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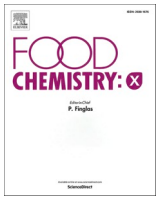

# Effects of maltodextrin and protein hydrolysate extracted from lotus seed peel powder on the fat substitution and lipid oxidation of lotus seed paste

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ABSTRACT

The fat substitution of maltodextrin from lotus seed peel powder (LSP-MD) and the lipid oxidation inhibitory effect of protein hydrolysate (LSP-PH) on lotus seed paste were investigated in this study. The LSP-MD with a dextrose equivalent value of 2.28 showed the smallest specific volume, strongest water-holding capacity and retrogradation. This LSP-MD effectively maintained the sensory quality, hardness and elasticity of low-fat lotus seed paste during storage at 25 °C. For protein hydrolysate, LSP-PH with a hydrolyzation degree of 13.45 % had the strongest DPPH· scavenging capacity and ferric reducing antioxidant power, which was further confirmed by FTIR spectra that enzymatic hydrolysis of LSP protein could facilitate the transformation of β-sheet into β-turn. Following 15 days of storage, supplementation with 0.5 % LSP-PH reduced the peroxide value and acid value of lotus seed paste, suggesting its excellent inhibitory effect on lipid peroxidation via interacting with hydrophobic polyunsaturated fatty acids.

## 1. Introduction

Lotus (*Nelumbo nucifera*), a perennial aquatic herb belonging to the Nymphaceae family, is widely existed in Australia, China, Russia and other countries (Liu et al., 2023). The mature fruit of lotus is lotus seed, which can be utilized to prepare lotus seed paste with a refreshing and sweet taste. Based on the various purposes, it can be divided into baking type used for making pastries, bread, mooncakes, etc., and frozen beverage type used for making ice cream, ice products, etc., as well as lotus seed paste for frozen food. As a popular food filling, lotus seed paste requires a smooth and shiny cut section without sticking to knives or teeth, and thus the oil content of the filling should account for about 30 % (Dong et al., 2020). Besides, the traditional lotus seed paste has high levels of sugar, fat and calorie, which can be easily spoiled via promoting its lipid oxidation and microorganism proliferation (Sharma, Gautam, Adhikari, & Karki, 2017). The excessive consumption of fat and sugar also triggers some detrimental effects on human wellness such as hyperlipidemia, obesity, and coronary disease. However, the majority of consumers are not accustomed to low-fat or fat-free foods lacking a delicate and greasy texture. Hence, the utilization of fat substitutes (e.g., maltodextrin) and natural antioxidants (e.g., protein hydrolysate) to simulate the texture of oil and extend the shelf life of lotus seed paste is urgently needed.

As a subclass of fat substitutes, maltodextrin, known as enzymatic or water-soluble dextrin, is a hydrolyzed product of starch with a low hydrolyzation degree (DH) (Hedayatnia et al., 2016). Generally, the dextrose equivalent (DE) of maltodextrin is less than 20. The maltodextrin with a low DE value of 2–5 has the advantages of low sweetness, excellent digestion and absorption, low permeability, prevention of dental caries, and forming a texture and taste similar to fat, which can be used as a fat substitute (Wangsakan, Chinachoti, & McClements, 2003). Furthermore, maltodextrin can improve the structure of water phase to form gels with three-dimensional network structure, thus retaining most of the water to exhibit good fluidity, further producing a creamy sense of lubrication and delicacy similar to that of fat, which can be used as a thickener and stabilizer (Kanyuck, Mills, Norton, & Norton-Welch, 2019). For instance, AbuDujayn et al. (2023) confirmed that supplementation with maltodextrin could maintain the color, texture and

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overall acceptability of panned bread via altering the physical characteristics and inhibiting the aging of wheat starch. Similarly, maltodextrin has been demonstrated to be effective in partially replacing the fat of instant coffee creamer with low stickiness and excellent acceptance comparable with the commercial creamer (Hedayatnia et al., 2016). Another report also found that maltodextrin could strengthen the gel structures of emulsion to retain volatiles, particularly the more lipophilic compounds, within the gels (Mao, Miao, Yuan, & Gao, 2018).

In order to promote the sensory quality (e.g., texture or taste), nutritional, and functional (e.g., antioxidant) characteristics of food, some specific proteases are also usually utilized to hydrolyze food protein. The obtained protein hydrolysate mainly exerts antioxidant effect by chelating metal ions, eliminating free radicals, and enhancing the decomposition of peroxides (Hougaard, Pindstrup, Arneborg, Andersen, & Skibsted, 2016; Liao et al., 2020). For instance, protein hydrolysate from silver carp fin could reduce the levels of free fatty acid, peroxide value and TBARS via antioxidant mechanism, thereby alleviating the lipid oxidation of bighead carp fillets (Zhang et al., 2020). Similarly, casein and whey protein hydrolysates have been demonstrated to be effective in significantly inhibiting the formation of TBARS to ameliorate the lipid peroxidation of zebrafish larvae (Carrillo, Guzman, & Vilcacundo, 2017).

Lotus seed peel powder (LSP), a by-product in lotus seed manufacturing, is mainly composed of lotus seed peel and a few lotus seed kernels (Xu, Gao, Liu, & Gong, 2022). However, LSP is usually used as feed, fertilizer or discarded directly with low additional value, which can be further utilized as a potential source of natural starch, protein, and phenolics. For instance, catechin, quercetin, kaempferol, and procyanidin B2 were identified as the dominant phenolics of lotus seed peel (Xu et al., 2022). More importantly, lotus seed and its peel have been found to be abundant in starch (26.29–38.90 %) and protein (16.53–19.05 %) by emerging literatures (Liu et al., 2023; Shahzad et al., 2021; Zhu, 2017). For example, the lotus seed red-peel extract could effectively alleviate oxidative stress, lipid peroxidation, and symptom of obesity via regulating lipoprotein lipase activity (Xu et al., 2022). Besides, lotus seed flour with a protein content of 16.53 % was successfully used as wheat replacer in cookies to enhance the antioxidant effects of products, thus maintaining the color of cookies and increasing the acceptability of consumers (Shahzad et al., 2021). However, reports regarding the characteristics and applications of LSP starch and protein in food industry are still scarce.

Nowadays, some works have been conducted on the chemical components (e.g., phenolic and polysaccharides) and biological activities (e.g., antioxidant and hypolipidemia) of lotus seed (Cao et al., 2018; Shahzad et al., 2021; Xu et al., 2022; Zhu, 2017). However, little information is available about the functional components of lotus seed peel and its application as natural fat substitutes and antioxidants in food processing. Therefore, the present study aimed to prepare and characterize the chemical composition of maltodextrin (LSP-MD) and protein hydrolysate (LSP-PH) from lotus seed peel powder, and investigate their substitution rate of fat (SRF) and inhibition of lipid oxidation effects on lotus seed paste, respectively. The main hypothesis is the positive influence of LSP-MD and LSP-PH from lotus seed peel powder on the fat substitution and lipid oxidation of lotus seed paste and, as a result, on extending the shelf life of lotus seed paste. This work firstly proposed the fat substitution effect of LSP-MD and the lipid oxidation inhibitory ability of LSP-PH, which would provide a theoretical basis for the application of lotus seed peel powder as a fat substitute and a potential antioxidant to preserve food products.

## 2. Materials and methods

### 2.1. Materials and reagents

Lotus seed and LSP were provided by Hongxinglong Xianglian Food Co., Ltd. (Xiangtan, Hunan Province, China). Folin-Ciocalteu and DPPH reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 2,4,6-tripyridyltriazine (TPTZ) reagent was provided by Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Alkaline protease (from *Bacillus subtilis*) was obtained from Novozymes A/S (Copenhagen, Denmark). Thermostable α-amylase (from *Aspergillus oryzae*) was provided by Ryon Biotechnology Co., Ltd. (Shanghai, China). White granulated sugar and sunflower seed oil were purchased at local Bubugao Supermarket (Changsha, Hunan Province, China). All of the other reagents were of analytical grade, and provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

### 2.2. Determination of chemical components in LSP

The moisture, protein and starch levels of LSP were determined by the Approved Methods of AACC Method 44–16, Method 46–10, and Method 76–11 (AACC, 2000), separately, as reported before. The soluble sugar content was determined by phenol–sulfuric acid method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956).

### 2.3. Preparation of maltodextrin from LSP (LSP-MD)

Briefly, LSP was dissolved in deionized water and adjusted to pH 6.8. Then, proper amount of thermostable α-amylase was added and kept at different temperatures for a certain period of time. The specific reaction conditions were listed in Table S1. Following enzymatic hydrolysis for the corresponding time, the enzyme was completely inactivated at 100 °C for 5 min. The obtained mixtures were centrifuged, a portion of supernatant was used for determining dextrose equivalent (DE), while the others were lyophilized by vacuum freeze dryer to yield LSP-MD.

### 2.4. Characterization of LSP-MD

#### 2.4.1. Determination of DE

The DE of LSP-MD was measured according to the titration method. In short, maltodextrin solution (10 mL) and 0.1 mol/L iodine solution (25 mL) were mixed thoroughly. Then, 0.1 mol/L NaOH solution (40 mL) was added, and the mixture was sealed and kept at dark for 10 min. After the further addition of 0.2 mol/L sulfuric acid (6 mL), 0.1 mol/L $Na_2S_2O_3$ standard solution was utilized to titrate this mixture with 0.5 % starch solution as indicator. Deionized water was used as the blank control. The DE value was calculated according to Eq. (1):

$$DE = 9 \times \frac{C \times (V_0 - V_1)}{m_1} \quad (1)$$

where C is the concentration of $Na_2S_2O_3$ standard solution, $V_0$ and $V_1$ are the volumes (mL) of $Na_2S_2O_3$ standard solution consumed in the blank control, and sample groups, respectively, and $m_1$ is the weight of LSP-MD sample (g).

#### 2.4.2. Determination of specific volume

Briefly, 1 g of LSP-MD (m) with different DE values was added into a 10 mL measuring cylinder and shaken well. The volume of LSP-MD (V) was read from the concave surface of measuring cylinder. The specific volume of sample was calculated according to Eq. (2):

$$\text{Specific volume } (cm^3/g) = V/m \quad (2)$$

#### 2.4.3. Determination of water-holding capacity

In short, 1 g sample ($m_0$) was dissolved in 5 mL deionized water, shaken thoroughly, and kept for 30 min. Afterward, the mixture was centrifuged at 6,800 g for 5 min, the precipitate was collected and weighed as $m_1$. The water-holding capacity of sample was calculated according to Equation (3):

$$\text{Water-holding capacity } (\%) = (m_0 - m_1)/m_0 \times 100 \quad (3)$$

#### 2.4.4. Determination of retrogradation

A certain amount of sample was prepared into 1 % solution, and then was incubated in boiling water with thorough stirring to gelatinize for 20 min. After natural cooling to room temperature, the gelatinized solution was poured into graduated tubes with plugs, and then kept for 0, 1, 2, 3, 4, and 5 days, respectively. The volume changes in the supernatant of this solution were recorded each day. The retrogradation properties of LSP-MD was evaluated by the ratio of supernatant volume to total volume under the same conditions.

### 2.5. Preparation of protein hydrolysate from LSP (LSP-PH)

Briefly, LSP was extracted by 0.03 mol/L $Na_2CO_3$-$NaHCO_3$ buffer solution (pH 10.5) in a solid–liquid ratio of 1:1.75 at 20 °C for 1 h with an interval stirring per 10 min, and then sonicated for 7.5 min. After 5 min of centrifugation at 4,500 g, the supernatant was adjusted to pH 2.0, and centrifuged (1,100 g, 10 min) again. The obtained precipitate was washed by deionized water to be neutral, and dried at 50 °C to obtain LSP protein. Afterward, the LSP protein solution (20 g/L, pH 9.0) and alkaline protease (6477 U/g) were mixed and incubated at 49.5 °C water bath for 15, 30, 60, 90, and 120 min, respectively. During the hydrolysis process, 0.5 mol/L NaOH was utilized to maintain the constant pH value of reaction solution as 9.0. The obtained mixtures were kept in boiling water bath for 5 min to halt the reaction, cooled to room temperature, and then centrifuged at 1,100 g for 10 min. The obtained supernatants were evaporated at 45 °C and lyophilized by vacuum freeze dryer to yield LSP-PH with different hydrolyzation degree (DH).

### 2.6. Characterization of LSP-PH

#### 2.6.1. Determination of DH

To our knowledge, the DH of protein hydrolysate was proportional to the consumption of base used for maintaining the constant pH value of reaction solution, which was measured by pH-stat method as reported previously (Van der Plancken, Van Remoortere, Indrawati, Van Loey, & Hendrickx, 2003). As described in Section 2.5, the volume of 0.5 mol/L NaOH solution used for keeping constant pH value was expressed as B (mL). The DH of LSP-PH was calculated according to Eq. (4):

$$\mathrm{DH}\,(\%) = \frac{\mathrm{B} \times \mathrm{N_b}}{\alpha \times \mathrm{m_p} \times \mathrm{h_{tot}}} \times 100 \tag{4}$$

where $N_b$ represents the concentration of the NaOH solution (mol/L), $m_p$ represents the weight of the protein substrate (g), $h_{tot}$ represents the total number of peptide bonds in the protein substrate (meqv/g protein). Besides, $\alpha$ represents the average degree of dissociation of the $\alpha$-$NH_3^+$, which was calculated according to Eq. (5):

$$\alpha = \frac{10^{\mathrm{pH-pK}}}{1 + 10^{\mathrm{pH-pK}}} \tag{5}$$

where pH represents the average pH of the α-amino groups liberated during the hydrolysis.

#### 2.6.2. DPPH radical scavenging capacity assay

The DPPH radical scavenging capacity assay was carried out as explained by a published report (Yu et al., 2021). Appropriately diluted samples and DPPH ethanol solution were fully mixed and incubated for 30 min. A series of diluted vitamin C solutions were used as positive control. Their absorbances were monitored by a UV–vis spectrophotometer (Purkinje T6-1650E, Beijing Purkinje General Instrument Co., Beijing, China) at 517 nm. The DPPH· scavenging capacity was calculated according to Equation (6) (Liu et al., 2015):

$$\mathrm{Inhibition}\,(\%) = \left[1 - \left(\mathrm{A_{sample}} - \mathrm{A_{background}}\right)/\mathrm{A_{control}}\right] \times 100 \tag{6}$$

where $A_{sample}$, $A_{background}$ and $A_{control}$ are the absorbances of sample, background and control, respectively.

#### 2.6.3. Ferric reducing antioxidant power (FRAP) assay

FRAP assay was conducted according to a previous study with little modification (Chen et al., 2021). In brief, sample solution (2 mL) and FRAP working solution (3 mL) were incubated for 10 min in the dark, and the absorbance was read at 593 nm using the UV–vis spectrophotometer. Vitamin C solutions were utilized as positive control. Final results were expressed as mg VC equivalent (VCE)/g DW.

#### 2.6.4. Fourier transform infrared spectroscopy (FTIR)

The functional groups and chemical structures of protein hydrolysate were detected by FTIR. In brief, these samples were mixed with potassium bromide, grounded into powder, and pressed into tablets (1–2 mm). The obtained tablets were scanned at a wavelength of 4000–400 $cm^{-1}$ and a resolution of 4 $cm^{-1}$ for 36 times.

### 2.7. Preparation of lotus seed paste

In brief, 150 g lotus seed was soaked in 300 mL of deionized water for 12 h, and then cooked at 100 °C for 2 h. The cooked lotus seed was cored, crushed, homogenized, and passed through a 60-mesh sieve. The sieve-through lotus seed powder (100 g), white granulated sugar (100 g) and sunflower seed oil (30 g) were added into a pot and stir-fried at 100 °C until the slurry gradually thickened. Afterward, 50 g of sunflower seed oil was added and stir-fried again until the formation of viscous shape. Finally, the obtained mixture was immediately cooled to prevent the surface hardening of lotus seed paste.

### 2.8. Effect of LSP-MD on the quality of lotus seed paste

#### 2.8.1. LSP-MD treatment and substitution rate of fat (SRF)

Briefly, 0, 8, 16, 24, and 32 g of LSP-MD with a DE value of 2.28 were utilized to partially replace the second added sunflower seed oil during the preparation of lotus seed paste. Their SRF values were 0 %, 10 %, 20 %, 30 %, and 40 %, respectively.

#### 2.8.2. Sensory evaluation

Each assessor used a “double blind” method for sensory evaluation and testing scores. Briefly, the prepared lotus seed pastes were cooled to room temperature and randomly numbered by staff. The meaning of these numbers or any hints cannot be told evaluators. Then, the numbered samples were placed on each clean white porcelain plate to visually observe their shape and color by 9 well-trained and experienced evaluators (six females and three males) at the same time in different evaluation rooms. Besides, one of the samples was cut into quarters to observe the internal tissue and to evaluate the aroma, taste and touch. After evaluating this sample, the assessors rinsed their mouth with distilled water before starting to evaluate the next sample. Based on the domestic trade industry standards of China, the standard of GB/T 21270–2007 for food fillings, and the actual situation in the experimental operation, the sensory evaluation standards for lotus seed paste were listed in Table S2.

#### 2.8.3. Determination of hardness and elasticity

The elasticity and hardness of lotus seed paste were measured using the TA-XT Plus texture analyzer with a probe type of P/36R. The specific parameters were as follows: test type was TPA full texture analysis, while the pre-test speed, test speed, post-test speed, test time, pressed depth, and test force were 2.0 mm/s, 3.0 mm/s, 2.0 mm/s, 5 s, 50 %, and 5 g, respectively.

### 2.9. Effect of LSP-PH on the lipid oxidation of lotus seed paste

#### 2.9.1. LSP-PH treatment

In brief, 0 %, 0.25 %, 0.5 %, 0.75 %, and 1 % of LSP-PH with a DH

value of 13.45 % were added in the preparation of lotus seed paste. The stored lotus seed paste for 0 and 15 days was sampled for chemical analyses below.

#### 2.9.2. Determination of peroxide value (POV)

The POV of sample was measured using titration method as explained by AOAC (2010). In short, 5 g of lotus seed paste and 30.0 mL of trichloroacetic acid-acetic acid solution (v:v, 1:3) were thoroughly mixed at room temperature to completely dissolve the oil. Then, 1.0 mL of saturated KI solution was added and reacted in the dark for 3 min. After the addition of 100 mL deionized water, this mixture was immediately titrated with $Na_2S_2O_3$ standard solution (2 mmol/L) until the solution turning into light yellow. At this time, 1 mL of starch indicator was added and continued titrating until the disappearance of blue color. Deionized water was utilized as the blank control group. The POV of sample was calculated according to Eq. (7):

$$\text{POV (g/100 g)} = \frac{(V_1 - V_0) \times C \times 0.1269 \times 100}{m_1} \times 78.7 \quad (7)$$

where $V_0$ and $V_1$ are the volumes (mL) of $Na_2S_2O_3$ standard solution consumed in the blank control, and sample groups, respectively, C is the concentration of $Na_2S_2O_3$ solution (mol/L), $m_1$ is the sample weight (g).

#### 2.9.3. Determination of acid value

The measurement of acid value was conducted according to AOAC method (AOAC, 2010). Briefly, 10 g of lotus seed paste was extracted by 150 mL of petroleum ether for 12 h, and then filtered with fast filter paper. The filtrate was evaporated to yield oil. Afterward, 2 g of oil sample and 50 mL of neutral ether-ethanol solution (v:v, 2:1) were fully mixed via a vortex. Following the addition of 3 drops of phenolphthalein indicator, this mixture was titrated with 0.05 mol/L KOH solution to the endpoint. The acid value of sample was calculated according to Eq. (8):

$$\text{Acid value (mg KOH/g)} = \frac{V \times C \times 56.1}{m_1} \quad (8)$$

where V represents the volume of NaOH solution (mL), C represents the concentration of NaOH solution (g/mL), $m_1$ represents the sample weight (g).

### 2.10. Statistical analysis

All the data were presented as mean ± SD (n = 3). Analysis of variance (ANOVA) and Duncan's multiple comparison was analyzed by SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set as $p < 0.05$.

## 3. Results and discussion

### 3.1. Chemical components of LSP

It can be seen from Table S3 that the protein, starch, soluble sugar, and moisture contents of LSP were 16.78 % ± 0.12 %, 38.24 % ± 0.34 %, 19.54 % ± 0.32 %, and 12.34 % ± 0.18 %, respectively. Whereas, some previous reports found that the protein, starch, and moisture levels of lotus seed were 16.53 %, 55.77 %, and 9.35 %, separately (Shahzad et al., 2021; Zhang et al., 2015). The various parts, varieties, and analytical methods might be responsible for this difference. Overall, the present result suggested starch and protein as the dominant components of LSP. Hence, the starch and protein in LSP were utilized to prepare maltodextrin and protein hydrolysate, respectively, in the present study.

### 3.2. Characteristics of LSP-MD

#### 3.2.1. The DE values

As shown in Fig. 1A, the DE values of LSP-MD in groups A, B, and C were 2.28 ± 0.13, 3.01 ± 0.31, and 4.32 ± 0.19, separately. In order to screen the optimal DE and reaction condition, the specific volume, water-holding capacity and retrogradation were used to assess the properties of LSP-MD.

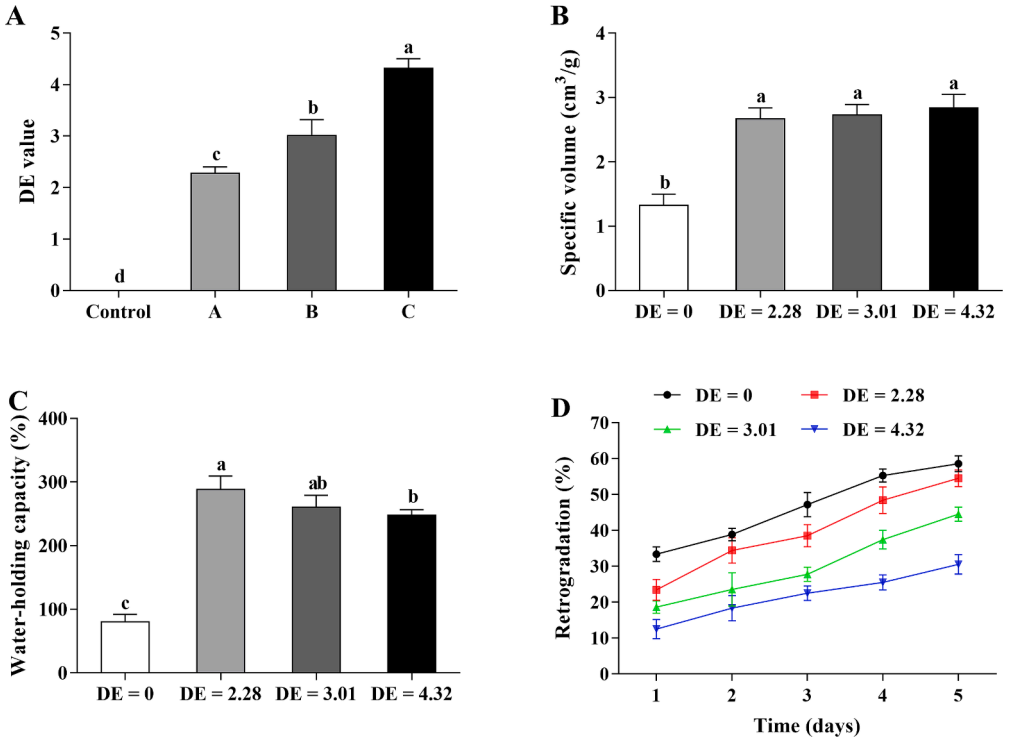


**Fig. 1.** The characteristics of LSP-MD evaluated by DE values (A), specific volume (B), water-holding capacity (C), and retrogradation (D). Different lowercase letters in the same treatment group indicate significant differences ($p < 0.05$).

### 3.2.2. Specific volume

Obviously, LSP-MD with different DE values exhibited a good dose-dependent association with its specific volume (Fig. 1B). In contrast with the original LSP starch, the maltodextrin with low DE value prepared from the controlled hydrolysis of starch had a significant increase in the specific volume (2.67–2.84 $cm^3/g$), which was twice of the original starch (1.32 ± 0.18 $cm^3/g$). This phenomenon would not only benefit the increment of unit product volume, but also help to reduce its energy level per unit volume. However, there was no significant difference in the specific volume among maltodextrins with different DE values.

### 3.2.3. Water-holding capacity

It can be seen in Fig. 1C that the water-holding capacity of LSP starch (DE = 0) with a concentration of 20 % at room temperature was relatively low (80.43 % ± 11.86 %), while appropriate α-amylase hydrolysis remarkably augmented its water-holding capacity. The hydrogen bonding between raw starch molecules can form large particles and bundle-like structures, resulting in a smaller spatial specific surface area, which is not conducive to their binding with water molecules (Donmez, Pinho, Patel, Desam, & Campanella, 2021). After enzymolysis, the maltodextrin, characterized by increased specific surface area, will be easier to absorb water molecules to form a reticulate gel, thus intercepting the water in the network structure and enhancing its water-holding capacity (Kanyuck et al., 2019). As the DE value further increases, the water-holding capacity of LSP-MD showed a downward trend. This may be ascribed that the ascended DE value of maltodextrin, accompanied by the increment of reducing sugar, triggers the dissolution of reducing sugar in water and the decrease of molecular weight of maltodextrin, thus resulting in the reduced reticular gel nodes and water-holding capacity (Wangsakan et al., 2003). Overall, good water-holding capacity of maltodextrin indicates its ability to retain more water, which exhibits great fluidity with similarity to fat in texture and taste, and thus being utilized to simulate the lubrication and viscosity of fat.

### 3.2.4. Retrogradation

After starch is gelatinized, if its volume reaches a certain level, the large particles and bundle-like structures within the amylose and the linear fractions of amylopectin will form sedimentation (Donmez et al., 2021). As depicted in Fig. 1D, LSP-MD with different DE values exhibited a good time-dependent association with its retrogradation. Specifically, the raw LSP starch (DE = 0) had the highest retrogradation in each time point. As the hydrolyzation degree increases, the retrogradation of LSP-MD showed a downward trend. This phenomenon might be explained by the fact that the greater DH could damage the amylose part of the starch and increase the proportion of branched chain molecules, thus leading to the diffusion of starch molecules to avoid their retrogradation (Kapusniak & Nebesny, 2017). Besides, the diffusive starch molecules can promote the formation of a gel network structure between the solution and the precipitation that traps the water molecules within the gel, thereby producing a viscosity similar to oil (Kanyuck et al., 2019). According to the results of specific volume, water-holding capacity and retrogradation, the LSP-MD with a DE value of 2.28 was utilized as a substitute to reduce the additive amount of oil in the lotus seed paste.

## 3.3. Effect of LSP-MD on the quality of lotus seed paste

### 3.3.1. Sensory preference of lotus seed paste

The effect of LSP-MD on the sensory evaluation of lotus seed paste was summarized in Fig. 2A. Overall, the aroma, color, texture, and section structure scores of lotus seed paste were inversely associated with the substitution rate of fat (SRF) by LSP-DM, while its taste and touch evaluation showed a fluctuation trend. When the proportion of other formulas remained unchanged, the lotus seed paste of 20 % SRF group had the closest aroma, color, taste and touch, texture, section structure, and total scores to those of the control group (SRF = 0 %). As the SRF of lotus seed paste continued to increase, all sensory indicators showed a downward trend until minimum value. Similar phenomenon was found by AbuDujayn et al. (2023) in which supplementation with maltodextrin could maintain the color, texture and overall acceptability of panned bread. Therefore, the addition of LSP-MD with a SRF of 20 % was the most suitable dose for maintaining the sensory quality of lotus

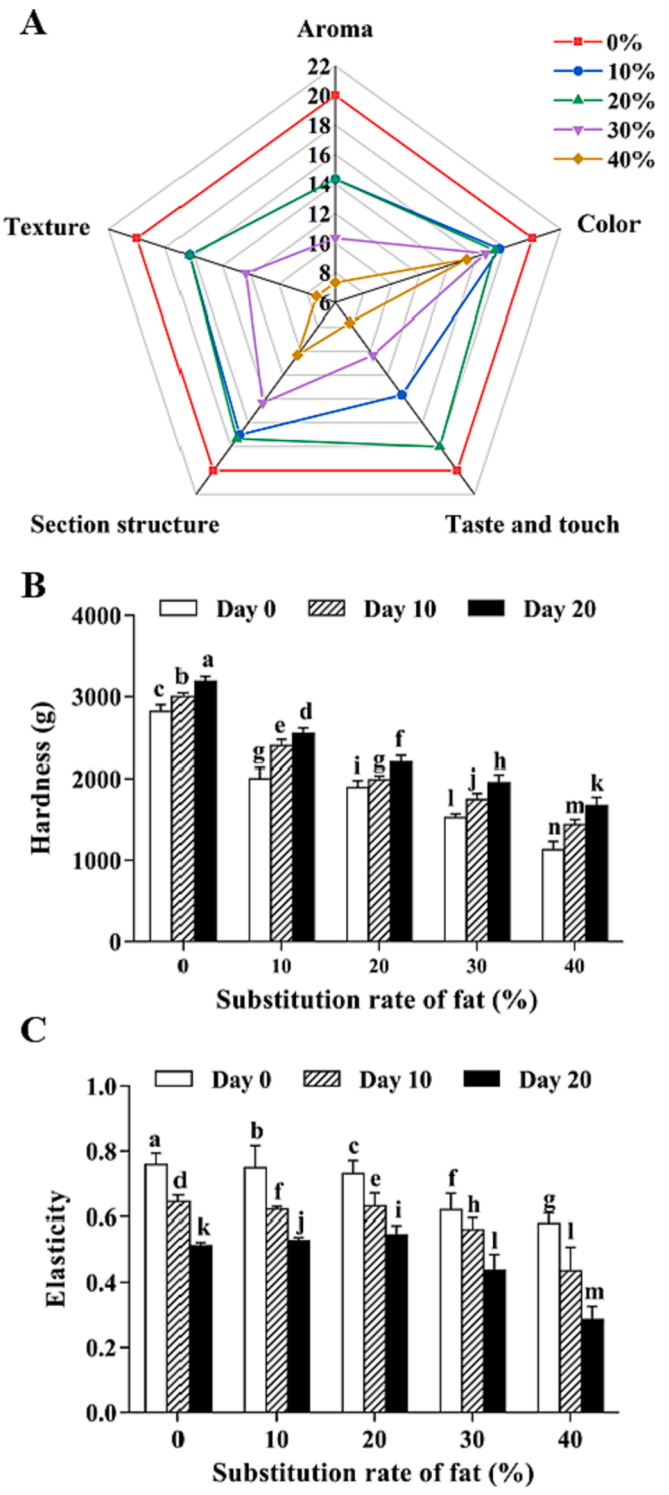


**Fig. 2.** Effect of LSP-MD on the sensory preference (A), hardness (B) and elasticity (C) of lotus seed paste. Different lowercase letters in the bar indicate significant differences ($p < 0.05$).

seed paste.

### 3.3.2. Hardness and elasticity of lotus seed paste

During the 20 days of storage, the hardness of the lotus seed pastes with different SRF all showed an obvious increasing trend (Fig. 2B), which could be explained by the retrogradation of starch molecules at room temperature with the extension of storage time. In terms with the different treatment groups, it can be seen that the hardness of lotus seed paste in the control group (SRF = 0 %) at each time point was all significantly higher than those of LSP-MD treated groups, indicating its severe starch retrogradation and poor sensory quality. Fortunately, the supplementation with LSP-MD remarkably inhibited the increment of hardness of lotus seed paste during the storage in a dose-dependent manner. This could be the reason that the addition of maltodextrin in the low-fat lotus seed paste system can form a weak gel with three-dimensional network structure (Pourmohammadi, Abedi, Hashemi, & Torri, 2018), thus avoiding the coalescence and flocculation between oil droplets and starch molecules, and eventually maintain the hardness and stability of lotus seed paste during storage.

Elasticity refers to the property of a sample that returns to its original state after the removal of external force, which is the height ratio of the second deformation to the first deformation (Steigmann, 2020). As depicted in Fig. 2C, the elasticity of the lotus seed paste in each group all showed an obvious downward trend in the 20 days of storage, which might be attributed to the starch aging and protein depolymerization caused by water recrystallization (Gao, Zeng, Qin, Zeng, & Wang, 2023; Niu, Zhang, Xia, Liu, & Kong, 2018). For the different sample groups, the lotus seed paste treated by 10 % and 20 % SRF of LSP-MD on Day 0 exhibited no significant differences in elasticity compared to the control group. As the SRF further increases, the elasticity of lotus seed pastes at each time point all showed a decreasing trend, indicating that the structure of lotus seed paste was damaged with poor recovery ability to some extent after exogenous force. On the 20th day, the elasticity of lotus seed paste in the 20 % SRF of LSP-MD group was stronger than that of the control and other LSP-MD groups. Based on the results of sensory evaluation, hardness and elasticity, LSP-MD (DE = 2.28) with an SRF of 20 % was the optimal additive amount to substitute part of oil in the lotus seed paste.

## 3.4. Characteristics of LSP-PH

### 3.4.1. The DH values

As shown in Fig. 3, the DH values of LSP-PH were dose-dependently augmented with the extension of enzymolysis time. Concretely, the DH values significantly increased from 8.62 % ± 0.49 % (15 min, LSP-PH1) to 16.37 % ± 0.85 % (60 min, LSP-PH3; $p < 0.05$), and then gradually ascended from 18.72 % ± 0.45 % (90 min, LSP-PH4) to 19.41 % ± 0.85 % (120 min, LSP-PH5) without significant difference ($p > 0.05$). In order to screen the optimal DH and hydrolysis time, DPPH· scavenging capacity and FRAP assays were used to evaluate the antioxidant activity of LSP-PHs.

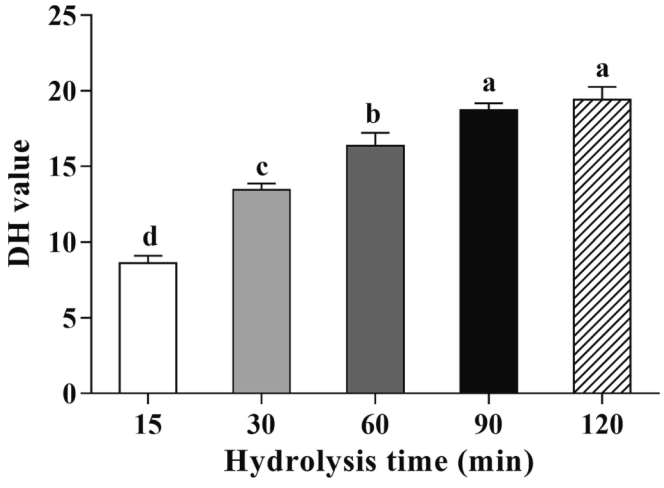


**Fig. 3.** The hydrolyzation degree (DH) of protein hydrolysate from LSP (LSP-PH) in the different enzymatic hydrolysis time. Different lowercase letters in the bar indicate significant differences ($p < 0.05$).

### 3.4.2. Antioxidant activity

Overall, LSP-PHs with different DH values showed good linear dose–effect relationships with their corresponding DPPH· scavenging rates and FRAP values (Fig. 4). Specifically, the DPPH· scavenging rate of LSP-PH2 with a DH of 13.45 % at 0.06 mg/mL was 80.53 % ± 1.66 %, which exceeded that of LSP-PH1 (78.34 % ± 3.28 %) and LSP-PH3 (75.43 % ± 1.86 %; Fig. 4A). Generally, a higher median inhibitory concentration ($IC_{50}$) suggests a stronger DPPH· scavenging capacity. The $IC_{50}$ values of samples for scavenging DPPH· were ranked as follows: LSP-PH2 (0.18 ± 0.02 mg/mL) < LSP-PH1 (0.22 ± 0.01 mg/mL) < LSP-PH3 (0.24 ± 0.01 mg/mL), which were significantly lower than that of the isolate (3.56 ± 0.01 mg/mL) and hydrolysate (4.7 ± 0.6 mg/mL) of lotus seed protein (Yu et al., 2021). Similarly, the capacity of samples at 0.06 mg/mL to reduce $Fe^{3+}$ was in a decreasing order of LSP-PH2 (1481.04 ± 90.63 mg VCE/g DW) > LSP-PH3 (1369.28 ± 89.65 mg VCE/g DW) > LSP-PH4 (1324.47 ± 98.31 mg VCE/g DW) > LSP-PH5 (1298.20 ± 64.10 mg

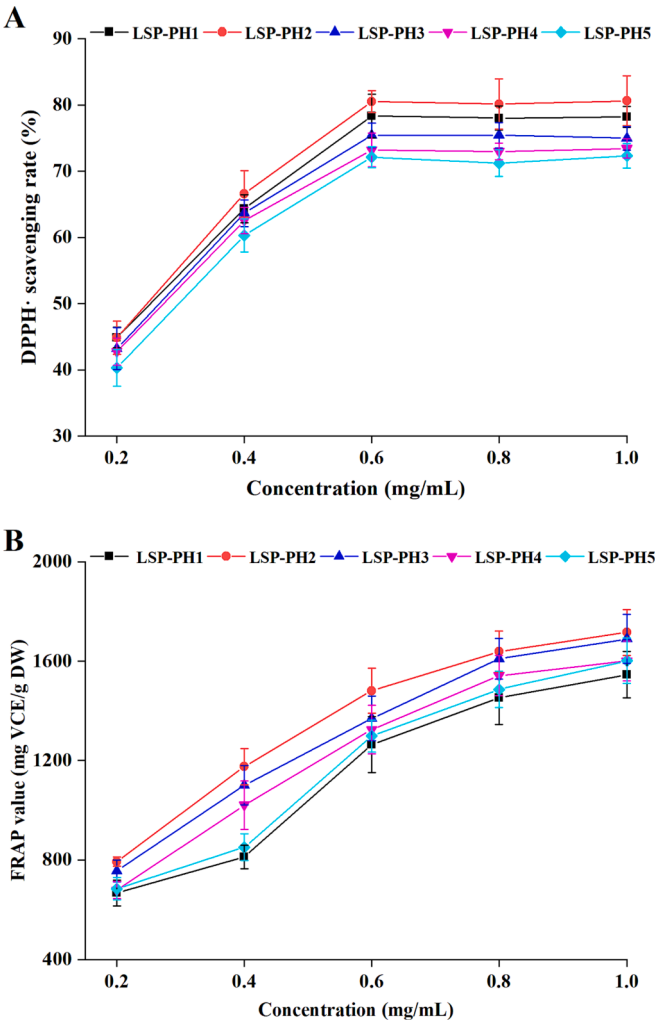


**Fig. 4.** The antioxidant activity of LSP-PH with different DH evaluated by DPPH radical scavenging capacity (A), and ferric reducing antioxidant power (FRAP; B).

VCE/g DW) > LSP-PH1 (1263.35 ± 113.07 mg VCE/g DW; Fig. 4B). Besides, a previous study confirmed the stronger $Fe^{3+}$ reducing power of lotus seedpod proanthocyanin-whey protein isolate conjugate (691.85 ± 4.54 μg/mL) than that of whey protein isolate (10.43 ± 0.26 μg/mL) (Chen et al., 2021). These results indicated that LSP-PH2 had stronger DPPH· scavenging capacity and ferric reducing antioxidant power than the other LSP-PHs, which might be ascribed to its higher hydroxyl groups caused by moderate hydrolysis. Thus, the LSP-PH2 with a DH of 13.45 % was further characterized by FTIR below.

### 3.4.3. Estimation of secondary structure of LSP-PH by FTIR

The FTIR spectra and secondary structure composition of LSP-PH2 were summarized in Fig. 5. According to our results shown in Fig. 5A-C and the previously published studies, it can be therefore inferred that 1614.8 $cm^{-1}$, 1627.7 $cm^{-1}$, 1631.5 $cm^{-1}$ and 1639.3 $cm^{-1}$ could be assigned to β-sheet structures, 1645.8 $cm^{-1}$ and 1652.8 $cm^{-1}$ belonged to random coil structures, 1658 $cm^{-1}$ and 1662.4 $cm^{-1}$ could be classified to α-helix structures, while peaks at 1670.5 $cm^{-1}$, 1673.9 $cm^{-1}$, 1684.2 $cm^{-1}$ and 1685.5 $cm^{-1}$ were β-turn structures (De Meutter & Goormaghtigh, 2021; Farrell, Wickham, Unruh, Qi, & Hoagland, 2001). As depicted in Fig. 5D, the secondary structure of LSP protein dominantly consisted of β-sheet (49.33 % ± 2.18 %), α-helix (22.85 % ± 1.12 %), random coil (17.80 % ± 3.40 %), and β-turn (10.02 % ± 3.30 %). However, the β-sheet, α-helix, and random coil of LSP-PH reduced by 23.21 %, 24.72 %, and 19.47 %, respectively, while β-turn increased by 119.16 %. Generally, the β-turn structure mainly exists on the surface of molecules instead of inside the molecules, which exhibits stronger hydrophilicity than the entire molecule (De Meutter & Goormaghtigh, 2021). The augmented β-turn level in LSP-PH may be explained by the fact that more hydrophilic amino acid residues were formed during the alkaline protease hydrolysis of LSP protein. To the best of our knowledge, α-helix and β-sheet involves a large number of hydrogen bonds, endowing the ordered secondary structure of proteins with considerable rigidity (Leader & Milner-White, 2011). Whereas, hydrogen bonds and other interactions do not exist in the random coil, resulting in greater degrees of freedom of each residual bond in the peptide segment without rigidity, thus exhibiting great flexibility (Liu, Wang, Barrow, & Adhikari, 2014). The β-turn is between the two characteristics mentioned above, which shows a combination of rigidity and softness. After enzymatic hydrolysis of LSP protein, the β-sheet was transformed into β-turn and random coil, triggering the exposure of hydrophobic amino acid residuals and the interaction between antioxidant peptides and hydrophobic polyunsaturated fatty acids (Ahmad, Fatima, Khan, & Khan, 2010), thereby inhibiting lipid peroxidation and improving the antioxidant activity of protein. Therefore, the LSP-PH with a DH of 13.45 % was further used as a supplement to inhibit the lipid oxidation of lotus seed paste in this study.

## 3.5. Effect of LSP-PH on the lipid oxidation of lotus seed paste

### 3.5.1. POV of lotus seed paste

The POV reflects the degree of primary oxidation of lipids, which is formed by the combination of unsaturated double bonds in lipids and oxygen. A larger POV indicates higher levels of intermediate products accumulated during the lipid oxidation process, which can gradually divide into small substances with the progress of oxidation (Chen et al.,

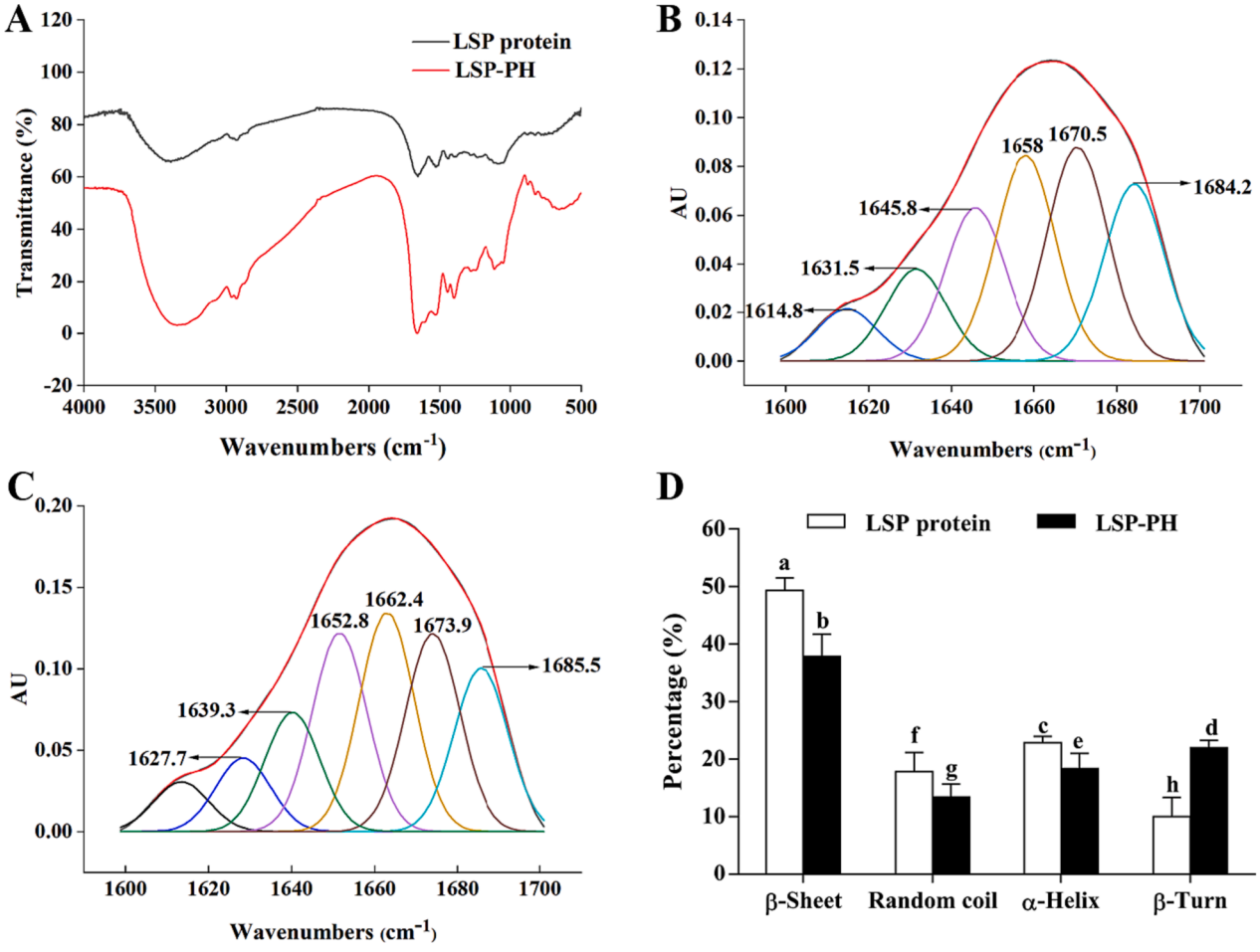


**Fig. 5.** The estimation of secondary structure of LSP-PH: FTIR spectra (A), infrared fitting diagrams of LSP protein (B) and LSP-PH (C), and secondary structural composition of proteins (D).

2022). As shown in Fig. 6A, the initial POV of lotus seed paste was significantly decreased by the addition of LSP-PH or $V_C$ ($p < 0.05$). Following 15 days of storage, the POV of each group showed an upward trend., which were in an increasing order of $V_C$ group (0.45 ± 0.04 g/kg) < 1 % LSP-PH group (0.47 ± 0.03 g/kg) < 0.75 % LSP-PH group (0.48 ± 0.04 g/kg) < 0.5 % LSP-PH group (0.51 ± 0.07 g/kg) < 0.25 % LSP-PH group (0.65 ± 0.02 g/kg) < control group (0.70 ± 0.01 g/kg). Overall, the inhibitory effect of LSP-PH on the lipid peroxidation of lotus seed paste was comparable to the $V_C$ group. Similarly, protein hydrolysate from silver carp fin has been confirmed to be effective in ameliorating the lipid oxidation by decreasing the levels of free fatty acid, POV and TBARS in bighead carp fillets via antioxidant mechanism (Zhang et al., 2020).

### 3.5.2. Acid value of lotus seed paste

Acid value is one of the indicators to measure the rancidity degree of lipids. It can be seen from Fig. 6B that the initial acid values of each group were basically consistent without significant difference ($p > 0.05$). After 15 days of storage, the acid values of each group all significantly augmented, in which 0.5 % LSP-PH group (1.13 ± 0.03 mg KOH/g) and $V_C$ group (0.98 ± 0.04 mg KOH/g) were the lowest, followed by 0.75 % LSP-PH group (1.24 ± 0.03 mg KOH/g), and that of control group (1.52 ± 0.03 mg KOH/g) were the highest. As the storage proceeds, the lotus seed paste may come into contact with air and gradually decompose lipid into free fatty acids to increase the acid value and reduce the storage stability of products (Zhao et al., 2022). Fortunately, LSP-PH could decline the acid value of lotus seed paste by 25.7 % to inhibit the lipid oxidation throughout storage to some extent, which was in great concordance with the results of POV assay mentioned above (Fig. 6A). The reason might be that enzymatic hydrolysis of LSP protein could facilitate the transition of β-sheet into β-turn and random coil, further triggering the exposure of hydrophobic amino acid residuals and the interaction between antioxidant peptides and hydrophobic polyunsaturated fatty acids (Ahmad et al., 2010), thus inhibiting the lipid peroxidation of lotus seed paste. A similar finding was reported that casein and whey protein hydrolysates could significantly prevent the formation of thiobarbituric acid reactive substances (TBARS) to alleviate the lipid peroxidation of zebrafish larvae (Carrillo et al., 2017). According to the results of POV and acid value, 0.5 % LSP-PH (DH = 13.45 %) was the optimal additive amount to inhibit the lipid oxidation of the lotus seed paste, thus extending its shelf life to some degree.

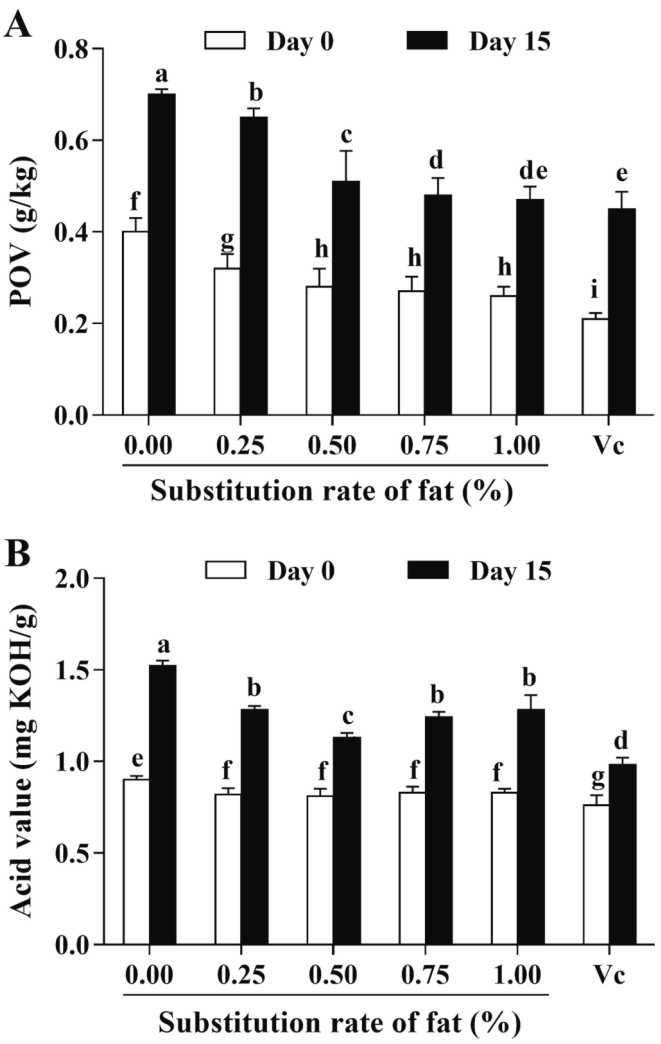


**Fig. 6.** Effect of LSP-PH on the fat oxidation of lotus seed paste during storage evaluated by POV (A), and acid value (B). Different lowercase letters in the same treatment group indicate significant differences ($p < 0.05$).

## 4. Conclusion

This study confirmed that the LSP-MD with a DE value of 2.28 was the most suitable fat substitute to obtain low-fat lotus seed paste. Throughout the 20 days of storage at 25 °C, this LSP-MD with a SRF of 20 % could effectively maintain the sensory quality, hardness and elasticity of lotus seed paste. For protein hydrolysate, LSP-PH with a DH of 13.45 % exhibited the strongest antioxidant activity, which was further demonstrated by FTIR spectra that enzymatic hydrolysis of LSP protein could facilitate the transition of β-sheet into β-turn, further triggering the exposure of hydrophobic amino acid residuals with hydroxyl groups. After 15 days of storage, intervention with LSP-PH significantly decreased the POV and acid value of lotus seed paste, which indicated its excellent inhibitory effect on the lipid peroxidation via interacting with hydrophobic polyunsaturated fatty acids. The present findings proposed that LSP-MD and LSP-PH could reduce the oil usage of lotus seed paste and inhibit its lipid oxidation, respectively, which would provide a theoretical basis for the application of lotus seed peel powder as a substitute for fat and a potential antioxidant in food products.

## CRediT authorship contribution statement

**Na Deng:** Methodology, Data curation, Software, Writing – original draft. **Zhao Li:** Methodology, Investigation, Data curation, Formal analysis. **Hui Li:** Resources, Visualization, Writing – review & editing. **Yongjian Cai:** Supervision, Visualization. **Changzhu Li:** Project administration, Software. **Zhihong Xiao:** Conceptualization, Visualization. **Bo Zhang:** Conceptualization, Validation. **Miao Liu:** Methodology, Resources. **Fang Fang:** Supervision, Resources. **Jianhui Wang:** Conceptualization, Visualization, Funding acquisition, Supervision, Writing – review & editing.

## Declaration of Competing Interest

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

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100967.

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