The antioxidant capacity and nutrient composition characteristics of lotus (*Nelumbo nucifera* Gaertn.) seed juice and their relationship with color at different storage temperatures

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ABSTRACT

The effects of different storage temperatures on the nutritional quality, color, and antioxidant capacity of lotus seed juice and the correlations between various physicochemical indices and antioxidant capacity during storage were investigated in this study. The results showed that the overall retention rate of various nutrients and antioxidant activity in lotus seed juice under low-temperature storage was better than that under 37 °C storage. Meanwhile, temperature had a significant effect on increasing the browning of lotus seed juice and the change in *L**. The results of Pearson correlation and redundancy analysis (RDA) showed that the reduction in antioxidant activity in lotus seed juice aggravated the browning index of the system at high temperatures. The color changes in the system were closely related to the clarity of lotus seed juice and aging of starch at low temperatures.

Introduction

Presently, with the continuous increase in consumer demand for functional foods, such as the increased level of lactose intolerance among the population and concerns for animal protection and environmental issues, the types of functional foods are also becoming increasingly abundant. This trend is driving food processing industries to focus on nutritional products derived from natural plant sources (Aydar, Tutuncu, & Ozcelik, 2020). Plant-based foods are an essential and sustainable option for human nutrition, and the plant-based beverage industry has always had a high potential for global growth, especially in countries where mammalian milk products are scarce and expensive, and plant-based beverages are a more affordable option (González-Monroy, Rodríguez-Hernández, Ozuna, & Sosa-Morales, 2018). Beverages made from ingredients extracted from different types of plants are called plant-based beverages, and they are usually rich in protein, dietary fiber, fat, vitamins, and phytochemicals, but the

beneficial bioactive compounds in plant-based beverages are easily lost during processing, and more research is needed on the browning capacity of plant-based beverages during storage and the relationships among ingredients. Interactions between different compounds can affect the absorption of themselves and other compounds co-occurring in foods and can have an impact on some chemical, physical, nutritional and organoleptic properties of foods (Cianciosi, Forbes-Hernandez, Regolo, Alvarez-Suarez, Navarro-Hortal, Xiao, et al., 2022; Danila Cianciosi, Forbes-Hernández, Giampieri, Zhang, Ansary, Pacetti, et al., 2019).

Plant beverages are complex systems composed of multiple nutrients. Therefore, destabilization phenomena, such as precipitation, emulsification, and dehydration separation, are more likely to occur during heating or storage, are detrimental to the storage stability of these beverages, and direct affect their quality and nutritional properties. Browning of beverages during storage is an important food quality consideration in the food industry, and non-enzymatic browning reactions are usually the cause of undesirable color changes in beverages

that have been autoclaved during storage. The main factors for the non-enzymatic browning of beverages during storage are storage temperature, storage time, oxygen content during storage, and different plant-based raw materials. In this case, the combination of storage time and temperature is an important variable that needs to be controlled (Ioannou, Hafsa, Hamdi, Charbonnel, & Ghoul, 2012).

The effects of temperature on different nutrients in plant-based beverages are different. When the storage temperature is above 30 °C, the browning rate of orange juice is accelerated. At the same time, with increasing storage temperature, the decomposition rate of ascorbic acid in beverages gradually accelerates. The decomposed products easily react with amino acids to form red and yellow substances, resulting in the browning of beverages (Klimczak, Małecka, Szlachta, & Gliszczyń-ska-Świgło, 2007; C. Wang, Yang, & Li, 2021). However, cold storage has no significant effect on the flavonoid concentrations in soy protein beverages, and thermal treatment does not reduce the content of soy flavonoid aglycones (Ruiz de la Bastida, Peirotén, Langa, Curiel, Arqués, & Landete, 2022). Therefore, how changes in food nutrients under high-temperature or low-temperature storage conditions affect the colors or browning of food also needs to be studied further.

Lotus (*Nelumbo nucifera Gaertn.*) seeds are mature seeds of the aquatic herb lotus and are rich in starch carbohydrates, proteins, lipids, and other nutrients (such as vitamins and minerals) needed by the human body but also contain small amounts of minerals, poly-saccharides, flavonoids, superoxide dismutase, and other active sub-stances (Yu, Wei, Liu, Dong, Hao, Zhang, et al., 2022). The presence of phenolic compounds endows lotus seeds with various biological activities, such as hypoglycemic, antiaging, gastrointestinal flora regulation, anti-inflammatory, antiviral, and immune regulation activities (Punia Bangar, Dunno, Kumar, Mostafa, & Maqsood, 2022). Therefore, lotus seed has been used as a dietary supplement to obtain health benefits for residents in Asia, Southeast Asia (China, India, and Russia), and the northern parts of Australia and North America for thousands of years (Lei, Zhang, Wang, Zheng, Miao, & Lu, 2022). Recently, the types and quantities of processed lotus seeds have been increasing, mainly including lotus seed bread, noodles, cookies, and canned lotus seeds (Punia Bangar, Dunno, Kumar, Mostafa, & Maqsood, 2022). There are also products corresponding to lotus juice beverages made from raw lotus seed materials directly pulped with water. Nevertheless, there is a lack of systematic research on the causes of browning of lotus seed juice beverages during storage at a high or low temperature after autoclaving. Therefore, this manuscript primarily studies the nutrient changes that occur during the storage of lotus seed juice (lotus seed juice beverages without additives) at different storage times and the relationships between the changes in nutrients in these beverages and antioxidant activities or browning during storage. Furthermore, the classical approaches of browning kinetic modeling (zero-order, half-order, first-order, and combined reaction modeling) were used to simulate the changes in color value parameters during the storage of lotus seed juice to predict the quality changes of lotus seed juice at different temperatures.

Materials and methods

Materials

Flash-frozen fresh lotus seeds were obtained from Green Acres Food Co., ltd. (Fujian, China). Coomassie brilliant blue protein reagent was obtained from Beijing Solarbio Technology Co., ltd., 95 % ethanol was obtained from Xilong Scientific Co., ltd., phenol, ethyl ether, and concentrated sulfuric acid were obtained from Sinopharm Group Chemical Reagent Co., ltd., Folin-Ciocalteu, anhydrous sodium carbon-ate, and catechol were obtained from Shanghai Macklin Biochemical Technology Co., ltd., AAPH (2, 2′-azobis(2-amidinopropane)dihydro-chloride) was obtained from Qiaoyi Biotechnology (Shanghai) Co., Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was

obtained from Aldrich Chemical Co., TPTZ, and sodium fluorescein was purchased from Sigma Chemical Co. All other chemicals used were of analytical grade.

Preparation of lotus seed juice beverages

After lotus seeds were selected, peeled, and cored, they were beaten in a blender (PRO-12S, German Pool ltd., Hong Kong, China) 2 min × 2 times with a material-to-liquid ratio of 1:10. The gelatinized lotus seed juice experienced high-pressure homogenization (SRH 40–100, Shanghai Shenger Homogenizer Co., ltd, China) and was homogenized at 60 MPa for 3 min. The homogenized lotus seed juice was filled and autoclaved at 121 °C for 15 min in a vertical autoclave (YSQ-30, Shanghai Boxun Industry & Commerce Co., ltd, China). The sterilized lotus seed juice was stored at 4 °C and 37 °C for 0, 1, 2, 3, 4, 5, and 6 months and then removed for measurement.

Determination of the nutrient composition

50 mL of lotus seed juice was centrifuged (centrifugal separator, Allegra X-30R benchtop, Beckman Coulter, Brea, CA, USA). Soluble protein content in supernatant, polysaccharide content uncentrifuged filtrate, and total phenolic content in supernatant were measured with Coomassie Brilliant Blue Reagent, phenol–sulfuric acid, and Folin-Ciocalteu reagent, respectively (Chen, et al., 2015; Lin, Yang, Chi, & Ma, 2020; Oliveira-Alves, Pereira, Pereira, Ferreira, Mecha, Silva, et al., 2020). The absorbance was measured at 595 nm, 490 nm, and 760 nm with an ultraviolet spectrophotometer (UV-1800 ultraviolet spectro-photometer, Meipuda Instruments ltd., Shanghai, China). The results are expressed as μg of protein equivalent per mL of sample (μg/mL), mg of glucose equivalent per mL of sample (mg/mL), and μg of gallic acid equivalent per mL of sample (μg GAE/mL).

Lipid and ash content were obtained following the Chinese national standard GB/T5009.6–2003 (Soxhlet extractor method) and GB-5009.4–2016 (Sundrasegaran & Mah, 2020).

Determination of enzyme activity

Polyphenol oxidase (PPO) activity assay

PPO was determined according to the method of Tiptiri-Kourpeti et al. (Tiptiri-Kourpeti, Fitsiou, Spyridopoulou, Vasileiadis, Iliopoulos, Galanis, et al., 2019) and modified appropriately. A 1.5 mL sample was weighed with an analytical balance (BSA 124S, Sartorius, Germany) and placed into a 50 mL conical flask, and 10.00 mL of phosphate buffer solution and 2.00 mL of catechol solution were added in sequence. After thorough mixing, the solution was reacted in a thermostatic oscillator (SHA-C, Guohua Electric Co., ltd., Changzhou, China) at 37 °C for 15 min and then quickly placed into an ice bath (Ice maker, IMS-20, Xueke Electric Appliances Co., ltd., Changshu, China) for 3 min.

The mixture was centrifuged at 6400 × g for 10 min at 4 °C, and the supernatant was collected. The absorbance of the blank solution was measured at 410 nm. The equation for calculating the PPO activity is shown below in Equation (1).

$$X = \frac{A}{0.001 \times m \times t} \tag{1}$$

In Equation (1) shown above, *X* denotes the enzyme activity of the sample (U), 0.001 corresponds to the enzyme activity conversion factor, *m* denotes the sample amount taken (mL), and *t* represents the reaction time (min).

Superoxide dismutase (SOD) activity assay

SOD activity was detected by the hydroxylamine method, and an appropriate amount of lotus seed juice was placed into a centrifuge tube and centrifuged at 2800 × g for 10 min. The reagents were added in sequence according to the manufacturer's instructions provided with the

reagent kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). After mixing the reagents, the absorbance was measured at 550 nm. The equation for calculating the SOD activity is shown below in Equation (2).

$$X = \frac{A_{control} - A_{measure}}{A_{measure}} \div 50\% \times \frac{V_1}{V_2} \div c \qquad (2)$$

In Equation (2) shown above, X denotes the enzyme activity of the sample (U/g), $A_{control}$ corresponds to the absorbance value of the control tube, $A_{measure}$ denotes the absorbance value of the sample (mL), V_1 represents the total volume of the reaction solution (mL), V_2 indicates the amount of the sample (mL), and c represents the homogenate concentration (mg/mL).

Peroxidase (POD) activity assay

POD activity was measured by colorimetry. An appropriate amount of lotus seed juice was placed into a centrifuge tube and centrifuged at 2800 × g for 10 min. The reagents were added in sequence according to the manufacturer's instructions for the reagent kit (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China), with deionized water used as the control sample. The absorbance at 550 nm was measured. The equation for calculating POD activity is shown in Equation (3).

$$X = \frac{A_{measure} - A_{control}}{12} \times \frac{V_1}{V_2} \div t \div c \times 1000 \qquad (3)$$

In Equation (3) shown above, X denotes the enzyme activity of the sample (U/mg), $A_{control}$ corresponds to the absorbance value of the control tube, $A_{measure}$ denotes the absorbance value of the sample (mL), V_1 represents the total volume of the reaction solution (mL), V_2 indicates the amount of sample (mL), and c represents the homogenate concentration (mg/mL), t represents the reaction time (min).

Determination of antioxidant capacity

Antioxidant capacity was determined by measuring oxygen radical absorbance capacity (ORAC).

The ORAC assay was performed essentially as described by Huang (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002). In summary, AAPH (2,2′-azobis (2-amidinopropane) dihydrochloride, Qiaoyi Biotechnology (Shanghai) Co) was dissolved in 10 mL of 75 mM phosphate buffer (pH 7.4) to a final concentration of 153 mM and was prepared fresh prior to use. This phosphate buffer was used to prepare a fluorescein stock solution (4×10^{-3} mM), which was stored and diluted with the phosphate buffer above. Then, 150 μL of sodium fluorescein solution was added to the experimental wells of all microplate readers (Tecan, Raleigh, NC). A total of 25 μL of 75 mM phosphate buffer (pH 7.4) was added to the blank wells, 25 μL of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Aldrich Chemical Co.) at different concentrations was added to the standard wells (the concentrations were linear within 0–100 μM), and 25 μL of lotus seed juice was added to the sample wells. An excitation wavelength of 485 nm and an emission wavelength of 535 nm were used. The reaction was started by adding 25 μL of AAPH reagent with a shaking duration of 8 s, kinetic cycle of 40, and kinetic interval of 127 s. The *AUC* and net *AUC* values of the standards and samples were determined as shown in Equation (4) and (5), respectively. The results are expressed as ORAC values, which are equivalent to Trolox antioxidant capacity values.

$$AUC = 0.5 + (R_2/R_1) + (R_3/R_1) + (R_4/R_1) + \cdots + 0.5(R_n/R_1) \qquad (4)$$

$$NetAUC = AUC_{sample} - AUC_{blank} \qquad (5)$$

In Equation (4) and (5) shown above, R_1 denotes the initial fluorescence reading at the start of the reaction and R_n represents the fluorescence reading at the last measurement.

Reducing power measured as ferric reducing antioxidant power (FRAP)

A FRAP assay was performed according to the method of Benzie (Iris F.F. Benzie, 1996) et al. with some modifications. The stock solutions (pH 3.6) included 300 mM acetate buffer (prepared from 3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$), a 10 mM solution of TPTZ (2,4,6-tripyridyl-*s*-triazine, Sigma Chemical Co.), a 40 mM solution of HCl and a 20 mM solution of $FeCl_3 \cdot 6H_2O$. Fresh reaction reagents were prepared as required by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution. Ten microliters of lotus seed juice was added to 1 mL of deionized water and reacted with 1.8 mL of FRAP solution for 10 min at 25 °C. Then, the sample absorbance was read at 593 nm. The standard curve was linear between 700 μM and 4800 μM Trolox. The results of the total antioxidant capacity are expressed in mmol Fe(II)/g.

Determination of the browning indices and color value

Ten milliliters of lotus seed juice was centrifuged at 6400 × g for 20 min at 4 °C, the supernatant was filtered with a 0.45 μm filter membrane, and the absorbance of the filtrate was measured at 420 nm with an ultraviolet spectrophotometer (UV-2100 ultraviolet spectrophotometer, Meipuda Instruments ltd., Shanghai, China). The degree of browning was represented by the absorbance value at 420 nm (Landl, Abadias, Sárraga, Viñas, & Picouet, 2010), and the test was performed with deionized water as the blank.

Color measurements were performed using a CS-200 spectrophotometer (Hangzhou CHNSpec Technology Co., ltd., Hangzhou, China). White calibration was used for instrument standardization. The L^*, a^*, b^* color space was used for the measurement. The values L^*, a^*, and b^* indicate luminosity on a green (−) to red (+) axis, and chromaticity on a blue (−) to yellow (+) axis, respectively. The total color change (ΔE) was calculated using Equation (6):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (6)$$

where L^*, a^*, and b^* indicate lightness, red-green coloration, and yellow-blue coloration, respectively.

Kinetic modeling

Kinetic models were developed using a three-step procedure. Reaction rate constants were determined by fitting the experimental data to zero-order (Eq. (7)), first-order (Eq. (8)) and combined (Eq. (9)) kinetic models (Chen, et al., 2015).

$$C = C_0 + k_0 t \qquad (7)$$

$$C = C_0 \exp(k_1 t) \qquad (8)$$

$$C = k_0/k_1 - (k_0/k_1 - C_0)exp(-k_1 t) \qquad (9)$$

In Equation (7), (8), and (9) shown above, C is the parameter studied (i.e., A420 nm, L^*, a^*, b^*) at any given reaction time, C_0 is the initial value of the untreated sample (L_0, a_0, b_0), t indicates the storage time, and k_0 and k_1 are reaction rate constants.

The Arrhenius model (Eq. (10)) was used to describe the effect of temperature on the reaction rate constants:

$$k = k_0 exp(-E_a/RT) \qquad (10)$$

where k is the equilibrium constant, k_0 is the exponential constant, E_a is the activation energy (kJ/mol), R is the constant (8.314×10^{-3} kJ/moL·K), and T is the absolute temperature (K).

Equation (11) takes the logarithm of both sides of Equation (10):

$$-\ln k = \ln k_0 + E_a/RT \qquad (11)$$

where k is the reaction constant of the model at an absolute

temperature of 310 K, k_0 is the kinetic constant of the joint reaction, E_a is the activation energy (kJ/mol), R is the constant (8.314×10^{-3} kJ/moL·K), and T is the absolute temperature (K). E_a is calculated according to Formula (10).

Results and discussion

Changes in soluble protein content

Lotus seeds contain protein contents that range between 16 and 28 %, and the total protein content can be divided into different components according to solubility, including albumin (41.6 %), globulin (26.6 %), glutenin (18.0 %), and prolamin soluble proteins (6.0 %) (Punia Bangar, Dunno, Kumar, Mostafa, & Maqsood, 2022). In Fig. 1A and B, the soluble protein content of lotus seed juice showed a slow rising trend at first and then decreased with prolonged storage time, but it did not increase significantly ($p > 0.05$) under storage at 37 °C. The maximum value was reached at 2 months of storage. This may be due to the release of free protein in lotus seed cells at the beginning of storage, leading to an increase in soluble protein content. In addition, the decomposition of insoluble proteins into soluble proteins may also promote an increase in soluble protein content. The soluble protein content decreased significantly ($p < 0.05$) after 2 months of storage and reached its lowest value after 6 months.

The decreased protein content may also be due to the formation of a more significant number of noncovalent hydrophobic bonds (such as van der Waals forces, hydrophobic interactions, ionic bonds, and hydrogen bonds) in lotus seed juice, which promotes interactions among polyphenols and proteins in the samples, thereby forming insoluble complexes (D. Cianciosi, et al., 2022). Moreover, lotus seed starch could also interact with lipids or proteins through hydrophobic interactions to form starch-protein-lipid complexes (Lin, Yang, Chi, & Ma, 2020), resulting in reductions in protein content.

In addition, plant proteins are more greatly denatured at high temperatures, and the denaturation, aggregation, or hydrolysis of proteins leads to a decreased soluble protein content in the system (Ahmadian-Kouchaksaraei, Varidi, Varidi, & Pourazarang, 2014). Therefore, the protein content decreases relatively more at high temperatures than at low temperatures.

Changes in polysaccharide content

Plant polysaccharides can scavenge oxygen free radicals in the body as free radical scavengers and biological antioxidants, which include superoxide, anions, hydrogen peroxide, and hydroxyl radicals. Lotus seed polysaccharide is a glycoprotein that has been indicated to have certain effects in improving immunity, regulating intestinal flora, and inhibiting aging (Zhang et al., 2023).

As Fig. 1C and D indicate, the polysaccharide content of stored lotus seed juice showed a trend of increasing first and then decreasing and tended to be stable in the late storage period (5–6 months). The polysaccharide content increased to their highest value at 1 month of storage. The increase in total sugar content in the early stage of storage may be due to the further destruction of the lotus seed matrix, which leads to degradation of cell wall pectin, cellulose, and hemicellulose, promoting the release of some neutral sugars (Atencio, Verkempinck, Bernaerts, Reineke, Hendrickx, & Van Loey, 2022). At high temperatures, soluble polysaccharides were released more strongly than at low temperatures.

Subsequently, the polysaccharide content of lotus seed juice decreased significantly ($p < 0.05$) and reached their lowest value at 6 months, a decrease of 9.56 %. With an extension of storage, polysaccharides are often consumed as energy storage materials, and the Maillard reaction with free amino acids may lead to a decrease in polysaccharides (Chantakun, Nilsuwan, Tagrida, Sumpavapol, & Benjakul, 2022).

Changes in total phenolic content

Phenolic compounds are widely distributed biologically active natural metabolites in nature and have been proven to be one of the main substances that determine antioxidant capacity (Punia Bangar, Dunno, Kumar, Mostafa, & Maqsood, 2022). As shown in Fig. 1E and F, the total phenolic content of lotus seed juice decreased over time during a 6 month storage period, and the decreasing trend of total phenolic content was not significant ($p > 0.05$) during the first 3 months under high-temperature storage or during the first 5 months under low-temperature storage. The lower total phenolic content in the early storage stage may be due to the low dissolved oxygen content in lotus seed juice and the oxidative degradation of phenolic compounds (Chen, Pang, Zhao, Gao, Liao, Wu, et al., 2015).

However, the total phenolic content subsequently decreased significantly ($p < 0.05$), and the content of soluble polysaccharides decreased more at high temperatures. This decreasing effect was more pronounced with increasing time (Purewal, Kamboj, Sandhu, Kaur, Sharma, Kaur, et al., 2022). Phenolic substances are prone to oxidation and polymerization during storage, reducing the number of free hydroxyl groups and causing a decrease in the measured total phenolic content (Liu, Wang, Li, Bi, & Liao, 2014). The total phenolic content of most plant beverages decrease with prolonged storage time, even under low-temperature storage conditions (Klimczak, Małecka, Szlachta, & Gliszczyńska-Świgło, 2007; Piljac-Žegarac, Valek, Martinez, & Belščak, 2009).

Changes in the lipid content of insoluble components in lotus seed juice

Most fruit and vegetable juices are low in lipids (Tiptiri-Kourpeti, Fitsiou, Spyridopoulou, Vasileiadis, Iliopoulos, Galanis, et al., 2019). The lipid content of lotus seeds is approximately 0.2–3 %, and linoleic acid is the main fatty acid present (Punia Bangar, Dunno, Kumar, Mostafa, & Maqsood, 2022). Lotus seed juice was autoclaved, resulting in a lower lipid content as a result of the dilution effect. Because of the low lipid content, lipid retention was mainly observed in lotus seed sediment. As shown in Fig. 1G and H, at the beginning of storage, the lipid content in the sediment of the lotus seed juice was 0.1 %, which indicates that the lipids in the sediment may have been effectively released into the lotus seed juice. However, the lipids disappeared from the system after storage, which may be due to the formation of a complex between the lipids of lotus seeds and the starch of lotus seeds, causing the lipids to be embedded in starch (Ye, Hu, Luo, McClements, Liang, & Liu, 2018). This process accelerated after high-pressure homogenization treatment. Furthermore, since lipids are insoluble in the aqueous phase, some of the lipids released and transferred to the aqueous phase may form micellar structures with other released intracellular active components (Atencio, Verkempinck, Bernaerts, Reineke, Hendrickx, & Van Loey, 2022). Taken together, the lipids release from the sediment were slower at low temperatures.

Changes in ash content

Ash is the inorganic substance that remains in food after calcination and is an important indicator used to measure the total content of inorganic components in foods. As Fig. 1I and J indicate, under storage conditions of 4 °C and 37 °C, the ash content of lotus seed beverages showed a trend of first decreasing and then increasing with prolonged storage times, and the lowest values all occurred at 3 months.

The decline in the ash content of stored lotus seed juice may be caused by the Maillard reaction between the reducing sugars and amino acids or proteins resulting in the formation of compounds that bind minerals tightly, thus reducing the ash content (de Albuquerque, Escalona-Buendía, de Magalhães Cordeiro, dos Santos Lima, de Souza Aquino, & da Silva Vasconcelos, 2021).

The content of oxidizing substances in lotus seed juice may increase with prolonged storage times, and the oxidizing substances may absorb
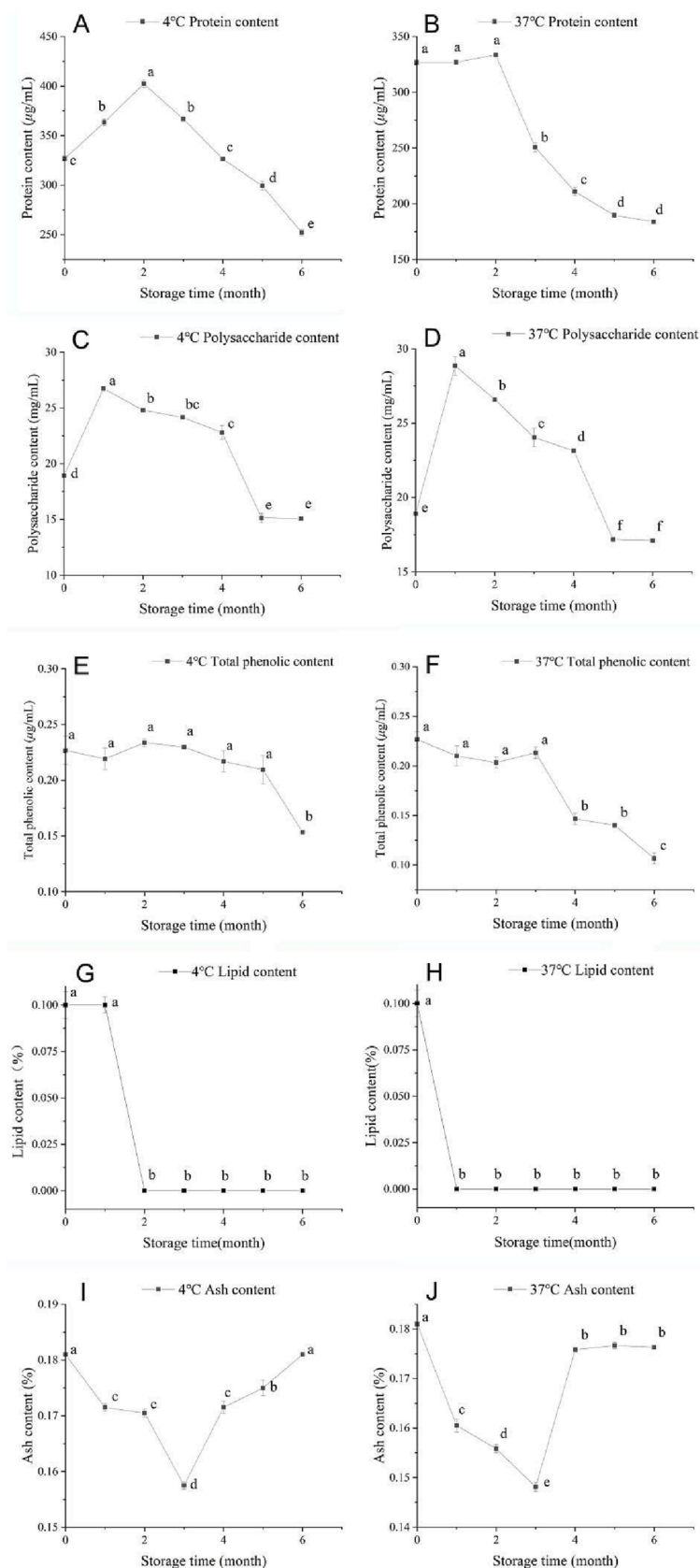
Fig. 1. Nutrient components of lotus seed juice under different storage temperatures (4 °C and 37 °C). (A) Soluble protein content at 4 °C; (B) Soluble protein content at 37 °C; (C) Polysaccharide content at 4 °C; (D) Polysaccharide content at 37 °C; (E) Total phenolic content at 4 °C; (F) Total phenolic content at 37 °C; (G) Lipid content at 4 °C; (H) Lipid content at 37 °C; (I) Ash content at 4 °C; (J) Ash content at 37 °C.

the carbon dioxide generated by the decomposition of organic matter to form carbonate, which increases the inorganic components and leads to increases in ash content during longer storage periods. This is also probably due to decreases in the protein and lipid content of lotus seed juice in later in the storage period, so the percentage of ash in lotus seed juice increases (Ahmadian-Kouchaksaraei, Varidi, Varidi, & Pourazarang, 2014). There were no significant changes in the ash content after 4 months of storage at 37 °C ($p > 0.05$). Interestingly, the temperature did not affect the progression of this oxidation reaction, but the reaction was faster at higher temperatures.

Changes in biological enzyme activity

As shown in Fig. 2, except for polyphenol oxidase (PPO), superoxide dismutase (SOD) and peroxidase (POD) were substantially inactivated

after autoclaving, but they maintained very low specific enzyme activities, which slowly decreased with increasing storage time. Lower activity or inactivation of antioxidant enzymes is expected in lotus seed juice after autoclaving heat treatment because the heat treatment is sufficient to inactivate or degrade these enzymes (Provesi, Dias, & Amante, 2011). Interestingly, the specific activity changes of endogenous enzymes from different plant sources after high-temperature sterilization are different, and some endogenous enzyme activities can be retained. For example, the PPO activity of high-temperature sterilized apple juice was entirely lost after 7 days of storage (Chen, et al., 2015), while the PPO activity in sterilized lotus seed juice was retained at low levels, and this activity showed an upward trend at 1 month, indicating that the storage environment was helpful in activating the source of the polyphenol oxidase enzyme of lotus seeds, preventing PPO from being completely inactivated.
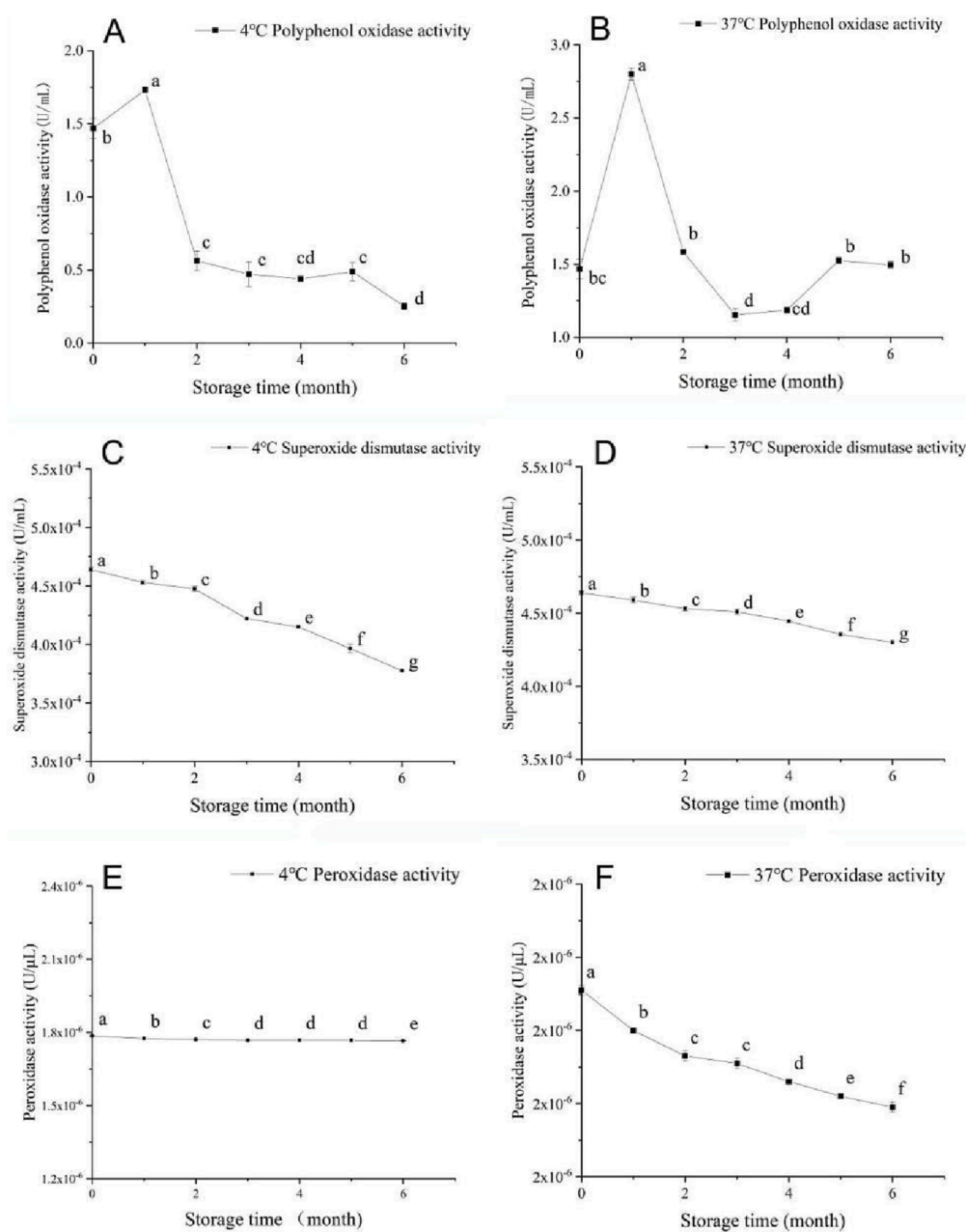
Fig. 2. Biological enzyme activities of lotus seed juice under different storage temperatures (4 °C and 37 °C). (A) PPO activity at 4 °C; (B) PPO activity at 37 °C; (C) SOD activity at 4 °C; (D) SOD activity at 37 °C; (E) POD activity at 4 °C; (F) POD activity at 37 °C. (PPO: polyphenol oxidase; SOD: superoxide dismutase; POD: peroxidase).

There are two main reasons for this: (1) the PPO structure in lotus seeds is relatively stable, but the enzyme structure is further stretched within a short period after autoclaving, which exposes the active sites. (2) Lotus seeds contain certain amounts of phenolic substances. Phenolic substances are the substrates for the PPO reaction, and the higher the substrate concentration, the easier it is for the enzyme to react with the substrate to form a complex, which may not be easily passivated by heat and ultrahigh pressure (Illera, Chaple, Sanz, Ng, Lu, Jones, et al., 2019). The enzyme activities gradually decreased after 1 month of storage, which was related to the gradual inactivation of the enzyme, and this may also be due to the consumption or destruction of phenolic substrates in lotus seeds aggravated by browning (Rios-Corripio, Welti-Chanes, Rodríguez-Martínez, & Guerrero-Beltrán, 2020). After autoclaving, the activities of PPO, SOD, and POD stored at low temperature were poor, and their inactivation was faster, which may be related to the inhibition of enzyme activities at low temperature (Basak, Mahale, & Chakraborty, 2022).

Changes in the antioxidant capacities of lotus seed juice during storage

The antioxidant capacity was measured as the oxygen radical absorbance capacity (ORAC).

The ORAC is mainly aimed at determining hydrogen atom transfer (HAT) capacity, scavenging of peroxy radicals generated in the process of antioxidant and substrate competition, preventing the destruction of fluorescent probes, and the change in fluorescence intensity is related to the antioxidant ability of the sample. It can accurately determine the antioxidant activities of water-soluble and lipid-soluble plant extracts, and the biological correlations are higher than those obtained from other antioxidant determination methods. During the 6-month storage period, the ORAC of lotus seed juice stored at 4 °C maintained high activity and even increased slightly (Fig. 3A). This shows that low-temperature storage may be beneficial for maintaining the antioxidant capacity of lotus seed juice.

The ORAC of lotus seed juice stored at 37 °C showed a sharp decline in the first 3 months and then stabilized (Fig. 3B). Higher temperatures have a more significant impact on the absorption capacity of oxygen free radicals. It is speculated that changes in the absorption capacity of oxygen free radicals may also be caused by the degradation of antioxidant components in lotus seed juice (Oliveira-Alves, Pereira, Pereira, Ferreira, Mecha, Silva, et al., 2020).

Reducing power measured as ferric reducing antioxidant power (FRAP)

FRAP is based on the determination of electron transfer (ET) capability and the capacity of an antioxidant to reduce an oxidant, which changes color when reduced. The degree of color change is correlated with the antioxidant concentration of the sample. According to Fig. 3C and D, during the 6-month storage period, the reducing power of lotus
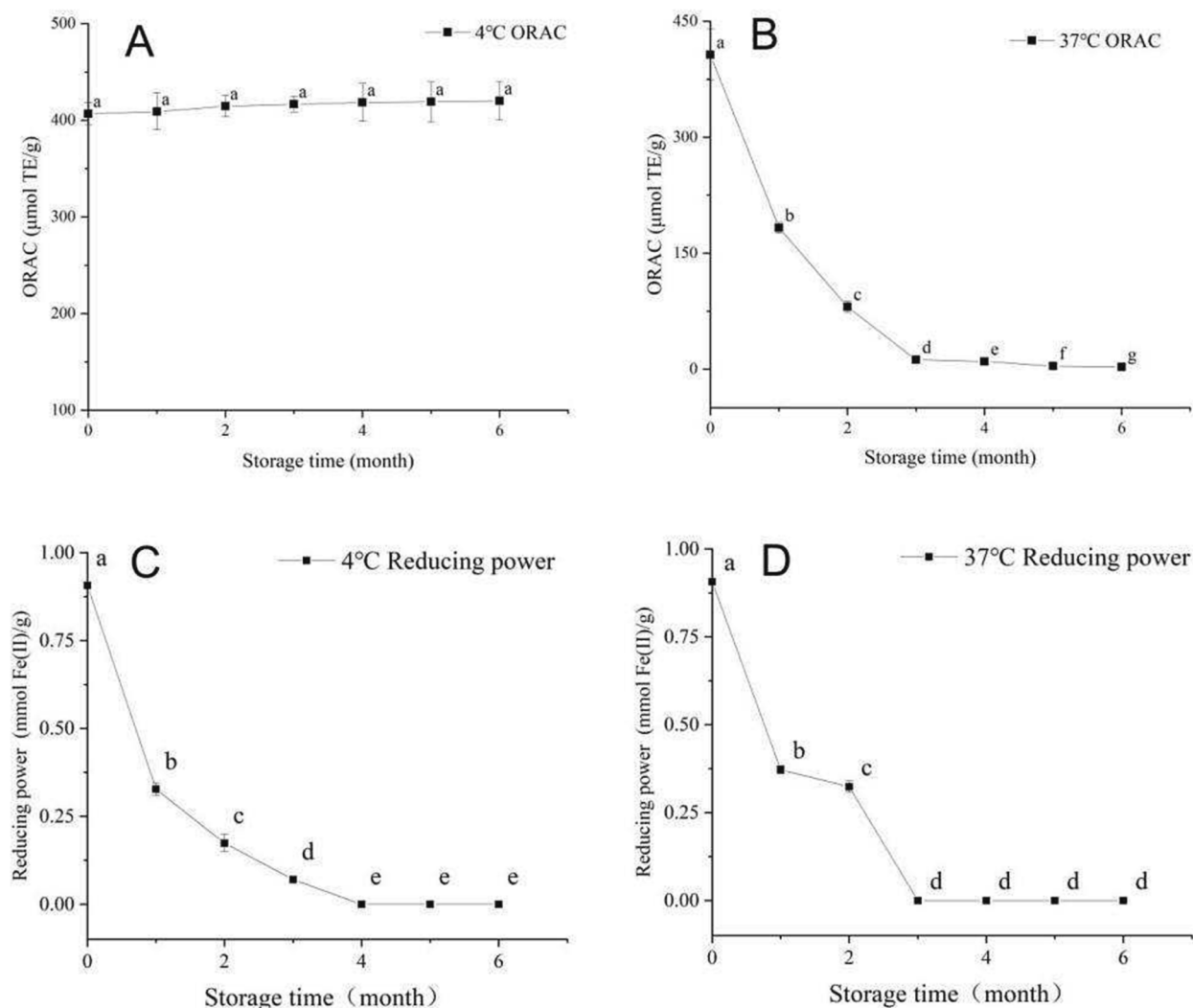

Fig. 3. Antioxidant capacities of lotus seed juice under different storage temperatures (4 ℃ and 37 ℃). (A) ORAC activity at 4 ℃; (B) ORAC activity at 37 ℃; (C) FRAP activity at 4 ℃; (D) FRAP activity at 37 ℃. (ORAP: oxygen radical absorbance capacity; FRAP: ferric reducing antioxidant power).

seed juice decreased with prolonged storage time, and the lotus seed juice lost its reducing power after 4 months and 3 months of storage at 4 ℃ and 37 ℃, respectively, which may mainly have been due to the degradation of various antioxidant components. The decrease in antioxidant activity may have been due to the combined effects of different compounds (such as phenolic compounds, proteins, and polysaccharides), including synergistic and antagonistic effects (Rios-Corripio, Welti-Chanes, Rodríguez-Martínez, & Guerrero-Beltrán, 2020). The reducing power of FRAP in vitro was determined in a study of cornelian cherry (e.g., *Cornus mas* L.) juice and was positively correlated with the contents of polyphenols and other biological compounds (Gastol, Krosniak, Derwisz, & Dobrowolska-Iwanek, 2013).

Browning kinetics study of lotus seed juice during storage

Changes in browning index

The browning index can be used as a parameter that reflects the quality of the color of plant beverages (Z. C. Wang, Yin, Ao, Yin, Ren, & Lu, 2022). As shown in Fig. 4A and B, during storage at low temperatures for 4 months, the browning index decreased significantly ($P < 0.05$) and then increased slowly. This browning may be caused by the oxidation of phenolic compounds and further polymerization with other phenolic substances to form molecular polymers with a dark color. The browning index of lotus seed juice was higher under high-temperature storage. It showed a significant increase ($P < 0.05$) with increased storage time, which may be affected by the enzymatic browning caused by the residual activity of PPO in sterilized lotus seed juice. In addition, the chemical properties of phenols are more easily oxidized to benzoquinone at high temperatures. Benzoquinone is prone to react with other endogenous molecules with nucleophilic groups and is rapidly self-polymerized resulting in browning (Landl, Abadias, Sárraga, Viñas, & Picouet, 2010).

Changes in L^*, a^*, b^*, and $\triangle E$

L^*, a^*, and b^* represent the light-shade value, red–green value, and yellow–blue value of lotus seed juice, respectively. The higher the L^* value is, the whiter the juice is, and the lower the L^* value is, the blacker the juice is. The lower the a^* value is, the greener the juice is, and the higher the a^* value is, the redder the juice is. The lower the b^* value is, the bluer the juice is, and the higher the b^* value is, the yellower the juice is.

As shown in Fig. 4C-J, the L^* value of lotus seed juice at storage temperatures of 4 ℃ and 37 ℃ first increased and then decreased with prolonged storage time. In the early storage period, the brightness of the lotus seed juice increased due to the precipitation of colored suspended matter. The change in the polyphenol content in lotus seed juice may also affect the change in the L^*/L_0^* value. As a result of the non-enzymatic browning reaction in the later storage period, the color gradually darkened, and the brightness decreased. The L^* value was low under high-temperature conditions, and the browning reaction may be violent under high temperatures, so it was transformed into a reaction system dominated by browning.

The a^*/a_0^* values of lotus seed juice at storage temperatures of 4 ℃ and 37 ℃ increased at 4 months and 2 months, respectively, and turned red, and the increase was greater at high temperatures. However, it decreased significantly after 4 months ($P < 0.05$), and the decrease was greater at low temperatures, indicating that the lotus seed juice turned green. With prolonged storage times, the b^*/b_0^* values of lotus seed juice at two storage temperatures gradually increased, and the increase was relatively large at 4 °C, indicating that the color of lotus seed juice system turned yellow during the storage period, which may be predominantly the effect of non-enzymatic browning.

The value of $\triangle E$ is an essential indicator to evaluate the overall change in color of lotus seed juice. Generally, when $\triangle E > 3.5$, it is easy to observe the change in color of the beverage (Z. C. Wang, Yin, Ao, Yin, Ren, & Lu, 2022). The $\triangle E$ value of lotus seed juice stored at two

temperatures was higher than 3.5, so the change in color of the system was visible. The color values of lotus seed juice stored at 4 ℃ and 37 ℃ increased continuously within 4 months and 3 months, respectively, which may be related to the short-term retrogradation and precipitation of lotus seed starch in the lotus seed juice. The juice tended to be clear, and the color increased compared to that at the beginning of storage. A small amount of residual oxygen in lotus seed juice, non-enzymatic reactions, incomplete inactivation of enzymes (such as PPO), and degradation and aggregation of pigments with increasing storage time may lead to color darkening and lower $\triangle E$ (Chen, et al., 2015). Compared to high-temperature storage, the $\triangle E$ and browning index of juice in low-temperature storage decreased to a greater extent, but the $\triangle E$ in low-temperature storage was higher. This indicates that the gelation behavior (such as starch aging) or precipitation (sedimentation of suspended matter) of macromolecules in lotus seed juice under low-temperature storage may have a more significant impact on the color fluctuation of the system (Laorko, Tongchitpakdee, & Youravong, 2013).

Kinetic analysis of L^*, a^*, b^*, ΔE, and browning index

Zero-order, first-order, and combined kinetic models are often used to quantify changes in food quality-related indicators (Wang, Hu, Chen, Wu, Zhang, Liao, et al., 2005). Zero-order, first-order, and combined reaction kinetic models were used to fit and analyze the color difference values (L^*, a^*, b^*, and $\triangle E$) of lotus seed juice during storage at 4 °C and 37 °C. The results are shown in Table 1.

The correlation coefficients of the combined kinetic model at storage temperatures of 4 °C and 37 °C are significantly better than those of the zero-order and first-order models. The combined model could better explain the dynamic changes in the browning index, L^*, b^*, and $\triangle E$ of lotus seed juice under different storage conditions at different temperatures than the zero-order and first-order models. The fitting correlation coefficient R^2 of a^* is low, so the kinetic model could not be used to fit it, which may be related to the less significant fluctuation of a^* and the more obvious influence of particle precipitation.

The reaction constant k value of L^*, b^*, and $\triangle E$ at 4 °C was higher, the change in brightness, yellow–blue value, and chromatic aberration of lotus seed juice under low-temperature storage conditions were more significant, and the change rate was higher. The k value of the browning index of lotus seed juice at 37 °C was higher, which indicated that high-temperature storage promoted an increase in the browning rate of lotus seed juice. The difference in the kinetic rate constant may be due to the difference in the residual oxygen content in storage bottle and the difference in the rate of color change (Chen, et al., 2015).

The activated energy (E_a) range of the reaction is generally between 40 and 400 kJ/mol. When Ea is <40 kJ/mol, the reaction rate is higher, i.e., the lower Ea is, the easier the reaction (Z. C. Wang, Yin, Ao, Yin, Ren, & Lu, 2022). The higher E_a is, the more temperature sensitive the reaction rate constant k, i.e., the more significant the effect of temperature is on k (Laorko, Tongchitpakdee, & Youravong, 2013). It can be seen from the results that the activated energies of the browning index, L^*, b^*, and $\triangle E$ are 30.47, 15.04, 21.55, and 16.14 kJ/mol, respectively, all of which are <40 kJ/mol, indicating that the system has high reactivity. The browning index, light–shade value, red–green value, and color fluctuations of the lotus seed juice were more likely caused by the two storage temperatures; the brightness change most likely occurred most commonly, and temperature had the most significant effect on the browning index of lotus seed juice.

Correlation between the nutrient Compositions, color in lotus seed juice and antioxidant activity indicators (ORAC and FRAP)

By examining the Pearson correlation analysis and redundancy analysis (RDA) results of the color (browning index, L^*, a^*, b^*, and $\triangle E$), nutrient components (e.g., ash, protein, polysaccharide, lipid and total phenolic content), and antioxidant capacities (ORAC and FRAP) of lotus
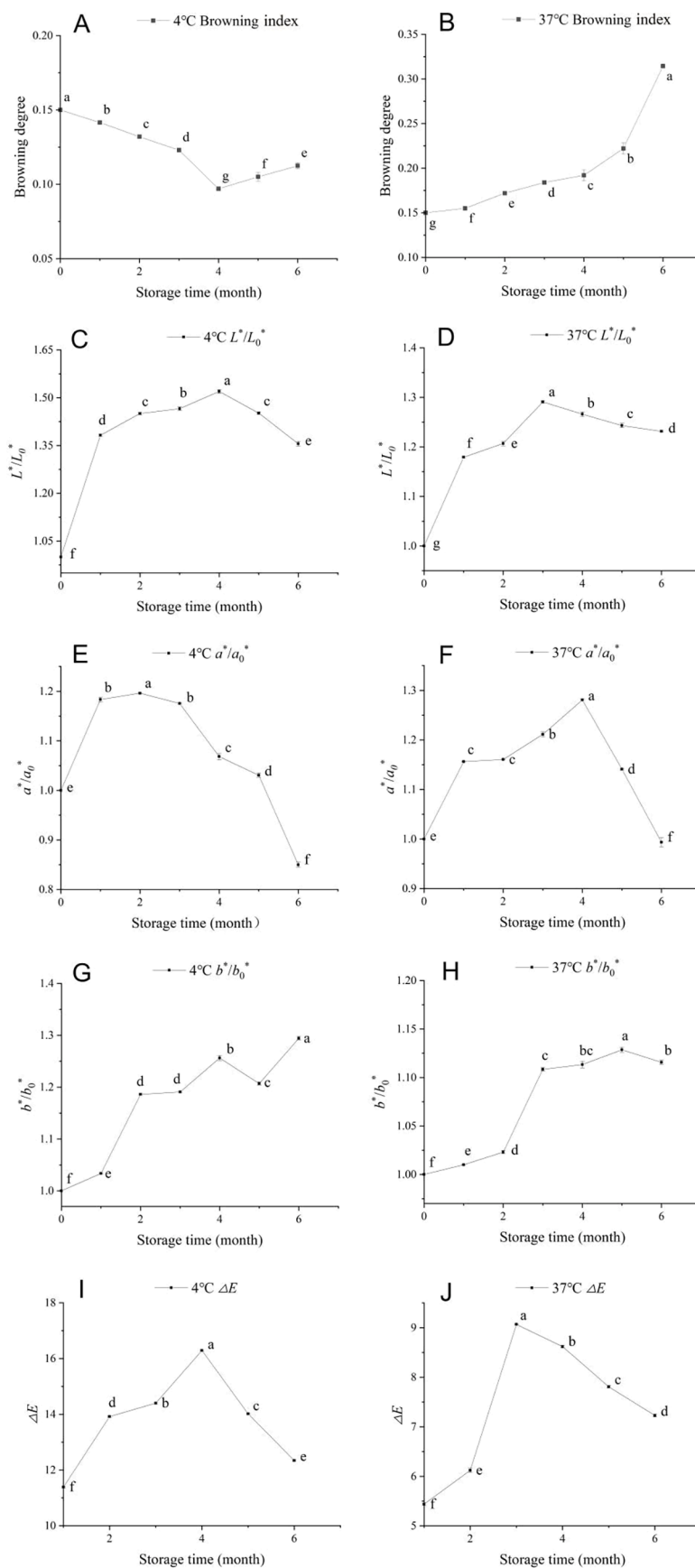
Fig. 4. Color values of lotus seed juice under different storage temperatures (4 ℃ and 37 ℃). (A) Browning index at 4℃; (B) Browning index at 37 ℃; (C) L^*/L_0^* value at 4 ℃; (D) L^*/L_0^* value at 37 ℃; (E) a^*/a_0^* value at 4 ℃; (F) a^*/a_0^* value at 37 ℃; (G) b^*/b_0^* value at 4 ℃; (H) b^*/b_0^* value at 37 ℃; (I) $\triangle E$ value at 4 ℃; (J) $\triangle E$ value at 37 ℃.

Table 1
Kinetic parameters of L^*, a^*, b^*, $\triangle E$, and browning index of lotus seed juice under different storage conditions (4 °C and 37 °C).

Reaction order	Parameter	Temperature (°C)	k (months^{-1})	R^2	E_a (kJ/mol)
$n = 0$	Browning index	4°C	$k_0 = -0.09$	0.757	30.47
		37°C	$k_0 = 0.018$	0.731	
	L^*	4°C	$k_0 = 1.352$	0.324	15.04
		37°C	$k_0 = 0.937$	0.504	
	a^*	4°C	$k_0 = 0.278$	0.288	32.26
		37°C	$k_0 = 0.030$	0.005	
	b^*	4°C	$k_0 = 0.658$	0.818	21.55
		37°C	$k_0 = 0.288$	0.823	
	$\triangle E$	4°C	$k_0 = 0.197$	0.047	16.14
		37°C	$k_0 = 0.387$	0.265	
$n = 1$	Browning index	4°C	$k_0 = -0.068$	0.793	30.47
		37°C	$k_0 = 0.098$	0.796	
	L^*	4°C	$k_0 = 0.050$	0	15.04
		37°C	$k_0 = 0.008$	0	
	a^*	4°C	$k_0 = 0.007$	0	32.26
		37°C	$k_0 = 0.029$	0	
	b^*	4°C	$k_0 = 0.045$	0.787	21.55
		37°C	$k_0 = 0.021$	0.817	
	$\triangle E$	4°C	$k_0 = -0.038$	0	16.14
		37°C	$k_0 = -0.021$	0	
Combined	Browning index	4°C	$k_0 = 0.022$, $k_1 = 0.243$	0.828	30.47
		37°C	$k_0 = -0.090$, $k_1 = -0.615$	0.975	
	L^*	4°C	$k_0 = 0.884$, $k_1 = 1.964$	0.925	15.04
		37°C	$k_0 = 0.441$, $k_1 = 1.131$	0.928	
	a^*	4°C	$k_0 = -176.822$, $k_1 = 18.204$	0.068	32.26
		37°C	$k_0 = -39.813$, $k_1 = 3.838$	0.335	
	b^*	4°C	$k_0 = 4.522$, $k_1 = 0.263$	0.889	21.55
		37°C	$k_0 = 1.670$, $k_1 = 0.102$	0.835	
	$\triangle E$	4°C	$k_0 = 193.788$, $k_1 = 3,348$	0.90	16.14
		37°C	$k_0 = 91.909$, $k_1 = 1.476$	0.950	

seed juice during storage at 4 °C and 37 °C, certain correlations between the nutrient components, color, and antioxidant capacity were found. The results are shown in Fig. 5.

The FRAP value in the lotus seed juice stored at 4 °C was extremely significantly positively correlated with lipid content, PPO, and SOD ($p < 0.001$), but it was significantly negatively correlated with the ORAC value ($p < 0.01$) (Fig. 5A). Compared to high-temperature storage, the activities of PPO and SOD enzymes were lower at low temperatures, which was not conducive to maintaining the FRAP activity of lotus seed juice in the first two months of storage. The ORAC had the lowest correlations with other antioxidant indicators. Different from other methods, ORAC analysis takes into account the kinetic effects of antioxidants, which explains the difference between the results (Dudonne, Vitrac, Coutiere, Woillez, & Merillon, 2009). Interestingly, compared to high-temperature storage, this difference was exacerbated under low-temperature conditions, which may be related to the increased phase separation of active ingredients in lotus seed juice under low-temperature conditions (FRAP, ORAC, and lipid content were inversely correlated). Although the active substance was better retained, the antioxidant activity was reduced due to poor solubility.

The browning index of lotus seed juice stored at 4 °C and 37 °C was negatively correlated with ORAC ($p < 0.05$), indicating that ORAC was the crucial factor controlling the browning of lotus seed juice (Fig. 5A and B). The browning index was positively associated with the activity of PPO, SOD, proteins, and lipids at low temperatures. The poor SOD

activity had little effect on the system, but PPO may have promoted the enzymatic browning of lotus seed juice at low temperatures. The a^* value was the most significantly positively correlated with the content of soluble proteins, polysaccharides, and total phenols ($p < 0.001$), all of which may form complexes and precipitate in the later storage period, resulting in the system turning green. The b^* value was the most significantly negatively correlated with FRAP ($p < 0.001$), and a reduction in antioxidant activity of FRAP would cause the system to turn yellow. In addition, the L^* value was extremely significantly negatively correlated with lipid content ($p < 0.001$), and lotus seeds starch may associate with lipids to form resistant starch precipitates of type RS5 in low-temperature storage, which could help to improve the brightness of the lotus juice supernatant. The change in $\triangle E$ of the system under low-temperature storage was extremely significantly correlated with the L^* and browning index ($p < 0.001$), indicating that the change in juice color was related to the clarity of the system and the degree of oxidative browning.

The ORAC and FRAP values in lotus seed juice stored at 37 °C were significantly positively correlated with the soluble protein, lipid, total phenol, and SOD content ($p < 0.01$) (Fig. 5B). These are significant active ingredients with antioxidant activity, and the reduction in these ingredient contents under high-temperature storage conditions is closely related to the decrease in antioxidant activity of the system. Many studies have demonstrated that the total polyphenol content of plant extracts and plant beverages are positively correlated with the antioxidant activities of the systems (Chen, et al., 2015; de Oliveira, de Souza, de Lima, Dos, Viera, Queiroga, et al., 2021; Roy, Koide, Rao, Okubo, Ogasawara, & Juneja, 2010). However, the polymerization of proteins with phenolic compounds and lipids may be the primary reason for the decreased total phenolic content. Therefore, there was an extremely significantly positive correlation between the total phenolic and soluble protein content in lotus seed juice($p < 0.001$). Nutrient interactions in lotus seed juice result in decreased ORAC and FRAP of lotus seed juice (Chen, et al., 2015). The browning index, a^* value, and b^* value of lotus seed juice were extremely significantly negatively correlated with ORAC and FRAP ($p < 0.001$); therefore, the Maillard reaction or non-enzymatic browning caused by reduced antioxidant activity exacerbates the browning index of lotus seed juice. The $\triangle E$ of the system under high-temperature storage was the value with the greatest correlation with ORAC and FRAP values ($p < 0.001$), indicating that the color change in lotus seed juice was related to the deepening degree of oxidation of the system.

The browning index and $\triangle E$ of lotus seed juice stored at 4 °C and 37 °C were used as dependent variables, and the remaining indicators were used as independent variables for redundancy analysis (RDA) (Fig. 5C and D). The measured independent variables could explain 100 % of the color changes (browning index and $\triangle E$) in lotus seed juice from the resulting variation in variance. The angle between the dependent and independent variables in Fig. 5C and D indicates the magnitude of the correlation. When the angle is acute, the correlation is positive (the smaller the acute angle is, the greater the correlation), and when the angle is obtuse, the correlation is negative (the larger the obtuse angle is, the greater the correlation). The browning index was highly positively correlated with PPO and SOD under 4 °C storage, $\triangle E$ was highly negatively correlated with FRAP, and lipids affected the L^* value. Moreover, $\triangle E$ was highly negatively correlated with ORAC and FRAP. The above results were consistent with the results of Pearson correlation analysis. The length of the blue arrow represents the contribution of the independent variable to all dependent variables, and the longer the length is, the greater the contribution of the factor to the dependent variable. Polysaccharides, proteins, FRAP, L^*, a^*, lipids, PPO, SOD, and ashs contributed more to the color of lotus seed juice when it was stored at 4 °C. Polysaccharides, protein, total polyphenol, FRAP, ORAC, L^*, a^*, b^*, lipid, and SOD have a greater impact on the color of lotus seed juice at 37 °C. The r^2 value in Table S1 shows that FRAP and L^* best explain the color change in the lotus seed juice under 4 °C storage, and changes
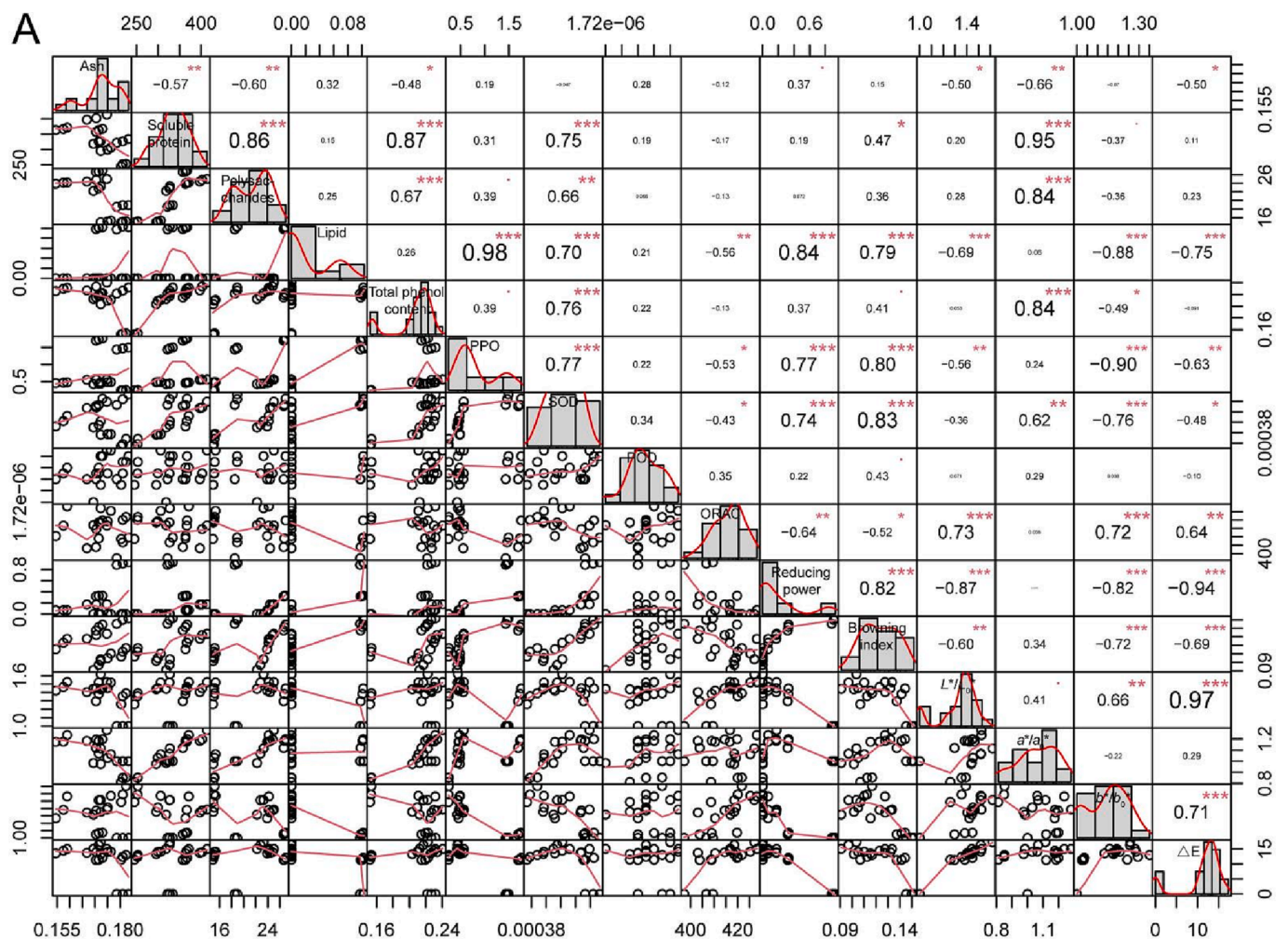
Fig. 5. Correlation analysis between the nutrient compositions, color in lotus seed juice and antioxidant activity indicators from different storage temperatures ((A) 4 °C; (B) 37 °C; significant correlations are indicated by *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$)). Redundancy analysis between the nutrient compositions, antioxidant properties and color of lotus seed juice from different storage temperatures ((C) 4 °C; (D) 37 °C), an ANOVA test was conducted and the significance was obtained for the two RDA models ($p < 0.001$).

in antioxidant activities (FRAP and ORAC) can best explain the color change under 37 °C.

The dots with different colors represent color change in lotus seed juice under the dependent variable. The color of lotus seed juice was different after 1 month of storage at 4 °C, and the colors were obviously different between 1, 2, and 3 months and 4, 5, and 6 months of storage. There was also a difference after 1 month of storage, which was smaller than that at 4 °C and was significantly different between 1, 2, 3, and 4 months and 5 and 6 months at 37 °C.

In conclusion, the color change in lotus seed juice under low-temperature storage may be related to suspended solid precipitation and the long- and short-term aging of starch. The short-term aging of starch and precipitation of suspended solids were completed within 1 month, and the long-term aging was completed after 3 months. In addition, the color change was also affected by the browning of lotus seed juice, which was related to the residual PPO and SOD enzyme activities at low temperatures. However, the color change in lotus seed juice under high-temperature storage is mainly affected by the browning index (while the browning of lotus seed juice may be primarily affected by the change in the nutrient content of lotus seed juice by the antioxidants in the system). The color change was slow in the first 4 months, but sped up after 5 months.

The color change in lotus seed juice was dependent on the browning

index of the clarified liquid and the floatation of suspended solids (including particles and gels). Under low-temperature storage conditions, antioxidant active substances were easier to retain and maintain their antioxidant capacity. Soluble starch in lotus seed juice was more likely to form gels, and suspended particles were prone to precipitation, which improved the brightness of the supernatant. The high-temperature storage depended on the antioxidant capacity of the lotus seed juice system, and the color changes were mainly related to the oxidation reaction.

Conclusions

The changes in nutrients, color, and antioxidant capacity of lotus seed juice during storage at different storage temperatures (4 °C and 37 °C) were investigated, and the correlations between color, various chemical indicators and antioxidant activity during storage were determined. With prolonged storage times, the nutrient components of lotus seed juice stored at different temperatures had different levels of loss, but the retention rate of the nutrient components of lotus seed juice was higher under low temperatures (4 °C). On the other hand, the inactivation rate of the biological enzymes of lotus seed juice at low temperature was faster, but 4 °C storage helped to maintain the antioxidant capacity (ORAC and FRAP) in the system. Additionally, the
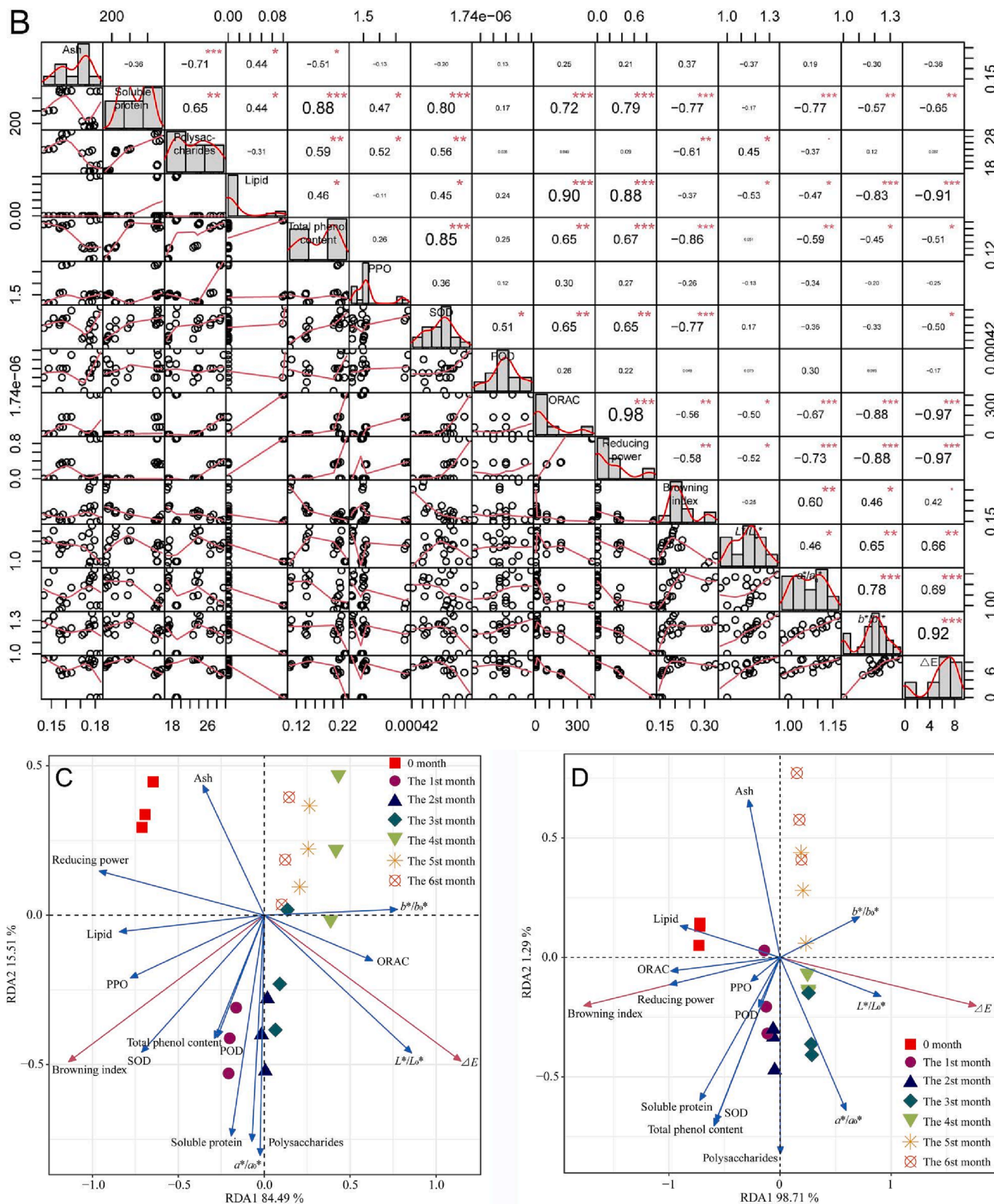
Fig. 5. (*continued*).

color changes in lotus seed juice were measured, and L^*, a^*, and $\triangle E$ at both storage temperatures first increased and then decreased with prolonged storage time, and b^* increased gradually. Furthermore, through the combined kinetic analysis of browning, temperature was found to have the most significant effect on the browning of lotus seed juice, and the color indicator that was most likely to change was brightness (L^*).

Pearson correlation analysis and RDA showed a strong correlation between the nutritional quality, color, and antioxidant capacity of lotus seed juice during storage. Several active components with significant antioxidant properties (lipids, soluble proteins, total phenols, PPO, and SOD) were extremely correlated with FRAP and ORAC to varying degrees. At the same time, the changes in FRAP and $L*$ could best explain the color changes in lotus seed juice stored at low temperatures (4 ℃), and the color indicators of lotus seed juice at high temperatures (37 ℃) were significantly negatively correlated with ORAC and FRAP ($p < 0.001$), which indicated that color changes in the lotus seed juice system were extremely correlated with the precipitation of suspended matter and aging of starch at low temperatures. At the same time, the color changes were significantly correlated with a decrease in antioxidant activity (affected by changes in nutrient contents) at high temperatures. This study elucidates the interplay between the nutrient composition, color, and antioxidant properties of plant-based beverages during storage, which may contribute to the development of plant-based starch and protein-based beverages.

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

No data was used for the research described in the article.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100669.

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