Effects of curdlan on the texture and structure of Alaska pollock surimi gels treated at 120°C

Yinong Wei, Tao Zhang, Fanqianhui Yu, Yong Xue, Zhaojie Li, Yuming Wang, and Changhu Xue

Department of Food Science and Engineering, Ocean University of China, Qingdao, PR China

ABSTRACT

The effects of curdlan (2%, 3% and 4%) on the gel properties of Alaska surimi using high-temperature treatment were examined in this study. Curdlan treatment improved the gel strength of surimi in a concentration-dependent manner. Based on the results of dynamic rheology and differential scanning calorimetry (DSC), curdlan promoted the stability of proteins in the gel. Moreover, curdlan facilitated the interaction between gel proteins and actomyosin, thereby preventing the aggregation and denaturation of protein and increased the thermal transition temperature. The results of scanning electron microscopy showed that the addition of curdlan induced the formation of a more ordered and denser gel matrix and the fibrils in the three-dimensional network became more delicate. Therefore, surimi samples with curdlan may hold more moisture and exhibit improved transfer of free water to bound water, leading to a higher water-holding capacity (WHC).

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Introduction

In recent years, consumers have demanded more convenient foods, including ready-to-eat (RTE) products, which do not require any preparation before consumption. Surimi has distinct gelling properties and can be used as a food base in RTE seafood products because it is a concentrate of salt-soluble myofibrillar proteins. Surimi gel, prepared from deboned and washed fish paste, has been used to produce restructured seafood analogs or gel-based food products, such as Japanese traditional gels, called ‘Kamaboko’. Additionally, surimi-based products have become common due to their unique textural properties and high nutritional value.

Myosin and actomyosin are the main components responsible for gelation, the most important functional property of surimi. Solubilized proteins first expand to create a continuous matrix and then undergo thermal aggregation and cross-linking, finally forming solid and elastic surimi gels with a three-dimensional muscle protein network. Rearrangement of the hydrogen bonds, covalent bonds and hydrophobic interactions within proteins affects the formation of the network.

To produce instant surimi products, the components must have a long shelf life, which can be realized by sterilization. After sterilizing at 120°C, the products can be stored at a normal temperature and exhibit a much longer shelf life than the untreated products. Therefore, studies on the influence of high temperature on surimi gels are needed. The gel properties of surimi-based foods are particularly affected by the heat-processing steps used during the conversion of raw surimi paste to cooked gel. Previous studies demonstrated that high-temperature treatment (≥100°C) could markedly decrease the breaking force, breaking deformation and gel strength, thus damaging the texture of the products. Therefore, because consumers are more likely to purchase high-
quality surimi products, new methods are needed to produce surimi-based products with a long shelf life and good textural properties in order to meet this public demand.

Many different components, including non-muscle protein and various hydrocolloid polysaccharides, have been used to improve the gel properties. Non-muscle proteins, e.g. protein isolates from black beans, can increase the water-holding capacity (WHC) and gel strength of sardine surimi and the gel becomes denser with a more ordered structure. Food hydrocolloids, such as polysaccharides and proteins, can contribute to the structure and functional properties of many processed foods. Leloup and others introduced complex gel systems made up of protein and polysaccharide. They showed that the length of the polysaccharide chain exceeded a certain range during the heating process; thus, the steric effects of long-chain polysaccharides block the aggregation of protein. The protein–water–polysaccharide complex is quite different from that of each absolute system and some changes in the composition, structure and functional properties of the protein particles are observed. Thus, the addition of polysaccharides may influence protein gelation properties, thereby affecting surimi gelation. Several hydrocolloids, including starch, carrageenan, xanthan gum and konjac, are used as gel binders, texture stabilizers and fat substitutes in the production of surimi gel products to improve the mechanical properties of surimi gels. Additionally, Herranz and others showed that deacetylated konjac glucomannan (KGM) improved the thermostability of gels, and Barrera and others found that the LM35 pectin could decrease expressible water and increase the levels of shears stress and hardness.

Curdlan is a linear glucan, interconnected by β-(1→3)-D-glucans without branching. As a water-insoluble microbial exopolysaccharide, curdlan is generated by microorganisms, such as Alcaligenes faecalis. Because of its distinct thermal gel properties, curdlan has extensive applications in the food industry, particularly in meat products. Curdlan suspensions may form both thermally reversible and thermally irreversible gels under different heating temperatures. The former (low-set gel) appeared when heated to 60°C and subsequently cooled. In contrast, a firm, resilient, high-set gel is formed at temperatures higher than 80°C. In the low-set gel, the curdlan micelles are cross-linked with molecules of a single helix through hydrogen bonds; however, cross-linking is also observed in curdlan micelles containing triple-stranded helixes with hydrophobic interactions in high-set gels. Owing to variations in the gelation mechanisms, these two types of gels have been added to different types of foods, such as noodles, bean curd and low-fat meat products. However, few studies have investigated the applications of curdlan in fish meat gel-based seafood products.

In our previous work, we showed that the deacetylation of KGM has an active effect on gel strength by retarding the protein denaturation of Alaska surimi significantly under high-temperature treatment. Thus, the purpose of the present study was to investigate the effects of curdlan at different concentrations on the physicochemical properties of surimi gels treated at a high temperature (120°C). We also aimed to clarify the recombination mechanism of the surimi gel–curdlan complex system.

Materials and methods

Materials

Frozen Alaska Pollock surimi (grade AAA) was purchased from JINCAN Foods Co., Ltd. (Qingdao, Shandong, China). Surimi was stored at −20°C until use. Food-grade curdlan was provided by Zhongke Biotechnology Co., Ltd. (Shandong, China). All of the chemicals used in this experiment were analytical grade and purchased from Sigma (St. Louis, MO, USA) or Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of curdlan and surimi gel

Curdlan powder was added to water and stirred for 10 min. The suspension was then kept until further use. Frozen Alaska Pollock surimi (250 g) was first semi-thawed for 3–4 h at 4°C and cut into small strips (approximately 3 cm cubes). Surimi cubes were then chopped in a Stephan vertical vacuum cut mixer
(Model UM 5; Stephan Machinery Co., Hameln, Germany) at a speed of 1500 rpm for 4 min. A chilling medium (ethanol:water, 95:5) was continuously circulated to keep the sample temperature below 4°C during chopping. Sodium chloride (3 wt%) was added and mixed with the surimi at a speed of 1800 rpm for 2.5 min. Subsequently, the mixture was chopped for an additional 3 min under 0.5 bar pressure to remove air pockets with or without the curdlan suspension. The moisture level was adjusted to 75%. The final curdlan concentrations were 0%, 2%, 3% and 4% (w/w, based on the surimi gel). The surimi sol was squeezed into plastic casings (3 cm i.d.), in which both ends were fastened tightly.

High-temperature treatment

Surimi samples were sterilized at 120°C. An electric/steam amphibious sterilization retort (JINDING Food Machinery Co., Ltd., Zhucheng, Shandong, China) was used for high-temperature treatment. The desired temperature was preset to ensure the accuracy of the sterilization temperature. During the heating process, counter-pressure was applied by adding compressed air into the retort in order to prevent casings from bulging. This counter-pressure was also preset. After sterilization, the temperature was cooled down via closing the steam source and adding cooled water into the spray tube. The surimi gels were heated under the same counter-pressure (0.12 MPa) until their central temperature reached 120 ± 1°C and then held for 15 min. The temperature was monitored using an Ellab TrackSense-Pro (Henglv Engineering Co., Ltd., Hubei, China). The samples were arranged in ice water for chilling and finally kept at 4°C until analysis.

Instrumental texture analysis

A TMS-PRO texture analyser (Food Technology Co., USA) was used to measure the textural properties of surimi gels. The gels were equilibrated at room temperature (25°C) before analysis. Cylindrical samples (2.5 cm in length) were prepared, placed in a texture analyser and tested by a spherical plunger (5 mm in diameter, 60 mm/min depression speed). The choices of breaking force and deformation were based on the first force peak.\(^{[27]}\)

Determination of WHC

Surimi gels were cut into several pieces, one of which was randomly chosen to analyse its water content (X\(_1\) %). Three pieces of Whatman filter paper and two pieces of filter paper were each placed under and above the surimi gels. The samples were subjected to a pressure of 5 kg for 2 min and the water content (X\(_2\) %) was then measured again. The WHC was calculated using the following equation:

\[
\text{WHC} = \left[1 - \frac{(X_1 - X_2)}{X_1}\right] \times 100\%
\]

Rheological measurements

The dynamic viscoelasticity, namely the storage modulus (G’) of curdlan-containing surimi samples, was measured using an MCR101 rheometer (Anton Paar Ltd., Austria) with a parallel plate (50 mm in diameter) and having a 1.0-mm gap. Dynamic temperature sweep measurements were conducted at a heating rate of 1°C/min and a frequency of 1.0 Hz, maintaining the amplitude strain at a constant value (2%) within the LVE region, which was determined by stress sweep tests.

Differential scanning calorimetry

DSC measurements were performed using a Netzsch DSC 200PC (Netzsch, Bavaria, Germany). About 10 mg of surimi paste was sealed in an aluminium pan and an empty pan was used as a reference. We set the scan temperature from 25°C to 120°C, at a heating rate of 5°C/min.
Nuclear magnetic resonance spin-spin relaxation (T2) measurements and post-processing

NMR relaxation measurements were performed using a Niumag Benchtop Pulsed NMR Analyzer PQ001 (Niumag Electric Corporation, Shanghai, China) operating at a resonance frequency for protons of 22.6 MHz. The 3-cm-long sample was placed in a glass tube (15 mm, i.d.) and inserted in the NMR probe. The spin-spin relaxation time (T2) measurements were performed by the Carr–Purcell–Meiboom–Gill (CPMG) sequence with a \( \tau \)-value (time between 100°C and 180°C pulse) of 100 \( \mu \)s and 8 scan repetitions. The interval time was set to acquire 5000 echoes for each 2.5 s.

MultiExp Inv Analysis software (Niumag Electric Corporation, Shanghai, China) was used to perform the distributed exponential fitting of the CPMG decay curves. Time constants of each process were calculated from the peak position and the area under each peak (corresponding to the proportion of water molecules exhibiting that relaxation time) was calculated through cumulative integration. Additionally, the width of the relaxation population was determined by the standard deviation of the observed relaxation times for the given peak.

Scanning electron microscopy

The microstructures of the surimi gels were analysed using SEM. The surimi gels were cut into pieces with 2–3 mm thickness and fixed with a 3% glutaraldehyde solution. Before dehydration in a gradient ethanol series of 50%, 70%, 80%, 90% and 100% (v/v), samples were washed for 1 h in distilled water and then mounted on bronze stubs and sputter-coated with gold. Finally, SEM (JEOLJSM-5800 LV; Tokyo, Japan) was applied to observe the specimens at an acceleration voltage of 15 kV.

Statistical analysis

The experiments were run in triplicate and the data were calculated as means ± standard deviations. Differences were compared by the least significant difference (\( p < 0.05 \)). Statistical analysis was performed using ORIGIN software (Version 8.0; Microcal Software Inc., Northampton, MA, USA).

Results and discussion

Breaking force and deformation

Gel strength is a key parameter used to determine the quality of fish meat gel-based seafood products. The breaking force (Fig. 1a) and deformation (Fig. 1b) of the surimi gels with curdlan (2%, 3% and 4%) are depicted in Fig. 1. According to previous studies, surimi gels deteriorated rapidly as the heating temperature rose to 120°C. High-temperature treatment may mitigate the gel strength and disrupt the gel structure of surimi. However, in the presence of curdlan, the

![Figure 1](image_url)

Figure 1. The breaking force (a) and deformation (b) of curdlan-contained surimi gel.

properties of the surimi gel were improved compared with those of the control. As shown in Fig. 1, the breaking force increased as the concentration of curdlan increased and the highest value was obtained at 4% curdlan. However, deformation of the surimi samples containing curdlan increased with a slight gradient compared with that of the control.

Surimi gels are sensitive to heat-induced gelation. According to a previous analysis using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), high-temperature treatment (120°C) abolished myosin heavy chain (MHC) levels and reduced the actin content, producing gels with poor properties. As shown in Fig. 1, curdlan improved the breaking force and deformation of the surimi gels compared with those of the control. Wu and others studied the effects of curdlan on the rheological properties of restructured Ribbonfish meat gel and found that curdlan has positive effects on increasing the cross-linking density in the complex gel networks when combined with fish meat, resulting in an ordered and stable three-dimensional gel structure, thus improving its properties. The good gel properties of samples with curdlan could be attributed to the interaction between curdlan gel and fish meat protein in the complex matrix during the gelation heating process. Curdlan in meat products exhibits strong thermo-irreversibility because it can inhibit the formation of hydrogen bonds during cross-linking in junction zones and strongly entrap released or free water within its gel structure when heating.

**Dynamic rheology**

G' is the stored energy caused by elastic deformation in a viscoelastic material during the process of deformation. We tested the storage modulus to study the effects of curdlan on the dynamic rheological properties of surimi gel. Temperature curves of G' during the heating process were compared to determine the dynamic viscoelasticity after the addition of 0%, 2%, 3% and 4% curdlan (Fig. 2). In the control group, we found that G' reached a first peak at 29.8°C and dropped to the minimum G' value rapidly when the temperature reached 54°C. With the rise of temperature, slight fluctuations in G' were observed. G' reached a second peak at 86.8°C and then decreased thereafter. These changes in G' were caused by denaturation and cross-linking of several compositions in fish meat proteins at different heating temperatures. The initial increase in G' at low temperatures could be related to the molecular interactions of actomyosin, which formed a weaker three-dimensional gel network, and the second peak of G' could be explained by the formation of a highly elastic protein gel through denaturation of myosin and actomyosin. When the heating temperature was above 100°C, the G' decreased in all samples.

**Figure 2.** Changes in the storage modulus (G') of surimi with or without curdlan as a function of temperature.
because high-temperature treatment destroyed the non-covalent interactions of protein and polymerized non-enzymatic myosin in the surimi gel. Zhang and others\textsuperscript{[10]} suggested that this polymerization might prevent fish meat proteins from forming high-quality network during the process of thermal gelation.

As shown in Fig. 2, similar trends for the storage modulus $G'$ curves were observed for surimi samples with curdlan. As the temperature increased, the $G'$ first increased and then decreased, forming the first peak. When the temperature reached 55°C, a nadir was observed. The second peak was obtained between 85°C and 95°C. However, compared with the control group, the values of $G'$ increased as the concentration of curdlan increased. Although curdlan increased the values of $G'$, as shown in Fig. 2, the overall profiles of the storage modulus were not altered. Thus, the protein network still predominated in the polysaccharide and protein complex system when the gel formed and curdlan functioned by improving or promoting the formation of surimi gels. The reason for the increase in the first peak may be due to the onset of the swelling of curdlan molecules,\textsuperscript{[34,35]} accompanied by breaks in some inter- or intramolecular hydrogen bonds.\textsuperscript{[36]} Additionally, as the temperature increased, the second peak of $G'$ values increased compared with the control, indicating the formation of thermo-irreversible gels through hydrophobic interactions between curdlan molecules\textsuperscript{[37]} and synergy with proteins.

**Effects of curdlan on the endothermic transitions of surimi protein by DSC**

The formation of surimi gels involves the heat-induced denaturation of different components of myofibrillar protein and the denaturation temperatures of different proteins can vary widely. Thus, heat uptake of the various proteins represented by endothermic peaks was detected using DSC; these heat uptake peaks were expected to represent the points at which the hydrogen bonds broke and the ordered network structure of the protein gradually unfolded during the denaturation process.\textsuperscript{[38]} Figure 3 shows the different DSC thermograms of surimi pastes with or without curdlan. In the control group, the first peak corresponded to the endothermic transition of myosin, the second peak was related to the endothermic transition of actin and the third peak in meat systems corresponded to sarcoplasmic proteins.\textsuperscript{[39]} However, since the sarcoplasmic proteins were removed during the washing process, its transition temperature was not observed in our thermograms.

![Figure 3. Differential scanning calorimetry (DSC) thermograms of surimi pastes with and without curdlan.](image-url)
In the DSC thermograms of surimi samples with or without curdlan, we could clearly distinguish two thermal denaturation transitions. The first peak, appearing at approximately 31 ± 1°C, corresponded to myosin and was not altered following the addition of curdlan. However, as the concentration of curdlan increased, the value of the second peak gradually became higher. The transition temperatures were 90.3°C for the control and 91.7°C, 96.9°C and 97.9°C for 2%, 3% and 4% curdlan, respectively. Based on these results, curdlan perhaps did not alter the thermal transition temperature of myosin, but could interact with actin, thereby improving the transition temperature. Because the molecular weight of actin is much lower than that of myosin and because curdlan can form a stable thermo-irreversible gel above 70°C, the added curdlan may bind with actin easily and form a stable gel when the heating temperature reaches 80°C, contributing to the improvement of the thermal transition temperature of actin. Murray and others[40] considered the transition temperature as an indication of the rupture of hydrogen bonds and protein aggregation based on hydrophobic interactions; hence, we speculated that curdlan may react with actin through hydrogen bonding, thereby increasing the transition temperature.

**Microstructure of surimi gels with curdlan**

To confirm the effects of curdlan on the formation of surimi gels, the microstructures of four surimi samples were observed. As shown in Fig. 4, all samples formed a three-dimensional network structure, which contributed to the elastic characteristics of the gel. However, surimi gel containing curdlan exhibited a more compact gel matrix than the control, for which the protein gel was much rougher and looser with larger cavities. The addition of curdlan produced smaller pores and a more compact network. Moreover, as the curdlan content increased, fibrils in the three-dimensional protein network became more delicate, inducing a more ordered and denser restructured gel network. Thus, combined with DSC and rheology results, our findings suggested that curdlan could interact with actomyosin, effectively preventing protein aggregation and improving the gel structure.

![Figure 4. Microstructure (8000×) of surimi gels with or without curdlan. (a) Control, (b) with 2% curdlan, (c) with 3% curdlan, (d) with 4% curdlan.](image-url)
**Water-holding capacity**

WHC is an important parameter associated with the stability of surimi gel-based restructured seafood. Because of its high WHC, fish meat protein can bind with abundant amounts of water and therefore preserve moisture during the heating process.\(^{[26]}\) The effects of curdlan on the WHCs of surimi gels are shown in Fig. 5. The WHCs of gels increased as the content of curdlan increased, suggesting that more water was bound to or reserved in the network.\(^{[41]}\) Funami and others\(^{[31,42]}\) reported that curdlan in meat absorbed free or released water during the heating process and held water well within the gel structures. The water-binding capacity of protein gels is affected by several factors, including hydration, hydrogen bonds and hydrophobic interactions.\(^{[25]}\) The addition of curdlan may lead to the formation of more hydrogen bonds and hydrophobic interactions. Moreover, according to SEM analysis, curdlan could make the gel structure become dense and uniform, which may increase the WHC of the gel.\(^{[43,44]}\) However, an obvious increase in WHC between surimi gels containing 3% or 4% curdlan under heat treatment at 120°C was observed in this study. Hu and others\(^{[45]}\) speculated that the dilution effect of curdlan at higher levels may lower the number of water-binding sites in proteins to some extent, thus reducing the ability of binding and interaction between muscle proteins and water molecules.

**The T2 time of surimi gels with curdlan under high-temperature treatment**

Low-field NMR (LF-NMR) technology can be used to assay the moisture distribution and state of products during the storage process at the molecular level, which can accurately reflect the moisture migration state in foods. Choi and Kerr\(^{[46]}\) studied the moisture content and molecular liquidity of wheat starch through\(^{[1]}\) H-NMR, which has been widely used in the study of moisture content in meat muscle.\(^{[47]}\) NMR transverse relaxation is related to WHC and can be used as an important indicator to assess the WHC of surimi gel.\(^{[48]}\) NMR transverse relaxation can easily distinguish different WHCs of the gel samples. Therefore, it is necessary to analyse the T\(_2\) to investigate the water distribution in surimi gels.

The distributed T\(_2\) relaxation times of the surimi gels with or without curdlan under high-temperature treatment are shown in Fig. 6. The T\(_2\) was characterized by a small population with a relaxation time of a few milliseconds; additionally, a major population was observed at around 30–130 ms and a minor population was observed at about 300–1500 ms. The first peak T\(_{21}\) was in the range of 0–10 ms. This result could be explained by the close association of the bound water with the large molecules, like proteins in the gel system or protons located on macromolecular structures plasticized by water.\(^{[49]}\) T\(_{22}\), the second peak, represented the immobilized water, whereas the third peak T\(_{23}\) represented free water.

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*Figure 5. WHCs of curdlan-containing surimi gels.*
As shown in Fig. 6, most water in surimi gels was immobilized; as curdlan increased, the peak of $T_{22}$ became higher and the position of each peak of $T_2$ moved to the left, indicating that free water transformed to bound water. Additionally, the relaxation time of $T_{22}$ became shorter with the addition of curdlan, clarifying that water became less movable in the surimi gels, consistent with the WHC results.

**Conclusion**

Curdlan affected the gel properties of surimi samples under high-temperature treatment. Curdlan could form thermo-irreversible gels at high temperatures and interact with surimi protein, thereby increasing the gel strength. According to the results of dynamic rheology and DSC, during the heating process, the addition of curdlan did not influence the denaturation of myosin but did affect the interaction with actin and increased its thermal transition temperature. In surimi products, curdlan promoted the cross-linking density of the complex gel network, resulting in a more uniform and stable three-dimensional network, thus allowing the surimi gel to hold more free or released water. Moreover, as the curdlan content increased, the effect on the protein gels was more significant. Based on the above-mentioned results, curdlan could be considered an alternative to enhance the gel properties or improve the quality of surimi products because it could markedly strengthen the qualities of Alaska surimi treated with high temperature (120°C).

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