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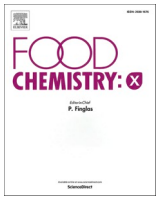

Differences in storage stability of cow's milk-based and goat's milk-based infant formulas

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ABSTRACT

Goat's milk-based infant formula (YIF) has the advantages of high nutritional value and hypoallergenicity, and fewer studies have been conducted on YIF. Therefore, this study examined the changes in physicochemical and functional properties of cow's milk-based infant formula (ZIF) and YIF during 6 months of storage at 25 °C, 37 °C, and 50 °C, respectively. The results showed that YIF had higher pH (7.26), wettability, protein oxidation, good wettability, and lower lactose crystallinity and lipid oxidation compared to ZIF. Further analysis revealed that the higher pH significantly accelerated the rate of the Maillard Reaction (MR), resulting in significantly higher browning of YIF than ZIF, and this difference was statistically significant ($p < 0.05$). This is one of the reasons for the decrease in solubility of YIF. This paper explores the differences in storage stability of ZIF and YIF and provides theoretical guidance for the selection of milk-based ingredients.

1. Introduction

Commercial breastmilk substitutes for infant formulas (IFs) that are designed to meet the nutritional needs of non-breastfed infants and are suitable for infant feeding, based primarily on the composition of breastmilk as the nutritional basis(Hernell, 2012; Prosser, 2021). In recent years, YIF has become increasingly attractive to some consumers (Chen et al., 2022). Goat's milk is similar in composition to breast milk, is nutrient-rich, has more beta-casein and less α_{S1}-series-casein, and has smaller fat globule diameters than cow's milk. As a result, goat's milk is better digested and absorbed by the infant's gastrointestinal tract than cow's milk and is also of interest to consumers interested in hypoallergenic products(Prosser, 2021). And goat's milk is rich in biologically active compounds and functional ingredients, which also provides greater possibilities for its use in special medical formulas for infants (Nayik et al., 2021).

Infant formula (IF), due to its special composition, is highly susceptible to quality deterioration reactions, such as particle caking, browning, protein oxidation, lipid oxidation, etc., when it is thermally processed or stored under high-temperature and high-humidity environmental conditions, which will lower the quality of IF. ZIF and YIF differ in protein content and type(Chen et al., 2022), and both of these differences can have a significant impact on the physical stability of the IFs, resulting in different levels of protein oxidation between the two. Levels of protein carbonyls and protein sulfhydryl groups represent useful markers of protein oxidation in dairy products. In addition, lipid oxidation can lead to the deterioration of high-quality foods, such as the development of off-flavors, nutrient loss, and undesirable compounds (Lai & Paterson, 2020). Evaluation of milk powder lipid oxidation by surface free fat content, peroxide value (PV), and malondialdehyde (MDA)(Burg et al., 2010). The factors that affect the quality of milk powder are mainly external environmental factors, such as storage temperature, humidity, etc., but also the differences in the composition of infant formula(Lloyd et al., 2009; Nunes et al., 2021). Therefore, it is important to study the quality changes of cow's milk-based and goat's milk-based IFs during storage.

Currently, there is an increasing global demand for fortification and improvement of ZIF and YIF, and it is important to understand the relationship between milk-based ingredients and quality stability. However, there is a paucity of literature on assessing the physical

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properties and degree of oxidation of produced YIF. This paper investigates the changes in pH, browning, lactose crystallization, surface morphology, protein oxidation, lipid oxidation, wettability, and solubility of two IFs stored at three temperatures (25 °C, 37 °C, and 50 °C) for 6 months. This study will systematically analyze the effects of physical, chemical, and functional properties of IF during storage, providing a more comprehensive theoretical basis for product development and rational storage of milk powder.

2. Material and methods

2.1. Chemicals and reagents

ZIF (lactose 56.95 g/100 g, protein 13.1 g/100 g, fat 24.65 g/100 g), YIF (lactose 53.96 g/100 g, protein 11.23 g/100 g, fat 25.50 g/100 g). Acetic acid, Anhydrous ethanol, sodium hydroxide (NaOH), Sodium dihydrogen phosphate (NaH_2PO_4), purchased from Tianjin Tianli Chemical Reagent Co., Ltd. Cumene hydroperoxide (CHP, 80 %), Purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Isopropanol, Ferrous sulfate, purchased from Tianjin Hengxing Chemical Reagent Manufacturing Co., Ltd. Tris(hydroxymethyl)aminomethane (Tris), Guanidine hydrochloride, Glycine, purchased from Beijing Lanjieke Technology Co., Ltd. Urea, Hydrochloric acid, Isooctane, 1-Butanol, Barium chloride, Ammonium thiocyanate, Ethyl acetate, purchased from Tianjin Yongda Chemical Reagent Co., Ltd. Hexane (HPLC ≥97 %), Methanol (HPLC ≥99 %), purchased from Tianjin Star Mark Technology Development Co., Ltd. Trichloroacetic acid (TCA), 2,4-dinitrophenylhydrazine (DNPH, HPLC ≥98 %), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, HPLC ≥98 %), purchased from Shanghai Macklin Biochemical Technology Co., Ltd. Drugs not labeled as HPLC above are analytical grade products.

2.2. Storage conditions

The ZIF and YIF used in this study were from the same factory and produced at the same time. In order to evaluate the effect of temperature on the physicochemical properties and functional properties of ZIF and YIF, the two IFs were stored in an incubator at 25 °C, 37 °C, and 50 °C, for 6 months, respectively, where the humidity in the incubator is 75 %.

2.3. Lactose crystallinity

X-ray diffraction (XRD) measurements were carried out using an X-ray diffraction system (Empyrean, Malvern Panalytical) with an anode current of 35 mA and an accelerating voltage of 40 kV. Cu-Kα radiation (40 kV, 35 mA). 1.00 ± 0.01 g of sample powder was placed in a sample holder and scanned from 10° to 30° at 0.02°/s for a total acquisition time of 19 min. Lactose peaks were identified according to available chemical databases using the Highscore software, and the results were reported as a percentage of total lactose.

2.4. Surface morphology analysis

A scanning electron microscope (SEM, SU8010, Hitachi Limited) was used to observe the particle morphology of milk powder. The sample stage was placed in an ion sputtering apparatus for 2 × 30 s for gold spraying. The gold-sprayed sample was then placed in the vacuum chamber of the SEM with the sample stage for sample observation, and the accelerating voltage was set to 15 kV.

2.5. pH

A pH meter (SevenCompact S210, Mettler Toledo) was used for the determination of sample pH. The IF was dissolved in warm water (40–45 °C) and measured after the sample reached room temperature.

2.6. Browning index

The degree of color change of the experimental IFs prepared during the study period was measured using a Hunter colorimeter (ZE6000, Nippon Denshoku). The resulting L*, a* and b* values were used to calculate the Browning index (Tham, Yeoh, & Zhou) of the powders. The BI was calculated by Eqs. (1) and (2)(Nasser et al., 2017).

$$BI = \frac{[100 \times (Z - 0.3)]}{0.17}\# \quad (1)$$

where

$$Z = \frac{(a^* \times 1.750 \times L^*)}{(5.645 \times L^* + a^* - 3.012 \times b^*)}\# \quad (2)$$

Where L* represents the light and dark chromaticity values of the sample, a* value represents the red-green chromaticity value of the sample, and b* value represents the yellow-blue chromaticity value of the sample.

2.7. Determination of carbonyl content

A slight modification of the method described by Berton et al. (2012). The DNPH method was used by dissolving the extracted proteins in phosphate buffer solution (10 mM, pH 7.0) and stirring at room temperature until the solution was homogenized. After mixing, 700 μL of the protein suspension was taken and mixed with 2 mL of 10 mM 2,4-dinitrophenylhydrazine, and the mixed samples were reacted under dark conditions at room temperature for 2 h. The proteins were then precipitated by the addition of 900 μL of 40 % TCA, and the mixture was centrifuged at 10,000 rpm/min for 10 min at 4 °C, the supernatant discarded, and the protein extracted was analyzed by using 1 mL of anhydrous ethanol/ ethyl acetate solution (1,1, *v*/v) repeated three times, and then the precipitate was dissolved in 2 mL of 6 M guanidine hydrochloride solution with shaking. The absorbance at 370 nm was recorded to determine the carbonyl concentration. The results were expressed as nmol of carbonyl per mg of soluble protein using a molar extinction coefficient of 22,000 M^{-1} cm^{-1} of protein.

2.8. Determination of sulfhydryl content

Another indicator of the degree of protein oxidation is the susceptibility of sulfur-containing side chains of amino acid residues, i.e., by detecting changes in the sulfhydryl content of proteins. The sulfhydryl content of the emulsion was determined by weighing 1.00 ± 0.01 g of milk powder dissolved in water using the DTNB method(Tian et al., 2023). 0.02 mL of a 4 mg/mL solution of DTNB was added to a Tris-Gly-Urea solution (10.4 g Tris, 6.9 g Gly, 1.2 g EDTA, 480 g Urea, dissolved in water and volume adjusted to 1000 mL, pH 8.0).

The emulsion to be measured was diluted with phosphate buffer (10 mM, pH 7.0), and 0.5 mL of the sample was mixed with 5 mL of Urea buffer (8 M) solution, then 20 μL of 4 mg/mL DTNB solution was added, and the absorbance was measured at 412 nm for 30 min in a water bath at 25 ± 1 °C. The sulfhydryl content results were expressed as nmol sulfhydryl/mg protein and calculated using the molar extinction coefficient of 13,600 M^{-1} cm^{-1} of protein.

2.9. Determination of surface free fat

Surface fat is the fat covering the surface of the IF particles that is not encapsulated inside the milk powder particles, which is easily eluted by organic reagents. A 2.00 ± 0.01 g milk powder sample was placed on filter paper in a glass funnel and washed four times with 5 mL hexane after every 30-s interval. The filtrate was collected in an aluminum dish that had been dried to a constant weight, and the sample was dried in an electric thermostatic drying oven (101-0AB, Tianjin Taisite Instrument

Co., Ltd.) at 80 °C until the weight was constant. The surface free fat was calculated by Eq. (3).

$$\text{Surface free fat}(g/100g) = \frac{M_{Al+F} - M_{Al}}{M_s} \times 100\# \quad (3)$$

where: M_{Al+F} is the total weight of the aluminum dish and surface fat (g); M_{Al} is the weight of the aluminum dish (g); M_s is the weight of the milk powder sample (g).

2.10. Determination of peroxide value

Accurately weigh 1.00 ± 0.01 *g* of IFs in a centrifuge tube, re-dissolve the sample with 2 mL of ultrapure water, add 10 mL of the configured organic solution of isooctane/isopropanol mixture with a volume ratio of 3:1, and vortex the sample sufficiently (2 × 30 s) so that hydroperoxides were extracted into the organic solution. After centrifugation of the mixed sample (3400 ×*g*, 5 min), 1 mL of the upper solution was transferred to a 15 mL glass test tube, and 2.8 mL of a 2:1 methanol/1-butanol mixed organic solution, 15 μL of ammonium thiocyanate (3.94 M), and 15 μL of ferrous ion solution were added sequentially (need to be prepared on the spot). Equal volumes of barium chloride solution (0.132 M, dissolved in 0.4 M hydrochloric acid solution) and ferrous sulfate solution (0.144 M) were mixed to obtain ferrous ion solution. The mixed solution in a glass test tube was vortexed thoroughly (2 × 30 s), and the absorbance value of the reaction solution was measured at 510 nm after standing for 20 min at room temperature. The hydroperoxide concentration (μmol/kg) in the samples was calculated from standard curves made from different concentrations of CHP.

2.11. Determination of MDA

MDA is a lipid peroxidation product. All samples were assayed using the commercial testing kit (Jiancheng Biotechnology Co. Ltd., Nanjing, China) according to manufacturer instructions. The absorbance of the diluted MDA solution was read at 532 nm by a microplate reader (Thermo Fisher, Waltham, MA, United States).

2.12. Determination of wettability

Referring to the study by S. Wu et al. (2019), the IF was compressed for 10 s using a manual press at 50 MPa to form a round flake sample. Milli-Q water (10 μL) was dropped on the disc surface. Contact angle measurements were performed by means of an optical tensiometer (Theta, KSV Instruments Ltd., Helsinki, Finland), which in turn evaluates the wetting properties of milk powder samples.

2.13. Determination of solubility

Weigh 5.00 ± 0.01 g of the sample in a 50 mL centrifuge tube and dissolve the sample with 30 mL of water at 25 °C to 30 °C. Shake the centrifuge tube for 3 min to fully dissolve it. Place in a centrifuge and centrifuge at 5000 rpm for 10 min to precipitate the insoluble material. Decant the supernatant and wipe the wall of the tube with a cotton ball. Repeat the above steps 2–3 times until the liquid is clear after centrifugation. The precipitate was rinsed into a weighing dish of known mass with 1 mL of water and transferred to an oven at 100 °C to dry to a constant weight (the difference in mass between the last two times was not more than 2 mg). The solubility was calculated by Eq. (4).

$$X = 100 - \frac{(m_2 - m_1) \times 100}{(1 - B) \times m}\# \quad (4)$$

where: X - the solubility of the sample in grams per hundred grams (g/100 g); m - the mass of the sample in grams (g); m_1 - the mass of the weighing dish in grams (g); m_2 - the mass of the weighing dish and the insoluble material after drying in grams (g); B - the sample moisture in grams (g), the moisture content of the sample is determined by a moisture activity meter (LabMaster-aw neo, Novasina AG, Switzerland).

2.14. Statistical analysis

The data were presented as mean ± standard deviation (SD) for each technical repeat. One-way analysis of variance (ANOVA) was analyzed using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, USA), SPSS 26.0 software (IBM SPSS Inc., Chicago, USA), and Origin 2024 (Origin Lab Inc., USA). Differences were considered significant at p-value <0.05.

3. Results and discussion

3.1. Physical changes in ZIF and YIF during storage

The changes in crystallinity, surface morphology, pH value, and browning degree of ZIF and YIF during storage are shown in Figs. 1 to 4, respectively.

Due to the rapidity of the spray drying process, most of the lactose and proteins in IF powders produced by spray drying are present in an amorphous glassy state. The glassy state of lactose is highly hydrophilic and thermodynamically unstable(Phosanam et al., 2021). The initial lactose crystallinity of ZIF was 7.07 %, and that of YIF was 6.86 %. The initial lactose crystallinity of ZIF was higher than that of YIF, owing to the higher initial lactose content of ZIF than that of YIF(Phosanam et al., 2020), which resulted in an increase in the amount of amorphous lactose that can be converted to crystalline lactose. Also, milk powders with high fat content may reduce water absorption and delay lactose crystallization(Tham et al., 2017). The effect of temperature on lactose crystallinity of ZIF and YIF were shown in **Fig. 1**. It can be observed that the lactose crystallinity of both IFs is significantly increasing with increasing storage temperature and storage time. This is in line with the study on crystallinity of IF by Arissara Phosanam et al.(Phosanam et al., 2021). The higher mobility of lactose molecules at high temperatures favors their crystallization. In **Fig. 1 (B)**, the lactose crystallinity of YIF decreased at the 6th month of storage at 50 °C, probably due to the intensification of the reaction between proteins and lactose, which produced MR products that affected the normal crystallization process of lactose, leading to a decrease in crystallinity.

Observations were made on two IFs, ZIF and YIF, stored at temperatures of 25 °C, 37 °C, and 50 °C for 6 months **(Fig. 2)** to show the effect of temperature on the surface morphology of the powders. Both IFs particle morphologies initially appeared as smooth spherical shapes, which is consistent with the results of previous studies on milk powder particle morphology(Qí et al., 2024). Observation at lower magnification reveals that, with the prolongation of storage time and increase in temperature, the two IFs, ZIF and YIF, show an obvious tendency to aggregate, and the pores of the two IFs increase. Storage of high-fat milk powder at high temperatures results in the release of oil from the internal particles to the surface, which increases the surface fat coverage and causes the powder particles to promote their aggregation via fat binding(Phosanam et al., 2020). Changes in surface morphology from smooth spherical to distinctly wrinkled (**Fig. 2** yellow arrows) and rough crystalline surfaces (**Fig. 2** red arrows) were observed in all samples at higher magnifications. The wrinkled appearance of milk powder particles may be contracted by the interaction of proteins and lactose (Conceição et al., 2024). It is also possible that fat is released from the core of the granule, resulting in a wrinkled surface appearance(Saxena et al., 2020). Both milk powders initially contain lactose in an amorphous state, as there is no observable crystal structure on the surface of the powder even at higher magnifications. By SEM, clear flaky crystals were observed at 50 °C compared to the two IFs stored at 25 °C and 37 °C.

Fig. 3 shows the variation of pH values of cow-based and goat-based infant formulae. The initial pH of the two samples, Z and Y, were 6.74

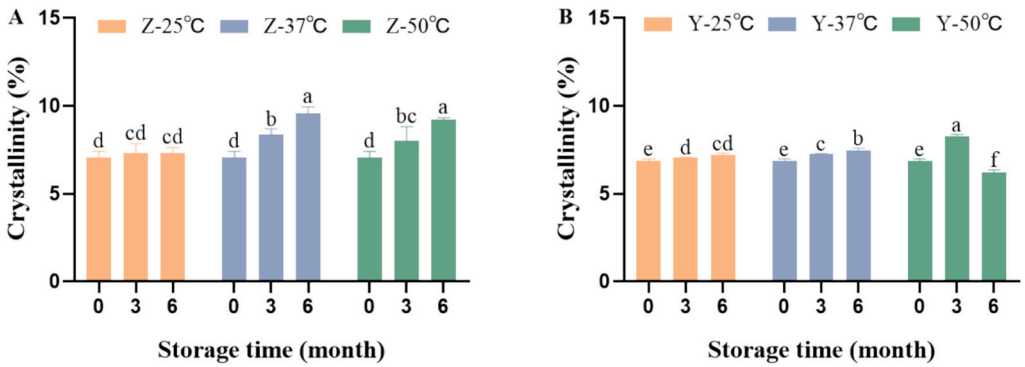

Fig. 1. Lactose crystallinity (%) of ZIF (A) and YIF (B) stored at 25°C, 37°C, and 50°C for 6 months.

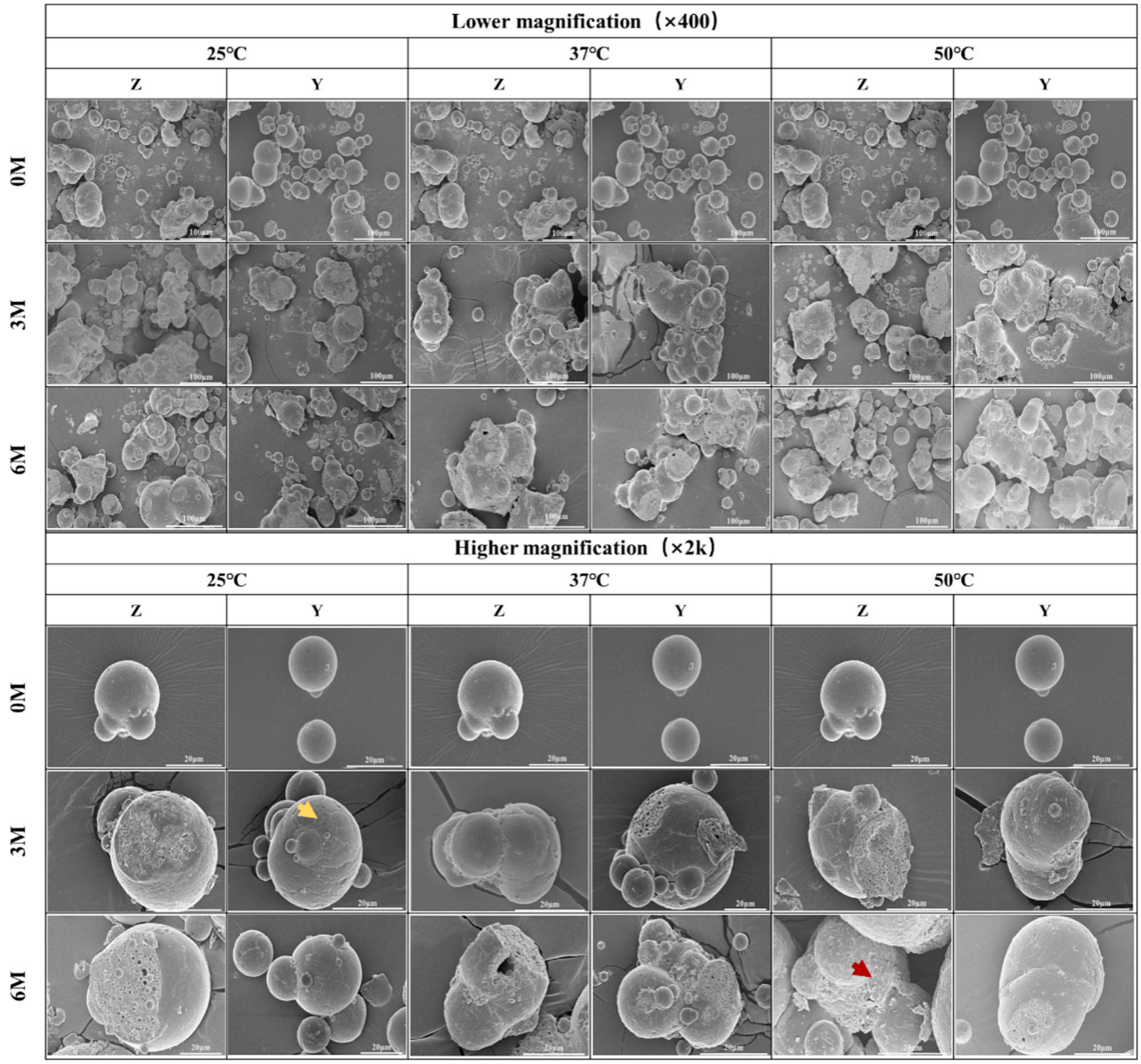

Fig. 2. Changes in surface morphology of ZIF and YIF stored for 6 months at 25°C, 37°C, and 50°C were observed at lower magnification (×400) and higher magnification (×2k). Scale bars are 100 μm and 20 μm, respectively.

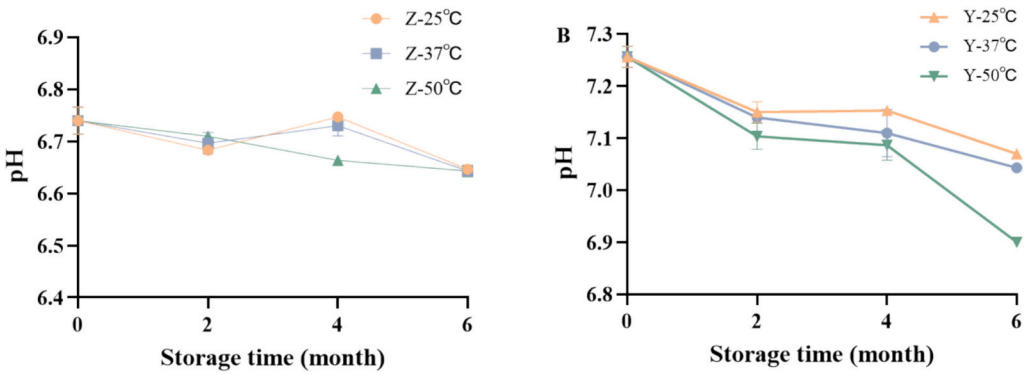

Fig. 3. Changes in pH of ZIF (A) and YIF (B) stored at 25°C, 37°C, and 50°C for 6 months.

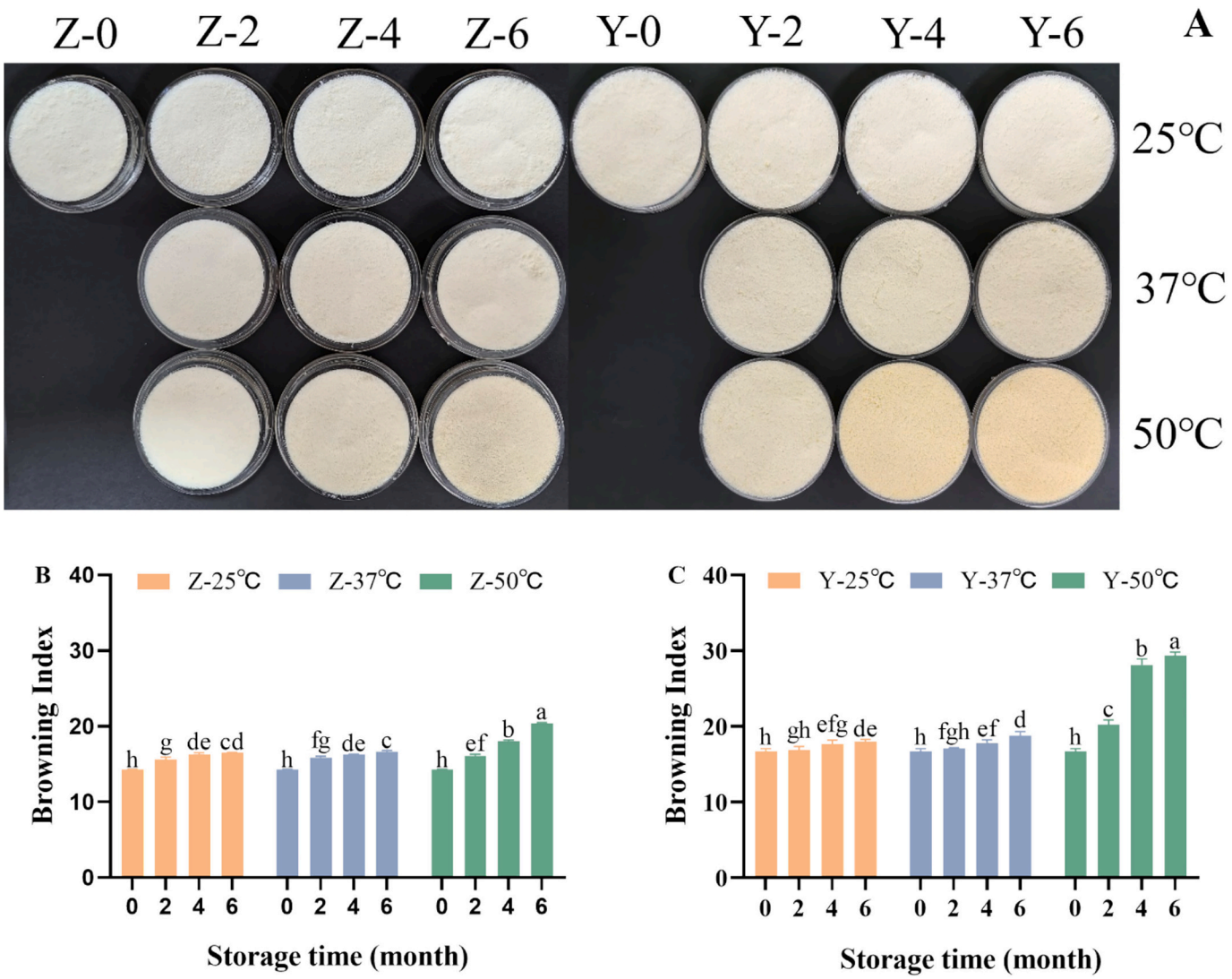

Fig. 4. Visualization images of color change (A) and browning index (B) (C) of ZIF and YIF after 6 months of storage at 25°C, 37°C, and 50°C.

and 7.26, respectively, and the initial pH of YIF was higher than that of ZIF, which was weakly alkaline. The two samples were stored at 25 °C, 37 °C, and 50 °C for 6 months. At 25 °C, the pH of ZIF decreased by 1.34 % and that of YIF decreased by 2.62 %; at 37 °C, the pH of ZIF decreased by 1.48 % and that of YIF decreased by 3.03 %; at 50 °C, the pH of ZIF decreased by 1.48 % and that of YIF decreased by 4.96 %. As the storage temperature increased, the degree of pH decrease increased for both ZIF and YIF, and the rate of decrease was higher for YIF than for ZIF at higher temperatures. This is mainly due to the fact that degradation of Amadori products results in the formation of acids such as formic acid, acetic acid, glyoxal, methylglyoxal, etc. These acidic compounds lead to a decrease in the pH of the milk powder in storage(Theng et al., 2024). In addition, reducing sugars consume the amino groups of proteins as the system undergoes the MR, which decreases the pH of the overall system.

At higher pH, proteins and lactose are presented in a more reactive form, which can accelerate the rate of the MR, leading to a faster rate of pH drop(J. Wu et al., 2022).

The visually observed changes in the color of ZIF and YIF with storage time at different storage temperatures were shown in **Fig. 4 (A)**. As expected, browning was apparently strongly influenced by the set storage temperature. Significant browning of the powder occurs when storage temperatures increase. The brown color of the powder of the IF stored at 50 °C was significantly deepened to the extent that it could be clearly detected even by the naked eye, and the color of YIF was darker than that of ZIF.

The variation of browning index with storage time for both ZIF and YIF stored at different temperatures is shown in Fig. 5 below. Two milk powders, ZIF and YIF, stored at 25 °C, changed from 14.24 and 16.70 to 16.51 and 17.96, respectively, after 6 months of storage. At the end of accelerated storage, the browning index of ZIF and YIF changed to 16.62 and 18.78 at 37 °C, and the browning index of ZIF and YIF changed to 20.43 and 29.36 at 50 °C, respectively. The results showed that the browning index of IF was strongly influenced by storage temperature. The browning index increased significantly ($p < 0.05$) with increasing storage temperature. The increase in temperature accelerates the MR and leads to the production of more brown powder(Sen et al., 2024).

From **Fig. 4 (B) and (C)**, it can be seen that the browning index of YIF is higher than that of ZIF, indicating that the browning degree of YIF is higher than that of ZIF, i.e., YIF has a higher degree of MR than ZIF. Browning of milk powder during storage is mainly caused by the MR(Ho et al., 2019). Higher pH **(Fig. 3)** results in faster formation of the Amadori product, and compounds produced by different routes are involved in further reactions leading to the formation of higher products, resulting in browning of the milk powder(J. Wu et al., 2022). It has been reported that an increase in temperature and further development of the MR can lead to changes in food properties such as color, flavor, nutritional value, and shelf life(Aktağ et al., 2019).

3.2. *Degree of protein oxidation of ZIF and YIF during storage*

The formation of protein carbonyl (PC) is the result of the oxidation of some amino acid residues, and PC can be produced by oxidative cleavage of PC into peptide backbones via the α-amidation pathway or by cleavage associated with the oxidation of glutamyl residues

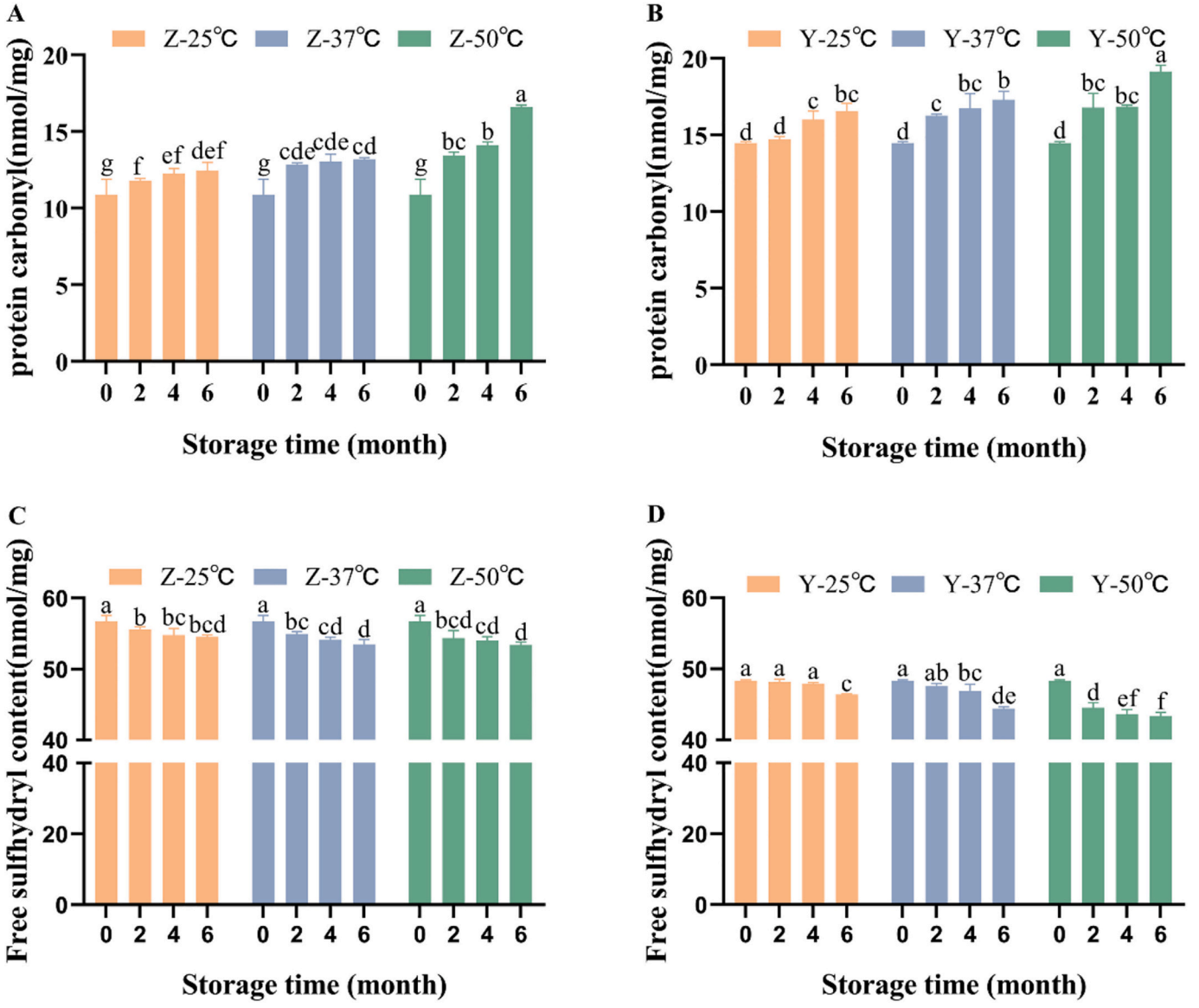

Fig. 5. Protein oxidation states of ZIF and YIF: (A) protein carbonyl compounds of ZIF, (B) protein carbonyl compounds of YIF, (C) sulfhydryl content of ZIF, and (D) sulfhydryl content of YIF.

(Stadtman & Levine, 2000). Sulfhydryl group (—SH) is one of the important functional groups in proteins, mainly found in cysteine residues, and the sulfhydryl group content can characterize the degree of protein aggregation in samples(Li et al., 2021). The changes in protein oxidation of the two milk powders were determined by carbonyl and sulfhydryl groups. There was a significant difference between the protein carbonyl and free sulfhydryl contents of ZIF and YIF ($p < 0.05$). With the prolongation of storage time and the increase of temperature, the carbonyl content of the IFs gradually increased and the free sulfhydryl content gradually decreased, which indicated that the proteins were constantly being oxidized.

The initial carbonyl content of ZIF and YIF was 10.87 nmol/mg and 14.46 nmol/mg, respectively, and increased to 12.45 nmol/mg and 16.54 nmol/mg under the storage condition of 25 °C, respectively; under the storage at 37 °C, the carbonyl content increased to 13.19 nmol/mg and 17.29 nmol/mg, respectively; under the high temperature storage at 50 °C, the carbonyl content increased to 16.60 nmol/mg and 19.12 nmol/mg, respectively. From the above data, it can be seen that the carbonyl content of YIF is higher than that of ZIF. Goat's milk contains higher levels of β-casein than cow's milk(Chen et al., 2022), and it has been reported that randomly curled α-casein and β-casein appear to be the two proteins most likely to form carbonyl groups(Dalsgaard et al., 2007). Whereas high concentrations of casein exacerbate free radical chain reactions and increase oxidative damage, and carbonyl accumulation on casein and macromolecular aggregate is produced by heat treatment(Scaloni et al., 2002).

The initial free sulfhydryl content of ZIF and YIF were 56.71 nmol/mg and 48.30 nmol/mg, respectively. The free sulfhydryl content decreased to 54.55 nmol/mg and 46.39 nmol/mg, respectively, when stored at 25 °C; the sulfhydryl content decreased to 53.51 nmol/mg and 44.38 nmol/mg for storage at 37 °C, respectively; the free sulfhydryl content decreased to 53.38 nmol/mg and 43.36 nmol/mg with a rate of decrease of 5.88 % and 10.22 %, respectively, when stored at 50 °C. From the above data, it can be seen that the free sulfhydryl content of YIF is lower than that of ZIF, and the rate of decrease of YIF is higher than that of ZIF. The initial free sulfhydryl content of ZIF was higher than that of YIF. This may be due to the denaturation of β-lactoglobulin in ZIF during processing, which led to an increase in the free sulfhydryl content; the effect of heat treatment on free sulfhydryl groups was mainly focused on β-lactoglobulin, which is the main whey protein in cow's milk powder, and is highly reactive due to the fact that the free sulfhydryl group is hidden in its folding structure(Xiong et al., 2021), whereas the β-lactoglobulin in goat's milk powder content is relatively low compared to cow's milk powder. At higher temperatures, the decrease in free sulfhydryl content may be caused by oxidation, as air is still present in the milk powder canister during the heating process (Lyster, 1964). Heat treatment of proteins leads to oxidation of free sulfhydryl groups and/or intermolecular exchange of SH-SS. Free sulfhydryl groups in milk powders decreased after heat treatment because glycosylation reactions in milk powders cause excess free sulfhydryl groups to reorganize and form new disulfide bonds, and protein aggregation and unfolding may be more intense with increasing levels of glycosylation, which may account for the lower levels of reactive sulfhydryl groups(Zhao et al., 2020).

By studying the changes in the carbonyl and sulfhydryl contents of ZIF and YIF, the results showed that the degree of oxidation of YIF was higher than that of ZIF.

3.3. Lipid oxidation of ZIF and YIF during storage

Surface free fat, which is fat that is not encapsulated inside the milk powder particles, is an undesirable characteristic, and the amount of surface free fat affects many of the functional properties of the powder (Masum et al., 2020). The surface free fat contents of unstored ZIF and YIF were 0.451 g/100 g and 0.292 g/100 g, respectively. The initial surface free fat content of YIF was lower than that of ZIF. Goat milk powder has a higher content of β-casein, which is preferentially adsorbed on the surface of fat globules in milk powder containing β-casein and α_{S1} series-casein(Chen et al., 2022). From **Fig. 6 (A)(B)**, it can be seen that the surface fat content of both samples showed an increasing trend with increasing storage time. ZIF was stored at 25 °C, 37 °C, and 50 °C for 6 months, and its surface free fat increased to 0.46 g/100 g, 0.51 g/100 g, and 0.60 g/100 g, respectively. The surface free fat of YIF increased to 0.41 g/100 g, 0.49 g/100 g, and 0.54 g/100 g when stored at 25 °C, 37 °C, and 50 °C for 6 months, respectively. The increase in temperature significantly affected the free fat content on the surface of both IFs. In contrast, the surface free fat of YIF was lower than that of ZIF during storage, probably due to the lower degree of lactose crystallization than that of ZIF. The surface free fat of YIF was lower than that of ZIF during storage. Increased lactose crystallization in milk powder during storage forces fat to migrate from the inside of the particle to the surface, resulting in increased surface free fat content(Saxena et al., 2020). Surface free fat is recognized as a major factor in the caking of IF during storage.

Determination of PV value reflects the amount of hydroperoxides produced during storage and can be used to initially determine the degree of fat oxidation in milk powder. The initial PV of ZIF and YIF were 0.20 meq/kg and 0.10 meq/kg, respectively, which shows that the initial PV of YIF is lower than that of ZIF. As can be seen from **Fig. 6 (C) and (D)**, during the 6 months of sample storage, the PV of both samples showed an increasing trend with the increase in storage time, and the changes in PV were correlated with the storage temperature. The PV of ZIF stored at 37 °C and 50 °C and YIF stored at 50 °C showed a tendency to increase rapidly and then decrease, attributed to the unstable nature of hydroperoxides(An et al., 2018), indicating the progression of lipid oxidation from primary to secondary oxidation stages(Mahmoodani et al., 2018). And higher temperatures tend to trigger the onset of peroxide earlier and increase its rate of increase.

MDA is one of the end products of lipid peroxidation, and it gives more insight into the degree of oxidation of the two formulas. The results of ZIF and YIF stored at three temperatures for 6 months are shown in **Fig. 6 (E) (F)**. The initial MDA values were 35.14 nmol/mL and 22.22 nmol/mL for ZIF and YIF, respectively. The lower lipid oxidation end products of YIF than ZIF were related to the lower oxidation of YIF than ZIF in the primary stage of lipid oxidation. It is also possible that casein in goat's milk can form a dense interfacial layer around the oil droplets, and this interfacial layer may prevent lipid oxidation of casein in goat's milk(Smialowska et al., 2017). From the figure, it can be seen that the MDA values of both IFs were increasing when stored at 25 °C; when stored at 37 °C, the MDA values of ZIF showed a tendency of increasing and then decreasing, whereas the MDA values of YIF showed a tendency of increasing, and the MDA values of both IFs stored at 50 °C showed a tendency of increasing and then decreasing. The decrease in the MDA values of the two IFs may be due to the fact that MDA is a volatile product that is volatilized after accumulating(Almansa et al., 2013).

By studying the changes in surface free fat, PV, and MDA content on the surface of two IFs, ZIF and YIF, the results showed that the degree of lipid oxidation of YIF was lower than that of ZIF.

3.4. Changes in wettability of powders during storage

Powder wettability is related to the water adsorption capacity of the powder surface. This property can be indirectly expressed by the contact angle; the smaller the contact angle, the better the wettability, and vice versa(Sharma et al., 2012). As can be seen in **Fig. 7 (A) and (B)**, the contact angle of YIF is lower than that of ZIF, indicating that the wettability of sample YIF is better than that of sample ZIF. The free fat content on the surface of YIF is lower at the beginning of storage. When ZIF and YIF were stored at 25 °C, 37 °C, and 50 °C for 6 months, the contact angles of ZIF increased by 0.97°, 3.04°, and 7.08°, respectively, and the contact angles of YIF increased by 2.62°, 3.71°, and 8.14°, respectively. The contact angles of both ZIF and YIF increased

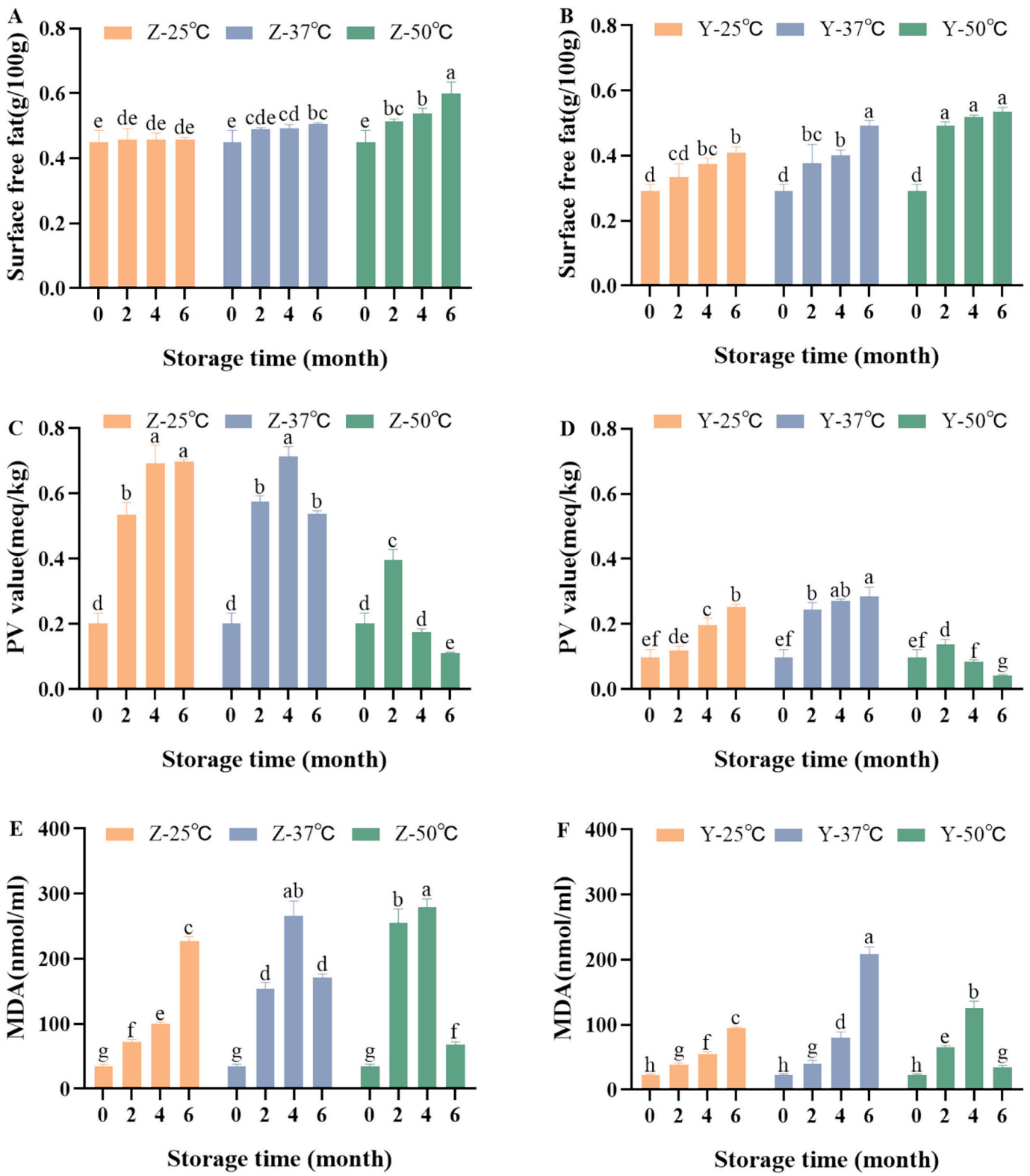

Fig. 6. Lipid oxidation status of ZIF and YIF: (A) surface free fat of ZIF, (B) surface free fat of YIF, (C) PV value of ZIF, and (D) PV value of YIF, (E) MDA content of ZIF, (F) MDA content of YIF.

significantly with increasing storage time and temperature, indicating that the wettability of both samples decreased because the fat content of the surface of the milk powder increases the hydrophobicity of the surface, which leads to a larger contact angle between the surface of the powder and the osmotic water, thus leading to a decrease in the wettability of the milk powder(Ho et al., 2021).

3.5. Solubility of powders during storage

Solubility is a key functional characteristic of the IFs because powdered milk is usually re-solubilized in water prior to use, and all the physicochemical characteristics discussed in the previous sections (pH, crystallinity, degree of browning, protein oxidation, lipid oxidation, and

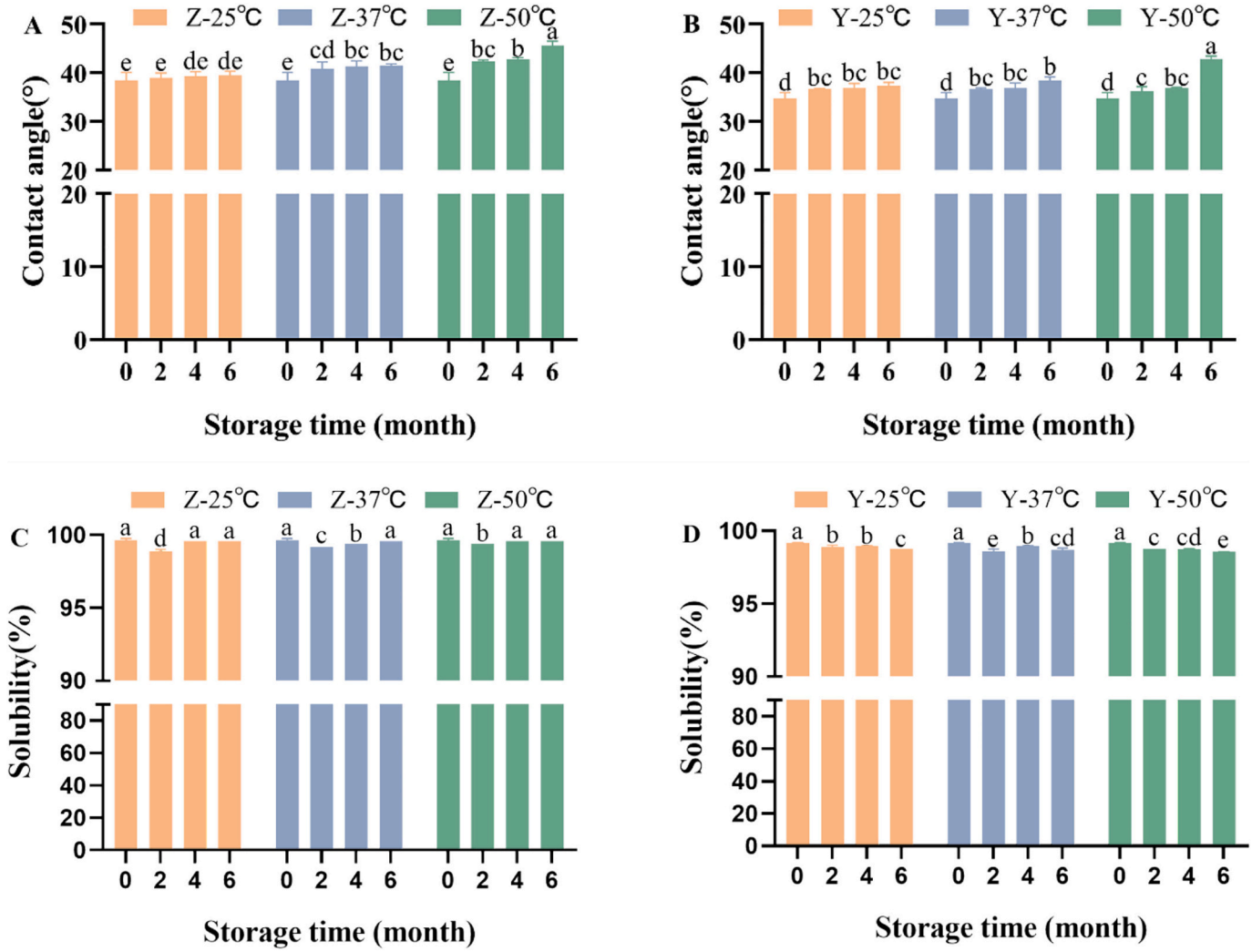

Fig. 7. Changes in functional properties of ZIF and YIF after 6 months of storage at 25°C, 37°C, and 50°C: (A) contact angle of ZIF (°); (B) contact angle of YIF (°); (C) solubility of ZIF (%); (D) solubility of YIF (%).

wettability) combine to determine their solubility. **Fig. 7 (C) and (D)** shows the solubility of ZIF and YIF stored at 25 °C, 37 °C, and 50 °C for 6 months. The initial solubilities of the ZIF and YIF were 99.66 % and 99.16 %, respectively, indicating that the two IFs were almost completely soluble in water. The initial solubility of YIF was slightly lower than that of ZIF. The particle size of ZIF was 281.53 μm, and that of YIF was 336.50 μm. The initial particle size of YIF was higher than that of ZIF, and the larger particle size indicated more serious aggregation of milk powder particles. And the higher content of β-casein in goat's milk powder and the larger size of the formed casein micelles can also explain the occurrence of this phenomenon(Ye et al., 2019).

The solubility of ZIF showed a fluctuating trend throughout the storage time, and there was no significant difference in the solubility of ZIF at the end of storage **(Fig. 7 C)**. The solubility of YIF had a significant decreasing trend at the end of storage, and the solubility went down to drop 98.77 %, 98.70 %, and 98.56 % at 25 °C, 37 °C, and 50 °C for 6 months, respectively. It may be related to the higher content of β-casein in YIF than ZIF, which allows more opportunities for the production of casein micelles, ultimately leading to a more pronounced decrease in the solubility of YIF compared to ZIF(Le et al., 2013). Casein content and cross-linking have a strong influence on the reduction of milk powder solubility. The solubility of proteins in milk may be closely related to the oxidized state of the proteins, as oxidation enhances protein interactions and aggregation, which can reduce the solubility of milk powders (Scheidegger et al., 2013).

3.6. Principal component analysis (PCA)

The results of the previous analysis showed that the physicochemical and functional indicators measured in this study were changed to different degrees at the three storage temperatures, and there were differences in the trends of the indicators, so the PCA method was used to quantify the degree of contribution of the indicators to the changes in the quality of the infant formula, and the results of the study were visualized in the PCA plots.

A variance of 80 % (PC1: 54.2 %, PC2: 25.8 %) is explained in **Fig. 8**, indicating reliable clustering. The graph shows a clear separation between the groups of ZIF and YIF, indicating a large difference between the two IFs. The correlation between the indicators of the two milk powders can also be visualized in **Fig. 8 (B)(C)**. In **Fig. 8 (B)**, the surface free fat of ZIF shows a strong positive correlation with the contact angle, which is consistent with the results of the analysis of wettability described above. In **Fig. 8 (C)**, the free sulfhydryl content of YIF shows a strong correlation with solubility, and the positive correlation between protein carbonyl content and browning index is strong in both milk powders, which is also in line with the analysis of protein oxidation results and solubility above.

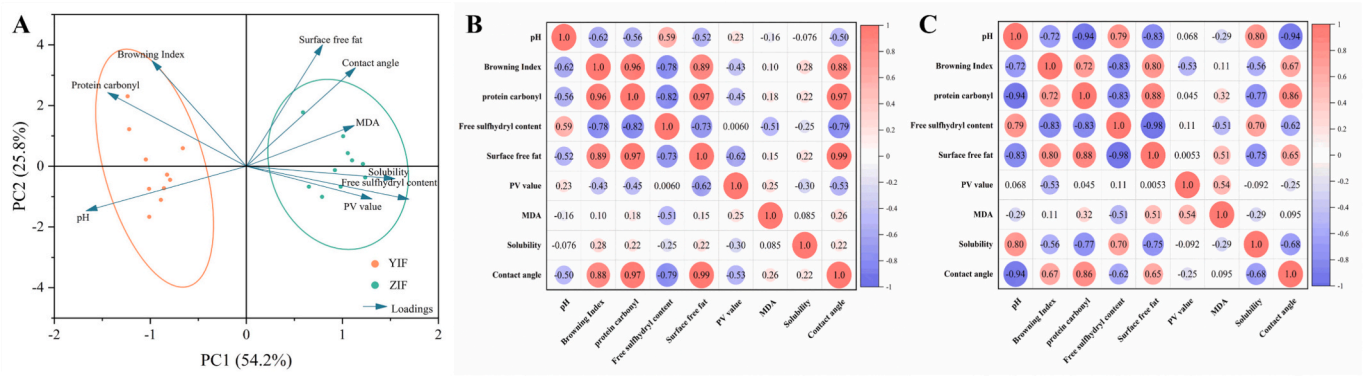

Fig. 8. Principal Component Analysis (PCA) biplot (A) and thermograms (B and C) of the results of the quantitative analyses of pH, Browning Index, Protein carbonyl content, Free sulfhydryl content, Surface free fat, PV value, MDA, Contact angle, and Solubility, where (B) is the thermogram of ZIF and (C) is the thermogram of YIF.

4. Conclusion

In this study, ZIF and YIF stored at different temperatures (25 °C, 37 °C, and 50 °C) for 6 months were investigated in terms of physical, chemical, and functional properties. High-temperature storage conditions significantly reduced the storage stability of both IFs. The surface morphology of both milk powder particles changed from smooth spherical shape to rough, irregular and agglomerated phenomenon during storage, and the surface appeared to be porous with flaky crystals. YIF with higher pH values increased the degree of browning of the powder, and the degree of browning of YIF was positively correlated with protein oxidation, with a strong correlation between the two, both of which also led to a decrease in the solubility of YIF. In contrast, YIF has a lower degree of lipid oxidation than ZIF, where the lower surface fat content results in better wettability of YIF. Because ZIF had a smaller particle size and less protein oxidation, its solubility is maintained at about 99 % throughout the 6-month storage period. This study provides better insights into how product ingredient selection and storage temperature affect the quality of infant formula. And it lays the foundation for the next research on the mechanism of protein oxidation and MR in cow-based and goat-based IFs.

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CRediT authorship contribution statement

Longyu Wan: Writing – original draft, Validation, Methodology, Investigation, Data curation. **Wen Tu:** Writing – review & editing, Validation, Formal analysis. **Jiaxin Zhang:** Writing – review & editing, Data curation. **Jiayue Yang:** Writing – review & editing, Formal analysis. **Xu Wang:** Methodology, Data curation. **Jian He:** Writing – review & editing, Methodology. **Chaoxin Man:** Project administration, Data curation. **Qianyu Zhao:** Validation, Data curation. **Feng Zhao:** Writing – review & editing, Supervision, Project administration. **Yujun Jiang:** Supervision, Project administration.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102275.

Data availability

Data will be made available on request.

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