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Synergistic interactions of deacetylated konjac glucomannan and fish mince on rheological and textural improvement of newly restructured Malaysian fish sausage (*keropok lekor*)

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Abstract

The present work investigated the effects of deacetylated konjac glucomannan (KGM) at varying concentrations (3.26, 2.38, and 1.42%) on the rheological, textural, and microstructural properties of *keropok lekor* restructured with reduced fish mince levels (50, 45, and 40%). Amplitude sweep tests revealed that all KGM-containing samples exhibited higher storage modulus (G'), loss modulus (G"), and critical strain (γ_{cr}) values than those of the control (70% fish mince). At 50% fish mince, samples with 3.26 and 2.38% KGM showed the highest γ_{cr} (2.54%), whereas samples with 1.42% KGM did not show significant structural improvement. A synergistic effect was observed at 45% fish mince with 3.26% KGM, yielding higher G' and G" than the 50% fish mince formulation. Dynamic rheological and textural properties showed more significantly enhanced structural formation, particularly at higher fish mince and KGM concentrations. SEM imaging confirmed that higher fish mince levels resulted in more compact gel structures. At 40% fish mince, all formulations with varying KGM levels showed poor structural properties. Overall, KGM acted as effective filler, enhancing viscoelasticity and structure at 45 and 50% fish mince levels, with the 45% fish mince and 3.26% KGM formulation emerging as a promising option for reducing fish content without compromising quality.

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Introduction

Seafood product restructuring has been employed as a method to create seafood analogues, aiming to produce products that do not rely on animal sources. This approach addresses the limited supply of high-value seafood products, and the need to process underutilised resources, which often face low acceptability due to high bone content or from the by-products of fish processing, such as muscle trimmings or sawdust from frozen fish (Ramírez *et al*., 2011). This process commonly incorporates hydrocolloids, such as konjac glucomannan. Konjac glucomannan (KGM) is a neutral polysaccharide that offers thickening and gelling properties that are widely being used as a new filler in seafood product restructuring (Herranz *et al*., 2013; Ran and Yang, 2022; Zhang *et al*., 2023a). KGM is extracted from the tubers of the *Amorphophallus konjac* plant in the form of powder or flour through a purification process. This water-soluble polysaccharide consists of β-1, 4 linked D-glucose and D-mannose in a molar ratio of 1:1.6 or 1:1.4, and an acetyl group side chain (5 - 10%). The average molecular weight of KGM typically varies between 500,000 and 2,000,000 due to the different species of konjac plants, cultivation areas, processing technologies, and storage times (Ye *et al*., 2021). KGM is known for its health benefits, such as lowering blood sugar and cholesterol levels, aiding in weight loss, and boosting immune and digestive functions. KGM has been consumed for centuries, primarily to improve the texture of foods, such as tofu, noodles, and jellies.

Native KGM is a natural form of konjac glucomannan that can be deacetylated by removing the acetyl groups with dietary alkali, such as sodium carbonate (Wu *et al*., 2020; Yan *et al*., 2021; Ran *et al*., 2022). Deacetylated KGM has been shown to

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improve the gel strength and firmness of surimi by enhancing the hydrophobic interactions between proteins, resulting in a denser and more compact network structure in the gels. These findings suggest that deacetylated KGM modifies protein aggregation, and promotes interactions and cross-links between protein molecules (Yuan *et al*., 2019). However, excessive deacetylation can lead to poor texture in surimi, causing the gel structure to weaken and collapse. Additionally, the rheological and gel properties of KGM are affected by the degree of deacetylation (Wu *et al*., 2020; Cao *et al*., 2022). The deacetylation degree of KGM is limited to 50.72% for surimi products as the gel strength decreases, and the water distribution becomes uneven when the deacetylation degree exceeds 50.72% (Yan *et al*., 2021).

Soy protein isolates (SPI) are highly purified soy protein products containing over 90% protein (O′ Flynn *et al*., 2021; Ma *et al*., 2022). They are affordable and highly sustainable, and are often used to enhance nutrition, and reduce costs in various foods by replacing dairy and meat proteins (Zheng *et al*., 2021). In the food industry, SPI serves as a versatile functional ingredient, acting as a binder, emulsifier, foaming agent, and gelling agent. Production methods, such as alkali extraction and isoelectric precipitation, affect SPI functionality. For instance, strong gelling is useful in meat replacements, whereas partially hydrolysed SPI is suitable for products requiring lower viscosity. The protein content of SPI is approximately 88.3 g per 100 g, with superior digestibility compared to other protein sources, such as pea protein. This makes SPI crucial in meat analogues, typically replacing 30 - 40% of the meat. Studies have shown that SPI maintains sensory qualities, and improves protein levels in products such as breakfast sausage and surimi (Lee *et al*., 2017). Incorporating SPI into surimi enhances its gel characteristics and sensory retention, creating a denser gel network with improved strength (Somjid *et al*., 2022; Zhang *et al*., 2023b).

Malaysian fish sausage, commonly known as *"keropok lekor",* is a culturally significant food product widely consumed on the east coast of Malaysia, particularly in the states of Terengganu, Kelantan, and Pahang. Traditionally, k*eropok lekor* is prepared using a mixture of fish mince (approximately 70%), sago flour, and permitted flavour enhancers such as sugar, salt, and monosodium glutamate (Nur Liyana *et al*., 2018). Favoured across Malaysia's multiracial communities, it is particularly associated with fishing towns, where it is regularly consumed as a side dish. The most commonly used fish species in *keropok lekor* are round scads and sardines, valued for their sweeter taste and fewer bones compared to other varieties.

In recent years, concerns regarding food security have intensified efforts to identify sustainable alternatives to animal proteins. In response, researchers have increasingly explored the substitution of animal-derived ingredients with plant-based proteins in various food systems. A previous study reported that reducing the fish mince content in *keropok lekor* to 50%, and replacing it with different types of starch resulted in lower consumer acceptability, primarily due to undesirable increases in textural hardness (Nur Liyana *et al*., 2018). Despite these challenges, continued reliance on high proportions of fish mince in *keropok lekor* production poses sustainability concerns related to fish supply (Murad *et al*., 2017). Overfishing has been identified as a key contributor to the depletion of fish stocks in Malaysian waters, which, in turn, has driven up market prices (Yeong, 2022). According to the National Fishermen's Association, illegal fishing activities have also exacerbated the recent surge in fish prices (Yeong, 2022). Furthermore, restricted fishing activities during the monsoon season and rising seafood prices present additional challenges to the fisheries sector.

To address these concerns, and promote the sustainability of seafood-based products, the present work aimed to develop a restructured version of *keropok lekor* with reduced fish mince content while maintaining desirable textural attributes. This was achieved by incorporating deacetylated KGM and SPI as functional ingredients. The SPI content was held constant across all formulations, whereas the fish mince was gradually reduced to 50, 45, and 40%, with corresponding adjustments in the deacetylated KGM. The feasibility of these reformulated products as lower-fish-content alternatives was assessed through rheological analysis, textural profile evaluation, and microstructural characterisation, and compared against a control sample. These novel formulations have the potential to support the development of high-protein, lower animal-based content foods, thereby contributing to more resilient and sustainable food systems through strategic restructuring of ingredients.

Materials and methods

Materials

The main ingredients for *keropok lekor* preparation were round scad *(Decapterus russelli)* mince, tapioca, and sago starch, all of which were purchased from Maperow Sdn. Bhd. Kuala Terengganu, Malaysia. Salt, sugar, and monosodium glutamate (MSG) were purchased from a local market in Kuala Terengganu, Malaysia. SPI and KGM powder (80% purity) were purchased from Thong Sheng Food Technology and Ingredient Sdn. Bhd., Penang, Malaysia. Sodium carbonate (Na_2CO_3) was purchased from Bischem Technology Sdn. Bhd., Selangor, Malaysia. All the ingredients used in the *keropok lekor* formulations were of food grade.

Production of keropok lekor

The traditional formulation with 70% fish mince, as described by Nur Liyana *et al*. (2018), was used as the control sample (Table 1A). The restructured *keropok lekor* formulations were developed by reducing the fish mince content to 50, 45, and 40%, as shown in Table 1B. To improve the texture, and maintain structural integrity, KGM was added at 3.26, 2.38, and 1.42% (w/w) of the total formulation, respectively. Preliminary trials indicated that exceeding 3.26% of KGM resulted in poor dough cohesion, making the mixture unsuitable for shaping. SPI was incorporated at a fixed level of 4% in all restructured formulations to compensate for the reduction in protein content caused by the partial substitution of fish mince. Deacetylation of KGM was carried out using 0.5% Na_2CO_3, and crushed ice was used to compensate for the weight difference, and to control the temperature during mixing.

Table 1A. Formulation of control *keropok lekor,* which was modified from Nur Liyana *et al.* (2018).

Ingredient	Percentage (%)
Fish mince	70.0
Starch	13.8
Salt	2.0
Sugar	1.0
Monosodium glutamate (MSG)	0.3
Crushed ice	12.9

Table 1B. Restructured formulations of *keropok lekor*.

Formulation/ Ingredient (g)	F50: DK3.26	F50: DK2.38	F50: DK1.42	F45: DK3.26	F45: DK2.38	F45: DK1.42	F40: DK3.26	F40: DK2.38	F40: DK1.42
Fish mince	50	50	50	45	45	45	40	40	40
KGM	3.26	2.38	1.42	3.26	2.38	1.42	3.26	2.38	1.42
Starch	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Soy protein isolate (SPI)	4	4	4	4	4	4	4	4	4
SPI water hydration	12	12	12	12	12	12	12	12	12
Crushed ice	8	8	8	13	13	13	18	18	18
Iced water	10.74	11.62	12.58	10.74	11.62	12.58	10.74	11.62	12.58
Salt	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sugar	1	1	1	1	1	1	1	1	1
Monosodium glutamate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium carbonate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

KGM = deacetylated konjac glucomannan; F50:DK3.26 = fish mince 50% + deacetylated KGM 3.26%; F45:DK2.38 = fish mince 45% + deacetylated KGM 2.38%; and F40:DK1.42 = fish mince 40% + deacetylated KGM 1.42%.

The control sample was prepared by blending all ingredients in a food processor (MK-5087M Panasonic, Osaka, Japan) for 3 min. For the structured *keropok lekor*, SPI was hydrated using water at three times its weight for 30 min, following the method described by Ran *et al*. (2022). Subsequently, all ingredients, except the KGM, were added into the food processor. After 1 min of blending, the KGM was gradually incorporated, and mixing continued for an additional 2 min. The resulting dough was shaped into cylinders (6 cm in length and 1 cm in diameter), and boiled at 100°C for 10 min.

Determination of deacetylation degree

The degree of deacetylation was measured based on the method described by Hu *et al*. (2019) with slight modifications. The deacetylated KGM was prepared before measuring the degree of deacetylation. First, 30 g of KGM was dispersed in 200 mL of an aqueous ethanol solution (50%, v/v) in a Schott bottle. The capped bottle was then placed in an ambient shaker incubator (SI-50, Protech, Malaysia) set at 40°C and 150 rpm for 30 min to allow the KGM to swell. Sodium carbonate (Na_2CO_3) was subsequently dispersed into the mixture, and the reaction was continued in an incubator shaker for 24 h at 40°C. Three samples with different amounts of sodium carbonate (4.6, 6.3, and 10.6 g) were prepared. These amounts corresponded to the ratios of Na_2CO_3 to KGM prepared for the restructured *keropok lekor*. After the incubation period, the sample was washed successively with 200 mL each of 50, 75, and 95% ethanol, and then dehydrated with 200 mL of anhydrous ethanol. This washing step was intended to remove excess alkali from the sample. Once the sample was dehydrated, excess ethanol was evaporated in a fume hood. The process continued with vacuum drying for 6 h at 40°C to obtain a powdered form of deacetylated KGM.

To determine the degree of deacetylation, 0.5 g of the sample was weighed and mixed with 10 mL of a 75% ethanol solution in a Schott bottle. The mixture was heated in a water bath (Memmert, Germany) at 40°C for 30 min, and then allowed to cool to room temperature. After cooling, 0.5 mL of 0.5 M NaOH solution was added, and the mixture was saponified in an incubator shaker (40°C, 150 rpm) for 24 h. The excess alkali was then titrated with 0.1 M HCl, using phenolphthalein (0.05% in 50% ethanol solution) as an indicator. A blank (without the sample) was titrated in parallel to serve as a control.

The acetyl content (W_0) was calculated using Eq. 1:

$$W_0 = \frac{(V_2 - V_1) \times N_{HCl} \times M_{acetyl}}{m_s} \times 100\% \quad \text{(Eq. 1)}$$

where, V_2 = volume of HCl consumed by the blank in litres (L); V_1= volume of HCl consumed by KGM sample in litres (L); N_{HCl} = normality of HCl; M_{acetyl} = molar mass of acetyl group and 43 g/mol; and m_s = mass of sample in gram (g).

The deacetylation degree (DD) was calculated using Eq. 2:

$$DD = \frac{W_0 - W}{W_0} \times 100\% \quad \text{(Eq. 2)}$$

where, W_0 = whole acetyl group content of native KGM; and W = acetyl group content when groups were partially removed by controlling the reaction conditions. The degrees of deacetylation for the restructured formulations with 3.26, 2.38, and 1.42% deacetylated KGM were 29.36(± 3.03)%, 34.81(± 5.99)%, and 77.75(± 8.11)%, respectively.

Rheological analysis

The effects of deacetylated KGM concentrations on the rheological properties of *keropok lekor* at different fish mince levels were assessed using the amplitude sweep test, frequency sweep test, and dynamic mechanical thermal analysis (DMTA). The experiments were carried out using the Discovery™ Hybrid Rheometer HR-30 by TA Instrument (New Castle, Delaware, USA).

i) Amplitude sweep test

An amplitude test was conducted to comprehend the linear viscoelastic region of the samples. The sample underwent amplitude sweep analysis using a rheometer, with the stress controlled at 25 ± 0.01°C, and the strain starting from 0.01 to 100%. The distance between the sample stage and parallel plate (diameter of 40 mm) was settled at 1.0 mm (Ran and Yang, 2022). The amplitude test served two purposes: to obtain the rheological properties of the sample, and to measure the damage to the material characteristics.

ii) Dynamic mechanical thermal analysis (DMTA)

The paste of the SPI-deacetylated KGM was equilibrated at 25°C for 2 min on the sample plate of the rheometer. The strain setup was 1.0% and 10 rad/s of angular frequency for three stages: the heating stage from 25 to 90°C at a rate of 2°C per min, the equilibrium stage at 90°C for 10 min, and the cooling stage from 90 to 25°C at 2°C per min. The sample was then measured using a rheometer. It was placed at the bottom of the rheometer with silicone oil around the sample to seal and prevent the sample from evaporating, and to create a form that fell around the gap. This analysis was to measure the variation in the storage of modulus G' and loss modulus G'' of restructured *keropok lekor* with different concentration levels of deacetylated KGM.

Texture profile analysis

Texture profile analysis was performed to evaluate the effect of different concentrations of deacetylated KGM at different fish mince levels. Texture analyser (TA.XT2i Texture Analyser, Stable Micro Systems Co. Ltd., Godalming, UK) was used to determine the hardness and chewiness of the *keropok lekor samples*. The parameter settings were as follows: load cell, 25 kg; compression platen, P.75; constant rate, 1 mm/s; trigger force, 10 g (2 s); pre- and post-test speed, 3 mm/s; and return distance, 35 mm. Samples (at room temperature) were cut into small pieces (2.5 cm in length), and placed centrally under the cylinder probe (P/36R with a 5 kg load cell).

Statistical analysis

Statistical analysis was performed using the MiniTab 2 Statistical Software (State College, Pennsylvania). One-way analysis of variance (ANOVA) was used, and significant differences were determined using Tukey's *post-hoc* test at $p < 0.05$ significance level.

Results and discussion

Amplitude sweep test

Amplitude sweep testing was conducted to evaluate the viscoelastic properties of the restructured *keropok lekor* dough formulated with varying levels of deacetylated KGM at different fish mince proportions (50, 45, and 40%). In this analysis, the strain (γ) applied was varied while the frequency remained constant. The results revealed the relationship between storage modulus (G'), loss modulus (G"), and strain (γ). Three regions were identified: the linear viscous region (LVR), the linear viscoelastic region (LVER), the non-linear viscoelastic region (NLVER), and the critical strain (γ_{cr}). LVR refers to a range of deformation conditions in which the sample exhibits purely viscous behaviour. LVER is the first to be determined during the oscillation stress sweep, followed by NLVER, which starts once G' deviates by more than 5% (Abbastabar *et al*., 2015). The critical strain, γ_{cr}, indicates the limit for LVER, where the sample deviates within 5%, and further increases in strain result in a non-linear response. Critical strain (γ_{cr}) is a measurement that indicates shape retention and structural strength under external stress (Hesarinejad *et al*., 2014). It shows the sensitivity of G' to high strain effects, and begins to decrease.

Figure 1 illustrates the changes in viscoelastic moduli as affected by the incorporation of the deacetylated KGM at varying levels of fish mince. Overall, all samples containing deacetylated KGM exhibited increases in critical strain (γ_{cr}), storage modulus (G'), and loss modulus values (G"). The critical strain for the control *keropok lekor* (70% fish mince, without the deacetylated KGM) was 1.01%. Regardless of fish mince content, all restructured samples showed higher critical strain values than the control. Notably, samples formulated with 2.38 and 3.26% deacetylated KGM at 50% fish mince demonstrated the highest γ_{cr} value (2.54%), while other restructured samples exhibited a critical strain of 1.60%. These findings indicated a synergistic effect between fish protein content and deacetylated KGM concentration in enhancing the structural resistance of *keropok lekor* dough under oscillatory stress. Similar trends in the increase in critical strain due to deacetylated KGM incorporation were reported by Ran and Yang (2022).

The storage modulus (G') reflects the elastic response of a material, and represents the amount of energy stored during deformation, indicating changes in the sample elasticity (Yu *et al*., 2017). The loss modulus (G") quantifies the viscous dissipation of energy under deformation, reflecting the viscous characteristics of the material (Hu *et al*., 2019). In all samples, G' values exceeded G" values, indicating typical viscoelastic solid-like behaviour. The restructured samples containing 2.38 and 3.26% deacetylated KGM exhibited significantly higher G' and G" values than both the 1.42% deacetylated KGM formulation and the control across all fish mince levels. The sample containing 1.42% deacetylated KGM showed no improvement in moduli values, suggesting that this concentration was insufficient to enhance the structural network of the *keropok lekor* dough.

At 50% fish mince, both 2.38 and 3.26% deacetylated KGM produced similar G' and G" values, suggesting no additional benefit from increasing deacetylated KGM beyond 2.38%. In contrast, at 45% fish mince, the formulation containing 3.26% deacetylated KGM exhibited

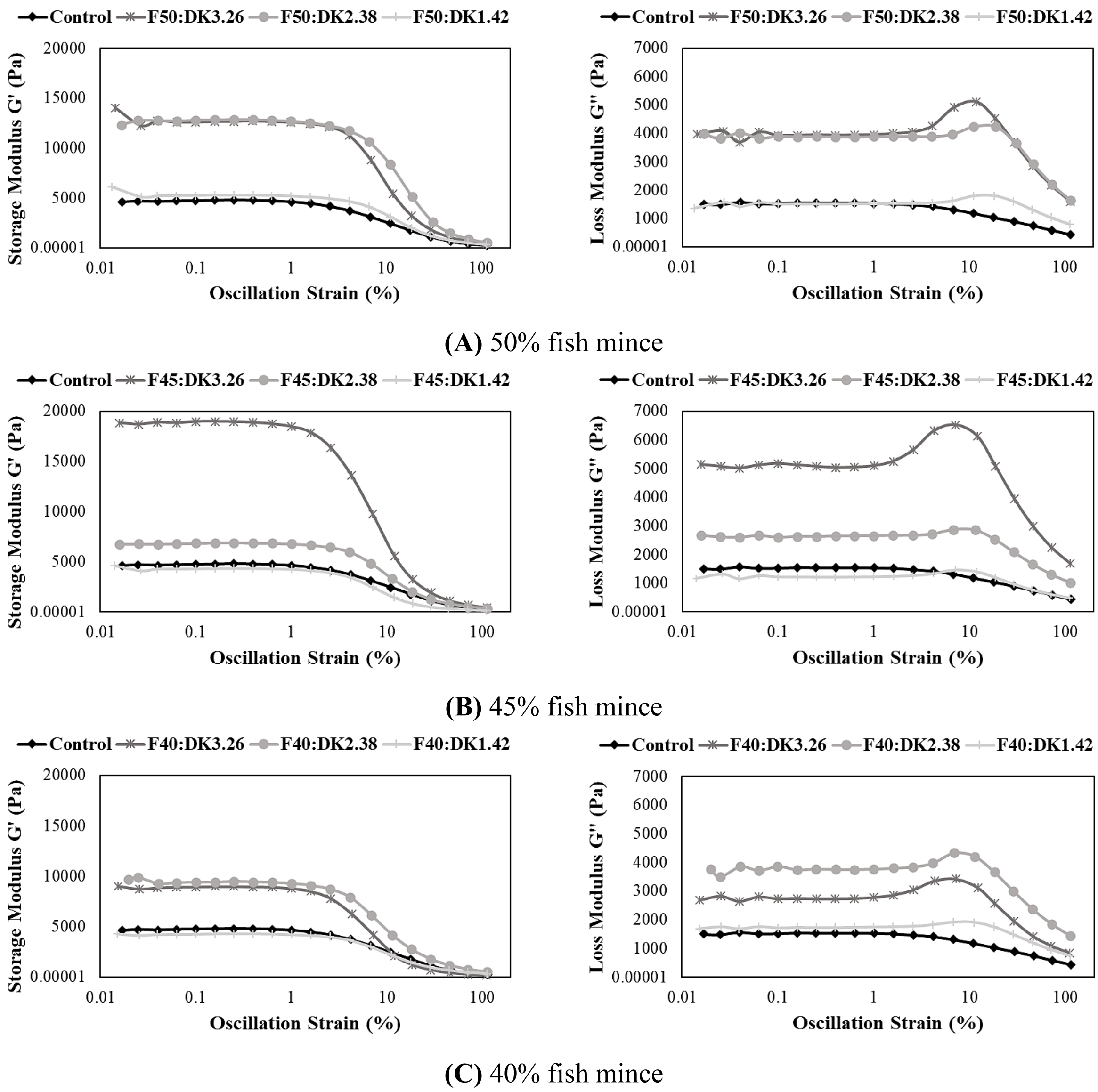


Figure 1. Changes in storage modulus (G') and loss modulus (G'') of restructured *keropok lekor* as affected by KGM concentrations at different fish mince ratios. Control sample was traditional formulation with 70% fish. F50:DK3.26 = fish mince 50% + deacetylated KGM 3.26%; F45:DK2.38 = fish mince 45% + deacetylated KGM 2.38%; and F40:DK1.42 = fish mince 40% + deacetylated KGM 1.42%.

significantly higher moduli than all other samples, even exceeding those observed at 50% fish mince. This indicated a pronounced synergistic interaction between the reduced fish mince level (45%) and 3.26% deacetylated KGM, leading to superior structural development and enhanced energy storage capacity.

Formulations containing 3.26 and 2.38% deacetylated KGM had DD of 29.36 and 34.81%, respectively. At these DD levels, the presence of acetyl groups enhanced the hydrogen bonding capacity with water (Yuan *et al*., 2019). Hydrogen bonding is a critical force for maintaining the folding structure of a protein (Sano *et al*., 1994). However, the strong water-binding ability of deacetylated KGM can limit the protein from fish mince to partially unfold (from the effect of mechanical shear and hydration during mixing), thereby reducing the formation of hydrogen bonds between carbonyl groups and the amino acid of the protein. In addition, differences in the amount of crushed ice used to compensate for the reduced fish mince may influence protein behaviour. In the 45% fish mince formulation, the higher water content facilitated greater protein unfolding, and the formation of hydrogen bonds with water compared to the 50% fish mince formulation. At 45% fish mince, optimal hydrogen bonding from the deacetylated KGM enhanced the formation of a cohesive polysaccharide-protein-water network, markedly improving the texture.

All the restructured samples experienced notable bulge in G” at the maximum strain amplitude (γ_{max}), indicating strain hardening, a phenomenon associated with increased resistance to deformation. This behaviour has been reported in other protein-gum systems (Mirarab Razi *et al*., 2018; Ran and Yang, 2022). Chemeda *et al*. (2015) also critically discussed strain hardening in the context of kaolinite particle interactions affected by hydrated lime ($Ca(OH)_2$). In the present work, this phenomenon was attributed to the intensified interactions among KGM-containing particles, resulting in a more compact and reinforced structural network (Solo-de-Zaldívar *et al*., 2014).

Dynamic rheology of restructured keropok lekor as affected by deacetylated KGM concentration at different fish mince levels

The effects of deacetylated KGM and varying fish mince levels on the dynamic rheological properties of *keropok lekor* are presented in Figure 2. The G’ of the control sample (70% fish mince, no deacetylated KGM) initially decreased up to approximately 50°C, followed by a rapid increase at neat 80°C, and then decreased towards the end of the heating process. This behaviour aligned with previous findings by Renkema and Van Vliet (2002), who reported that heat-induced gelation of myofibrillar proteins involved a decrease in G’ due to protein unfolding or denaturation, followed by an increase in G’ as aggregation and rearrangement led to gel network formation. The initial G’ decrease was attributed to the thermal denaturation and separation of the myosin tail region, which increased molecular mobility (Liu *et al*., 2007).

In general, the restructured samples exhibited a trend similar to that of the control sample, except for the rapid increase in moduli at temperatures above 50°C. While the control sample showed a sharp increase in G’ value from 50 to 80°C, indicating a typical gelation event where myosin denatures and aggregates to form a stable gel (Yuan *et al*., 2019), the restructured samples displayed more gradual changes, with G’ curves nearly plateauing within this range. This suggested that early-stage gelation may have already occurred in these samples, likely facilitated by the presence of deacetylated KGM. At the early stage of heating, proteins in the restructured samples may partially unfold and compete with KGM for water binding, limiting their ability to undergo the rapid aggregation typically observed in the control. The presence of deacetylated KGM may thus stabilise the protein network earlier in the heating process, decreasing the mobility of the protein molecule, and reducing the observable G’ increase during the heating temperature range.

Regardless of the deacetylated KGM concentration, formulations with higher fish mince levels consistently exhibited higher G’ and G” values, reflecting greater structural integrity due to higher protein content. Notably, the addition of deacetylated KGM enhanced the viscoelastic properties across all formulations, as evidenced by the higher G’ values compared to the control. This enhancement aligned with the findings of Yuan *et al*. (2019), who reported improved gelation in surimi containing deacetylated KGM. Upon heating, the hydrogen bonds between the carbonyl and amino groups within the protein chains are disrupted, leading to complete unfolding and exposure of the hydrophobic regions, which in turn drives protein aggregation. The deacetylated KGM may act as filler in the composite gel, affecting the exposure and aggregation of the hydrophobic amino acid residues of the proteins. KGM could form an irreversible gel after the partial removal of the acetyl group, and improve hydrophobic interactions between KGM molecules, resulting in enhanced aggregation of fish protein.

The degree of deacetylation (DD) is also important in structural development, as reported by Yuan *et al*. (2019) and Yan *et al*. (2021). Since a low DD of KGM was used in the present work, the ability to enhance the structural formation was due to the concentrations of KGM rather than the DD of deacetylated KGM. The deacetylated KGM likely functioned primarily as filler, with higher concentrations exerting a more substantial effect on structural reinforcement. This “concentration effect” likely enhances texture by crowding protein particles closer together, promoting cross-linking and gel strengthening (Yan *et al*., 2021). As a result, formulations with higher KGM concentrations displayed increased G’ values, even at reduced fish mince levels. This trend persisted even at 40% fish mince level, although the overall structural strength was reduced due to lower protein availability. These findings highlighted the importance of sufficient protein content in achieving optimal texture in

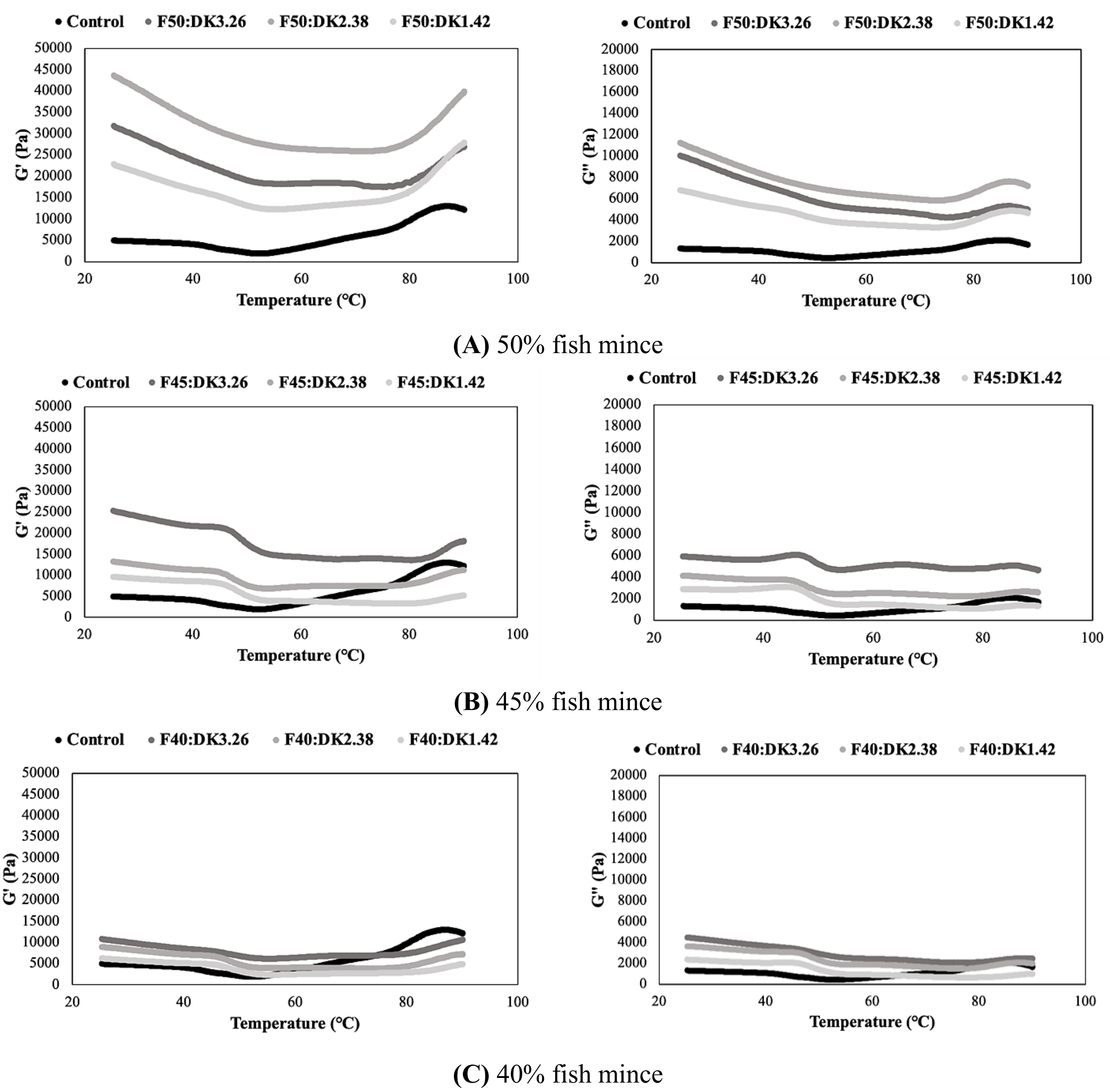


Figure 2. Changes in storage modulus (G') and loss modulus (G') of restructured *keropok lekor* at different temperatures as affected by KGM concentrations at different fish mince ratios. Control sample was traditional formulation with 70% fish. F50:DK3.26 = fish mince 50% + deacetylated KGM 3.26%; F45:DK2.38 = fish mince 45% + deacetylated KGM 2.38%; and F40:DK1.42 = fish mince 40% + deacetylated KGM 1.42%.

restructured *keropok lekor*, even when polysaccharide-based structuring agents were employed.

Effect of deacetylated KGM on texture of restructured keropok lekor

The effects of deacetylated KGM at varying fish mince levels on the texture of cooked *keropok lekor* were evaluated to complement the findings of the rheological analyses. Texture was assessed based on hardness and chewiness, as shown in Figures 3A and 3B, respectively. The results demonstrated that increasing the fish mince level corresponded to significant improvements in both parameters. Notably, at the 50% fish mince level, restructured samples containing 3.26% deacetylated KGM exhibited significantly higher hardness and chewiness than the control, suggesting that this formulation produced a firmer and more elastic texture. In contrast, the same concentration of deacetylated KGM incorporated at 45% fish mince yielded a less pronounced textural enhancement,

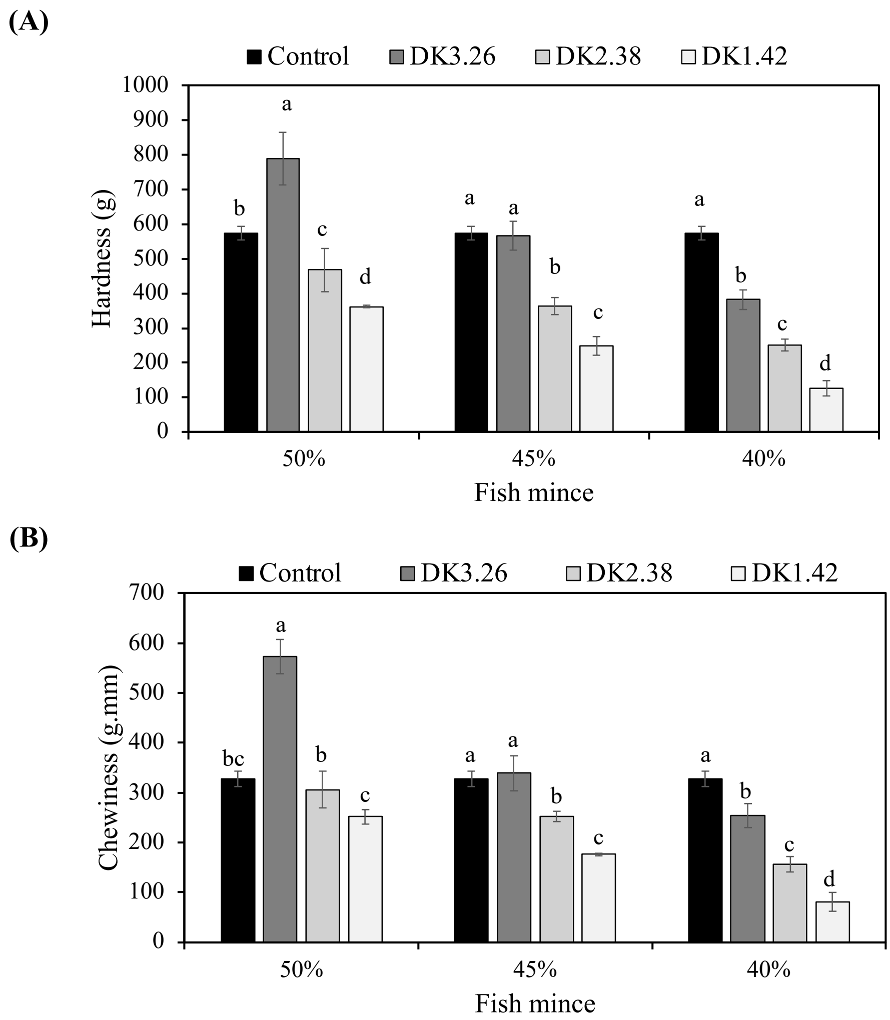


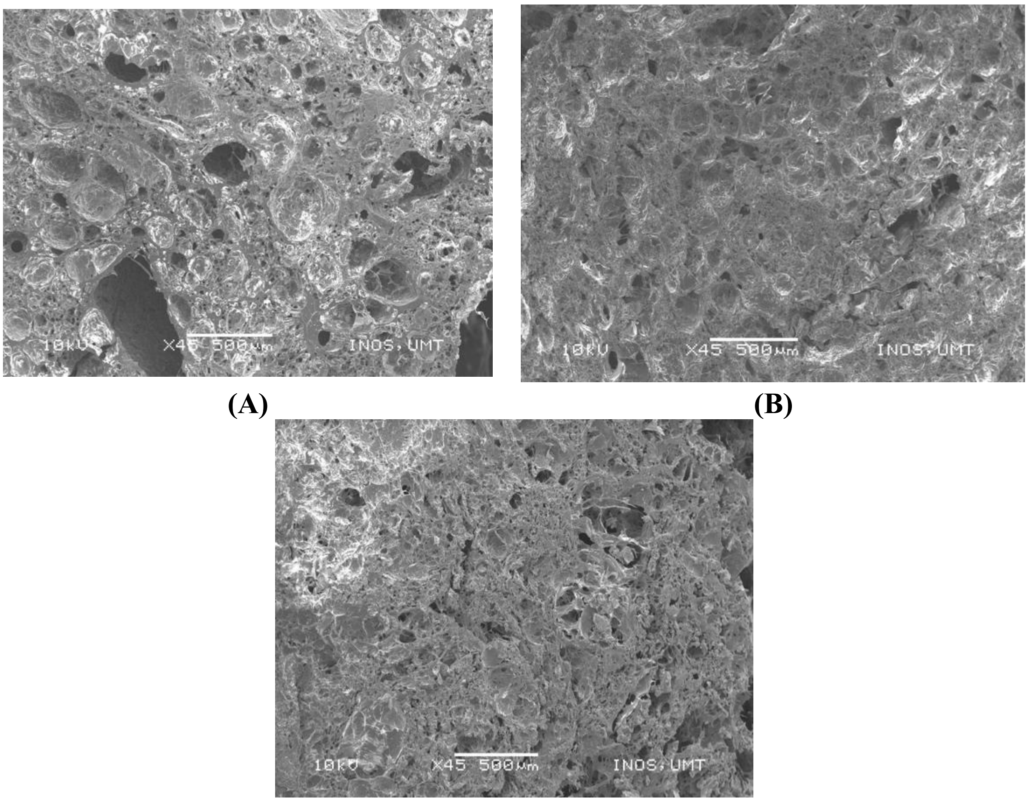
Figure 3. Hardness **(A)** and gumminess **(B)** of restructured *keropok lekor* as affected by KGM concentrations at different fish mince levels. Control sample was traditional formulation with 70% fish. DK3.26 = 3.26% deacetylated KGM; DK2.38 = 2.38% deacetylated KGM; and DK1.42 = 1.42% deacetylated KGM.

indicating that sufficient protein content was necessary to maximise the structuring effect of KGM. The combined effects of protein aggregation and deacetylated KGM incorporation likely contributed to the development of a denser and more cohesive matrix in the 50% fish mince formulations. Higher fish mince content showed a greater effect on protein aggregation, combined with the effect of KGM, resulting in a stronger texture. However, it is important to note that excessive hardness may negatively affect consumer acceptance, suggesting the need for further investigation through sensory evaluation, which was not part of the present work. As highlighted by Nur Liyana *et al*. (2018), increased hardness in *keropok lekor* has been associated with decreased consumer preference. While hardness influences bite resistance, chewiness is a critical determinant of mouthfeel and eating quality (Varela *et al*., 2021).

Reducing the fish content to 45% produced a restructured product with textural properties comparable to those of the control, highlighting the potential of this formulation to maintain desirable eating quality while reducing the fish mince content. At 40% fish mince level, however, all samples exhibited significantly softer textures regardless of KGM concentration, indicating insufficient protein content to enhance interactions to form a stronger gel network. In such low-protein formulations, the inclusion of higher levels of soy protein isolate (SPI) is recommended. Previous studies have demonstrated that SPI can induce myofibrillar proteins to form a compact gel network structure (Li *et al*., 2021; Somjid *et al*., 2022).

Microstructure of restructured keropok lekor

Figure 4 shows the microstructure of the restructured *keropok lekor*, visualised using SEM for samples containing 45 and 50% fish mince with 3.26% KGM. These samples were selected based on the improved properties observed in the rheological and textural analyses presented earlier. The SEM images revealed that the sample with 50% fish mince had a more compact structure than both the 45% fish mince sample and the control, which supported its firmer texture. At this level, fish proteins likely competed with KGM for water, resulting in tighter structures with smaller pores. Previous studies have primarily focused on how increasing the DD in KGM could lead to more compact gels (Yuan *et al*., 2019; Yan *et al*., 2021). However, the present work highlighted that increasing fish mince alone while keeping the KGM concentration and DD constant could also create a denser structure. During gelation, fish protein needs water to unfold, migrate, and form hydrophobic interactions, which drive the formation of a dense gel network (Yan *et al*., 2021). Therefore, higher protein content contributed to the tighter gel network observed in the 50% fish mince sample.



(A) **(B)**

(C)

Figure 4. Scanning electron microscope (SEM) images of control *keropok lekor* sample **(A)**, 45% fish mince of restructured *keropok lekor* containing 3.26% KGM **(B)**, and 50% fish mince of restructured *keropok lekor* containing 3.26% KGM **(C)**.

Conclusion

The rheological and textural properties of the restructured *keropok lekor* samples were strongly influenced by both the concentration of deacetylated KGM and the level of fish mince. Deacetylated KGM primarily acted as structural filler, as evidenced by the increased viscoelastic moduli, enhanced textural properties, and the more compact microstructure observed in SEM images at higher concentrations. This effect was more pronounced when paired with higher fish mince content, suggesting a synergistic interaction that enhanced structural development. Although the degree of deacetylation (DD) of KGM varied with concentration, its influence was less significant than that of concentration, likely due to the relatively low DD values used. Although the 1.42% KGM sample exhibited the highest DD (attributed to a higher alkali-to-KGM ratio), it failed to contribute meaningfully to network formation compared to higher KGM concentrations. This reinforces the conclusion that, in this context, KGM functions more effectively as filler than as a gelling agent, an effect driven by DD. Furthermore, samples formulated with only 40% fish mince demonstrated poor structural integrity regardless of KGM level, highlighting the need for additional protein such as soy protein isolate (SPI) to enhance gel formation through improved interactions with KGM.

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