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Properties and bioactivity of carrageenan, myofibril, and collagen-based smoked edible films

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ABSTRACT

The objective of this study is to develop and evaluate the properties of smoked edible film (EF) composed of carrageenan, myofibril, and collagen. The smoked EF was prepared by incorporating 0.8% liquid smoke. The analysis focused on various parameters including pH, physical properties such as thickness, solubility, tensile strength, elongation percentage, and water vapor transmission rate (WVTR). Sensory evaluation was also conducted to assess the texture attributes of the coated product, including wateriness, firmness, elasticity, hardness, and juiciness. The findings revealed that the concentration of the ingredients influenced the thickness of the EF, with myofibril proteins exhibiting higher concentrations compared to carrageenan and collagen. Both collagen and myofibril demonstrated maximum solubility at a concentration of 6%, while carrageenan achieved optimal solubility at concentrations ranging from 2 to 2.5%. Carrageenan exhibited significantly higher tensile strength compared to myofibril and collagen, whereas collagen demonstrated greater elasticity than carrageenan and myofibril protein. Moreover, myofibril protein film exhibited a lower water vapor transmission rate compared to carrageenan and collagen films. In terms of sensory assessment, carrageenan displayed high elasticity and juiciness, while collagen and myofibril showed high firmness and hardness. All EFs showed better antioxidant activity compared to Trolox (EC50 < 95.57 μg/mL).

KEYWORDS

Smoked Edible Film, Carrageenan, Myofibril, Collagen, Characteristic.

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INTRODUCTION

Over the past two decades, packaging materials derived from petrochemical polymers have gained significant popularity. This can be attributed to their numerous benefits, including flexibility, strength, transparency, and affordability. However, it is important to acknowledge the drawbacks associated with plastic polymers as well. One significant concern is the potential transmission of monomer contaminants into the packaged contents. Additionally, plastic waste is known for its non-biodegradable nature, posing environmental challenges. Increased human population gives a consequence of increased non-biodegradable wastes (Bishop et al., 2021). On the other hand, the wastes of fisheries product are still high, i.e., 20 – 30%, meaning that about 1.3 million tons of waste are produced from 6.5 million tons of Indonesian landed fish every year. This is the reason why research on the utilization of fisheries waste is important to be conducted (Thirukumaran et al., 2022). Edible film (EF) is an up-to-date material generated after biodegradable packaging materials. The advantages are the materials can be eaten together with the food and applied as coating materials for many kinds of food, especially snacks. According to (Donhowe & Fennema, 1994), EFs are classified into three categories based on their component properties: hydrocolloids (containing proteins, polysaccharides, or alginates), lipids (constituted by fatty acids, acylglycerols or waxes), and composites (made by combining substances from the two categories). Two kinds of hydrocolloids usually used are proteins and polysaccharides. Fish meat contains about 15 – 24% of protein and still becomes a main source of protein in Indonesia (Karl et al., 2014). Bones and skins can also be utilized as sources of gelatin and collagen.

According to Nurilmala et al. (2022), fish skin contains thick connective tissues and collagen. It is a potential source of collagen as an alternative to mammalian skin especially pig skin which is not "halal" for Muslim society. Fish protein is a main component of surimi dominated by myofibril protein consisting of myosin, actins, and actomyosin. In response to this, the belly meat of Black Marlin contains high myofibril protein and the potential as a material for EF or coating.

Liquid smoke is a condensate of smoke originating from the pyrolysis process of wood, as an alternative to conventional fish smoking process. Problems of traditional smoked fish are high contents of polycyclic aromatic hydrocarbon (PAH), especially benzopyrene, low edible portion, no processing standard, flavor varied, difficulty in packaging, low performance, and short shelf life. Liquid smoke is an alternative because PAH content is low, the concentration is easily controlled, and the quality of the product can be standardized, easy to produce, needs simple equipment only, and is easily found in the market (Xin et al., 2021). Smoked EF can be used for food, especially easily oxidized food. The compounds resulting from the smoking process, especially phenol, have antimicrobial and antioxidant agents (Lingbeck et al., 2014; Soares et al., 2016). Therefore, this research aims to produce and analyze the physical characteristics of smoked EFs made from carrageenan, collagen, and myofibril protein of black marlin industrial waste. Moreover, the radical scavenging activities of the produced EFs are also evaluated in this study.

MATERIALS AND METHODS

Fresh Black Marlin (Makaira indica) skin and belly meat was taken from the Bersehati Fish Market of Manado, which was further identified and authenticated at the Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado 95115, Indonesia. Liquid smoke was produced using smoke condensation equipment (Patent P00201405308) consisting of three main parts: the fuel combustion furnace, the smoke distribution or separation section, and the condenser part where cooling is carried out, to accelerate smoke condensation; with coconut shells as fuel (Berhimpon et al., 2018; Dien et al., 2019). The smoking process uses raw materials as much as 10 kg with a smoking duration of 4 to 6 hours at a combustion furnace temperature of 400 – 600 °C. The results obtained include liquid smoke of 1900 – 2290 mL with a concentration of 7.5 – 23% and by-products in the form of residual activated charcoal of 2.5 – 3.5 kg.

Processing Procedure of EF or Coating

Collagen extraction from Black Marlin skin used a method modification of Li et al. (2013) and Nagai & Suzuki (2000), while extraction of myofibril from fish meat employed a modified method of Heruwati et al. (2017). Carrageenan was extracted from seaweed Kappaphycus alvareziii, following Montolalu et al. (2008). The EF was produced using a modification of Santoso's method (Santoso et al., 2007).

Treatments

Three EF materials were used, i.e., collagen and myofibril from the waste of Black Marlin, and carrageenan. Five concentrations were set, 2%, 4%, 6%, 8%, and 10%, for collagen and myofibril, and five concentrations for carrageenan, 1.5%, 2%, 2.5%, 3%, and 3.5%. All EFs were added with 0.8% liquid smoke.

Sample Analysis

Analyses were done for physical characteristics i.e., pH with a pH meter, thickness using a micrometer with an accuracy of 0.001 mm (ISO 4591, 1979), solubility (Gontard et al., 1992), tensile strength and % elongation (SNI 0778, 2009), and water vapor transmission rate (WVTR) using WVTR Tester (ASTM E-96-99).

Antioxidant Assay

The DPPH radical scavenging activity test was employed to evaluate the antioxidant properties. The test followed the procedure outlined in a previous study (Wagey et al., 2023). Carrageenan, myofibril, and collagen EF were combined with 3 mL of the DPPH reagent in a testing vial. After cooling to room temperature, the mixtures were left undisturbed for 30 minutes. The change in DPPH concentration was measured by determining the absorbance at 517 nm. To ensure the reliability of the data, each sample was analyzed in triplicate (n = 3). Trolox, a known antioxidant compound, served as a positive control in the DPPH test. The EC50 (half-maximal effective concentration ratio) was utilized to assess the radical scavenging capability of all tested samples, including Trolox. EC50 signifies the concentration of a sample that leads to a 50% reduction in the initial radical concentration. The inhibition of the DPPH assay was expressed as a percentage and calculated using the following formula: The absorbance of the blank (A0) was subtracted from the absorbance of the standard or sample (A1).

$$\text{Antioxidant Activity (\%)} = [(A0 - A1)/A0] \times 100\% \qquad (1)$$

Statistical Analysis

A completely randomized trial with a factorial design was used to analyze the data. Two separate experiments were done with the treatment of collagen and myofibril and the treatment of carrageenan and myofibril. Both experiments used 2 replications. The ANOVA (F test) was used to assess the treatment effect, which was then continued with the Duncan test.

RESULTS AND DISCUSSION

pH

The higher the concentration of collagen, myofibril, and carrageenan, the higher the pH will be. ANOVA, however, showed that the treatments did not significantly affect pH (P > 0.05). The range of pH was very small, only 7.6 to 7.9.

Solubility

The solubility of EF ranged from 35.4% to 55% for collagen material, 33.3% to 50% for myofibril, and 72.08 to 94.2% for carrageenan. Compared to EF made from collagen and myofibril to carrageenan, the solubility of EF made of collagen and myofibril was low in the water at room temperature. The higher solubility for collagen EF occurred at the concentration of 4% and 6% and that for myofibril EF was at the concentration of 6%. ANOVA also showed no significant effect of the treatments on the solubility (P > 0.05).

Thicknesses

The EF thickness ranged from 0.159 mm to 0.211 mm for collagen, 0.186 mm to 0.223 mm for myofibril, and 0.120 to 0.168 mm for carrageenan. The possibility of protein denaturation of protein could cause the EF of collagen and myofibril to become thicker. The thickness curve is presented in Figure 1. The higher the concentration is, the higher the thickness will be. ANOVA indicated that the concentrations gave a highly significant effect (P < 0.01).

Tensile Strengh

The tensile strength of EF ranged from 21.54 kg/cm2 to 38.33 kg/cm2 for collagen, 20.800 kg/cm2 to 94.74 kg/cm2 for myofibril, 99.88 to 260.78 kg/cm2 for carrageenan (Figure 2). The tensile strength of collagen EF was lower than that of myofibril and carrageenan. ANOVA showed that the effect of protein types on thickness was highly significant (P < 0.01), while the effect of concentration and interaction between a kind of proteins and concentration were non-significant (P > 0.05).

Percent of Elongation

The range of elongation percentage was from 69.945% to 165.745% for collagen, 16.795% to 41.075% for myofibril, and 9.33 to 24.0% for carrageenan (Figure 3). The elongation percentage of collagen EF was higher than that of myofibril and carrageenan. It means that the collagen EF was more flexible. ANOVA revealed that the effect of kind proteins, concentrations, and interaction between all kinds of proteins and concentrations were highly significant (P < 0.01).

Water Vapor Transmission Rate (WVTR)

The range of WVTR of EF was from 1450.665 to 1648.0 g/m2 per 24 h for collagen, 1205 to 1391g/m2 per 24 h for myofibril, and 1916.665 to 2266.0 g/m2 per 24 h for carrageenan (Figure 4). WVTR of EF made from carrageenan and collagen was higher than that of myofibril. It means that myofibril EF is better than collagen and carrageenan in preventing water vapor. ANOVA showed that the effect of biopolymer types and concentrations was highly significant (P < 0.01) while the interaction between protein types and concentrations was significant (P < 0.05).

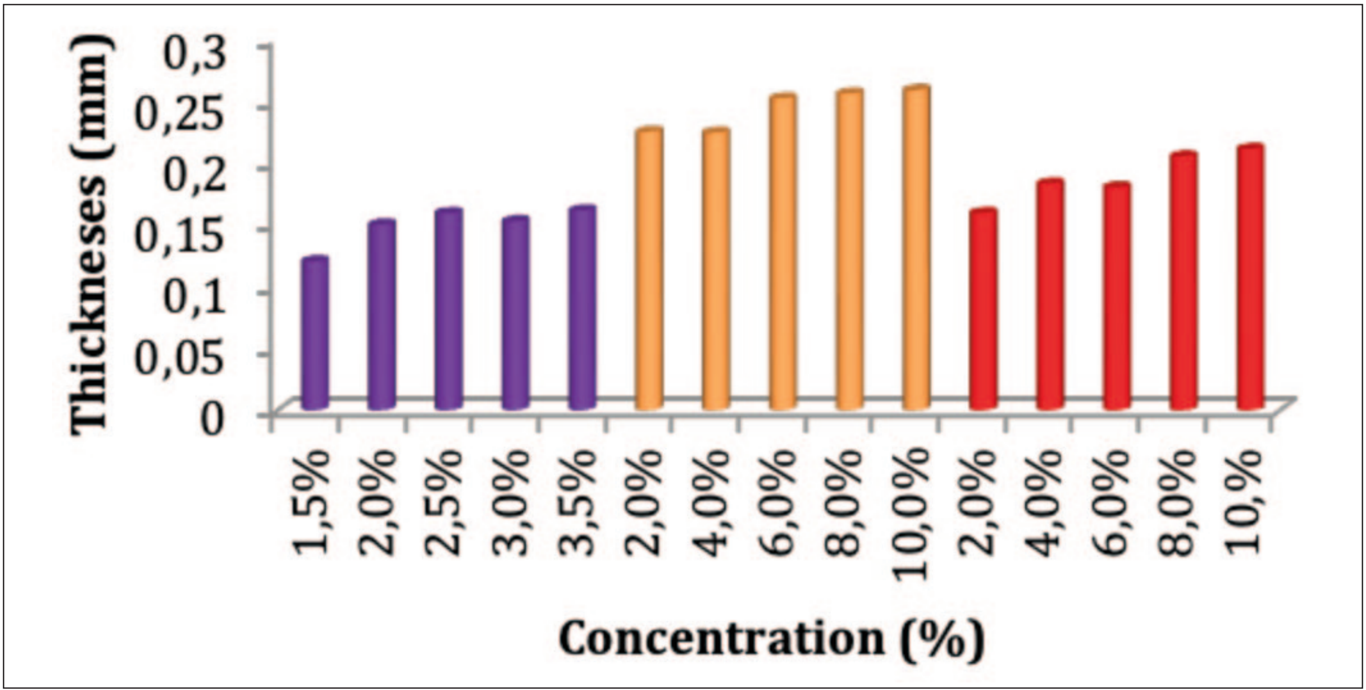

Figure 1. Thicknesses of EF from Carrageenan (Purple), Myofibril (Orange), and Collagen (Red)

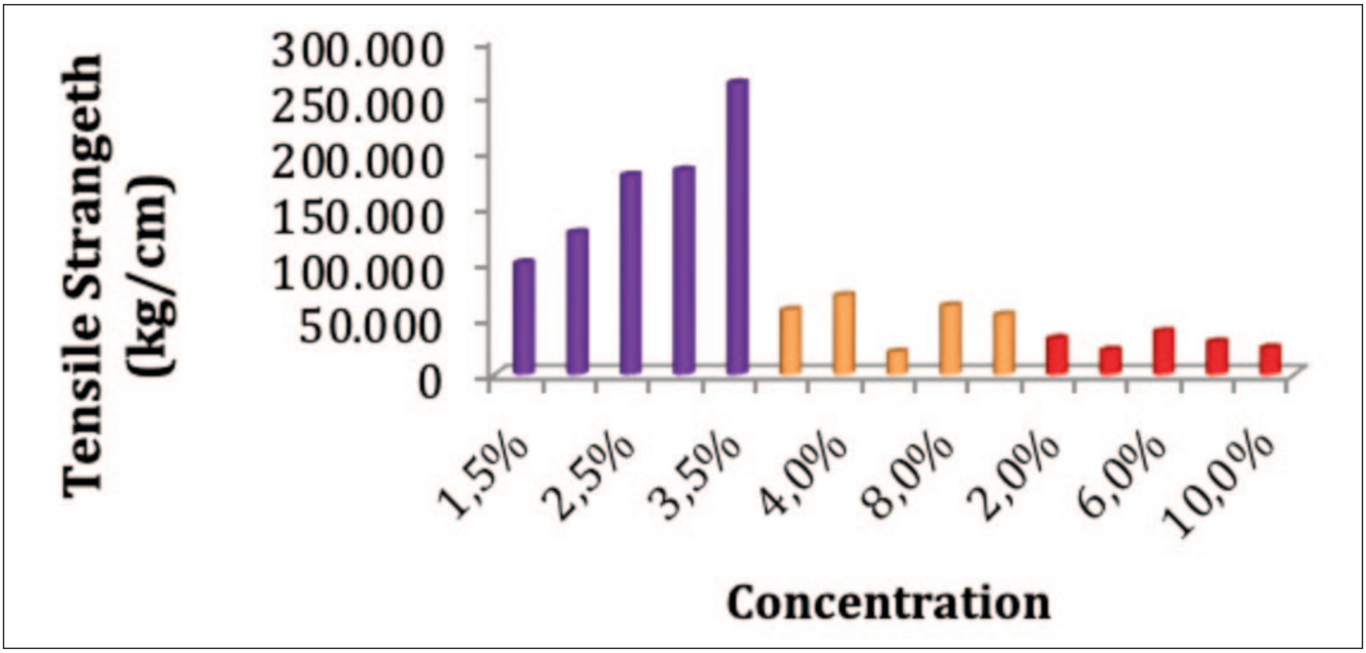

Figure 2. Tensile strength of EF from Carrageenan (Purple), Myofibril (Orange), and Collagen (Red)

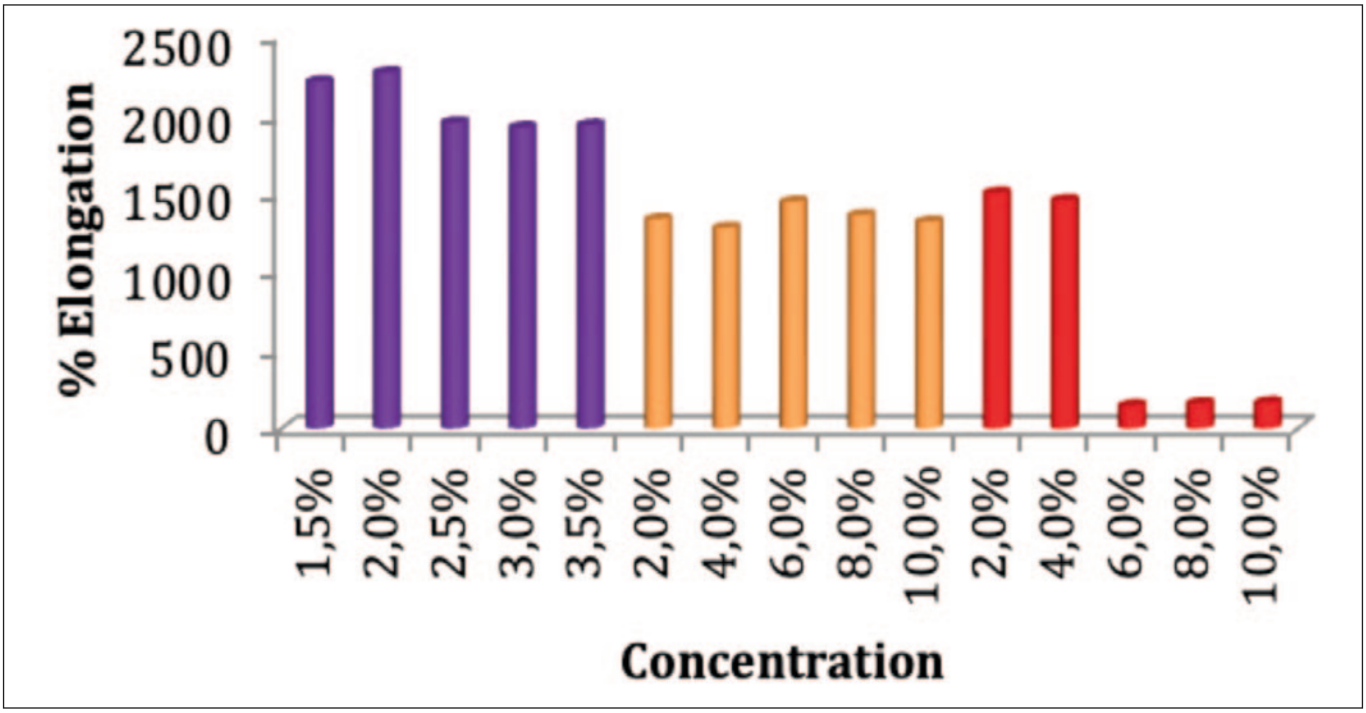

Figure 3. Percent of elongation of EF from Carrageenan (Purple), Myofibril (Orange), and Collagen (Red)

Radical Scavenging Activity

From Figure 5, it is seen that all EFs had a lower EC50 value compared to the Trolox as the control (EC50 Trolox = 95.57 μg/mL). It can be inferred that EFs from carrageenan, myofibril, and collagen have better antioxidant potency in terms of DPPH radical scavenging activity compared to the standard drug Trolox. Between all EFs, EF myofibril has the highest effective concentration in terms of DPPH inhibition activity (EC50 EF Myofibril = 49.33 μg/mL), followed by EF collagen and EF carrageenan (EC50 EF Collagen = 60.94 μg/mL, EC50 EF Carrageenan = 61.47 μg/mL), meaning that EF myofibril exhibits best antioxidant activity.

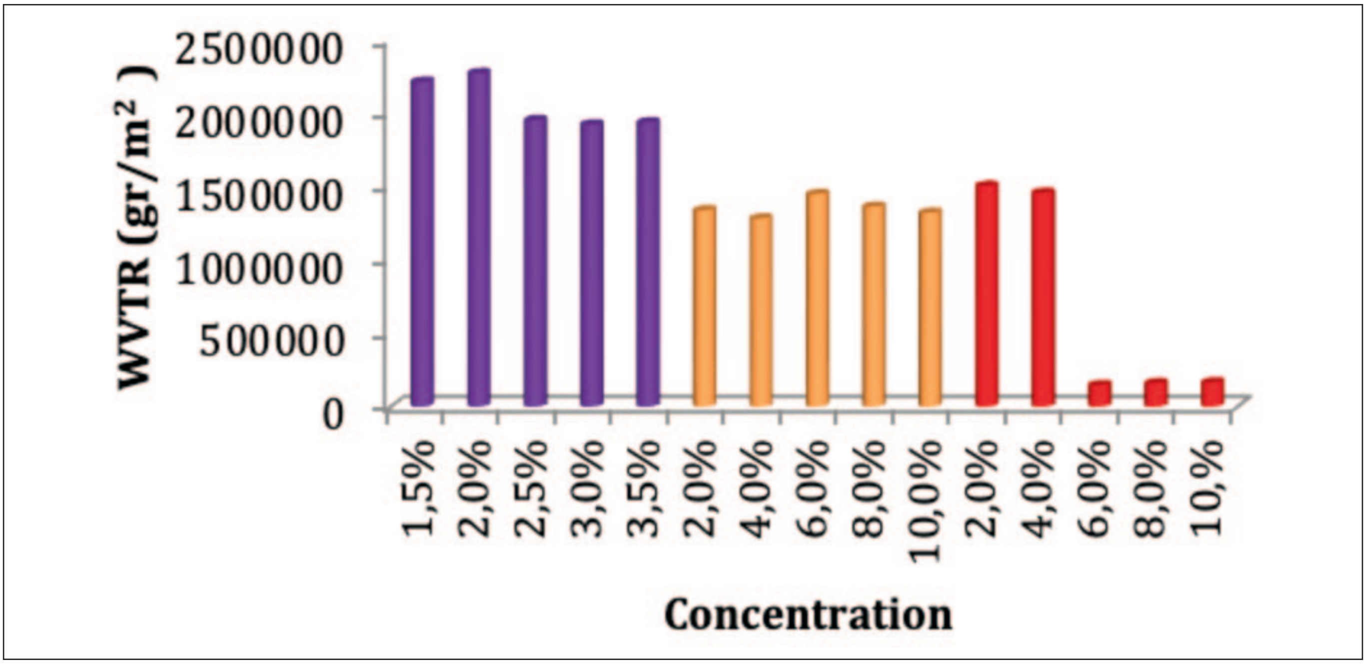

Figure 4. WVTR of EF from Carrageenan (Purple), Myofibril (Orange), and Collagen (Red)

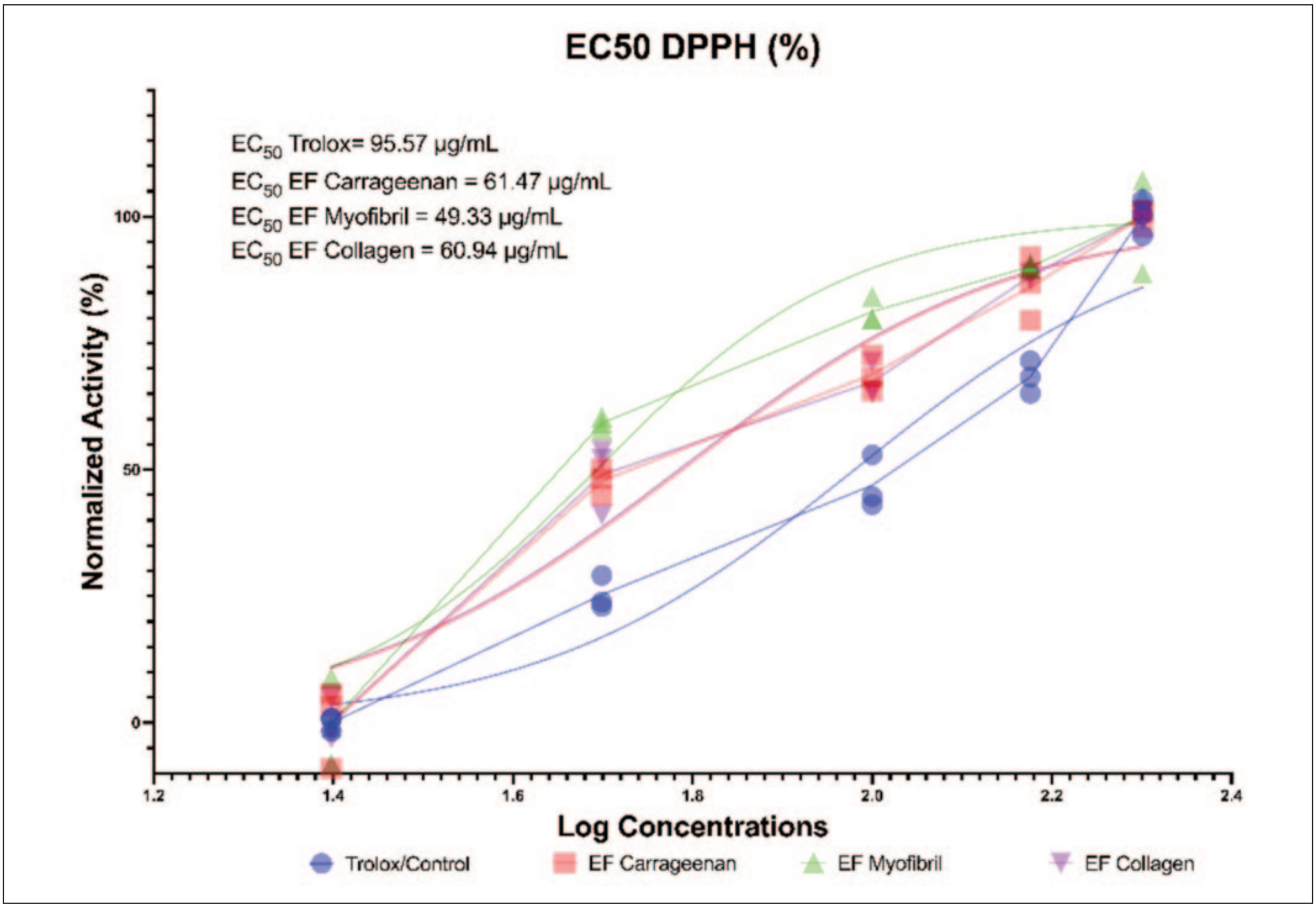

Figure 5. Antioxidant Activity of EF from Carrageenan, Myofibril, and Collagen

The utilization of EF layers derived from fish waste or marine waste has significant importance in various applications. First, it addresses global problems in waste management by converting by-products from the fish and marine industries into edible films, which promotes sustainability and reduces environmental impact. Secondly, EF coating provides a protective barrier for food products, extending their shelf life and preventing damage. It is very beneficial in maintaining the freshness of perishable products, such as fruits, vegetables, and meat, by reducing moisture loss and inhibiting microbial growth. In addition, EF coating can enhance the sensory properties of food, providing an additional layer to taste, texture, and appearance. This EF coating can serve as a carrier of bioactive compounds, such as antioxidants or antimicrobial agents, providing functional benefits to food (Lingbeck et al., 2014; Soares et al., 2016). The use of fish waste or marine waste in edible film coating presents an innovative and sustainable approach to waste reduction, food preservation, and value addition in the food industry (Ibrahim et al., 2022).

EF production using carrageenan, myofibril, and collagen produces EFs with a pH range between 7.6 to 7.9. The pH of a film is an important parameter because it affects the structural integrity, stability, and functionality of the film (Gioffrè et al., 2012). In these cases, films produced using carrageenan, myofibril, and collagen have a slightly alkaline pH, exhibiting relatively neutral to slightly alkaline properties. The pH of the film is influenced by the pH of the starting materials used in the formulation of the film. Carrageenan, a type of polysaccharide derived from seaweed, has a pH value of around 8.5 (Irawan, 2021), while myofibrils, a component of proteins found in muscle tissue, usually have a pH close to neutral (Sun & Holley, 2011). Collagen, a protein abundant in connective tissue, has a pH range between 5.6 to 7.4 (Latorre et al., 2016). The pH range of an edible film has implications for the functionality and application of the film. For example, films with a slightly alkaline pH are generally more stable and more resistant to degradation than films with acidic properties. This pH range also indicates that the films may have enhanced resistance to microbial growth, which is advantageous for food packaging applications (Goel et al., 2021). In some cases, a certain pH range is desirable to ensure compatibility with certain types of food or prevent unwanted chemical reactions (Zhao et al., 2020).

Solubility is one of the important characteristics of edible films that can affect the application and stability of the film. In this study, EFs derived from collagen and myofibril exhibited lower solubility when exposed to water at room temperature, compared to EF from carrageenan (72.08 to 94.2%). As a comparison, Tamaela & Lewerissa (2007) found that the solubility of EF from carrageenan has a range between 71.3% to 96.3%. The high solubility of carrageenan could result from carrageenan that is a water-soluble polymer with a linear chain of partially sulfated galactan that presents high potential as film-forming material, while collagen and myofibril are proteins in which heating at 80 °C during the EF processing makes them be partly denaturized (Suwa et al., 2016). Furthermore, the high solubility of carrageenan can be due to the hydrophilic (easily soluble in water) nature of carrageenan. When carrageenan is used as the main ingredient in the manufacture of edible film, the film has a strong affinity with water, making it more easily dissolved (Alam et al., 2019). The solubility of edible films made of myofibrils and collagen (proteins abundant in connective tissue) tends to be lower, as these proteins have hydrophobic properties (Piez & Trus, 1977). The higher solubility of edible carrageenan film can provide advantages in practical applications. More soluble films can easily decompose and dissolve in water, thereby reducing the risk of environmental contamination and optimizing the use of films as environmentally friendly packaging materials. In addition, high solubility can also affect the texture and organoleptic properties of the film when used in food products (Kirtil et al., 2021). Nevertheless, it is important to note that the solubility of edible films is determined not only by the type of material used, but also by a variety of other factors, including film formulation, processing, and environmental conditions. Therefore, for specific applications, it is important to consider all these factors comprehensively to select materials and design suitable edible films.

EFs from myofibril and collagen are the thickest in this study's findings. These findings suggest that EF's thickness varies depending on the type of material used. The possibility of protein denaturation in collagen and myofibrils can cause the film thickness to become thicker (Schmid et al., 2014). Changes in film thickness can be influenced by several factors, such as the concentration of the material, the nature of the material itself, and the interaction between the materials used. In these cases, the increased concentration in the film formulation results in a film of greater thickness. This can be due to the presence of stronger molecular interactions or different physical influences on the structure of the film with an increase in the concentration of the material. Proteins such as collagen and myofibrils tend to interact and form denser aggregates or tissues at higher concentrations, which in turn can lead to an increase in film thickness (Yang et al., 2016). However, it is important to note that the thickness of the film has implications on the nature and performance of the film. Greater thickness can affect the texture, mechanical strength, permeability rate of water vapor and gas, and physical stability of the film. In some applications, such as food packaging, the appropriate film thickness can be an important factor in maintaining product quality and freshness. However, film thickness can also affect the solubility, solubility, and functional properties of films in different environments (Das et al., 2022).

The results of this study revealed that the tensile strength of EF varies depending on the type of material used. Tensile strength is an important parameter that describes the extent to which edible films can withstand tensile loads before they crack or break. In this study, films made of carrageenan have higher tensile strength compared to films of collagen and myofibrils. This may be due to differences in the physical and structural properties of each of these proteins. Collagen, as an abundant protein component in connective tissue, has lower elastic properties and lower tensile strength compared to myofibrils and carrageenan (Kwansa et al., 2016). Myofibril, which is a component of protein in muscle tissue, has better elastic properties and higher tensile strength (Washio et al., 2019) while carrageenan, as a natural polysaccharide extracted from seaweed, has strong mechanical properties and high tensile strength (Briones et al., 2004). The results of ANOVA analysis showed that the effect of protein type on film thickness was significant, but the effect of concentration and interaction between protein type and concentration was not significant. This suggests that the tensile strength of the film is more influenced by the type of protein used than the concentration of the material. In the context of practical application, the tensile strength of the edible film is an important parameter to be considered. Films with high tensile strength can provide better protection against physical damage and provide safety in the packaging and use of food products. Therefore, the selection of the right material and understanding of the tensile strength characteristics of edible films are very important in the development of quality films.

The results of this study show variations in the percentage of EF elongation depending on the type of material used. The elongation percentage is a parameter that describes the extent to which the film can stretch before it cracks or breaks. The results showed that collagen EF has a higher percentage of elongation compared to myofibrils and carrageenan EF. This shows that collagen films have better softness and flexibility than myofibrils and carrageenan films. Furthermore, ANOVA revealed that the effect of protein type, concentration, and interaction between all protein types and concentrations had a very significant influence on the percentage of elongation ($P < 0.01$). The increase in the percentage of elongation in the EF collagen film can be due to the intrinsic nature of the collagen itself. Collagen, as a component of proteins in connective tissue, has a fibril structure that gives it high softness and elasticity (Kirkness et al., 2019; Shoulders & Raines, 2009). This makes the EF collagen film more able to stretch and return to its original shape without significant damage. This discovery has important implications for the development of edible film applications. Films with a high percentage of elongation can provide better flexibility in the packaging and use of food products. This is especially important in the context of applications that require the film's ability to adjust to changes in product shape or position, such as unusually shaped packaging or products that are prone to changes in size or shape.

WVTR is a parameter that describes how easily water vapor can pass through the film. The higher the WVTR, the higher the film's ability to release or absorb moisture. The results showed that EF myofibrils had a lower WVTR compared to EF collagen and carrageenan. This suggests that myofibrils have better properties in preventing moisture transmission, which could mean they are more effective in protecting products from environmental moisture (Gaikwad et al., 2019). ANOVA showed that the effect of biopolymer type and concentration had a very significant influence on WVTR ($P < 0.01$), while the interaction between protein type and concentration had a significant effect ($P < 0.05$). These results suggest that the type of material and concentration used in edible film manufacturing affect the WVTR of the film. The increase in WVTR in EF carrageenan and collagen films can be due to the intrinsic properties of these materials. Carrageenan is a polysaccharide that can absorb water well (Alam et al., 2019), while collagen has a high affinity for water (Gonzalez & Wess, 2013). Both properties can cause films made of carrageenan and collagen to have a higher ability to absorb and release moisture. Meanwhile, EF myofibrils, which are made of muscle protein components, may have a denser structure and be less reactive to water vapor, resulting in a lower WVTR (Piez & Trus, 1977). These findings have important implications for edible film applications. Films with lower WVTR can be used to protect moisture-sensitive food products, such as dry food or perishable products due to moisture exposure (Gaikwad et al., 2019). In this case, EF myofibrils show better potential in maintaining product quality because they have a lower WVTR.

Antioxidant testing in this study found that EF made from ingredients such as carrageenan, myofibril, and collagen has strong antioxidant potential. A lower EC50 value indicates that the EF has a better ability to capture free radicals, which is an indicator of antioxidant activity. EF myofibrils exhibit the best antioxidant activity with the lowest EC50, suggesting that they may be an attractive option for product development with antioxidant properties. It is important to note that Trolox was used as a control in this study because it is known to have strong antioxidant activity. In this regard, the EF tested showed better antioxidant activity than Trolox, demonstrating its potential use as a natural antioxidant agent that can be used in food or health supplement applications. These findings have important implications for the development of EF-based products (Rangaraj et al., 2021). By having strong antioxidant activity, EF from carrageenan, myofibrils, and collagen can be used as natural additives to improve the stability and oxidative resistance of food products. In addition, EF can also potentially be an active ingredient in the formulation of health supplements or beauty products.

CONCLUSIONS

Edible films (EF) could be produced from collagen, myofibril, and carrageenan of Black Marlin waste. Nevertheless, research on the application of the EF or coating to food needs to be done. The pH and solubility of EF made from collagen, myofibril, and carrageenan were the same. Their thickness EF was similar as well but seemed to rise with increased concentration. The tensile strength of myofibril EF was better than that of collagen and there are no differences by concentration. The percent elongation of collagen EF was higher than that of myofibril and carrageenan and sharply rose with increased concentration. The WVTR of collagen EF was slightly higher than that of myofibril and carrageenan. All EF from all kinds of proteins showed better antioxidant activity compared to Trolox (EC50 < 95.57 μg/mL). All EFs have been successfully characterized and should be utilized based on the intended purpose or characteristics.

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REFERENCES

1. Alam, J., Alhoshan, M., Shukla, A. K., Aldalbahi, A., & Ali, F. A. A. (2019). k-Carrageenan – A versatile biopolymer for the preparation of a hydrophilic PVDF composite membrane. European Polymer Journal, 120, 109219. https://doi.org/10.1016/j.eurpolymj.2019.109219

2. Berhimpon, S., Montolalu, R. I., Dien, H. A., Mentang, F., & Meko, A. U. I. (2018). Concentration and application methods of liquid smoke for exotic smoked Skipjack (Katsuwonus pelamis L.). International Food Research Journal, 25(5).

3. Bishop, G., Styles, D., & Lens, P. N. L. (2021). Environmental performance of bioplastic packaging on fresh food produce: A consequential life cycle assessment. Journal of Cleaner Production, 317, 128377. https://doi.org/10.1016/j.jclepro.2021.128377

4. Briones, A. V, Ambal, W. O., Estrella, R. R., Pangilinan, R., De Vera, C. J., Pacis, R. L., Rodriguez, N., & Villanueva, M. A. (2004). Tensile and Tear Strength of Carrageenan Film from Philippine Eucheuma Species. Marine Biotechnology, 6(2), 148–151. https://doi.org/10.1007/s10126-003-0005-9

5. Das, D., Panesar, P. S., Saini, C. S., & Kennedy, J. F. (2022). Improvement in properties of edible film through non-thermal treatments and nanocomposite materials: A review. Food Packaging and Shelf Life, 32, 100843. https://doi.org/10.1016/j.fpsl.2022.100843

6. Dien, H. A., Montolalu, R. I., & Berhimpon, S. (2019). Liquid smoke inhibits growth of pathogenic and histamine forming bacteria on skipjack fillets. IOP Conference Series: Earth and Environmental Science, 278(1), 12018. https://doi.org/10.1088/1755-1315/278/1/012018

7. Donhowe, G. I., & Fennema, O. R. (1994). Edible films and coatings: Characteristics, formation, definitions, and testing methods. In J. Krochta, E. Baldwin, & M. Nisperos-Carriedo (Eds.), Edible Coatings and Films to Improve Food Quality (pp. 11–17). Technomic Publ. Co., Inc., Lancaster, PA.

8. Gaikwad, K. K., Singh, S., & Ajji, A. (2019). Moisture absorbers for food packaging applications. Environmental Chemistry Letters, 17(2), 609–628. https://doi.org/10.1007/s10311-018-0810-z

9. Gioffrè, M., Torricelli, P., Panzavolta, S., Rubini, K., & Bigi, A. (2012). Role of pH on stability and mechanical properties of gelatin films. Journal of Bioactive and Compatible Polymers, 27(1), 67–77. https://doi.org/10.1177/0883911511431484

10. Goel, N., Fatima, S. W., Kumar, S., Sinha, R., & Khare, S. K. (2021). Antimicrobial resistance in biofilms: Exploring marine actinobacteria as a potential source of antibiotics and biofilm inhibitors. Biotechnology Reports, 30, e00613. https://doi.org/10.1016/j.btre.2021.e00613

11. Gontard, N., Guilbert, S., & Cuq, J.-L. (1992). Edible Wheat Gluten Films: Influence of the Main Process Variables on Film Properties using Response Surface Methodology. Journal of Food Science, 57(1), 190–195. https://doi.org/10.1111/j.1365-2621.1992.tb05453.x

12. Gonzalez, L. G., & Wess, T. J. (2013). The effects of hydration on the collagen and gelatine phases within parchment artefacts. Heritage Science, 1(1), 14. https://doi.org/10.1186/2050-7445-1-14

13. Heruwati, E. S., Murtini, J. T., Rahayu, S., & Suherman, M. (2017). Pengaruh Jenis Ikan dan Zat Penambahan Terhadap Elastisitas Surimi Ikan Air Tawar. Jurnal Penelitian Perikanan Indonesia, 1(1), 86–94.

14. Ibrahim, I. D., Hamam, Y., Sadiku, E. R., Ndambuki, J. M., Kupolati, W. K., Jamiru, T., Eze, A. A., & Snyman, J. (2022). Need for Sustainable Packaging: An Overview. In Polymers (Vol. 14, Issue 20). https://doi.org/10.3390/polym14204430

15. Irawan, I. (2021). Characteristics of Kappaphycus alvarezii carrageenan from different cultivation locations. Berkala Perikanan Terubuk, 49(2), 902–908.

16. Karl, H., Lehmann, I., Manthey-Karl, M., Meyer, C., & Ostermeyer, U. (2014). Comparison of nutritional value and microbiological status of new imported fish species on the German market. International Journal of Food Science & Technology, 49(11), 2481–2490. https://doi.org/10.1111/ijfs.12543

17. Kirkness, M. W. H., Lehmann, K., & Forde, N. R. (2019). Mechanics and structural stability of the collagen triple helix. Current Opinion in Chemical Biology, 53, 98–105. https://doi.org/10.1016/j.cbpa.2019.08.001

18. Kirtil, E., Aydogdu, A., Svitova, T., & Radke, C. J. (2021). Assessment of the performance of several novel approaches to improve physical properties of guar gum based biopolymer films. Food Packaging and Shelf Life, 29, 100687. https://doi.org/10.1016/j.fpsl.2021.100687

19. Kwansa, A. L., De Vita, R., & Freeman, J. W. (2016). Tensile mechanical properties of collagen type I and its enzymatic crosslinks. Biophysical Chemistry, 214–215, 1–10. https://doi.org/10.1016/j.bpc.2016.04.001

20. Latorre, M. E., Lifschitz, A. L., & Purslow, P. P. (2016). New recommendations for measuring collagen solubility. Meat Science, 118, 78–81. https://doi.org/10.1016/j.meatsci.2016.03.019

21. Li, Z.-R., Wang, B., Chi, C., Zhang, Q.-H., Gong, Y., Tang, J.-J., Luo, H., & Ding, G. (2013). Isolation and characterization of acid soluble collagens and pepsin soluble collagens from the skin and bone of Spanish mackerel (Scomberomorous niphonius). Food Hydrocolloids, 31(1), 103–113. https://doi.org/10.1016/j.foodhyd.2012.10.001

22. Lingbeck, J. M., Cordero, P., O'Bryan, C. A., Johnson, M. G., Ricke, S. C., & Crandall, P. G. (2014). Functionality of liquid smoke as an all-natural antimicrobial in food preservation. Meat Science, 97(2), 197–206. https://doi.org/10.1016/j.meatsci.2014.02.003

23. Montolalu, R. I., Tashiro, Y., Matsukawa, S., & Ogawa, H. (2008). Effects of extraction parameters on gel properties of carrageenan from Kappaphycus alvarezii (Rhodophyta). Journal of Applied Phycology, 20(5), 521–526. https://doi.org/10.1007/s10811-007-9284-2

24. Nagai, T., & Suzuki, N. (2000). Isolation of collagen from fish waste material — skin, bone and fins. Food Chemistry, 68(3), 277–281. https://doi.org/10.1016/S0308-8146(99)00188-0

25. Nurilmala, M., Suryamarevita, H., Husein Hizbullah, H., Jacoeb, A. M., & Ochiai, Y. (2022). Fish skin as a biomaterial for halal collagen and gelatin. Saudi Journal of Biological Sciences, 29(2), 1100–1110. https://doi.org/10.1016/j.sjbs.2021.09.056

26. Piez, K. A., & Trus, B. L. (1977). Microfibrillar structure and packing of collagen: Hydrophobic interactions. Journal of Molecular Biology, 110(4), 701–704. https://doi.org/10.1016/S0022-2836(77)80086-7

27. Rangaraj, V. M., Rambabu, K., Banat, F., & Mittal, V. (2021). Natural antioxidants-based edible active food packaging: An overview of current advancements. Food Bioscience, 43, 101251. https://doi.org/10.1016/j.fbio.2021.101251

28. Santoso, B., Manssur, A., & Malahayati, N. (2007). Karakteristik sifat fisik dan kimia edible film dari pati ganyong. Seminar Hasil-Hasil Penelitian Dosen Ilmu Pertanian Dalam Rangka Seminar Dan Rapat Tahunan (Semirata) Badan Kerjasama Perguruan Tinggi Negeri (BKS PTN) Wilayah Barat.

29. Schmid, M., Krimmel, B., Grupa, U., & Noller, K. (2014). Effects of thermally induced denaturation on technological-functional properties of whey protein isolate-based films. Journal of Dairy Science, 97(9), 5315–5327. https://doi.org/10.3168/jds.2013-7852

30. Shoulders, M. D., & Raines, R. T. (2009). Collagen Structure and Stability. Annual Review of Biochemistry, 78(1), 929–958. https://doi.org/10.1146/annurev.biochem.77.032207.120833

31. Soares, J. M., da Silva, P. F., Puton, B. M. S., Brustolin, A. P., Cansian, R. L., Dallago, R. M., & Valduga, E. (2016). Antimicrobial and antioxidant activity of liquid smoke and its potential application to bacon. Innovative Food Science & Emerging Technologies, 38, 189–197. https://doi.org/10.1016/j.ifset.2016.10.007

32. Sun, X. D., & Holley, R. A. (2011). Factors Influencing Gel Formation by Myofibrillar Proteins in Muscle Foods. Comprehensive Reviews in Food Science and Food Safety, 10(1), 33–51. https://doi.org/10.1111/j.1541-4337.2010.00137.x

33. Suwa, Y., Nam, K., Ozeki, K., Kimura, T., Kishida, A., & Masuzawa, T. (2016). Thermal denaturation behavior of collagen fibrils in wet and dry environment. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 104(3), 538–545. https://doi.org/10.1002/jbm.b.33418

34. Tamaela, P., & Lewerissa, S. (2007). Karakteristik edible film dari karagenan. Fakultas Perikanan Dan Ilmu Kelautan Universitas Patimura. Ambon.

35. Thirukumaran, R., Anu Priya, V. K., Krishnamoorthy, S., Ramakrishnan, P., Moses, J. A., & Anandharamakrishnan, C. (2022). Resource recovery from fish waste: Prospects and the usage of intensified extraction technologies. Chemosphere, 299, 134361. https://doi.org/10.1016/j.chemosphere.2022.134361

36. Wagey, B. T., Gunawan, W. B., Lasabuda, R., Mayulu, N., Al Mahira, M. F. N., Lailossa, D. G., Riswanda, F., Berta, E. L., Dewa, P. M., Yudisthira, D., Alisaputra, D., Arnamalia, A., Sabrina, N., Taslim, N. A., Hayes, C., & Nurkolis, F. (2023). New insight on antioxidants and anti-obesity properties of two Indonesian seagrass Thalassia hemprichii and Zostera marina: an integrated molecular docking simulation with in vitro study [version 1; peer review: awaiting peer review]. F1000Research, 12(727). https://doi.org/10.12688/f1000research.135221.1

37. Washio, T., Shintani, S. A., Higuchi, H., Sugiura, S., & Hisada, T. (2019). Effect of myofibril passive elastic properties on the mechanical communication between motor proteins on adjacent sarcomeres. Scientific Reports, 9(1), 9355. https://doi.org/10.1038/s41598-019-45772-1

38. Xin, X., Dell, K., Udugama, I. A., Young, B. R., & Baroutian, S. (2021). Transforming biomass pyrolysis technologies to produce liquid smoke food flavouring. Journal of Cleaner Production, 294, 125368. https://doi.org/10.1016/j.jclepro.2020.125368

39. Yang, H., Xu, S., Shen, L., Liu, W., & Li, G. (2016). Changes in aggregation behavior of collagen molecules in solution with varying concentrations of acetic acid. International Journal of Biological Macromolecules, 92, 581–586. https://doi.org/10.1016/j.ijbiomac.2016.07.080

40. Zhao, X., Xing, T., Xu, X., & Zhou, G. (2020). Influence of extreme alkaline pH induced unfolding and aggregation on PSE-like chicken protein edible film formation. Food Chemistry, 319, 126574. https://doi.org/10.1016/j.foodchem.2020.126574