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Effects of water absorption of soybean seed on the quality of soymilk and the release of flavor compounds

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The water absorption of soybeans during soaking is directly related to the quality characteristics and the flavor properties of soybeans for processing. In this paper, the effects of water absorption of soybean seed on the quality of soymilk and the release of flavor compounds were investigated during soaking at 4 °C, 25 °C, and 50 °C at different pH values. The results showed that the water absorption rate increased as the soaking temperature and pH increased, while the equilibrium value was relatively stable. Peleg's equation with good fitting of the absorption kinetics was used to predict the hydration characteristics of undehulled soybean. MALDI-TOF/TOF-MS results showed that the major released proteins are basic 7S globulin, which is released in large amounts at high temperature. The water absorption of soybean seed significantly enhanced the extraction yields of protein, fat and solids of the prepared soymilk, and alkaline soaking pH further promoted the extraction of proteins and solids. A high soaking temperature can significantly decrease the required soaking time; however, it is unfavorable to the extraction yields of fat, proteins and solids, as well as the whiteness values and the particle sizes. The beany odor compounds of soymilk mainly consisted of hexanal, *trans*-2-hexenal, 1-octene-3-ol, hexanol, and 2-pentylfuran, and their contents were positively correlated with soaking temperature. A good balance of soymilk quality and flavor compound release can be achieved with soaking conditions of 25 °C and pH 9.

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1. Introduction

Soymilk is rich in protein, vitamins, and isoflavones and provides a balanced amino acid composition; thus, it has long been recognized as a good plant source of whole protein. Soy beverages are the most important traditional beverages that are consumed widely in Asian countries; extensive evidence has indicated the strong relationship between soy food consumption and health-promoting effects.[1,2] Soymilk is also used as an important milk substitute for milk allergy patients or people with lactose intolerance.[3]

Currently, with the emergence of soygurt and many functional foods, high soymilk quality is becoming an increasing concern. Soymilk preparation is a key step in the processing of soy products, and soybean soaking is an important step in the preparation of soymilk. Soybean soaking is a traditional process that is mainly used to soften the texture of soybeans and to facilitate their subsequent processing. Soaking temperature and soaking time are the two key factors affecting the water absorption of soybeans[4] and the quality of the subsequent soy products, such as soymilk, tofu, and soygurt. Soaking at 40 °C to 60 °C can decrease lipoxygenase activity and thus improve the digestibility of soybean proteins.[5] However, high soaking temperatures induce greater loss of solids, such as proteins and isoflavones, into the aqueous medium.[4] Prolonged soaking times are not necessary to generate additional softening of the grains because excessive soaking often results in bacterial growth and does not contribute to further water absorption.[6]

The degree of water absorption in the soybean soaking process directly affects the texture of the soybeans and their grinding characteristics during processing. Pan *et al.* found that the grinding characteristics of soybeans were only related to the equilibrium water absorption rate, regardless of soaking conditions.[4] Water absorption of legumes has been investigated using several rather complex models. Joshi *et al.* studied the water absorption of three different types of lentils at different soaking temperatures; they reported that the water absorption process can be fitted using the Mitscherlich model at high temperatures (50 °C and 85 °C), while this model is only acceptable for "Boomer" lentils at room temperature.[7] Another typical fitting model is the Peleg equation, which has been used in recent years to model the water absorptions of different grains and foods during soaking;[8–10] meanwhile, the

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relationship between the kinetic parameters and temperature can be further fitted by the Arrhenius equation or an empirical formula.[11]

Because most research focuses on the characteristics of water absorption, solid loss, moisture content, textural characteristics and grinding properties of soybeans, there is still a lack of systematic investigation of the subsequent quality of soybean products, such as soymilk and tofu. Giri *et al.* showed that soybeans became easier to grind after soaking in carbonate solution compared with immersion in deionized water or NaOH solution; the prepared soymilk was found to have high protein content and viscosity as well as less beany flavor.[12] There is still controversy about whether the extraction rate of proteins and solids is the highest when the maximum water absorption rate is reached, and how the composition of soymilk changes.[13,14]

The flavor of soymilk is an important factor affecting its acceptance. Many factors affect the flavor of soymilk, such as storage conditions, enzymes, and soaking conditions.[15] Several methods have been used to reduce off-flavors of soybeans, including vacuum deodorization treatment and adding other flavor substances; pretreatment strategies mainly focus on how to prevent or reduce the formation of off-flavor compounds, such as blanching enzymes, dry bean refining, anaerobic pulping, and soaking treatment with acid or alkali solution. Badenhop *et al.* showed that when the soaking temperature was 50 °C, the content of 1-octene-3-ol in soymilk increased with soaking time.[16] Ha *et al.* found that boiling soybeans in $NaHCO_3$ solution can effectively inhibit the production of daidzein and genistein and decrease the formation of several volatile organic compounds in soybeans.[17] However, the relationship between water adsorption and the quality and flavor release of soymilk is still poorly understood, and systematic study is required.

In this paper, the effects of soaking conditions on the kinetics of water absorption of soybeans were firstly studied to predict the adsorption equilibrium of soybeans at different pH values and temperatures. Then, the effects of soaking conditions on the release of proteins were further investigated using SDS-PAGE and MALDI-TOF/TOF-MS. Based on the above results, the quality of prepared soymilk was carefully studied with regard to the extraction yield of fat, proteins and solids of soymilk, as well as the changes in color and particle size. Finally, the volatile compounds in soymilk were evaluated by headspace GC-MS analysis.

2. Materials and methods

2.1 Materials

Dry soybeans (*Glycine max*) were purchased from a local supermarket and stored at room temperature in a dark room. All other reagents were of analytical grade.

2.2 Soaking of dry soybeans

20 g dry soybeans were placed in a beaker containing 100 mL of deionized water and incubated at different soaking temperatures (4 °C, 20 °C, 30 °C, 40 °C, 50 °C) and soaking times (0 to 24 h). Soaking experiments at pH 3 and pH 9 were carried out using 200 mM pH 3 citrate buffer solution or 0.5% $NaHCO_3$ (pH 9) solution.

The moisture content (M_t) of the soybean seed was expressed as the difference in the soybean seed before and after soaking, as follows:

$$M_t = \frac{W_t - W_0}{W_0} \times 100 \quad (1)$$

where t is the soaking time (h); W_t is the mass of the soybeans after soaking; and W_0 is the mass of the soybeans before soaking.

2.3 Analysis of soaking data with soaking models

Peleg's model[18] was used to model the water absorption of soybean seed during soaking. The equation is expressed as follows:

$$M_t = M_0 + \frac{t}{k_1 + k_2 t} \quad (2)$$

where M_t is the moisture content at time t (%), M_0 is the initial moisture content (%), t is time (h), and k_1 and k_2 are the Peleg rate and Peleg capacity constant, respectively. k_1 is negatively correlated with sample water absorption rate (h %$^{-1}$), while k_2 is positively correlated with sample water absorption capacity (%$^{-1}$). When the soaking time is infinite, the equilibrium moisture content of the sample $M_e = M_0 + \frac{1}{k_2}$ can be obtained.

2.4 Preparation of soymilk

The soaked soybeans were mixed with water at a 1 : 9 mass ratio and pulped for 30 s using a JYL-C012 Joyoung Soymilk Maker (Joyoung, Hangzhou, China). Then, 500 mL of the mixture was filtered through a Buchner funnel to obtain the raw soymilk. The raw soymilk was heated at 95 °C for 10 min to obtain the cooked soymilk.

2.5 SDS-PAGE and mass spectrometry of proteins

The soaking solutions with different soaking conditions were collected and freeze-dried. Then, the protein distributions were analyzed by Tricine-SDS-PAGE according to our previous work.[19] All the samples were dissolved in 2.0 mL loading solution and then reduced with 2% (v/v) 2-mercaptoethanol in boiled water for 3 to 5 min. 10 μL of sample solution were loaded per well. Protein molecular weight markers were included in each gel. The electrophoresis was conducted using a DYY-8c vertical electrophoresis apparatus provided by LiuYi Biotechnology, Co., Ltd, (Beijing, China) at a constant voltage of 30 V for the stacking gel (4%) and 100 V for the separation gel (16%). The gel was stained with 0.05% Coomassie Brilliant Blue G-250.

The unknown proteins in the soaking solutions were identified by mass spectrometry (MALDI-TOF/TOF-MS).[20] Briefly, the protein spots on the Tricine-SDS-PAGE gel were excised and destained using 30% (v/v) acetonitrile containing 100 mM NH_4HCO_3. The destained gel was then washed with 100% acetonitrile and incubated in 10 μL reducing solution (100 mM DTT and 90 μL of 100 mM NH_4HCO_3) for 30 min at 56 °C. After washing with 100% acetonitrile, 70 μL of 100 mM NH_4HCO_3 and 30 μL of 200 mM iodoacetic acid were added, and the mixture was incubated for 20 min in the dark. After dehydrating the sample with 100% acetonitrile, 5 μL of 10 μg mL^{-1} of trypsin were added and the solution was incubated at 37 °C for 20 h to induce protein hydrolysis. The resulting peptide solution was incubated at 30 °C until a dry powder was obtained, and then the powder was dissolved in 3 μL of 0.1% (v/v) TFA. For MALDI-TOF/TOF analysis, 0.7 μL of the sample and 0.7 μL of 4-hydroxy-α-cyanocinnamic acid (used as a matrix) were loaded on a MALDI target and air-dried. Mass spectrometric analysis was performed in batch mode using an ABI 4700 Proteomics Analyzer (Ultraflex TOF/TOF, Bruker Daltonik GmbH, Bremen, Germany). Mass spectrum data were analyzed using 203 flex-Analysis software; then, we searched for plant proteins in the NCBInr database, and only those protein hits were evaluated.

2.6 Measurement of volatile flavor substances

Volatile substance analysis was performed according to the method reported by Achouri *et al.*[21] with some modifications. Carboxen–polydimethylsiloxane (CAR–PDMS) fiber (85 μm) was used to perform headspace solid-phase microextraction (HS-SPME). 5 mL sample solution and 1 μL of 2-methyl-3-heptanone (0.25 mg mL^{-1}) were placed into 10 mL glass vials, and a gas-tight syringe was used for sample preparation at 40 °C for 30 min.

A gas chromatograph-mass spectrometer (GC-MS, 1200 L, Varian, USA) equipped with a DB-WAX column (0.25 μm, 30 m × 0.25 mm) was used to analyze the volatile substances. The temperature was programmed as follows: the initial temperature was maintained at 40 °C for 3 min and then increased to 100 °C at 6 °C min^{-1}. The temperature was then increased to 230 °C at 10 °C min^{-1} and held for 7 min. The adsorbed volatiles in the fiber were desorbed without splitting at 260 °C for 7 min. Compounds were identified based on the National Institute of Standards and Technology (NIST) database through a Saturn mass spectra library search.

2.7 Color analysis of soymilk

Raw soymilk and cooked soymilk were placed in cuvettes, and their surface colors were determined with a colorimeter (Minolta, CR-310, Ramsey, NJ) using the Hunter Lab color scale. Before each test, the colorimeter was calibrated using a Minolta standard-white reflector, and the light source was a pulsed xenon lamp. Data were represented by the L^* (brightness/darkness), a^* (redness/greenness) and b^* (yellowness/blueness) values of the international color system. Each sample was tested in duplicate.

2.8 Particle sizes of soymilk

The particle sizes of raw soymilk and cooked soymilk were measured using a Zetasizer Nano ZS instrument (Malvern, UK). This system employs a 633 nm laser and a fixed scattering angle (173°) that is sufficiently high to justify neglect of the contribution from rotational diffusion effects to the autocorrelation files. The protein with a 1.450 refractive index (RI) was selected as the material, and water was selected as the dispersant (0.8872 cP, RI: 1.330). The soymilk was diluted 100 times before the experiment, and each sample was tested at 25.0 ± 0.1 °C. Each test was performed in duplicate.

2.9 Statistical analysis

All the measurements were performed three times with parallel samples. Statistical significance analysis ($p \leq 0.05$) was performed using the SPSS 13.0 statistical analysis program. The data shown in the corresponding figures are the mean values with the standard deviation.

3. Results and discussion

3.1 Kinetics of water absorption of soybean seed

Fig. 1 shows the water absorption rate as a function of soaking temperature and time. The maximum water absorption was around 130%, reached after soaking at 4 °C for 24 h, 20 °C for 12 h, 30 °C for 9 h, 40 °C for 5 h, and 50 °C for 3 h, respectively. This value is very similar to the 129% value reported by Chopra *et al.*[22] The water absorption curves display typical water intake behavior in legumes.[23,24] The soaking process of dry soybeans mainly depends on the driving force induced by the difference in moisture content at the saturation time and at a certain time; therefore, the equilibrium at different temperatures should theoretically be roughly equal, as shown in Fig. 1. The moisture content decreased slightly after soaking for 3 h at 50 °C; this may be due to the release of other substances, such as solid matter, from the soaked soybeans. Similar results have been reported for other plant seeds, such as pasta sorghum, milled rice, and barley seeds.[25–27]

The water absorption curves were fitted with Peleg's equation, eqn (2), and the related parameters are shown in Table 1. The coefficients of regression, R^2, for the four different temperatures varied from 0.966 to 0.995. This indicates a very good fit of the model to the experimental data and suggests that Peleg's equation is well suited to describing the water absorption of soybean seed during soaking. The results also showed

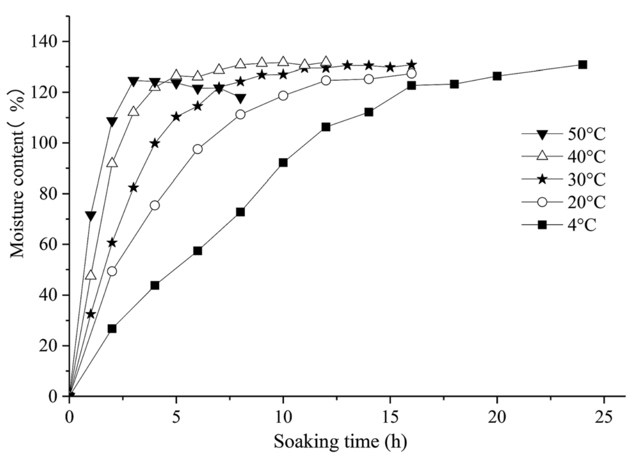

Fig. 1 Moisture content of soybeans at different soaking temperatures and times.

Table 1 Peleg's parameters of soybean seed soaked at different temperatures

Peleg's equation constant	Temperature (°C) 4	20	30	40	50
k_1	0.0674	0.0272	0.0170	0.0093	0.0049
k_2	0.0044	0.0059	0.0062	0.0065	0.0073
R^2	0.9869	0.9950	0.9815	0.9711	0.9656
W_0	14.84	36.83	58.86	107.76	204.50
M_e	226.24	169.20	160.26	153.85	137.55

that k_1 decreased with increasing temperature, while the initial water absorption rate W_0, equal to $1/k_1$, increased with increasing temperature. Here, the Arrhenius equation was additionally used to construct the relationship between the constant k_1 and temperature:[28]

$$\ln\frac{1}{k_1} = \ln k_0 - \frac{E}{R}\times\frac{1}{T} \tag{3}$$

Then:

$$\ln\frac{1}{k_1} = 20.75 - 5019.06\times\frac{1}{T}\left(R^2 = 0.9901\right) \tag{4}$$

It can be seen that the linear coefficient of the equation is good ($R^2 = 0.9901$); thus, the Arrhenius equation can better express the influence of temperature during soybean soaking.

There is no unified description of the relationship between the capacity constant K_2 and temperature depending on the sample material. A simple linear fit was applied to obtain the following equation:

$$k_2 = 6.9152\times10^{-5}\ \mathrm{T} - 0.0146\ (R^2 = 0.9476) \tag{5}$$

In this study, k_2 increased with increasing temperature, which is consistent with the results previously reported by Jideani *et al.*[29]

To verify the effectiveness of Peleg's equation, we further investigated the water absorption of soybean seed (with or without dehulling) at 4 °C, 25 °C and 50 °C, as shown in Fig. 2.

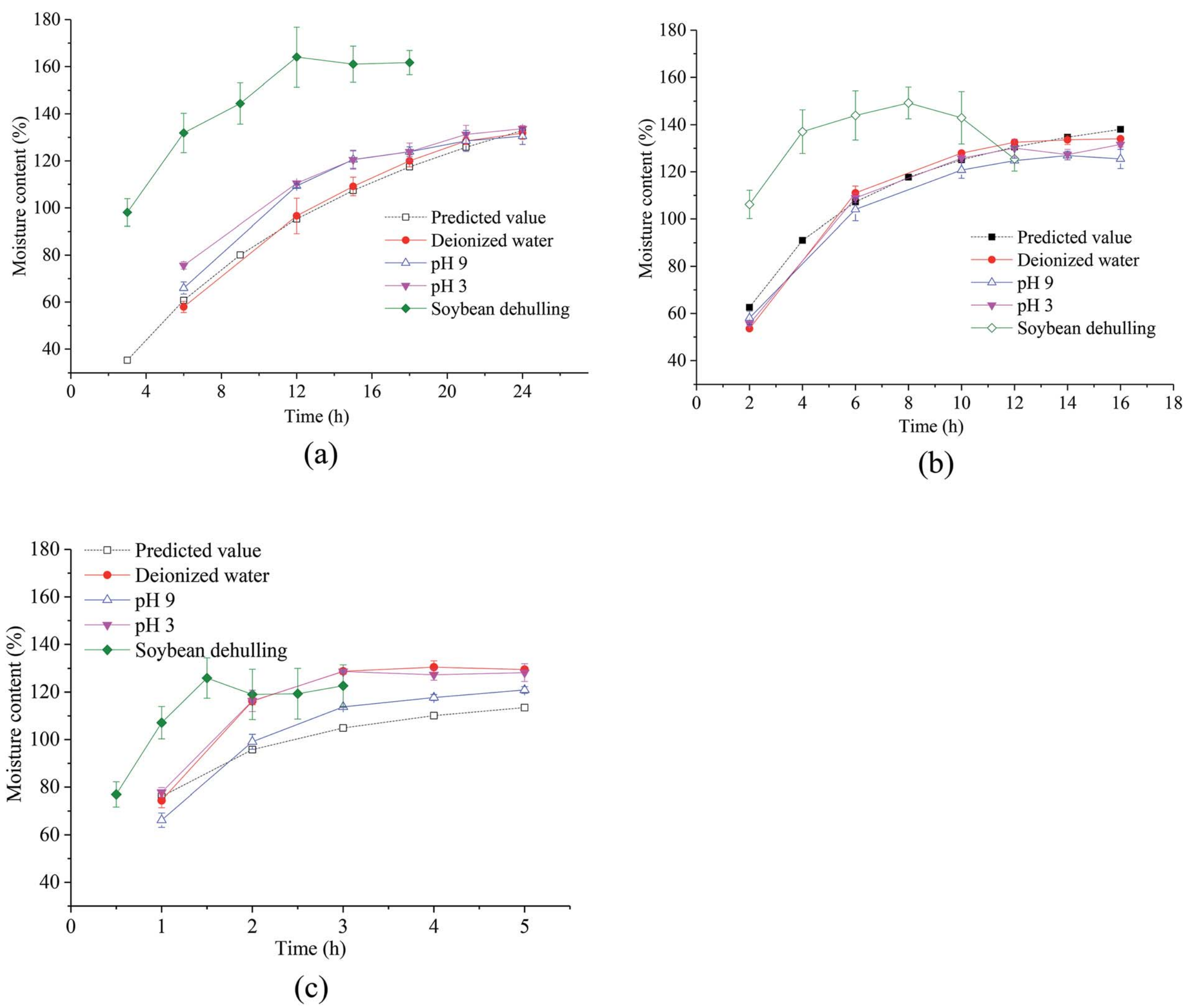

Fig. 2 Moisture absorption characteristics and predicted Peleg models fitted on undehulled and dehulled soybeans during soaking at different temperatures: (a) 4 °C; (b) 25 °C; (c) 50 °C at different soaking pH values.

At 4 °C and 50 °C, good fitting of the water absorption curves for undehulled soybean with Peleg's equation was obtained in neutral pH conditions (deionized water). For the 4 °C soaking treatment, the water absorption became faster when soaking in acid or alkaline pH solution; for the 50 °C soaking treatment, the water absorption became slower in alkaline soaking solution; there was no significant difference in water absorption ($p > 0.05$) between different soaking pH values for the 25 °C soaking treatment. Therefore, there were some deviations from the predictions by Peleg's equation when the pH or temperature was changed. However, different soaking treatments achieved very similar equilibrium values, suggesting the independence of the water adsorption saturation of soybeans. For the 25 °C soaking treatment, Peleg's equation could also be used to predict the water absorption of undehulled soybeans at three different pH values, and the changes in pH had insignificant effects ($p > 0.05$) on the process of water absorption. These results show that the process of water absorption varied with the soaking conditions (pH, temperature), while the water adsorption equilibrium value remained relatively stable and was not affected by these factors.

However, the fitting equation was not suitable for dehulled soybeans because a large deviation ($p < 0.05$) from the predicted values was observed. As shown in Fig. 2, at the soaking temperatures of 4 °C, 25 °C and 50 °C, the water absorption rates of the soybeans after dehulling were greater ($p < 0.05$) than those of the undehulled soybeans, while the time required to reach water adsorption equilibrium greatly decreased due to the absence of the seed coat (which prevents the free intake of water).

3.2 Influence of soaking conditions on the release of protein components

Although different soaking treatments can obtain very similar equilibrium values of water adsorption, the effects of soaking conditions on the release of components are still unclear. The released protein components after soaking were investigated using SDS-PAGE, and the results are shown in Fig. 3. It was found that after soaking at low temperatures (4 °C and 25 °C, Fig. 3b and c), some known and unknown proteins were found in the soybean seed exudates. According to their molecular weights and soybean protein isolate (SPI) compositions (Fig. 3c), the known protein bands corresponded to the α, α′, and β subunits of β-conglycinin (7S globulin) and the AB (acidic-basic) and B (basic) subunits of glycinin (11S globulin), similar

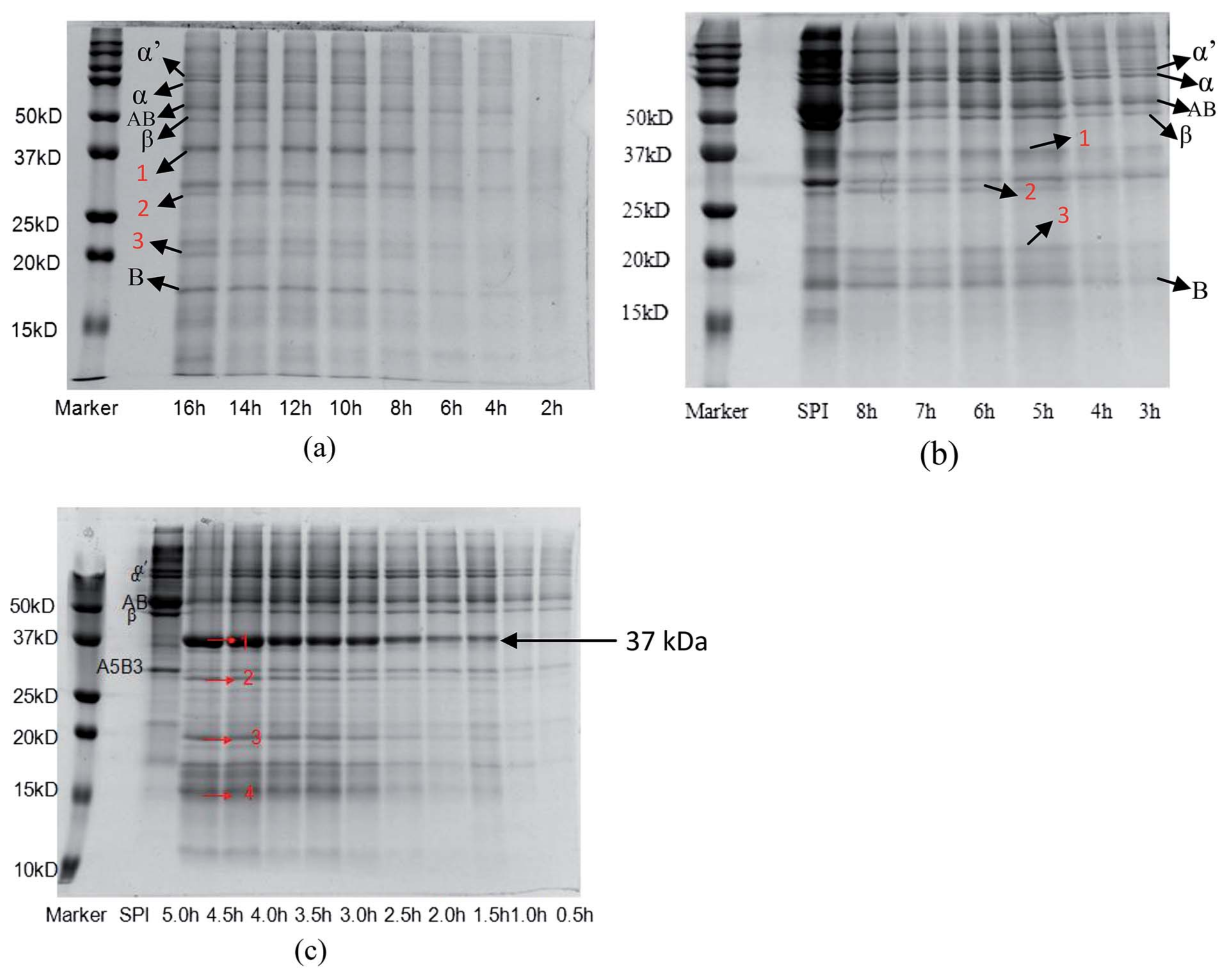

Fig. 3 SDS-PAGE of proteins released from soybean seeds incubated in warm water at (a) 20 °C; (b) 40 °C; (c) 50 °C.

to results observed in other studies.[30,31] With increasing soaking time, the release of 7S and 11S proteins increased. As is well known, 7S and 11S proteins are seed storage proteins that are stored in organelles. In dry soybean seeds, these proteins are protected by the seed coat. However, after soaking in warm water, our confocal laser scanning microscopy results (unpublished data) showed that the structure of the seed coat became increasingly loose, and some space between cells appeared after a certain soaking time. Therefore, 7S and 11S proteins will be released by the force of the concentration difference, and the release rate depends on the soaking temperature and soaking time.

Apart from the seed storage proteins, the unknown proteins found in the soybean seed exudates are named bands 1, 2, 3 and 4 (Fig. 3c) in this paper, respectively. Especially, the concentration of band 1 (around 37 kDa) was greatly enhanced after soaking at high temperature (50 °C). These unknown protein bands were further identified by MALDI-TOF/TOF-MS. As shown in Table 2, bands 1–4 were matched to basic 7S globulin (*G. max*), *endo*-1,3-beta-glucanase, 24 kDa seed coat protein precursor (*G. max*), and seed maturation protein PM22 (*G. max*), respectively. The 24 kDa seed coat protein precursor (SC24) is present in the aleurone layer or parenchyma of the seed coat; it is a newly discovered plant defense protein with carboxylate binding activity.[32] The 37 kDa protein was identified as alkaline 7S globulin (Bg7S), which is an extracellular matrix protein;[33] it was released in large amounts at high temperature, indicating that the cell wall structure is destroyed and that water is more likely to enter the cells. Thus, the water absorption rate of the soybeans accelerated at 50 °C. Bg7S is rapidly released when soybean seeds are immersed in water at 50 °C to 60 °C.[34,35] When the soaking temperature was less than 40 °C, a small amount of Bg7S was released (as seen in Fig. 3a and b), which is consistent with previous reported results.[36] However, protease inhibitors were not detected in the soybean seed exudates in the present case, although they were reported in studies by Palavalli *et al.*[36] and Hirano *et al.*[34]

3.3 Effects of soaking conditions on the quality of soymilk

3.3.1 Extraction yields of fat, proteins and solids in soymilk. Before preparing soymilk, the beating time should be optimized to obtain a high soymilk yield. In our preliminary experiment, considering the relationships between beating time, soaking conditions, and protein and solid extraction rates, a 30 s beating time was selected for the next experiment.

Fig. 4 shows the fat extraction yields of soymilk as a function of soaking time at various soaking pH values and temperatures. For soaking at pH 9, the maximum fat extraction yields were 80.93% (4 °C), 79.02% (25 °C), and 73.81% (50 °C) after 18 h, 12 h and 3 h of soaking, respectively. The corresponding water absorption rates were 123.89%, 124.76% and 113.79%, respectively. When soaked in pH 3 solution, the maximum fat extraction yields were 79.31% (4 °C), 79.52% (25 °C), and 65.39% (50 °C) after 24 h, 10 h and 3 h, respectively; the water absorption rates were 133.68%, 125.74% and 128.68%, respectively. Therefore, the maximum fat extraction yield was lowest at 50 °C and pH 3; meanwhile, at 4 °C and pH 9, the maximum fat extraction yield was the highest. These results also showed that the extraction of fat had a relatively positive correlation with the soaking time and was negatively correlated with the soaking temperature.

At soaking temperatures of 4 °C and 25 °C, there was no significant difference ($p > 0.05$) in the fat extraction yields at different pH values. For 50 °C soaking treatment, the fat extraction yields were lower at pH 3 and pH 9 than that at neutral pH, especially at pH 3 ($p < 0.05$). At high temperature and acidic pH, some fat compounds may be lost due to hydrolysis; meanwhile, under alkaline conditions, fat saponification can occur at pH 9, which can also decrease the fat extraction yield. At soaking temperatures of 4 °C and 25 °C, the hydrolysis and saponification reactions cannot readily occur; thus the fat extraction yields did not appear to be affected by the changes in pH.

As shown in Fig. 5, the protein extraction yield increased gradually with increasing soaking time for soybeans soaked at 4 °C. Soaking at pH 3 or pH 9 obviously improved the protein extraction yields compared with neutral soaking pH. For the 25 °C and 50 °C soaking treatments, compared to neutral soaking pH, soaking at alkaline pH (pH 9) improved the protein extraction yield of soymilk, while soaking at acid pH (pH 3) significantly decreased ($p < 0.05$) the protein extraction yield. The highest and lowest maximum protein extraction yields were 80.06% and 65.01% when soaking at 25 °C, pH 9 and 50 °C, pH 3, respectively. In an acidic environment, the formation of 11S globulin aggregates is promoted by hydrogen bonding[37] and the dissolution rate will decrease during grinding of soaked soybeans, resulting in a decrease in the protein extraction yield. On the other hand, increased viscosity of the slurry soybean mixture after grinding was observed after soaking at pH 3, which is disadvantageous for protein extraction.

Fig. 5 shows that the protein concentration of soymilk showed a similar trend to that observed in the protein

Table 2 Proteins identified in the soybean seed exudates after SDS-PAGE separation and MALDI-TOF/TOF MS

Band	Protein ID	M_r[a]	pI[a]	NBCInr accession no.
1	Basic 7S globulin (*G. max*)	47 105	8.68	gil356505878
2	*Endo*-1,3-beta-glucanase	36 638	8.88	gil351723211
3	24 kDa seed coat protein precursor (*G. max*)	24 611	9.14	gil351721970
4	Seed maturation protein PM22 (*G. max*)	16 735	5.16	gil351727629

[a] Molecular weights (M_r) and isoelectric points (pI) are given as theoretical values.

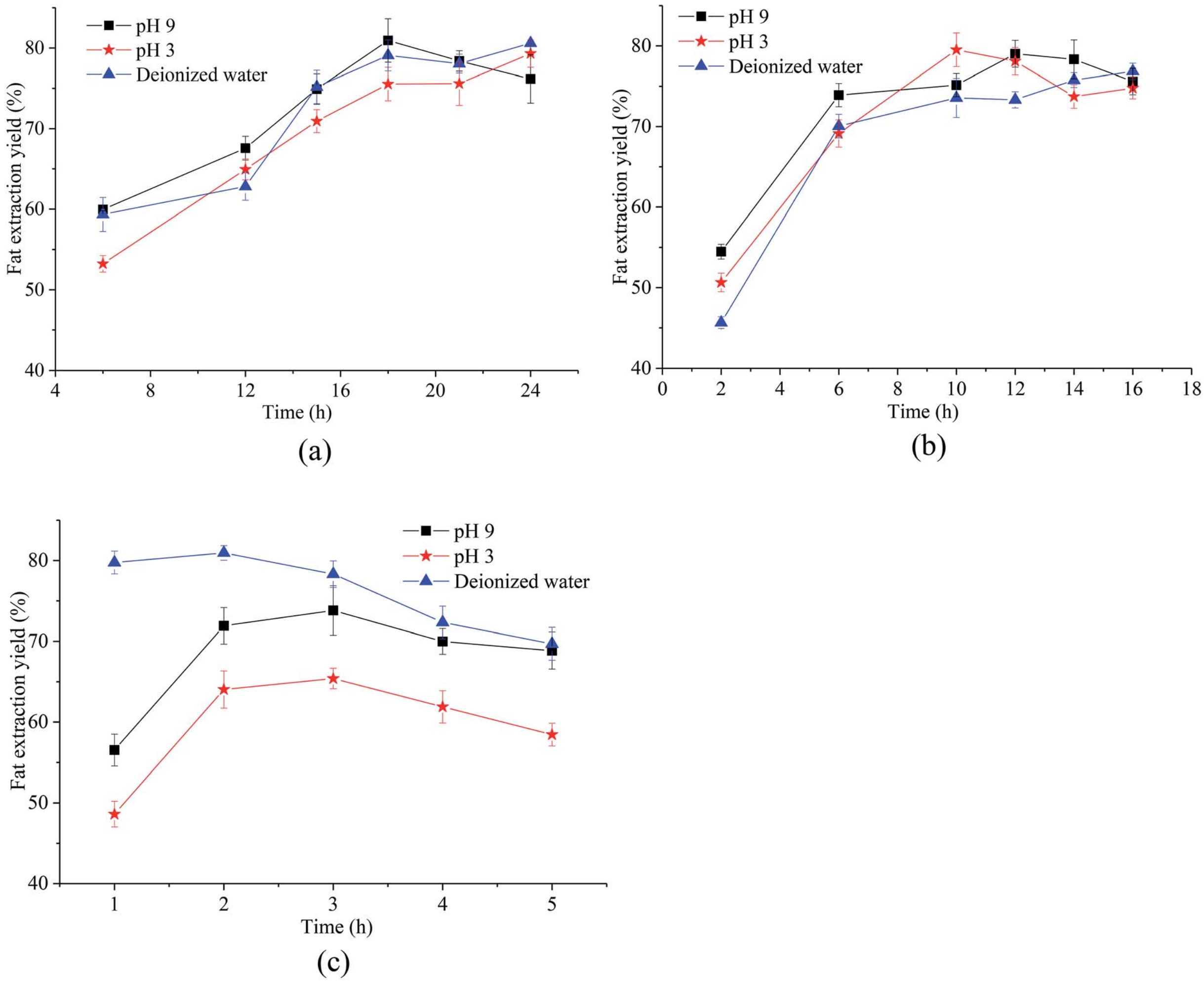

Fig. 4 Effects of the pH value of the soaking water on the extraction rate of fat from soymilk: (a) 4 °C; (b) 25 °C; (c) 50 °C.

extraction yield for soybeans soaked at 4 °C. At soaking temperatures of 25 °C and 50 °C, there was no significant difference ($p > 0.05$) in protein concentration at the different pH values, while a lower protein extraction yield was obtained from soaking at pH 3.0. This resulted in a lower weight of soybean milk than those obtained at pH 9 and neutral pH, as shown in Fig. 6. In contrast, a higher weight of soymilk was obtained at pH 9 due to the high solubility of extracted protein.

Fig. 6 shows the changes in the solid extraction yields as a function of pH and soaking time. For soaking at pH 9, the maximum solid extraction yields were 66.35%. 67.30% and 58.24% after 15 h, 10 h and 2 h of incubation at 4 °C, 25 °C, and 50 °C, respectively. When soaked at pH 3, the maximum solid extraction yields were 66.33%, 61.05% and 55.67%, respectively. Therefore, the maximum solid extraction yield was the lowest at 50 °C and pH 3; meanwhile, at 25 °C and pH 9, the maximum solid extraction yield was the highest. When the soaking temperature was 4 °C, the maximum solid extraction yields at pH 3 and pH 9 were higher than those from neutral soaking, and the time to reach the maximum decreased. When the soaking temperature was 50 °C or 25 °C, the solid extraction yield decreased in the following order: pH 9 > deionized water > pH 3, showing that alkaline soaking pH enhanced the extraction of solids. This result was very similar to the observed protein extraction yield results because the solids mainly consist of proteins. However, other components, such as saccharides, polyphenols, and isoflavones, also contributed to the solid components[38] and showed different effects on the extraction yield.

3.3.2 Color and particle size of soymilk. Color is also an important property in evaluating the quality of soymilk. Under the conditions in which the highest extraction yield of protein was obtained, the influence of soaking conditions on the color of soymilk was studied using a colorimeter. The L^* value represents the whiteness of the soymilk. Fig. 7 shows that the whiteness of the soymilk decreased as the pH value of the soaking solution increased. The highest whiteness of the soymilk was observed at 25 °C soaking temperature, whereas 50 °C soaking treatment significantly decreased the whiteness value ($p < 0.05$). As stated above (Fig. 4), the obtained soymilk had a low fat content at a soaking temperature of 50 °C; thus, the oil body (white color) content decreased, and the whiteness decreased. The b^* value of cooked soymilk was higher than that of raw soymilk, indicating that the color of cooked soymilk

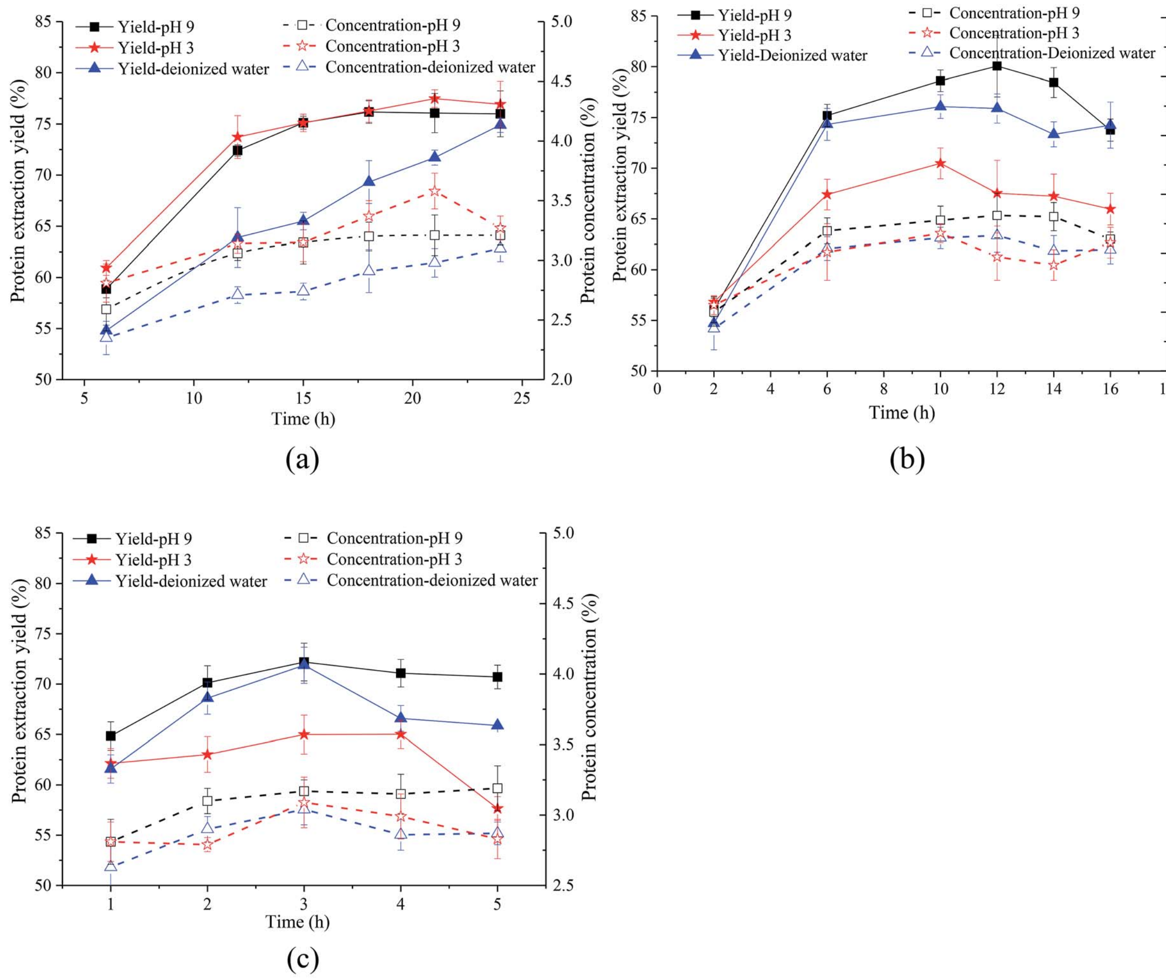

Fig. 5 Effects of the pH value of the soaking water on the concentration and protein extraction yield of soymilk at (a) 4 °C; (b) 25 °C; (c) 50 °C.

tended toward yellow. The b^* value of the raw soymilk obtained from soaking at pH 9 was significantly higher ($p < 0.05$) than that obtained under other soaking conditions; meanwhile, it did not change greatly after heating of the soymilk. The a^* value represents redness or greenness; the cooked soymilk showed a lower a^* value than the raw soymilk and thus looks greener than the latter.

Fig. 8 shows a comparison of the particle sizes of the raw and cooked soymilk as affected by different soaking temperatures and pH values. In soymilk, particles dispersed in the aqueous phase have different characteristics, such as oil droplets, native protein aggregates (protein bodies), and other aggregates formed from oil droplets and proteins and/or polysaccharides.[39,40] For cooked soymilk, the particle size was larger than that of raw soymilk, as shown in Fig. 8. This may be due to aggregation of particles caused by denaturation of proteins, where intermolecular disulfide bonds and hydrophobic bonds between protein molecules may be formed, and the colloidal particles are coalesced to some extent. As stated above (Fig. 4–6), when the soybeans were soaked at 50 °C and pH 3, the obtained raw soymilk showed the lowest fat, protein and solid yields, which may be unfavorable to form a stable emulsion; at the same time, the polysaccharides may be involved in the loss of solids which also play important roles in the stability of the emulsion. Therefore, raw soymilk obtained at 50 °C and pH 3 had higher colloidal particle sizes, and larger aggregates were more likely to form after cooking at 95 °C for 10 min (Fig. 8). With increasing soaking pH, the particle size of the cooked soymilk decreased gradually. This may be due to the fact that the acid soaking pH causes the pH of the obtained soymilk to be closer to the isoelectric point (pH 4.5) of the soy proteins (which can more readily form large aggregates), while the alkaline soaking pH produced the opposite effect.

3.4 Effects of soaking conditions on the release of volatile flavor compounds in soymilk

It has been reported that the volatile compounds in soymilk mainly consist of aldehydes, alcohols, ketones, esters, phenols, and acids; these contribute to mushroom flavor, grass flavor, fat smells, fresh vegetable flavors, rose flavor, and fruit flavors.[21] Kühn *et al.* and Lei *et al.* pointed out that any factors affecting the hydrophobic interactions of a protein surface will affect the binding of volatile flavor compounds.[41,42] Around fifty-seven compounds were identified in the headspace of the soymilks studied in the present work; these were identified as aldehydes,

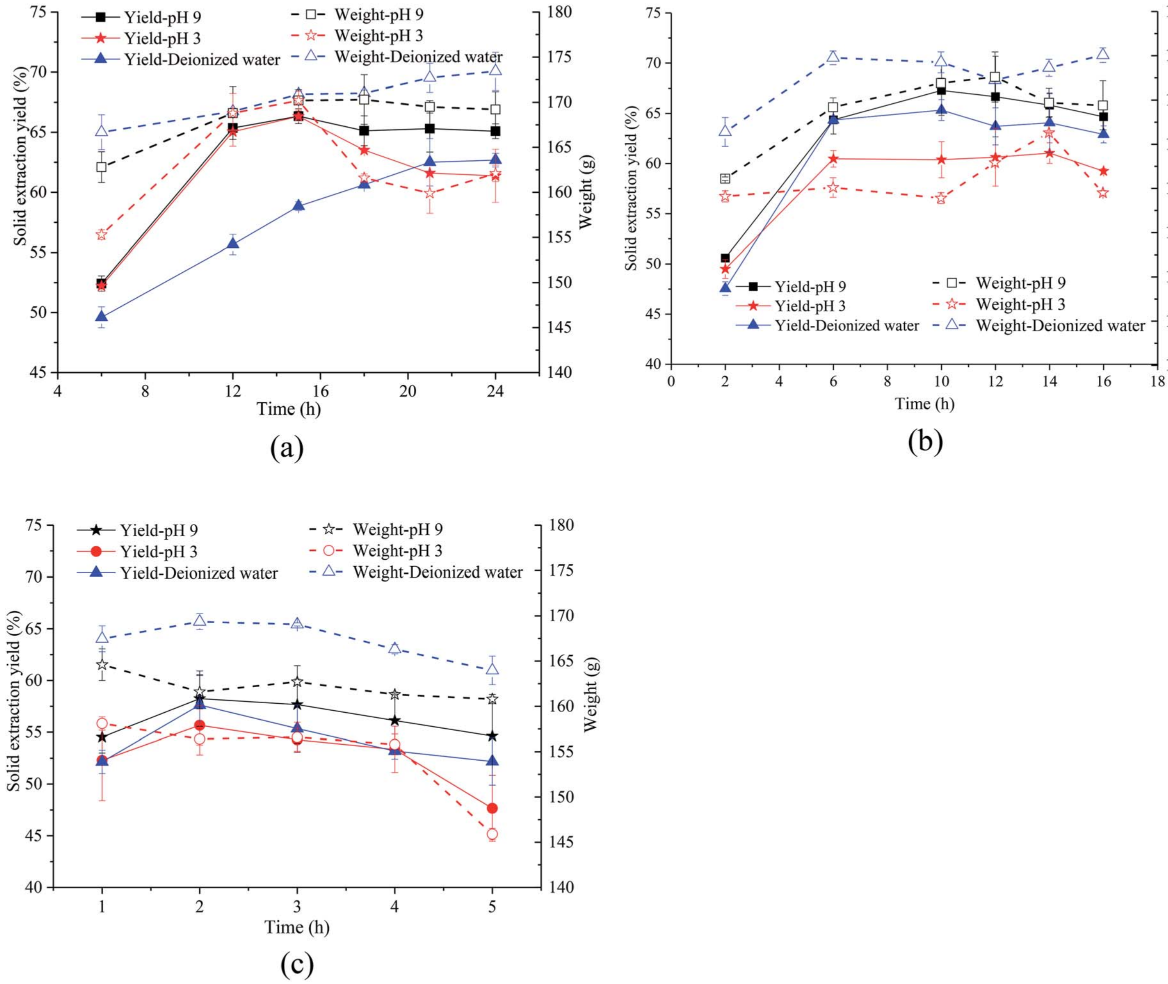

Fig. 6 Effects of the pH value of the soaking water on the extraction rate of solids and the weight of soymilk at (a) 4 °C; (b) 25 °C; (c) 50 °C.

ketones, alcohols, furans, hydrocarbons, esters and acids (Fig. 9). Table 3 shows the quantitative analysis of the typical odor components in soymilk.[43]

3.4.1 Aldehydes. As shown in Table 3, hexanal was the most abundant odor component identified, followed by *trans*-2-hexenal. Other compounds were also detected at low levels, such as heptanal, octanal, benzaldehyde and pentanal (data not shown). Fig. 9 illustrates that the total concentration of aldehydes varies greatly with soaking temperature and pH. It was found that the raw soymilk treated at 50 °C with deionized water achieved the highest level of aldehydes, while the lowest concentration of aldehydes was observed at soaking conditions of 25 °C and pH 9. Among the aldehydes identified in soymilk, hexanal has a low detection threshold and is considered to be the main contributor to sensory off-flavors, such as beany and grassy flavors.[44] In addition, high temperature can promote the activity of lipoxygenase, which can generate the oxidation products of unsaturated lipids by catalysis. At a high soaking temperature of 50 °C with deionized water, the hexanal level was significantly higher ($p < 0.05$) than under other soaking conditions, indicating that these conditions are very suitable for the lipoxygenase-induced oxidation reaction. *trans*-2-Hexenal is a leaf aldehyde which has a very strong green leaf odor, pentanal has a fatty taste, and benzaldehyde has a bitter almond flavor. After soaking at 50 °C in deionized water, the level of *trans*-2-hexenal reached 82.22 μg L^{-1}, which is significantly higher ($p < 0.05$) than the other soaking conditions.

3.4.2 Alcohols. Most volatile alcohols have grassy and cardamom flavors; they are the second most abundant volatile compounds in soymilk. As shown in Fig. 9, the total concentration of aldehydes was greatly influenced by the soaking temperature and pH. The concentration of alcohols achieved the maximum after soaking at 50 °C in deionized water, while it reached a minimum at 4 °C and pH 3. When the soaking temperature increased, the concentration of alcohol substances first increased and then decreased. Among the volatile alcohols, 1-octene-3-ol is oxidized from linoleic acid and confers a mushroomy off-flavor.[45] After immersion at 4 °C and pH 3, the content of 1-octene-3-ol was only 79.75 μg L^{-1}; meanwhile, it reached 910.48 μg L^{-1} after soaking at 50 °C in deionized water, which is nearly 12-fold the content of the former. Additionally, the hexitol content of the soymilk obtained at 25 °C and pH 9 is obviously higher than those obtained under the other conditions.

3.4.3 Other volatile compounds. Among the other volatile compounds, furan exhibits an unpleasant odor in soymilk,

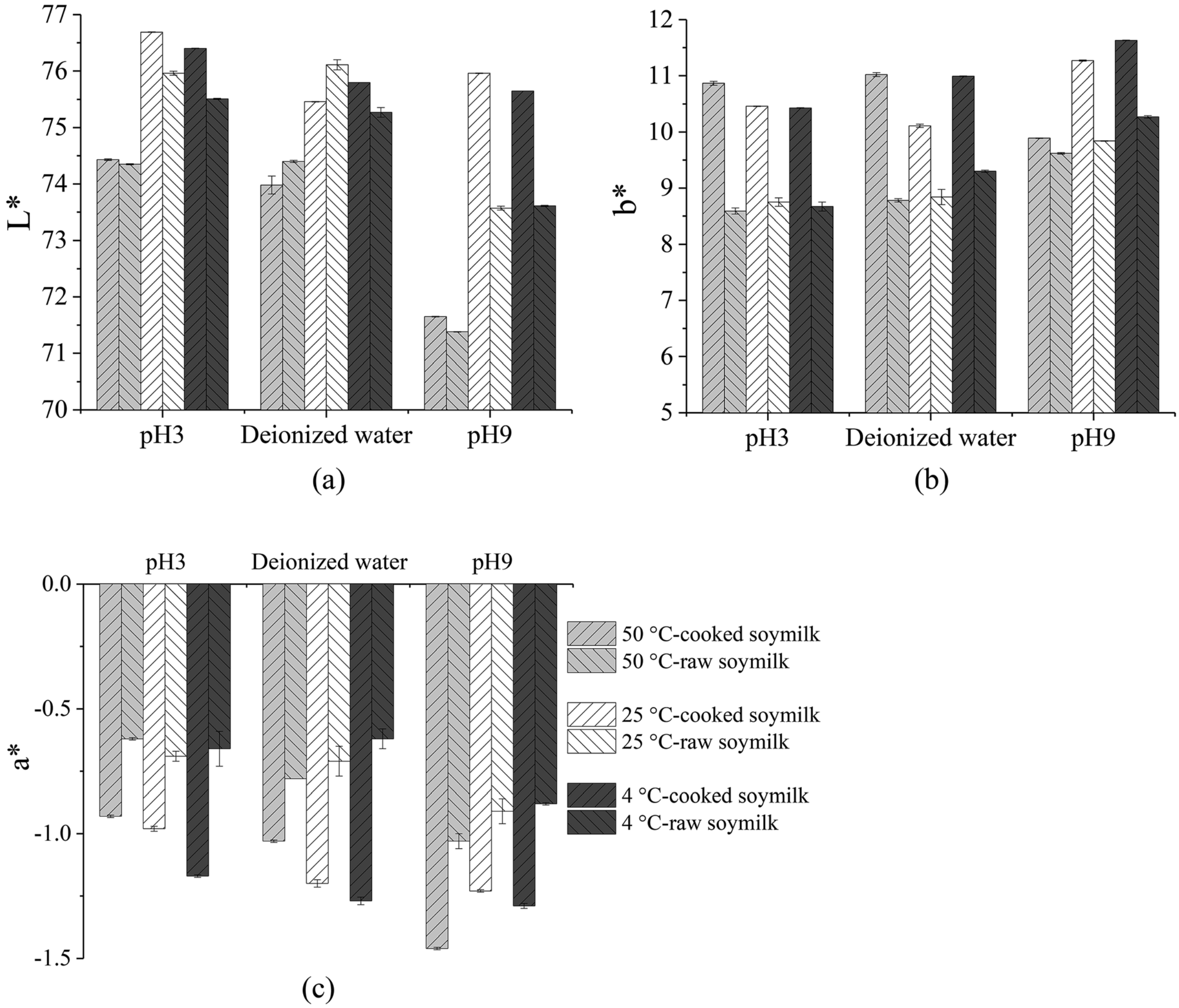

Fig. 7 Effects of soaking conditions on the color of soymilk: (a) L^* value; (b) b^* value; (c) a^* value.

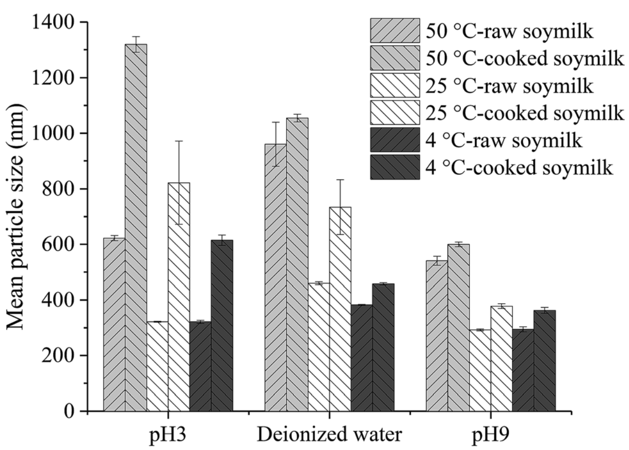

Fig. 8 Effects of soaking conditions on the particle size of soymilk.

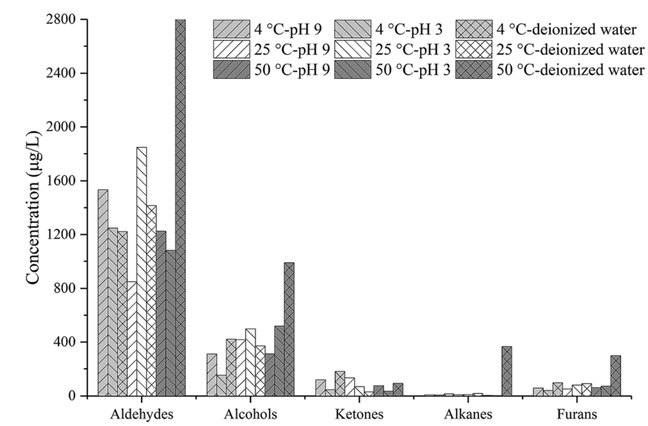

Fig. 9 Effects of soaking conditions on the volatile compounds of soymilk.

which is mainly generated from oxidation of unsaturated fatty acids or the Maillard reaction; it is also related to the color of soymilk. Especially, 2-pentylfuran is considered to be the main contributor of grassy or beany flavors that cause soymilk to taste unpleasant or unacceptable.[46] After soaking at 50 °C in deionized water, the content of 2-pentylfuran in soymilk reached 281.45 μg mL^{-1} (Table 3), which is significantly higher ($p < 0.05$) than that of the other soaking conditions. Ketones and alkanes

Table 3 Main identified volatile compounds in soymilk determined by GC-MS[a]

	Concentration (μg L^{-1})				
Soaking conditions	Hexanal	*trans*-2-Hexenal	1-Octene-3-ol	Hexanol	2-Pentylfuran
4 °C-pH 9	1280.69^{b}	17.05^{bc}	168.36^{c}	118.03^{ab}	57.00^{c}
4 °C-pH 3	1028.58^{b}	28.45^{b}	79.75^{d}	43.01^{c}	92.73^{b}
4°C-deionized water	886.05^{bc}	21.76^{b}	294.41^{b}	83.55^{b}	33.73^{c}
25 °C-pH 9	510.14^{c}	26.05^{b}	255.45^{bc}	141.23^{a}	50.03^{c}
25 °C-pH 3	1560.0^{ab}	34.20^{b}	387.69^{b}	91.15^{b}	70.59^{b}
25 °C-deionized water	1108.11^{b}	19.22^{b}	237.39^{c}	111.24^{ab}	86.13^{b}
50 °C-pH 9	920.96^{b}	18.65^{bc}	270.94^{bc}	26.47^{cd}	61.70^{bc}
50 °C-pH 3	719.81^{bc}	13.29^{c}	478.36^{b}	17.71^{d}	67.92^{bc}
50 °C-deionized water	1834.31^{a}	82.22^{a}	910.48^{a}	28.11^{cd}	281.45^{a}

[a] a–dDifferent letters in the same column are significantly different ($p < 0.05$).

are the other two volatile compounds detected at low levels. Ketones are mainly derived from linoleic acid and have creamy or fruity aromas; they make little contribution to the off-flavor of soymilk compared to aldehydes and alcohols. Alkanes are mainly composed of olefins and alkanes; because of their low concentration and high detection thresholds, they also contribute little to the off-flavor of soymilk.

4. Conclusion

The effects of water absorption of soybeans during the soaking and heating processes in soymilk production on the protein, fat and solid extraction of soymilk and their relationships to the release of volatile flavor compounds were investigated in this study. The water absorption rate increased with increasing soaking temperature and pH, and similar maximum moisture contents were obtained after reaching the saturation soaking time. Peleg's equation with good fitting of the absorption kinetics can be used to predict the hydration characteristics of soybean soaking under other conditions (different pH values and temperatures). Basic 7S globulin was identified as the most released protein during soaking; it showed the highest content after 50 °C soaking treatment. For raw soymilk, the relatively highest extraction yield of proteins, fat and solids was obtained after soaking at 25 °C and pH 9; they had no positive correlation with water adsorption. Hexanal, *trans*-2-hexenal, 1-octene-3-ol, hexanol, and 2-pentylfuran were the main identified volatile off-flavor compounds of soymilk; their contents significantly increased after soaking at high temperature. Soaking at low temperature (4 °C and 25 °C) with deionized water or pH 3 solution enables the soymilk to retain relatively low odor profiles compared to the other soaking conditions.

Conflicts of interest

There are no conflicts to declare.

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