

Review Article

Physical Characteristics of Biocomposite Edible Films Based on Fish Gelatin and Nanochitosan with the Addition of Beeswax: A Review

ABSTRACT

Edible films is a thin layer that can be eaten directly with the food it is coated with and is biodegradable, so it is used as an alternative to plastic packaging. The components of the edible film consist of hydrocolloids (proteins and polysaccharides), lipids (fatty acids), and composites (a combination of hydrocolloids and lipids). Common physical characteristics of edible films are thickness, water vapor transmission rate and solubility. Several studies have shown that the addition of beeswax to gelatin and non-occhitosan edible films can produce films with good physical characteristics. The addition of a greater concentration of beeswax can increase the thickness value and decrease the value of the transmission rate of water vapor in the film, so that the resulting film can be used as food packaging.

Keywords edible film, physical characteristics, beeswax, gelatin and chitosan

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1. INTRODUCTION

Edible films a thin layer that functions as packaging for food products that can be eaten directly with the food ingredients it is coated with. Edible film is an environmentally friendly packaging because it easily decomposes naturally or is biodegradable, so it is used as an alternative to plastic packaging Rusli et al.[1]. Edible films are generally made from natural materials such as polysaccharides, proteins, fats or a combination of several materials (composites), with or without the addition of other materials such as plasticizers and surfactants. Cerqueira et al. [2]. Edible films made from only one material still have some drawbacks, so to fix them by combining hydrocolloid bolimers with lipids. Composite edible films are film which is formed from a combination of hydrocolloid biopolymers with lipids, which can increase the advantages and cover the disadvantages of each of these films Ismaya et al.[3]. The constituents of biocomposite edible films include hydrocolloids (gelatin and chitosan), and lipids (beeswax).

Gelatin is a protein derivative derived from animal bones and skin through an extraction process Rizki et al.[4]. Fish gelatin has a high content of glycine, proline, and hydroxyproline so it is more flexible and easy to apply in food ingredients Wang et al.[5]. Mohammadi et al.[6], states that films made of gelatin have good optical properties, but have deficiencies in mechanical properties and

water barriers, so that they can be detrimental film gelatin is used as food packaging. Gelatin films have weaknesses in physical properties such as water vapor barrier and thermalShankar et al.[7]. One way to improve the physical properties of fish gelatin films is by adding other biopolymers that come from renewable resources and have film-forming properties such as chitosan.Jridi et al.[8].

Chitosan is a chitin derivative which is the most abundant polysaccharide on earth after cellulose, has hydrophobic properties and can form film layers. Modification of chitosan in nano form aims to streamline the absorption of chitosan by expanding the surface of the chitosan.Nasution et al.[9]. Nanochitosan can be used as a nanofiller in making edible films, because it can improve the mechanical properties, film color and water vapor permeability of the resulting edible films compared to ordinary edible films.Jeevahan and Chandrasekaran[10].Haghighi et al.[11], explained that edible films based on gelatin and chitosan have a good barrier against gases such as CO₂ and O₂, but still have weaknesses in mechanical properties and water vapor barrier properties. The poor water vapor barrier properties of edible films can be improved by adding lipids because they are hydrophobic, for example, like beeswaxNadiwilastio et al.[12].

Candlebees is a type of lipid that can be used in making edible films because it has hydrophobic properties that function to inhibit water vapor that diffuses throughfilm. The use of the right concentration of beeswax will be able to improve the physical and mechanical capabilities of edible films in protecting packaged food productsMudaffar[13].Testing the physical and mechanical characteristics of the edible film can be used as a reference in adjusting it to the product to be packaged Safitri et al.[14].Based on this information, it can be seen that biocomposite edible films made from nanochitosan, gelatin, and beeswax can improve physical properties, especially the rate of water vapor transmission so that they have the effect of extending the shelf life and improving the quality of the products they are packaged.

2. COMPONENTS OF EDIBLE FILM

The components of the edible film consist of hydrocolloids (proteins and polysaccharides), lipids (fatty acids), and composites (a combination of hydrocolloids and lipids) Mudaffar [13].Rohim et al.[15]states that hydrocolloid polymers consist of proteins (gelatin, casein, soy protein, corn protein and wheat gluten) and carbohydrates (starch, alginate, pectin, arabic gum and other carbohydrate modifications). Lipid groups commonly used in the manufacture of edible films such as waxes, beeswax, glycerol and fatty acids. Making edible films can also be made with or without the addition of plasticizers such as glycerol, sorbitol, sucrose, and others.Cerqueira et al.[2].The addition of plasticizers to the manufacture of edible films is necessary to increase the elasticity and flexibility of edible filmsRusli et al.[1]. The following components make up edible film, which can be seen in Table 1.

Table 1. Edible Film Composition Components

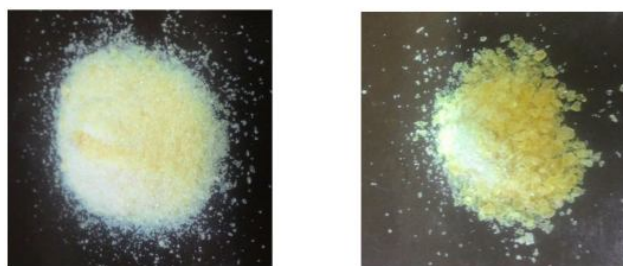
Component	Material Type	Example
The main constituent of edible films	Animal protein	Whey protein, collagen, gelatin, casein, egg white protein, fish myofibrillar protein, feather keratin.
	Plant proteins	Soy protein, corn zein, wheat gluten, pea protein, rice bran protein, cottonseed protein, peanut protein.
	Linear and neutral polysaccharides	Agar, curdlan, cereal b-glucan, methylcellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, pullulan, konjac glucomannan, inulin.
	Polysaccharide liner anions	Sodium alginate, propylene glycol alginate, carrageenan, pectin, gellan gum, carboxymethylcellulose or cellulose gum.
	Cationic linear polysaccharides	Chitosan
	Fat	Waxes (beeswax, paraffin, carnauba wax, candelilla wax, rice

	Resins	bran wax), acetoglycerides. Shellac, terpene, asafoetida, benjoin, chicle, guarana, myrrh, opoponax, sandarake, styrax
<i>Plasticizers</i>	Polyol	Glycerol, propylene glycol, polypropylene glycol, sorbitol, polyethylene glycol, corn syrup
	Other	Sucrose and water
Additives	Flavor	Oil based flavors, citrus, mint, volatile oils
	Antimicrobial	Organic acids (acetic, benzoic, lactic, propionic, sorbic), fatty acid esters (glyceryl monolauric); Polypeptides (lysozyme, peroxidase, lactoferrin); nitrites and sulfites, chitosan, bacteriocins (nisin, pediosin), parabens, liquid smoke, sodium chloride.
	Antioxidant	Ascorbic acid, 4-hexylresorcinol, amino acids (cysteine and glutathione), citric acid.
	Nutrition	Vitamin E, calcium, zinc, aluminium
	Emulsifier	Lecithin, mono and diglycerides, mono and diglyceride esters, Fatty sucrose esters, fatty alcohols, fatty acids
	Lipid emulsion	Edible wax, fatty acids
	probiotic organisms	Bifidobacterium (Bifidobacterium lactis Bb-12)
Plant essential oil	Cinnamon, oregano, lemongrass, savory, sweet inula, vanillin, cloves, lemongrass	

Source:Erkmen and Barazi[16].

2.1 FISH GELATIN

Gelatin is a protein derivative derived from animal bones and skin through an extraction process. Generally, gelatin is produced from materials that are high in collagen content such as those found in animal bones and skin Rizki et al.[13]. The gelatin extraction process can be carried out using an acid or alkaline process, but the gelatin extraction process is safer, faster and preferred by the industry, namely using the citric acid process. Nurilmala et al.[17]. The characteristics of gelatin are bright yellow or transparent to near white, in the form of sheets, powder or flour-like, stems, like leaves, soluble in hot water, glycerol and citric acid and other organic solvents. Gelatin can expand and absorb water 5-10 times its original weight Gunawan et al.[18]. Gelatin can be used in the food and non-food industries due to the unique properties of gelatin. The characteristic properties of gelatin are that it can change reversibly from sol to gel form, expands in cold water, can form films, affects the viscosity of a material and can protect the colloidal system. Nurilmala et al.[19]. Gelatin in non-food products is used in the pharmaceutical and medical industries, the cosmetic industry and the photography industry. The food industry utilizes gelatin to be used as an additional ingredient, namely as an emulsifier, stabilizer, foaming agent, encapsulant and film-forming agent. Cahyaningrum et al.[13]. The following fish gelatin can be seen in Figure 1.



(a) (b)

Figure 1. Extraction of Catfish Skin Gelatin with (a) Acid Process; (b) Base Process

Source: Nasution et al.[21]

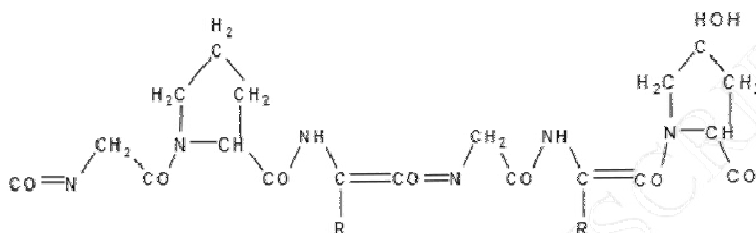


Figure 2. Chemical Structure of Gelatin

Source: Hassan et al.[22]

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2.2 NANOCHITOSAN

Chitosan is a natural polysaccharide resulting from the deacetylation process (removal of the $-\text{COCH}_3$ group) of chitin. Chitin is the main constituent of the exoskeleton of crustacean aquatic animals such as crabs and shrimp. Chitin is composed of several units of N-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucopyranose) which are connected linearly via $\beta(1\rightarrow4)$ bonds Rohim et al.[15]. Chitosan which has changed its size to nano aims for wider utilization with a particle size range of 10-1000 nm Suptijah et al.[23]. Chitosan modified into a nano form is used as an additional filler in edible formulas so that it will produce nanocomposites to increase antibacterial activity. This is due to the large surface area and positive charge of the amine groups of chitosan nanoparticles Rochima et al.[24].

Nanochitosan can be formed using various methods, but in the manufacture of stable and high quality nanochitosan usually requires a method that is quite difficult. Determining the use of the method used in the manufacture of nanochitosan depends on factors including the desired particle size, chemical and thermal stability of the particles, the kinetic profile of the particles and the toxicity of the residue associated with the final product. Rochima et al.[24]. Suptijah et al.[23], stated that the method of making nanochitosan is simpler and the process is more efficient, namely using the ionic gelation method.

The ionic gelation method is a method for making nanochitosan based on ionic interactions between amino groups in chitosan and negatively charged polyanions Taurina et al.[25]. According to Nadia et

al.[26]The ionic gelation method is a polyelectrolyte combination between positively charged chitosan and negatively charged tripolyphosphate. The addition of tripolyphosphate (TPP) and surfactant (Tween 80) can strengthen the mechanical properties of chitosan which is easily brittle and can form ionic cross-links between chitosan molecules, while reducing the particle size with a magnetic stirrer, can distribute a more homogeneous particle size. The size of nanochitosan particles using the ionic gelation method can produce very small particles that do not polymerize and do not cause enlarged (micro) particles, which have sizes ranging from 400-450 nm by reducing the particle size using a magnetic stirrer.Suptijah et al.[23].

2.3 BEESWAX

Beeswax is a product of the leftover feeling of honey which is processed first from beehives to produce wax. Remnants of beehives that have been treated usually still contain as much as 30% pure beeswaxBogdanovs[27]. The advantage of beeswax as a raw material for edible film is that it is classified as food grade, its availability throughout the year, its use is still very limited, and the price is still relatively cheap and easy to obtain.Santoso[28]. The content of beeswax is composed of free fatty acids, free fatty alcohols, and mixturesFloros et al.[29]. Beeswax is a hydrophobic agent consisting of a mixture of esters, hydrocarbons, fatty acids, alcohols, and others.Perez et al.[30]. The following beeswax content can be seen in Table 2.

Table 2. Beeswax Content

Content	Amount %
Monoester	35
Dieter	14
Triester	3
Hydroxy monoester	4
Hydroxy polyester	8
Esters	1
Polyester acid	2
Hydrocarbons	14
Free acid	12
Alcohol	1
Etc	6
Total	100

Source: Bogdanov[27]



Figure 3. Beeswax

Source: Bogdanov[31]

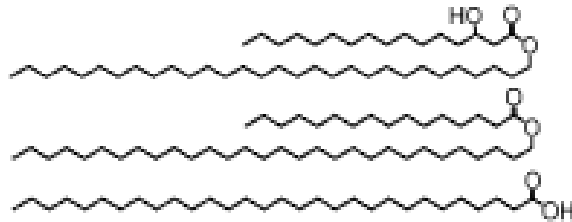


Figure 4. Chemical Structure of Beeswax
Source: Floros et al. [29]

3. PHYSICAL CHARACTERISTICS OF EDIBLE FILM

Characteristics of edible film is a key success factor in making edible film as good packaging Dwimayasanti[32]. Good packaging is packaging that can control the permeability of water vapor and gas, is easy to decompose, and is able to maintain the quality or quality of food ingredients during the storage period because they are antimicrobial. Aguirre et al.[33]. Characteristics of edible films that are commonly used are physical and mechanical characteristics. Analysis of the physical and mechanical characteristics of edible films includes: thickness, tensile strength, elongation percentage, solubility, water vapor transmission rate and water content Diova et al.[34]. The physical characteristics of the edible film can determine the flexibility of the packaging, the smaller the tensile strength value and the greater the elongation value, the more applicable the edible film will be. Mechanical characteristics can determine the quality of the packaging, the smaller the water vapor transmission rate is, the better the quality of the edible film will be.[35].Nurinda et al.[36]edible filmswhich is used as food packaging must meet the quality standards set by the Japanese Industrial Standard (1975). The following are the standard characteristics of edible films, which can be seen in Table 3.

Table 3. Edible Film Characteristic Standards

Characteristic Parameters	JIS standard
Thickness	max. 0.25mm
Water Vapor Transmission Rate	Mom. 7 g/m ² / 24 hours
Tensile strength	Min. 0.3 MPa
Elongation	Min.70%.

Source:JIS (1975) in Nurindraet al.[36]

3.1 THICKNESS

Film thickness is an important parameter in determining the feasibility of edible films as packaging for food products because thickness can affect the physical and mechanical properties of other edible films, such as tensile strength, elongation, solubility and water vapor permeability.Rusli et al.[1].Factors that can affect the thickness of the edible film include the area of the printed plate and the volume of the suspension, the constituent components, and the drying and addition of glycerol. Wijayani et al.[37]. The smaller the thickness of the edible film, the faster water will penetrate the edible film, but the higher the thickness, the better it will be to hold water vapor. Wijayani et al.[37].

3.2 WATER VAPOR TRANSMISSION RATE

The water vapor transmission rate is a test carried out to find out how resistant edible film is in holding water. Zahra et al.[38].The value of the water vapor transmission rate can determine the quality of the edible film as packaging, because the lower the value of the water vapor transmission rate, the better the quality of the edible film because it can protect the product, slow down the oxidation reaction, and can maintain the water content in the product. Wijayani et al.[37]. The value of the water vapor transmission rate is influenced by several factors including the structure of the edible film constituents and the concentration of the plasticizer used by Togas et al.[39].

3.3 SOLUBILITY

Solubility is a test parameter for edible films to determine how soluble the film is when consumed and also as a determining characteristic of biodegradable films when used as food packaging.[40].Cerqueira et al.[2].InTogas et al.[39]., States thatThe solubility percentage of an edible film can be used as an indicator to measure water resistance, film integrity and biodegradability of the edible film when used as a packaging material. The solubility of edible films is influenced by several factors, including the source of the basic ingredients for making edible films and the added plasticizersTogas et al.[39].

4.EDIBLE FILMBIOCOMPOSITE WITH THE ADDITION OF BEESWAX

Edible filmsbiocomposite is an edible film composed of a combination of hydrocolloid biopolymers and lipids. The combination of these biopolymers can complement and cover the deficiencies of each of these biopolymers, thereby increasing the characteristics of the resulting edible composite film.Santoso et al.[41].Edible filmswith the addition of lipids such as beeswax can improve physical properties in resisting the transmission rate of water vapor and can affect the thickness, tensile strength and elongation percentage of edible films. The hydrophobic nature of beeswax serves to increase the moisture barrier properties of the edible film, so that the edible film produced is better. Kanani et al.[42].

Edible films Lipid-based (waxes and resins) have a high efficiency of preventing moisture loss and adsorption due to their low permeability and hydrophobicity, but their appearance is generally opaque and unattractive as packaging materials Tola et al.[43]. The lower the value of the water vapor transmission rate, the better the quality of the edible film because it can protect the product, slow down the oxidation reaction, and can maintain the water content in the product. Wijayani et al.[37]. The small value of the water vapor transmission rate indicates that the film is better when used as a packaging material because less water vapor can penetrate into the film and the moisture content of the packaged material does not increase Purnavita and Ayu[44].

The addition of beeswax as a plasticizer to chitosan-based edible films can affect the physical characteristics of chitosan edible films, such as decreased tensile strength but on the other hand can increase the thickness and elongation percent values. Chitosan-based edible films without the addition of beeswax plasticizer tend to be thin and brittle, while edible films with the addition of beeswax plasticizer are thicker in proportion to the increase in beeswax concentration. Nabila et al.[45]. Nandiwilastio et al.[12], stated that edible films chitosan-based with the addition of zinc oxide and beeswax nanoparticles can significantly affect the physical and mechanical properties of the film. Significant changes were found in the water vapor transmission rate parameter which decreased to 10.38 g/m² hour and the film thickness increased by 90.48 μm, and has a fairly stable elongation value of more than 40%. Following are some studies regarding the addition of beeswax to biocomposite edible films, which can be seen in Table 4.

Table 4. Biocomposite Edible Film with the Addition of Beeswax

No.	Ingredients	The Best Beeswax Concentration	Results	Reference
1	Carrageenan and Beeswax	0.8%	<ul style="list-style-type: none"> • thickness increases 0.1534mm • water vapor transmission rate decreases 25.3411 g/m²/hour • solubility 56.142% 	Togas et al.[45]
2	Carrageenan and Beeswax	0.4%	<ul style="list-style-type: none"> • thickness increases 0.0705mm • water vapor transmission rate decreased 2.1797 (10g/30Min) 	Diova et al.[34]
3	Suweg Starch and Beeswax	1%	<ul style="list-style-type: none"> • thickness increases 0.193mm • water vapor transmission rate decreases 25.3411 g/m²/hour • solubility 56.142% 	Safitri et al.[14]
4	sago starch, Gelatin and Beeswax	1%	<ul style="list-style-type: none"> • thickness increases 0.129mm • water vapor transmission rate decreases 25.3411 g/m²/hour 	Mudaffar[13]

5. CONCLUSION

The addition of beeswax to gelatin and chitosan-based biocomposite edible films can produce edible films with good physical characteristics. The addition of a greater concentration of beeswax can

increase the thickness value, and reduce the value of the transmission rate of water vapor on the film, so that the resulting edible film can be used as food packaging.

REFERENCES

1. Rusli, A., M. Methusalach, and MM Tahir. 2017. Characterization of Carrageenan Edible films Plasticized with Glycerol. *Journal of Processing of Indonesian Fishery Products*. 20(2): 219. <https://doi.org/10.17844/jphpi.v20i2.17499>
2. Cerqueira, MA, AI Bourbon, AC Pinheiro, JT Martins, BWS Souza, and JA Teixeira. 2011. Galactomannans use in the development of edible films / coatings for food applications. *Trends in Food Science and Technology*. 22(12): 662–671. <https://doi.org/10.1016/j.tifs.2011.07.002>
3. Ismaya, FC, NH Fihtriyah., TY Hendrawati. 2021. Production and Characteristics of Edible Film from Nata De Coco and Glycerol. *Technology Journal*. 13(1): 81-88.
4. Rizki, AD, Fahrizal, and N. Arpi. 2021. Laboratory Scale Increase to Pilot Plant (Scale Up) Gelatin Production Based on Tuna (*Thunnus sp.*) Skin and Scale Waste. *Agricultural Student Scientific Journal*. 6(4): 447–451.
5. Wang, H., F. Ding, L. Ma, and Y. Zhang. 2021. Edible films from chitosan-gelatin: Physical properties and food packaging applications. *Food Bioscience*. 40: 100871. <https://doi.org/10.1016/j.fbio.2020.100871>
6. Mohammadi, R., MA Mohammadifar, M. Rouhi, M. Kariminejad, AM, Mortazavian, E. Sadeghi, and S. Hasanvand. 2018. Physico-mechanical and structural properties of eggshell membrane gelatin-chitosan blend edible films Authors: *International Journal of Biological Macromolecules*. 1–24. <https://doi.org/10.1016/j.ijbiomac.2017.09.003>
7. Shankar, S., LF Wang, and JW Rhim. 2019. Effect of melanin nanoparticles on the mechanical, water vapor barrier, and antioxidant properties of gelatin-based films for food packaging applications. *Food Packaging and Shelf Life*. 21:100363. <https://doi.org/10.1016/j.fpsl.2019.100363>
8. Jridi, M., S. Hajji, H. Ben Ayed, I. Lassoued, A. Mbarek, M. Kammoun, N. Souissi, and M. Nasri. 2014. Physical, structural, antioxidant and antimicrobial properties of gelatin-chitosan composite edible films. *International Journal of Biological Macromolecules*. 67(14): 373–379. <https://doi.org/10.1016/j.ijbiomac.2014.03.054>
9. Nasution, Z., H. Agusnar, Z. Alfian, and B. Wirjosentono. 2013. Effect of Chitosan Viscosity of Various Molecular Weights on the Making of Chitosan Nanoparticles Using an Ultrasonic Bath. *Unimal Chemical Technology Journal*. 2(2): 68–79.
10. Jeevahan, J., and M. Chandrasekaran. 2019. Nanoedible films for food packaging: a review. *Journal of Materials Science*. 54(19): 12290–12318. <https://doi.org/10.1007/s10853-019-03742-y>
11. Haghghia, H., S. Biard, F. Bigi, R. De Leoa , E. Bedin, F. Pfeifer, HW Siesler, F. Licciardello, and A. Pulvirenti. 2019. Comprehensive Characterization of Active Chitosan-Gelatin Blend Films Enriched with Different Essential Oils. *Food Hydrocolloids*. 95(1): 33–42.
12. Nandiwilastio, N., TR Muchtadi, NE Suyatma, and S. Yuliani. 2019. The Effect of

- Addition of Beeswax and Zinc Oxide Nanoparticles on the Physical and Mechanical Properties of Chitosan-Based Films. *Journal of Food Technology and Industry*. 30(2): 119-126.
13. Mudaffar, RA 2018. Characteristics of Composite Edible Films from Sago Starch, Gelatin and Beeswax (Beeswax). *Journal of Tabaro Agriculture Science*. 2(2): 247–256. <https://unanda.ac.id/ojs/index.php/jtas/article/view/134>
 14. Safitri, ELD, W. Warkoyo, and R. Anggriani. 2020. Study of the Physical and Mechanical Characteristics of Edible Film Based on Suweg Starch (*Amorphophallus paeoniifolius*) with Variations of Beeswax Concentrations. *Food Technology and Halal Science Journal*. 3(1): 57–70. <https://doi.org/10.22219/fths.v3i1.13061>
 15. Rohim, M., L. Destiarti, and TA Zaharah. 2015. Organoleptic Test of Chitosan Coated Tofu Products. *Journal of Equatorial Chemistry*. 4(3): 54–58.
 16. Erkmen, O., and AO Barazi. 2018. General Characteristics of Edible Films. *Journal of Food Biotechnology Research*. 2(13): 1–4. <http://www.imedpub.com/journal-food-biotechnology-research/>
 17. Nurilmala, M., MT Nasirullah, T. Nurhayati, and N. Darmawan. 2021. Physical-Chemical Characteristics of Gelatin from the Skins of Catfish, Tilapia, and Tuna. *Gajah Mada University Fisheries Journal*. 23(1): 71–77. <https://doi.org/https://doi.org/10.22146/jfs.59960>
 18. Gunawan, F., P. Suptijah, and Uju. 2017. Extraction and Characterization of Mackerel Skin Gelatin (*Scomberomorus commersonii*) from the Bangka Belitung Islands Province. *JPHPI*. 20(3): 568–581.
 19. Nurilmala, M., AM Jacoeb, and RA Dzaky. 2017. Characteristics of Yellowfin Tuna Skin Gelatin. *JPHPI*. 20(2): 339–350.
 20. Cahyaningrum, R., KK Safira, GN Lutfiyah, SI Zahra, and AA Rahasticha. 2021. The Potential of Gelatin From Various Sources in Improving the Characteristics of Marshmallow: Review. *Pasundan Food Technology Journal*. 8(2): 39–44. <https://doi.org/10.23969/pftj.v8i2.4035>
 21. Nasution, AY, Harmita, and Y. Harahap. 2018. Characterization of Gelatin Extracted from the Skin of Catfish (*Pangasius hypophthalmus*) with Acid and Base Processes. *Pharmaceutical Sciences and Research (PSR)*. 5(3): 142–151.
 22. Hassan, B., SAS Chatha, AI Hussain, KM Zia, and N. Akhtar. 2018. Recent advances on polysaccharides, lipids and protein based edible films and coatings: A review. *International Journal of Biological Macromolecules*. 109:1095–1107. <https://doi.org/10.1016/j.ijbiomac.2017.11.097>
 23. Suptijah, P., AM Jacoeb, and D. Rachmania. 2011. Nano Chitosan Characterization of Vannamei Shrimp (*Litopenaeus vannamei*) Shells with Ionic Gelation Method. *Journal of Processing of Fishery Products*. 14(2): 78–84. <https://doi.org/10.17844/jphpi.v14i2.5315>
 24. Rochima, E., E. Fiyanih, E. Afrianto, IM Joni, U. Subhan, and C. Panatarani. 2018. Effect of Adding Nanochitosan Suspension on Edible Coatings on Antibacterial Activity. *Journal of Processing of Indonesian Fishery Products*. 21(1): 127. <https://doi.org/10.17844/jphpi.v21i1.21461>
 25. Taurina, W., R. Sari, UC Hafinur, S. Wahdaningsih, and Isnindar. 2017. Optimization of Stirring Speed and Duration of Nano Chitosan Size of 70% Ethanol Extract of Siamese Orange Peel (*Citrus nobilis* L. var *Microcarpa*).

- Traditional Medicine Journal. 22(1): 16–20.
26. Nadia, LMH, P. Suptijah, and B. Ibrahim. 2014. Production and Characterization of Nano Chitosan from Tiger Shrimp Shells with the Gelation Method. *JPHPI*. 17(2): 119–126.
 27. Bogdanov, S. 2016. Beeswax: Production, Properties, Composition, Control (Chapter 1).
 28. Santoso, B. 2006. Composite Characteristics of Edible Film of Kolang-Kaling Fruit (*Arenga Pinnata*) and Beeswax (Beeswax). *Journal of Food Technology and Industry*. 17(2): 125–135.
 29. Floros, MC, L. Raghunanan, SS Narine. 2016. A Toolbox for the Characterization of Bio-based Waxes. *European Journal of Lipid Science and Technology*. 1–32.
 30. Perez, VLD, MT Cifuentes, AP Franco, CE Pérez-cervera, and RD Andrade-pizarro. 2020. Development and characterization of edible films based on native cassava starch, beeswax, and propolis. *NFS Journal*. 21:39–49. <https://doi.org/10.1016/j.nfs.2020.09.002>
 31. Bogdanov, S. 2009. Beeswax: Uses and Trade (Chapter 1). ResearchGate, Switzerland.
 32. Dwimayasanti, R. 2016. Utilization of Carrageenan as Edible Film. *ocean*. 41(2): 8–13.
 33. Aguirre-joya, JA, M. A DLZapata, OB Alvarez-perez, C. Torres-león, D. E Nieto-oropeza, J. M Ventura-sobrevilla, M. A Aguilar. 2017. Chapter 1 - Basic and Applied Concepts of Edible Packaging for Foods. *Food Packaging and Preservation*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-811516-9/00001-4>.
 34. Diova, DA, Y. Darmanto, and L. Rianingsih, L. 2013. Characteristics of Carrageenan Semirefined Composite Edible Film from *Euचेuma Cottonii* Seaweed and Beeswax. *Journal of Processing and Biotechnology of Fishery Products*. 2(3): 1–10.
 35. Irawan, S. 2010. Effect of Glycerol on Physical/Mechanical Properties and Barrier Edible Film from Chitosan. *Journal of Chemistry and Packaging*. 32(1): 6–12.
 36. Nurindra, AP, MA Alamsjah, and Sudarno. 2015. Characteristics of Edible Film from Lindur Mangrove Propagul Starch (*Bruguiera gymnorrhiza*) with the Addition of Carboxymethyl Cellulose (CMC) as a Plasticizer. *Journal of Fisheries and Marine Sciences*. 7(2): 125–132.
 37. Wijayani, KD, YS Darmanto, and E. Susanto. 2021. Characteristics of Edible Film from Different Fish Skin Gelatin. *Journal of Fisheries Science and Technology*. 3(1): 59–64.
 38. Zahra, H., Ratna, and AA Munawar. 2020. Production of Corn Starch-Based Edible Film Using Variations of Glycerol as a Plasticizer. *Agricultural Student Scientific Journal*. 5(1): 514–515.
 39. Togas, C., S. Berhimpon, R. Iwan Montolalu, AD Henny, and M. Feny. 2017. Physical Characteristics of Carrageenan and Beeswax Composite Edible Films Using the Nanoemulsion Process. *JPHPI*. 20(3): 468–477.
 40. Widodo, LU, SN Wati, and APNM Vivi. 2019. Making Edible Film From Yellow Pumpkin and Chitosan With Glycerol As a Plasticizer. *Journal of Food Technology*. 13(1): 59–65. <https://doi.org/10.33005/jtp.v13i1.1511>
 41. Santoso, B., D. Amilita, G. Priyanto, H. Hermanto, and S. Sugito. 2018.

Development of Composite Edible Film Based on Corn Starch with the Addition of Palm Oil and Tween 20. *Agritech*. 38(2): 119–124. <https://doi.org/10.22146/agritech.30275>

42. Kanani, N., Ekasari, Wardalia, A. Subkhan, and R. Rizky. 2018. The Effect of Adding Glycerol and Beeswax on Weight Loss of Sapodilla Fruit Typical Banten. *Conversion Journal*. 7(2): 37-44
43. Tola, PS, S. Winarti, AD Isnaini. 2021. The Effect of Composition of Barley Starch (*Setaria italica* L.) and Beeswax and Sorbitol Concentration on Edible Film Characteristics. *Journal of Food Technology*. 15(2): 14–25.
44. Purnavita, S., and A. Anggraeni. 2019. The Effect of Adding Beeswax and Glycerol on the Characteristics of Polyblend Glucomannan-Polyvinyl Alcohol (PVA). *Chemical Engineering Innovation*. 4(2): 33–39.
45. Nabila, SDP, R. Kusdarwati, and Agustono. 2018. The Effect of Adding Beeswax as a Plasticizer on the Physical Characteristics of Chitosan Edible Films. *Fisheries and Marine Scientific Journal*. 10(1): 34–39.

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