Review Article

EFFECT OF BEESWAX ADDITION IN THE MECHANICAL AND ANTIMICROBIAL PROPERTIES OF EDIBLE FILM BASED ON FISH GELATIN AND NANOCHITOSAN: A REVIEW

ABSTRACT

Beeswax is a lipid component obtained from the dregs of cooked honey and then filtered to obtain wax. The advantages of beeswax as a raw material for edible film are that it is classified as food grade, available throughout the year, use is still limited, price is relatively cheap, and easy to obtain. This article aims to provide a review of the effect of adding beeswax to fish gelatin and nanochitosan edible films on mechanical characteristics (tensile strength and percent elongation) and antibacterial activity (inhibition zone test).

Keywords: Edible film, Gelatin, Nanochitosan, Beeswax

1. INTRODUCTION

The use of plastic in everyday life causes the accumulation of waste which results in new problems because plastic is difficult to decompose. About 79% of plastic waste accumulates in landfills and in nature. Only 9% has been recycled and 12% destroyed (incineration) [1]. “Biodegradable packaging materials have become important to replace synthetic packaging materials. These biodegradable materials are sometimes consumed with products or can be broken down by microorganisms without producing harmful environmental emissions. Edible films have unique advantages over synthetic polymers such as biodegradability and biocompatibility. Edible film has another advantage, namely that it can act as a carrier for various additives such as antimicrobials and antioxidants” [2]. “Some natural ingredients such as proteins, polysaccharides, lipids, and others can be used for the development of biodegradable packaging. Biodegradable packaging materials are enriched with bioactive ingredients, namely antimicrobials, antioxidants, vitamins, flavonoids, etc. to further enhance the functionality of the ingredients” [3].

“Gelatin is a denatured protein obtained by partial thermal hydrolysis of collagen. Gelatin has excellent gelling ability and, therefore, is used in the food, pharmaceutical and nutraceutical industries” [4]. “Gelatin-based films and coatings have a poor barrier against moisture due to their hydrophilic nature. Water vapor permeability can be increased by adding essential oils and lipids” [3]. “One of the effective strategies used to improve the physical performance of gelatin film is to decompose the composite film by mixing gelatin with other biopolymers. Mixing can be done with materials that exhibit film-forming properties and are derived from renewable resources such as chitosan” [5].

“Chitosan is a natural polymer obtained by deacetylation of chitin, which is a by-product of the fishing industry. Chitosan is one of the most studied polysaccharides for active packaging as films and coatings due to its antimicrobial, antifungal and film forming properties” [6]. Chitosan modified into nanoparticles has been found to be a highly developed tool for removing contaminants from water. Nano-structured chitosan has hydrophilicity, high biocompatibility, non-toxic, biodegradable, and antimicrobial properties [7]. “Chitosan nanoparticles have been successfully used as a filler to improve the mechanical and barrier properties as well as the stability of thermo films, reduce solubility and produce a more compact and denser material” [8].

“The addition of lipids in the gelatin film aims to strengthen the barrier properties of the film. The addition of lipids serves to provide excellent water and oxygen barrier properties” [10]. Beeswax is a lipid component obtained from the dregs of cooked honey and then filtered to obtain wax. The advantages of beeswax as a raw material for edible film are that it is classified as food grade, available all year round, use is still limited, price is relatively cheap, and easy to obtain [11]. Beeswax is a hydrophobic agent consisting of a mixture of esters, hydrocarbons, fatty acids, alcohols, and others [12]. 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 products [13].

2. BIODEGRADABLE FILM PACKAGING

“Biodegradable packaging materials have been categorized as films and coatings. Films are layers prepared separately by pouring and drying into suitable forms as stand-alone structures. The prepared film is then used to wrap food or to be placed between layers of food products” [14]. According toSharma et al.[15] “coating is a thin layer of material that can be directly applied to food products which functions as a barrier between the product environment during transportation, processing and storage”. “The coating is applied either by dipping the product into the coating solution or by spraying it directly onto the surface of the product” [3].

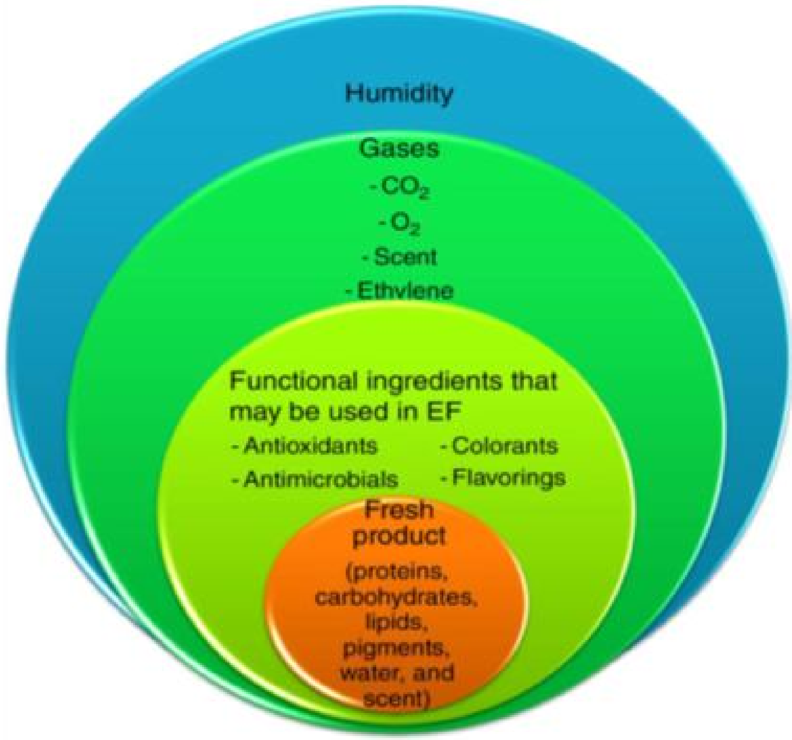


Figure 1. Edible film packaging and its functions
source: Aguirre et al. [16]

Based on researchŁupina et al.[4] "The use of polymeric biomaterials to prepare edible packaging films has become a widespread trend in the food packaging industry and has received significant attention in recent years". "Edible films can be divided into three categories based on the raw materials used, namely polysaccharide films, protein films, and mixtures of edible film compounds" [17].

*"Edible films*Biocomposites are films composed of a combination of hydrocolloid biopolymers and lipids. The combination of the two biopolymers can cover and complement the deficiencies of each of these biopolymers" [18]. "Active packaging materials consisting of bionanocomposites play multiple roles, including protecting food ingredients from the external environment, as well as increasing their shelf life by maintaining their quality. This smart packaging material prevents oxidation of food products, thereby delaying their spoilage. Active packaging ingredients can also act as moisture barriers, antimicrobial agents, freshness indicators, and so on" [19].

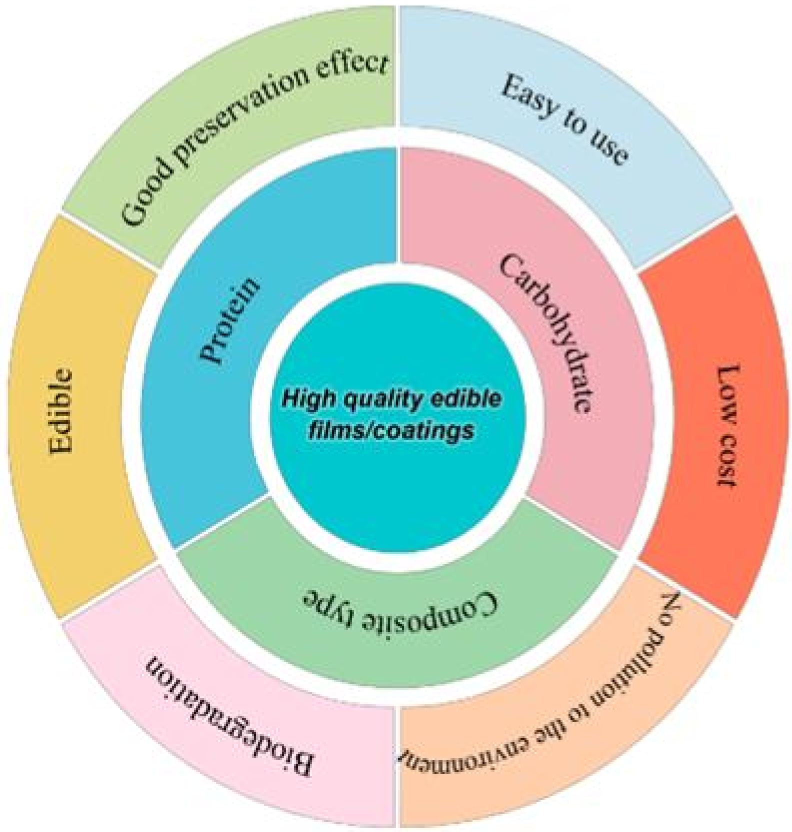


Figure 2. Characteristics of edible film/coating for high quality
Source: Lu et al. 2022 [20]

"The characteristics of edible films and coatings are influenced by several parameters such as the type of film-forming material composition, the conditions under which the film is made such as; type of solvent, pH of medium, temperature and type and concentration. Water vapor permeability or water vapor transmission rate. additives (plasticizers, antimicrobials, antioxidants, crosslinking agents or emulsifiers" [13].

3. EDIBLE FILM FORMULATIONS

*Edible films*is an alternative packaging that can be applied to foodstuffs because it has properties that can be decomposed naturally (biodegradable) so it is environmentally friendly, made of materials that are safe for health so that it can be eaten directly with the food it is coated with [21]. There are several factors that affect the structure and properties of films, such as polymer sources, polymer chemical structures, types of plasticizers, component concentrations, and drying temperatures [22].

*"Film*Hydrocolloids (protein and polysaccharide based) have good gas barrier properties (oxygen, carbon dioxide) even lower than plastic films and adequate barrier properties for lipids but not for water vapour. Edible films and lipid-based coatings (such as waxes and resins) are the most efficient film-forming materials for preventing moisture loss and gain due to their low water vapor permeability and hydrophobic properties, but their appearance is mostly opaque and unattractive as packaging materials" [23]. The main constituent components of edible films consist of three groups, namely hydrocolloids

(proteins and polysaccharides), lipids (fatty acids), and composites (combinations of hydrocolloids and fatty acids) [24].

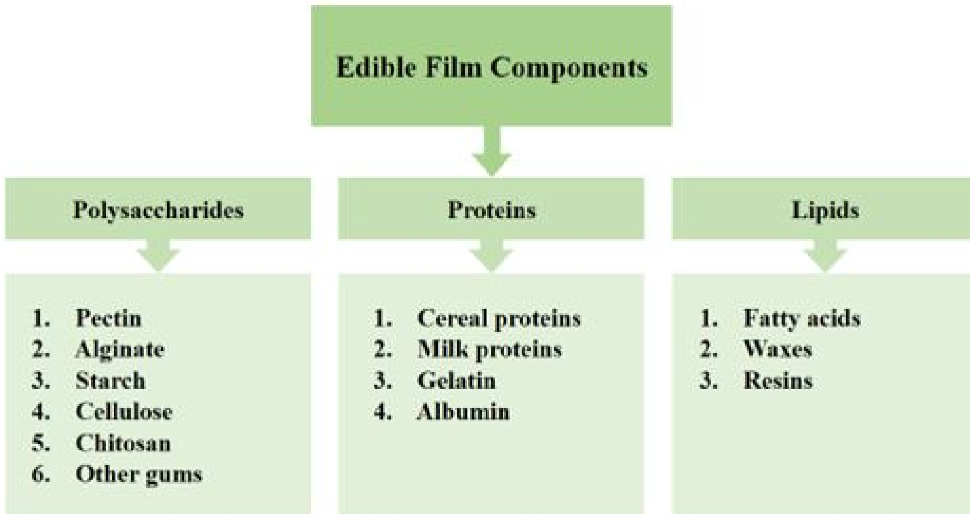


Figure 3. General Components of Edible Film Composition
Source :Abdollahzadeh et al.[25]

*"Edible films*can be produced by proteins (gluten, collagen, gelatin, keratin, casein and soy), polysaccharides (cellulose, tapioca, alginate, pectin and carrageenan), lipids (waxes, triglycerides, oils and fatty acids) and composites. The addition of additives such as plasticizers, antioxidants, vitamins, antimicrobials, essential oils, and chemical preservatives are used to increase the protective properties of edible films and coatings" [23]. The addition of plasticizers to the manufacture of edible films is necessary to increase the elasticity and flexibility of edible films [21].

3.1 GELATINE

"Gelatin is a denatured protein obtained by partial thermal hydrolysis of collagen. Gelatin has excellent gelling ability and, therefore, is used in the food, pharmaceutical and nutraceutical industries" [4]. "Gelatin is a naturally occurring polypeptide-based polymer, which is derived from collagen but has a lower sequence than collagen, due to the lack of a triple helix. Apart from its biodegradability and biocompatibility, other beneficial properties of gelatin are its good solubility in water and its ability to form strong hydrogen bonds" [26].

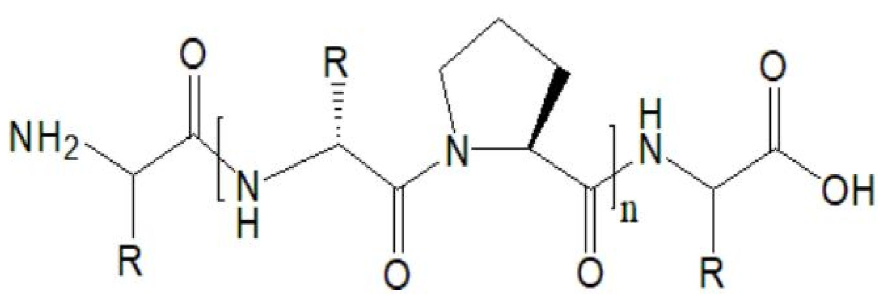


Figure 4. The chemical structure of collagen

Source: Lu et al. [20]

Gelatin is a polymer with abundant resources, relatively low cost and excellent functional properties. Gelatin has become one of the most commonly used biomaterials for preparing biodegradable packaging materials. Gelatin films have poor water barrier and mechanical properties, which are the most detrimental problems in the application process [27].

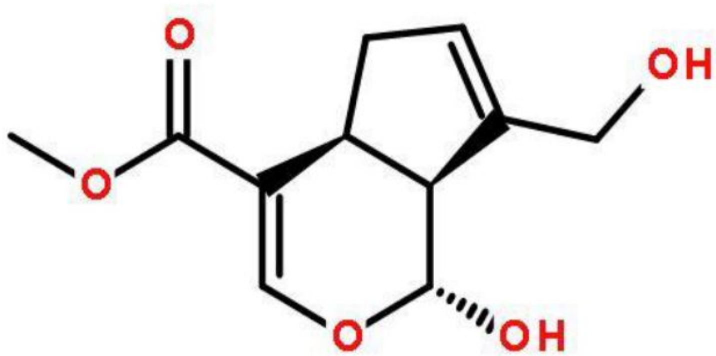


Figure 5. The chemical structure of gelatin
Source: Lu et al. [20]

*"Film*The edible ingredients obtained from gelatin can be used as surface coatings to protect food against oxygen and light because of their abundance, excellent biocompatibility and weak biodegradability and antigenicity" [28]. "The films produced from gelatin as a whole have suitable optical properties, but have weak mechanical properties and water barrier. These two properties are the main drawbacks of gelatin-based films for applications such as coatings and food packaging" [29]. Gelatin eggshell membranes have been mixed with other biopolymers, such as chitosan to overcome the limitations of gelatin and increase the functionality of hydrocolloid films [30]. Gelatin and chitosan based films used for coating or packaging can maintain food quality during storage,

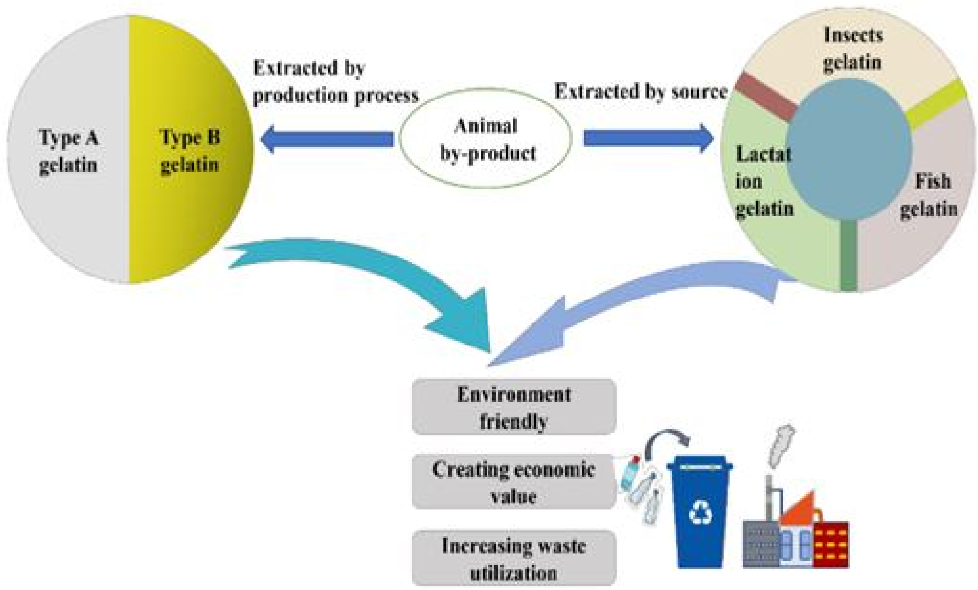


Figure 6. Schematic representation of gelatin extraction based on different sources and methods
Source: Lu et al. [20]

"In general, two different types of gelatin can be produced based on the pre-treatment of collagen: type A gelatin and type B gelatin. Type A is acid treated gelatin, which has an isoelectric point at pH 6 to 9 and is most often used for less cross-linked collagen. covalent found in pig skin. Type B is an alkaline treated gelatin which is isoelectric point at pH 5 and can be applied to the more complex collagen found in bovine skin" [32]. According toAramwit et al.[33] "nanoparticles in type B gelatin have a higher degree of cross-linking, so the degradation rate is slower than nanoparticles in type A gelatin" [34].

3.2 FISH GELATINE

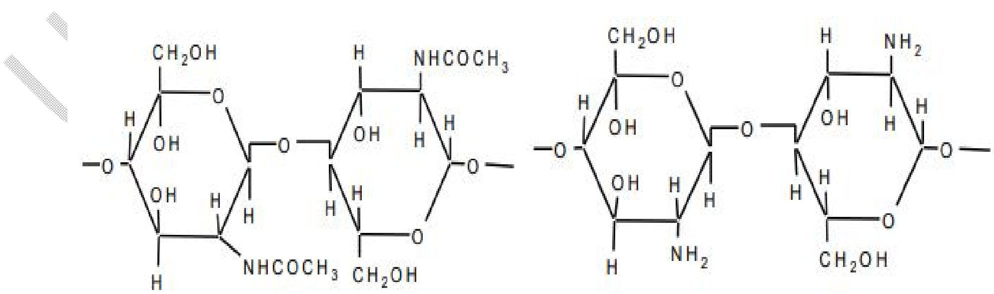
Gelatin can be sourced from the hydrolysis of collagen, which is the main protein found in animal meat, skin or bones, one of which is fish skin. Utilization of fish skin is considered more economical and reduces waste from fishery activities that are not utilized properly [35]. "Films based on fish gelatin have relatively poor water vapor permeability, mechanical properties and water resistance, which may limit their use as potential packaging materials. One of the effective strategies used to improve the physical performance of gelatin film is to decompose the composite film by mixing fish gelatin with other biopolymers. Chitosan is one of the bioplomers that exhibits film-forming properties and comes from renewable resources" [5].

Figure 7. Fish Gelatin
source:Nasution et al.[36]

“Gelatin has the advantages of excellent biocompatibility and biodegradability, wide source, and low cost, gelatin is widely used as an edible food packaging material. In addition, its barrier properties and air permeability make it advantageous for food packaging applications extending the shelf life of foods. Fish gelatin, in particular, is receiving increasing attention from both the Islamic and Jewish markets, as it is a raw material that can be extracted from the abundant fish waste, such as skin and bones, produced by the fish processing industry. It can be used as an edible film, but its mechanical properties and water barrier are inferior to synthetic commercial films, which limits its application in the food and biomedical industries” [17]. Fish gelatin is easy to apply in foodstuffs,

3.3. CHITOSAN

Chitosan is a cationic polysaccharide isolated from chitin deacetylation. Chitosan films have been widely studied in food packaging, due to their advantages, namely good biocompatibility, mechanical properties and antibacterial capacity [27]. Chitin is a naturally occurring biopolymer that is abundantly present in crustacean exoskeletons, fungal cell walls and other similar biological compounds. Chitin has a structure (β-(1-4)-2-acetamido-D-glucose. Its structure is similar to cellulose with the hydroxyl groups replaced by acetamide groups in each monomer [37].Wu[38] Chitosan was obtained by deacetylation of chitin, consisting of -(1→ 4)-2-amino-2-deoxy-D-glucose monomer. Chitosan and chitin are biodegradable polymers that have excellent film formation capabilities and excellent antimicrobial activity [39].



A B.
Figure 8. Chemical Structure of Chitin (A) and Chitosan (B)
Source :Hassan et al.[40]

“Chitosan, obtained from chitin, which is naturally present in the exoskeleton of crustaceans, is one of the most abundant biopolymers. Chitosan based materials have been used for various biomedical applications. Chitosan and chitin have important advantages such as biodegradability, non-toxicity, biocompatibility and functional properties as a bacteriostatic and fungal stat” [41]. Chitosan synthesis generally goes through the processes of demineralization, deproteination and deacetylation [42].

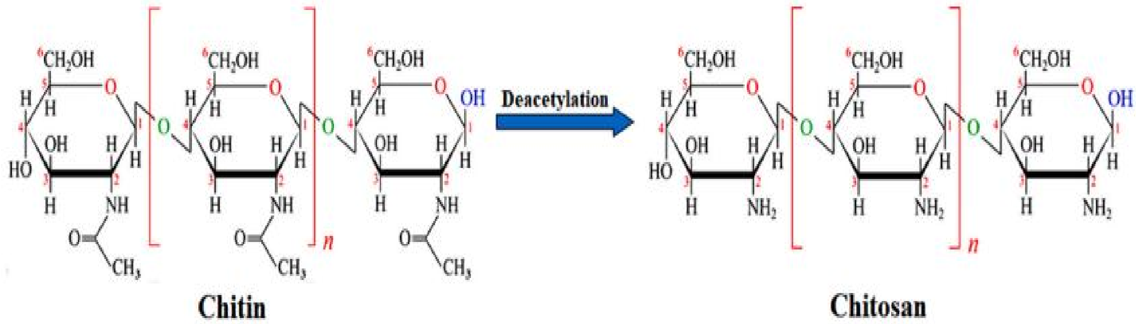


Figure 9. Chitin Molecular Deacetylation Reaction to Chitosan
Source:Sirajudheen et al.[43]

“Chemically, chitosan is a natural polymer, consisting of -(1,4)-linked N-acetyl-D-glucosamine and D-glucosamine units. Chitosan based composites are edible films due to their special characteristics including suitable mechanical properties, excellent film forming capacity and lower gas transfer. Chitosan can be used in making edible films to cover food and design packaging structures” [39].

Figure 10.chitosan
Source:Darwesh et al.[44]

Chitosan is a non-toxic, degradable, biocompatible and biologically versatile polysaccharide that can rapidly form films. Liu et al.[45] studied “the effect of chitosan with different molecular weights and degrees of deacetylation on gelatin/chitosan composite films and showed that chitosan with higher molecular weights and degrees of deacetylation could effectively improve the physicochemical properties of composite films” [17]. “Malleability is another important desirable characteristic of food packaging films. Chitosan film is less flexible and has poor mechanical properties, especially in the ability to stretch. One of the promising ways to overcome the poor mechanical properties is to modify the surface chemistry and molecular structure of chitosan” [46].

3.4 CHITOSAN NANOPARTICLES

"Chitosan nanoparticles combined with natural nanocomposite films resulted in significant changes in increasing thermal stability and improving the mechanical properties of biopolymer nanocomposite films" [47]. "This is due to the precise proportion of nanoparticles and the great tendency through interfacial communication between the polymer lattice and the dispersed nanoparticles" [48].

"The use of nanoparticles in packaging materials can increase the antibacterial properties of food packaging. The main reasons for the effectiveness of nanoparticles in possessing antibacterial properties are the high surface-to-volume proportion and enhanced surface reactivity of the nano-sized antibacterial agents. Researchers have found that nanoparticles such as zinc oxide and chitosan nanoparticles exhibit excellent antimicrobial activity against gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli) by forming large areas of inhibition zones" [49].

Nanoparticle-tethered chitosan has been found to be a highly developed tool for removing contaminants from water. Chitosan in an acidic environment, the amino chitosan functional groups are easily protonated and bind to the anionic portion of organic pollutants. Nano-structured chitosan has hydrophilicity, non-toxicity, biodegradability, and potential as an antimicrobial.[17].

Nanoparticles are proven to be able to overcome the weaknesses in biodegradable films. The addition of nanoparticles is able to produce materials with better mechanical reinforcement, higher thermal stability and barrier properties as well as lower moisture sensitivity. Chitosan nanoparticles have extensive antimicrobial properties besides having a high surface area and charge density [8].

3.5 BEESWAX

"Waxes are hydrophobic substances consisting of long and medium chains of carbon atoms. Waxes can be synthetic or natural depending on their source, namely long-chain carboxylic acids, long-chain esters, hydrocarbons, long-chain alcohols, sterols, and others" [50]. "Beeswax is a natural animal wax, which is produced by honey bees and can be characterized as inert with high plasticity. It exhibits viscoelastic behavior due to the presence of fatty acids" [51]. "Beeswax has a low melting point. Beeswax is the most commercially used natural wax and its application in food products is unlimited" [50].

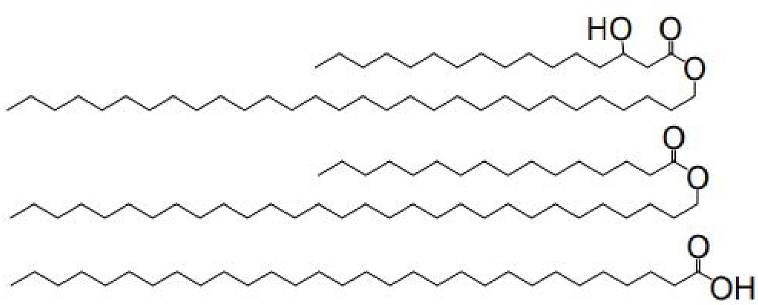


Figure 11. Main Elements of Beeswax Structure
Source:Floros et al.[52]

Beeswax is a complex mixture (more than 300 components) of hydrocarbons, free fatty acids, fatty acid esters and fatty alcohols, diesters and exogenous substances. The composition of beeswax is: hydrocarbons (12%–16%) with dominant chain lengths C27–C33, especially heptacosane, nonacosane, hentriacontane, pentacosane, and tricosane, free fatty acids (12%-14%), with chain lengths C24-C32 , free fatty alcohols (ca. 1%) of C28–C35 linear waxy monoesters and hydroxymonoesters (35%–45%) with chain lengths generally C40–C48, which are principally derived from palmitic acid, 15-hydroxypalmite and oleic, wax esters complexes (15%-27%) containing 15-hydroxypalmic acid or diols, which, through their hydroxyl groups, are linked to other fatty acid molecules, exogenous substances which are mostly residues of propolis, pollen, small pieces of component factors of interest and pollution. The composition of beeswax can vary between and among different families and different breeds of bees, because the possibility of wax production is closely related to the genetics and diet of bees [53].

Figure 12. Beeswax
Source :Bogdanovs[54]

Beeswax is a complex product that is secreted in a liquid form by special wax glands in the abdomen of the younger worker bees (between 12 and 18 days old, that is, at the end of the period in which the bee acts as a nurse). Beeswax hardens in contact with air. Pure beeswax is almost white in color only after contact with honey and pollen, after about four years the yellowish color varies and turns brown due to the cocoons it contains [53].

4. EFFECT OF ADDITIONAL BEESWAX ON EDIBLE FILM FISH GELATIN-NANOCHITOSANE

4.1 Mechanical Properties

As a food product packaging, good mechanical properties such as good tensile strength and elongation in edible films are needed to withstand external pressures, such as environmental factors and maintain toughness, as well as their role as a barrier to water vapor [30]. Packaging films must have adequate mechanical performance to protect food, maintain integrity and extend the shelf life of food. Therefore, the mechanical properties of edible films are an important aspect to pay attention to. Naturally based films must have suitable mechanical properties and must withstand the same external forces. Common external forces such as friction may occur in the processing, subsequent shipment, handling, storage and application of packaged foods, where the mechanical properties of the film indicate its ability to withstand these forces or stresses.

Sufficient mechanical strength and flexibility are required for packaging films to withstand external stresses, as well as to maintain integrity and barrier properties during packaging. Higher mechanical strength results in stronger films [55]. The mechanical properties reflect the film's ability to protect the integrity of the food. The important parameters of the mechanical properties are tensile strength and elongation at break. Tensile strength describes the mechanical resistance of a film due to cohesive forces between chains or internal stresses, while elongation break measures its plasticity, which is the capacity of a film to elongate before breaking [56].

Film packaging must be resistant to damage and must be flexible, so that it does not break if deformation occurs, thereby maintaining the structure of the food and protecting it from environmental influences. In general, the important mechanical properties of film as a measure of film quality are tensile strength and percent elongation (elongation at break). This mechanical property describes the film's ability to maintain film integrity when applied to food products. The mechanical properties of a bio-nanocomposite material are highly dependent on the morphology of the interface, such as filler shape, size, size distribution, adequacy of dispersion and the degree of adhesion between the filler and the polymer matrix. Elongation at break is the percentage of maximum elongation when the tensile stress is obtained before the film breaks. Elongation has an inverse relationship with tensile strength in most cases [57]. The elongation shows the change in the maximum length of the biodegradable film when obtaining a tensile force until the biodegradable film breaks compared to its initial length [58]. Percent elongation is the maximum change in length before the edible film breaks [59].

4.1.1 Tensile Strength

Tensile strength is the amount of force required for bioplastics to break. A low value of the tensile strength of a bioplastic indicates that the bioplastic is easily damaged and a high value of tensile strength indicates that bioplastics can protect the product from mechanical disturbances in the form of friction or impact on the product [60]. Tensile strength is the maximum tensile force that a film can withstand until it breaks [61]. Tensile strength is an important parameter for an edible film. Edible films with high tensile strength will be able to protect products well from mechanical damage [59].

Research conducted byNabila et al.[59] regarding the effect of adding beeswax as a plasticizer on the physical characteristics of chitosan edible films where increasing the concentration of beeswax plasticizer can reduce tensile strength. Based on research by Diova et al. [62] the use of beeswax concentrations up to a level of 0.3% has an effect on the characteristics of the resulting edible film, namely increasing the tensile strength value. The best formulation is the addition of 0.3% beeswax. Based on researchTogas et al.[63] The addition of beeswax had a significant effect on the tensile strength of edible films with the best formulations being 4.5% carrageenan and 0.8% beeswax. Based on research by Safitri et al. [64] the addition of beeswax increased at a concentration of 2% and decreased at a concentration of 3%. The higher addition of beeswax to the edible film causes the higher tensile strength and after that it decreases. The concentration of the addition of 1% (w/v) beeswax was the best treatment

Mudaffar[13] examined the characteristics of composite edible films from sago starch, gelatin and beeswax. The addition of the concentration of beeswax can increase the tensile strength with the best formulation of the addition of 1% beeswax. The concentration of beeswax plays a role in the formation of a strong edible film. The greater the concentration of beeswax, the higher the tensile strength of the edible film produced. This is because beeswax has a crystalline phase where there is attraction between adjacent fatty acids in the crystal, causing the amount of polymer matrix to increase and forming a film with a tight polymer structure so that the resulting film is not easily torn.Diova et al.[62] added that the stronger the edible film that is formed, the more difficult it is for a film to stretch or elongate so that it will reduce the percentage of elongation. The presence of plasticizers also affects the tensile strength of edible films, where plasticizers added to polymers can overcome brittleness, provide flexibility and increase hardness [63].

4.1.2 Percent Elongation

The tensile strength of a film is very important to note because it plays a very important role when a film is applied. In addition, due to the tensile strength at break it shows the resilience of a film which is the maximum stretch that can be accepted by the film before it breaks when it is stretched. High tensile strength is generally very important for an edible film to withstand normal pressure during treatment, transfer or transportation, and detention of a food ingredient. Edible film must be able to withstand the pressure during use in food handling. High tensile strength or tensile strength is generally desired but the occurrence of changes must be managed according to the application of the film [62].

Research conducted byNabila et al.[59] who examined the effect of adding beeswax as a plasticizer on the physical characteristics of chitosan edible films where increasing the concentration of beeswax plasticizer increased the percent elongation value. According toTogas et al.[63] The addition of beeswax had a significant effect on the percent elongation of edible films with the best formulations being 4.5% carrageenan and 0.8% beeswax. According to Diova et al. [62] the use of beeswax concentrations up to 0.3% gave an effect on the characteristics of the resulting edible film, namely increasing the percentage of elongation. The best formulation is the addition of 0.3% beeswax.

Mudaffar[13] examined the characteristics of composite edible films from sago starch, gelatin and beeswax. The concentration of 1% beeswax can reduce the percent elongation and transmission rate of water vapor of sago starch edible film. The greater the concentration of beeswax, the lower the percent elongation of the edible film produced. According to Safitri et al. [64] the addition of beeswax increased at a concentration of 2% and decreased at a concentration of 3%. The higher addition of beeswax to the edible film causes the percent elongation to be higher and after that it decreases. The concentration of the addition of 1% (w/v) beeswax was the best treatment. The presence of beeswax also has a significant effect on mechanical properties as evidenced by a decrease in tensile strength and an increase in film elongation [65].

According toNabila et al.[59] the addition of beeswax had a significant effect on the percent elongation of edible films where the addition of beeswax at a concentration of 1% to 4% increased the percent elongation value however, at an additional concentration of 5% there was a decrease in the percent elongation value. The composition of the edible film mixture exceeds the saturation point so that the excess beeswax is in a separate phase outside the film constituent phase which causes the film to become increasingly inhomogeneous. This causes a decrease in the intermolecular forces between the chains. The analysis showed that the most effective addition of beeswax to increase elongation was not more than 4% by weight per volume.

4.2 Anti activitybacteria

Antibacterial compounds are substances that can inhibit or kill growth by interfering with the metabolism of harmful bacteria. One of the pathogenic bacteria is S. aureus where this bacterium is commonly found in semi-wet products such as sausages, nuggets and fish sticks. Staphylococcus aureus is a gram-positive bacterium commonly found in the respiratory tract, human digestive tract and in the air or the surrounding environment. Escherichia coli is a gram-negative bacterium that generally has a three-layered cell wall structure, namely the cytoplasmic membrane, outer membrane, and peptidoglycan layer, which is usually found in fishery products and the human body. The size of the inhibition zone is affected by the sensitivity of the test organism, the culture medium and the incubation conditions. diffusion speed of the antibacterial compound and the concentration of the antibacterial compound. A small inhibition zone indicates lower antibacterial activity, while a large inhibition zone indicates greater antibacterial activity [67].

Antioxidant and antibacterial packaging are the main categories of active packaging and are very promising for extending the shelf life of food products [4]. The anti-bacterial test serves to determine the activity of the edible film constituents against the test bacteria, because the antibacterial activity of the edible film constituents is shown by the appearance of an inhibition zone or clear area around the paper disk. If the inhibition zone does not appear, it is assumed that there is no inhibition zone on the film [68].

Based on research resultsDarmanto et al.[68] gelatin-chitosan film (GI 4%-Ch 3%) showed antibacterial properties with an inhibition zone of 0.1 cm. The mechanism of chitosan's antimicrobial activity against Staphylococcus aureus is carried out by an inhibition mechanism originating from its polymer structure. Chitosan has a positively charged amine group that can interact with a negatively charged molecule, such as protein from microbes, thereby inhibiting microbial growth [69]. The mechanism of the antimicrobial activity of chitosan against Staphylococcus aureus is that chitosan will form a polymeric membrane on the surface of Staphylococcus aureus cells so that it will inhibit nutrients from entering the cell. This is due to the presence of amine groups in chitosan which have a cationic charge which can bind food sources for these bacteria such as alginate, pectin, protein, and inorganic polyelectrolytes such as polyphosphates. The antibacterial activity of chitosan against Staphylococcus aureus increases with increasing the molecular weight of chitosan. In addition, the antibacterial activity of chitosan is affected by the degree of deacetylation, the concentration in solution, and the pH of the medium [68]

Inhibition by nanochitosan in S. aureus bacteria is carried out by forming a polymer membrane on the surface of the cell so that it blocks nutrients from entering the cell, while the inhibition of nanochitosan in E. coli is thought to be through the mechanism of substance entry into the cell thereby disrupting bacterial metabolism. Chitosan has a bactericidal effect against E. coli bacteria, and inhibits the growth of Listeria monocytogenes [70].

According toNorma et al.[42] Edible film formulations with a combination of 2% chitosan and 4% beeswax showed the smallest increase in microbial count compared to other edible film formulations. The variation of the beeswax used, namely 4 and 4.5%, did not have a significant effect on the TPC analysis. Based on research resultsNaiu et al.[69] Edible film gelatin-CMC-beeswax enriched with 0.3% beeswax was still able to inhibit microbial growth until the end of the observation allegedly due to the hydrophobic nature of beeswax and partially hydrophobic CMC properties. Based on researchHermayasari et al.[71] increasing the concentration of honeycomb wax on edible coatings has a significant effect on reducing the value of microbial contamination. The concentration of 20% beeswax wax is the optimum concentration in providing an inhibitory effect on total bacteria and Staphylococcus aureus in ground beef jerky.

8. CONCLUSION

Based on the review, it can be concluded that beeswax is a potential biopolymer for edible films. The addition of beeswax to edible films can have a significant effect on mechanical properties (tensile strength and percent elongation) and antibacterial activity. The addition of beeswax has an effect on increasing the tensile strength of edible films. As the beeswax concentration increases, the percent elongation value decreases, but in some cases it can increase the elasticity of the edible film. This depends on the constituent materials and the type of plasticizer used. The addition of Beeswax is proven to be able to inhibit antibacterial activity on edible films, however, it is necessary to carry out more in-depth studies on testing antimicrobial activity on edible films.

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