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Research Article

Insight into extruded soy protein isolate for improving hardening of high protein-nutrition bars during storage

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Abstract: Most high-protein nutrition bars (HPNBs) would harden during storage, seriously affecting the acceptability of consumers. In this work, soy protein isolate (SPI), extruded at 50, 75, 100, 125, and 150 ℃, was formulated for HPNBs to investigate whether extrusion protein could relieve hardening and improve quality characteristics of HPNBs during 45 days of storage at 37 ℃. HPNBs prepared with extruded SPI were notably softer. And they had higher sensory scores than HPNBs produced with unextruded SPI during storage ($P < 0.05$). But there were no significant differences in hardness and total color change with the increase of extrusion temperature after 45 days of storage ($P > 0.05$). According to the correlation analysis, HPNBs prepared by SPI extruded at 50 ℃ had the best physicochemical properties. This study provides an effective way to relieve the hardening of HPNBs during shelf life or even longer.

Keywords: high-protein nutrition bars; hardening; extrusion; soy protein isolate

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1 Introduction

More recently, there has been a significant growth of interest in high-protein nutrition bars (HPNBs). HPNBs contain 15%–45% protein, 10%–50% carbohydrates, and 10%–15% lipids which can rapidly supply sufficient protein and energy for organism requirements[1]. Thus, they are extensively used for nutrition supplements, muscle building and weight losing[2]. HPNBs are usually formulated with dairy and soy proteins[3]. Among them, soy protein isolate (SPI) is derived from oil crops and the protein content is more than 90%[4]. Owing to its good nature and low cost, SPI is an excellent alternative to dairy proteins such as casein and whey protein isolate for HPNBs[5]. Compared with dairy proteins, HPNBs formulated by SPI probably have a good performance. SPI may be conductive to reduce water activity in HPNBs system and modify the textural parameters of HPNBs. And SPI-contained HPNBs show higher brightness than those with others, such as whey protein, rice protein, wheat protein, and so on[6]. Moreover, HPNBs have a unique cereal flavor when use SPI as the main gradient[7].

Despite these advantages, the texture and flavor of all HPNBs probably change during long-term storage[8]. Hardening is the most influential one among these changes, which negatively affects the shelf stability, food quality and customer acceptance of HPNBs[9]. At present, the main mechanisms for the hardening of HPNBs are sugar crystallization[10], water migration[11], protein self-aggregation[12], phase separation[13] and Maillard reaction[14]. In fact, the hardening of HPNBs is the result of a combination of these mechanisms[15]. The hardening can be separated into two stages, i.e., the early stage and the mid-late stage of storage. Water migration and protein self-aggregation are important factors that lead to the hardening of HPNBs with SPI during the earlier period of storage[16]. Protein aggregation, phase separation, and Maillard reaction are considered as main factors affecting the hardening process during the later period of storage[17]. Currently, the varieties and contents of sugar have been improved, so sugar crystallization is not the main mechanism to increase the hardness of HPNBs.

Extrusion is a high-temperature short-time (HTST) process, which is an effective way to modify food structures[18]. In the extruding process, the structure and properties of SPI are changed because of the high temperature and shear force[19]. On the one side, after extrusion treatment, proteins are cross-linked by a disulfide bond, which leads to the decrease of the free sulfhydryl group of SPI, and the internal aggregation of protein in HPNBs is reduced[20]. Additionally, protein particles are easy to hydrate after extrusion, which helps part of the particles collapse and prevents the complete particles from absorbing water from other components during storage[21]. Based on these changes, it is presumed that extrusion pretreatment would be viable to improve the properties of HPNBs with SPI during storage.

Full studies have been published on extrusion applied to HPNBs with dairy proteins[22]. It has been pointed out that extrusion changes the wettability of the dairy proteins and reduces the content of the free sulfhydryl group and free amino group, leading to a lower protein reactivity in HPNBs system during storage[21]. HPNBs prepared with extruded dairy proteins are less prone to

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phase separations during storage[23]. However, the quality characteristics of SPI-extruded HPNBs during storage are rarely reported. Thus, the changes in color, hardening, and sensory properties of SPI-extruded HPNBs during storage were amply investigated in this test. Meanwhile, the influences of extrusion pretreatment on the internal structure of SPI in different temperatures were also analyzed, to further understand the principle of improving HPNBs hardening by extrusion. Moreover, the physicochemical properties of HPNBs after long-term storage were visualized in a heat map. This work potentially provides a new approach to the production of anti-hardening HPNBs and promotes the growth of plant-based foods.

2 Materials and methods

2.1 Materials and chemicals

SPI (protein 92.2%, fat 0.2%) and fructose syrup were purchased from Henan Qianweiyuan Food Additives Co., Ltd. (Zhengzhou, China). Glycerol was obtained from Tianjin Zhonghe Shengtai Chemical Co., Ltd. (Tianjin, China). Tris(hydroxymethyl) aminomethane (Tris), ethylene diamine tetraacetic acid (EDTA) and glycine (Gly) were obtained from Biotopped Boaotuoda Technology Co., Ltd. (Beijing, China), and β-mercaptoethanol was obtained from Amresco Co. (Solon, America). 8-Anilino-1-naphthalene sulfonic acid (ANS) was obtained from Sigma Co. (St. Louis, MO, America). Urea was obtained from Tianjin Chemio Chemical Reagent Co., Ltd. (Tianjin, China). Sodium dodecyl sulfate (SDS), 5,5'-dithiobis (DTNB), ophthaladehyde (OPA) were obtained from Beijing Boaotuoda Technology Co., Ltd. (Beijing, China). All chemicals used in this study were of analytical grade.

2.2 Preparation of extruded SPI

SPI with 40% moisture content was fed into a twin-screw extruder (Process 11, Thermo Fisher Scientific, China) at a rate of 60 r/min. Moreover, this extruder has 8 independent heating zones (7 built-in heating zones, 1 external die heating zone)[24]. The first zone, the second zone, the third zone, the seventh zone and the eighth zone were remained at 25, 35, 45, 45, and 25 ℃, respectively. The barrel temperature of zones 4–6 was set to 50, 75, 100, 125, and 150 ℃, respectively, to get the SPI at different extrusion temperatures. After that, they were freeze-dried by a vacuum freeze dryer (Songyuan Huaxing Technology Development Co., Ltd., Beijing, China) and marked as SPE 50 ℃, SPE 75 ℃, SPE 100 ℃, SPE 125 ℃ and SPE 150 ℃.

2.3 Preparation of high-protein nutrition bars

The HPNBs were formulated to contained 45 g protein per 100 g. Extruded or unextruded SPI, fructose syrup and glycerol were mixed in a ratio of 9:8:3[25] and knead them into doughs. The doughs were uniformly packed into molds and sealed with parafilm, then equilibrated at 25 ℃ for 0.5 h, and kept in an incubator at 37 ℃. Samples were removed from incubator on 0, 3, 7, 14, 21, 28, 45 days for testing.

2.4 Free sulfhydryl group content analysis

The content of free sulfhydryl group of protein and HPNBs was measured according to the modified method of Ellman[26]. Disolved 15 mg of the samples in Tris-Gly buffer (0.086 mol/L Tris, 0.09 mol/L Gly and 0.04 mol/L EDTA in water to make 1 L at pH 8.0), 50 μL of 4 mg/mL Ellman reagent (50 mL Tris-Gly and 0.2 g DTNB) was added at room temperature and the obtained mixture was kept for 15 min in the dark. The absorbance was measured at 412 nm by a spectrophotometer UV-Vis (TU-1800, General Instrument, China). Samples and standard control were prepared with the buffer instead of DTNB. The content of free sulfhydryl group was calculated using the following formula:

$$\text{Free sulfhydryl group content } (\mu\text{mol/g}) = \frac{73.53 \times A_{412\,\text{nm}} \times D}{\rho} \quad (1)$$

Where $A_{412\ \text{nm}}$ is the UV absorbance at 412 nm, ρ is the mass concentration (g/mL) of the sample, and D is the dilution factor. $73.53 = 10^6/(1.36 \times 10^4)$, 10^6 is for conversions from the molar basis to the μmol/L basis and from mg solids to g solids, 1.36×10^4 is the molar absorptivity coefficient of DTNB (L/(mol·cm)).

2.5 Free amino group content analysis

The content of free amino group was measured following the OPA method[27]. Firstly, 40 mg of OPA, 12.5 mL of 20% SDS, 500 μL of β-mercaptoethanol, and 25 mL of 0.1 mol/L sodium tetraborate were added in 1 mL methanol, then making the volume to 250 mL. Subsequently, the sample (100 μL) which contained 0.2 mg/mL protein was blended into 3 mL OPA. After it was kept for 5 min in the dark, the absorbance was measured at 340 nm by a spectrophotometer (TU-1 800, General Instruments LLC., General Instrument, Beijing, China). *L*-Leucinestandard curve (0.1–0.5 mg/mL) was applied in this experiment for calibration curve: $y = 1.362x - 0.009\ 4$ ($R^2 = 0.999\ 7$), where x is *L*-leucine concentration (mg/mL), y is the absorbance.

2.6 Determination of surface hydrophobicity (H_0)

The surface hydrophobicity was measured by fluorescence spectrophotometer (F-4500, Hitachi, Japan) according to ANS fluorescence method[28]. Samples were diluted to 0.1, 0.05, 0.025, and 0.012 5 mg/mL, and then mixed with 20 μL 8 mmol/L ANS. After keeping in dark for 15 min, samples were measured at 390 (excitation) and 470 nm (emission). The surface hydrophobicity was obtained by the slope of fluorescence intensity and the mass of protein solution curve.

2.7 Color analysis

The color was measured by a colorimeter (WSC-S, Precision Scientific Instrument Co., Ltd., Shanghai, China). The lightness (L^*), redness/greenness (a^*) and yellowness/blueness (b^*) values were obtained. The total color difference (ΔE) between samples was calculated using the following formula:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (2)$$

Where L^*_0, a^*_0, and b^*_0 are the values of SPI-contained HPNBs and L^*, a^*, and b^* are the values of SPI-extruded HPNBs.

2.8 Hardening analysis

Samples were divided into 1 cm high cylinders with 1 cm diameter. The hardening of samples was measured according to the texture profile analysis (TPA) by a texture analyzer (TA.XT.plus, Stable Micro System, Inc., Surrey GU7 1YL, UK) with 36 mm diameter cylindrical probe P/36R. All experiments were performed in triplicate.

2.9 Scanning electron microscopy test

The microstructure of the samples were analyzed by a scanning electron microscopy (S-3400, Hitachi, Japan)[29]. Initially, each flake sample (0.1 g) was fixed with 2.5% aqueous glutaraldehyde solution

for 24 h at 4 ℃. The samples were shaken periodically in order to prevent the sample from sticking on the bottom of the container. After fixation, samples were rinsed in phosphate buffer 3 times for 15 minutes each time. Concentrations of ethanol solutions were increased in turn: 30%, 50%, 70%, 80%, 90%, 100% and 100%. The samples were dried for 1 h to remove the water and then observed at 500 × magnification.

2.10 Sensory evaluation

Evaluators were randomly selected and trained to score the HPNBs from four indicators (appearance, texture, flavor, and taste) using 1–10 points. The scores were categorized into 4 levels: excellent (8–10 points), good (5–7 points), medium (3–4 points) and poor (1–2 points)[30]. However, the traditional sensory evaluation methods have a certain fuzziness due to the influence of some factors such as the region, habits and hobby. Fuzzy mathematics can eliminate these influences. Thus, fuzzy mathematics was applied in this study.

2.11 Fuzzy mathematical modelling

The steps of fuzzy mathematics comprehensive evaluation are as follows[31]: The evaluated factors of HPNBs were color (U_1), texture (U_2), flavor (U_3), and taste (U_4). So the evaluated factor set was $\boldsymbol{U} = \{U_1, U_2, U_3, U_4\}$ = {appearance, texture, flavor, taste}. Moreover, the comment set of HPNBs was $\boldsymbol{V} = \{V_1, V_2, V_3, V_4\}$ = {excellent, good, medium, poor}. The 8–10 score was excellent, 6–8 score was good, 4–6 score was medium, 2–4 score was poor. To make the difference more obvious, intermediate value for each grade was determined as the final value, i.e., $\boldsymbol{V} = \{9, 7, 5, 3\}$.

Frequency statistics was adopted to confirm the weights, and the weight set was $\boldsymbol{X} = \{x_1, x_2, x_3, x_4\} = \{0.22, 0.31, 0.23, 0.24\}$.

The sensory scores were transformed into a fuzzy matrix R. The fuzzy relationship evaluation set was $\boldsymbol{Y} = \boldsymbol{X} \times R$. The final scores in the fuzzy comprehensive evaluation were $\boldsymbol{T} = \boldsymbol{Y} \times \boldsymbol{V}$.

2.12 Statistical analysis

The results were showed as mean ± standard deviation, and each test was repeated three times ($n = 3$). SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) was used for analysis of variance (ANOVA). Duncan test was used to analyze the significant differences of the average at $P < 0.05$. Origin 2017 (OriginLab, Northampton, MA, USA) was used for plotting. Moreover, principal component analysis (PCA) was used to downscale the data. Following the principle of eigenvalues more than 1, several principal components were selected and visualized to explore the relationships among samples as well as between samples and variables.

3 Results and discussion

3.1 Free sulfhydryl group of SPI

Free sulfhydryl group and disulfide bond are key to the protein aggregation, which can cause hardening of HPNBs[32]. Figure 1A shows the effects of extrusion temperature on the free sulfhydryl group in SPI. The free sulfhydryl group of SPI notably declined after extrusion ($P < 0.05$). Similarly, Alonso et al.[33] reported that free sulfhydryl group in pea and kidney bean was reduced after extrusion pretreatment. It was also found that protein aggregation occurred after high-intensity homogenization[34]. Hence, a possible explanation was extrusion promoted the cross-linking between the free sulfhydryl group and resulted in a decrease in the free sulfhydryl group[35]. As the extrusion temperature raised, free sulfhydryl group of SPE significantly reduced ($P < 0.05$). The free sulfhydryl group of SPE 150 ℃ was reduced by 74.12%. It probably meant more disulfide bond formation and/or protein denaturation at high extrusion temperature[21].

3.2 Free amino group of SPI

Glycosylation reaction between reducing sugar and free amino group generates insoluble aggregates during storage, resulting in the degradation of food quality[36]. Figure 1B shows the effects of extrusion temperature on free amino group in SPI. The free amino group of SPE was significantly less than SPI ($P < 0.05$). The result might be caused by high temperature, pressure, and shear force, which would change the structure of SPI, thereby resulting in the reduction of amino groups[37]. Furthermore, the free amino group of SPE significantly declined when the extrusion temperature increased ($P < 0.05$). A previous study also showed that free amino group of whey protein isolate had a downward trend when the extrusion temperature raised through 50 to 130 ℃[35]. The free amino group in SPE 150 ℃ reduced by 22.38% in comparison with SPI, due to the protein-to-protein interactions at high extrusion temperature[38]. Compared with Figures 1B and 2B, the free amino group of HPNBs prepared with extruded and unextruded SPI did not change on day 0 of storage. With the increase in storage time, the free amino group of HPNBs decreased.

3.3 Surface hydrophobicity of SPI

Surface hydrophobicity is a most extremely structure-related factor which influence the functional properties of proteins in many areas[39–40]. Figure 1C shows the effect of extrusion temperature on surface hydrophobicity in SPI. At first, the surface hydrophobicity of SPI is high because its solubility in water was very low. Moreover, the surface hydrophobicity of SPE was notably less than SPI ($P < 0.05$). The reduction could be attributed to protein aggregates

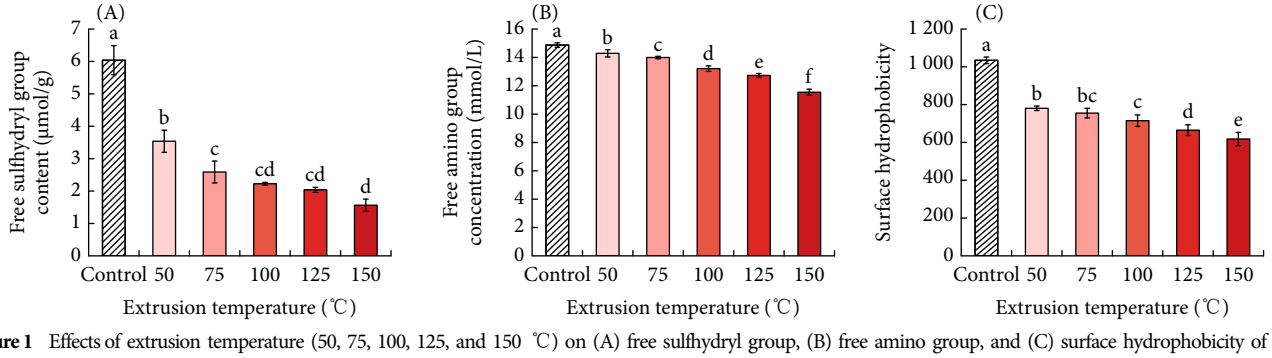

Figure 1 Effects of extrusion temperature (50, 75, 100, 125, and 150 ℃) on (A) free sulfhydryl group, (B) free amino group, and (C) surface hydrophobicity of SPI. Different lowercase letters (a–f) indicate significant differences in different extrusion temperatures ($P < 0.05$).

and protein-protein interactions after extrusion, covering the most hydrophobic sites inside the aggregate, which led to a decrease in surface hydrophobicity of SPI[41–42]. As the extrusion temperature increased, the surface hydrophobicity in SPE greatly reduced ($P < 0.05$). The surface hydrophobicity of SPI was reduced by 50.32% after extrusion at 150 ℃. A similar result was also obtained by Ma et al.[43]. Furthermore, more SPI congregated through hydrophobic interactions after high-temperature extrusion, which encapsulated more hydrophobic groups within the aggregates[44]. Therefore, extruded SPI was less reactive in HPNBs during storage and was less likely to self-aggregation by hydrophobic interactions, thus alleviating hardening.

3.4 Free sulfhydryl group of HPNBs during storage

Figure 2A shows the content of free sulfhydryl group of HPNBs prepared with extruded and unextruded SPI during storage at 0, 3, 7, 14, 21, 28 and 45 days. With the extension of storage, the free sulfhydryl group of each HPNBs was reduced significantly ($P < 0.05$). The same results were also found by Wang et al.[45]. It could be attributed to protein self-aggregation through the formation of disulfide bonds during storage in HPNBs, which led to an obvious decline in free sulfhydryl group[46]. And the free sulfhydryl group of HPNBs prepared with SPI, SPE 50 ℃, SPE 75 ℃, SPE 100 ℃, SPE 125 ℃ and SPE 150 ℃ was reduced by 49.77%, 40.55%, 34.36%, 41.92%, 42.04% and 33.33% after 45 days of storage, respectively. Clearly, the consumption of the free sulfhydryl group in SPI-extruded HPNBs was less than that in SPI-contained HPNBs after 45 days of storage. It might be the reason that the free sulfhydryl group of SPI that participated in the self-aggregation was reduced after extrusion (according to Figure 1A). This followed that protein self-aggregation was reduced in SPI-extruded HPNBs during storage, thus mitigating the hardening of HPNBs.

3.5 Free amino group of HPNBs during storage

Figure 2B shows the free amino group of HPNBs prepared with extruded and unextruded SPI during storage at 0, 3, 7, 14, 21, 28 and 45 days. Obviously, the free amino group of each HPNBs significantly depressed during storage ($P < 0.05$). Previously, Banach et al.[47] also found the same trend. This finding might be attributed to the Maillard reaction between the free amino group and reducing sugar during storage in the HPNBs system, which led to the decrease of free amino group[48]. Moreover, the free amino group in HPNBs prepared with SPI, SPE 50 ℃, SPE 75 ℃, SPE 100 ℃, SPE 125 ℃ and SPE 150 ℃ was decreased by 86.28%, 74.58%, 77.93%, 80.68%, 81.85% and 82.67% after 45 days of storage, respectively. This indicated that the consumption of free amino groups in SPI-extruded HPNBs was less than that in SPI-contained HPNBs. This was probably owing to fewer free amino groups in the extruded SPI participated in the Maillard reaction (according to Figure 1B). Therefore, the Maillard reaction between the free amino group and the reducing sugar was inhibited after extrusion, which reduced the formation of insoluble aggregates, thus alleviating hardening.

3.6 Hardness of HPNBs during storage

Hardness as a critical indicator effects the customer acceptance of HPNBs[49]. Figure 2C shows the hardness of HPNBs prepared with extruded and unextruded SPI during storage at 0, 3, 7, 14, 21, 28 and 45 days. The hardness of HPNBs increased in each group with the extension of storage. There were no remarkable differences between HPNBs on day 0. After 3 days of storage, the hardness of HPNBs increased within a short time. As observed by Tolstoguzov[50], the results were mainly caused by the migration of substances such as moisture and glycerol in the system. While stored at 37 ℃ for 3–45 days, the hardness of HPNBs increased and reached the maximum after a 45-day storage. However, the significant differences were not found in each group ($P > 0.05$).

The HPNBs with extrusion were remarkably softer than SPI-contained HPNBs even after a long-term (45 days) storage ($P < 0.05$). It might be due to the decrease of free sulfhydryl group and amino group of SPI in HPNBs after extrusion (shown in Figures 1A and 1B). Additionally, after the extrusion pretreatment, the hardening caused by self-aggregation was relieved because there was a decrease in the free sulfhydryl group content. Meanwhile, the reduction of free amino group after extrusion slowed down the glycosylation in HPNBs, mitigating the hardening of HPNBs during storage[51]. Moreover, the surface hydrophobicity of SPI was reduced by extrusion (shown in Figure 1C), which was beneficial to slow the phase separation in the HPNBs system during storage. The hardness of HPNBs was also decreased.

3.7 Color of HPNBs during storage

Color change can also be another important factor for the quality decline of HPNBs except hardening[52]. ΔE of HPNBs during storage was presented in Table 1. The ΔE of HPNBs significantly raised when the extrusion temperature increased to 125 and 150 ℃. It was attributed to high temperature promoted Maillard reaction between the free amino group and reducing sugar in HPNBs system, which could generate melanoidins and change the color of HPNBs[53].

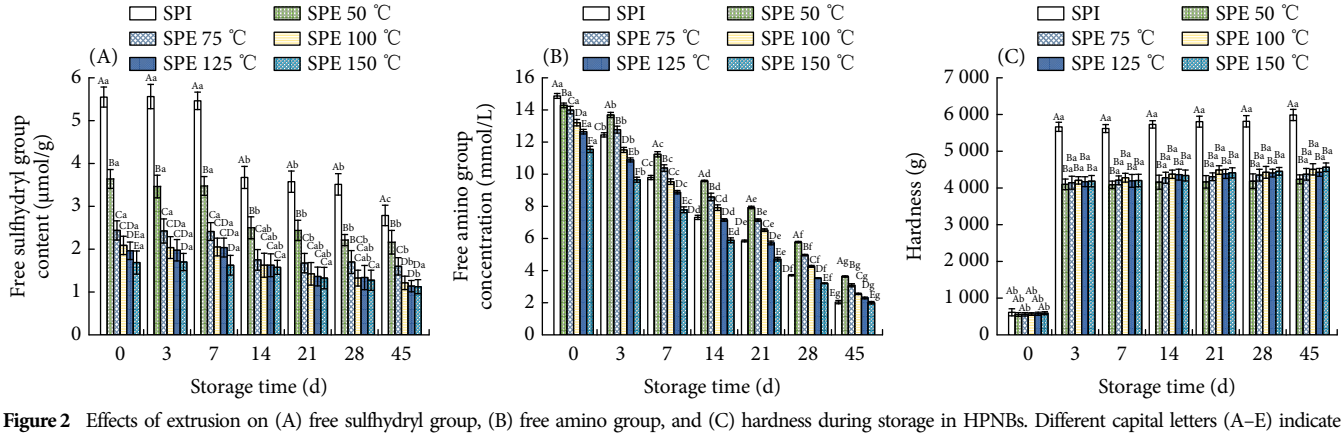

Figure 2 Effects of extrusion on (A) free sulfhydryl group, (B) free amino group, and (C) hardness during storage in HPNBs. Different capital letters (A–E) indicate significant differences in different extrusion temperatures ($P < 0.05$), and different lowercase letters (a–g) indicate significant differences in different storage days ($P < 0.05$).

Table 1 The Δ*E* change of HPNBs prepared with extruded and unextruded SPI on 0, 3, 7, 14, 21, 28 and 45 days.

Extrusion temperature (℃)	0 d	3 d	7 d	14 d	21 d	28 d	45 d
50	0.70 ± 0.60^{ab}	0.85 ± 0.39^{a}	1.93 ± 0.58^{a}	1.95 ± 0.61^{a}	1.89 ± 0.63^{a}	1.39 ± 0.31^{a}	2.04 ± 0.25^{a}
75	1.36 ± 0.75^{ab}	1.10 ± 0.18^{a}	1.45 ± 0.30^{ab}	1.60 ± 0.80^{ab}	1.85 ± 0.03^{b}	1.17 ± 0.18^{b}	1.97 ± 0.37^{a}
100	1.70 ± 0.80^{ab}	1.19 ± 0.14^{a}	1.11 ± 0.47^{ab}	1.89 ± 0.11^{bc}	1.24 ± 0.09^{c}	1.08 ± 0.06^{b}	2.09 ± 0.21^{a}
125	2.59 ± 0.48^{a}	1.88 ± 0.29^{a}	0.83 ± 0.21^{b}	1.35 ± 0.38^{c}	1.11 ± 0.14^{c}	1.06 ± 0.60^{b}	2.04 ± 0.13^{a}
150	2.32 ± 1.03^{a}	1.83 ± 0.30^{a}	0.80 ± 0.50^{b}	1.13 ± 0.25^{c}	1.11 ± 0.09^{c}	1.03 ± 0.17^{b}	1.99 ± 0.09^{a}

Note: Different letters indicate significant differences in different extrusion temperatures ($P < 0.05$).

Nevertheless, there were no significant differences in Δ*E* of HPNBs between changes of extrusion temperature after 45 days of storage ($P > 0.05$).

3.8 Microstructure of HPNBs during storage

Scanning electron microscope (SEM) has been widely used for studying the structure and distribution of materiel in many areas[54–55]. Figure 3 shows the morphological structure of HPNBs prepared with SPI and SPE 150 ℃ through the storage of 3, 21, 45 days. At early stage of storage (3 and 21 days), the microstructure between HPNBs prepared with SPI and SPE 150 ℃ was different due to extrusion pretreatment. Noticeably, there were more circular pores on the surface of HPNBs prepared with SPI (shown in Figures 3A and 3B). For HPNBs prepared with SPE 150 ℃ (shown in Figures 3a and 3b), the surfaces were rough and showed a large blocky structure. However, there were no significant differences in microstructure between HPNBs prepared with SPI and SPE 150 ℃ after stored for 45 days ($P > 0.05$). The structure of HPNBs was relatively tight after storage of 45 d (shown in Figures 3C and 3c). The physicochemical properties of proteins were tightly related to protein-small molecule interactions[56]. Protein in the HPNBs system gradually absorbed water, swelled from the liquid continuous phase, blended to form a cross-linked network structure, and finally made the structure tight[23].

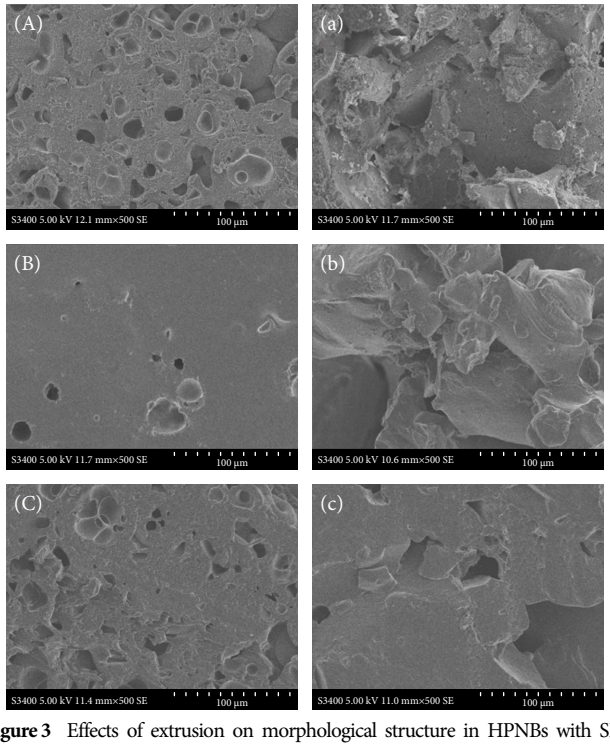

Figure 3 Effects of extrusion on morphological structure in HPNBs with SPI (A, B, C) and SPE 150 ℃ (a, b, c) during storage for 3, 21, and 45 days.

3.9 Sensory evaluation of HPNBs during storage

Sensory evaluation is a key process in control of food quality[57]. Table 2 shows the sensory scores of HPNBs prepared with extruded and unextruded SPI. Obviously, the sensory scores of HPNBs prepared with SPE 50 ℃, SPE 75 ℃ and SPE 100 ℃ were higher than SPI-added HPNBs on day 0 of storage. It indicated that low temperature extrusion could improve the sensory properties of HPNBs. Onyeoziri et al.[58] also reported that extrusion could enhance the sensory characteristics of instant porridge. However, the sensory scores of HPNBs prepared with SPE 125 ℃ and SPE 150 ℃ were lower than HPNBs prepared with SPI on day 0 of storage. It might be due to the Maillard reaction and some relative interactions at high temperature and pressures, resulting in poor sensory properties of HPNBs[59]. Moreover, the sensory scores of SPI-extruded HPNBs are higher than SPI-contained HPNBs after stored 45 days. Besides HPNBs prepared with SPE 50 ℃ had the highest sensory score after a long-term storage (45 days).

Table 2 Comprehensive score of fuzzy evaluation of HPNBs on 0, 3, 7, 14, 21, 28 and 45 days.

Extrusion temperature (℃)	0 d	3 d	7 d	14 d	21 d	28 d	45 d
Control	7.12	8.46	8.41	8.19	7.46	6.97	5.93
50	7.26	8.67	8.69	8.58	8.31	7.52	6.48
75	7.19	8.61	8.63	8.46	7.77	7.37	6.34
100	7.15	8.51	8.57	8.52	7.83	7.32	6.29
125	7.02	8.52	8.52	8.62	7.94	7.38	6.29
150	7.02	8.57	8.57	8.41	7.72	7.47	6.29

3.10 Correlation analysis

With the PCA, factors of HPNBs during storage were transformed and downscaled. As shown in Figure 4A, the contribution rates of PC1 and PC2 were 74.4% and 24.8%, respectively. PC1 and PC2 reflected 99.2% of the total amount of information provided by the original data. For PC1, hardness, Δ*E* and sensory scores were the characteristic indexes. For PC2, the free amino group and the free sulfhydryl group were the characteristic indexes. The hardness was more closely related to PC1 and the free amino group was more closely related to PC2. Compared with the free amino group, the free sulfhydryl group of HPNBs had more influence on the hardness of HPNBs. The sample points of the HPNBs before and after extrusion were distributed on both sides of the vertical axis, indicating significant differences ($P < 0.05$) in these factors between HPNBs prepared with extruded and unextruded SPI.

For a more visual observation of the distribution of experimental data, a heat map was used to transform data into chroma. Row standardization was also adopted to make the differences between data more significant. Figure 4B indicated that sensory scores of

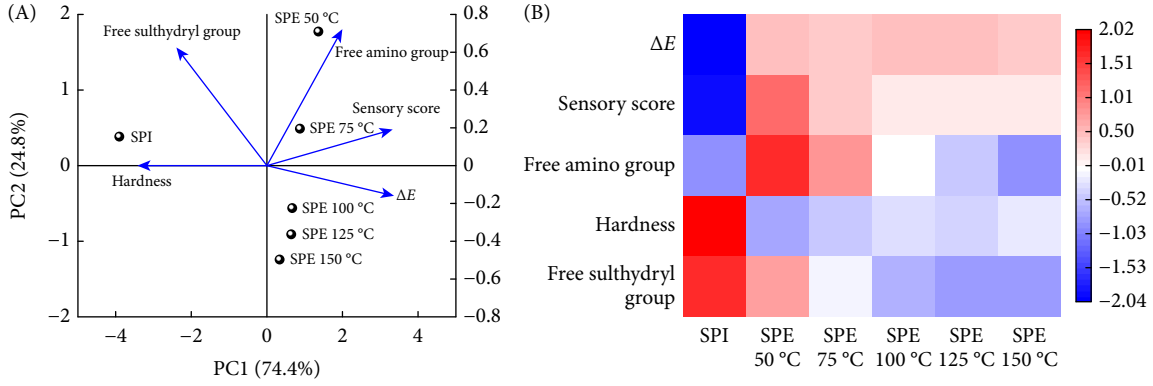

Figure 4 (A) PCA and (B) heatmap of HPNBs with extruded or unextruded SPI after storage for 45 days.

HPNBs prepared with SPE 50 ℃ were higher than other HPNBs after stored for 45 days. Besides, after 45 days of storage, the hardness of HPNBs made with SPE 50 ℃ was lower than that of other HPNBs. In summary, HPNBs prepared with SPE 50 ℃ had better quality characteristics even after a long-term storage (45 days).

4 Conclusion

In conclusion, SPI was less reactive in HPNBs and then relieved the hardening of HPNBs during storage due to lower free sulfhydryl group, free amino group, and surface hydrophobicity after extrusion. Thus, the SPI-extruded HPNBs were softer than SPI-contained HPNBs even after 45 days of storage. Additionally, SPI-extruded HPNBs had better sensory properties than SPI-contained HPNBs. Moreover, the HPNBs prepared with SPE 50 ℃ showed the best quality characteristics among SPI-extruded HPNBs after a long-term storage (45 days). This work has revealed that anti-hardening HPNBs can be produced by the application of extrusion pretreatment. In further research, the effects of extrusion on other characteristics (such as nutritional value, flavor, and so on) of HPNBs would be required to develop high-quality HPNBs.

Conflict of interest

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

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