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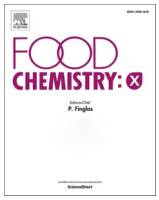

New insights into the off-flavor improvement of soymilk by three grinding processing: Dry-blanching grinding, wet-blanching grinding, and wet-anaerobic grinding

Xiaoxiao Feng [a], Yiwen Zhu [a], Yufei Hua [b,*]

[a] Department of Food Science & Technology, School of Agriculture & Biology, Shanghai Jiao Tong University, Shanghai 200240, PR China
[b] State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, PR China

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ABSTRACT

Advances in grinding strategies have been beneficial to eliminating the off-flavor of soymilk and improving the quality soy products. Herein, four grinding processing, dry-blanching grinding (D-BG), wet-blanching grinding (W-BG), wet-anaerobic grinding (W-AG) and traditional grinding (TG) were employed and found to impose a significant impact on off-flavor components, accompanied by changes of hydroperoxides and free radicals. The results showed that all three methods could significantly hinder the formation of C6 aldehydes. C8 Alcohols and (E)-2-heptenal could be removed by D-BG, but lipids in dehulled soybean were prefer to be oxidized during storage, resulting in the accumulation of hydroperoxides and radicals. W-BG and W-AG have higher levels of 1-octen-3-ol, and soaking at an alkaline pH and increasing the number of rinses is beneficial for its removal. Gas chromatography-olfaction-mass spectrometry (GC-O-MS) combined with sensory evaluation showed that off-flavor profile of D-BGS, W-BGS and W-AGS was different. D-BG and W-AG possessed better flavor quality.

1. Introduction

Soymilk itself, a vegetable protein beverage made from soybeans, is gaining wide acceptance worldwide for its health benefits in preventing heart disease, cancer, osteoporosis, syndrome, and aging (Jia et al., 2020; Liu, 2016; Ramdath et al., 2017). At the same time, soymilk is the starting material not only for traditional soy foods, such as tofu, yuba, but also for many emerging plant-based products, such as soy yogurt, soy cheese, and soy ice cream (Lee et al., 2014; Tunick & Van Hekken, 2015; Zhou et al., 2019). However, soybean products have been limited, particularly in western societies, due to its "beany" flavour (Yu et al., 2018). Short chain (ranging from five to eight carbons) aliphatic aldehydes, alcohols, and ketones are largely responsible for the "beany" flavour (Lampi et al., 2020; Stolterfoht et al., 2019). In general, these volatile compounds are formed when seeds are soaked in water and disrupted for processing. Several approaches have been tested to suppress the formation of off-flavor compounds (Arora & Damodaran, 2010; Fischer et al., 2022; Mellor et al., 2010; Zhang et al., 2018). Recently, many grinding technologies have been put forward in terms of inhibiting the off-flavor of soymilk, including dry dehulled soybeans blanching, wet-blanching and anaerobic grinding.

Blanching soybeans is a commonly used technique for soymilk preparation (Xie & Fretzdorff, 1992). Lv et al. (2011) believed that blanching soybeans in boiling water for 10 min could completely block the lipoxygenases (LOXs) reaction and reduce the content of hexanal. However, blanching could not completely remove the "beany" flavor, soymilk still contained high content of 1-octen-3-ol, (E)-2-nonenal and (E, E)-2,4-decadienal. Studies have shown that LOXs and other enzymatic reactions occur during the soaking process. Our previous study showed that the soaking stage can lead to the large formation of 1-octen-3-ol, 3-octanol and (E)-2-heptenal, meanwhile, LOXs pathway products (hexanal and (E)-2-hexenal) were begin to produce (Feng et al., 2022). So, controlling soaking conditions, such as soaking pH and temperature, soybean-water ratio, rinsing times and blanching conditions are important aspect in improving off-flavor quality of soymilk. Dry blanching grinding is one of the common strategies used to reduce the off-flavor. Nowadays, the emerging cold grinding technologies, such as anaerobic grinding (Kaharso et al., 2021), have been promoted. Kaharso et al. (2021) found that the content of volatiles in anaerobic soymilk (75.04 μg/mL) was lower than that of traditional soymilk (966.65 μg/

* Corresponding author at: State Key Laboratory of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, Jiangsu Province, PR China.
E-mail address: hzlandfxx09011217@126.com (Y. Hua).

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mL), and anaerobic grinding could significantly reduce lipid oxidation. To date, few reports are available on the flavor properties of of anaerobically ground soymilk.

Lipid hydroperoxides are the primary oxidation products formed in lipid oxidation reactions (Cao et al., 2021). Hydroperoxides are highly reactive and can be further decomposed into secondary oxidation products, such as aldehydes, ketones, or react with other components of food (Ito et al., 2015; Steltzer, 2012). Analysis of hydroperoxides can further reflect the reaction mechanism of the lipid oxidation front in food (Koch et al., 2022; Santas et al., 2014). Studies have shown that lipid peroxidation is such a process which produces many different lipid-derived radicals (Saporito-Magrina et al., 2017; Yin et al., 2011). However, there are few reports on the use of free radical detection methods to study lipid oxidation in soymilk.

The objective of this research is to investigate the impact of different processing methods on the off-flavor compounds, lipid hydroperoxides, and free radicals of soymilk. The study compares traditional grinding (TG), dehulled dry-blanching grinding (D-BG), wet-blanching grinding (W-BG), and wet-anaerobic grinding (W-AG) methods. The study also evaluates the soybean milk samples using a combination of intelligent and artificial senses to determine the impact of different conditions on the quality of processed soymilk. The findings of the current study will provide baseline data and new insights for soymilk industries in developing the best soymilk processing technology to provide high-quality soymilk to consumers.

2. Materials and methods

2.1. Materials

Soybean [*Glycine* max (L.) Merr.] seeds were purchased from local market (*Wuxi,* China) in 2021 and stored in 4 ℃ refrigerator until use. Hexanal (>98%), heptanal (>97%), octanal (>97%), nonanal (>95.0%), (E)-2-hexenal (99%), (E)-2-heptenal (>95.0%), (E)-2-nonenal (>98%), (E)-2-octenal, (97.0%), 1-octen-3-ol (>95.0%), 3-octanol (>97.0%), 1-octen-3-one (>95.0%), (E,E)-2,4-decadienal (>90.0%), (E,E)-2,4-nonadienal (>90.0%), 2-pentyl furan (>97%), 3-metyl butanol (>97%) and 2-methyl-3-heptanone (99%) were purchased from Sigma-Aldrich Co., Ltd (St. Louis, MO, USA). 1,2-Dimyristoyl-*sn-glycero*-3-phosphatidylcholine (DMPC, 99%), 15-hydrogen peroxide (15-HPED) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) was purchased from Sigma-Aldrich, further purified with activated carbon/benzene, and stored at −20 °C before use.

2.2. Preparation of soymilk

Four different processing technologies were employed to prepare soymilk. The detailed description of these technologies is given below.

2.2.1. Dry-blanching method grinding (D-BG)

Soybean seeds were oven dried at 80 °C for 2 h and dehulled. The dehulled soybean seeds (intact cotyledons) were selected and placed at 25 °C (exposed to the air for accelerating oxidation), then a portion was taken out every 4 days, filled with nitrogen and stored at 4 °C. Hundred gram of dehulled soybean seeds were blanched in 100 ℃ for 5 min at a soybean to water ratio of 1:7 (w/w), respectively. Then, boiling water was added to a total mass of 800 g, the mixture was hot ground for 2 min, and then filtered with a 100-mesh sieves to obtain a slurry, named dry-blanching grinding soymilk (D-BGS). The filtering step was done by the same person until no soymilk was pressed out to maintain the consistency. Soymilk from dehulled soybeans with different storage times was prepared as above.

2.2.2. Wet-blanching grinding (W-BG)

To prepare each batch of soymilk, 100 g of soybean seeds were placed in a beaker containing 700 mL deionized water, incubated at room temperature (25 ℃) and different pH values (pH 7.0, pH 9.0, pH 10.0) for 12 h. The hydrated soybeans were drained, rinsed for 0, 1 2, 3 times, and then blanched with deionized water at a temperature (70, 80, 90, 100 ℃) for 1, 2, 3, 5, 8, and 10 min. The mixture was then transfer to a blender (SQ2119, XIBEILE Group Ltd., Shanghai, China) and hot ground for 3 min with a bean-to-water ratio of 1:7 (w/w). In order for the grinding temperature to be accurately maintained at desired temperature, the blender was preheating twice with boiling water, and immediately, blanched soaked soybeans with blanching water were added. All of these temperatures were determined in preliminary tests. After grinding, the soymilk was manually filtered through a 100-mesh sieves, named wet-blanching grinding soymilk (W-BGS).

2.2.3. Wet-anaerobic grinding (W-AG)

All the soaking and grinding procedures were same as W-BG, except blanching. The entire process is carried out in a nitrogen-filled glove bag. After filtration, the soymilk was obtained, named wet-anaerobic method grinding soymilk (W-AGS).

2.2.4. Traditional grinding (TG)

100 g of soybean seeds were placed in a beaker containing 700 mL DI water, incubated at 25 ℃ for 12 h, and drained, rinsed for 3 times, and then cold grinding for 3 min with a bean-to-water ratio of 1:7 (w/w). After filtration, the soymilk was obtained, named traditional grinding soymilk (TGS). TGS was selected as a control.

2.3. Headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC–MS) analysis

Soymilk (5.0 mL) obtained above were transferred to glass vials (20 mL, Agilent, USA) separately and to each vial 10 μL of 2-methyl-3-heptanone (Internal Standard, IS, 320 μg/mL) was added. The volatiles were collected at 40 ℃. The SPME fiber (50/30 μm DVB/Carboxen/PDMS, preconditioned at 250 °C for 30 min immediately prior to use) was exposed to the headspace of 1 cm from the sample surface and stirring extracted at 40 ℃ for 30 min. A GC–MS system (QP 460, Bruker, Germany) equipped with a DB-WAX column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, Agilent, USA) was used to analyze the volatile compounds. The compounds were positively identified by comparison of their mass spectra, RI and flavor properties with those of authentic standards. A compound was tentatively identified upon analysis without an authentic standard using the previously mentioned criteria only. For quantification, A series of concentrations of the standard compounds with an added internal standard 2-methyl-3-heptanone was established.

2.4. Aroma extract dilution analysis by gas chromatography–olfactometry-mass spectrometry (GC-O-MS)

The key odor active volatiles in soymilk were analyzed by GC-O-MS. The volatiles were separated by a DB-WAX capillary column and entered into the MS and the olfactometer (Sniffer-8226, Shimadzu, Kyoto, Japan) at a ratio of 1:1 (v/v). The odor intensity is based on a 5-point scale, and the inspectors record the odor intensity according to their perception of the intensity of the odor. The sample was diluted by increasing the GC inlet split ratio from 4:1 to 16:1, 64:1, 256:1.

2.5. Odor-activity value (OAV) analysis

To evaluate the contribution of key volatile odor compounds to soymilk, odor activity value (OAV) was defined as the ratios of the concentration of one flavor calculated on basis of the standard curve to its corresponding threshold in water (Gemert, 2011). Volatile compounds with OAV > 1 may have a major contribution to soymilk, whereas compounds with OAV less than 1 indicate a minor contribution.

2.6. Sensory evaluation

Sensory evaluation was performed according to previous study with minor modification. A total of 80 trained panelists from Jiangnan University were invited for this study, including 45 females and 35 males, 23–28 years of age. Prior to the sensory evaluation, panelists were trained in the sensory evaluation of food products, and received an additional training of soymilk, and also reached an agreement on the definition and specific attributes of soymilk flavor. Six typical flavor reference samples were prepared, which were green, fruity, fatty, cucumber, mushroom and flower flavor. Paper ballots with rating scales were used, and a scoring system of 0–5 points was used to represent ''none'' to ''high intensity'' of a flavor that was perceived. Samples (20 mL) were dispensed in 30 mL portion cups labeled randomly with 3-digit codes, covered, and kept at 25 ℃. The panelists evaluated the samples independently according to the intensity of a specific flavor sensed and were given a 3 min rest between individual samples.

2.7. Hydroperoxide analysis and electron spin resonance (ESR) analysis

Hydroperoxides were analyzed according to methods previous reported (Feng et al., 2022).

The lipid radicals were extracted based on the method reported (Feng et al., 2020).

2.9. Statistical analysis

All of the experiments were performed in triplicate, and the mean values of triplicate experiments were used. Statistical significance analysis ($p < 0.05$) was performed using SPSS 22.0. The differences were compared by one-way analysis of variance (ANOVA) at $p = 0.05$. Values marked with different letters were different significantly ($p < 0.05$).

3. Results and discussion

3.1. Identification of off-flavor compounds in four varieties of soymilks

Table 1A showed that 22, 29 and 26 kinds of volatile compounds were detected in D-BGS, W-BGS and W-AGS, respectively. Different kinds of volatile compounds had different effects on the flavor of D-BGS, W-BGS and W-AGS. Saturated aldehydes (hexanal, heptanal), alkenals ((E)-2-hexenal, (E)-2-decenal), and alkadienals (E, E)-2,4-nonadienal and (E, E)-2,4-decadienal) imparted green and fatty flavor. C8 alcohols (1-octen-3-ol and 3-octanol) and 1-octen-3-one imparted typical mushroom flavor. Meanwhile, (E)-2-heptenal and (E, Z or E, E)-2,6-nonadienal contributed a fruity flavor. Notably, hexanal showed the highest FD value (256) in W-BGS compared to that in D-BGS and W-AGS. In W-BGS and W-AGS, FD values of 1-octen-3-ol were 64 and 256, respectively, higher than that in D-BGS, so it contributed greatly to the flavor of mushroom and earthy. In addition, (E)-2-heptenal with a higher FD value of 16 in W-BGS, which may lead to a fruity flavor in W-BGS.

According to the differences of formation mechanisms, volatile components of soymilk are mainly divided into four categories, namely C6 aldehydes (hexanal and (E)-2-hexenal), C8 alcohols (1-octen-3-ol and 3-octanol), (E)-2-heptenal and others volatiles, which contained mainly (E)-2-octenal, (E)-2-nonenal, (E, E)-2,4-decadienal, 2-pentyl furan. Other volatiles are widely accepted from lipid autooxidation or photooxidation. The contents of volatiles in soymilk obtained from different

Table 1A
Identification of off-flavor compounds in soymilk.

[a]No.	Compounds	[b]RI	[c]FD factor			[d]Oder description	[e]Identification
			D-BGS	W-BGS	W-AGS		
1	pentanal	896	4	4	4	Grass, green	MS, RI, O, S
2	hexanal	1089	64	256	64	Grass, green	MS, RI, O, S
3	heptanal	1184	4	4	4	Citrus-like, green	MS, RI, O, S
4	octanal	1293	ND	4	16	Fruity, fatty	MS, RI, O, S
5	nonanal	1398	4	4	4	Citrus, tallowy, floral	MS, RI, O, S
6	decanal	1496	4	4	4	Fatty, floral, green	MS, RI, O, S
7	benzaldehyde	1520	4	4	4	Almond	MS, RI, O, S
8	(E)-2-hexenal	1165	4	4	4	Grass, green	MS, RI, O, S
9	(E)-2-heptenal	1326	4	16	4	Fruity, oily	MS, RI, O, S
10	(E)-2-octenal	1428	4	4	4	Green, fatty	MS, RI, O, S
11	(Z)-2-nonenal	1533	4	4	16	Earthy, fatty	MS, RI, O
12	(E, E)-2,4-heptadienal	1492	ND	4	ND	Fatty, nutty, oily, rancid	MS, RI, O, S
13	(E, Z or E, E)-2,6-nonadienal	1786	ND	4	ND	Cucumber	MS, RI, O
14	(E, E)-2,4-nonadienal	1780	ND	16	4	Fatty, green, waxy	MS, RI, O, S
15	(E, E)-2,4-decadienal	2003	ND	4	4	Deep-fried, fatty	MS, RI, O, S
16	3-methyl butanol	958	4	4	4	Fruity	MS, RI, O, S
17	1-pentanol	1021	4	4	4	Fatty, floral, green	MS, RI, O
18	1-hexanol	1367	4	4	4	Grass, green	MS, RI, O, S
19	2-ethyl hexanol	1390	4	4	4	Grass, green	MS, RI, S
20	1-octen-3-ol	1510	4	64	256	Mushroom, Earthy	MS, RI, O, S
21	3-octanol	1582	4	4	4	Mushroom, Earthy	MS, RI, O, S
22	1-nonanol	1560	4	4	4	Flowery	MS, RI, O, S
23	(E)-2-nonen-1-ol	1622	4	4	4	Earthy, fatty	MS, RI, O
24	2-methyl furan	1042	ND	4	4	Grass, green	MS, RI, O
25	2-ethyl furan	1150	ND	4	4	Grass, green	MS, RI, O
26	2-penyl furan	1237	16	16	ND	Butter,fruity, green bean	MS, RI, O, S
27	1-octen-3-one	1533	64	16	4	Mushroom, Earthy	MS, RI, O, S
28	6-methyl-5-hepten-2-one	1480	4	4	4	Fatty	MS, RI, O, S
29	5-methyl-3-heptanone	1392	4	4	4	Green, fatty	MS, RI, O

ND, not detected.

[a] Odorants were consecutively numbered according to their retention indices on capillary DB-WAX.

[b] RI were determined using a homologous series of *n*-alkanes (C7-C40) on DB-WAX capillary columns.

[c] FD factor was determined by AEDA.

[d] Odor description was detected by GC-O.

[e] MS, identified by NIST 14 mass spectral database; RI, agreed with the retention indices published in literature; O, agreed with the odor characteristics published in literature; S, the authentic standard chemicals.

grinding methods were shown in supporting information (Table S1, S2, S3 and S4).

3.2. Effect of grinding processing condition on off-flavor compounds

3.2.1. Storage of dehulled soybean on off-flavor of D-BGS

D-BG processing method is used to improve off-flavor of soymilk. Dehulled soybean seeds, also called soybean cotyledons, were used for D-BGS preparation. However, in actually, dehulled seeds inevitably undergo a storage period before processing, which could lead to be oxidized due to the loss of seed coat (Achouri et al., 2008). As shown in Fig. 1A. Compared to the TGS, the contents of C6 aldehydes, C8 alcohols, (E)-2-heptenal and other volatiles in D-BGS (0 d) were significantly reduced, and were 57.3 μg/kg, 8.8 μg/kg, 10.1 μg/kg and 74.0 μg/kg cotyledon, respectively. Since hypocotyls were removed along with the coat during peeling, the contents of C8 alcohols and (E)-2-heptenal in soymilk were lower, 8.8 μg/kg and 10.1 μg/kg, respectively. In addition, when blanching, the rapid inactivation of glycosidase inhibited the

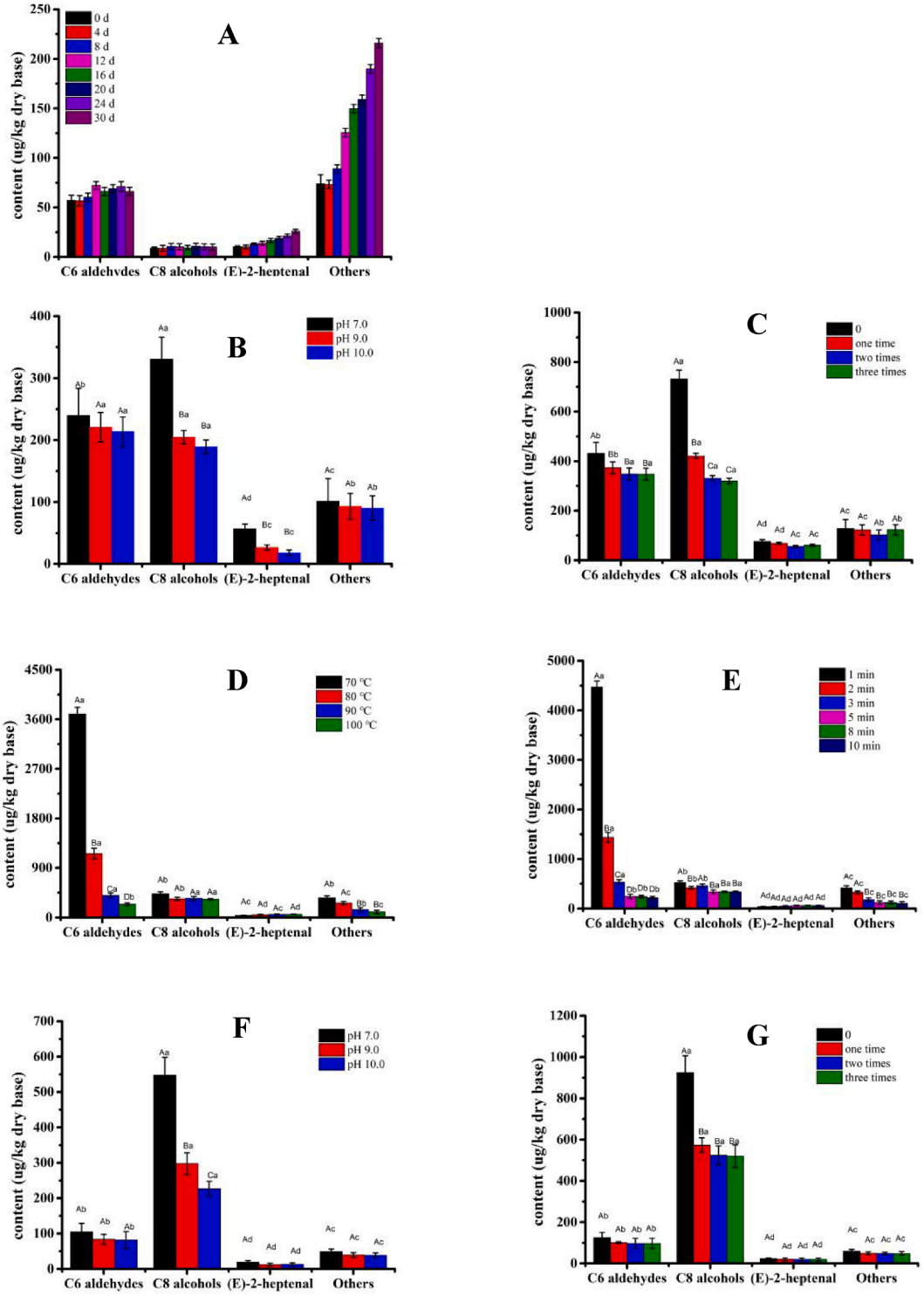

Fig. 1. Effect of processing technologies on soymilk volatiles. Effect of storage time (A) on off-flavor compounds in D-BGS. Effects of soaking (B), rinsing (C) and blanching (D, E) conditions on off-flavor compounds in W-BGS. Effects of soaking (F) and rinsing (G) conditions on off-flavor compounds in W-AGS.

hydrolysis of C8 alcohol-β-primeveroside, resulted in decreasing in C8 alcohols. Nevertheless, with an increase in storage time, the content other volatiles significantly increased, and the content of (E)-2-heptenal also showed an increasing trend, but with a lower increased absolute content. C6 aldehydes and C8 alcohols remained unchanged. The results showed that when dehulled soybean was fully exposed to air or light, the autooxidation and photooxidation were accelerated. Both (E)-2-octenal and 2-pentyl furan can make soymilk a taste of fatty, which is not conducive to the overall flavor acceptability of soymilk.

In conclusion, storage of dehulled soybeans can have negative effects on D-BGS preparation due to the increased oxidation of the soybean cotyledons. It is important to minimize the storage time of dehulled soybeans before processing to ensure optimal flavor in the final product.

3.2.2. Effects of soaking, rinsing and blanching conditions on off-flavor of W-BGS

Herein, effects of soaking pH, rinsing times and blanching conditions on the off-flavor compounds of W-BGS were investigated, and the results were presented in Fig. 1B, 1C, 1D and 1E, respectively. The content of C8 alcohols decreased significantly after immersion at pH 9.0, followed by (E)-2-heptenal (Fig. 1B). It was reported that the optimal pH of soybean endogenous β-glucosidase was 5.5, and the activity reduces to 30% ~40% when pH is 9.0 (Hsu et al., 2015; Ismail & Hayes, 2005). Therefore, when soybeans were soaked under alkaline conditions, the activity of endogenous β-glycosidase reduced, resulting in the content of C8 alcohol lower than that of at pH 7.0. In addition, Fe^{2+} was reported less stable under alkaline conditions, which would decrease the degradation rate of 12-PEOOH sharply(Feng et al., 2020), so the formation of (E)-2-heptenal was inhibited. The content of C6 aldehydes also showed a slight decrease in alkaline soaking compared with neutral soaking, but there was no significant difference. The content of other volatiles was not affected by the soaking pH. As shown in Fig. 1C, after rinsing, the contents of four off-flavor components were decreased, especially C8 alcohols, followed by C6 aldehyde. Studies have shown that C8 alcohols were formed in large quantities during soaking and were easily leached into the soaking water (Feng & Hua 2022). In this study, about 30% of C8 alcohols were removed after one time of rinsing, and >50% of C8 alcohols were removed after two times of rinsing. And the content of C6 aldehyde was reduced by about 15% after two times of rinsing. No significant effect on the content of (E)-2-heptenal and other volatiles. Fig. 1D and 2E showed the effects of blanching temperature and blanching time on the off-flavor components of W-BGS, respectively. With the increase of blanching temperature, the contents of C6 aldehydes decreased significantly. Compared with 70 °C, C6 aldehydes was reduced by 69% after blanching at 80 °C, 89% at 90 °C, and to less than 1% at 100 °C. The results showed that blanching, especially 100 °C treatment, could significantly reduce the contents of C6 aldehydes. However, the contents of C8 alcohols and (E)-2-heptenal did not decrease after blanching. Within 1 to 5 min, the contents of C6 aldehydes and other volatiles decreased significantly with the increase of blanching time, and remained constant at 5 to 10 min. Similarly, blanching time had no effect on the contents of C8 alcohols and (E)-2-heptenal (Fig. 1E).

3.2.3. Effects of soaking and rinsing conditions on off-flavor of W-AGS

The key of anaerobic grinding in improving off-flavor of soymilk is to remove or reduce the concentration of oxygen. Under hypoxia condition, the LOX pathway is inhibited, so the content of C6 aldehyde in W-AGS was significantly lower than that of TGS. Fig. 1F showed the effect of soaking pH on off-flavor contents of W-AGS. Soaking at pH 9.0 reduced the C8 alcohols content by 45% and at pH 10.0 by 59%. The content of C8 alcohols of rinsed soybeans was significantly reduced compared with that of un-rinsed soybeans. And after the first and second rinsing, the content of C8 alcohols decreased by 38% and 45%, respectively.

3.3. Effect of grinding processing condition on lipid hydroperoxides

3.3.1. Effect of storage of dehulled soybean on lipid hydroperoxide of D-BGS

According to the findings presented in Fig. 2A, the hydroperoxide content of D-BGS (0 d) was significantly lower than that of TGS. This can be attributed to the rapid deactivation of LOX and HPL during blanching, which inhibited the formation of enzyme-catalyzed hydroperoxide during grinding. However, the hydroperoxide content in D-BGS increased significantly with the longer storage time of dehulled soybeans. Specifically, the levels of 9-PCOOH and 9-HPOD began to rise after 8 days of storage, and continued to increase with longer storage time. When the storage time exceeds 16 d, 13-PCOOH, 13-HPOD and 12-PEOOH also showed small increase trend (Fig. 2A). Studies have shown that lipid autoxidation was more likely to occur in 9-position carbon atoms of unsaturated fatty acids(Morita & Tokita, 1993) and it was speculated that the significant increase of 9-hydroperoxide content may be related to the accelerated rate of lipid autoxidation.

3.3.2. Effects of soaking, rinsing and blanching conditions on lipid hydroperoxides of W-BGS

The study investigated the impact of soaking pH, rinsing, and blanching on the lipid hydroperoxide in W-BGS. The results were presented in Fig. 2B, 2C, 2D, and 3E, respectively. Under alkaline conditions, the degradation rate of 12-PEOOH was lower. Therefore, 12-PEOOH content of W-BGS prepared by alkaline soaking was higher than that by neutral soaking (Fig. 2B). Compared to unrinsed soaked soybeans, rinsing treatment removed only small amounts of 9-HPOD and 13-HPOD and had no effect on esterified hydroperoxides, indicating that free hydroperoxides were more likely to diffuse into water. As shown in Fig. 2C and 2D, contents of 13-PCOOH, 9-PCOOH and 9-HPOD decreased significantly when the blanching temperature was higher than 80 °C. Compared with 70 °C, blanching at 90 °C could reduce the contents of 13-PCOOH, 9-PCOOH and 9-HPOD to 60%, 35% and 35%, respectively. After blanching at 100 °C, the contents of three hydroperoxides were reduced by 80%, 50% and 43%, respectively, which was similar to the results of C6 aldehydes. Blanching had no effect on the content of 12-PEOOH. Within 1 to 5 min, the contents of 13-PCOOH, 9-PCOOH and 9-HPOD decreased significantly with the increase of blanching time. From 5 to 10 min, 9-PCOOH showed an increasing trend, which may be related to lipid autooxidation or hydroperoxide isomerization (Fig. 2E).

3.3.3. Effects of soaking and rinsing conditions on lipid hydroperoxides of W-AGS

Except for 12-PEOOH, the content of hydroperoxide in W-AGS was less than 1 μmol/kg. The 12-PEOOH content of soybean soaked in alkaline condition was higher than that soaked in neutral condition. However, the content of other hydroperoxides did not change (Fig. 2F). As shown in Fig. 2G, small amounts of 9-HPOD and 13-HPOD were removed by rinsing, while the content of esterified hydroperoxides was almost unchanged, consistent with the results of the effects of rinsing conditions on W-BGS.

3.4. Effect of grinding processing condition on free radicals

A certain number of free radicals could be detected in D-BGS (Fig. S1 (a)), W-BGS (Fig. S1(b)) and TGS (Fig. S1(d)), but no signal in W-AGS (Fig. S1(c)). By computer fitting the ESR spectra of D-BGS, four different kinds of radicals were obtained, which were identified as peroxyl radical (DMPO-LOO·, α^N = 13.24G, α^H = 11.39G), long chain alkoxyl radical (DMPO-LO·, α^N = 13.01G, α^H = 6.82G, 1.73G), long-chain carbon centered lipid free radicals (DMPO-L·, α^N = 14.8G, α^H = 20.98G), DMPO radicals (DMPO-X·; α^N = 15.1 G). These four free radicals also were detected in W-BGS. Five different radical adducts were observed in TGS, and they were DMPO-X·, DMPO-L·, DMPO-LOO·, short-chain alkoxyl

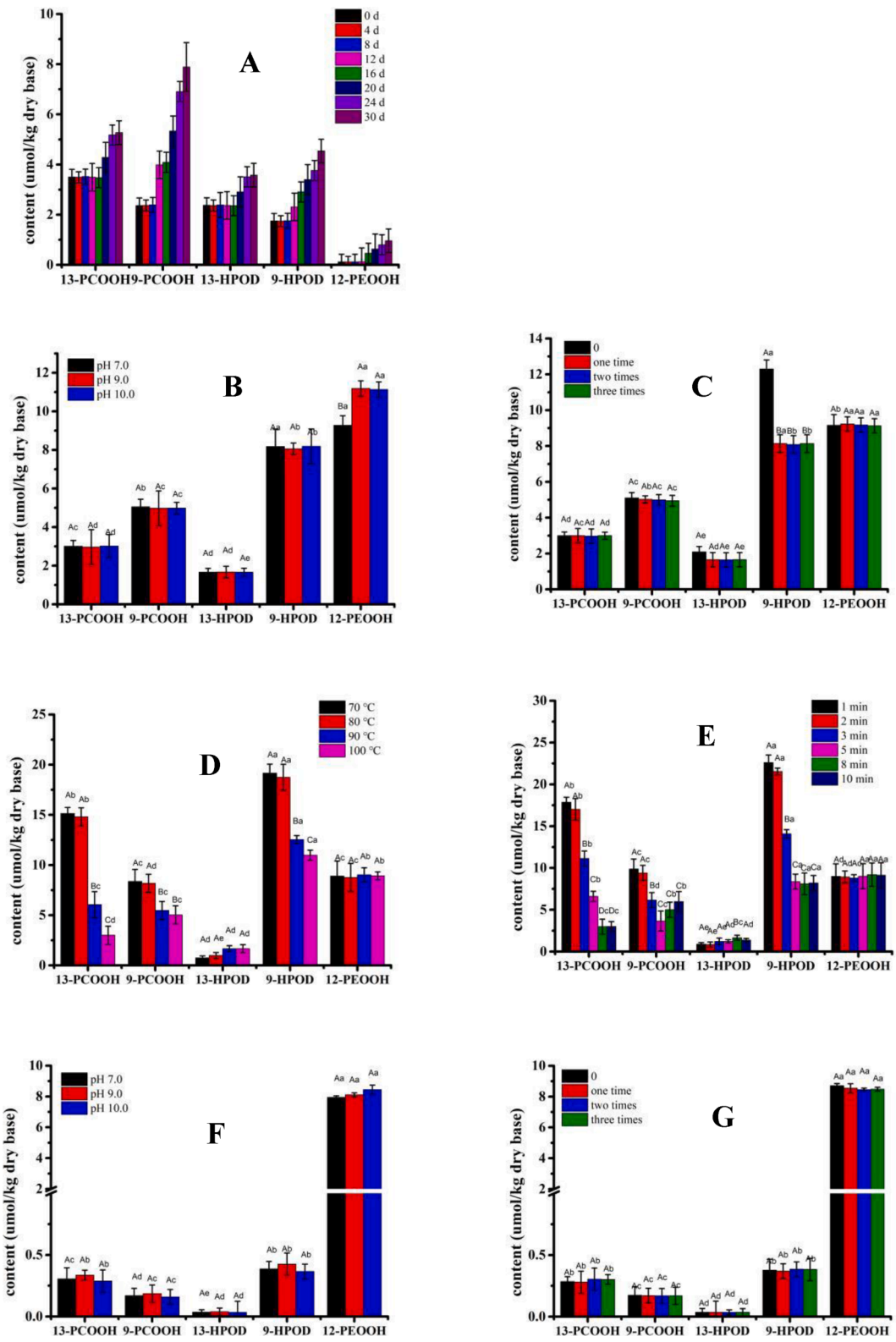

Fig. 2. Effect of processing technologies on soymilk hydroperoxides. Effect of storage time (A) on hydroperoxides in D-BGS. Effects of soaking (B), rinsing (C) and blanching (D, E) conditions on hydroperoxides in W-BGS. Effects of soaking (F) and rinsing (G) conditions on hydroperoxides in W-AGS.

radical (DMPO-RO·, α^N = 12.2G, α^H = 9.7G, 1.5G) and short-chain carbon centered radical (DMPO-R·, α^N = 14.6G, α^H = 22.3G), respectively. Due to the low signal strength of some free radical species, the accuracy of computer fitting was not reliable. Therefore, the influence of different processing methods and different processing conditions on free radicals was studied by analyzing the change of total amount of free radicals (total amounts of spins).

3.4.1. *Storage of dehulled soybean on total amounts of spins of D-BGS*

From 1 to 20 days, the total amounts of spins increased slowly, and

increased significantly after 20 days (Fig. 3A). Exposed to the air, the lipid autooxidation rate of dehulled soybeans was accelerated. The results showed that the content of 9- hydroperoxide in D-BGS increased significantly during storage. There was a correlation between free radicals and the storage time during the storage of dehulled soybeans, and the total amounts of spins could to a certain extent reflect the degree of lipid autoxidation.

3.4.2. Effects of soaking, rinsing and blanching conditions on total amounts of spins of W-BGS

Free radicals could be formed during immersion. However, most free radicals were very reactive with other components and caused a series of unfavorable effects, resulting in lower amounts actually captured and measured. Fig. 3B, 3C, 3D and 3E respectively showed the effects of soaking pH, rinsing times and blanching conditions on the total amounts of spins in W-BGS. The total amounts of spins did not change under alkaline pH immersion (Fig. 3B). And in rinsed soybeans, total amounts of spins were reduced compared to unrinsed soybeans (Fig. 3C). Higher than 90℃ blanching treatments of soybeans, the total amounts of spins did not decrease, but showed a significant increase trend (Fig. 3D), indicating that the formation rate of free radicals was greater than its decay rate. In addition, the total amounts of spins decreased firstly and then increased with the increase of blanching time (Fig. 3E). It was speculated that blanching treatment leads to thermal oxidation of lipids, which induced free radical chain reaction.

3.5. Orthogonal partial least squares discrimination analysis

A distance of observation (DModX) analysis was also used to identify and eliminate any outliers. 'DCrit (critical value of DModX)', derived from the F-distribution, calculates the size of observational area under analysis. The DModX plot of the PCA data indicates that no samples exceeded the threshold for rejecting a sample. The threshold for a moderate outlier is considered when the sample DModX value is twice the DCrit at 0.05, which, in this instance, was 2.778 (DCrit = 1.389) (Fig. 4). In order to discriminate the soymilk samples, an OPLS-DA model was applied to these data comparing the different processing samples. The subsequent OPLS-DA score scatter plot and OPLS-DA loading scatter plot are presented in Fig. 4, and this illustrates a clear separation between the two groups, with R^2X, R^2Y and Q^2 values of 0.968, 0.99 and 0.975, respectively. This is indicative of a model that reasonably fits the data and has moderately strong predictive capability.

3.6. Key odor active volatiles of D-BGS, W-BGS, W-AGS and TGS

The composition and content of odor active off-flavor compounds of OVA > 1 in three kinds of soymilks were significantly different (Table 1B). The total amounts of off-flavor compounds in D-BGS were 149.28 ± 5.34 μg/kg, among which the content of 2-pentyl furan was the highest, followed by hexanal. The total amounts of off-flavor compounds in W-BGS and W-AGS were 726.26 ± 77.52 μg/kg and 685.70 ±

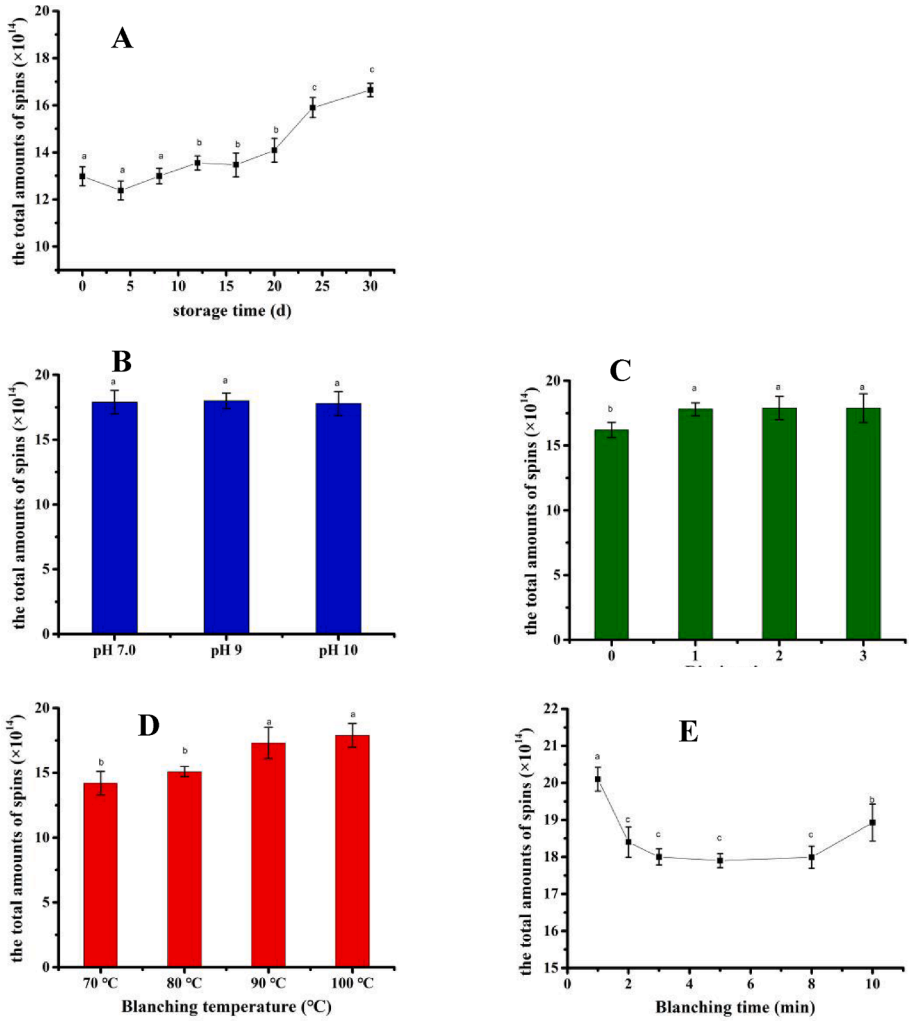

Fig. 3. Effect of processing technologies on soymilk free radicals. Effect of storage time (A) on hydroperoxides in W-BGS. Effects of soaking (B), rinsing (C) and blanching (D, E) conditions on hydroperoxides in W-BGS.

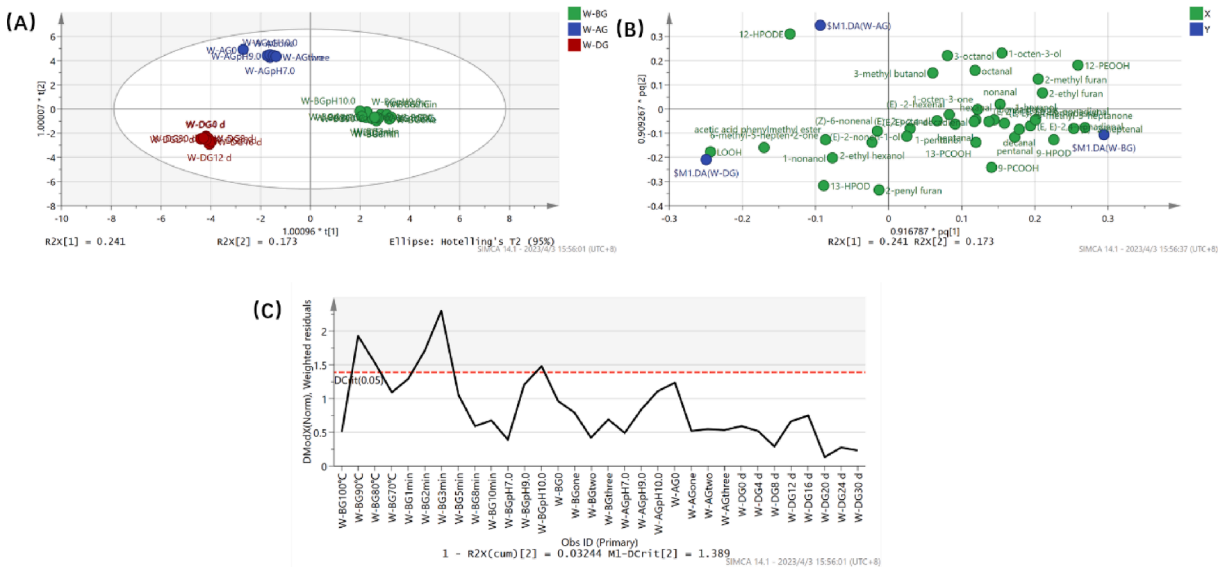

Fig.4. Multivariate analysis of off-flavors, hydroperoxides and free radicals of soymilk with different processing methods. A, Scores of orthogonal partial least squares discrimination analysis (OPLS-DA), $R^2X = 0.968$, $R^2Y = 0.99$, $Q^2 = 0.975$; B, Distance of observation (DModX) plot, the red dotted line represents DCrit value (1.389) of this model; C, Loading scatter plot of OPLS-DA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

42.71 μg/kg, respectively, which were much higher than that in D-BGS. The content of hexanal in W-BGS was the highest, followed by 1-octen-3-ol and 2-pentylfuran, and the content of 1-octen-3-ol in W-AGS was the highest of 526.10 ± 35.07 μg/kg. Dry blanching may lead to the oxidative degradation of lipids, resulting in the formation of 2-pentyl furan. The soaking process in wet blanching and anaerobic grinding provided a favorable environment for the hydrolysis of C8 alcohol-β-primeveroside and the oxidation reaction mediated by metal ions and LOX, thus easily led to the formation of off-flavor compounds such as 1-octen-3-ol, hexanal and (E)-2-heptenal. Six key odor active volatiles (OAV > 1), hexanal, (E)-2-octenal, (E)-2-nonenal, 1-octen-3-ol, 2-pentylfuran and 1-octen-3-one, were identified. The OAV value of hexanal in W-BGS was 48.67 ± 3.12, while that in D-BGS and W-AGS were 11.52 ± 0.77 and 17.19 ± 1.15, respectively. 1-Octen-3-ol had the highest OAV value (58.01 ± 23.38) in W-AGS, and the lowest OAV value (1.26 ± 0.39) in D-BGS. The flavor contribution rate of 1-octen-3-one was the largest (58.42%±0.91%), followed by hexanal (17.53%±2.08%) in D-BGS. A total of 11 odor active volatiles were detected in W-BGS, among which hexanal contributed the most (30.11%±1.00%), followed by 1-octen-3-ol (23.93%±4.57%). The contribution rate of 1-octen-3-ol (61.51%±6.04%) was the highest in W-AGS. The main odor characteristics of D-BGS were fatty, green and mushroom flavor, and fatty flavor was mainly from the high content of 2-pentyl furan. W-BGS showed a variety of odor characteristics, mainly reflected in green and mushroom flavor, corresponding to the content of hexanal and 1-octen-3-ol respectively. For W-AGS, the main odor characteristics were fruity and mushroom. C6 aldehydes based green flavor was the core flavor feature of TGS. The results showed that three grinding techniques could be used for off-flavor improvement by reducing grassy flavor, and make the overall flavor characteristics of soymilk more diversified.

3.7. Sensory evaluation of D-BGS, W-BGS, W-AGS and TGS

From Fig. 5B, the sensory scores of the three kinds of soymilk were significantly different, among which W-AGS had the lowest overall score, followed by D-BGS. W-BGS had the highest overall score. Green flavor was the highest in W-BGS, followed by fatty and mushroom flavor. In W-AGS, mushroom flavor scored highest, followed by flower and fruity flavor. The results of sensory evaluation were consistent with those of GC-O-MS. The flavor quality of the three kinds of soymilk were significantly better than that of TGS.

4. Conclusion

This study explored the new technology of soybean milk flavor improvement from the perspectives of off-flavors, lipid hydroperoxides and free radicals, which provided a new insight for the improvement of soymilk flavor quality and had important practical guiding significance for the improvement of soymilk process strategy. (i) D-BG could remove C8 alcohols and (E)-2-heptenal in soymilk. However, lipids were prone to be oxidized during storage of dehulled soybean. With the increase of storage time, 2-pentyl furan and (E)-2-octenal increased. The contents of 1-octen-3-ol in W-BGS and W-AGS were the highest, followed by hexanal and (E)-2-heptenal. Alkaline soaking and rinsing were beneficial to the removal of 1-octen-3-ol. Wet blanching could significantly inhibit the reaction of LOX pathway, especially boiling for 5 min. (ii) Blanching would cause lipid non-enzymatic reactions, resulting in the accumulation of 9-hydroperoxides and free radicals in soymilk. 9-Hydroperoxides and free radicals also showed a correlation with storage time during the storage of dehulled soybeans. (iii) The results of GC-O-MS and sensory evaluation showed that the main odor characteristics of D-BGS were fatty, green and mushroom, and that of W-BGS was green and mushroom flavor, W-AGS was fruit and mushroom flavor. Overall, D-BGS and W-AGS had better flavor. Fully understanding the formation mechanism of flavor substances during the soaking and refining process is of great significance to the improvement of the processing technology of low-beany-flavored soymilk and the development of industrial production. Exploring the relationship between volatile components, lipids, hydroperoxides and free radicals will be our next research target.

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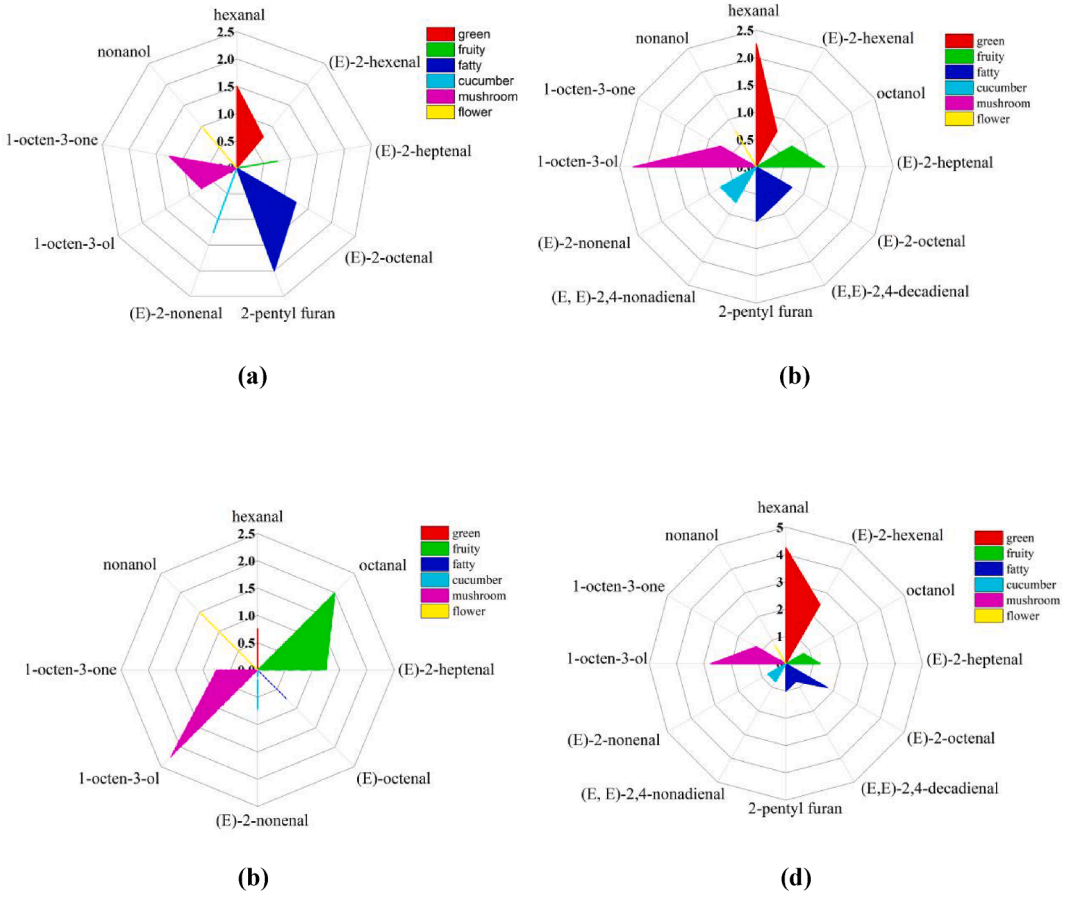
Table 1B
Key odor active volatiles of OAV > 1 and their contribution in soymilk.

Volatile compounds	[a]Threshold in water (μg/kg)	W-BGS			D-BGS			W-AGS		
		[b]Concentration (μg/kg)	[c]OAV	[d]Contribution (%)	Concentration (μg/kg)	OAV	Contribution (%)	Concentration (μg/kg)	OAV	Contribution (%)
hexanal	4.5	210.92 ± 14.06	48.67 ± 3.12	30.11 ± 1.00	51.85 ± 3.46	11.52 ± 0.77	17.53 ± 2.08	77.35 ± 5.16	17.19 ± 1.15	18.23 ± 0.30
heptanal	3	4.25 ± 0.28	1.42 ± 0.09	0.91 ± 0.03	ND	ND	ND	4.15 ± 0.28	1.38 ± 0.09	1.47 ± 0.02
octanal	0.7	0.93 ± 0.06	1.33 ± 0.09	0.85 ± 0.03	ND	ND	ND	2.91 ± 0.19	4.16 ± 0.28	4.41 ± 0.07
(E)-2-heptenal	13	56.92 ± 3.79	4.38 ± 0.29	2.81 ± 0.09	ND	ND	ND	19.22 ± 1.28	1.48 ± 0.10	1.57 ± 0.03
(E)-2-octenal	3	3.04 ± 0.20	1.01 ± 0.07	0.65 ± 0.02	3.14 ± 0.21	1.05 ± 0.07	1.59 ± 0.19	7.50 ± 0.50	2.50 ± 0.17	2.65 ± 0.04
(E)-2-nonenal	0.4	0.98 ± 0.07	2.44 ± 0.16	1.57 ± 0.05	1.33 ± 0.09	3.33 ± 0.22	5.07 ± 0.60	1.94 ± 0.13	4.86 ± 0.32	5.15 ± 0.08
(E,E)-2,4-nonadienal	0.1	1.20 ± 0.08	12.00 ± 0.80	7.71 ± 0.26	ND	ND	ND	ND	ND	ND
(E,E)-2,4-decadienal	0.03	0.23 ± 0.02	8.52 ± 0.57	5.47 ± 0.18	ND	ND	ND	ND	ND	ND
1-octen-3-ol	1.5	320.8 ± 21.39	37.26 ± 14.26	23.93 ± 4.57	8.80 ± 0.59	1.26 ± 0.39	1.91 ± 1.06	526.10 ± 35.07	58.01 ± 23.38	61.51 ± 6.04
2-pentyl furan	5.9	63.7 ± 4.25	10.80 ± 0.72	6.93 ± 0.23	60.03 ± 4.23	10.17 ± 0.68	15.48 ± 1.83	ND	ND	ND
1-octen-3-one	0.003	0.09 ± 0.00	29.67 ± 0.64	19.06 ± 0.21	0.12 ± 0.00	38.40 ± 0.34	58.42 ± 0.91	0.01 ± 0.00	4.73 ± 0.32	5.02 ± 0.08

Values represent the means ± SD (n = 3).
ND: not detected.
[a] Odor thresholds were from reference (Gemert, 2011).
[b] The average concentration of sample.
[c] OAVs were calculated by dividing the concentrations by the odor thresholds.
[d] The percentage of OAV for each compound.



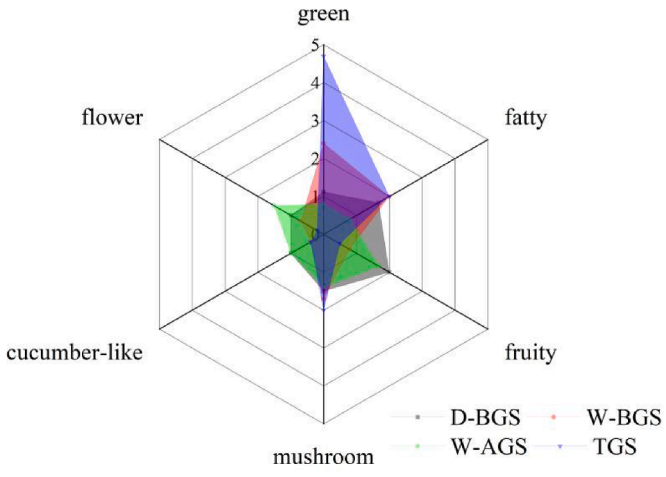


Fig. 5. **(A)** GC-O analysis of D-BGS、W-BGS、W-AGS compared with TGS. **(B)** Sensory evaluation of four soymilks.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Xingfei Li reports financial support was provided by Jiangnan University.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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