



Effects of daylily extract microcapsule on the quality and gel properties of steamed fish cake-a surimi-based product

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ARTICLE INFO

Handling Editor: Dr. Xing Chen

Keywords:

Daylily
Steamed fish cake
Food quality
Gel properties
Mechanisms

ABSTRACT

This study evaluated the effects of 0.95% daylily powder (m/m, 0.95%-DL), 0.5% daylily extract (m/m, 0.5%-DLE), and 0.5% daylily extract microcapsules (m/m, 0.5%-DLEMC) on the storage stability, gel properties, and microstructure of steamed fish cake. The results showed that 0.5%-DLEMC exhibited superior performance in maintaining product quality, significantly enhancing gel strength and reducing cooking loss. Detailed analysis revealed that both 0.5%-DLE and 0.5%-DLEMC promoted protein unfolding, leading to a structural transition from random coil to β -sheet, and increased the formation of disulfide and non-disulfide covalent bonds. These molecular interactions contributed to the formation of a more uniform and dense gel network, improving the overall gel properties. The overall findings suggest that the use of 0.5% DLEMC manifests potential in improving the quality of surimi-based products and would provide guidance for the implementation of DLEMC as an innovative additive in the food industry.

1. Introduction

Chinese fish cake, valued for its elasticity, tenderness, and lower calorie content, offers a refined flavor profile compared to fried or dried fish products (Park et al., 2014). The growing demand for ready-to-eat goods, such as fish cakes and other surimi-based products, is driven by their convenience in preparation and their rich content of healthy proteins, essential minerals, and vitamins (Sampels, 2015). However, products are inevitably subjected to chemical and structure changes caused by choppers and cooking machines, resulting in chemical and structural changes which including lipid oxidation and protein denaturation or aggregation during storage. These changes can negatively affect product characteristics such as color, gel strength, and pH, ultimately reducing the quality and shelf life of fish cakes and other surimi-based products (Khalid et al., 2023; Orlie & Bolumar, 2019). Therefore, efforts to improve the quality and gel properties of fish cakes are crucial and remain a focal point of ongoing research.

Prior studies have demonstrated that the application of antioxidants can effectively slow down the changes in protein properties (denaturation and aggregation) and lipid oxidation (Amaral et al., 2018;

Maqsood et al., 2014). Daylily, scientifically named *Hemerocallis citrina Baroni*, is a plant that has both medicinal and culinary benefits. Its medicinal value is derived from the presence of bioactive chemicals that possess antioxidant effects and several other features such as anti-tumor and anti-oxidation (Cichewicz et al., 2004; J. Wang et al., 2018). Other studies also found that the phenolic components in daylily could inhibit the growth of bacteria that cause food spoilage (Hao et al., 2022). Based on the reported antioxidative and antibacterial properties of daylily, it is postulated that the compounds present in daylily may enhance the quality of steamed food items, such as steamed fish cake. However, using daylily powder in fish cakes imparts a yellow hue, which is often considered less desirable compared to other food additives with lighter colors. Daylily extract (DLE), in contrast, offers a lighter color and superior antioxidant properties, potentially making it more acceptable to consumers as a fish cake additive. Moreover, microencapsulation of bioactive substances, using gelatin and carboxymethyl cellulose (CMC) as wall materials, can improve the stability of these compounds and control their release over time (Kuang et al., 2010). CMC has also been reported to positively influence the gel characteristics of fish cake, although the effects of daylily extract microcapsule (DLEC) prepared

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<https://doi.org/10.1016/j.crfs.2024.100837>

Received 21 June 2024; Received in revised form 3 August 2024; Accepted 4 September 2024

Available online 14 September 2024

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with CMC on quality and gel properties are yet to be fully explored (C. Zhang et al., 2023).

In our previous study, 0.95% daylily powder (m/m, 0.95%-DL) was the optimal amount during the process optimization of fish cake preparation. On the other hand, 0.95%-DL, 0.5%-DLE and 0.5%-DLEMC showed similar extract rates, while DLE and DLEC presented different antioxidant activity, with the ABTS* and DPPH* radical scavenging activity: DLEMC > DLE > DL powder (Fig. S1). Based on this background, propose to investigate the impact of three different types of daylily additions on the quality and gel properties of fish cake. This study will include a detailed analysis of protein structure, conformation, and the key chemical forces at play, aiming to develop a methodology and theoretical foundation for enhancing the quality and gel properties of steamed fish cake. Ultimately, this research seeks to facilitate the broader application of daylily in the production of surimi and surimi-based products.

2. Material and methods

2.1. Materials

The frozen surimi was acquired from HONGHU Xinhongye Food Co., Ltd. (Jinzhou, Hubei, China). The Daylily was acquired from HUBEI Jinxuan Food Co., Ltd. (Jinzhou, Hubei, China). Food-quality gelatin and carboxymethyl cellulose (CMC) were acquired from Henan Wanbang Industrial Co., Ltd. (Zhengzhou, Henan, China). The ingredients, including starch, pork, cooking wine, monosodium glutamate, and sugar, were purchased at a grocery (Wuhan, Hubei, China). All other compounds and solvents of analytical grade were employed in this investigation.

2.2. Daylily extract and microcapsule preparation

Daylily powder: the daylily was crushed using a multifunctional grinder (De'Longhi Appliances (Shanghai) Co., Ltd, China), manually sieved through a 200-mesh sieve, put the powder into a sealing bag and stored in a dryer for later use.

Daylily extract was obtained according to a previous report (Hao et al., 2022). Briefly, the daylily powder was manually sieved again through an 80-mesh sieve. Then, 100g of sieved powder was extracted at 25 ± 2 °C with 1L distilled water for 12 h, simultaneously stirred at 100 rpm. The chamomile extract was subsequently obtained by centrifuging at $2,4200 \times g$ for 20 min, and it was freeze-dried at 60 °C for 48 h to form the powder, which was daylily extract.

Daylily extract microcapsule was prepared according to a previous report (Duhoranimana et al., 2018). At first, Gelatin and CMC powders were dissolved in deionized water with a biopolymer content of 1% (w/w). The solution was agitated with a gentle motion at 60 °C for 2h. Subsequently, gelatin and CMC solutions were combined to create Gelatin/CMC mixes at a specified ratio of 1% (w/w). The obtained dispersions underwent centrifugation at 5000 rpm for 30 min at ambient temperature. This process was carried out to eliminate any insoluble particles or air bubbles, resulting in the formation of uniform solutions. Daylily extract was added to the homogeneous solutions and emulsified using a high-speed dispersing device. The emulsification mixture was maintained at a temperature of 45 °C with a stirring rate of 400 rpm for a duration of 30 min at the appropriate pH level. Acetic acid (10%, v/v) was applied dropwise during the manufacture of coacervated microcapsules to precisely tune the pH value to 4.5. Subsequently, an ice-water bath was employed to lower the temperature to a level under 15 °C for a duration of 30 min. After the chilling process, a 10% (v/v) solution of NaOH was applied to modify the pH value to 6.0. Subsequently, glutamine transferase was added at a ratio of 25 U/g (based on the weight of gelatin) and incubated at room temperature with a stirring rate of 400 rpm for 3h. The microcapsule suspension was stratified, and the supernatant was discarded. The liquid coacervates were obtained by

pumping and filtering the sediment, followed by freeze-drying to obtain solid microcapsules.

2.3. Steamed fish cake preparation

Fish cake pastes were created according to our process optimization (data was not shown). Cake past samples were created employing various daylily: no daylily (control), 0.95%-DL (m/m, %), 0.5%-DLE (m/m, %), and 0.5%-DLEC (m/m, %). The frozen surimi was thawed in a refrigerator at 4 °C for 10 h and then diced for a time of 5 min. Surimi was further processed by adding 1.0% salt and chopping it for an additional 5 min. Then 8.0% starch, 1.0% cooking wine, 0.6% monosodium glutamate, 0.5% TG enzyme, 3% SPI, 1% white sugar, an appropriate amount of egg white, onion, and ginger water as well as different daylily (include daylily powder, extract and extract microcapsule) were added, and chopped again for 15min. The surimi paste was kept at a temperature below 10 °C. The mixture was added to a plastic patty to make individual fish cakes, and the shaped fish cake was heated in a two-stage water bath; gel formation was conducted at 40 °C for 30min and steamed at boiling water condition for 30 min. The products were cooled in ice water (<10 °C) and stored at 4 °C for further analysis.

2.4. The quality of chilled fish cake

2.4.1. Whiteness

The fish cake color was quantified employing a colorimeter (CR-410, Japan) according to the technique outlined by Li et al., with some adjustments (Yi et al., 2020). Briefly, each sample was cut into 1cm × 1cm × 1 cm and tested five times. During each operation, The equipment was calibrated utilizing white and black reference standards. The whiteness and chromatism (ΔE) were ascertained employing the subsequent equation, respectively:

$$\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

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

where L^* , a^* , and b^* denote the color parameters lightness, redness/greenness, and yellowness/blueness, respectively.

2.4.2. pH

Three replicates of minced fish cake were produced for pH measurement. Each replicate consisted of mixing a 10 g specimen with 100 mL of distilled water. Subsequently, the mixture was homogenized by employing a stomacher at a speed of 8000 rpm for 2 min. After filtering, a digital pH meter (ST2100/E, USA) was subsequently employed to estimate the pH of the filtrate (Kang et al., 2021).

2.4.3. Total volatile base nitrogen (TVB-N) value

The TVB-N of the fish cake was evaluated with the methodology described by our previous report (Hou et al., 2021). The samples, weighing 10 g, were mixed thoroughly with 100 mL of sterile water. The resulting liquid was then used to measure the TVB-N content. Each sample was approximated using three replicates.

2.4.4. Bacterial enumeration

Typically, 25 g of diced specimens were diluted with 225 mL of sterile saline solution and subsequently homogenized using a stomacher. Incubation of the sample was carried out using a plate count agar (PCA) at 37 °C for 48 h. Each sample was approximated using three replicates.

2.5. Cooking loss

The cooking loss (CL) of fish cake gels was assessed employing the methodology developed by Zhang et al. (F. H. Zhang et al., 2013). The

fish cake pieces, each weighing about 5 g, were measured and assigned the label C_1 . The specimens were placed within a plastic centrifuge tube, which encompassed three layers of filter paper, and subjected to centrifugation for 20 min at a force of 3000 g utilizing a freezing centrifuge at a temperature of 4 °C. The mass of the specimens after centrifugation is referred to as C_2 . The CL was determined employing the subsequent equation:

$$CL (\%) = (C_1 - C_2) / C_1 \times 100$$

2.6. Microstructures

The microstructure change of fish cake gel specimens induced via the daylily treatment was analyzed via Scanning electron microscopy (SEM). Surface morphology was executed as presented in our prior investigation (Hou et al., 2023). In summary, the samples were sliced to a thickness of 1 cm and underwent fixation with a solution comprising 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h at ambient temperature. This was then followed by a further 12 h at a temperature of 4 °C. Subsequently, the specimens underwent a triple washing process using distilled water, followed by dehydration in ethanol. The ethanol concentrations used were progressively increased to 30%, 50%, and 70%, with each concentration being applied for a duration of 15 min. Ultimately, the specimens were submerged in iso-amyl acetate for 20 min. The specimens underwent freeze-drying and were then coated with gold employing a sputter-coating approach. The specimens underwent analysis employing the SEM (Nova Nano-SEM450, FEI, American). The analysis was conducted at an acceleration voltage of 20 KV.

2.7. Gel strength

Gel strength is ascertained by multiplying the force necessary to fracture the gel and the distance throughout which the gel fractures, and it is estimated employing the technique developed by Pi et al. with the use of a texture analyzer that is fitted with a trigger-type spherical probe P/0.25S (Pi et al., 2022). The fish cake specimens were sliced into cubes of 2 cm × 2 cm × 2 cm, and the experimental parameters were as follows: The starting velocity before the experiment was 5 mm/s. During the experiment, it decreased to 1 mm per s and then reverted back to 5 mm/s during the post-test. The compression distance measured 15 mm. The trigger type was set to automatic, with a trigger force of 5.0 g. All specimens are estimated in a minimum of 6 parallel measurements.

2.8. Dynamic rheological determination

The rheological characteristics of fish cake gel, with varying amounts of day lily, were measured using a Discovery TA DHR2 rheometer (Xu et al., 2021). The paste samples were placed onto parallel plates that had a diameter of 40 mm and a spacing of 1 mm. Before conducting the research, the samples were tightly sealed using liquid paraffin to prevent them from drying out. Configuration parameters: The temperature will increase from 20 to 75 °C at a rate of 2 °C/min. The scanning frequency will be fixed at 1 Hz, and the strain will be set at 1%. The storage modulus (G') and loss modulus (G'') were ascertained while conducting a temperature scan.

2.9. Chemical interaction

The chemical interaction among protein molecules in fish cake gels was examined using the approach previously presented (Zhang et al., 2013). Typically, 2 g of a complex consisting of surimi and daylily were added to solutions containing 10 mm of NaCl with concentrations of 0.05 mol/L (SA), 0.6 mol/L (SB), 0.6 mol/L with the addition of 1.5 mol/L urea (SC), 0.6 mol/L with the addition of 8 mol/L urea (SD), and 0.6 mol/L with the addition of 8 mol/L urea and 0.5 mol/L

β -mercaptoethanol (SE). The heterogeneous solutions were thoroughly mixed at a speed of 4000 rpm for 5 min. They were then subjected to a reaction at 4 for a period of 1 h. Subsequently, the mixture underwent centrifugation at a speed of 8000 rpm for 10 min to achieve separation. The protein content in the supernatant was ascertained by employing the biuret technique. An estimation was made of the presence of ionic bonds, hydrogen bonds, hydrophobic interaction forces, and disulfide bonds in the specimens. Each sample underwent three iterations of computations.

2.10. Analysis of myofibrillar protein from fish cake samples

2.10.1. Extraction of myofibrillar protein

Briefly, fish cake samples (100 g) were homogenized in 0.1 M phosphate buffer in a solid-liquid ratio of 1:8 for 5 min. Subsequently, the homogenate underwent centrifugation at a velocity of 9000 rpm for 10 min at 4 °C, and the supernatant was eliminated. The precipitate was washed and resuspended with PBS buffer (0.1M, pH = 7.4) and incubated at 4 °C for 1h to fully dissolve the salt-soluble protein. Subsequently, the solution was centrifuged at 9000 r/min for 20 min at 4 °C and repeated the process. The final sediment was dispersed in the previously described solution, referred to as myofibrillar protein. The protein concentration was measured using the Buggy et al. technique (Buggy et al., 2018), and the myofibrillar protein concentration was set at 1.0–1.2 mg/mL for the assessment of other indicators.

2.10.2. In vitro pepsin digestibility

The technique of in vitro pepsin digestion was mentioned in the research conducted by Minekus et al. (Minekus et al., 2014) with slight modification. The concentration of myofibrillar protein was mitigated to 2.5 mg/mL by diluting it with a phosphate buffer solution containing 10 mmol/L of phosphate ions at a pH of 7.0. A total of 5 mL diluted sample was transferred into a plastic centrifuge tube, shaking continuously until it dissolved completely. Placing it in a refrigerator at 4 °C for 1 h and centrifuging at 4 °C for 20 min (4500 r/min) to determine the protein content in the supernatant before and after centrifugation, in vitro protein digestibility was calculated as follows:

$$\text{Digestibility (\%)} = \frac{M1}{M2} \times 100\%$$

where M1 is the concentration of protein before centrifugation and M2 is the concentration of protein after centrifugation.

2.10.3. Protein secondary and tertiary structures

The obtained myofibrillar proteins were freeze-fried to powder, and NEXUS-670 Fourier transform infrared spectrometer was used to collect FTIR spectra of freeze-dried materials at room temperature. The freeze-dried samples, weighing 1.0 mg, were finely pulverized with KBr, weighing 100 mg, and thereafter formed into pellets. The FTIR spectra were collected within the wavenumber range of 4000 to 4000 cm^{-1} , with a data collection rate of 4 cm^{-1} per point and a total of 32 scans.

Tertiary structure of diluted myofibrillar proteins (0.1 mg/mL) were determined using an NICOLET 6700 spectrofluorometer (Thermo, USA). The excitation wavelength was set at 280 nm, with emission spectra recorded from 300 to 450 nm at 1 nm/s. Both sample and background data were collected under identical conditions.

2.10.4. SDS-PAGE

The protein profiles of the fish cake specimens were analyzed using SDS-PAGE, using a previously established procedure (Hou et al., 2023). The myofibrillar protein suspension, generated in section 2.9.1, was combined with protein loading buffer in a 4:1 ratio by vigorously mixing for 1 min using a vortex. The proteins were denatured by heating a metal bath for 5 min 10 μL of the finished mixture was applied to the polyacrylamide gels, and the electrophoresis was conducted using a

continuous current of 15 mA for about 1.5–2.0 h. The gels were dyed and captured employing a Universal Hood II gel imager.

2.11. Sensory evaluation

The sensory examination of fish cake samples was conducted using a previously published method with minor adjustments. A group of eight expert evaluators and physically fit persons between the ages of 18 and 40 assessed the color, aroma, flavor, consistency, and overall preference of the specimens employing a standardized 100-point scale.

2.12. Statistical analysis

SPSS software (version 26.0, Chicago, USA) was employed to analyze the data that was obtained. A significant variation among specimens was denoted by $P < 0.05$, and statistical variations were ascertained employing Duncan's multiple-range test with a one-way ANOVA.

3. Results and discussion

3.1. Implications of quality

The whiteness of fish cake specimens is a crucial criterion for indicating food quality and customer preference, and the effect of daylily addition on the color of fish cake samples is shown in Fig. 1a (Zhang et al., 2015). There was no significant implication on the whiteness value of 0.5%-DLE and 0.5%-DLEMC group, indicating that the aforementioned addition method would not influence customer acceptance. However, the value of the 0.95%-DL group significantly mitigated ($P < 0.05$). This was likely associated with the fact that the predominant hue

of daylily pollen is mostly yellow. Compared with 0.95%-DL group, the total color difference (ΔE) value of 0.5%-DLE and 0.5%-DLEMC group decreased significantly ($P < 0.05$), manifested that the daylily introduction forms would induce the change of ΔE value. Furthermore, the total color difference of the 0.5%-DLEMC group couldn't be recognized by the naked eye as $\Delta E < 2$ ($\Delta E = 1.71$), manifested that the implication of 0.5%-DLEMC introduction on fish cake color was ignorable and would be more easily accepted by consumers.

The pH, a reliable parameter for assessing food stability, is closely associated with microbial and chemical reactions that contribute to the deterioration of food quality. As shown in Fig. 1b, the pH values of all samples initially exhibited an increasing tendency, followed by a subsequent decrease, ultimately indicating a state of mild acidity. The pH of the four groups approached neutrality, with the exception of 0.5%-DLEMC, which exhibited a higher pH than the other three groups on the third day of storage. This disparity in pH levels during the initial three-day period can be attributed to the utilization of protein by microorganisms during their growth, resulting in the production of alkaline nitrogenous compounds such as amines, trimethylamines, and other spoiled products. In the later stages of storage, the pH begins to decline due to the metabolic activity of microorganisms that break down the fish cake, resulting in the production of byproducts such as CO_2 and water. These byproducts contribute to the formation of acidic compounds, which lower the pH and lead to a deterioration in the gel texture of the fish cake. On the 12th day, the pH value of the 0.5%-DLEMC group was significantly higher than that of the other experimental groups, indicating that this microencapsulation form can prolong the release of bioactive substances in the extract and inhibit microbial metabolism.

TVB-N is one of the commonly used markers for ascertaining the freshness of meat products. Protein in fish cake is decomposed by

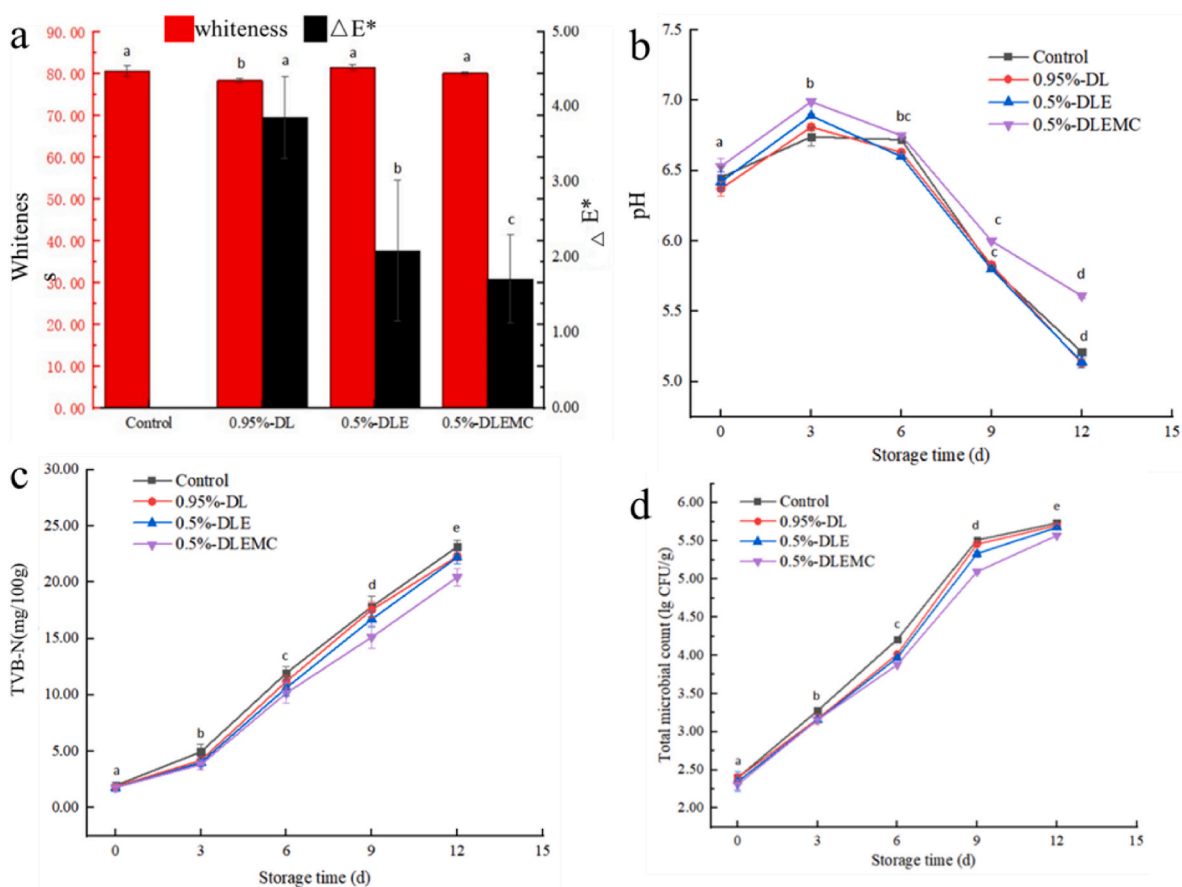


Fig. 1. Effect of different daylily addition on the quality of steamed fish cake samples, including a (whiteness, total color difference, ΔE), b (pH), c (TVB-N) and d (Total microbial count). In Fig. 1a, different letters indicate significant differences ($P < 0.05$).

microorganisms and enzymes during storage, producing alkaline nitrogen-containing substances such as ammonia, amines, aldehydes, ketones, etc. More of these compounds and a higher TVB-N value indicated more amino acid decomposition and more severe spoilage of fish cake. The TVB-N levels of all specimens were constant at 3.8–5 mg/100 g throughout the first three days, and a dramatic increasing trend was observed in the subsequent period (Fig. 1c). At day 12, all groups, except for 0.5%-DLEMC, were recorded the upper limit of fresh surimi (20 mg/100 g), indicating that the 0.5%-DLEMC solution demonstrated superior effectiveness in preserving the freshness of the fish cake.

Initial total bacterial counts of daylily fish cake and control group (CG) all presented relatively lower levels; an overall significant elevation was seen in all groups over the whole storage period (Fig. 1d). However, the increasing rates of daylily fish cake samples were mitigated contrasted with CG, indicated the bacteria growth was suppressed. According to the guidelines set by the National Food Safety Standard for animal-derived aquatic goods (GB 1013–2015), the maximum allowable bacterial count for cooked animal-derived aquatic products is 5.0 lg cfu/g. The negative control, 0.95%-DL, and 0.5%-DLE all attained a level of 5.0 lg cfu/g by Day 9. However, the 0.5%-DLEMC reached its maximum by Day 12. The antibacterial activity of 0.5%-DLEMC seemed to be the most effective, resulting in a delay of deterioration by about 3 days. Based on the above chemical and biological analysis, the shelf-life of steamed fish cake under 0.5%-DLEMC addition was prolonged for 3 days, and it is recommended 0.5%-DLEMC as a preservative on steamed fish cake in this study.

3.2. Gel strength

Gel strength is a critical indicator of surimi quality, with higher values reflecting superior product characteristics (Cao et al., 2018). The impact of daylily incorporation on the gel strength of fish cake samples was assessed on days 0 and 6, as shown in Fig. 2a. At day 0, the gel strength of 0.5%-DLE and 0.5%-DLEMC samples increased significantly by 31.4% and 34.26% ($P < 0.05$), while the samples of the 0.95%-DL group increased by 13.2% (from 350.01 g*cm to 396.34 g*cm). At 6th-day, the gel strength of 0.95%-DL, 0.5%-DLE and 0.5%-DLEMC samples were found to be 367.25 g*cm, 371.42 g*cm and 391.39 g*cm, which were significantly elevated contrasted with CG (300 g*cm, $P < 0.05$). Previous studies have demonstrated that polysaccharides enhance the hardness and gel strength of meat products by promoting protein unfolding and myosin molecules interactions (He et al., 2023). Furthermore, the gel strength reached a maximum in the addition of 0.5%-DLEMC since the daylily extract microcapsule was prepared with sodium carboxymethyl cellulose (CMC) and gelatin, which further contributed to the observed increase in gel strength (C. Zhang et al.,

2023).

3.3. Cooking loss

Cooking loss refers to the hindrance in water content in surimi gels that occurs during the cooking process, and the value of different fish cake samples are depicted in Fig. 2b. Without daylily addition, cooking loss was recorded to 5.04% and 4.68% at day 0 and 6, respectively. However, with the addition of daylily, cooking loss decreased significantly ($P < 0.05$) and was found to be 4.31%, 3.71%, and 3.26% with respect to 0.95%-DL, 0.5%-DLE and 0.5%-DLEMC group at day 0. On the 6th day, the cooking loss in the 0.5%-DLEMC group was reduced by 32.5% compared to the control group (CG), making it the lowest among all groups. This reduction suggests that daylily enhances the water retention capacity of surimi gels. Typically, a decrease in cooking loss corresponds to a denser and more organized gel structure, leading to increased gel strength (Mi et al., 2021). The impact of daylily addition showed a consistent effect in reducing cooking loss and enhancing gel strength. It is hypothesized that the polysaccharides in daylily exert a protective effect against muscle tissue dehydration by forming hydrogen bonds with water molecules, thus minimizing water loss during cooking (Fan et al., 2017). Moreover, the formation of a more organized and condensed gel network allows for the retention of more water molecules, ultimately improving the gel's water-holding capacity (Chen et al., 2020). Additionally, other research has shown that the reduction in cooking loss of surimi gels can be attributed to the presence of oxidized cellulose nanofibrils, which create smaller pores and increase electrostatic repulsion via the -COO- groups, while also exposing more hydrophilic groups, further supporting the finding that 0.5%-DLEMC presented the smallest cooking loss (Piao et al., 2022).

3.4. Rheological properties

The dynamic rheology curves of the daylily-fish cake gel during heating were analyzed to assess its viscoelastic properties, specifically the storage modulus (G') and loss modulus (G''). All samples followed a similar pattern, with two distinct stages observed: both G' and G'' gradually decreased between 25 °C and 50 °C, reaching their minimum values around 50 °C. This trend is likely due to the disruption of hydrogen bonds and the aggregation of myofibrillar proteins (Fig. 3) (X. Wang et al., 2016). In fish cake specimens, the gel network structure was disturbed, and the value of G' declined due to protein degradation caused by endogenous proteolytic enzymes (Gani and Benjakul, 2018). As the temperature rose, G' also increased and eventually leveled out at 75 °C. This indicates that the protein established a stable gel structure that cannot be reversed after undergoing aggregation and crosslinking

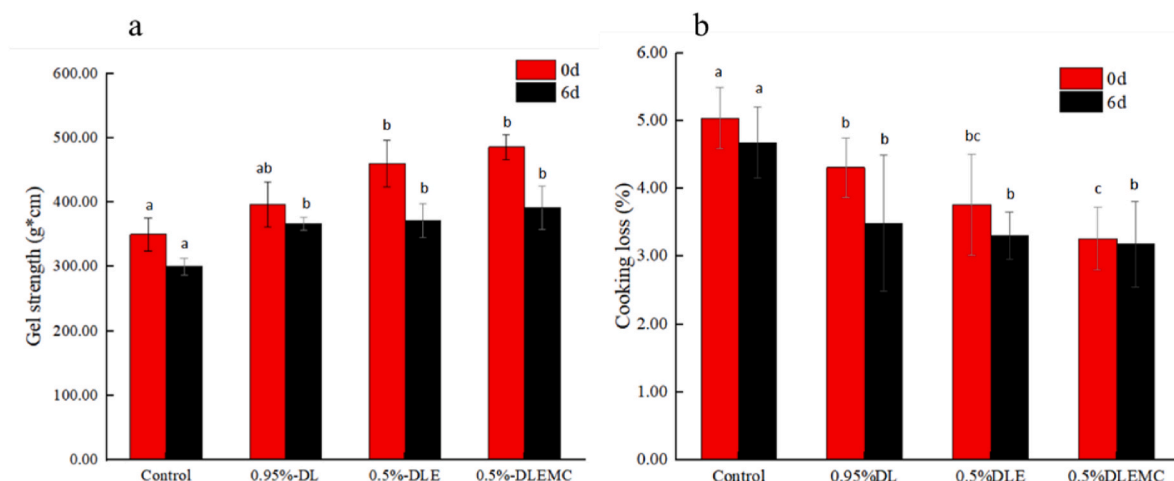


Fig. 2. Effect of different daylily addition on the gel strength (a) and cooking loss (b) of fish cake samples at day 0 and 6, respectively.

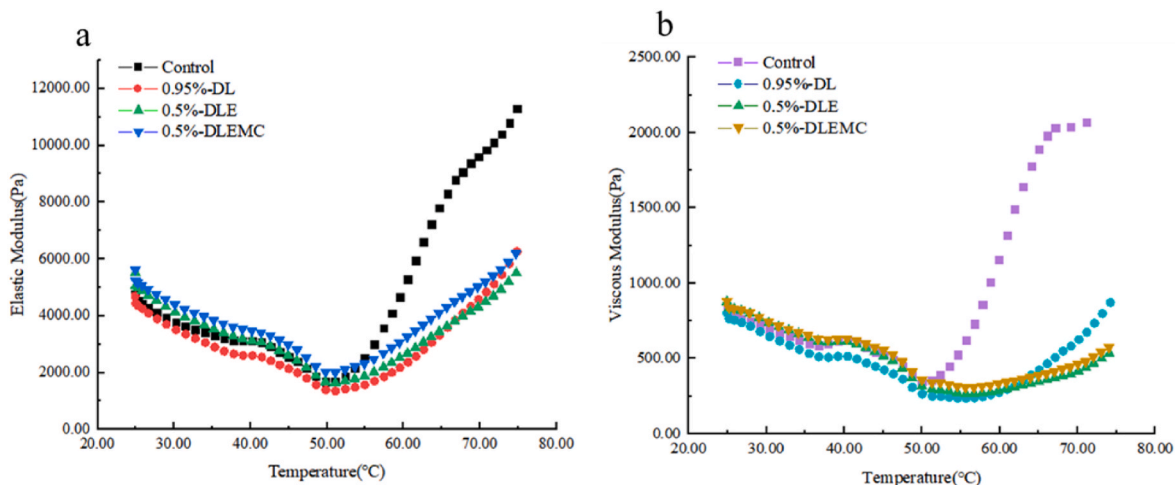


Fig. 3. Changes in storage modulus (G' , a) and loss modulus (G'' , b) of fish cake with or without different daylily addition during the heating.

due to additional heating (Fig. 3a). The aggregation was caused by conformational changes from hydrophobic interactions and disulfide bonds, which create cross-links for elasticity in the gel structure. Moreover, the holistic curve G' was much lower than that of G' suggesting the superior elasticity of fish cake gels (Fig. 3b).

On the other hand, G' values of 0.5%-DLE and 0.5%-DLEMC were slightly greater than that of CGs during the initial heating phase (25 °C–50 °C), which can probably be ascribed to the proteins in 0.5%-DLE and 0.5%-DLEMC are more likely to interact with the proteins in fish cake gels and the early gel network is formed by the weak bonding

between protein molecules. Afterward, G' values of the daylily addition group were lower than those of the CG and were found to be 11300 Pa, 6260 Pa, 5510 Pa, 6250 Pa of CG, 0.95%-DL, 0.5%-DLE and 0.5%-DLEMC group when the temperature reached 75 °C, respectively. Additional research has shown a strong correlation between the G' of surimi gel and its gel strength (C. Zhang et al., 2023; F. H. Zhang et al., 2013). However, there was a variance between the final value G' of fish cake gels and the gel strength. Specifically, the G' value in the CG was higher than that in other groups. Higher than in the other groups. The reduction in protein concentration in surimi gels should be considered

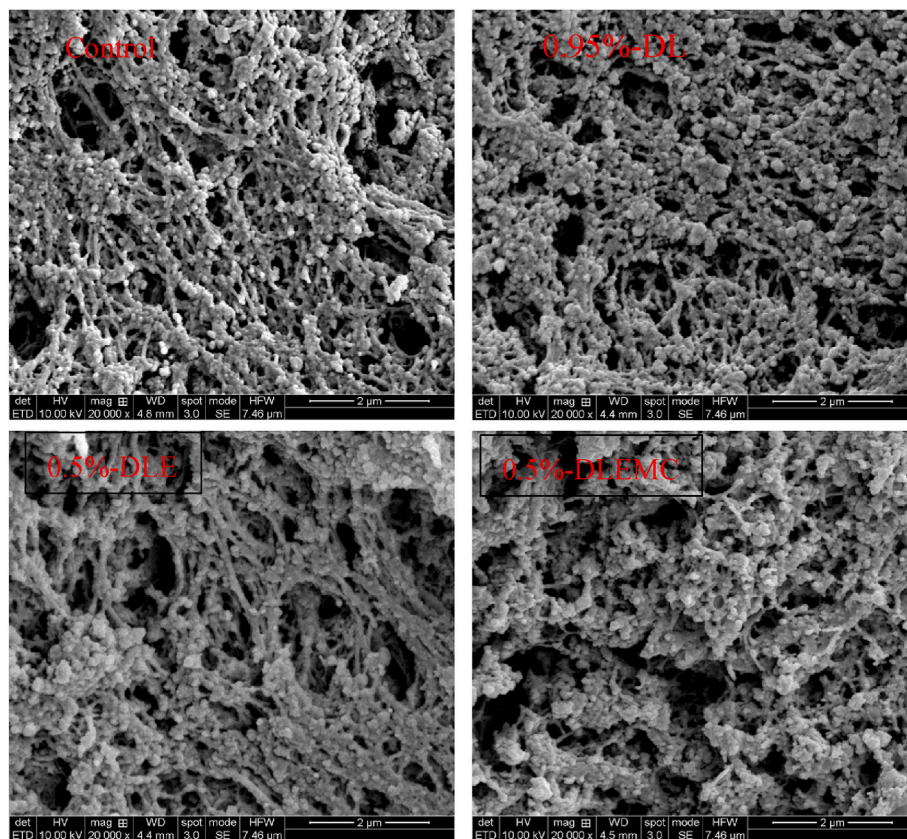


Fig. 4. Scanning electron microscopy (SEM) photographs ($\times 200$) of different fish cake/daylily composite gels. Control, fish cake without daylily addition; 0.95%-DL: fish cake with 0.95% (m/m) daylily powder addition; 0.5%-DLE, fish cake with 0.5% (m/m) daylily extract addition; 0.5%-DLEMC, fish cake with 0.5% (m/m) daylily extract microcapsule.

when adding 0.95%-DL, 0.5%-DLE, and 0.5%-DLEMC, in order to maintain consistent moisture levels. Additionally, gel properties were used to assess the static behavior of the surimi gel, while rheological properties were used to evaluate its dynamic behavior (Yang et al., 2020).

3.5. Effects of daylily addition on microstructure

Fig. 4 displays the microstructures of fish cake samples, both with and without the inclusion of daylily. The results indicated that daylily was sporadically present throughout the gel structure, acting as a physical filler. Furthermore, the inclusion of daylily facilitated the creation of a much smoother gel with more consistent and smaller holes. In contrast, the CG manifested a disorganized gel network with a high level of porosity. As the pore size of surimi gel decreased, its capacity for water retention enhanced, concurrently demonstrating an augmentation in elasticity (Y. Wang et al., 2023). In addition, the samples containing 0.5%-DLEMC exhibited a network topology that was much more intricate and compact, resulting in a decrease in the quantity and size of protein aggregates with respect to the other three groups, making it possess higher gel strength. It was assumed that daylily and sodium carboxymethyl cellulose-induced protein cross-linking improved the irregular aggregation to participate in gel formation, therefore improving its network structure.

3.6. In vitro pepsin digestibility

The pepsin digestibility rate reflects the quantity of ϵ -(γ -Glu)-Lys non-disulfide covalent bonds, which are formed by endogenous TGase and are key chemical linkages in the development of surimi gels (Benjakul et al., 2001). As solubility declines, there is an elevation in the amount of non-disulfide covalent bonds, leading to a rise in surimi gel strength. Prior research has shown that phenolic compounds can stimulate the formation of covalent bonds between protein molecules, thereby reducing the solubility of myofibrillar proteins (Zhou et al., 2019). In comparison to the CG, the daylily addition group exhibited lower pepsin digestibility rates (Fig. 5a). Moreover, the addition of 0.5%-DLEMC significantly reduced the solubility of surimi protein gel ($P < 0.05$). This difference may result from the synergistic effect of CMC (microencapsulated wall material) and daylily extract, which promotes protein-protein interactions, including the formation of non-disulfide covalent bonds, thereby enhancing surimi gel properties.

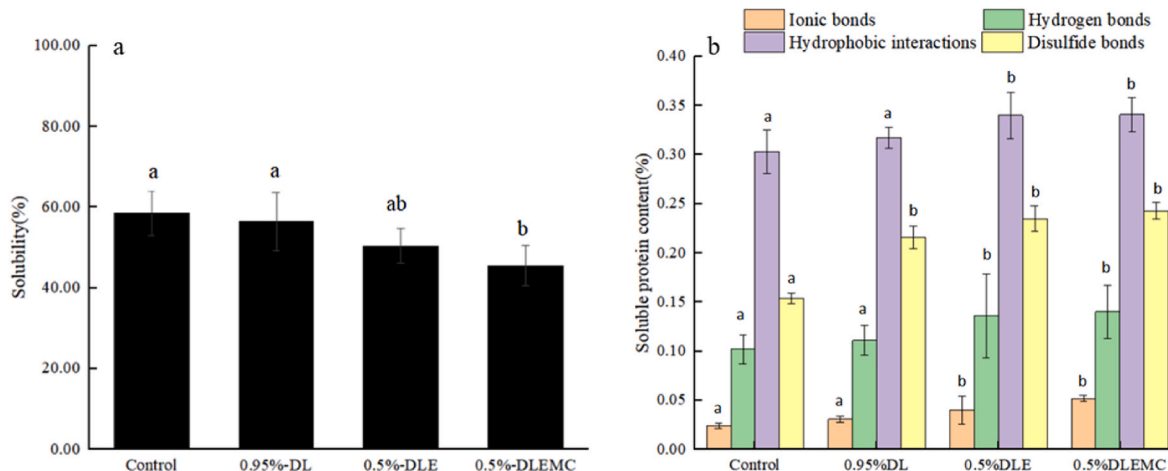


Fig. 5. In vitro pepsin digestibility (5a) and chemical forces (5b) of fish cake samples prepared with daylily; different letters indicate significant differences ($P < 0.05$).

3.7. Chemical interaction

The structural framework of the surimi gel system is primarily preserved by chemical interactions among proteins, both within and between molecules, including hydrogen bonds, ionic bonds, hydrophobic interaction, and disulfide bonds. The chemical interaction between the fish cake gel and daylily is illustrated in Fig. 5b. The gel network structure of fish cake gels is primarily maintained by hydrophobic bond and disulfide bonds, which play a crucial role in the chemical forces involved (X. Wang et al., 2020). The inclusion of daylily resulted in a significant increase in both ionic bonds and disulfide bonds ($P < 0.05$). The addition of daylily to the fish cake may enhance the creation of ionic bonds in the surimi protein, resulting in a modest increase in gelation.

Conversely, disulfide bonds possess higher bond energy compared to non-covalent bonds and are essential for forming a stable gel network. Therefore, it is suggested that the daylily promotes the formation of disulfide linkages among protein molecules, leading to a significant enhancement in the gel properties of the fish cake. The addition of daylily may alter the composition of surimi protein, increasing the presence of sulfhydryl groups and converting them into disulfide bonds. This process enhances the chemical cross-linking of surimi protein, resulting in the formation of a dense gel network structure (Buamard et al., 2017). In addition, the concentration of disulfide bonds in the 0.95%-DL, 0.5%-DLE, and 0.5%-DLEMC groups rose by 39.9%, 52.3%, and 57.3%, respectively, compared to the CG. The significant elevation in disulfide bonds in the daylily group was comparable to the changes seen in cooking loss and gel strength.

3.8. Alterations in the secondary structures of myofibrillar proteins

As shown in Fig. 6a, all fish cake gels, whether they included daylily or not, exhibited comparable characteristic peaks without any additional peaks, suggesting the absence of chemical interactions between surimi and daylily. The Amide I band, which occurs at a wavelength range of 1600–1700 cm^{-1} , is strongly associated with different protein secondary structures. Specifically, the α -helix structure is centered around 1650–1658 cm^{-1} , the β -sheet structure is centered around 1665–1680 cm^{-1} , the β -turn structure is located around 1680 cm^{-1} , and the random coil structure is centered around 1660–1665 cm^{-1} (Zhuang et al., 2018). To further substantiate the effects of adding daylily on the oxidative secondary structure of a myofibrillar protein, we utilized the second derivative of the amide I band. This allowed us to differentiate the overlapping peaks in this area and enhance the accuracy of the spectral analysis.

The four indicated contents of 0.95%-DL did not manifest

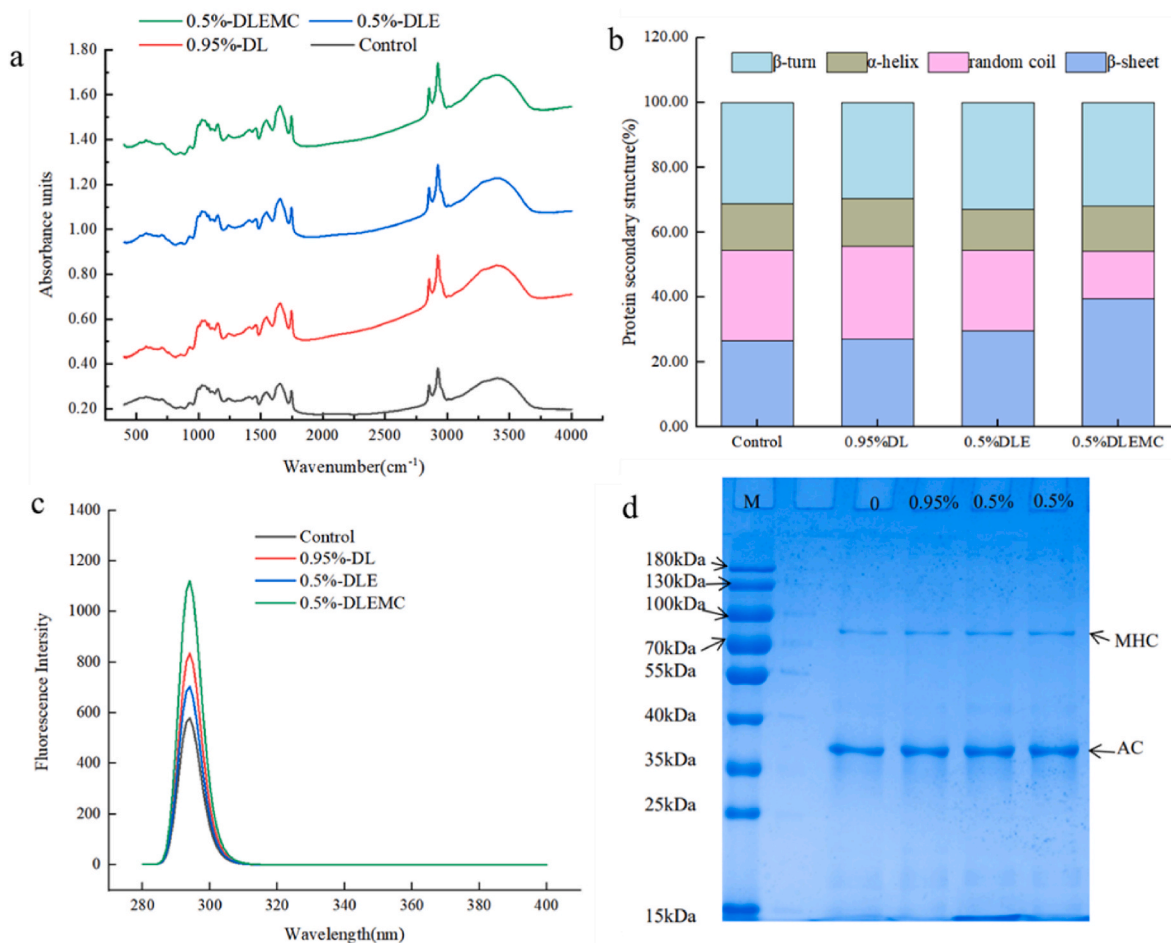


Fig. 6. Flourier transform infrared spectroscopy(a), relative content (%) of protein secondary structures(b), fluorescence spectrum(c) and SDS-PAGE pattern of different fish cake/daylily composite gels.

statistically significant alterations as compared to the CG ($P > 0.05$). The percentage of disordered secondary structure (random coil) dropped significantly in both the 0.5%-DLE and 0.5%-DLEMC groups, from 27.88% to 24.94% and 14.55% respectively ($P < 0.01$). Conversely, the proportion of ordered secondary structure (β -sheet) rose significantly, from 26.57% to 29.48% and 39.52% in the two groups, respectively ($P < 0.01$) (Fig. 6b). The findings demonstrated that the addition of DLE and DLEC facilitated the conversion of random coil structure to β -sheet structure in fish cake gels. Prior research has shown that the disordered structure of random coli has a detrimental implication on the creation of a cohesive gel network structure, mostly because of its irregular shape. Conversely, the gelation capacity is favorably associated with the β -sheet concentration (Mi et al., 2021), confirming that in the current investigation, the quality of fish cake gels was significantly enhanced in the 0.5%-DLE and 0.5%-DLEMC groups. Consequently, the higher β -sheet richness and lower random coil content in DLE/DLEMC-enhanced fish cake samples resulted in a denser gel network structure.

3.9. Intrinsic fluorescence intensity

Endogenous amino acids, such as tryptophan, tyrosine and phenylalanine, are sensitive to the polarity of their surroundings, leading to changes in intrinsic fluorescence intensity when exposed on the surface (Poowakanjana et al., 2012). The peak emission wavelength of myofibrillar protein is 290 nm (Fig. 6c). The addition of different forms of daylily did not cause a significant shift in the maximum emission wavelength; however, the fluorescence intensity increased compared to

the control group (CG). This suggests that the introduction of daylily induced myofibrillar protein denaturation and unfolding, promoting the exposure of endogenous amino acids to a polar environment. The 0.5%-DLEMC group exhibited the strongest fluorescence intensity, indicating that the microencapsulated daylily extract is more likely to interact with the protein, providing additional binding sites.

3.10. SDS-PAGE

SDS-PAGE was applied to characterize the implication of daylily addition on the myofibrillar protein in fish cake gel. The MHC bands of myofibrillar proteins in each group were thinner and shallower (Fig. 6d). It was assumed that MHC was cross-linked through intermolecular covalent bonds during high-temperature gelation of fish cake, and the aggregate could not enter the separation gel due to its excessive molecular weight (Xia et al., 2011). On the other hand, compared with CG, MHC, and actin bands were deeper and thicker, indicating that daylily components promoted the cross-link between MHC and AC and formed a stable three-dimensional network structure.

3.11. Sensory evaluation

Table 1 presents the sensory assessment of fish cake samples with and without the inclusion of daylily. The findings revealed that the inclusion of daylily had little impact on color but had a significant influence on the aroma, flavor, texture, and overall desirability ($P < 0.05$). The inclusion of 0.95%-DL and 0.5%-DEL resulted in a drop in the color score of the fish cake sample. This decrease may be attributed to the

Table 1
Sensory analysis of fish cake gel with different daylily addition. Different letters (a-c) indicated significant difference.

	odor	color	texture	smell	taste	overall acceptability
Control	15 ± 0.57 ^a	17 ± 0.82 ^b	15 ± 0.58 ^a	15 ± 0.58 ^a	15 ± 0.58 ^a	15 ± 0.69 ^a
0.95%-DL	18 ± 0.58 ^a	15 ± 0.63 ^b	17 ± 0.82 ^b	18 ± 0.58 ^a	17.17 ± 0.90 ^b	16 ± 0.58 ^a
0.5%-DLE	18 ± 0.58 ^a	16 ± 0.58 ^b	17 ± 1.0 ^c	17 ± 0.58 ^a	18 ± 0.58 ^a	17 ± 0.82 ^b
0.5%-DLEMC	17 ± 0.58 ^a	17 ± 0.63 ^b	17 ± 0.82 ^b	17 ± 0.82 ^b	18 ± 0.58 ^a	17 ± 0.58 ^a

prominent yellow hue of daylily powder. However, the color score of the sample with 0.5%-DLEMC remained unchanged, which aligns with the pattern shown in Fig. 1a. Daylily significantly improved the smell, taste, texture, and overall acceptability scores of the fish cake ($P < 0.05$); 0.5%-DLE and 0.5%-DLEMC fish cake samples presented the best taste and overall acceptability. Hence, daylily extract has promising potential to enhance the texture and taste of surimi or surimi-based goods.

3.12. Mechanism analysis of daylily to improve the gel quality of fish cake gel

The gel properties of fish cake were notably improved by the addition of daylily extract, especially daylily extract microcapsule. The proposed mechanism illustrating the enhancement of fish cake gel quality through the addition of daylily extract is depicted in Fig. 7. First, daylily extract and daylily extract microcapsules with high crystallinity uniform size could act as reinforcing fillers in the fish cake. This leads to the creation of a dense and sturdy gel network, resulting in an improvement in the gel strength of the fish cake. This enhancement is supported by the observed increase in gel strength and the SEM outcomes. Furthermore, the FTIR study revealed that both daylily extract and daylily extract microcapsule facilitated the transformation of protein structure from random coil to β -sheet when subjected to heat. The primary driving force behind this process, as shown by chemical force analysis, was the disulfide bond. The enhancement of β -sheet formation and disulfide bond formation promotes the aggregation of unfolded protein molecules, increasing the compactness of the gel network and

improving gel quality. Furthermore, daylily enhances the effectiveness of gel formation in surimi by influencing the activity of naturally occurring enzymes. In this study, the inclusion of 0.5%-DLE and 0.5%-DLEMC led to an increase in TGase activity and promoted the exposure of additional TGase binding sites on the protein, facilitating the formation of more non-disulfide covalent connections. The presence of polysaccharides in daylily also aids in protein unfolding and enhances the interaction between myosin molecules, as evidenced by the increase in intrinsic fluorescence intensity and chemical interaction analysis. These combined effects synergistically contribute to enhancing the gel properties of the fish cake samples.

4. Conclusion

This study demonstrates that daylily extract microcapsules (DLEMC), as a novel additive, significantly outperform both daylily extract (DLE) and daylily powder (DL) in maintaining the quality and enhancing the gel properties of steamed fish cake without altering sensory attributes. The incorporation of 0.5%-DLEMC not only improved gel strength and water-holding capacity but also promoted the formation of non-disulfide and predominant disulfide bonds within the fish cake gel, leading to a more homogeneous and compact gel network structure. Additionally, DLEMC showed the potential to significantly increase the β -sheet content. These molecular interactions and structural modifications contributed to a more stable and resilient gel network, highlighting the role of DLEMC in enhancing the secondary and tertiary structures of proteins. The observed changes suggest that DLEMC facilitates protein

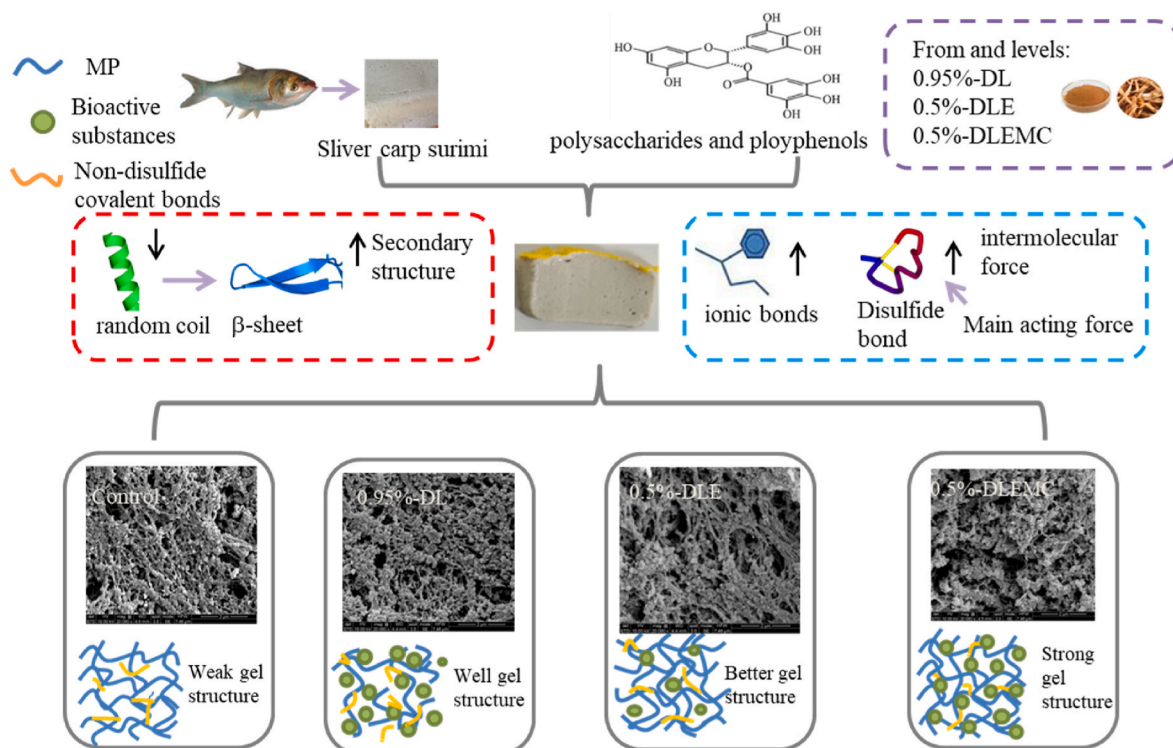


Fig. 7. Schematic mechanism for the interaction between DL, DLE and DLEMC and proteins in fish cake gels.

unfolding and subsequent reorganization into more stable configurations, which are crucial for improving the overall gel characteristics, and the findings suggest that DLEMC has significant potential to enhance the quality and structural integrity of surimi products, making it a promising candidate for future innovations in the food industry.

CRedit authorship contribution statement

Hongxun Wang: Conceptualization, Project administration, Writing – original draft. **Jie Li:** Methodology, Writing – original draft. **Tingting Liu:** Methodology, Data curation, Formal analysis. **Yahong Han:** Resources, Supervision, Project administration. **Wenfu Hou:** Resources, Supervision, Project administration, Writing – review & editing. **Yang Yi:** Validation, Visualization.

Declaration of competing interest

The authors declare that they have no known competing interests in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by The Key R&D Program of Hubei (2023BBB143) and the Key project of the science and technology plan of Hubei Provincial Department of Education (D20221613).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crfs.2024.100837>.

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