Agr. Nat. Resour. 55 (2021) 259–264

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Effects of deacetylation of konjac glucomannan and high temperature treatment on rheological and textural characteristics of threadfin bream surimi gels

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Article Info

Received 12 February 2021
Revised 20 March 2021
Accepted 16 April 2021
Available online 31 April 2021

Keywords:
Deacetylation,
Konjac glucomannan,
High temperature treatment,
Rheological characteristic,
Threadfin Bream surimi

Abstract

The effects were investigated of different degrees of deacetylation (DD) of konjac glucomannan (KGM) on the rheological and textural characteristics of threadfin bream surimi gels subjected to high temperature treatment. Different DD percentages of KGM (0%DD, 64%DD, 94%DD) were added with 2% surimi by weight and the samples were coded as SD0, SD1 and SD2, respectively. The control sample had no added KGM. In a temperature sweep test (4–120°C), the surimi with or without deacetylated KGM had higher storage modulus (G′) and loss modulus (G″) values than that of the control at all temperatures. SD2 had the highest G′ value at temperatures of 22–57°C and above 95°C. SD2 had the highest gel strength but the lowest expressible water content. During heating, the 94%DD KGM could readily disperse through the protein gel matrix, and after cooling it could increase the G′ value of the surimi gel. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns suggested that high temperatures may enhance myosin polymerization, as all samples completely lacked the myosin heavy chain band. Scanning electron microscopy imaging of SD2 showed a more dense and more uniform microstructure than for the other samples.

Introduction

Surimi is minced fish meat washed to remove most fats, water-soluble proteins and undesirable muscle components such as blood and pigments; it is stabilized using cryoprotectants before frozen storage (Iwata et al., 2001). Surimi consists mainly of concentrated myofibrillar proteins, primarily myosin and actin, which directly contribute to gel formation under heat treatment (Liu et al., 2014). Surimi is an intermediate product, usually further processed to make kamaboko products and seafood analogs such as imitation crab, fish ball, and fish stick (Xiong, 2004).

Sterilization is an effective method for producing long shelf life products that can be stored at room temperature (Zhang et al., 2016). However, sterilization has negative effects on products qualities such as texture, color, flavor, loss of nutritional values and damage to sensory quality (Jiang et al., 2018). Many studies have reported the effects of high temperature treatment on surimi gel properties. For example, Zhang et al. (2013) reported that high temperature treatment (≥100°C) under pressure (0.12 MPa) decreased the myosin heavy chain (MHC) and actin contents and affected the protein structure and texture of the surimi gels. The breaking force and gel strength decreased as the temperature increased from 100°C to 120°C. Zhang et al. (2016) demonstrated that a random coil of pollock surimi gel became damaged with increasing temperature, the network structure became fragile and holes became larger.

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https://doi.org/10.34044/j.anres.2021.55.2.14

Konjac glucomannan (KGM) is a neutral polysaccharide extracted from *Amorphophallus konjac* C. Koch and comprises β-1,4-linked glucose and mannose units (1:1.6) (Kato and Matsuda, 1969). The backbone consists of acetyl groups at the C-6 position of the side chain which frequently range from 1 per 9 sugar units to 1 per 20 sugar units (Chua et al., 2010). KGM is a unique gelation; the rheological properties of this gel vary with the kind of reagents used as a gelling agent and the gelation proceeds more completely as temperature increases (Maekaji, 1974). KGM molecules lose their acetyl groups under alkaline treatment and aggregate through linkages including hydrogen bonds (Maekaji, 1974). The molecules form a network structure and ultimately gel. Zhang et al. (2015) found that gelling of surimi (Alaska pollock) subjected to high temperatures was improved by the addition of deacetylated KGM. However, there have been no reports on the effects of high temperature treatment on threadfin bream surimi gels. The objectives of this study were to investigate the effects of different degrees of deacetylation of KGM on the rheological and physical properties of threadfin bream surimi gels under high temperature treatment.

Materials and Methods

Materials

Samples of frozen threadfin bream surimi (*Nemipterus hexodon*) grade SA were purchased from Andaman Surimi Industries Co., Ltd.; Samut Sakhon province, Thailand. KGM was purchased from Thai Food and Chemical Co., Ltd.; Bangkok, Thailand.

Preparation of deacetylated konjac glucomannan samples

Following the method of Du et al. (2012) with minor modification, KGM (30 g) was mixed with 100 mL of 50 vol% ethanol in an Erlenmeyer flask in a water bath shaker (SBD50; Heto; Denmark) at 190 revolutions per minute (rpm) and 40°C for 30 min. Then, 50 mL of NaOH solution at 0.01M or 1M were added to obtain difference percentages of the degree of deacetylation (%DD). The mixture was stirred at 40°C for 24 hr to allow deacetylation. To remove any alkali, the samples were immersed in 100 mL of 50 vol% ethanol for 20 min and filtered through a filter cloth using a suction pump set. This process was repeated until the pH of the filtrate was neutral. The sample was dehydrated using different gradients of ethanol (75 vol%, 95 vol%, absolute), followed by vacuum-drying at 40°C for 6 hr.

Determination of degree of deacetylation of konjac glucomannan

The %DD of the KGM was determined using titration, following the method of Chen et al. (2006). Dried samples (2 g) were mixed with 50 mL of 75 vol% ethanol in an Erlenmeyer flask, followed by stirring at 190 rpm and 40°C for 30 min. The mixture was cooled to room temperature. After that, 5 mL of 0.5M NaOH was added and then the mixture was stirred at 190 rpm and 40°C for 24 hr. The excess alkali was back-titrated using 0.1M HCl, with phenolphthalein as the indicator. The KGM samples were coded as D0 (native KGM), D1 (%DD = 64.08 ± 1.47) and D2 (%DD = 93.49 ± 1.04).

Preparation of surimi sol

Frozen surimi was partially thawed at 4 ± 2°C for 16–18 hr and then cut into pieces of approximately 1 cm × 1 cm × 1 cm. To prepare the sol, the surimi was chopped in a food processor (MCM64060; Bosch; Slovenia) for 1 min, followed by the addition of sodium chloride (2.5% by surimi weight) and KGM (2% by surimi weight), following Zhang et al. (2015) and further chopped for 4 min. Three different DD percentage (D0, D1 and D2) of KGM were added in surimi. Moisture content of the mixture was adjusted to 80% by the addition of iced water. The mixture was chopped for another 1 min. The temperature of the mixture was maintained below 12°C throughout the process. The surimi sol was stored at 4±2°C before analysis. All experiments were done in triplicate with three different lots of surimi following a randomized complete block design.

Preparation of surimi gel

The sol was stuffed into cellulose casing (2.5 cm internal diameter) using a stuffer (Model KM005, Kenwood; Turkey) and both ends were sealed tightly. The samples were heated at 120°C for 24 min using a water spray retort (PP500; OFM; Thailand) within 60 min after stuffing. After heating, all gel samples were cooled rapidly in iced water until the core temperature of the sample fell below 4±2°C. The samples were stored overnight at 4±2°C prior to analysis.

Rheological measurement

Dynamic viscoelastic measurements were carried out using a Bohlin Gemini 200 HR nano rotational rheometer (Malvern; UK) with parallel plate geometry and a 25 mm diameter and a 1.0 mm gap. The sol was placed on the stage and any excess was removed. The linear viscoelastic region was determined using a stress-controlled rheometer. Stress sweep measurements were performed by increasing the shear stress in the range 1–10,000 Pa. A shear stress of 100 Pa (within the linear viscoelastic region) was used in the temperature sweep test. Testing was conducted at an oscillation frequency of 0.1 Hz. The samples were heated from 4°C to 120°C at a heating rate of 6°C/min. The storage modulus (G′) and loss modulus (G″) were recorded during the temperature scanning process.

Determination of properties of surimi gel

Gel strength

The gels were equilibrated at ambient temperature before analysis and samples were cut into lengths of 2.5 cm. The breaking force (measured in grams) and the deformation (measured in centimeters) were measured using a texture analyzer (Model TA-XT2i; Stable Micro Systems; England) equipped with a spherical plunger (5 mm diameter). The gel strength was calculated by multiplying the breaking force by the deformation and expressed as grams.centimeters (Sutloet et al., 2019).

Expressible water content

The expressible water content of the surimi gels was measured following the method of Sutloet et al. (2018). Gel samples were cut into pieces 0.5 cm × 1 cm × 0.5 cm and weighed. The sample was placed between pieces of Whatman paper (No.1). A standard weight of 5 kg was placed on the top for 2 min then the sample was removed and weighed again. The expressible water content was calculated using Equation 1:

$$\textit{Expressible moisture content}\ (\%) = 100[(X - Y)/X] \quad (1)$$

where X is the prepressed weight and Y is the weight after pressing, both measured in grams.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The protein patterns of the gel were analyzed using the method of Laemmli (1970). The protein concentration was determined following Lowry et al. (1951), using bovine serum albumin as a standard protein. Preparation of solubilized samples, stacking and separating gels and analyzing of protein pattern was carried out following the method of Sutloet et al. (2019). Briefly, to prepare the solubilized sample, gel (0.5 g) was mixed with 20 mL of the extract solution (2% (weight per volume; w/v) in 60 mM Tris-HCl, pH 7.5). Then, the solubilized sample was mixed at a 1:1 (volume per volume; v/v) ratio with the sample buffer (0.5 M Tris-HCl, pH 6.8 containing 2% (w/v) sodium dodecyl sulfate, 10% (v/v) glycerol and 5% (v/v) β-mercaptoethanol) and heated at 95ºC for 5 min. Stacking gels and separating gels comprised 4% (w/v) and 10% (w/v) of the polyacrylamide, respectively. A sample (15 µL) was loaded onto each lane. The samples were subjected to electrophoresis at a constant current of 20 mA per gel using an electrophoresis unit (Mini-Protean II; Bio-rad Laboratories; USA).

Microstructure of surimi gel

The microstructure of the gels was determined using a scanning electron microscope (JSM - 5410LV; JEOL; Japan). Gels were fixed using 2.5% glutaraldehyde in phosphate buffer solution (pH 7.2) at 4°C for 24 hr. The samples were washed in phosphate buffer solution for two cycles (10 min/cycle) and then in distilled water for 10 min. The samples were dehydrated in a gradient ethanol series of 30% (v/v), 50% (v/v), 70% (v/v) and 95% (v/v) for three cycles (10 min/cycle). The samples were critical-point dried using a critical point dryer (CPD 020; Balzers; Germany). The dried samples were sputter-coated with gold and the specimens were observed using scanning electron microscopy (SEM) at an acceleration voltage of 15 kV (Sutloet et al., 2019).

Statistical analysis

Data were analyzed using analysis of variance. Duncan's new multiple range test was used to determine the differences between sample means at $p \leq 0.05$.

Results and Discussion

Effects of deacetylation of konjac glucomannan on rheology of surimi samples

The storage modulus (G′) and loss modulus (G″) of surimi during transition from sol to gel as a function of temperature are shown in Fig. 1. All surimi samples had higher values for G′ than G″ throughout the temperature range (4–120°C). In the control, G′ increased from 4°C and reached a first peak at 39°C. This increase at low temperature indicated the formation of a weaker, three-dimensional gel network through hydrogen bonding (Zhang et al., 2013; Lefèvre et al., 2008). The G′ dropped to a minimum at 68°C. These changes in G′ were attributed to proteinase in the fish muscles becoming activated in this temperature range. These enzymes may hydrolyze the protein network (the modori phenomenon), resulting in a decrease in G′ (Zhang et al., 2013). Then, G′ gradually increased and reached a second peak. Damodaram and Paraf (1997) reported that the increase in G′ could be related to an increase in the number of protein-protein interactions, resulting in the formation of a highly elastic protein gel. Mleko and Foegeding (2000) demonstrated that the increase in G′ at high temperature resulted from the denaturation of myosin and actomyosin. The G′ values of the surimi samples with different degrees of deacetylation produced the same trend as in the control throughout the temperature range. However, the G′ values of the first peak were different, being 35°C for surimi with native KGM, 34°C for surimi with 64%DD and 36°C for surimi with 94%DD. Zhang et al. (2015) concluded that the hydrophobic interactions became stronger as the %DD increased, indicating that the %DD played an important role in the gelation of the KGM.

As shown in Fig. 1, the control sample had the lowest G′ value throughout the temperature range. The viscoelastic solid-like behavior of surimi could be improved by the use of native or deacetylated KGM that might have been dispersed in a myofibrillar protein gel matrix, which acted as a filler in the protein matrix. Yuan et al.

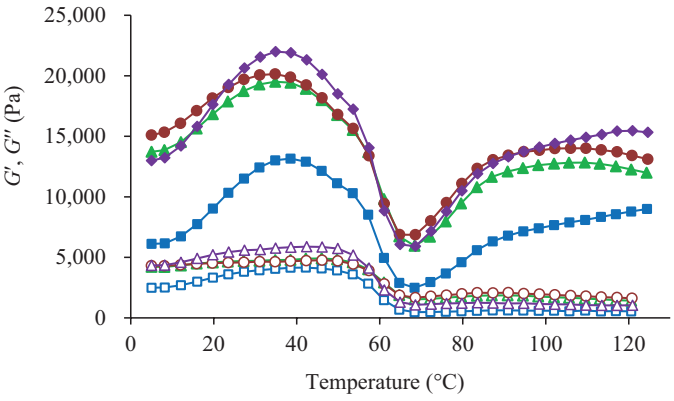

Fig. 1 Storage modulus (G′, solid symbol) and loss modulus (G″, open symbol) of surimi with different degrees of deacetylation (DD) of konjac glucomannan (KGM) during transition as a function of temperature, where □ = control (without KGM); △ = with native KGM (SD0); ○ = with deacetylated KGM, %DD of 64.08±1.47 (SD1); ◇ = deacetylated KGM, %DD of 93.49±1.04 (SD2)

(2019) reported that native and deacetylated KGM improved protein molecular cross-linking and the formation of surimi gel from silver carp (*Hypophthalmichthys molitrix*). As seen from Fig. 1, the G′ value of samples with D2 KGM trended highest in both the first peak (22–60°C) and second peak (95–120°C). Phisutthigoson and Sompongse (2020) found that D2 KGM had the lowest solubility (data not shown) because of removal of the acetyl groups that confer solubility. The D2 KGM had a weaker ability to bind water, so that competition to bind water between protein and D2 KGM might have been lower than for D0 KGM and D1 KGM. D2 KGM had a lower G′ value than D0 KGM and D1 KGM during heating (4–110C), but had the highest G′ value above 110°C. However, the G′ value of D2 KGM increased to the highest value when the temperature decreased during the cooling step (data not shown). D2 KGM had not only the lowest %DD but also had more liquid like-behavior than the other samples. Thus, D2 KGM could readily disperse through the protein gel matrix during heating, and after cooling, it could increase the G′ value of the surimi gel. The protein molecules of the sample with D2 KGM could easily interact with each other, forming a gel network and leading to an increase in G′. A similar trend was reported by Zhang et al. (2015), who demonstrated that the G′ values of surimi gels with native and deacetylated KGM (34%DD, 65%DD, 98%DD) were higher than that of the control. Therefore, surimi gel with added 98%DD had the highest G′ curve (specifically the highest G′ value was for the D2 KGM gel after cooling and a stronger surimi gel network with the lowest %DD of KGM).

Effects of deacetylation of konjac glucomannan on gel strength of surimi gels

Gel strength is one of the most important parameters for surimi quality (Arfat and Benjakul, 2012). Fig. 2 shows the gel strength of surimi gels with native and deacetylated KGM. The gel samples were coded as SD0 (surimi gels with native KGM), SD1 (surimi gels with 64%DD of KGM), and SD2 (surimi gels with 94%DD of KGM) and compared with a control without KGM. Phisutthigoson and Sompongse (2020) reported that samples set at 40°C for 30 min followed by heating at 90°C for 20 min had higher gel strength (data not shown) than high temperature-treated gels. This result was consistent with other research. For example, Zhang et al. (2018) found that the gel strength of surimi samples gradually decreased as the temperature increased from 100°C to 105°C and then sharply decreased as the temperature increased to 121°C. High temperature treatment (≥ 100°C) affected the protein structure and decreased the gel strength of surimi samples (Zhang et al., 2013). The main protein in the surimi gels is myofibril, which is composed of mainly myosin and actin. Protein is easily denatured under high temperature (100–121°C) conditions (Zhang et al., 2018). Ramírez et al. (2011) reported that surimi gels heated at 90°C for 30 min had the best textural properties.

As shown in Fig. 2, the degree of deacetylation of KGM had significant effects on the gel strength of the samples. The SD2 sample had the significantly highest gel strength. After deacetylation, the acetyl group content was reduced, which decreased steric hindrance (Li et al., 2014). Therefore, the surimi with 94%DD (SD2) had the lowest steric hindrance of the acetyl group of KGM. The 94% DD molecules had a smaller volume compared to the native and 64%DD of KGM so the protein molecules could move closer and more easily interact to form a gel network. The KGM molecules could more easily interact, producing a filler entrapped in the three-dimensional protein network. All these factors contributed to the stronger formation of the SD2 sample. SD0 and SD1 had significantly lower gel strength than SD2. Because native and 64%DD of KGM had more steric hindrance of the acetyl group, gelation of protein was prevented, reducing the gel strength.

Effects of deacetylation of konjac glucomannan on expressible water content of surimi gels

Fig. 3 shows the expressible water content of surimi gels with native and deacetylated KGM and of the control sample. The surimi

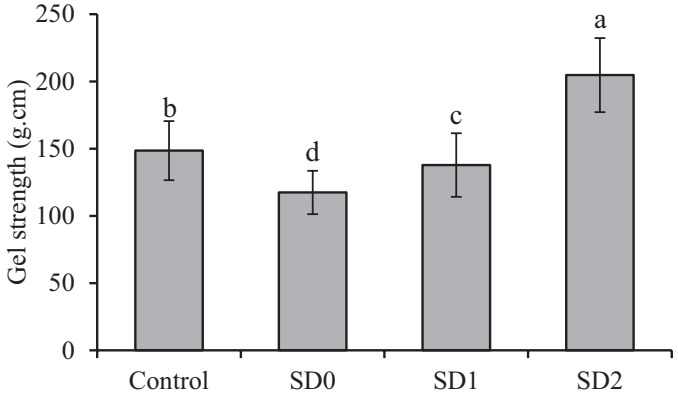

Fig. 2 Gel strength of surimi gels with different degrees of deacetylation of konjac glucomannan (KGM) and subjected to heat at 120°C for 24 min, where error bars = ±SD (n = 8); different lowercase letters above columns indicate significant ($p < 0.05$) differences; Control = surimi gel without KGM, SD0 = surimi gel with native KGM; SD1 = surimi gel with deacetylated KGM, %DD of 64.08 ± 1.47; and SD2 = surimi gel with deacetylated KGM, %DD of 93.49 ± 1.04

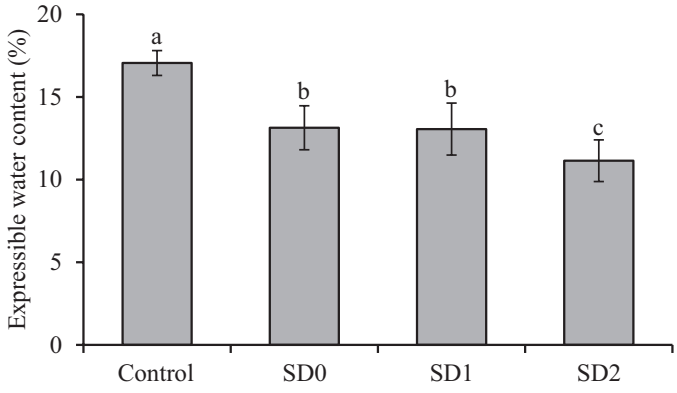

Fig. 3 Expressible water content of surimi gels with different degrees of deacetylation of konjac glucomannan (KGM) and subjected to heat at 120°C for 24 min, where error bars = ±SD (n = 5); different lowercase letters above columns indicate significant ($p < 0.05$) differences; Control = surimi gel without KGM, SD0 = surimi gel with native KGM; SD1 = surimi gel with deacetylated KGM, %DD of 64.08 ± 1.47; and SD2 = surimi gel with deacetylated KGM, %DD of 93.49 ± 1.04

gels with native (SD0) and deacetylated KGM (SD1 and SD2) had significantly lower expressible water contents than the control. The addition of KGM improved water retention because the KGM could absorb water and swell. Native KGM can hydrate rapidly, absorbing up to 200 times its weight in water, depending on purity (BeMiller, 2018). SD2 had the lowest ($p < 0.05$) expressible water content because this sample had a strong network, related to the gel strength in Fig. 2. However, the expressible water contents of SD0 and SD1 were not directly related to their gel strength. SD0 and SD1 had lower gel strengths, but the expressible water content was lower than for the control. Phisutthigoson and Sompongse (2020) found that native (SD0) and 64%DD KGM (SD1) exhibited 80% higher solubility, while 94%DD (SD2) had only 32% solubility (data not shown). As the presence of the acetyl group confers water solubility on KGM, the solubility of KGM decreased as the %DD increased (Du et al., 2012). Although the gel networks of SD0 and SD1 were weaker than that of the control, KGM could help water absorption into the gel matrix, due to their higher solubility as mention above. Du et al. (2012) reported that the acetyl group of KGM enhanced hydrogen bonding with water. Therefore, the expressible water content of the samples was lower than that of the control.

Effects of deacetylation of konjac glucomannan on protein patterns of surimi gels

The pattern of proteins extracted from surimi gel with native and deacetylated KGM was compared with that of control. The results are shown in Fig. 4. The MHC bands (200 kDa) disappeared, confirming the results of Runglerdkriangkrai et al. (2006). They reported that the MHC bands in fish ball subjected to high temperature treatment (116°C, 6 min) almost disappeared. High temperatures may enhance myosin polymerization and MHC polymers are located at the top of the gel. However, actin bands (45 kDa) still appeared. The degree of deacetylation had little effect on the protein patterns of the gels.

Effects of deacetylation of konjac glucomannan on microstructure of surimi gels

Fig. 5 shows the SEM micrographs of the surimi gel with native and deacetylated KGM and the control. Addition of KGM produced a denser and more uniform microstructure in the gels. However, the SD2 gel had a more uniform network structure with smaller holes than the other samples. This was consistent with its higher gel strength. The results were in agreement with those of Zhang et al. (2015), who reported that surimi gel with the highest DD of KGM (98%DD) had a denser and more uniform network structure with small holes. They hypothesized that the aggregation of protein became less serious as the DD of KGM increased.

The surimi samples added with KGM showed higher values for G′ than G″ throughout the temperature range (4–120°C) and the SD2 sample had the highest G′ trend. In addition, the SD2 sample had the highest gel strength but the lowest expressible water content with more dense and uniform microstructures compared to the others. Based on the sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern, the MHC band completely disappeared in all the samples.

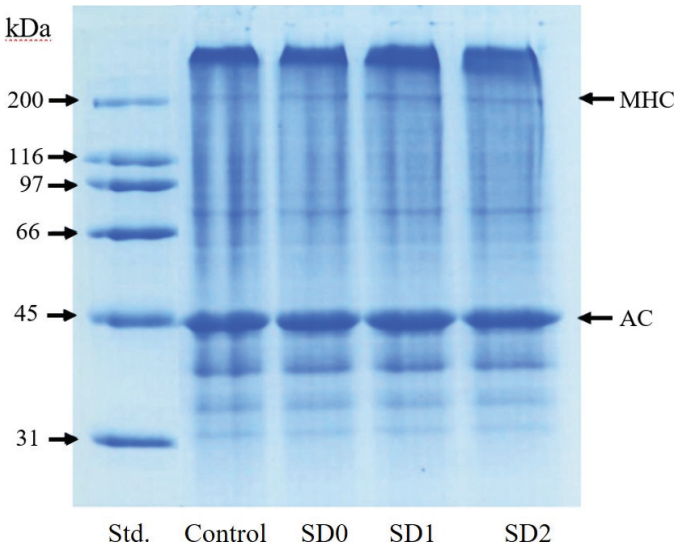

Fig. 4 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of surimi gels with different degrees of deacetylation of konjac glucomannan (KGM) and subjected to heat at 120°C for 24 min, where MHC = myosin heavy chain, AC = actin, Std. = standard protein, Control = surimi gel without KGM; SD0 = surimi gel with native KGM; SD1 = surimi gel with deacetylated KGM, %DD of 64.08 ± 1.47; and SD2 = surimi gel with deacetylated KGM, %DD of 93.49 ± 1.04

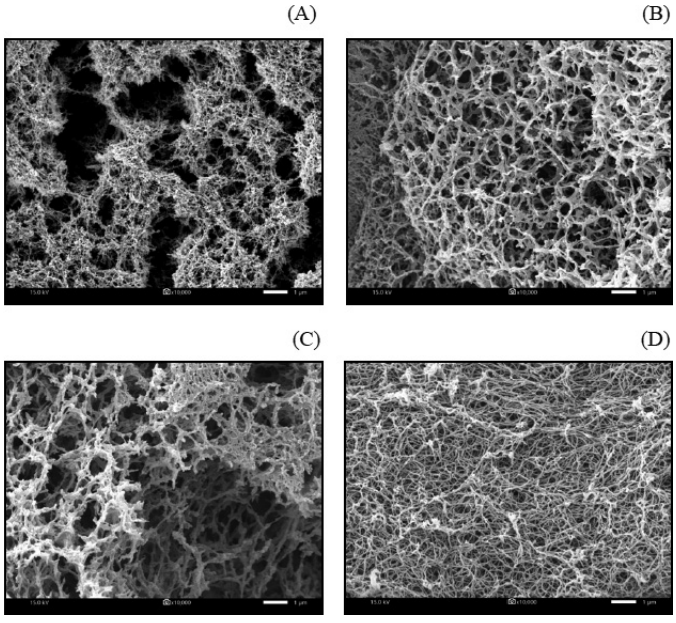

Fig. 5 Microstructure (magnification: 10,000×) of surimi gels with different degrees of deacetylation of konjac glucomannan (KGM) and subjected to heat at 120°C for 24 min: (A) surimi gel without KGM; (B) surimi gel with native KGM; (C) Surimi gel with deacetylated KGM, %DD of 64.08 ± 1.47; (D) Surimi gel with deacetylated KGM, %DD of 93.49 ± 1.04

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

Financial support was provided by the Thammasat University Research Fund under the TU Research Scholar, Contact No. TN15/2561 and a scholarship for Talented Student to Study Graduate Program in Faculty of Science and Technology, Thammasat University (2016 academic year).

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