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Understanding the role of $CaCl_2$ in salt substitute for low-salt and high-quality surimi products

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ABSTRACT

Salt substitute has been widely used to prepare low-salt foods due to potential health benefits, though the role of $CaCl_2$ in salt substitute and its unique impacts on food quality have been rarely investigated. In this study, comprehensive research has been conducted to elucidate the effects of replacing NaCl with varying concentrations of $CaCl_2$ on the surimi gel characteristics. The introduction of $CaCl_2$ interacted with surimi proteins differently from NaCl, thus leading to difference in protein aggregation behaviors and surimi gel properties. It has been found that a proper proportion of $CaCl_2$ for NaCl substitution could create salt bridges between surimi proteins more effectively, resulting in an ordered, smooth and dense gel network with an increased water holding capacity (WHC) and improved gel strength. Furthermore, TGase activated by Ca^{2+} boosted the formation of ε-(γ-glutamyl) lysine bonds, which cross-linked surimi proteins to form a firm gel with a better three-dimensional structure. However, replacing NaCl with excessive amount of $CaCl_2$ as divalent salts induced more serious protein aggregation, leading to water loss and gel properties deterioration. More specially, replacing NaCl with $CaCl_2$ at 50% showed the best performance, as evidenced by the most abundant disulfide bonds and hydrophobic interactions, highest hardness and chewiness, and greatest storage modulus. This study provided new insights on developing high-quality surimi gels with significantly reduced salt concentration and improved gel characteristics.

1. Introduction

Fish has become a significant and preferred dietary component of human beings due to good taste, unique textures and great health benefits (Walayat et al., 2023). Out of the 179 million tons of fish produced in 2018, 156 million tons were consumed as foods, equal to 20.5 kg per person. In addition, 17.1% of the world's protein supply was obtained from fish and fish products in 2017, which makes them the third-largest source of protein consumed globally after cereals and milk (Pankyamma et al., 2022). According to FAO, as the global population continues to grow, the overall demand for food will increase by at least 60% by 2030. The demand for resource-intensive food products, such as fish products, is expected to grow faster (Cooney et al., 2023).

Fish can be processed into restructured fish products such as surimi-based seafoods. Salt (NaCl) at a concentration of 2–3 g/100g is typically added during manufacturing of surimi seafood. The salt dissolves fish myofibrillar proteins in surimi, creating a viscous protein paste. During cooking, the surimi proteins crosslink to form an elastic surimi gel, providing final surimi seafood products with the proper texture and other sensory qualities. The salt concentration plays a significant role in the formation of gel properties of the surimi-based products, as it influences the dissolubility of surimi proteins. A reduction in the salt concentration can affect the functional and mechanical properties of the gel resulting into less desirable characteristics of the restructured surimi seafood (Tahergorabi and Jaczynski, 2012).

Over the past few years, a high daily NaCl intake has been linked to hypertension, stroke, kidney disease, and even more. In 2021 WHO reported that, approximately 1,28 billion adults worldwide were affected by hypertension, with higher prevalence in low- and middle-income countries. To address this issue, WHO has recommended food

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manufacturers to reformulate salt content in their products, aiming for a 30% reduction of global sodium intake by 2025, as well as to increase public awareness on the detrimental effects of excessive salt consumption. Excessive salt consumption is among the major contributors of high blood pressure which in turn increases the risk of cardiovascular diseases (CVD) and stroke. NaCl added during food production, preparation and serving is the primary source of dietary Na. Guidelines published by public health and regulatory authorities recommend a reduction of Na intake to 2 g/day or less (Greiff et al., 2015).

Reducing salt content in processed food products typically involves lowering the concentration of added salt (NaCl), replacing it with alternative chloride salts such as KCl, $CaCl_2$, and $MgCl_2$, as well as incorporating flavors and preservatives, or utilizing a combination of these methods. Most attention is on KCl. Many commercial goods partially replace NaCl with KCl due to potassium's antihypertensive properties and considerably higher than the maximum recommended intake of sodium (2300 mg/day and 4700 mg/day) (Tahergorabi and Jaczynski, 2012).

From the technological point of view, KCl has been used to ensure the ionic strength necessary to develop stable gels; however, its use alone results in a bitter, astringent and metallic taste (Horita et al., 2011). Salt replacements like $CaCl_2$, and $MgCl_2$ can lower meat sodium. However, there has been relatively little investigation on how these salts affect the stability and sensory acceptability of the end-products.

Chloride salts such as $CaCl_2$, $MgCl_2$, $ZnCl_2$, and NH_4Cl affect protein functioning during gelation. Divalent metal ions like Ca^{2+}, Mg^{2+}, and Zn^{2+} might cause changes in protein conformation by interacting with negative charges on forming a double layer of ionic groups, which decreases electrostatic interaction between protein molecules, resulting in the changes in protein-protein, protein-water interactions. Ca^{2+} can also enhance surimi's endogenous enzyme transglutaminase (endo-TGase) activity, which catalyzes the acyl transfer reaction between glutamine's γ-carboxyamide groups and lysine's ε-amino groups, forming stronger gels through ε-(γ-glutamyl) lysine cross-linking. Many studies have examined how $CaCl_2$ affects surimi gel's ε-(γ-glutamyl) lysine cross-linking and texture. In addition to ε-(γ-glutamyl) lysine cross-linking, other chemical forces including: hydrophobic interactions, hydrogen bonds, ionic bonds, and disulfide bonds contribute to the formation of surimi gel network. The relative contribution of each type of chemical interaction to surimi gel networks varies with protein properties, ambient circumstances, and gelation protocols, resulting in various textural features and water holding capacity of the gel. Examining the ways in which $CaCl_2$ affects chemical interactions and gel characteristics is essential for comprehending its effects on the production of surimi gel. Understanding protein and ion activities and interactions in surimi can help the surimi processing industry improve product quality and generate desirable products (Ding et al., 2011).

Given its crucial role in the human body, magnesium deficiency has been linked to various health issues such as cardiovascular diseases, hypertension, diabetes and migraines. It is also important for bone development and may enhance athletic performance. Studies have explored magnesium supplementation in relation to conditions such as cardiovascular disease, hypertension, diabetes, asthma, migraines and pregnancy. Consequently, substituting sodium with magnesium in fish and meat where salt formulations are needed, seems to be a beneficial approach. It not only reduces sodium intake, but also enriches the diet with magnesium which has minimal negative effects (Barat et al., 2012).

Until now, many chloride salts have been investigated as replacer for NaCl in food systems, such as $MgCl_2$, $ZnCl_2$ and $CaCl_2$. However, previous study found that $MgCl_2$ could cause off-flavors with additional sensations described as salty, metallic, astringent and sour when used as a substitute for NaCl (Lawless et al., 2003). Though $ZnCl_2$ showed less off-flavors issues as sodium substitute, its recommended dietary allowances (RDAs) for adults were only between 7 and 19 mg (Brown et al., 2004), and replacing NaCl with zinc salt would far exceed the maximum recommended intake of zinc (Yasir and Soottawat, 2013). Ca^{2+} has a lighter bad taste than other divalent cation salts in food systems (Harry et al., 2003), and it also plays an important function in supplementing dietary calcium and preventing osteoporosis. Moreover, its potential use as effective sodium replacer has also been verified by many available studies (Pi et al., 2022; Yu et al., 2022).

To prevent potential negative effects on customers, the probability of hazardous consequences must be taken into account and researched. Salt, known for imparting a salty flavor, is predominantly salty when in the form of LiCl and NaCl. Other mono and divalent salts may also impart a range of flavors including: bitter, salty, sour and stringent. Lawless and colleagues conducted research using a Duncan test to evaluate the salinity and bitterness of magnesium salts, both chloride and sulfate forms. They observed that the intensity of these flavors increased as the salts concentration was increased. Few variations were also observed between the flavors of NaCl and $MgCl_2$ salts with specific reference to sweetness, umami, sour and metallic flavors. Notably, Lawless and colleagues also found out that $MgCl_2$ could cause off-flavors when used as a substitute for NaCl, however, the presence of NaCl and other cations can mask these unpleasant flavors particularly bitterness (Lawless et al., 2003).

Osteoporosis, a condition characterized by weak and brittle bones, affects between 20 and 30 million Americans and is the underlying cause of the majority of bone fractures in the elderly. This condition often results from insufficient calcium intake. To address this, an increasing number of foods and beverages are being fortified with calcium to enhance their nutritional value. As part of the trend towards nutritionally fortified and multifunctional foods, the market has seen a rise in products that are calcium-enriched. The FDA permits the addition of nutrient fortifiers like calcium to food products when they are used to remedy a dietary deficiency that is well-recognized by the scientific community as leading to specific nutritional deficiency diseases. Consequently, the food industry has been actively producing a range of calcium-rich foods, supporting consumers in meeting their nutritional needs and in the prevention of osteoporosis. It is generally understood that divalent salts, which are commonly used to fortify foods with calcium, impart a complex flavor acceptable to most people. In these compounds, calcium serves as the cation paired with various anions (Ima Wijayanti and Soottawat, 2021).

Calcium is essential for human health. Understanding the flavor profile of calcium salts may lead to the development of better calcium-fortified foods. However, taste attributes of calcium and other divalent salts are not well documented. Calcium salts taste undesirable in solution, but their effect in the overall food flavor is still unclear. Mixture suppression due to the presence of other flavors in the dish could potentially reduce or mask the sensory impact of the calcium salts. Unlike some bitter compounds whose bitterness is effectively repressed by sodium ions, previous research found that NaCl and sodium gluconate can slightly mask the bitterness of $MgSO_4$. It is still questionable whether $CaCl_2$ and NaCl exhibit the same patterns of suppression. Investigation of the mixing effect of $CaCl_2$ with monogustatory tasters like sucrose, sodium chloride, and citric acid in straightforward systems may produce insights into flavor interactions (Lawless et al., 2003).

2. Materials and methods

2.1. Materials and reagents

$CaCl_2$ and NaCl, both of food-grade quality were purchased from the local market. Zhejiang Xingye Group Co., Ltd. (Zhoushan, China) supplied grade AAA frozen surimi made from red gurnard (*Chelidonichthys cuculus*). HCl, NaCl, CH_4N_2O and other required chemicals were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the reagents used in this experiment were of analytical grade and utilized directly without further treatment.

2.2. Preparation of surimi gels

In this study, the red gurnard surimi was chosen due to its easy availability and wide distribution (Shimada and Tsuruwaka, 2024). The frozen surimi was removed from the −80 °C environment and thawed at 4 °C overnight. Afterwards, it was cut into small pieces for subsequent chopping and mixing. The mixing was performed at 1000 rpm for 30 s and then at 3000 rpm for 1 min. To control the overall chloride salt concentration at 2.5%, different proportions of $CaCl_2$ (0, 0.4, 0.5, 0.6, 1.0) were weighed and added to the chopping machine in place of NaCl. It should be noted that the replacing ratio of NaCl by $CaCl_2$ at 0–1.0 in such an interval was decided based on previous findings (Ima Wijayanti and Soottawat, 2021; Ramírez et al., 2002). Ice water was added to adjust the water content of the surimi to 78% (w/w) (Yang et al., 2022). The paste was mixed for 3 min, while maintaining the temperature below 10 °C. The surimi paste was then poured into polyethylene casings with a diameter of 25 mm. The surimi paste-filled casings were then heated for 30 min at 40 °C, followed by 20 min at 90 °C, and subsequently cooled in ice water (Zhang et al., 2023). The resulting surimi gels were named Ca0, Ca0.4, Ca0.5, Ca0.6, and Ca1.0, with the number indicating the proportion of $CaCl_2$ added in place of NaCl, then stored overnight at 4 °C for further analysis.

2.3. Surimi gel characterization

2.3.1. Texture profile analysis (TPA)

The texture profile analysis (TPA) was carried out using the protocol outlined by Zhao et al. (2023a,b). The surimi gel was manually cut into the cylinder with dimensions of 25 mm in diameter and 25 mm in height. The TPA was conducted using a cylindrical P/36R probe with a diameter of 36 mm on the TA-XT Plus texture analyzer (Stable Microsy Stems Company, UK), set at a speed of 3 mm/s and with a compression distance of 5 mm (Wu et al., 2024). It should be noted that only a compression at 20% was employed in this study, which was decided by avoiding the breakage of the surmi gels (Zhao et al., 2023a,b).

2.3.2. Dynamic rheological measurements

The dynamic rheological measurement was performed using an AR2000ex dynamic rheometer (TA Instrument Ltd., New Castle, Delaware, USA). To prepare for the measurement, the surimi paste sample was positioned to maintain a 1 mm gap between two parallel splints. The sample was allowed to rest for 2 min to achieve equilibrium for analysis according to Zhang et al. (2013). The parameters were then set to increase the temperature from 20 °C to 90 °C at a rate of 5 °C/min while applying a 10 Pa stress and a frequency of 1 Hz. The storage modulus (G′) and the loss modulus (G″) were obtained.

2.3.3. Water holding capacity (WHC) determination

The surimi gel was chopped into tiny bits and weighed. The weight was expressed as W_1. The weighed sample was then wrapped in two filter papers and placed in a 50 mL centrifuge tube. The centrifuge tube containing the sample was centrifuged at 8000 rpm for 20 min. The mass of the surimi gel after centrifugation was weighed and expressed as W_2. The WHC was calculated according to the previously reported formula (Yan et al., 2020):

$$WHC(\%) = \frac{W_2}{W_1} \times 100 \quad (1)$$

2.3.4. Color parameters analysis

The color parameters of the surimi gel including lightness (L*), redness/greenness (a*), and yellowness/blueness (b*) values were obtained using a HunterLab Colorflex colorimeter (Hunter Associates Laboratory, VA, USA). The whiteness was calculated using the previously published formula (Thamyres César de Albuquerque et al., 2022) which is:

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

2.3.5. Chemical forces analysis

The methodology described by Yi et al. (2020) was used to evaluate the chemical forces between the protein molecules in the surimi gel. The surimi gel was soaked in different solutions made from different chemicals: 0.05 mol/L NaCl (SA), 0.6 mol/L NaCl (SB), 1.5 mol/L urea solution + 0.6 mol/L NaCl (SC), 8 mol/L urea solution + 0.6 mol/L NaCl (SD) and 8 mol/L urea solution + 0.6 mol/L NaCl + 0.5 mol/L β-mercaptothion (SE), homogenized for 3 min then incubated for 1 h at 4 °C. After incubation, the samples were centrifuged at 10000 r/min for 15 min. The chemical forces were determined by analyzing the previously reported variation in the protein content of the supernatant.

2.3.6. Low-field nuclear magnetic resonance (LF-NMR)

The measurement method refered to Liu et al. (2021) with some appropriate adjustments. The LF-NMR T_2 relaxation time was measured using a Carre-Purcelle-Meiboome-Gill (CPMG) RF pulse train with a MicroMR pulsed nuclear magnetic resonance analyzer (Niumag, Shanghai, China). The proton resonance frequency was set to 22 MHz. The Multi-index inverse analysis software (Niumag Co., Shanghai, China) was used to generate the T_2 relaxation time chart, and the corresponding proportions of the different relaxation components were obtained.

2.3.7. Raman spectroscopy

The methodology described by Leng et al. (2022) was used for Raman analysis. The Raman spectra were acquired using a LabRAM HR Evolution Raman spectrometer (Horiba Jobin Yvon SAS, France) equipped with a 785 nm laser source. A total of 5 scans were performed with a collection period of 40 s at a spectral resolution of 2 cm^{-1} between 250 and 2000 cm^{-1}.

2.3.8. Circular dichroism (CD) spectroscopy

Protein secondary structure was analyzed using a Chirascan circular dichroism (CD) spectropolarimeter (Applied Photonics Ltd., England). The myosin was extracted and then dissolved in 0.6 mol/L NaCl. The myosin solution was dialyzed for 12 h at 4 °C before performing the CD spectroscopy measurements. The circular dichroism spectra were recorded in the far ultraviolet range (190–300 nm) at a scan speed of 100 nm/min with a resolution of 0.5 nm.

2.3.9. Scanning electron microscope (SEM)

A gold coating approximately 3–5 nm thick was applied using a Cressington 208HR high resolution sputtering coater and refer to Yuan et al. (2021). The microscopic structure of surimi gel was then examined using a Hitachi S-4800 field emission scanning electron microscope.

2.3.10. Sensory evaluation

Sensory evaluation was carried out to assess the selected sensory attributes of the surimi gels: color, texture, taste, and aroma. Panelists were selected based on their availability, willingness to participate, and experience with sensory analysis. A total of five trained panelists conducted the evaluations, having been provided with specific instructions on sensory profiling techniques for surimi gels. Each sample was evaluated in triplicate to ensure reliability of the results. Uniform samples of cooked surimi with 1.0 cm thickness and 2.5 cm diameter, were served at room temperature on white paper dishes. The presentation to the panelists was randomized to minimize evaluative bias. The panelists used a 100-point standard to rate the samples as shown in Table 1 (Zhang et al., 2019).

Table 1
Score criteria on sensory.

Project	Standard for evaluation	Score/ point
Color	Greyish white and shiny	16~20
	Greyish white with slight yellow	11~15
	Yellow	6~10
	Dark yellow	1~5
Smell	Basically no unpleasant odor of fish	16~20
	A little of unpleasant odor of fish	11~15
	Unpleasant odor of fish	6~10
	Heavy unpleasant odor of fish	1~5
Taste	Delicious Taste with characteristic flavor of fish	23~30
	Good Taste with most characteristic flavor of fish	16~22
	Taste with a little of characteristic flavor of fish	8~15
	Rough Taste without characteristic flavor of fish	1~7
Texture	Compact structure with uniform small pores, the sample doesn't crack after press and rapidly recovery after press removed	23~30
	Compact structure with pores, the sample doesn't crack after press and can't recovery after press removed	16~22
	Loose structure with many uneven pores, the sample cracks after press	8~15
	Poor structure without gelation, the sample cracks after press	1~7

2.4. Statistical analysis

Except when otherwise indicated, each experiment was conducted in triplicate and repeated at least twice. Statistical analyses were conducted using a SPSS software to determine significant differences ($p < 0.05$). ANOVA was used to compare the means, followed by Duncan's multiple range test for post hoc analysis.

3. Results and discussion

3.1. Effects of different ratios of $CaCl_2$ replacing NaCl on dynamic rheological properties of surimi gels

The effects of adding different amounts of calcium salts on the dynamic rheology of surimi gel were studied. One of the most important qualities of surimi is its textural properties, which are the result of its elastic behavior. Therefore, the main method to evaluate the quality of surimi is rheological test (Lei et al., 2023). The storage modulus (G′) reflects the non-permanent deformation of the gel. G′ is often used to describe the transition from a sticky protein paste to a solid but elastic gel, closely related to the structure and mechanical properties of the gel. In contrary, loss modulus (G″) reflects the viscous properties of the gel (Tahergorabi and Jaczynski, 2012; Zhang et al., 2023).

As demonstrated in Fig. 1, all surimi gels exhibited a G′ significantly higher than their G″, confirming their typical viscoelastic solid-like behavior, which is independent to the $CaCl_2$ concentration (Campo-Deaño and Tovar, 2009). In addition, the rheological patterns of all the surimi samples were similar, which can be divided into three stages: gel weakening (<55 °C), gel strengthening (55–75 °C), and gel stabilizing (>75 °C). However, the change of G′ varies with the substitution ratio of calcium salt (Zhao et al., 2023). In Fig. 1a, compared to the starting G′ of about 0.04 Mpa at 20 °C in the CON group and about 0.02 Mpa in the Ca-salt added group, the G′ curve remained constant and rapidly decreased to a minimum at about 40 °C until it rose to 75 °C, and then decreased again due to protein cross-linking. Moreover, in the CON, Ca0, Ca0.4, and Ca0.5 groups, the G′ of surimi gels increased with the increase of Ca^{2+} ($p < 0.05$), indicating that calcium salts could improve the gel formation ability. However, G′ of Ca0.6 and Ca1.0 decreased, which indicated that excess Ca^{2+} led to deterioration of gel properties and they were not conducive to the improvement of gel properties. In contrast to others, as indicated by the highest G′, adding Ca0.5 sped up gelation (Zhang et al., 2023).

The G″ curve of the surimi gel followed a similar trend to that of G' (Fig. 1b), with an almost linear increase in G″ between 50 and 80 °C ($p < 0.05$). The gelation was accelerated by the addition of Ca^{2+} compared to Ca0. Addition of Ca^{2+} to surimi better entangled myosin molecules, which enhanced protein-protein interactions in surimi gels. The enhanced interaction between proteins promoted the unfolding and aggregation of proteins to form a cross-linked elastic hybrid gel. All these observations confirmed the higher gelation rate and better gel network of Ca0.5 by comparing the surimi gels containing different levels of Ca^{2+}. It could be interpreted that Ca^{2+} helped the myosin molecules to unfold and aggregate at an appropriate concentration, resulting in the formation of a good three-dimensional surimi gel network. This was supported by previous findings (Yongsawatdigul et al., 2005).

3.2. Effects of different ratios of $CaCl_2$ replacing NaCl on protein secondary structure in surimi gels

Circular dichroism (CD) spectroscopy was used to determine the relative percentages of protein secondary structures to analyze their alterations. As shown in Fig. 2a, $CaCl_2$ addition generally reduced the β-sheet content irrespective of the $CaCl_2$ concentration. For example, the percentage of the β-turn increased from 2% to 9.4% in Ca0.5, whereas the percentage of the β-sheet structure declined from 49.8% in the control to 35.1% in Ca0.5 ($p < 0.05$). Introducing $CaCl_2$ into the surimi activated the endogenous transglutaminase (endo-TGase) activity. This enzyme catalyzes acyl transfer reactions between glutamine and lysine, thus forming ε-γ-glutamyl-lysine bonds, which promoted protein gel formation and altered protein secondary structure (Piao et al., 2023).

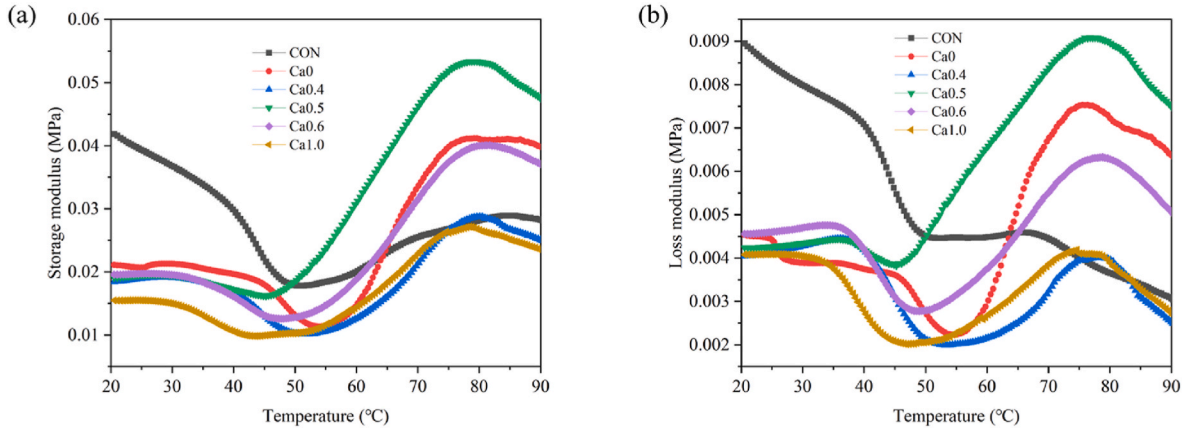

Fig. 1. Dynamic rheological characterization of surimi gels with different ratios of $CaCl_2$ replacing NaCl. Storage modulus G′ (a) and loss modulus G″ (b).

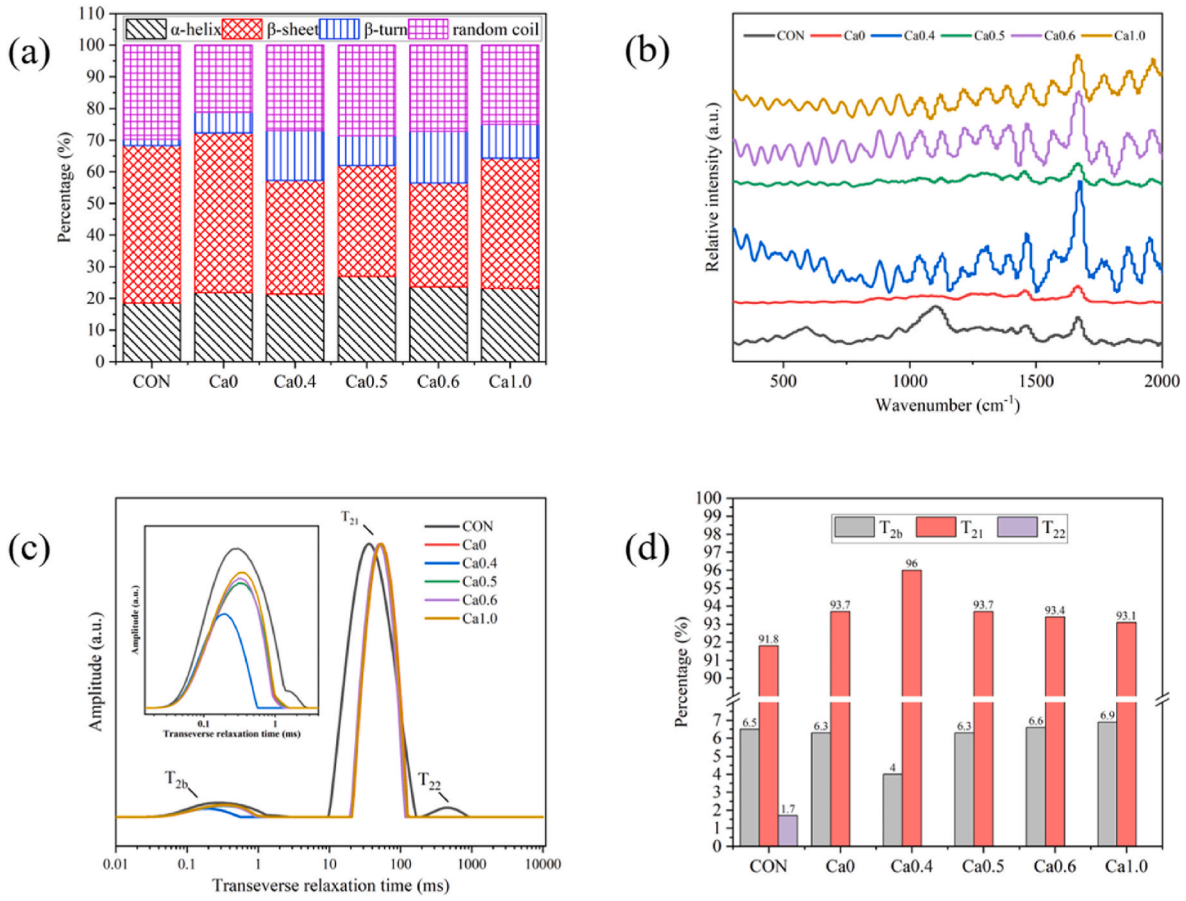

Fig. 2. Characterization of surimi gels with different ratios of $CaCl_2$ replacing NaCl. Protein conformational changes (a), Raman spectra (b), LF-NMR spectra (c) and water distribution (d).

It is well-known that the secondary structure of proteins is determined by hydrogen bonds and electrostatic interactions between their amino acids (Jiang et al., 2017). The salt ions can interact with oppositely charged amino acid residual groups of proteins, resulting in decreased electrostatic interactions and changing protein structure (Zhao et al., 2023). CON had the lowest α-helix content of 18.5 % among all samples, which was matched by the lowest content of β-turns with 2 %, while Ca0.5 had the highest α-helix content of 26.9 % ($p < 0.05$). As demonstrated in Fig. 2b, a characteristic peak at 1670 cm^{-1} is assigned to the amide I structure, with α-helix, β-sheet, and random coil bands at 1650-1660 cm^{-1}, 1665-1680 cm^{-1}, and 1660-1665 cm^{-1}, respectively. The amide I band of surimi gels exhibited maximum scattering between 1660 cm^{-1} and 1665 cm^{-1} with an increased concentration of $CaCl_2$ concentration (Fig. 2b), indicating changes in the structure of surimi proteins. The impact of replacing NaCl with different ratios of $CaCl_2$ on the secondary structure of surimi proteins were depicted in Fig. 2a. It can be seen that Ca0 had the highest percentages of β-sheet and β-turn, while the random coil structure was most abundant in Ca0.5. The differences in α-helix content and β-sheet content of the surimi gels might result from interactions among adsorbed protein molecules (Zhou et al., 2017).

3.3. *Effects of different ratios of $CaCl_2$ replacing NaCl on chemical forces present in surimi gels*

Unfolded myofibrillar proteins had extremely reactive surfaces. The reactive surfaces of nearby protein molecules interacted to create intermolecular linkages when surimi pastes are heated. When sufficient bonding occurred, gels were formed via the three-dimensional networks of bonding (Wang et al., 2023). The secondary structure of myofibrillar proteins mainly consisted of α-helices, β-folds, β-turns, and random coils, whereas the tertiary structure was a specific spatial conformation formed by further curling and folding through the side-chains sustained by secondary bonds (Zhang et al., 2016).

Of all molecular interactions, hydrogen bonding was considered to be the most important chemical force in stabilizing the secondary structure of water and proteins (Chen et al., 2023). In Table 2, the addition of calcium salts had no significant effect on the hydrophobicity

Table 2
Chemical forces present in surimi gels with different ratios of $CaCl_2$ replacing NaCl.

	Ionic bond (g/L)	Hydrogen bond (g/L)	Hydrophobic interaction (g/L)	Disulfide bond (g/L)
CON	0.19 ± 0.01^{e}	0.05 ± 0.02^{b}	0.33 ± 0.01^{c}	0.07 ± 0.02^{b}
Ca0	0.23 ± 0.01^{d}	0.06 ± 0.01^{b}	0.44 ± 0.03^{b}	0.16 ± 0.03^{a}
Ca0.4	0.27 ± 0.01^{c}	0.07 ± 0.01^{b}	0.46 ± 0.01^{b}	0.07 ± 0.01^{b}
Ca0.5	0.28 ± 0.00^{b}	0.07 ± 0.01^{ab}	0.54 ± 0.01^{a}	0.10 ± 0.01^{b}
Ca0.6	0.25 ± 0.01^{c}	0.07 ± 0.01^{b}	0.45 ± 0.01^{b}	0.08 ± 0.02^{b}
Ca1.0	0.30 ± 0.01^{a}	0.09 ± 0.01^{a}	0.44 ± 0.02^{b}	0.09 ± 0.02^{b}

*Different superscripts in the same column indicate significant differences at $p < 0.05$.

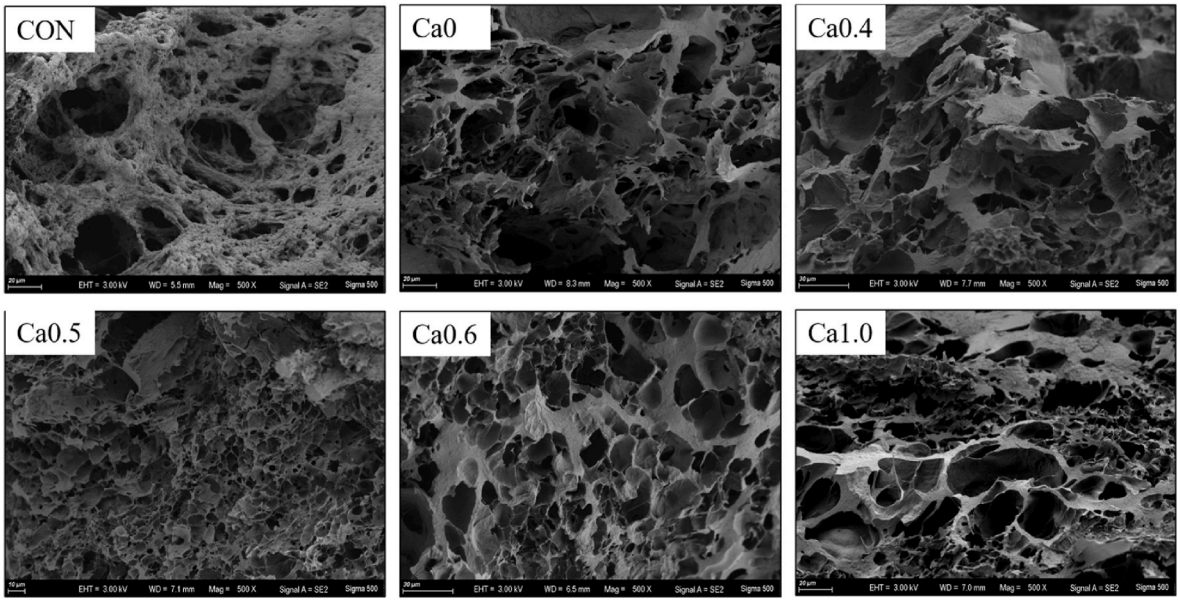

Fig. 3. Morphological profiles of surimi gels without salt and with the addition of different $CaCl_2$ concentrations.

of the gels, indicating that their contribution to the gels was unchanged. Secondly, the results showed that the disulfide bond content was similar in the other five groups, except for the Ca0 group, when compared to CON. The formation of disulfide bonds between the -SH groups of myosin molecules promoted the formation of protein gels and was considered to be the main covalent bond in surimi gels (Cao et al., 2024). However, disulfide bonds were not a major factor in this study. Hydrophobic interaction was another chemical interaction that stabilized the gel structure of surimi. Due to heat denaturation of proteins, more hydrophobic groups were exposed and these hydrophobic interactions drove to form a gel network (Zhang et al., 2023). The addition of Ca^{2+} increased the hydrophobic interaction from 0.33 mg/L for CON to 0.54 mg/L for Ca0.5 (Table 2). Based on these observations, the best performance of surimi gel was formed by replacing sodium chloride with 50% $CaCl_2$, where disulfide bonds and hydrophobic interactions were most abundant.

3.4. Effects of different ratios of $CaCl_2$ replacing NaCl on the appearance and microstructure of surimi gels

When considering the gelation process of surimi, both cations (Na^+ and Ca^{2+}) should be comprehensively considered, along with the anion Cl^- (Yan et al., 2020). Based on the above analysis of the effects of replacing NaCl with $CaCl_2$ on surimi gel, Fig. 3 showed that the microstructures of surimi gels were enhanced with increasing concentrations of $CaCl_2$. Contrary to the very porous network structure of the CON group, the surimi gel formed a denser and more continuous structure with the addition of $CaCl_2$, which was consistent with the results shown by the TPA (textural profile analysis) parameters as shown in Table 4. As the SEM images revealed, when the substitution of $CaCl_2$ was as high as 0.5, the gel surface became smoother and denser, with smaller pores uniformly distributed in the network, and the interconnecting channels increased significantly throughout the gel. However, there was no significant difference between the Ca1.0 and Ca0 groups, indicating that the high $CaCl_2$ content negatively affected the gel structure, resulting in a loose, rough and porous network with large and irregular pores. This might be due to the fact that excess Ca^{2+} led to protein aggregation, forming a larger channel and an inhomogeneous three-dimensional network (Yan et al., 2020). The amount of $CaCl_2$ instead of sodium chloride in surimi gels should be kept around 0.5 to eventually form the densest and most continuous three-dimensional gel network.

Certainly, addition of different ratios of $CaCl_2$ to replace NaCl in the surimi changed the interactions between protein molecules, which enhanced the filling of the structure voids within the gel to varying degrees and consequently increased its strength as shown in Fig. 3. This may be due to the changes in protein functional groups of the surimi which varied with the proportion of the two chloride salts added. Compared to other samples, the CON surimi gel demonstrated less complete myosin network formation, resulting in a disordered gel structure that had a negative impact on the gel's smoothness. Table 2 showed that Ca0.5 surimi gel had more hydrophobic amino acids, which contributed more to the gel texture. Protein denaturation exposed additional hydrophobic groups, which clustered on hydrophobic surfaces. Therefore, the surimi sample with Ca0.5 had a relatively compact gel structure (Yi et al., 2020).

3.5. Effects of different ratios of $CaCl_2$ replacing NaCl on the textural profile of surimi gels

Textural characteristics were critical to the gel quality of surimi

Table 3
Whiteness and WHC in surimi gels with different ratios of $CaCl_2$ replacing NaCl*.

	L	a	b	Whiteness	WHC (%)
CON	79.27 ± 0.41^{a}	-0.17 ± 0.05^{a}	9.54 ± 0.17^{a}	77.18 ± 0.36^{c}	62.78 ± 1.68^{c}
Ca0	76.07 ± 0.31^{d}	-1.72 ± 0.05^{e}	5.40 ± 0.17^{c}	75.41 ± 0.27^{d}	75.22 ± 1.35^{a}
Ca0.4	79.62 ± 0.32^{a}	-1.30 ± 0.04^{b}	6.07 ± 0.36^{b}	78.70 ± 0.35^{a}	67.67 ± 1.15^{b}
Ca0.5	78.66 ± 0.37^{b}	-1.41 ± 0.04^{c}	5.95 ± 0.28^{b}	77.80 ± 0.33^{b}	73.22 ± 0.77^{a}
Ca0.6	78.48 ± 0.38^{b}	-1.38 ± 0.03^{c}	6.01 ± 0.18^{b}	77.61 ± 0.37^{b}	66.11 ± 3.34^{bc}
Ca1.0	77.52 ± 0.28^{c}	-1.64 ± 0.04^{d}	4.97 ± 0.10^{d}	76.92 ± 0.27^{c}	73.78 ± 0.51^{a}

*Different superscripts in the same column indicate significant differences at $p < 0.05$.

Table 4
TPA parameters of surimi gels with different proportions of $CaCl_2$ replacing NaCl.

	Hardness (g)	Springiness	Cohesiveness (g × sec)	Gumminess (g)	Chewiness (g)	Resilience
CON	1747 ± 51^{d}	0.95 ± 0.00^{a}	0.81 ± 0.00^{a}	1418 ± 33^{e}	1349 ± 39^{e}	0.42 ± 0.00^{c}
Ca0	2490 ± 65^{b}	0.95 ± 0.00^{a}	0.80 ± 0.00^{b}	1999 ± 45^{c}	1889 ± 49^{c}	0.45 ± 0.00^{b}
Ca0.4	2526 ± 131^{b}	0.95 ± 0.00^{a}	0.80 ± 0.00^{b}	2017 ± 90^{bc}	1916 ± 94^{bc}	0.45 ± 0.00^{b}
Ca0.5	2873 ± 150^{a}	0.95 ± 0.00^{a}	0.80 ± 0.00^{b}	2286 ± 101^{a}	2177 ± 67^{a}	0.44 ± 0.00^{b}
Ca0.6	2629 ± 103^{b}	0.95 ± 0.00^{a}	0.80 ± 0.00^{b}	2104 ± 66^{b}	1997 ± 69^{b}	0.45 ± 0.00^{b}
Ca1.0	2193 ± 48^{c}	0.95 ± 0.00^{a}	0.81 ± 0.00^{a}	1782 ± 33^{d}	1696 ± 41^{d}	0.47 ± 0.00^{a}

*Different superscripts in the same column indicate significant differences at $p < 0.05$.

products as they can significantly influence consumer acceptance and preference. This study investigated the effect of different proportions of $CaCl_2$ as a substitute for sodium chloride on the properties of surimi gels, with TPAs measuring hardness, elasticity, stickiness, tackiness, chewiness and flexibility. Among these measured TPA parameters, hardness reflected the force required to bend surimi gels, while chewiness was the energy required to chew them into a swallowable state (Gani and Benjakul, 2018).

As shown in Table 4, the hardness and chewiness of surimi gels increased with the addition of $CaCl_2$. In this case, the increase in $CaCl_2$ concentration from 0 to 0.5 increased the hardness from 1747 to 2873 and chewiness from 1439 to 2177 ($p < 0.05$). The increase in these two values suggested that $CaCl_2$ facilitated molecular interactions and improved the density of the gel matrix, thereby improving the gel properties (Zhang et al., 2023). The observed changes in TPA parameters could be attributed to the formation of salt bridges within the gel, i.e., the increase in ionic reactions resulted in a compact network structure and increased elasticity of the surimi gel. In Table 4, Ca0.5 had the highest viscosity of 2286, which also proved to have the best gel properties, and these results were verified by scanning electron microscopy observations (Fig. 3).

Resilience and springiness reflected surimi gel's ability to deform and recover after being subjected to the external stresses (Hu et al., 2023). Unlike hardness and chewiness, the springiness and resilience of the surimi gels containing NaCl and $CaCl_2$ were stable, indicating that they retained their adhesion strength and immediate recovery capability well. Therefore, Ca0.5 was the optimal concentration to increase the strength of surimi gels.

3.6. Effects of different ratios of $CaCl_2$ replacing NaCl on WHC and water molecule distribution in surimi gels

Table 3 shows the effect of replacing NaCl with different proportions of $CaCl_2$ on the whiteness and WHC values of surimi gels. Calcium salts had a significant effect on the WHC values of surimi gels. As shown in Table 3, the water retention of CON was 62.78%. In the absence of salt, myosin could not form a complete three-dimensional network structure, resulting in lower WHC values than other samples with salt. The addition of Ca0.4 and Ca0.6 increased the water retention to 67.67% and 66.11%, respectively, but the water retention was lower compared to Ca0.5 at 73.22% ($p < 0.05$). While the myosin with Ca0.4 and Ca0.6 could form a network structure, certain aggregatons were observed ($p < 0.05$). These changes of microstructure and rheological properties indicated that the addition of $CaCl_2$ and NaCl promoted the formation of gels, while the excessive addition of $CaCl_2$ disrupted the gel formation structure (Hu et al., 2022).

The LF-NMR T_2 relaxation method was used to study the proton relaxation behavior of surimi gel. The resulting spectra (Fig. 2c) and water distribution (Fig. 2d) for surimi gels with varying proportions of $CaCl_2$ replacing NaCl were shown. Typically, the surimi exhibited two or three relaxation peaks, however in this study only the CON group exhibited T_{22} peak, indicating that it had higher free water content compared to the other groups. A possible explanation for the reduced relaxation time in the Ca0.4 group could be due to changes in the protein matrix caused by the addition of salt and the heating effect (cooking). It is increasingly known that chemical and diffusive proton exchange between water molecules and biopolymers such as proteins led to the relaxation behavior modifications (Dayanidhi and Madhurima, 2018; Mohanta and Jana, 2018).

When the $CaCl_2$ content was increased from 0 to 0.4%, the water content corresponding to T_{21} increased from 93.7% to 96%, however, a further increase of $CaCl_2$ concentration to 0.6 did not affect the T_{21} values, which exhibited mean values of 93.7% and 93.4% ($p < 0.05$). The effect of the divalent cation Ca^{2+} and the proton exchange on the muscle structure might account for the more open matrix structure (Greiff et al., 2015).

3.7. Effects of different ratios of $CaCl_2$ replacing NaCl on the sensory quality evaluation of surimi gels

Sensory evaluation of surimi gels with varying proportions of $CaCl_2$ replacing NaCl was presented in Table 5. Compared with Ca0 group, the color score of surimi gel decreased due to the addition of $CaCl_2$. However, the resulting slight yellow color was still acceptable by consumers. The addition of $CaCl_2$ did not significantly change the odor score when compared with the surimi gel of the CON group. However, as the concentration of $CaCl_2$ increased, the surimi gel's odor scores were significantly affected at Ca0.6 and Ca1.0. Meanwhile, the surimi gels' taste score was not significantly affected by the addition of $CaCl_2$, suggesting that $CaCl_2$ can be a suitable addition for the further development of salt substitute.

Furthermore, surimi gels' enhanced hardness, springiness, and chewiness were matched by texture scores that were greater for surimi gels containing various concentrations of $CaCl_2$ than Ca0 ($p < 0.05$) (Table 4). In general, the addition of $CaCl_2$ pronouncedly reinforced the texture of the surimi gel and slightly reduced the fishy smell while preserving the desirable taste of the fish. Therefore, the appropriate substitution of NaCl with $CaCl_2$ indicated a promising application in enhancing the flavor and texture of the surimi products (Zhang et al., 2019).

4. Conclusions

The results of this study indicated that the partial replacement of NaCl with $CaCl_2$ was effective in the production of low-salt high quality

Table 5
Sensory evaluation of surimi gels with different proportions of $CaCl_2$ replacing NaCl.

	Color	Smell	Taste	Texture
CON	16.0 ± 0.7^{b}	15.6 ± 0.5^{ab}	6.6 ± 0.5^{c}	14.8 ± 0.8^{c}
Ca0	13.8 ± 0.8^{c}	15.8 ± 2.2^{ab}	23.0 ± 1.2^{a}	23.6 ± 1.1^{b}
Ca0.4	18.8 ± 0.8^{a}	15.0 ± 1.4^{b}	24.2 ± 0.8^{a}	25.6 ± 0.9^{a}
Ca0.5	17.2 ± 0.8^{b}	16.0 ± 0.0^{ab}	24.2 ± 0.8^{a}	26.0 ± 1.0^{a}
Ca0.6	17.0 ± 0.7^{b}	17.0 ± 0.7^{a}	23.2 ± 0.8^{a}	24.0 ± 0.7^{b}
Ca1.0	17.0 ± 1.0^{b}	17.0 ± 0.7^{a}	20.2 ± 1.3^{b}	24.0 ± 1.2^{b}

*Different superscripts in the same column indicate significant differences at $p < 0.05$.

surimi products. The content of hydrophobic interactions in the Ca0.5 group was significantly higher ($p < 0.05$) than that in other groups. Hydrophobic interactions were among the most important chemical forces that maintained the structure of the surimi gels. An increase in the amount of $CaCl_2$ from 0 to 0.5 resulted in a dramatic increase in the gumminess of the surimi gels from 1418 g to 2286 g, confirming that Ca0.5 significantly enhanced the quality of the surimi gels. In addition, $CaCl_2$ addition at 0.5% did not affect the sensory properties of surimi gels significantly. Collectively, this study elucidated the mechanisms that $CaCl_2$ as a sodium salt substitute acted effectively in surimi gels, and found that 50% replacement was the best percentage, thus guiding the development of low-salt surimi products.

CRediT authorship contribution statement

Xinyan Tong: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Yijin Liu:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Ganping Wei:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Nasra Seif Juma:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing, Supervision. **Fang Tian:** Supervision, Validation, Writing – original draft, Writing – review & editing. **Dieynabou Diao:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing, Supervision. **Meiling Chen:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing, Supervision. **Bin Zheng:** Supervision, Validation, Writing – original draft, Writing – review & editing. **Yadong Zhao:** Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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