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pH-shift strategy improving the thermal stability and oxidation stability of rice starch/casein-based high internal phase emulsions for the application in fish cake

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ABSTRACT

The thermal stability of the different pH-shift rice starch/casein-based high internal phase emulsions (SC-HIPE) were evaluated in the present study to verify potential in improving the quality of fish cake. The results showed that the pH-shift treatment improved thermal stability (from 27.23% to 76.33%) and oxidation time (from 5.01 h to 6.86 h) of SC-HIPE, which showed the smaller droplet size (decreased from 15.14 to 1.64 μm) and higher storage module. The breaking force of FC with thermal stable SC-HIPE (average 64.95 g) was higher than that with thermal unstable SC-HIPE (51.05 g). The cohesiveness, adhesiveness and chewiness could be improved by adding thermal stable SC-HIPE, compared with pork fat. Additionally, combining sensory evaluation, the thermal stable SC-HIPE improved the gel quality, thus it could be completely replaced pork fat in the preparation of FC, which provided theoretical guidance for the preparation and application of fat substitutes.

1. Introduction

Jingzhou fish cake (FC) is one of the most famous traditional Chinese characteristic products, showing the characterization of elasticity and tenderness, and is popular among consumers (Du et al., 2022). This kind of FC contains 20%–30% pork back fat, which provides a unique flavor and texture (Guo et al., 2020). Meanwhile, pork fat would increase the risk of obesity, high blood cholesterol, and type-2 diabetes, etc. (Sacks et al., 2017). Therefore, it is also necessary for us to find suitable fat substitutes to prepare FC to improve health.

Some researchers have found that polysaccharide-based (Varga-Visi, Toxanbayeva, Andrássyné Baka, & Romvári, 2018) or/and protein-based fat substitutes (Akesowa, 2009) have specific efficacy in reducing fat content, but their replacement would result in changes in product, such as texture and flavor. To improve the ratio of fatty acids, maintain the flavor, texture, taste, and other qualities similar to the products, and inhibit fat oxidation, a more effective fat substitute is needed. At present, oil-based fat substitutes have attracted more and more attention in replacing animal fat. High internal phase emulsions (HIPEs) are semi-solid materials, which can be used to mimic the texture properties of saturated fatty acids. Liu, Guo, Wan, Liu, Ruan, and Yang (2018) found that wheat gluten protein stabilized O/W HIPEs ($\varphi = 75\%$) exhibited very similar droplet size distribution, rheological behavior, nearperfect thixotropic recovery, and tribological property, and showed similar sensory properties, creaminess, smoothness, and viscosity with mayonnaise as a useful fat replacer, both of them formed homogeneous oil-in-protein network microstructure. Liu et al. (2023) found that HIPEs prepared by cod protein or casein could replace pork fat in making FC by increasing the binding ability of protein and water to ensure that the gel network structure can better "capture" water and oil, and the replacement amount of different HIPEs was different. Xu et al. (2022) found that the hardness of chicken gels could increase by adding 30% egg yolk-modified starch complex stabilized HIPEs. Similar results also found that β-lactoglobulin nanoparticles stabilized HIPEs could decrease cooking loss, and the elasticity of minced meat products (Lan et al., 2021). According to the above studies, HIPEs could be used as fat replacers.

HIPEs prepared by rice starch/casein (SC-HIPE) had higher storage modulus, viscosity, and three interval thixotropy, including lower frequency dependence, which had been revealed to be able to 3D-printed foods (in our previous study). However, in order to expand its

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application in the food manufacturing industry, applying as fat substitute in making FC has great prospects.

Heating is one of the most important processes in making cooked surimi products, such as FC. Thus, as the fat substitute in making FC, the stability especially thermal stability of HIPEs is important. Recently, some studies have been done on improvement of the thermal stability of emulsions. The methods cover using co-emulsifier, Maillard-reacted, pH-shift, etc. The transglutaminase (TGase) cross-linked collagen fibres stabilized algal oil HIPEs showed good thermal stability (90 °C, 40 min) (Tian, Li, Li, & Wang, 2022). The transglutaminase crosslink with gelatin was used to stabilize food-grade HIPEs with high thermal stability at 90 and 121 °C for 20 min, showing a uniform droplet diameter (Du et al., 2021). Du et al. (2021) found that TG promoted the production of high weight molecular. The epigallocatechin gallate mixed with Maillard-reacted products of whey protein isolates and lactose (1:1 w/w) could stabilize emulsion with high thermal stability (Liu, Wang, Hu, Cai, & Qin, 2019). However, transglutaminase crosslink or Maillard-reacted took much more resource investment, and pH-shift was a more convenient and economical method. The pH-shift would allow protein to unfold and refold, and change the structure of protein, resulting in improving solubility and emulsifying properties of protein (Ryan & foegding, 2015). Abdollahi, Olofsson, Zhang, Alminger, and Undeland (2020) found that the solubility of protein in pH alkaline pH conditions was higher than that in extreme acid, which was agree with Abdollahi and Undeland (2018), and Marmon and Undeland (2010). Bi, Chi, Wang, Alkhatib, Huang, and Liu (2021) found that the flax gum mixed with soy protein isolate acid-induced emulsion gels showed high thermal stability by enhancing chemical stability of the protein.

In our previous study, the SC-HIPE showed poor thermal stability. Thus, in this study, the pH-shift strategy (pH 2.5, 6.5, 8.5, and 10.5) was used to adjust the SC water phase to improve the thermal stability of the SC-HIPE. And the oil phase prepared by mixing soybean oil and purple perilla seed oil (SPO) to reach the nutritional requirement of n6/n3 ratio of 5:1 was used. The droplets size, mechanical characterization, and oxidation stability of SC-HIPE were also revealed. Finally, the thermal stable or thermal unstable SC-HIPE was used in the preparation of FC as a substitute for pork back fat, and reveal if the thermal stability of SC-HIPE affected the texture properties and sensory evaluation of FC.

2. Materials and methods

2.1. Materials

Frozen silver carp surimi (Grade AA, 28% protein, 1% fat, 3% carbohydrate, and 3% sodium, w/w) was purchased from HUBEI Communications Investment & Laker Agriculture Technology Co., Ltd. (Qianjiang, Hubei, China). Casein was bought from Sigma-Aldrich (Shanghai, China), rice starch (3–5 μm) were obtained from Shanghai yuaye Bio-Technology Co., Ltd. Salt, soybean oil and pork back fat were purchased from a local supermarket (Dalian, China), and purple perilla seed oil (PPSO) was purchased from JD.com (China). All other used chemicals and solvents were of analytical grade in this study.

2.2. Fatty acid composition of soybean oil and purple perilla seed oil

The fatty acid composition of soybean oil (SO) and purple perilla seed oil (PPSO) was determined according to GB 5009.168–2016. The SO contained 55.1% linoleic acid (n6) and 7.21% linolenic acid (n3), PPSO contains 16.2% linoleic acid (n6) and 56% linolenic acid (n3) (the date was not showed). The mixture of SO and PPSO (SPO) was conducted according to the ratio of n6/n3 (5:1).

2.3. Preparation of HIPEs

The 5% rice starch and 3% casein complex (SC) was stirred for 12 h at room temperature to fully hydrate. Then, 6 molL^{-1} HCL was used to adjust pH of SC at 2.5, 6 molL^{-1} NaOH was used to adjust pH of SC at 6.5, 8.5, and 10.5. After, pH-shifted SC was heated at 100 °C for 30 min, cooled at room temperature, and was used to prepare HIPEs ($\varphi = 0.8$) by a T10 IKA homogeniser (IKA® Werke, Germany). The HIPEs prepared by different pH-shift SC were named pH 2.5, pH 6.5, pH 8.5, and pH 10.5, respectively. The HIPEs prepared by SC without pH-shifting was named untreated.

2.4. The characterization of HIPEs

The acoustic and electroacoustic spectrometer (DT-1202, DTI, USA) was used to measure the size of HIPEs under the emulsion measurement modes.

The rheometer (Discovery HR-2, TA, USA) was used to analyze the mechanical characterization of HIPEs with a parallel plate (116801, TA, USA). The samples were equilibrated at room temperature for 2 h before test. The strain sweep test, frequency sweep test, and steady shearing tests were used to reveal the rheological properties of HIPEs according to Du et al. (2021).

The thermal stability of HIPEs was tested with temperature sweep test by a rheometer (Discovery HR-2, TA, USA) equipped with a parallel plate (116801, TA, USA), or heated at 90 °C to observe the delamination. The temperature sweep test was tested when temperature rising range was from 25 °C to 90 °C at the rate of 5 °C/min under a fixed frequency (1 Hz).

The oil oxidation stability was measured by OXITEST (892 professional rancimat, Metrohm, China). The test parameter: The heat temperature was 110 °C with the gas flow rate of 20.0 L/h.

2.5. Preparation of fish cake

The fish cake (FC) was prepared according to our previous study with some modifications (Liu et al., 2023). The FC prepared by surimi (70 g/100 g) mixed with 30 g/100 g pork fat (named Pf), 30 g/100 g untreated SC-HIPE (named HIPE), 30 g/100 g pH 2.5 SC-HIPE (named 2.5HIPE), 30 g/100 g pH 6.5 SC-HIPE (named 6.5HIPE), 30 g/100 g pH 8.5 SC-HIPE (named 8.5HIPE), 30 g/100 g pH 10.5 SC-HIPE (named 10.5HIPE), respectively. The FC (named Native) without any oil was used as blank control, the FC with pork fat (Pf) was used as positive control.

2.6. Physical and texture properties of fish cake

The pH of uncooked and cooked FC was measure by homogenizing 10 g sample with 90 g water with T10 IKA homogeniser (IKA® Werke, Germany) (Techaratanakrai, Nishida, Igarashi, Watanabe, Okazaki, & Osako, 2011).

The cooked FC was balanced at 25 °C for 1 h before measuring. The texture analyzer (TA.XT.plus, Stable Micro Systems Ltd., UK) was used to analyze the texture properties including gel strength and TPA. The gel strength was measured under 5 g trigger force and 15 mm pressure distance by a spherical plunger (diameter: 5 mm, P/5 S) with 2 mm/s pre-test, 1 mm/s test, and 2 mm/s return speed. The TPA was measured by a spherical plunger (diameter: 50 mm, P/50) with 1 mm/s test speed. Each test was repeated at least three times.

The cooked FC was weighted (m_1), wrapped in 8 layers of filter paper and placed in a centrifugal tube. Then samples were centrifuged at 5000 g at 4 °C for 10 min. Finally, the samples were taken out and weighed (m_2). The water holding capacity (WHC) (%) = $m_2/m_1 \times 100$.

The moisture composition and proton magnetic resonance images (MRIs) were determined according to Liu (Y. Liu et al., 2023) by using low-field nuclear magnetic resonance (LF-NMR; Me-soMR23-060 V-I, Niumag Co., Ltd., China).

The contact angle of cooked FC was measured by a drop shape analyzer (DSA 25, KRÜSS, Germany) according to Gao, You, Yin, Xiong, and Liu (2023) described. The cooked FC was cut into slices, put on a glass slide, and placed on a movable platform. The 5 μL of ultrapure water was slowly dropped by a microsyringe on the surface of FC.

2.7. Sensory evaluation of fish cake

Sensory evaluation is very important for product evaluation. Two kinds of scoring methods were used for sensory evaluation. One was property evaluation rated the attributes of flavor (fishy odor), appearance (whiteness), texture (hardness, tendernesss, elasticity), tasty (acidity). One was preference evaluation, in which we asked 10 people working in related fields to likeness, fishy odor, acidity, brightness, elasticity and tenderness.

Each fish cake sample was cut into three identical pieces (3 cm × 1 cm × 1 cm) and labeled with randomly selected three-digit numbers. Ten untrained panelists (aged 23–35) were invited to evaluate. Before the panel evaluation, the panelists were given a briefing about the background of the project and instructions for the sensory evaluation procedure. A 5 point scale and 5 point JAR scale (based on the intensity of perception) was used for scoring by the panel.

2.8. Statistical analysis

All of the experiments were repeated at least three times. Data analysis was conducted using IBM SPSS 19.0 (SPSS Inc., Chicago, IL). A significant difference was defined as $p < 0.05$.

3. Results and discussion

3.1. Particle size distribution of HIPEs prepared with SC after different pH-shifting

The average droplet size of high internal phase emulsions (HIPEs) stabilized with different pH-shift (pH 2.5, pH 6.5, pH 8.5, pH 10.5) treated SC is shown in Fig. 1A. HIPEs prepared by SC after pH-shifting showed significantly lower than HIPEs prepared by SC without pH-shifting (named untreated). The average drop size of pH 2.5 was 4.05 μm, and that of pH 6.5, pH 8.5, pH 10.5 were 1.63, 1.90, and 2.08 μm, respectively, while that of untreated was 15.14 μm. The drop size decreased might because the structure of protein changed when unfold and refold occurred after pH-shifting, resulting in improving solubility and emulsifying properties of protein (Ryan & foegding, 2015). Guo, Wu, Du, Lin, Xu, and Yu (2021a) found that casein particles showed large size at pH 2, which might because the strong hydrogen bonds formed between amino acid residues by the protonated amino acid residues, and electrostatic interaction was disturbed (Chakraborty & Basak, 2007). Xu, Han, Chen, Kou, Gao, and Tang (2023) also found that HIPEs prepared with chitosan/gum arabic composite particles after pH5-shifting were significantly higher than that after pH7-shifting. In general, pH-shift treated SC could decrease the droplet size of SC-based HIPEs.

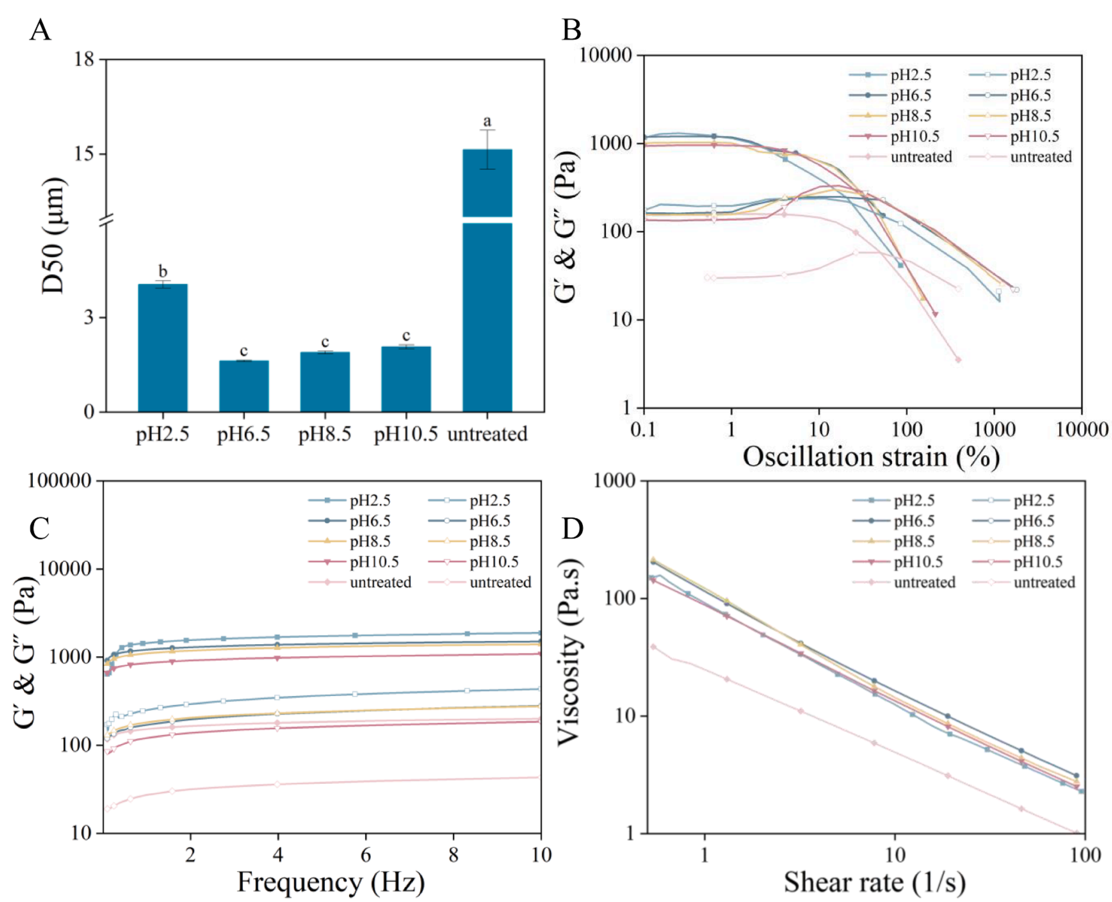

Fig. 1. The effects of pH-shift on size (A), strain sweep (B), frequency sweep (C), and shear rate sweep (D) of SC stabilized HIPEs. pH2.5: HIPEs prepared by SC after treating at pH 2.5; pH6.5: HIPEs prepared by SC after treating at pH 6.5; pH8.5: HIPEs prepared by SC after treating at pH 8.5; pH10.5: HIPEs prepared by SC after treating at pH 10.5; untreated: HIPEs prepared by SC without pH-shifting.

3.2. *Mechanical properties of HIPEs prepared with SC after different pH-shifting*

The mechanical properties of HIPEs were measured by rheology and the results are showed in Fig. 1B–D. The G’, G“, and viscosity could be characterized beneficially emulsion stability and droplet interaction (Zhu, Chen, McClements, Zou, & Liu, 2017). During measurement, strain should be applied to all samples in the LVE region to ensure that the samples were not damaged, thus obtaining correct data. (Zhang, Zhang, Hu, Xue, & Xue, 2020).

The storage modulus (elasticity modulus G’) and loss modulus (viscosity modulus G“) as a function of strain are showed in Fig. 1B, and the G’ of all HIPEs was much higher than the G” at the outset, indicating the high elasticity characterization, and the G’ for pH 2.5 was slightly higher, then pH 6.5, pH 8.5, and pH 10.5 in decreasing order. While, the G’ or G“ of HIPEs stabilized by SC after pH-shifting were much higher than untreated. Thus, the HIPEs stabilized by SC after pH-shifting possessed the higher elasticity than untreated. It might be due to the increased solubility of casein after pH-shifting, which could be better adsorb to the oil–water interface (Guo et al., 2021a). Furthermore, the forces between particles could be revealed by the length of LVR (Guo et al., 2021a). The LVR of HIPEs stabilized by SC after different pH-shifting was similar and all of them were longer than untreated, indicating the droplet–droplet forces in HIPEs stabilized by SC after pH-shifting were much stronger.

Fig. 1C shows the dependence of the frequency of HIPEs. The G’ was dominating G“ when frequency increased from 0.1 to 100 Hz, suggesting all HIPEs showed elasticity and a gel-like behavior (Xu, Liu, & Tang, 2019). The similar results in HIPEs stabilized by casein (Guo et al., 2021a), soluble starch/whey protein isolate complex (Guo et al.,

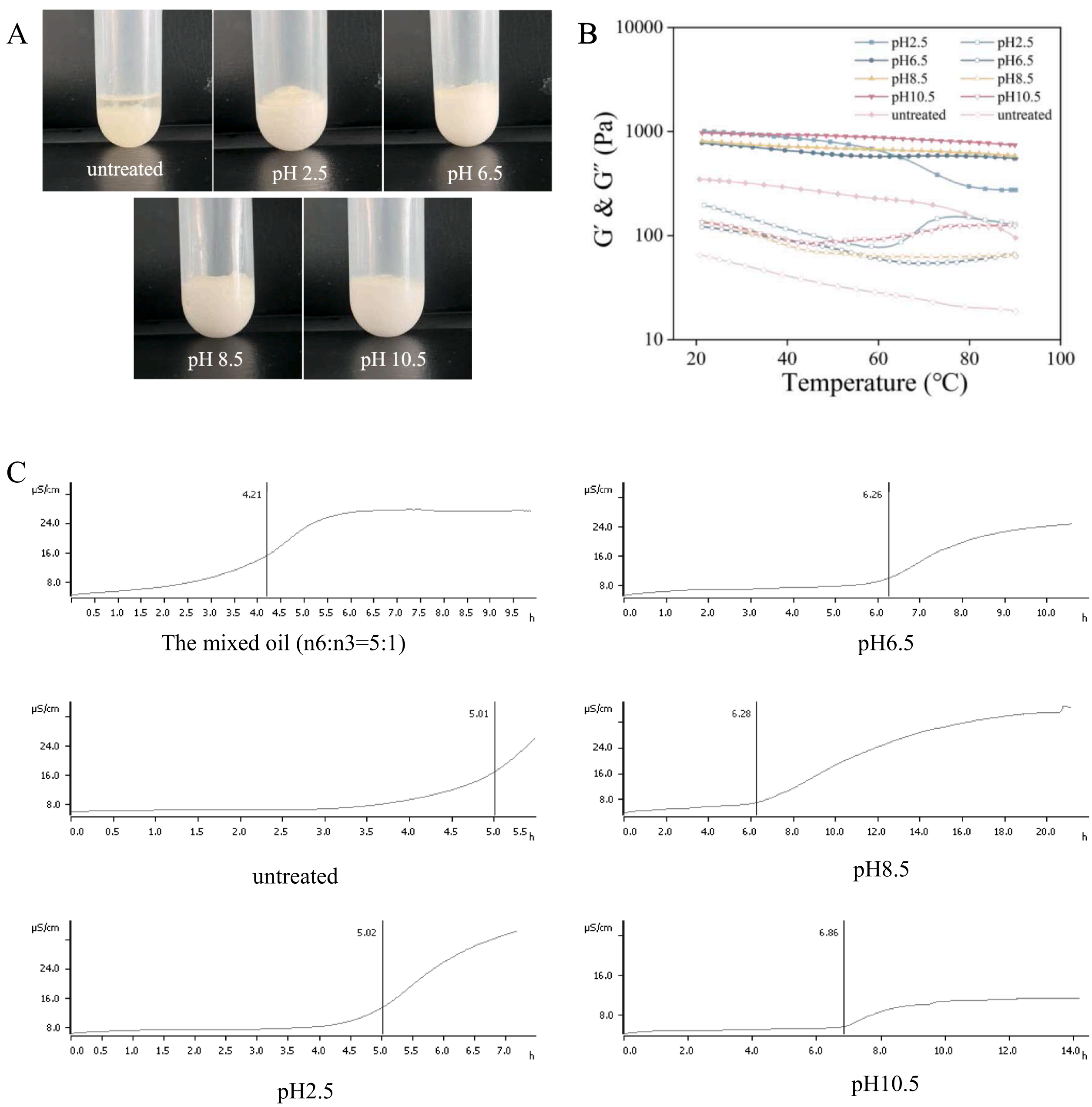

Fig. 2. The effects of pH-shift on the apparent condition after heating at 90 °C for 30 min (A), temperature sweep test (B), and oxidation stability (C) of SC stabilized HIPEs. pH2.5: HIPEs prepared by SC after treating at pH 2.5; pH6.5: HIPEs prepared by SC after treating at pH 6.5; pH8.5: HIPEs prepared by SC after treating at pH 8.5; pH10.5: HIPEs prepared by SC after treating at pH 10.5; untreated: HIPEs prepared by SC without pH-shifting.

2021b), and OSA starch/chitosan polysaccharide (Yan, McClements, Zhu, Zou, Zhou, & Liu, 2019). Additionally, the G' or G'' of HIPEs stabilized by SC after pH-shifting were much higher than that of untreated, which might due to the small droplet size of HIPEs (Xu et al., 2023), which was agree with the results of Fig. 1A.

When the shear rate was 50 s^{-1}, the viscosity of the HIPEs stabilized by SC after pH 6.5, pH 8.5, pH 10.5, and pH 2.5 decreased in order, and was obviously higher than untreated (Fig. 1D). Viscosity at 50 s^{-1} shear rate was highly correlated with sensory perception, such as mouthfeel, aroma, and taste (He, Hort, & Wolf, 2016). Guo el al. (2021a) though that the HIPEs with especial sensory gave them wide applications in the alternative of margarine, salad dresser and so on in the food industry.

3.3. Thermal and oil oxidation stability of HIPEs prepared with SC after different pH-shifting

Most cook foods endure heat treatment to achieve ready-to-eat, good taste and, longer shelf life, indicating heat stability is very important. The thermal stability of HIPEs is showed in Fig. 2A&B. As can be seen from Fig. 2A, untreated sample appeared stratification after heating at 90 °C for 30 min, while HIPEs stabilized by SC after pH-shifting showed unchanged apparent compared with fresh one. Additionally, the temperature sweep test was performed by rheology, and was also used to reveal the thermal stability of HIPEs. With the sweep temperature increasing, the curve of HIPEs stabilized by SC after pH-shifting (pH 6.5, 8.5, 10.5) was relatively flat, while the curve of untreated showed slow down (Fig. 2B), and the results was agree with Fig. 2A. Yang, Li and Tang (2020) found that insoluble soybean polysaccharide-protein composite nanoparticles with a diameter of 160 nm treated in the range of pH 2.0–12.0 and ionic strength 0–500 mmol/L could stabilize HIPEs with excellent thermal stability in the temperature range of 50–90℃.

Oxidation stability is of great important for the shelf-life of emulsion-based products (Shadman, Hosseini, Langroudi, & Shabani, 2017). Oxidation stability of HIPEs is shown in Fig. 2C. The oxidation of fresh mixture oil occurred after 4.2 h (110 °C), while the oxidation of HIPEs 5–7 h. The results showed that HIPEs could prolong the oxidation stability. Huang, Wang, and Tan (2021) also found that the HIPEs exhibit superior oxidation stability. The occurred oxidation time of pH 6.5, pH 8.5, and pH 10.5 was longer than pH 2.5 and untreated, which might because that the droplet size of pH 2.5 and untreated was much larger (Fig. 1A), the forces between particles was much smaller (Fig. 1B) and oxidation was more likely to occur.

3.4. The pH values of uncooked and cooked fish cake

The pH values might influence texture properties such as gel strength, water-holding capacity (WHC) of the heat-induced myofibrillar protein gels (Westphalen, Briggs, & Longergan, 2006). The pH values of uncooked and cooked FC are shown in Fig. 3. The uncooked and cooked pH of FC with 30% HIPEs prepared with SC after pH 10.5 (10.5HIPE) or pH 8.5 (8.5HIPE) treating showed highest, while the pH value of FC decreased with the decrease of pH values of SC. The result revealed that the pH of FC was related to HIPEs microenvironment. Additionally, the pH value of FC with HIPEs was higher than that with pork fat (Pf), and the pH value of FC without any liquid (Native) showed the lowest.

3.5. Mechanical characteristics of cooked fish cake

3.5.1. TPa

Textural properties play an important role in commodity value, acceptance, and consumer preferences, which are the primary determinant for the quality of surimi products (Yang, Li, Li, Sun, & Guo, 2020). The hardness, sprinting, cohesiveness, adhesiveness, and chewiness of FC with/without adding HIPEs or pork fat are shown in Fig. 4A–E. Compared with FC with 30% pork fat, the cohesiveness,

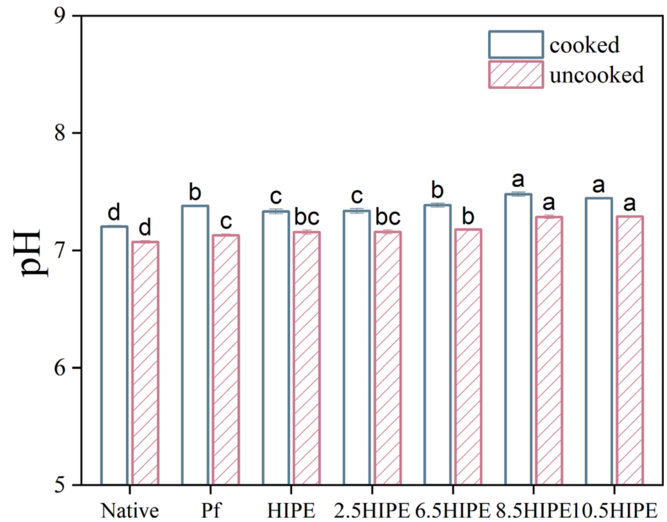

Fig. 3. The pH of fish cake with/without pork fat or HIPEs. Native: fish cake without any lipid; Pf: fish cake with pork fat; HIPE: fish cake with HIPE prepared by SC without pH-shifting; 2.5HIPE: fish cake with HIPE prepared by SC treating at pH 2.5; 6.5HIPE: fish cake with HIPE prepared by SC treating at pH 6.5; 8.5HIPE: fish cake with HIPE prepared by SC treating at pH 8.5; 10.5HIPE: fish cake with HIPE prepared by SC treating at pH 10.5.

adhesiveness, and chewiness were significantly ($p < 0.05$) increased when using HIPEs total replaced pork fat. The increased of texture of surimi gel might because that the HIPEs droplets were evenly distributed in the gel network, making network structure tighter and texture more rigid (Cao et al., 2021; Eisinaite, Juraite, Schroën, & Leskauskaite, 2017). While there was no significant difference in texture properties between FC with and without HIPEs. Yu et al. (2023) found that adding 16% HIPES stabilized by soybean phospholipid/ sodium caseinate could enhance the texture properties of surimi gel (Yu, Xiao, Song, Xue, & Xue, 2023). Thus, in our study, HIPEs could be effective replaced pork fat in making FC, and the adding amount reached 30%. Compared with FC after adding pH-shifted SC-HIPE (thermal stable), the hardness, adhesiveness, and chewiness of FC after adding native SC-HIPE (thermal unstable) were lower. Adding different pH-shifted SC-HIPE had a little different effect on TPA properties of FC (Fig. 4A-E). It might because of the different pH of surimi, which would affect the texture properties of surimi gel (Zhou, & Yang, 2019).

3.5.2. Gel strength

Gel strength is as important as texture in myofibrillar protein gels, which affects the quality of meat products (Walayat et al., 2021). Breaking force, breaking distance, and gel strength of FC are shown in Fig. 4F. The Pf showed the highest breaking force, while FC with HIPEs stabilized by SC after pH-shifting (2.6, 6.5, 8.5, 10.5) was slight higher than FC without any lipid. The gel strength of FC with HIPEs stabilized by SC after pH-shifting was close or even slightly better than FC with/without pork fat. However, Pei et al. (2023) used tilapia myofibrillar protein stabilized emulsion gel in surimi preparation, and found that with the increased amount of emulsion gel, the hardness and gel strength of surimi gel decreased. When the addition amount of tilapia myofibrillar protein stabilized emulsion gel arrived at 30%, the gel strength decreased about 53.86% (Pei et al., 2023). The different might because the characterization of HIPEs prepared with SC after pH-shifting was better and more suitable in making surimi gel than emulsion gel they prepared. Additionally, the gel strength of FC with HIPEs stabilized by SC without pH-shifting showed the lowest, which might be due that the HIPEs stabilized by SC without pH-shifting was unstable during heating (Fig. 2A&B), oil and water separation in HIPEs (Fig. 2A) showed strong fluidity, the formation of gel network was affected. In generally, thermal stability of HIPEs significantly affected the gel strength of FC.

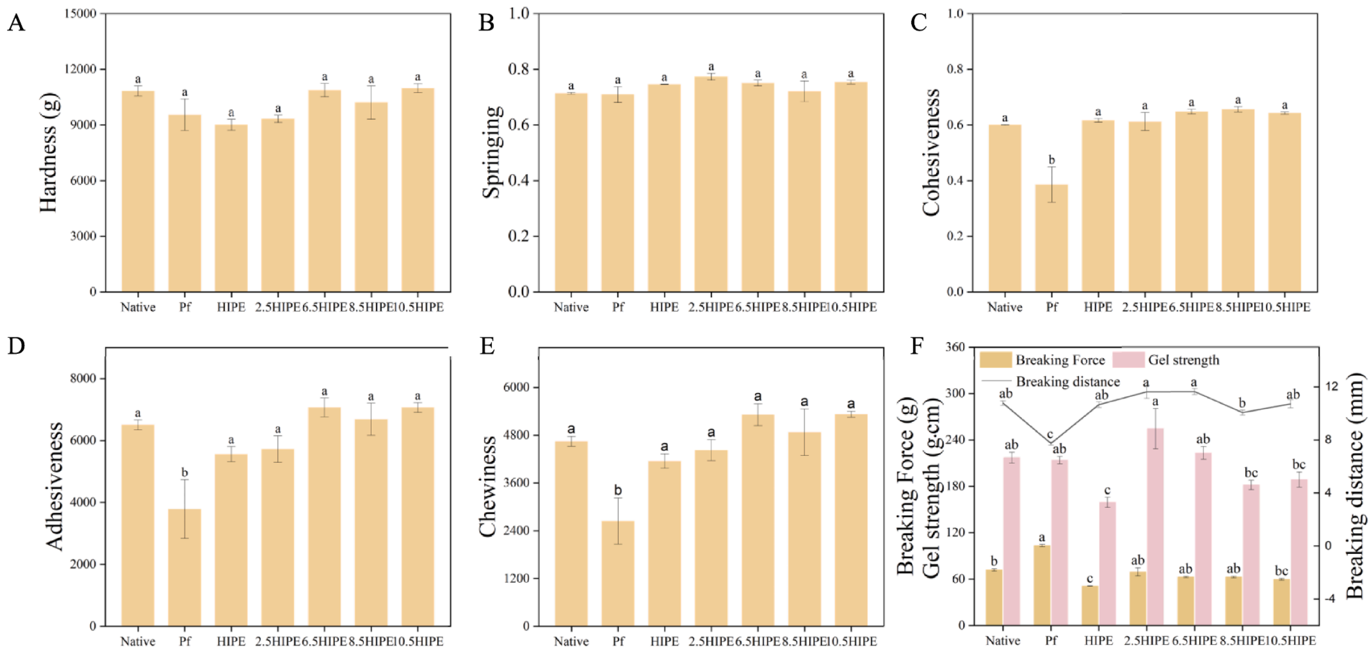

Fig. 4. The hardness (A), springing (B), cohesiveness (C), adhesiveness (D), chewiness (E), and gel strength (F) of fish cake with/without pork fat or HIPEs. Native: fish cake without any lipid; Pf: fish cake with pork fat; HIPE: fish cake with HIPE prepared by SC without pH-shifting; 2.5HIPE: fish cake with HIPE prepared by SC treating at pH 2.5; 6.5HIPE: fish cake with HIPE prepared by SC treating at pH 6.5; 8.5HIPE: fish cake with HIPE prepared by SC treating at pH 8.5; 10.5HIPE: fish cake with HIPE prepared by SC treating at pH 10.5.

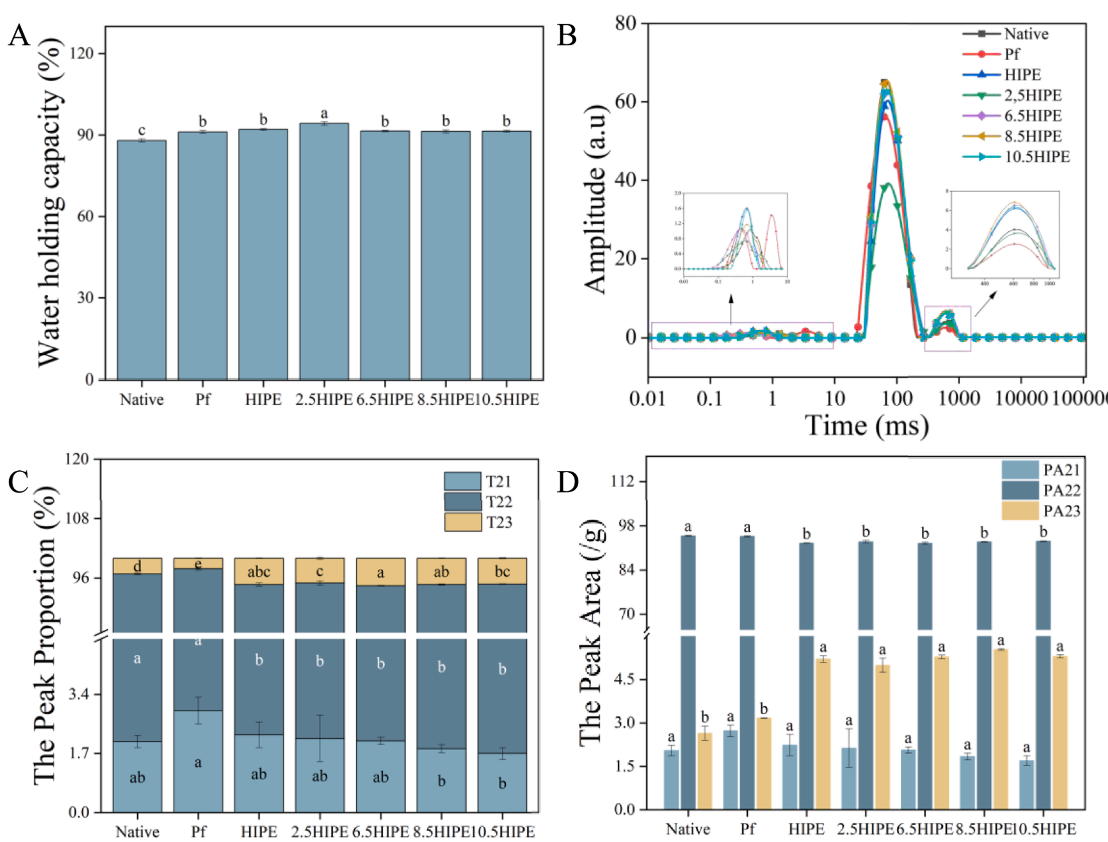

Fig. 5. The water holding capacity (A), and the distribution of oil and water (B–D) of fish cake with/without pork fat or HIPEs. Native: fish cake without any lipid; Pf: fish cake with pork fat; HIPE: fish cake with HIPE prepared by SC without pH-shifting; 2.5HIPE: fish cake with HIPE prepared by SC treating at pH 2.5; 6.5HIPE: fish cake with HIPE prepared by SC treating at pH 6.5; 8.5HIPE: fish cake with HIPE prepared by SC treating at pH 8.5; 10.5HIPE: fish cake with HIPE prepared by SC treating at pH 10.5.

3.6. The water holding capacity and the distribution of oil and water of fish cake

The structure of protein changed after heating, forming a three-dimensional matrix to trap water molecules during gelation, and affecting the water holding capacity (WHC) of surimi gel (Shi, Wang, Chang, Wang, Yang, & Cui, 2014). The WHC of FC with HIPEs or pork fat was significantly higher ($p < 0.05$) than FC without any lipid (Fig. 5A). The increased of WHC was similar with Pet et al. (2023) who found that the appropriate addition of lipids could enhance the formation of network structure, where the gel matrix filled with water and lipids (Youssef, Barbut, & Smith, 2011). Thus, HIPEs could be used to totally replace pork fat in making FC. FC after adding pH2.5-shifted SC-HIPE showed higher WHC than that after adding pH6.5, 8.5, 10.5-shifted SC-HIPE. It might because that different pH-shifted SC-HIPE changed the pH of surimi, which could be seen in Fig. 3. Surimi has better water holding capacity in acidic environment than in alkaline environment, which was agree with Zhou and Yang (2019), who also found that the expressible moisture content of surimi under alkaline treatment was higher than that under acidic treatment, that is the water holding capacity of surimi under acidic treatment was higher.

The LF-NMR analysis was used to reveal the state of the water and oil in the FC, and the results are shown in Fig. 5B–D. Fig. 5B shows the effect of HIPEs or pork fat on the T_2 distribution of FC. There were three peaks in T_2 distribution. The range of T_{21} relaxation time of FC without/with HIPEs was 0.02–4 ms, while that of Pf was 0.02–10 ms, which might because that pork back fat contained bound water, leading to a wide range. The range of T_{22} relaxation time of FC without/with pork fat was 30–200 ms, while that of FC with HIPEs was 30–300 ms, which might be due to the high oil content, agreeing with Wang, Su, Wang, and Nie, (2019) who found that longer T_2 components (54–58.6 ms, and 200–216 ms) were ascribed to the oil content. The range of T_{23} relaxation time of FC with/without pork fat was 300–1000 ms, and that of FC with HIPEs was 300–1100 ms. The water and oil distribution in the FC were calculated by accumulating peak proportion (Fig. 5C) and peak area (Fig. 5D) in spectrum. The peak proportion and average peak area of T_{23} in the FC after the addition of HIPEs were significantly higher ($p < 0.05$) than FC with/without pork fat, T_{22} was the opposite, while no significant ($p < 0.05$) change in T_{21} was observed. The ratio of bound water was<4% in all FC (Fig. 1B), which indicated that it was hardly affected by different pH-shifted SC-HIPE or pork fat. Over 90% immobilised water was observed in all FC, and accounted for the largest proportion of the total water. Zhao, Chen, Wongmaneepratip, He, Zhao, and Yang (2021) also found that bound water was<5% and the immobilised water was more than 90%. Free water is retained via capillary force between water and its surroundings, the increased peak proportion and average peak area of T_{23} might be due to the high water and oil content of HIPEs. Hence, it was suggested that the surimi gel matrix was uniformly filled with HIPEs droplets, and a dense three-dimensional network structure was formed, resulting in the non-obviously change of the distribution of water and oil. However, the thermal stability of HIPEs showed non-obviously effect on water distribution, which might because water and lipids were well dispersed and filled in the gel matrix as Pet et al. (2023) thought, and also found in the result of WHC (Fig. 5A).

The visualization image information of the internal tomography of the sample could be obtained by magnetic resonance imaging (MRI), providing the density and distribution diagram of the hydrogen proton, showing the content and distribution of water (or oil) in the sample (Zhao, 2004). Proton density weighted figures afforded valuable information on water and oil state in all FC samples are presented in Supplementary Fig. 1 (S1), and T_1 and T_2 represent short and long relaxation times, respectively. The red and blue colour means high density and low density of H proton, respectively (Zhou, Li, Fang, Mei, & Xie, 2020). It can be seen from the T_1 and T_2 pseudo-colour images, the internal structure of the FC was similar, and the difference in signal distribution of all samples was not obvious, because the protein forming three-dimensional gel network structure in FC did not change, SC-HIPE or pig backfat addition is inactive filling, which does not affect the protein. When the degree of protein denaturation was not consistent, the moisture in the sample changed significantly (Xia, Xu, Huang, Song, Zhu, & Tan, 2018). After the addition of pork fat or SC-HIPE with/without pH-shifting, the distribution of water and oil was more uniform, and showed high proton density, and the water holding capacity was better (over 90%), showing as Fig. 5A, which is consistent with the results of Zhao et al. (2021). Additionally, it was noted that the water and oil content in FC increased after adding the HIPEs (Fig. 5B–D), and the MRI showed high proton density (S1).

3.7. The water contact angle of fish cake

The surface hydrophilicity of FC could be determined by the contact angle analysis (Gao et al., 2023; Xu, Liu, Goff, & Zhong, 2020). The contact angle of FC with/without HIPEs or pork fat is illustrated in Supplementary Fig. 2A. The water contact angle was all lower than 60°, which was similar with Gao et al. (2023) who found in high intensity ultrasound surimi gel, indicating that the samples were hydrophilic. In our study, thermal stability of HIPEs did not affect the surface hydrophilicity of FC.

3.8. Sensory evaluation of fish cake

To determine if thermal stability of HIPEs would affect the evaluation and likeness of FC, and if HIPEs would be an effective fat substitute in making FC, the assessors were invited to evaluate all studied FC. Two sensory evaluation methods were used to evaluate the prepared edible FC (Supplementary Fig. 2B), including property evaluation (Fig. 6A) and preference evaluation (Fig. 6B).

The assessors were invited to evaluate the flavor (fishy odor), appearance (whiteness), texture (hardness, tendernesss, elasticity), tasty (acidity). The fishy odor of FC with HIPEs was significant ($p < 0.05$) lower than that without adding lipid (Native), and was slight lower than that with pork fat (Pf) (Fig. 6A), indicating that adding HIPEs could improve the flavor of FC, and its effect was also slightly better than pork fat, while the thermal stability of HIPEs showed little effect on the flavor of FC. The hardness of Pf was highest, and that of FC with HIPEs was similar with that of Native. However, according to evaluation, it could be found that the hardness of FC with the thermal unstable HIPEs showed the lowest.

The tender and elasticity of Pf showed the lowest, and that of FC with HIPEs were significant ($p < 0.05$) higher than Native. The acidity of FC was highest, which was agree with the results of pH we found (Fig. 3), Pf and FC with HIPEs decreased in turn.

It can be seen from property evaluation results, HIPEs can completely replace pork fat in making FC, decreasing the fishy odor and acidity, and improving tenderness, elasticity and whiteness on the premise of ensuring hardness (Fig. 6B). In addition, the thermal stability of HIPEs had an effect on hardness, and the hardness of FC with thermal unstable HIPEs was the lowest. A tight and stable texture could be produced by adding stable emulsion, enhancing a high score in sensory evaluation such as color, flavor, taste, and overall acceptance (Badar, Liu, Chen, Xia, & Kong, 2021).

According to the likeness, the more popular the overall likeness, brightness, elasticity, and tenderness were, the higher the scores were, while the higher scores fishy odor and acidity were, the less the likeness was. From the overall likeness score (Fig. 6B), FC with HIPEs stabilized by SC at pH 8.5 and pH 2.5 were the most popular, while FC are the least popular, and the likeness of Pf was slightly higher than Native. From the likeness of fishy odor and acidity, FC was the least popular, which might because that the fishy odor and acidity of FC was highest according the property evaluation. And assessors prefer FC with high brightness, tenderness and elasticity. The result of preference evaluation also

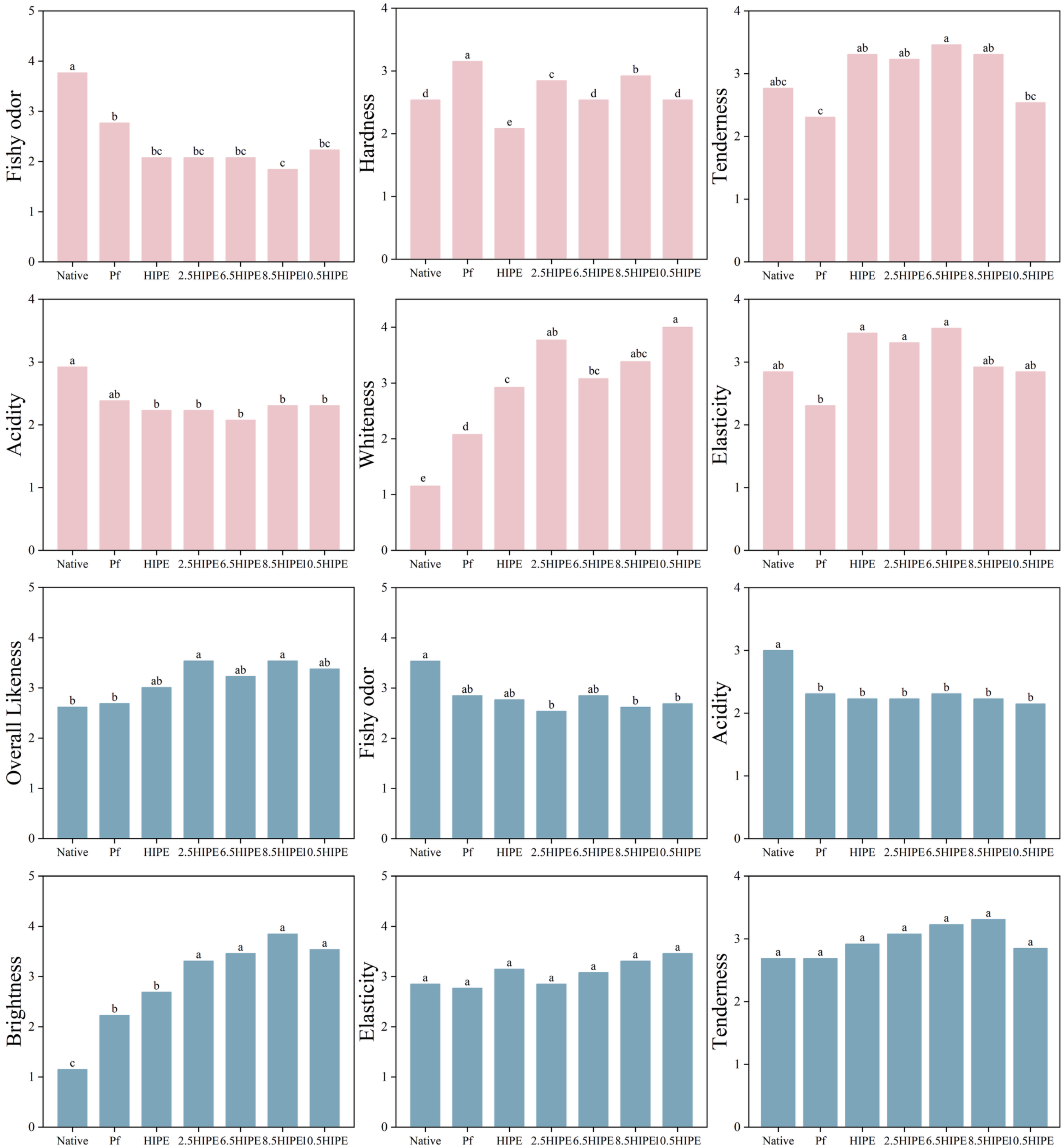

Fig. 6. The property evaluation (A) and preference evaluation (B) of fish cake with/without pork fat or HIPEs. Native: fish cake without any lipid; Pf: fish cake with pork fat; HIPE: fish cake with HIPE prepared by SC without pH-shifting; 2.5HIPE: fish cake with HIPE prepared by SC treating at pH 2.5; 6.5HIPE: fish cake with HIPE prepared by SC treating at pH 6.5; 8.5HIPE: fish cake with HIPE prepared by SC treating at pH 8.5; 10.5HIPE: fish cake with HIPE prepared by SC treating at pH 10.5.

showed that it was feasible and popular to prepare FC with HIPEs completely replacing pork fat.

3.9. Schematic mechanism of fish cake with/without pork fat or HIPEs and correlation analysis

The schematic model is shown in supplementary Fig. 3. The charge, and particle size of casein was changed after pH-shifting. As a result, the SC-based HIPEs showed high storage module, thermal stability and oxidation stability. Water droplets in the FC without lipids were evenly distributed in the gel network. After the addition of pork fat or SC-HIPE, they were used as inactive fillers and filled in FC gel network. Liu et al. (2023) found that pork fat and HIPEs filled in gel network structure. Xu, Yu, Xue, and Xue (2023) also found that emulsion addition allowed small oil droplets in surimi gels to be uniformly distributed in a dense and ordered protein network. The pH-shifted SC-HIPE showed good thermal stability, so SC-HIPE droplets filled in in the fish cake. However, the SC-HIPE without pH treatment had poor thermal stability, so the SC-HIPE droplets and water separated after heating, and were distributed in the myofibrillar gel network of FC, showing the similar water composition and distribution.

Correlation coefficients between the measured parameters in pH-shifting treatment and control group (untreated) of SC-HIPE and its application in FC were calculated (supplementary Fig. 4). Results indicated that pH-shifting was positively correlated ($p < 0.01$) with oxidation stability of SC-HIPE, and significantly correlated ($p < 0.05$) with thermal stability of SC-HIPE, pH of FC, hardness of FC, adhesiveness of FC, and chewiness of FC. Notably, thermal stability of SC-HIPE was positively correlated ($p < 0.01$) with oxidation stability of SC-HIPE and adhesiveness of FC, and it was significantly correlated ($p < 0.05$) with hardness, cohesiveness, and chewiness of FC. However, the particle size of SC-HIPE was significant negative correlation ($p < 0.05$) with brightness in sensory evaluation.

4. Conclusion

In this study, the effects of SC-HIPE with different thermal stability on the mechanical characteristics and sensory evaluation of fish cake (FC) were investigated. Rice starch/casein complex (SC) was adjusted by pH-shifting (2.5, 6.5, 8.5, and 10.5) could improve the thermal stability of SC-HIPE, comparing with SC without pH-shifting. Additionally, pH-shifting could enhance not only thermal stability, but also viscoelasticity and oxidation stability. Furthermore, different thermal stable SC-HIPE was used to replace pork fat in preparing FC, and compared with FC without lipid. The tests included gel strength, TPA and water holding capacity showed that adding SC-HIPE to FC could make up for the problems of breaking distance, cohesiveness, adhesiveness, and chewiness caused by adding pork fat. However, the addition of heat-unstable HIPEs can lead to a reduction in breaking force and gel strength. Moreover, according to the preference evaluation and property evaluation, the addition of SC-HIPE in FC was more popular than the addition of pork fat in FC because of its brightness, flavor, elasticity and tenderness. Interestingly, as can be seen in the property evaluation, adding the heat-unstable SC-HIPE led to a decrease in the predicted hardness, which was not found in TPA testing. Therefore, after sensory personnel training, the result of sensory evaluation was more accurate than the instruments. Thus, the texture characterization of products with subsequent instruments needs to be more accurate. In generally, FC prepared with HIPEs with good heat stability showed good results in both instrumental measurement and sensory evaluation, suggesting that high heat stability of SC-HIPE can be used to completely substitute for pork fat in the preparation of FC. According to the texture characteristics and sensory evaluation, HIPEs prepared by SC after pH8.5-shifting with high thermal stability and oxidation stability is the most suitable for preparing fish cakes. The results provide a theoretical basis for the complete replacement of high saturated fatty acid products, and make the products meet the dietary nutritional requirements of fat (n6/n3 = 5:1).

CRediT authorship contribution statement

Yu Liu: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Zhifeng Tan:** Writing – original draft. **Yizhen Huang:** Writing – original draft. **Jiaqi Liu:** Writing – original draft. **Xianbing Xu:** Writing – original draft. **Beiwei Zhu:** Writing – review & editing, Project administration. **Xiuping Dong:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition.

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

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100694.

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