当中和油的酸值(KOH)低于0.13 mg/g时,脱臭馏出物的酸值(KOH)在120 mg/kg左右,生育酚含量总体在4%～5%,但适当降低脱酸程度,可减少菜籽油中生育酚损耗。

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