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Effects of acid and alkaline treatments on physicochemical and rheological properties of tilapia surimi prepared by pH shift method during cold storage

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ABSTRACT

Microbial, physicochemical, rheological, and microstructural changes of surimi prepared by pH shift methods and the traditional water-washing method during cold storage were investigated. The starting aerobic mesophilic count (AMC) of pH shift surimi was around 1 log CFU/g lower than water-washed surimi, suggesting antimicrobial effects of the pH shift. All samples could be stored for 5 to 6 days based on the AMC results. Throughout the storage, the gel strength of alkaline-treated surimi increased from 204.2 to 491.9 g, while water-washed surimi decreased from 462.1 to 172.9 g. After the storage, alkaline-treated surimi showed lower total volatile basic nitrogen (TVB-N) value and smaller network hole size that was suitable for incorporation of moisture and starch. It also remained its rheological properties comparing with acid-treated surimi, with better odour properties, less protein degradation, and better network formation. The results indicate that alkaline-treated surimi is more suitable for cold storage.

1. Introduction

Surimi, an intermediate food ingredient for the production of fish balls or fish cakes, is usually made from repeatedly washed fish meat mince (Yoshie-Stark, Tsukamoto, Futagawa, Kubota, & Ogushi, 2009). It has a high content of myofibril proteins and low content of lipids; thus besides unique texture and savoury tastes, surimi-based food is also good for health (Liu, Zhang, Xue, & Xue, 2019). Before cooking, surimi pastes are usually blended with seasonings like sugar and salts, and other ingredients like flour or starch. There are numerous methods to optimise the texture quality of surimi gel or modify the myofibril proteins, such as adding polyphenols (Sun, Sun, Thavaraj, Yang, & Guo, 2017) or polysaccharides (Jeyakumari et al., 2016), pre-treating the proteins using high-intensity ultrasound (Li et al., 2020) or high pressure (Zhang, Yang, Zhou, Zhang, & Wang, 2017), and one innovative method called pH shift method that was found to be extremely effective in enhancing the gel quality (Panpipat & Chaijan, 2017; Zhou & Yang, 2019).

Concentrating fish proteins by washing requires a large amount of water, and the residual of sarcoplasmic proteins or other unwanted substances such as fish skins or fish bones remained in surimi all have detrimental effects on the gel quality. Besides, with more washing cycles, the yield of myofibril proteins was significantly lowered (Moosavi-Nasab, Alli, Ismail, & Ngadi, 2005). To address such issues, Herbert (1999) proposed the pH shift extraction method. At pH < 3.5 and pH $>$ 10.5, myofibril proteins are soluble in water and can be separated from other insoluble and unwanted substances. Then the targeted proteins, mainly myofibril proteins, are precipitated by adjusting pH to the isoelectric point (usually around pH 5.5) (Kristinsson & Liang, 2006). High extraction proficiency and extremely low lipid content are the primal advantages of the pH shift method. For Atlantic Croakers, acid and alkaline treatment had 78.7% and 65.0% protein recovery compared to 57.7% for conventional washing (Kristinsson & Liang, 2006). More than 70% of lipid reduction was obtained in both acid and alkaline treated surimi (Zhou & Yang, 2019).

On the other hand, as a protein-rich food, surimi must be kept at low temperatures to extend its shelf life. Traditionally, raw surimi is mixed with cryoprotectants before being stored at freezing temperature, but the proteins still suffer from freeze-denaturation, and constant freeze–thaw cycles are damaging to the gelling ability of proteins and final gel qualities (Jia et al., 2020). However, chilled surimi offers several advantages over frozen ones: low cost without the need of cryoprotectants and freezing; have the option of being sugar-free; improved consumer image as "never frozen" product and preserved with good gelling properties. This was demonstrated in Pacific Whiting surimi, where it

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had a shelf life of 5 days at 5 °C but retained its gel functionality and quality throughout storage (Pipatsattayanuwong, Park, & Morrissey, 1995). Gel shear stress was also largely greater than its frozen counterpart, showing improved quality. Sun et al. (2017) reported that grass carp surimi added with thinned young apple polyphenols could be stored at 4 °C for 7 days. Hajji, Hamdi, Boufi, Li, and Nasri (2019) found that surimi supplemented with chitosan nanoparticles increased the storage length to about 9 days. However, there were relatively few studies devoted to the storage of pH shift method prepared surimi.

The objective of this study was to investigate the effects of cold storage (4 ± 2 °C) on the pH shift method processed surimi in comparison with traditional water-washed surimi. The storage length was determined by the microbial analysis. Then the textural properties, colour changes, total volatile basic nitrogen (TVB-N) and the surimi rheological behaviour were examined to study the quality and the freshness of the surimi. Collectively, a gelation mechanism was proposed based on the observations. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) was used to validate the proposed mechanism.

2. Materials and methods

2.1. Preparation and storage of surimi

Raw surimi samples were prepared according to Zhou and Yang (2019), with slight modifications. Fresh tilapia mince was homogenised with chilled water (4 °C) at a ratio of 1:5 (w/v) for 2 min. Then the pH of the homogenate was adjusted to 2 or 12 using 6 mol/L HCl or 6 mol/L NaOH using a pH meter (Mettler Toledo, Zurich, Switzerland), respectively. The homogenates were then centrifuged using an Eppendorf Centrifuge 5810 R (with rotor F-34–6-38, Eppendorf, Hamburg, Germany) at 10,000*g* at 4 °C for 10 min. The pH of the supernatant was then adjusted back to the isoelectric point (pH 5.5) for precipitation. After centrifugation, the precipitate was collected as the acid and alkaline-treated surimi samples. For conventional water-washed surimi, fish mince was mixed with chilled water at a ratio of 1:3 (w/w) and washed for 10 min then centrifuged at 10,000*g* at 4 °C for 10 min. The precipitate was washed twice, where the last wash was with a 0.5% NaCl solution. The precipitate after centrifugation was collected as sample W (water-washed sample). After surimi processing, all samples were adjusted to around 80% moisture content and added with 2.5% (w/w) NaCl before storage and other analyses. All samples were stored at 4 ± 2 °C in a domestic fridge.

2.2. Microbial analysis

Surimi samples were analysed with a spread plate method from Zhao et al. (2019), with modifications. Each sample (5 g) was stomached with sterile peptone water (0.1%, 45 mL) in a stomacher bag. Serial dilution was performed and 100 μL of each dilution was used for plate spreading. Enrichment test was also applied to verify whether there were any bacteria survived. Standard plate count agar was used for the aerobic psychrotrophic count and aerobic mesophilic count, which were incubated at 4 °C for 7 days and 36 °C for 2 days, respectively. Yeast and mould growth was performed on potato dextrose agar and incubated at 25 °C for 3 days. Results of the aerobic mesophilic count (AMC), yeast and mould count (YMC), and aerobic psychrotrophic count (APC) were expressed as log colony forming units (CFU)/g sample.

2.3. Physicochemical properties and freshness of surimi

2.3.1. Texture profile analysis

Before texture profile analysis (TPA), all samples were mixed with 10%, 0.5%, and 5% (w/w) potato flour, sugar, and chilled water, respectively, according to previous processing procedures with modifications (Hwang, Choi, & Lee, 2013; Lazos, 1996; Yi et al., 2011). The mixture was blended in a food processor for 5 min and stuffed into a tube. Then all the samples were incubated in a 90 °C water bath for 30 min, then transferred to an ice water bath and incubated for 20 min. After setting at 4 °C overnight, samples were taken out of the tube and cut into a cylinder with 3 cm diameter and 2 cm length. Before analysis, all samples were subject to temperature equilibrium at 25 °C for 1 h.

Hardness, gumminess, chewiness, cohesiveness, resilience, and adhesiveness were analysed with a TA-XT2i texture analyser (Stable Micro System, Surrey, UK) with a 36 mm diameter flat top cylindrical probe. The test parameters were set as follows: test speed: 1.0 mm/s; distance: 8 mm (40% of original gel height), trigger force: 0.05 N (Sow & Yang, 2015).

Fracture gel analysis, including gel strength and elasticity, was analysed using the same equipment with a 5 mm diameter spherical headed probe. The maximum sustained force was used as the gel strength value. The test parameters were set as follows: pre-test speed: 5 mm/s; test speed: 1 mm/s; penetration distance: 15 mm (Zhou & Yang, 2019).

The expressible moisture was determined according to the method of Chaijan, Panpipat, & Benjakul (2010). The moisture on the surface of the freshly cut surimi gel was dried using tissue paper, then the mass of the gel was recorded, and the gel was placed on two pieces of filter paper and covered with another piece on the top. A standard mass (5 kg) was placed on the sample and held for 2 min. Then the compressed surimi gel was weighed again. Expressible moisture was calculated by the following equations:

$$\text{Expressible moisture}(\%) = \frac{\text{Original mass} - \text{Final mass}}{\text{Original mass}} \times 100\,\% \quad (1)$$

2.3.2. Colour changes

Colour analysis for surimi samples was conducted according to Panpipat and Chaijan (2017). L^*, a^* and b^* of the cooked surimi on day 0 and day 6 were determined with a colourimeter (CM-3500d, Konica Minolta, Inc., Japan). The whiteness value was calculated using the following equation:

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

where L^* is lightness; a^* is redness/greenness; b^* is yellowness/blueness.

2.3.3. TVB-N

The TVB-N value was determined according to a standard method using Kjeldahl Nitrogen apparatus (BÜCHI 321 Distillation Unit, Flawil, Switzerland) (Zhou et al., 2019). A blank test was conducted using the same volume of water with MgO. The value of TVB-N was calculated by the following equation:

$$\text{TVB-N}\ (mg/100\ g) = \frac{(V_1 - V_2) \times c \times 14}{m \times \frac{1}{10}} \times 100 \quad (3)$$

where V_1 is the amount of HCl (L) used in the sample titration, V_2 is the amount of HCl (L) used in the blank titration, c represents concentration (mol/L) of HCl, and m is sample mass (g).

2.3.4. Sensory evaluation

To further support the results from the texture profile analysis, a sensory evaluation was conducted to allow a direct touching and feeling of the treated surimi samples. It could provide a more authentic connection between the data obtained from texture profile analysis and the real human feeling of the samples. All surimi samples were subject to sensory evaluation via quantitative descriptive analysis, approved by university Institutional Review Board (No. NUS-IRB-2021-21). The surimi samples before and after storage were cut into 1 × 1 × 1 cm^3 cubes and assessed by trained panellists (12 members aged from 22 to 35 years

old from the Department of Food Science and Technology, National University of Singapore). Before the evaluation, all samples were equilibrated at room temperature for 20 min. All samples were coded with random three-digit numbers. The intensity of the sensory parameters was quantified on a scale from 1 to 9; hardness was rated by 1 for very soft, 5 for medium hardness, and 9 for a common fish ball hardness; springiness was rated by 1 for not springy at all, 5 for medium springy, and 9 for a common fish ball springiness; colour was rated by 1 for dark or greyish colour; 5 for slight dark but close to a common fish ball colour, and 9 for a common fish ball colour; expressible moisture was rated by 1 for excessive moisture oozing out after slight squeezing, 5 for little moisture oozing out after squeezing, and 9 for no or very little moisture oozing out after squeezing; overall appearance was rated as 1 for not resembling a fish ball at all, 5 for average resemblance, and 9 for extremely resemblance (Chen et al., 2019; Santana, Huda, & Yang, 2015). A control commercial fish ball sample, which was also stored and cooked under the same condition as all other samples, was presented simultaneously with the tested surimi samples.

2.4. Rheological behaviour

Temperature sweeps of surimi samples were measured as of Zhou and Yang (2019). About 0.5 g of surimi samples were spread on the sample holder under a 25 mm diameter parallel plate on the Anton Paar MCR-102 (Anton Paar, Ashland, VA, USA) stress-controlled rheometer. The gap between the plate and the sample holder was fixed at 0.5 mm and a thin layer of oil was added to prevent moisture loss. A temperature sweep was conducted from 5 to 90 °C with a heating rate of 2 °C/min, frequency of 0.1 Hz, and 1% strain. The storage modulus G' and loss modulus G'' were measured.

Frequency sweep was conducted after the temperature sweep at 25 °C. Before the measurements, a strain sweep of 0.01–100% was performed at a fixed frequency of 1 Hz to determine the linear viscoelastic region (LVR). G' and G'' were recorded against angular frequency (ω) ranging from 0.1 to 100 rad/s, with amplitude strain fixed at 1%, which was within the LVR (Chen et al., 2018).

2.5. Microstructures of the myofibril proteins of the cooked surimi

Myofibril protein was extracted according to Choi and Park (2002), with modifications. One gram of each sample (acid and alkaline-treated surimi) was homogenised with 2 mL of 0.6 mol/L NaCl and 20 mmol/L Tris-HCl (pH 7.0) for 30 s. The mixture was incubated for 1 h at 4 °C and centrifuged at 4 °C, 10,000*g* for 10 min, where the supernatant was collected as the myofibril solution. Bradford method was used to determine the protein concentration (Bradford, 1976).

The extraction of water-washed surimi was performed as described by Zhang et al. (2017), with modifications. One gram of the sample was added with 5 mL of low salt buffer containing 0.1 mol/L NaCl and 20 mmol/L Tris-HCl at pH 7.5 and homogenised. The homogenate was centrifuged at 4 °C, 10,000*g* for 10 min. The pellet was then homogenised in 10 mL of high salt buffer containing 0.6 mol/L NaCl and 20 mmol/L Tris-HCl (pH 7.0) and extracted for 1 h at 4 °C. After centrifugation at the same condition, the supernatant was collected as the myofibril solution. Bradford method was used to determine the protein concentration (Bradford, 1976). A standard curve $y = 0.4678x + 0.0115, R^2 = 0.9913$ was used, where x is the protein concentration (mg/mL), y is the absorbance at 595 nm, the range of x was 0–1 mg/mL and the range of y was 0–0.455.

For transmission electron microscopy (TEM), the extracted myofibril proteins were placed in a 90 °C water bath for 30 min, then it was cooled in an ice water bath for 20 min, to mimic the heating process of the surimi gels. Then the solutions were diluted to 0.05 mg/mL. Twenty microliters of the heated myofibril solution were fixed on a carbon-coated copper 400-mesh grid for 2 min. Redundant solutions were blot away and a drop of 3% tungsphosphoric acid was added on the grid to stain for 3 min. The copper grid was blot dry with filter paper strips and washed with deionised water until clean. Specimens were visualised using a JEOL JEM-3011 transmission electron microscope (JEOL Ltd., Tokyo, Japan) (Zhou & Yang, 2020).

2.6. Protein patterns

SDS-PAGE was conducted according to the method of (Laemmli, 1970) with modifications. Surimi samples were homogenised using a 5% SDS solution and boiled for 30 min for extraction and binding of proteins to SDS. The homogenate was centrifuged for 5 min at 10,000*g* and protein concentration was determined for the supernatant. Solution with 2 mg/mL protein was mixed with sample buffer at a ratio of 1:1. The mixture was boiled for 4 min before gel electrophoresis, where 7–10 μg of proteins were loaded into the well. A staining solution (0.1% Coomassie Blue R-250 (w/v)) and destaining solution (40% methanol, 10% acetic acid, 50% water (w/v)) were used for staining and removing stains of the gels. After taking the gel images, the band intensity of each band was determined using the software ImageJ.

2.7. Statistical analysis

At least three independent samples were prepared for each experiment, and the experiment was conducted in triplicate independently. Statistical analysis was performed using IBM SPSS Statistics Version 23 (International Business Machines Co. Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to determine the significant differences among surimi with different treatments on the same day, and paired *t*-test was used to determine the significant differences between surimi with the same treatments on the different days. The significant difference between means for ANOVA tests was compared using the least significant difference (LSD) and Duncan's multiple range test ($P < 0.05$).

3. Results and discussion

3.1. Microbial growth of surimi during cold storage

To determine the storage length of the surimi samples, microbial growth tests including aerobic mesophilic counts (AMC), yeast and moulds count (YMC), and psychrotrophic counts (APC) were determined. As shown in Fig. 1(a), the AMC of all samples exceeded 7 log CFU/g around day 6, which reaches the limit for fresh fish (International Commission on Microbiological Specifications for Foods, 1974), thus the study was conducted within the whole storage period of 6 days.

On day 0, the AMC counts for acid-treated surimi and alkaline-treated surimi were 3.34 and 3.49 log CFU/g, similar to the findings of Pipatsattayanuwong et al. (1995), which were significantly lower than the count value of water-washed surimi, 4.41 log CFU/g. This initial difference was attributed to the effects of extreme pH, which can help to reduce the microbial load during the pH shift process of acid-treated surimi and alkaline-treated surimi. However, the microbial grew rapidly in acid-treated surimi and alkaline-treated surimi after 2 days of storage, and the initial reduction of microbial loads didn't effectively prolong the storage period.

The YMC values on day 0 were 3.98, 3.60, and 4.86 log CFU/g for acid-treated surimi, alkaline-treated surimi, and water-washed surimi, respectively, which were similar to other reports (Feng, Ng, Mikš-Krajnik, & Yang, 2017; Lou, Zhai, & Yang, 2021). The counts of water-washed surimi were relatively higher than the counts of acid-treated surimi and alkaline-treated surimi. It was possible that during the washing steps, the minced tilapia fish was exposed to the air, allowing more microbial contaminations and increased the load. For APC, the counts on day 0 were lower than the detection limit, but on day 2 the count in water-washed surimi was again higher than the counts of acid-treated surimi and alkaline-treated surimi. Being the tropical fish, it was

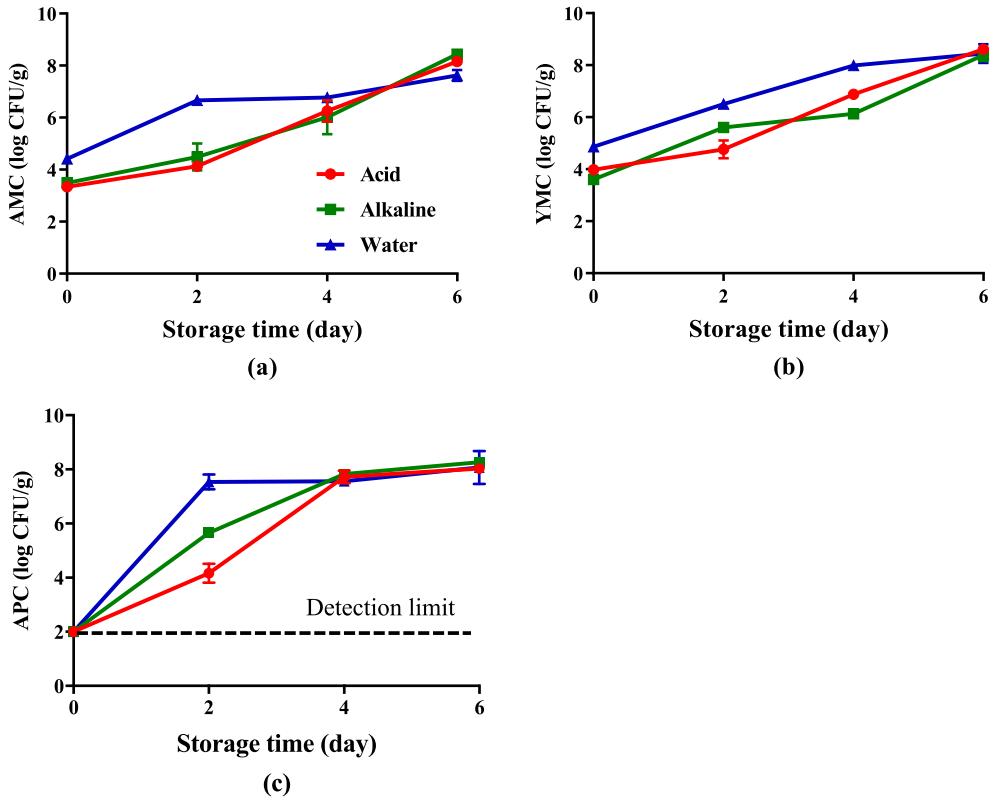


Fig. 1. Microbial growth of surimi with different treatments throughout the 6 days cold storage. (a) Aerobic mesophilic count (AMC); (b) yeast and mould count (YMC); (c) aerobic psychrotrophic count (APC). Acid: acid-treated surimi; Alkaline: alkaline-treated surimi; Water: water-washed surimi.

presumed that the initial load of aerobic psychrotrophic count on tilapia was much lower than other non-tropical fish. However, it was reported that the skin and the fillet of tilapia could be cross-contaminated or even self-contaminated with many foodborne pathogens (Gatti Junior, Assunção, Baldin, & Amaral, 2014), which led to the growth of the psychrotrophic count.

Similarly, Pipatsattayanuwong et al. (1995) found that fresh pacific whiting surimi could be stored for up to 5 days at refrigerated temperature (5 °C). The initial APC count was around 3.2 log CFU/g and increased significantly after 2 days. Although they only applied tests on fresh surimi, it was with no doubt that high protein products as surimi were prone to deterioration under refrigerated conditions. Although the protein contents in pH shift processed surimi were usually much higher than in fresh/water-washed surimi (Zhou & Yang, 2019), which might make acid-treated surimi and alkaline-treated surimi more susceptible to microbial degradation, both acid-treated surimi and alkaline-treated surimi stood for the same storage period as water-washed surimi. This result clearly indicated that acid-treated surimi and alkaline-treated surimi could be stored for the same period as water-washed surimi, while on the other hand, provided a view that pH shift method could be used not only a processing method but also a potential antimicrobial method in surimi processing.

3.2. Physicochemical properties and freshness of surimi before and after cold storage

3.2.1. Textural profiles of surimi

Cooked surimi was made from raw surimi paste on day 0 and day 6 of storage, respectively. Hardness, gumminess, chewiness, cohesiveness, resilience, adhesiveness, gel strength, elasticity, and expressible moisture were examined, the results are shown in Fig. 2(a)-(i).

On day 0, acid-treated surimi generally exhibited the worst value for all parameters, indicating the poor textural properties, which is mainly due to the more comprehensive effects on myofibril proteins caused by acid (Yongsawatdigul & Park, 2004). Alkaline-treated surimi showed the highest hardness, gumminess and elasticity on day 0, indicating a more organised gel structure. Water-washed surimi showed the best value in chewiness, cohesiveness, resilience, adhesiveness, and especially in gel strength and expressible moisture, suggesting that traditionally water-washed surimi properly held their structure under external pressure, and could hold water inside their gel matrices.

After 6 days of storage, acid-treated surimi remained the poor gel textural properties, while alkaline-treated surimi showed improved gel qualities. Compared between alkaline-treated surimi and water-washed surimi, the hardness, cohesiveness, adhesiveness, and especially gel strength of alkaline-treated surimi all significantly increased and were even better than those of water-washed surimi. Besides, the expressible moisture of alkaline-treated surimi on day 0 was higher than water-washed surimi, and then it decreased to the same level as water-washed surimi on day 6, showed an improved water holding capacity. The significant improvements in gel qualities of alkaline-treated surimi suggested that it could withstand the cold storage and preserve or even enhance its gelling ability after the cold storage condition. For instance, as the most important and the most prevalent parameter in evaluating gel quality, the breaking forces of alkaline-treated surimi and water-washed surimi on day 0 were 204.2 and 462.1 g, then on day 6 were 491.9 and 172.9 g, respectively. The drastic drop in water-washed surimi suggesting a major quality deterioration and it certainly wouldn't meet the consumer interests.

Liu et al. (2019) suggested that heat-induced gels had limited time for myofibril proteins to properly unfold and aggregates, while gels stored at cold storage condition had more time to thoroughly unfold the proteins and to allow gel network pre-aggregation. Hence, the day 0 heated gel properties were mainly depended on the instant protein statues after the processing procedures. This explains why on day 0, acid-treated surimi and water-washed surimi possessed the worst and

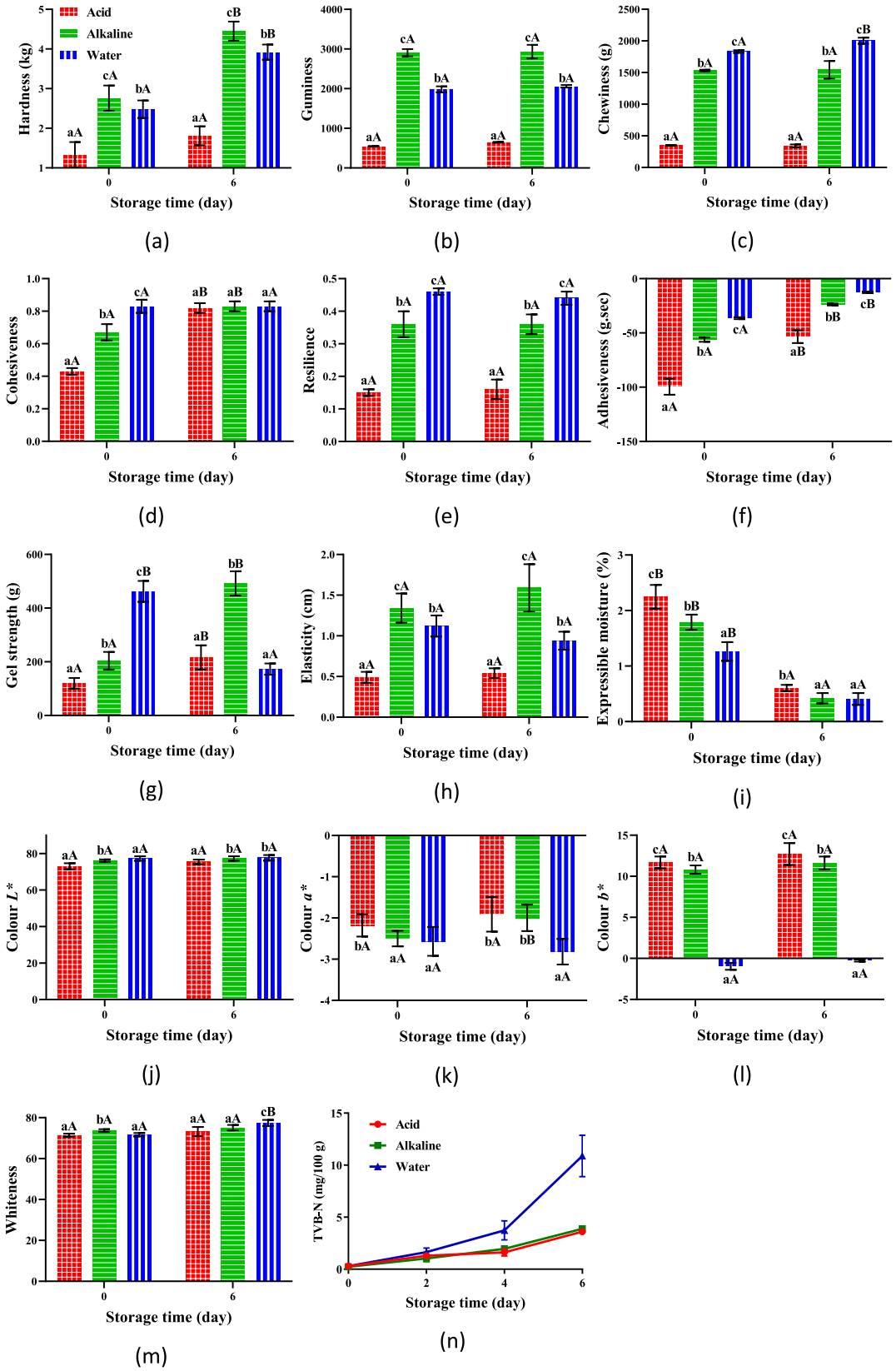


Fig. 2. Physicochemical properties of surimi with different treatments before and after 6 days of storage. (a) Hardness; (b) gumminess; (c) chewiness; (d) cohesiveness; (e) resilience; (f) adhesiveness; (g) gel strength (breaking force); (h) elasticity; (i) expressible moisture; (j) colour L^*; (k) colour a^*; (l) colour b^*; (m) whiteness; (n) TVB-N. Acid: acid-treated surimi; Alkaline: alkaline-treated surimi; Water: water-washed surimi. Values with different lowercase letters indicate significant differences within the same days, values with different uppercase letters indicate significant differences within the same treatment groups ($P < 0.05$).

best gel qualities, respectively. On day 0, due to the mild processing method, raw water-washed surimi remained the original structures of myofibril proteins, thus the heated gel exhibited good gel strength and water holding capacity (low expressible moisture). However, the 6 days storage allowed the growth of microbials, and the relatively high storage temperature (4 ± 2 °C) facilitated the protein oxidation, thus the gel quality of raw water-washed surimi on day 6 was greatly lowered and yielded low quality heated surimi gels.

As for acid-treated surimi, the effects of acid on myofibril proteins were found to be too profound and the structure of proteins was greatly disturbed (Zhou & Yang, 2019), thus it showed low quality at both day 0 and 6. Alkaline-treated surimi, on the other hand, didn't undergo the extreme process as acid treatment, but the proteins still underwent unfolding and refolding process induced by the changing pH, thus the gel quality on day 0 was lower than water-washed surimi. During the 6 days of storage, the myofibril proteins in raw alkaline-treated surimi had sufficient time to interact with each other and thoroughly solubilised due to the added salts. As mentioned in the discussion of microbial growth, some of the microbial cells could be killed due to the pH shift process, thus on day 6, the gel quality was improved and even elevated to surpass water-washed surimi.

3.2.2. Freshness of surimi

The changes in colour were the consequences of protein decomposition and denaturation which led to the loss of translucency of the products (Pipatsattayanuwong et al., 1995). It greatly influenced the appearance and acceptability of surimi products (Panpipat & Chaijan, 2017). Thus, it can be regarded as an indication of the freshness of the products.

The L^*, a^*, b^* values, and whiteness of all cooked surimi samples before and after treatments are shown in Fig. 2 (j)-(m). On day 0, alkaline-treated surimi had the highest lightness, acid-treated surimi showed the highest redness and yellowness. The highest overall whiteness was obtained by alkaline-treated surimi, which was also observed in other alkaline processed surimi (Chomnawang & Yongsawatdigul, 2013). Generally, acid-treated surimi showed a less white appearance, mainly due to its inability to thoroughly remove or denature myoglobin (Yongsawatdigul & Park, 2004). It was also found that the pH shift method could accelerate the oxidation of myoglobin (Yongsawatdigul & Park, 2004), thus both acid-treated surimi and alkaline-treated surimi showed much higher b^* value than water-washed surimi. On day 6, the general trend of all the parameters showed very little changes. The a^* of alkaline-treated surimi increased from −2.50 to −2.00, indicating more yellowness appeared, and the overall whiteness of water-washed surimi increased from 71.64 to 77.42. Acid-treated surimi showed almost no changes in any colour index during the 6 days' storage, probably due to the short length of storage. In general, cold storage didn't greatly change the appearance of all the surimi samples.

TVB-N value is another commonly used parameter in evaluating the freshness of surimi products (Zhou et al., 2019). During storage, amino acids, proteins, and nitrogen-containing compounds will decompose to form volatile bases (Zhao et al., 2019), a high TVB-N value indicates low freshness. As shown in Fig. 2(n), the TVB-N values of acid-treated surimi, alkaline-treated surimi, and water-washed surimi on day 0 were 0.289, 0.236, and 0.289 mg/100 g, respectively. Starting from day 2, water-washed surimi increased rapidly and on day 6, the value was 10.88 mg/100 g. The changes in acid-treated surimi and alkaline-treated surimi were much milder, the values on day 6 were 3.61 and 3.89 mg/100 g, respectively. According to Commission Regulation (EC) NO 2074/2005, the rejection level of TVB-N for fishery products was set as 25 – 35 mg/100 g, varies with fish species. After 6 days of storage, no sample exceeded the rejection level; however, the freshness decrease in water-washed surimi was quite evident. At low storage temperature, specifically 0 to 10 °C, one of the major component of the spoilage populations found in surimi-based imitation crab was *Pseudomonas* spp. (Yoon, Matches, & Rasco, 1988). It was possible that the extreme pH condition significantly reduced the population of this microbial species thus slowed down the production of TVB-N, showing that pH shift treatment helped to remain the freshness of surimi.

3.2.3. Sensory evaluation

Sensory evaluation is widely used to examine the intuitive changes of the fishery food quality during storage (Yu, Jiang, Xu, & Xia, 2017). The sensory evaluation scores are shown in Fig. 3. In general, a higher score indicated that the sample resembled more to a real fish ball product. On both day 0 and day 6, acid-treated surimi showed the lowest scores for most of the parameters. The alkaline-treated samples showed similar scores to the water-washed surimi and the commercial fish ball samples. On day 0, alkaline-treated surimi showed similar scores in springiness, colour, and overall appearance comparing with the water-washed surimi and the commercial fish ball sample. The hardness and the expressible moisture scores were lower, even though the alkaline-treated surimi showed the highest hardness texture profile on day 0, indicating that a lower but proper hardness in surimi products will be more preferable. In general, except for acid-treated surimi, the other three samples showed very similar scores for all parameters.

On day 6, all scores of acid-treated surimi remained low. Water-washed surimi was found to have lower hardness and overall appearance scores. The significant decrease indicated that there were obvious quality degradations during the storage. In contrast, the scores of alkaline-treated surimi remained almost the same as compared to the scores on day 0, also, it also remained a high value for the overall appearance score. The sensory characteristic results supported the texture profile analysis results from human being perspectives, and the alkaline-treated surimi was more satisfactory than all other samples after the cold storage.

3.3. Rheological behaviour

3.3.1. Thermal gelation of surimi

Temperature sweeps describe the gelation process of the surimi proteins during the heating process. G' measures stored energy as the elastic portion after deformation, and G'' measures the energy released as heat, or the viscous portion of the material (Zhang et al., 2017). As shown in Fig. 4(a) and (b), all three samples showed typical gelation profiles of fish protein samples. The major difference was that both G' and G'' values of acid-treated surimi and alkaline-treated surimi were significantly higher than water-washed surimi before and after storage, mainly due to the high gelling protein contents (myosin) presented (Zhou & Yang, 2019). Selected thermal transition points of G' of all samples are given in Table 1.

Between 30 and 40 °C, most surimi was found to show a slight increase in G' and G'' value to reach their first peak, and this is regarded as the "gel setting" stage. At this stage, myosin would unfold to allow organised aggregation, and the proteins lost its initial elasticity (Park, Yongsawatdigul, Choi, & Park, 2008). Only alkaline-treated surimi and water-washed surimi showed this increase in their spectrum. The absence in acid-treated surimi indicated that there was no or very little preliminary protein network was formed, where relatively weak intermolecular hydrogen bonds, or other non-covalent, short term intermolecular interactions initiated to hold this loose network (Zhang et al., 2013). From 40 to 45 °C, there was a decrease in G' for all samples. According to Sano, Noguchi, Tsuchiya, and Matsumoto (1988), the decrease in G' was due to the coil transformation of α-helix, where the fluidity of protein increased and reduced the viscoelasticity, this also explained the same decrease happened in G''. Starting from around 45 °C (for acid-treated surimi, it started from around 50 °C), all samples G' continued to increase till the end of temperature elevation. Major cross-linking and aggregates of myosin happened at this "gel strengthening stage". The firmness of the gel was greatly strengthened caused by the cross-linking interactions of the globular head portion of myosin (Stone & Stanley, 1992), and was mainly caused by protein aggregations

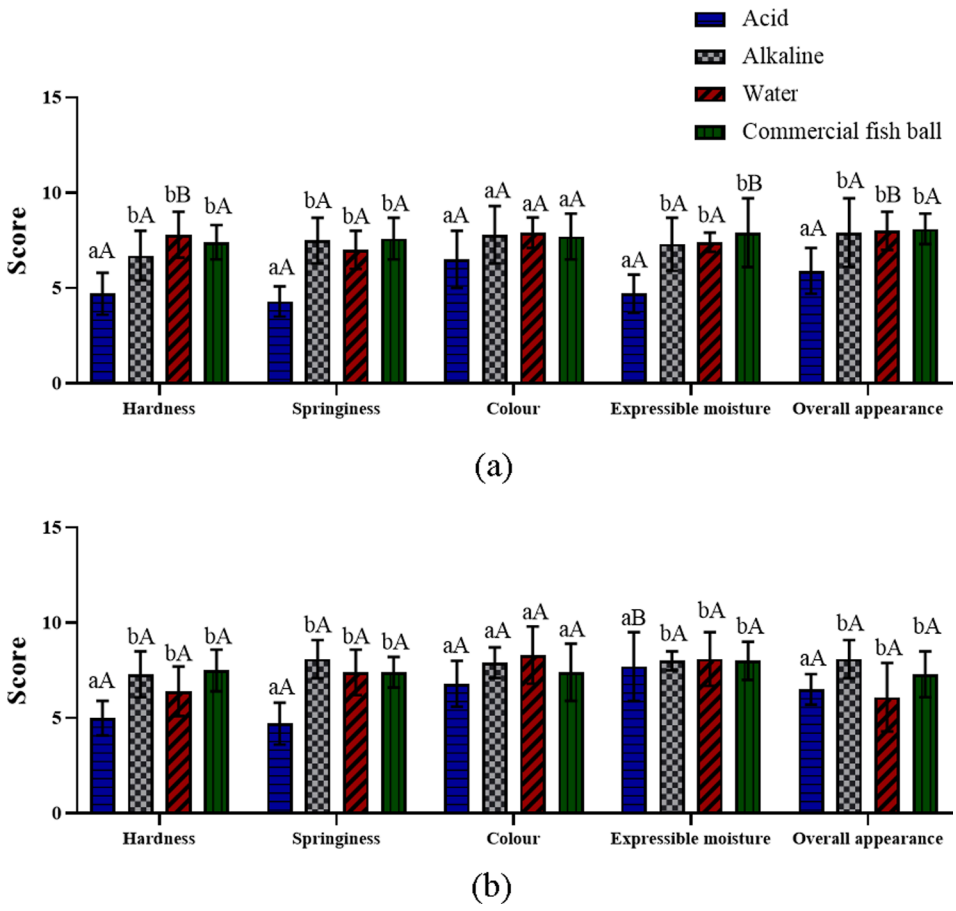


Fig. 3. Sensory evaluation scores of the pH-shift treated and water-washed surimi samples (a) before and (b) after storage. Values with different lowercase letters indicate significant differences within the same treatment groups, values with different uppercase letters indicate significant differences within the same days ($P < 0.05$).

(Takahashi, Kurose, Okazaki, & Osako, 2016). *G''* also showed similarly increment but started to decrease from around 65 °C, for water-washed surimi, the decrease started immediately from 45 °C, showing less viscosity portion was maintained in the forming gels.

From comparison between samples before and after storage, all samples at day 6 showed lower *G'* and *G''* values to their day 0 counterparts, suggesting that the 6 days' storage decreased their gelling ability. However, *G'* of alkaline-treated surimi at 90 °C decreased from 74.39 to 58.19 kPa before and after storage, while acid-treated surimi decreased from 112.04 to 46.45 kPa. This supported the fact that the gel quality of alkaline-treated surimi at day 6 didn't show a significant loss. The final value of *G'* of acid-treated surimi at day 0 was the highest, seemed to advise a better gel quality, however, as discussed above, the pre-gelling stages of acid-treated surimi didn't show the essential protein aggregation and organised matrix formation, thus the high value obtained was not very instructive in guaranteeing a better gel matrix formation. Notably, the texture profiles of all samples were tested with the pre-addition of potato flours, there would be interactions between the myofibril proteins and the constituents of flour (Jia et al., 2020). Also, texture profile samples were cooked at 90 °C, and the temperature sweeps were applied with 2 °C/min temperature elevation, thus the two different systems could bring discrepancy. Even so, the spectrum still provided valuable information in examining the gelation procedure rather than the final products.

Based on both the results from texture profile and rheological behaviour, it was presumed that the myofibril proteins in acid-treated surimi on day 0 were already greatly destructed, with little gelling activity remained. Under heat treatment, the high values in *G'* and *G''* were mainly contributed by the higher yield of myofibril proteins brought by pH shift method purification (Panpipat & Chaijan, 2016). During storage, protein activity continued to lose due to microbial activity and protein oxidation, thus the *G'* and *G''* value significantly decreased and as revealed by texture profiles, the textural properties of acid-treated surimi were much worse than the other two.

3.3.2. *Viscoelastic properties of surimi after gelation*

To better visualise and compare the viscoelastic properties of all heated surimi samples, changes of both *G'* and *G''* of acid-treated surimi, alkaline-treated surimi, and water-washed surimi against different angular frequencies of all samples were investigated. Fig. 4(c) shows the results at day 0, and Fig. 4(d) shows the changes after 6 days of storage. Classifications were made based on the trends of *G'* and *G''*: a dilute solution was attributed to spectrum with higher *G''* than *G'* over the entire frequency range; an entanglement network shows intersects of *G'* and *G''* at the middle of the frequency range; a weak gel shows larger *G'* and *G''* and they are almost parallel to each other; a strong gel shows larger *G'* than *G''*, while *G'* with a slope of 0 and *G''* shows a minimum at intermediate frequencies (Huang, Zeng, Xiong, & Huang, 2016). As shown in Fig. 4(c) and (d), *G'* and *G''* of water-washed surimi were parallel to each other before and after 6 days of storage, thus they were categorised as weak gel systems. Acid-treated surimi and alkaline-treated surimi showed intersects on both days which were regarded as an entanglement network. Besides, all samples showed increased *G'* and *G''* values with an increase of the frequency, exhibiting frequency dependence behaviour.

Generally, when frequency was low, the interval of imposed deformation was long enough for the proteins to entangle, thus the main response would be the viscous portion (*G''*). And at high frequencies, the

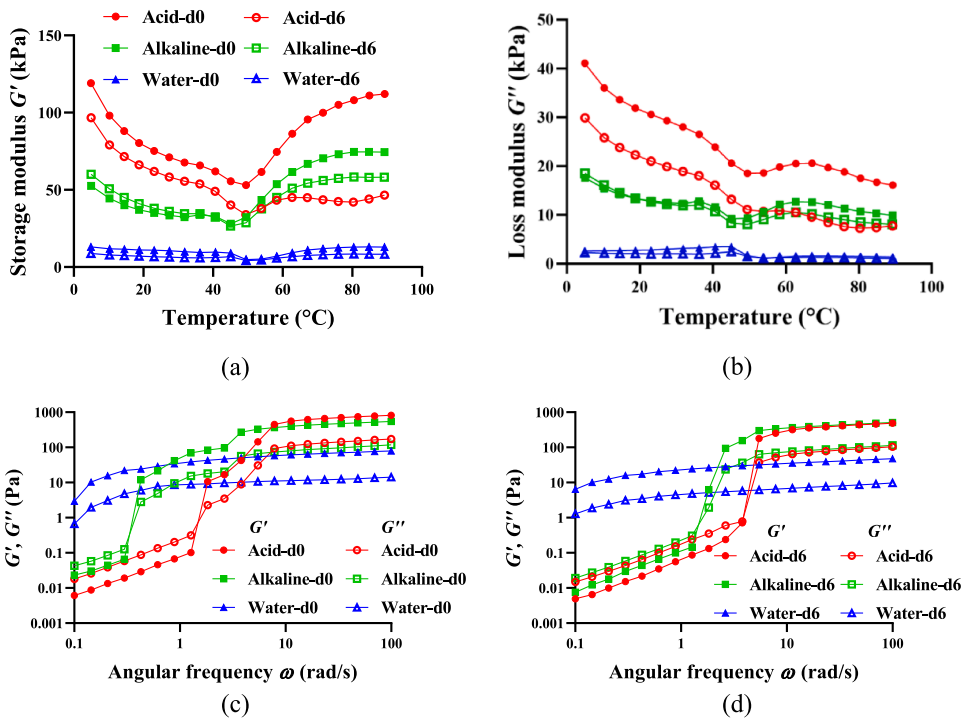


Fig. 4. Changes in (a) *G'* and (b) *G''* values of raw surimi with different treatments before and after storage; changes of *G'* and *G''* values of cooked surimi against different angular frequencies (c) before the storage and (d) after the storage. Acid: acid-treated surimi; Alkaline: alkaline-treated surimi; Water: water-washed surimi. d0: before storage; d6: after storage.

Table 1
Thermal transition points obtained from storage modulus (*G'*) of temperature sweeps.

Temperature (°C)	*G'* (kPa)					
	Acid-d0	Alkaline-d0	Water-d_0	Acid-d6	Alkaline-d6	Water-d_6
31	67.70 ± 1.20^{e}	32.32 ± 0.35^{c}	9.49 ± 1.08^{b}	55.58 ± 5.99^{d}	34.58 ± 4.10^{c}	6.06 ± 1.60^{a}
36	65.78 ± 0.13^{e}	33.77 ± 1.02^{c}	9.97 ± 0.74^{b}	53.80 ± 0.74^{d}	34.64 ± 0.20^{c}	5.88 ± 1.47^{a}
40	61.85 ± 2.17^{e}	32.81 ± 1.11^{c}	9.73 ± 1.93^{b}	49.07 ± 5.46^{d}	32.30 ± 1.08^{c}	6.21 ± 0.59^{a}
45	55.62 ± 4.39^{e}	28.06 ± 3.04^{c}	9.07 ± 0.38^{b}	40.16 ± 2.08^{d}	26.55 ± 3.63^{c}	6.67 ± 2.36^{a}
52	61.54 ± 0.10^{d}	43.38 ± 0.98^{c}	5.15 ± 1.55^{a}	37.88 ± 2.67^{b}	37.71 ± 1.87^{b}	4.54 ± 1.07^{a}
90	112.04 ± 5.99^{f}	74.39 ± 4.24^{e}	12.9 ± 2.07^{b}	46.45 ± 3.91^{c}	58.19 ± 0.97^{d}	8.32 ± 1.01^{a}

Different alphabets indicated a significant difference ($P < 0.05$) within the same temperature.
Acid: acid-treated surimi; Alkaline: alkaline-treated surimi; Water: water-washed surimi; d0: before storage; d6: after storage.

protein molecules associations were unable to disentangle at this short oscillation period, thus made the *G'* dominant (Chen et al., 2018)(Sow, Chong, Liao, & Yang, 2018). It was confirmed by textural profiles that acid-treated surimi exhibited very poor gel structure, thus acid treatment on myofibril proteins might have destroyed the formerly organised protein alignment thus proteins were more entangled after pH shift treatment and heating process, led to the behaviour of entanglement network in acid-treated surimi. Similarly, myofibril proteins in water-washed surimi were more in the original state, thus it showed weak gel system. However, the intersects of *G'* and *G''* in alkaline-treated surimi occurred earlier than in acid-treated surimi. When *G'* started to exceed *G''*, the gel was more solid-like (Huang et al., 2016), while acid-treated surimi continued to exhibit fluid-like structure. It was presumed that alkaline-treated surimi had a more organised network that can withstand the applied external frequency changes.

Notably, when combining the textural profiles and the rheological behaviour of surimi after heat treatments, it was found that even though water-washed surimi showed weak gel system after heating at both day 0 and day 6, the texture properties actually degraded from day 0 to day 6 while alkaline-treated surimi showed an inverse trend. As mentioned before, the 6 days of storage allowed the myofibril proteins in raw alkaline-treated surimi to interact with other myofibril proteins, flour protein molecules (mainly starch, gliadin, and gluten) and the added salt, thus it was presumed that when myofibril proteins were degraded, it made room for the incorporation of flour molecules to strengthen the overall network. Specifically, when starch was added to the system, it would swell and exert pressure on the gel matrix, thus made the matrix more compact (Kim & Lee, 1987). On the other hand, the microbial growth of water-washed surimi was already flourishing on day 2 (Fig. 1), the protein degradation was probably much earlier than in alkaline-treated surimi, thus the incorporation of flour didn't help in the texture qualities.

3.3.3. Microstructures of cooked surimi

Fig. 5 shows the microstructure images of the networks formed by cooked acid-treated surimi, alkaline-treated surimi, and water-washed surimi on both day 0 and day 6. Fig. 5(a) and (b) represent the microstructure changes of acid-treated surimi on both days. On day 0, both networks and single rods were seen. On day 6, there were very little networks shown, and most of the image was filled with rods with different lengths and coagulations. For alkaline-treated surimi, more networks were shown on both days comparing with acid-treated surimi.

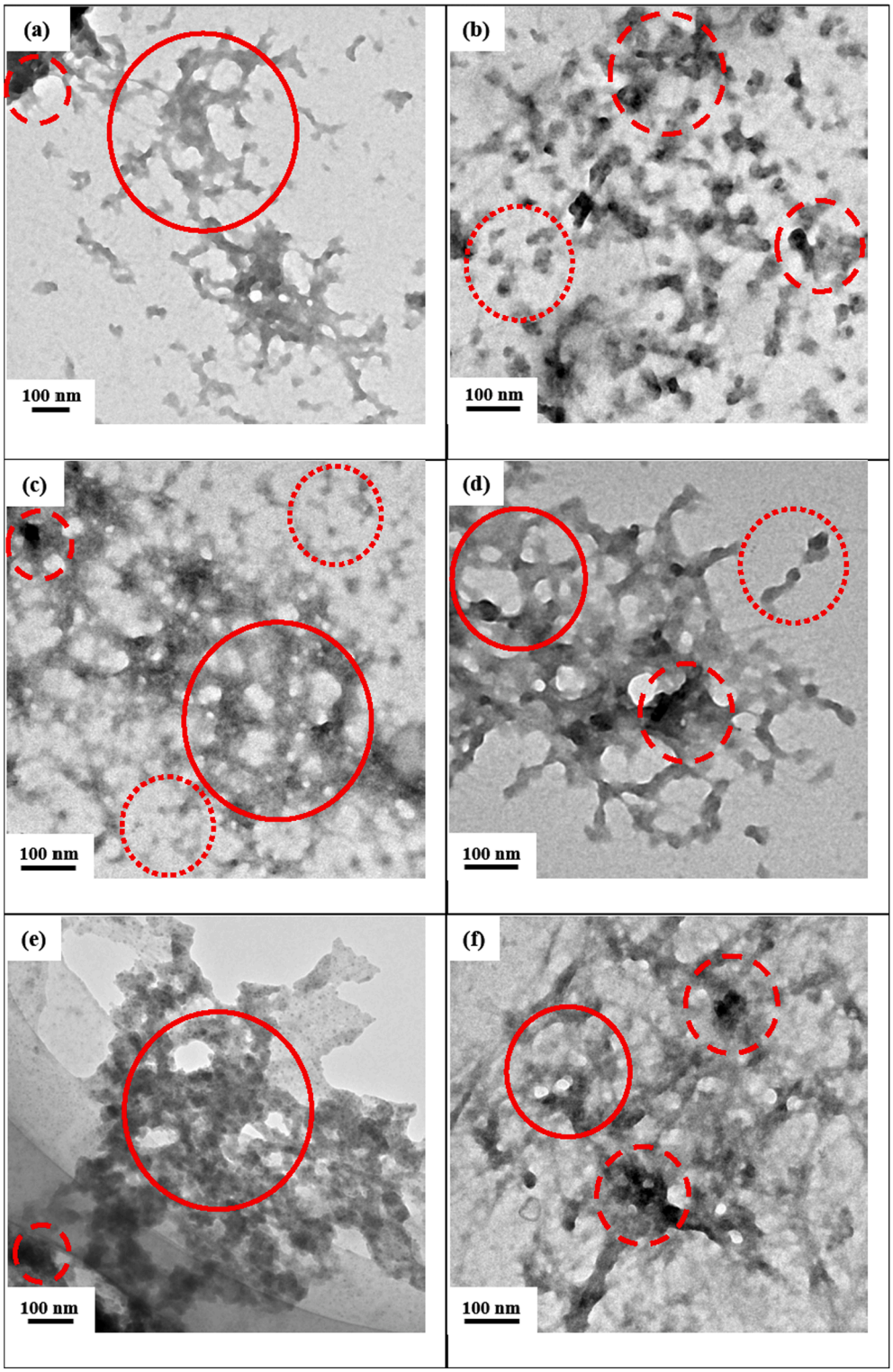


Fig. 5. Transmission electron microscope images of cooked surimi network before and after storage. (a) Acid-treated surimi before storage; (b) Acid-treated surimi after storage; (c) Alkaline-treated surimi before storage; (d) Alkaline-treated surimi after storage; (e) Water-washed surimi before storage; (f) Water-washed surimi after storage. ◯ indicates networks, ◌ indicate individual structures, ◌ indicates coagulates.

On day 6, the hollows seem to be more evenly distributed than on day 0, and there were fewer single rods and the networks seemed to be denser. This might be the evidence that alkaline-treated surimi underwent thorough unfolding during the 6 days storage, which allowed more pre-forming of gel network (Liu et al., 2019), and improved the cooked surimi texture properties on day 6. As for water-washed surimi, it showed quite organised networks on day 0 with almost no single rods, however, the networks on day 6 became unorganised and the numbers

of hollows were limited compared with alkaline-treated surimi, only a few hollows and more coagulations were shown in Fig. 5(f). When comparing the seeming disputes of texture properties and rheology results, it was presumed that the differences were mainly due to the differences in the network systems. Texture profiles were performed with a mixture of surimi and other ingredients, and rheological behaviours were examined with pure surimi. The TEM images showed that there were indeed differences in the hollows of the microstructures. The

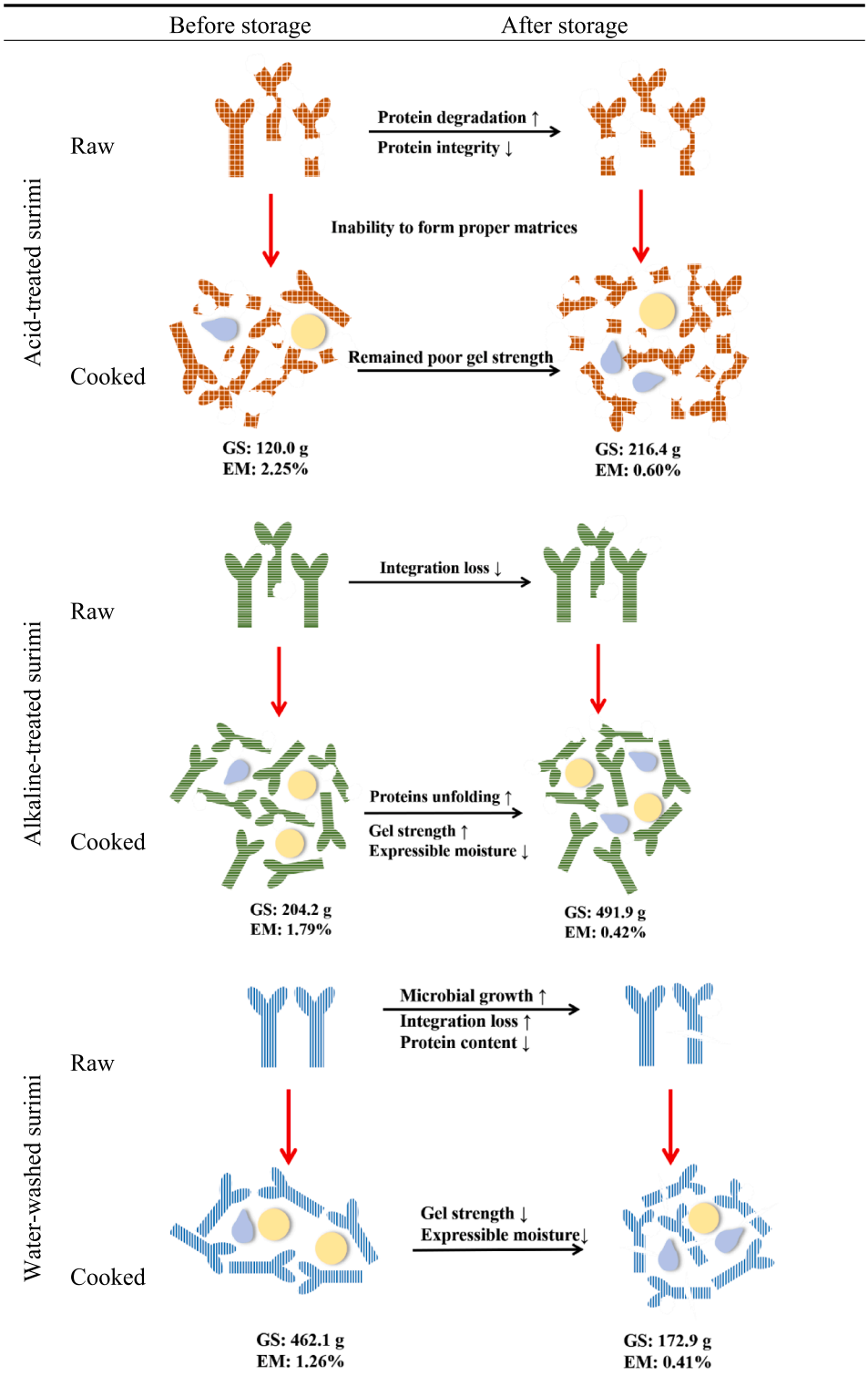


Fig. 6. Schematic illustrations of changes on the gelation portion of myofibril proteins and the heated gel matrices before and after storages. GS: gel strength, EM: expressible moisture. : myofibril proteins, different colour of the protein indicates different treatments; : moisture; : flour particles.

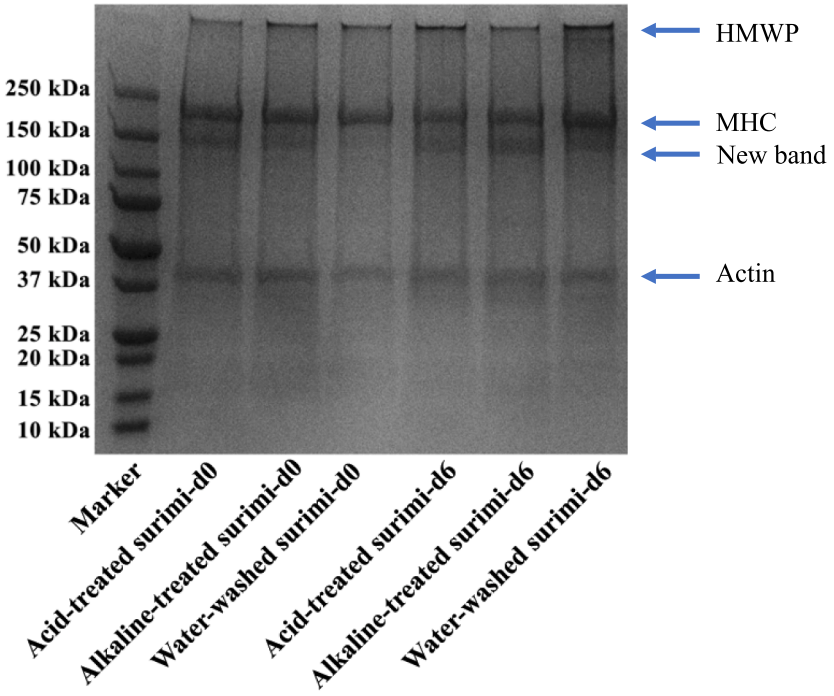
results might suggest that the pH shift method processed surimi, especially alkaline-treated surimi, showed more potential in real practice, where surimi was not used alone but mixed with many other ingredients. With more hollows, more ingredients can be incorporated.

3.3.4. Schematic illustration

Based on the observations, a schematic illustration to summarise the underlying mechanism is shown in Fig. 6. The freshly prepared acid-treated surimi was composed of greatly disrupted myofibril proteins. After heating, the formed matrices were very loose and weak, with many single protein molecules, supported by the TEM images. The poor matrices couldn't hold the incorporated moisture or flours, thus yielded poor gel quality and bad water holding capacity. After 6 days of storage, protein integrity continued to lose. After mixing and heating, as shown in Fig. 6(b), much fewer networks and more single particles were found. Even after incorporation of flour, the myofibril proteins, as the major gelling protein, didn't form a strong gel matrix to hold the moisture or other ingredients. The overall structure of the matrices was too weak to have proper respond to external forces. The results of TPA and rheological behaviour both confirmed that the texture and microstructure of acid-treated surimi were poor.

Alkaline-treated surimi showed much better quality than acid-treated surimi. After the pH shift process, there was also protein degradation. However, the disruption of the myofibrils of alkaline-treated surimi was lower, and the majority would still be intact myofibril proteins. According to the TEM image Fig. 5(c), networks with small hallows were shown. The hollows were able to hold some of the moisture or flours, but the interactions were weak, thus alkaline-treated surimi on day 0 didn't show the best gel quality. After 6 days of storage, due to the low degree of disruption and ability to kill microbials on day 0, some of the alkaline-treated surimi myofibril proteins unfold to an extend that facilitated gel formation. The microstructures showed in Fig. 5(d) contained both gel matrix and single particles, and the hollows in the networks were larger than the hollows on day 0. Larger hollows could hold more moisture and flour particles, thus the gel texture and microstructure on day 6 of alkaline-treated surimi was even better than on day 0.

As the original form of gelling proteins, myofibril proteins in water-washed surimi were good at forming gels and holding moistures, thus water-washed surimi showed good quality on day 0 after heating. Besides, the TEM showed the existence of hollows around or larger than 100 nm. Lin, Tay, Yang, Yang, and Li (2017) used atomic force microscope to confirm that the size of gliadin aggregates, one of the main ingredients in flour, was 100 – 200 nm, thus the hollows in water-washed surimi were able to hold the gliadin particles. With more gliadin held in the matrix, water-washed surimi exhibited the best gel



(a)

	Acid-d0	Alkaline-d0	Water-d0	Acid-d6	Alkaline-d6	Water-d6
HMWP	3.90 ± 0.07^{a}	4.05 ± 0.02^{a}	4.10 ± 0.09^{a}	5.01 ± 0.04^{c}	4.41 ± 0.14^{b}	5.15 ± 0.08^{c}
MHC	4.65 ± 0.10^{c}	4.85 ± 0.09^{c}	4.74 ± 0.05^{c}	4.45 ± 0.08^{b}	4.30 ± 0.03^{b}	3.85 ± 0.06^{a}
New band	4.15 ± 0.12^{b}	3.87 ± 0.14^{a}	N.D.	4.20 ± 0.10^{b}	4.15 ± 0.05^{b}	3.82 ± 0.07^{a}
Actin	3.55 ± 0.03^{a}	3.92 ± 0.01^{b}	3.49 ± 0.01^{a}	3.88 ± 0.02^{b}	3.74 ± 0.07^{a}	3.55 ± 0.05^{a}

(b)

Fig. 7. (a) Protein patterns of surimi with different treatments before and after 6 days of storage; (b) band intensity analysis (%) based on protein patterns images. Different letters indicating significant differences among all groups within the same protein type ($P < 0.05$). N.D.: not detected. Acid: acid-treated surimi; Alkaline: alkaline-treated surimi; Water: water-washed surimi. d0: before storage; d6: after storage.

strength and water holding capacity on day 0. After storage, myofibrils in water-washed surimi degraded during the 6-day storage, the TEM images showed that the gel matrices of water-washed surimi on day 6 were more disturbed than on day 0 but remained with connections and hollows. Although a good water holding capacity was found in water-washed surimi, the gel strength was significantly lowered. The microstructures showed in Fig. 5(f) had smaller hollows than those in Fig. 5 (e), it was possible that only small amount of moisture and flour particles could be incorporated properly in such small hollows. Gliadin with such large size was difficult to be incorporated into the hollows, thus the gel strength of water-washed surimi on day 6 was lower. Besides, the raw water-washed surimi usually had the lowest content of myofibril proteins compared to pH shift method processed surimi (Zhou & Yang, 2019), the gel-forming process was not as profound as acid-treated surimi and alkaline-treated surimi (as indicated by temperature sweeps).

3.3.5. *Mechanism validation by protein patterns*

As a widely used method for separating and investigating specific proteins based on their molecular weight, SDS-PAGE was applied to study all samples on day 0 and day 6. As shown in Fig. 7(a), two major bands were found to be myosin heavy chain (MHC) around 217.7 kDa and actin around 46.1–49.2 kDa. Although myofibril proteins are mainly composed of these two proteins, there were additional high molecular weight proteins (HMWP) at the top of the gel, and new bands below MHC (~150 kDa) found for different samples. The specific band intensity is shown in Fig. 7(b).

On day 0, the content of HMWP of all samples showed no significant differences. The most interesting band was the new band at 150 kDa, which was absent in the water-washed surimi-d0 sample or the content was too little to be detected. This was probably due to the hydrolysis of MHC, by which it would degrade the myosin protein to form a band at 120–160 kDa (Kristinsson & Liang, 2006), while in water-washed surimi, the proteins didn't undergo the extreme pH treatments, thus no new proteins were formed due to hydrolysis or water washing.

On day 6, the HMWP band intensities of acid-treated surimi, alkaline-treated surimi, and water-washed surimi increased from 3.90 to 5.01%, 4.05 to 4.41%, and 4.10 to 5.15%, respectively, suggesting that more random coagulations took place during the storage period for acid-treated surimi and water-washed surimi. As seen from TEM images, coagulations on day 0 of all samples were less than those on day 6, which agreed with the HMWP increments. For MHC, the band intensity of water-washed surimi significantly decreased from 4.74 to 3.85%, in the meantime the new band was found to be formed under MHC, indicating that the storage promoted protein degradation or hydrolysis in water-washed surimi. The major gel strength decrease found in water-washed surimi after the heating process on day 6 could be due to the high contents of protein coagulation and the degradation of gelling proteins (mainly MHC) that occurred during storage. The formation of an organised gel matrix requires organised aggregation of the gelling proteins, and the coagulations are usually randomly distributed and can be detrimental to the gel quality (Stone & Stanley, 1992). This agreed with the presumption in the mechanism that the myofibril proteins were broken thus lowered the gel qualities. Similarly, band intensities of MHC in acid-treated surimi and alkaline-treated surimi also decreased with the increases of new bands on day 6. However, alkaline-treated surimi initially showed a poorer gel quality than water-washed surimi on day 0, but the better preservation ability of alkaline-treated surimi endured the 6 days of storage, thus less HMWP was found, and the contents of MHC didn't decrease as much as water-washed surimi, thus the gel quality increased on day 6.

The results obtained from the gel electrophoresis further supported our proposed mechanism. The high content of HMWP found in acid-treated surimi and water-washed surimi supported the theory that the quality degradation was mainly due to the coagulation observed by TEM, and the new bands formed in water-washed surimi on day 6 could be the source of the adverse odours detected by TVB-N due to protein decomposition. As for alkaline-treated surimi, the storage didn't cause too much protein degradation, but to provide sufficient time for the protein to unfold (Liu et al., 2019) for a better gel formation.

4. Conclusions

The present study showed that, although the protein content in the surimi treated with the pH shift method was higher than the traditional surimi, all surimi samples obtained the same storage period. In general, no significant colour changes were found during the storage, and the TVB-N values were all within the acceptable range. However, surimi processed with acid method showed very poor gel quality before and after storage; surimi processed with alkaline method showed worse gel quality than water washed surimi before storage, but alkaline treatment decreased the initial microbial counts without disrupting the myofibril proteins as deep as acid treatment, thus the gel quality of alkaline-treated surimi after 6 days of storage was improved and exceeded water-washed surimi. After heating, alkaline-treated surimi showed more solid-like structure starting from low-frequency range. Overall, surimi processed with the alkaline method was the most suitable method if applied in real practice. The results indicated that when mixing the raw surimi with other essential ingredients of surimi products, alkaline-treated surimi could stand the long period storage with good gel strength and water holding capacity.

CRediT authorship contribution statement

Yige Zhou: Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing - original draft. **Jonathan Jia He Liu:** Resources, Data curation, Formal analysis, Investigation. **Ying Kang:** Resources, Data curation, Formal analysis, Investigation. **Hanjing Cui:** Resources, Data curation, Formal analysis, Investigation. **Hongshun Yang:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

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

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