Effects of calcium chloride as a salt substitute on physicochemical and 3D printing properties of silver carp surimi gels

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ABSTRACT
This work investigated the effects of calcium chloride (CaCl$_2$) as sodium replacement on the physicochemical properties of silver carp surimi. As NaCl replaced by 0.32% and 0.63% CaCl$_2$, surimi gels presented better textural properties, breaking force and rheological properties, while 0.95%, 1.27% and 1.58% CaCl$_2$ led to the deterioration of gel strength. With the addition of CaCl$_2$, water holding capacity (WHC) decreased significantly ($p < .05$), the $T_{23}$ relaxation time decreased and the proportion of $T_{23}$ increased. Compared with control, the sulphhydryl content in surimi gel with 0.63% CaCl$_2$ increased by 23.61% and the solubility decreased by 17.31%. Furthermore, the Raman spectra data demonstrated that more β-sheet structures and random coils were induced in surimi gels prepared with CaCl$_2$. Then surimi with and without CaCl$_2$ was used as food-ink for three-dimensional (3D) printing, it indicated that surimi with 0.32% and 0.63% CaCl$_2$ could effectively improve the 3D printability.

Efectos del cloruro de calcio como sustituto de la sal en las propiedades fisicoquímicas y de impresión en 3D de geles de surimi de carpa plateada

RESUMEN
Este trabajo investigó los efectos del cloruro de calcio (CaCl$_2$) en las propiedades fisicoquímicas del surimi de carpa plateada cuando es utilizado como sustituto del sodio. Al sustituir el NaCl por 0.32% y 0.63% de CaCl$_2$, los geles de surimi presentaron mejores propiedades de textura, fuerza de rotura y propiedades reológicas, mientras que su sustitución con 0.95%, 1.27% y 1.58% de CaCl$_2$ provocó el deterioro de la resistencia del gel. Con la adición de CaCl$_2$, la capacidad de retención de agua (WHC) disminuyó significativamente ($p < .05$), el tiempo de relajación $T_{23}$ disminuyó y la proporción de $T_{23}$ aumentó. En comparación con el control, el contenido de sulfhidrilo del gel de surimi con 0.63% de CaCl$_2$ aumentó en 23.61% y su solubilidad disminuyó en 17.31%. Además, los datos del espectro Raman Raman demostraron que en los geles de surimi preparados con CaCl$_2$ se indujeron más estructuras de lámina β y espirales aleatorias. Posteriormente, se utilizó el surimi con y sin CaCl$_2$ como tinta alimentaria para la impresión tridimensional (3D), encontrándose que el surimi con 0.32% y 0.63% de CaCl$_2$ podía mejorar eficazmente la imprimibilidad en 3D.

1. Introduction
Excessive sodium in diets is closely related to the development of health problems, such as raised blood pressure and cardiovascular disease (Zheng, Han, et al., 2019). The current recommendation sodium intake from the Word Health Organization (WHO) is less than 2 g/day/person (Verma & Banerjee, 2012), while in China, the average daily salt consumption from foods is higher than 12 g/day/person (Mente et al., 2018). Processed food is a major source of sodium consumption. Therefore, it is urgent to reduce the amount of sodium chloride (NaCl) in processed food products to meet the healthy needs of consumers.

Surimi is produced by solubilizing the myofibrillar proteins from fish meat, which has unique gelling properties. Gelation is one of the most important steps to achieve the desired texture. Salt is a crucial element to solubilize myofibrillar proteins, which are necessary for the gelation (Ruth et al., 2018). During heating process, the extracted proteins underwent aggregation and cross-linking, leading to the formation of a three-dimensional network (Y. Zhang et al., 2019). To fully solubilize the myofibrillar proteins and develop surimi products with good gel properties, a high concentration (3%) of NaCl is generally required (Yamada et al., 2020).

To manufacture sodium-reduced surimi products, researchers have studied salt reduction methods extensively. Recently, Salt substitutes are the most common option for reducing salt content in food products. Addition of calcium chloride (CaCl$_2$) can not only reduce sodium content, but also offer additional calcium, which helps to enrich the nutrition of food products (Caceres et al., 2007). Calcium chloride can significantly increase the hardness and chewiness of meat because that there are negative charges on the polypeptide chains of proteins, a double layer of ionic groups could be formed through the connection of Ca$^{2+}$, leading to improvement of protein interactions. In addition, endogenous transglutaminase activity of surimi could be
activated by Ca\(^2+\), which catalyzes the acyl transfer reaction between ε-amino groups of lysine and γ-carboxamide groups of glutamine, leading to the formation of ε-(γ-glutamyl) lysine (Arfat & Benjakul, 2013; Ding et al., 2011). Benjakul et al. (2010) found that CaCl\(_2\) could improve gel properties of kamaboko gels produced from goatfish. Ding et al. (2011) also found that the gel strength of yellowcheek carp and grass carp surimi gels significantly increased by adding low concentrations of CaCl\(_2\). A research conducted by Pan et al. (2017) indicated that adding appropriate concentration of CaCl\(_2\) could improve the textural properties of low-fat meat products.

Three-dimensional (3D) printing technology could generate complicated shapes based on the additive manufacturing (AM) process, using layer-by-layer deposition (Arianna et al., 2019). 3D printing provides novel food products with numerous advantages, such as creating personalized nutrition, intricate shapes and reducing waste in food supply chain (Y. Liu et al., 2020). Surimi is an ideal food ink for extrusion-based 3D printing. Wang et al. (2018) investigated the effect of different levels of NaCl on the printability of silver carp surimi gel.

Although there are researches on the application of CaCl\(_2\) during surimi processing, whether it is suitable to replace NaCl in surimi products at the equivalent total ionic strength remains to be explored (Arfat & Benjakul, 2013; Pan et al., 2017). Furthermore, the mechanism of the substitution on silver carp surimi and 3D printability of surimi gels with salt substitution has not been investigated yet. Therefore, the main purpose of this research was to study the physicochemical characteristics and 3D printing properties of surimi gels with reduced NaCl by its partial substitution with CaCl\(_2\).

## 2. Material and methods

### 2.1. Materials

Frozen silver carp surimi (grade AAA) was provided from Hubei Jingli Aquatic Food Co., Ltd. and kept at −18°C until needed. All reagents used in the study were of analytical grade. According to the manufacturer, moisture content of surimi grade AAA is less than 77.0 ± 0.5%, pH is in the range of 6.8–7.4, whiteness is among 50–53, and the gel strength is in the range of 400–550 g x cm.

### 2.2. Surimi gel preparation

According to the method described by Zhang et al. (2015), frozen surimi was thawed overnight at 4°C, then cut into small pieces and chopped for 3 min by a mincer (Joyoimg Co., Ltd., Shandong, China). After that, 3% NaCl or its substitutes (shown in Table 1) were added, and the mixtures were chopped for 5 min using the mincer. During chopping, the temperature was kept at below 10°C. The homogeneous sol was tightly sealed into a polyamides casing (2.5 cm in diameter) and setting at 40°C for 30 min, then subjected to heating at 90°C for 20 min. After cooling, the gels were stored at 4°C overnight.

### 2.3. Texture profile analysis (TPA)

As described by Xu et al. (2010), the textural properties of surimi gels were evaluated using the TA.XTPlus texture analyzer (Stable Micro Systems, Godalming, UK) with a 36-mm diameter cylindrical probe. Surimi gels were cut into cylindrical samples (height 2.5 cm) and tested under conditions as follows: the testing speed was 1 mm/s, compression was 50%, and the sensing force was 5.0 g. The Texture Expert software was used to calculate hardness, springiness, gumminess, cohesiveness and resilience. Each measurement was replicated 9 times.

### 2.4. Gel strength analysis

According to Oujifard et al. (2012), gel strength analysis was performed using a texture analyzer with a spherical head (P/S). Surimi gels were cut into cylindrical samples (height 2.5 cm). The constant speed was 1 mm/s, and compression was 50%. During determination, the breaking force and distance to rupture of samples were recorded. The gel strength was the result of multiplying the breaking force by the distance to rupture (g x cm). Nine replicates of the samples were analyzed.

### 2.5. Water holding capacity (WHC) analysis

WHC was measured according to the method of Pan et al. (2017). The gels were placed into centrifuge tubes and centrifuged at 10,000 × g for 10 min at 4°C. After that, the tubes were inverted to drain. The residual water in tubes and gel surface was carefully removed using dry filter paper. Then, the tubes with initial gel and centrifuged gel were accurately weighted. WHC (%) was calculated as the ratio of centrifuged gel weight relative to the initial gel weight multiplied by 100.

### 2.6. Rheological analysis

According to the method of Huang et al. (2016), surimi pastes were subjected to determine rheological properties. The storage (G’) and loss (G”) modulus were measured by a rheometer (DHR-3, TA, USA) equipped with parallel plate geometry (20 mm, 1 mm gap). Measurement was conducted at 1% strain and 1 Hz frequency. The surimi paste was poured onto the rheometer bottom plate, and the excess sample was removed. Temperature sweep tests (25 to 90°C) was carried out at 2°C/min. Silicone oil was used to cover the applied sample to avoid evaporation.

### 2.7. Low-field nuclear magnetic resonance (LF-NMR) relaxation time (T_2) measurements

LF-NMR \(^1\)H T_2 measurements were conducted according to Pan et al. (2017) with minor modification. A spectrometer (MesoMR23-060 V–I, Niumag Corporation, Shanghai, China)

### Table 1. Salt substitutes (%) in surimi gels at equal ionic strength (IS).

| Treatment       | Sodium Chloride IS (M) | % | Calcium Chloride IS (M) |
|-----------------|------------------------|---|-------------------------|
| Control         | 0.513                  | 3.00 | 0.427                   | 0.086 | 0.32 |
| T1              | 0.427                  | 2.50 | 0.342                   | 0.171 | 0.63 |
| T2              | 0.342                  | 2.00 | 0.256                   | 0.257 | 0.95 |
| T3              | 0.256                  | 1.50 | 0.171                   | 0.342 | 1.27 |
| T4              | 0.171                  | 1.00 | 0.085                   | 0.428 | 1.58 |
| T5              | 0.085                  | 0.50 |                         |       |     |
at 23.2 MHz was used. Glass tubes (25 mm in diameter) were used to place the samples (2 g). The T2 was measured using the Carr-Purcell-Meiboom-Gill sequence, with the following settings: 32 scans, 4500 echoes, 6.5 s between scans, with t-value (time between 90 and 180 pulses) of 200 ms. Multi Exp Inv Analysis software (Niumag Corporation, Shanghai, China) was used for data analysis.

2.8. Raman spectral analysis

Raman spectra of surimi gel were collected according to the procedure described by Yu et al. (2017). A Raman spectrometer equipped with a 632.8 nm excitation line was used. The line was focused on samples paved on glass slides by a microscope (Horiba Jobin Yvon S.A.S., France) equipped with a 50× objective. The spectra were recorded in the range from 400 to 3500 cm⁻¹. The conditions of each spectrum of samples were as follows: 1 scan, 2 cm⁻¹ resolution, 30 s exposure time, 2 min per spectrum and a sampling speed of 120 cm⁻¹/min, with data collected every 1 cm⁻¹. LabSpec version 5.0 was used to baseline-correct and smooth the spectra obtained.

2.9. Sulphydryl (SH) group contents analysis

Total sulphydryl group (-SH) content analysis was based on the method described by Frank and John (1995) with minor modifications. Samples (0.10 g) were homogenized in 15 mL of buffer solution (pH 8.0, 0.09 mol/L glycine, 0.086 mol/L Tris, 8 mol/L urea, 4 mmol/L EDTA), and stirred for 6 h at ambient temperature. Then the solution was centrifuged at 12,000 × g for 10 min. For each sample, 0.3 mL of 10 mmol/L DTNB (5,5’-Dithiobis-(2-nitrobenzoic acid)) was added into 3 mL supernatant. The absorbance was determined at 412 nm. The SH concentration was estimated by a molar extinction coefficient of 13,600 M⁻¹ cm⁻¹. Results are expressed as SH moles per 10⁶ g of protein.

2.10. Solubility analysis

According to Benjakul et al. (2004), surimi gels (1 g) were solubilized in 20 mL of 20 mmol/L Tris-HCl solution (pH 8.0, 8 mol/L SDS, 1% (w/v) urea, 2% (v/v) β-mercaptoethanol), then the solution was incubated at 100°C for 2 min. Before centrifugation at 10,000 × g for 30 min, the resulting homogenerate was stirred for 4 h at 25°C. Then 50% (w/v) cold TCA was added into supernatant (10 mL) to make the final concentration of the solution 10%, then kept at 4°C for 18 h. After that, the solution was centrifuged at 10,000 × g for 30 min, then the resulting pellet was washed by 10% TCA, and was solubilized by 0.5 mol/L NaOH. The total protein contents of the samples was measured by suspending gels in 0.5 mol/L NaOH directly and using Lowry methods (Lowry et al., 1951). The solubility was calculated as the percent of the total protein.

2.11. Three-dimensional printing process

A Shinnove-D1 3D printer (Shiyin Co., Ltd., Hangzhou, China) was used in this study. A cylindrical model (20 mm in diameter and 10 mm in height) was designed by Rhinoceros 5.0 software. According to Chen et al. (2021), printing parameters were set as follows: layer height, 0.5 mm; nozzle movement speed, 25 mm/s; nozzle diameter, 0.5 mm. The whole printing process was carried out around 25°C.

2.12. Statistical analysis

Analysis of variance (ANOVA) was used for data analysis. In order to evaluate the statistical significance (p < .05), comparisons of group means were analysed by Duncan’s multiple range test using SPSS 19.0 software (SPSS Inc., Chicago, IL). All measurements were performed on at least three times

| Table 2. Texture profile analysis (TPA) of surimi gels added of blends of salt substitutes. |
|--------------------------------------|----------------|----------------|----------------|----------------|
| Treatment   | Hardness (g) | Springiness | Cohesiveness | Gumminess | Resilience |
| Control     | 3085.15 ± 136.47⁣<sup>a</sup> | 0.95 ± 0.00⁣<sup>bc</sup> | 0.71 ± 0.01<sup>c</sup> | 2241.59 ± 40.84<sup>d</sup> | 0.36 ± 0.01<sup>a</sup> |
| T1          | 3916.68 ± 18.80<sup>bc</sup> | 0.92 ± 0.01<sup>bc</sup> | 0.68 ± 0.00<sup>c</sup> | 2320.29 ± 35.97<sup>c</sup> | 0.33 ± 0.00<sup>b</sup> |
| T2          | 3961.60 ± 143.75<sup>abc</sup> | 0.92 ± 0.01<sup>bc</sup> | 0.66 ± 0.00<sup>c</sup> | 2511.94 ± 29.68<sup>c</sup> | 0.33 ± 0.00<sup>b</sup> |
| T3          | 4075.40 ± 114.51<sup>c</sup> | 0.91 ± 0.00<sup>c</sup> | 0.63 ± 0.01<sup>c</sup> | 2633.80 ± 46.85<sup>c</sup> | 0.31 ± 0.01<sup>c</sup> |
| T4          | 3710.12 ± 67.93<sup>c</sup> | 0.91 ± 0.00<sup>c</sup> | 0.60 ± 0.00<sup>c</sup> | 2541.53 ± 31.70<sup>b</sup> | 0.30 ± 0.00<sup>c</sup> |
| T5          | 3759.43 ± 196.92<sup>bc</sup> | 0.91 ± 0.01<sup>bc</sup> | 0.57 ± 0.01<sup>f</sup> | 2158.86 ± 70.68<sup>d</sup> | 0.28 ± 0.00<sup>d</sup> |

Values are mean ± standard deviation (SD). Different letters (a–f) in the same column indicate significant differences (p < .05) according to Duncan’s test.

T1: 2.50% NaCl+0.32% CaCl₂; T2: 2.00% NaCl+0.63% CaCl₂; T3: 1.50% NaCl+0.95% CaCl₂; T4: 1.00% NaCl+1.27% CaCl₂; T5: 0.50% NaCl+1.58% CaCl₂.

Los valores representan la media ± desviación estándar (DE). Las distintas letras (a-f) en la misma columna indican diferencias significativas (p < .05) según la prueba de Duncan.

T1: 2.50% NaCl+0.32% CaCl₂; T2: 2.00% NaCl+0.63% CaCl₂; T3: 1.50% NaCl+0.95% CaCl₂; T4: 1.00% NaCl+1.27% CaCl₂; T5: 0.50% NaCl+1.58% CaCl₂.
an important role in the formation of gels with enhanced gel properties.

The highest springiness value occurred in control. It decreased significantly when NaCl was substituted by CaCl$_2$. However, no significant changes were found among the T2-T5 treatments ($p > .05$). Adding CaCl$_2$ contributes to increasing rigidity or brittleness of a low sodium surimi gel (Petcharat & Benjakul, 2017). In comparison with the control, samples with the addition of CaCl$_2$ showed lower cohesive-ness and resilience, which further reduced when more CaCl$_2$ existed in the surimi gel. Thus, adding a high amount of CaCl$_2$ negatively affected the textural character of the surimi gels as indicated by the loss in hardness, elasticity and cohesiveness. Similar results were also found by Ding et al. (2011), Horita et al. (2011), and Benjakul et al. (2010). According to these authors, the reduction of textural properties was attributed to a poor gel network.

3.2. Gel strength analysis

According to Figure 1a, compared with control, the breaking force of surimi gels increased significantly ($p < .05$) in the presence of 0.32% and 0.63% CaCl$_2$, while distance to rupture decreased. No significant change in breaking force ($p > .05$) was observed among the T3, T4, T5 and the control. Distance to rupture decreased significantly ($p < .05$) as more

![Figure 1. Breaking force, distance to rupture (a) and gel strength (b) of surimi gels added of blends of salt substitutes. Different letters on the top of SD bars are used to indicate significant differences (Duncan’s test, $p < .05$). T1: 2.50% NaCl+0.32% CaCl$_2$; T2: 2.00% NaCl+0.63% CaCl$_2$; T3: 1.50% NaCl+0.95% CaCl$_2$; T4: 1.00% NaCl+1.27% CaCl$_2$; T5: 0.05% NaCl+1.58% CaCl$_2$.](image-url)
NaCl was substituted by CaCl$_2$. Distance to rupture of T5 was reduced by 29.7% compared with the control. The results were consistent with the texture profile analysis, that the addition of CaCl$_2$ decreased the springiness of surimi gels.

Figure 1b shows that there was no significant difference of gel strength among the control and surimi gel with 0.32% and 0.63% CaCl$_2$. However, when 0.95%, 1.27% and 1.58% CaCl$_2$ were added, the gel strength of surimi gel decreased significantly ($p < .05$), indicating that excessive replacement of NaCl by CaCl$_2$ resulted in poor gel properties. Similar results were found by Hunt and Park (2013), who incorporated 2% CaCl$_2$ as a replacement for NaCl to decrease gel strength of Alaskan Pollock surimi gels. A reasonable explanation was that excessive addition of CaCl$_2$ resulted in a greater aggregation extent of the proteins, preventing network formation, leading to decreased gel strength (Jia et al., 2015).

### 3.3. Water holding capacity (WHC) analysis

WHC is usually used to evaluate interactions between protein and water. As shown in Figure 2, there was nonsignificantly ($p > .05$) effect on WHC for CaCl$_2$ at low concentration. However, as more NaCl was substituted by CaCl$_2$, the amount of removal water increased significantly ($p < .05$). Compared with control, the WHC of T5 decreased from 96% to 81%. A similar result was reported by Martínez-Alvarez and Gómez-Guillén (2013), in which the WHC of the surimi gels was reduced with the addition of CaCl$_2$. Pan et al. (2017) also found that 0.10 M and 0.12 M CaCl$_2$ led to a significant decrease in WHC. NaCl could make myofibrillar proteins swell and break into actomyosin, myosin, and other protein complexes. Low content of NaCl reduced the interactions between protein and protein or protein and water (Kong et al., 2008). Barbut (1994) believed that a high concentration of CaCl$_2$ induced excessive aggregation of the protein, resulting in the formation of coarse protein networks in the gel system. Therefore, the weak gel matrix led to a decrease in WHC.

3.4. Rheological analysis

During the heating process, surimi presented typical viscoelastic solid-like behavior. Figure 3 shows that the storage modulus of the surimi was significantly affected by the addition of CaCl$_2$. Similar $G'$ curves were observed for the control and surimi pastes with low CaCl$_2$ content (T1, T2, and T3), presenting the typical four-stage rheological behavior of myosin (Liu, Bao, et al., 2014). As the temperature increased, $G'$ showed a slight acceleration slope first then declined dramatically. After that, the value of $G'$ underwent a fast growth then a slight increase until the temperature reached 90°C. The temperature at which the $G'$ presented the minimum value was defined as the critical temperature ($T_d$) (Yu et al., 2017). However, for T4 and T5, $G'$ curves presented a three-stage change. It first decreased slightly then declined sharply with the temperature increased. Then it kept increasing until the heating process was stopped. Before temperature reached 50°C, sample T2 presented the highest $G'$ value among all treatments, while surimi with a high content of CaCl$_2$ showed lower $G'$ values. Before the temperature reached 55°C, the $G'$ values of control, T1 and T2 were higher than those of T3, T4 and T5. When the temperature kept increasing, the $G'$ of T2, T3, T4 and T5 increased sharply and presented significantly higher values than T1 and control. As the concentration of CaCl$_2$ increased, for T$_d$ of samples, it declined from 59 to 47 °C from 0 to 1.58%. A reasonable explanation of the decrease was that heating had a greater impact on protein in surimi, demonstrating that the thermal stability of proteins decreases in response to a high concentration of CaCl$_2$. During the heating process, denaturation of components of myofibrillar protein was important to the formation of surimi gel (Reed & Park, 2011). The dissociation of actin–myosin and denaturation of light meromyosin led to the initial decrease of G’ (Jia et al., 2016). As the temperature was further increased, heavy chain myosin and actomyosin began to denature, thermo irreversible gel network was formed, leading to the increase of G’ (Mleko & Foegeding, 2000). Similar rheological properties were
reported by Yin and Park (2014), the $G'$ value for surimi with 1% nano-scaled fish bone (NFB) was higher than other groups. The first peak temperature of $G'$ was also changed with the addition of NFB.

### 3.5. LF-NMR spin-spin relaxation time ($T_2$) measurements

LF-NMR was applied to estimate the changes in water distribution in the surimi gels. $T_2$ relaxation curves of samples with different treatments are presented in Figure 4. As shown in Figure 4, the water populations were centered at approximately 1–10, 20–100 and 200–2200 ms, which respectively referred as $T_{21}$, $T_{22}$ and $T_{23}$. In protein foods, it has been proposed that $T_{21}$ represents bound water, which is trapped within the protein-dense myofibrillar network. $T_{22}$ is ascribed to immobilized water, which located in tertiary and quaternary protein structures with high myofibrillar protein densities. $T_{23}$ represents more mobile water located outside the myofibrillar network (Sanchez-Alonso et al., 2014; Zhang et al., 2016).

Table 3 shows the relaxation time changes of the surimi gels. $T_{21}$ position of control was similar to that of T1, T2 and T4 ($p > .05$). $T_{22}$ and $T_{23}$ tended to shift to lower values in surimi gels with a high concentration of CaCl$_2$. For samples with 1.58% CaCl$_2$, the

![Figure 3. Dynamic rheometry measurements of surimi gels added of blends of salt substitutes by temperature sweep. T1: 2.50% NaCl+0.32% CaCl$_2$; T2: 2.00% NaCl+0.63% CaCl$_2$; T3: 1.50% NaCl+0.95% CaCl$_2$; T4: 1.00% NaCl+1.27% CaCl$_2$; T5: 0.05% NaCl+1.58% CaCl$_2$.](image)

![Figure 4. Representative of $T_2$ relaxation times in surimi gels added of blends of salt substitutes. T1: 2.50% NaCl+0.32% CaCl$_2$; T2: 2.00% NaCl+0.63% CaCl$_2$; T3: 1.50% NaCl+0.95% CaCl$_2$; T4: 1.00% NaCl+1.27% CaCl$_2$; T5: 0.05% NaCl+1.58% CaCl$_2$.](image)
position of $T_{22}$ was 80% lower than control. The decrease in the $T_{22}$ relaxation time indicated that more mobile water existed inside the gels. That is to say, the interactions between gel network and water tended to be weaker, demonstrating that the network structure of surimi gel was changed by adding CaCl$_2$.

The peak area proportion of $T_{22}$ did not significantly change among the surimi gel samples. $T_{22}$ represents immobilized water, it accounted for 94–98% of the total signal. The existence of CaCl$_2$ decreased the proportion of $T_{22}$. For the proportion of $T_{2b}$, there was no obvious change between the control and the T1 treatment. However, it significantly increased in the other CaCl$_2$ treated samples ($p < 0.05$), particularly in the corresponding low-sodium-treated samples. The proportion of $T_{2b}$ in sample T5 was about 14 times that of the control, indicating that surimi gels with more NaCl restricted water movement. Pan et al. (2017) also reported that more free water existed in the gel network of the high-Ca-treated groups. That was also consistent with the WHC results.

### 3.6. Raman spectral analysis

Raman spectra (400–3,500 cm$^{-1}$) of surimi gel were collected and presented in Figure 5a. Information about the polarity of protein microenvironment can be provided by Raman spectrum bands of some aromatic amino acid side chains. When buried tryptophan (Trp) residues from hydrophobic microenvironment are exposed, the hydrophobic interaction of proteins will change (Kobayashi et al., 2017). As shown in Figure 5b, the normalized intensity of the 760 cm$^{-1}$ bands tended to be lower when NaCl was replaced by CaCl$_2$ in the surimi gels, indicating that adding CaCl$_2$ could induce more Trp residues to be exposed, leading to stronger hydrophobic interactions among protein molecules.

The vibration of the p-substituted benzene ring of tyrosine (Tyr) residues resulted in two peaks occurred in the Raman spectrum at 830 cm$^{-1}$ and 850 cm$^{-1}$. The local micro-environment around Tyr residues can be indicated by the intensity ratio of the doublet bands ($I_{850}/I_{850}$) (Zhang et al., 2016). When the Tyr residues are exposed to the polar environment, the value of $I_{850}/I_{850}$ is in the range of 0.90 to 2.50. A value of 0.70–1.0 indicates that the Tyr residues are buried in the hydrophobic environment (Pan et al., 2017). In our case, the value of $I_{850}/I_{850}$ of the control was 1.11, and there was an increase as CaCl$_2$ was added ($p < 0.05$) (Figure 5b). The $I_{850}/I_{850}$ ratio increased as more NaCl was replaced by CaCl$_2$, which ranged from 1.16 to 1.73 for T1-T5, indicating that a higher concentration of CaCl$_2$ led to a more polar environment (Zhang et al., 2015).

The Raman bands in the range of 1600–1700 cm$^{-1}$ (Amide I) is the most important region to indicate variations in protein secondary structure (Zhou, Zhao, et al., 2014). It consists of C = O stretching, C-N stretching, C-C-N bending, and N-H in-plane bending vibrations (Herrero et al., 2008; Shao et al., 2015). Generally, the amide I band in 1650–1658 cm$^{-1}$ and 1665–1680 cm$^{-1}$ were assigned to α-helix and β-sheet respectively, the band near 1680 cm$^{-1}$ and 1660–1665 cm$^{-1}$ attributed to β-turn and random coil structures respectively (Zheng, Di, et al., 2019). As shown in Figure 5a, the control presented a characteristic peak at 1667 cm$^{-1}$, indicating a predominance of β-sheets. The amide I band of the T1-T5 surimi gel samples exhibited maximum scattering in the range of 1669–1673 cm$^{-1}$. It increased with more NaCl replaced by CaCl$_2$, demonstrating a high proportion of β-sheet structure. This result indicates the formation of a more ordered proteins structure as CaCl$_2$ was added.

According to Nunez-Flores et al. (2018), the relative contributions of protein secondary structure were estimated by deconvolving and fitting the amide I band. A quantitative estimate of the α-helix, β-sheet, β-turn, and random coils is presented in Figure 5c. The proportion of the α-helix structure was higher in control than that of the other groups, while the amount of random coil was less. The proportion of α-helix structures in samples T1-T5 decreased from 24.02% to 13.15%, accompanied by an increase of the β-sheet from 40.42% to 47.88%. The highest amount of random coil in T4 was nearly two fold than control. Based on transformation of the peak position and changes in secondary structural content, we assumed that CaCl$_2$ induced an increase in the β-sheets and random coils of myosin, which was similar to that reported by Barrett et al. (1978).

The amide III bands also provide information to estimate the secondary structure of the protein backbone. The α-helix usually has a band centered at about 1,275 cm$^{-1}$, while the high β-sheet content is illustrated by a more intense band near 1238–1245 cm$^{-1}$. The random coil structure usually appears near 1250 cm$^{-1}$ (Xiong et al., 2016). As shown in Figure 5a, bands that appeared at 1243–1245 cm$^{-1}$ demonstrated the existence of the β-sheet structure in control, T1 and T5. Bands near 1,250 cm$^{-1}$ were present in samples with a high concentration of CaCl$_2$ (T2, T3, and T4), indicating a predominance of the random coil structure. This result coincided with what was obtained from the amide I analysis.
3.7. **Sulphydryl (SH) group contents analysis**

Figure 6 shows the changes in SH group contents for surimi gels added of blends of salt substitutes. SH content first increased significantly then decreased as CaCl\(_2\) was added. For T2, T3 and T5, SH group content decreased with the concentration of CaCl\(_2\) increasing in surimi gel. When CaCl\(_2\) was added from 0.32% to 1.58%, the total sulphydryl content decreased by 26.74%. A decrease in total sulphydryl groups...
indicated that CaCl₂ induced the unfolding of myosin and actin during the heating process, promoting the exposure of free SH groups, which underwent disulfide interchanges, leading to the conversion of free SH groups into the disulfide bonds (Liu, Gao, et al., 2014; Zhou, Lin, et al., 2014). The formation of disulfide bonds could contribute to the improved textural properties (Yin & Park, 2014). Similar results were reported by Yongswatdigul (2005) and Ding et al. (2011), that the SH groups of myosin continuously decrease as CaCl₂ concentration was increased.

3.8. Solubility analysis

The ε-(γ-Glu)-Lys non-disulfide covalent bond induced the formation of protein-protein interactions, resulting in low solubility of protein gels, which was in relation to the gel matrix formation (Benjakul et al., 2004; Rawdkuen et al., 2004). Compared with the control, Solubility decreased significantly (p < .05) as the concentration of CaCl₂ was increased to 0.63% (Figure 7). When NaCl was further replaced by CaCl₂, the solubility increased significantly.
(p < .05). The solubility of T4 and T5 was significantly greater than the other samples, suggesting fewer non-disulfide covalent bonds. Ding et al. (2011) also reported that endo-TGase activity in grass carp could be activated by a low concentration of CaCl₂, while adding excessive concentration of CaCl₂ would lead to a negative effect on the formation of ε-(γ-Glu)-Lys covalent bonds. On the other hand, a NaCl deficiency inhibits the extraction of myofibrillar protein, inhibiting the formation of non-disulfide covalent bonds (Gordon & Barlow, 1992).

3.9. 3D printing properties of surimi gel

The printability of surimi gels added of blends of salts substitutes was determined. As shown in Figure 8, it can be observed that the control group, T1 and T2 group presented a smooth and compact surface with a well-defined network. With more NaCl was substituted by CaCl₂, the printed samples tended to be more uneven and less continuity. When the amount of CaCl₂ was 1.58%, the printed surimi (T5) obtained the least uniform shape during 3D printing, leading to the poorest printing accuracy. Wang et al. (2018) also found that the printed constructs exhibited a smoother surface texture with more NaCl addition.

4. Conclusions

The partial substitution of NaCl by CaCl₂ appears to be a reliable strategy to reduce the sodium in surimi products. The addition of 0.32% and 0.63% CaCl₂ positively promoted the functional properties of surimi gels because it led to higher hardness, rheological properties and 3D printing capability. Those properties were ascribed to the formation of disulfide bonds, non-disulfide covalent bonds, stronger hydrophobic interactions, and higher β-sheet content. There was no significant difference of WHC between control and surimi with 0.63% CaCl₂. When NaCl was excessively substituted by 0.95%, 1.27% and 1.58% CaCl₂, the gel properties of surimi gels tended to decrease. In conclusion, appropriate replacement of NaCl by CaCl₂ improved the gel properties of surimi gels and can be used 3D printing ink to create low sodium 3D surimi products.

Disclosure statement

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