

QUALITY CHANGES Of *Kappaphycus alvarezii* JELLY CANDY COATED WITH GELATIN-CHITOSAN-NANOCHITIN COMPOSITES-EDIBLE FILM DURING STORAGE

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Received Agust 23-2022; Received in revised from January 12-2023; Accepted January 20-2023

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ABSTRACT

This study aims to analyze changes in the quality of *Kappaphycusalvarezii* seaweed jelly candy packaged in edible film based on chitosan-nano chitin gelatin composite during storage. The research method used was a laboratory experimental method. The research stage consisted of preliminary research aimed at determining the formula for edible film gelatin-chitosan enriched with nanochitin. The main research was applying the best formulation of edible film gelatin- chitosan-nanochitin to seaweed jelly candy which was then stored for 60 days that was converted to 15 days in a climatic chamber at 40 °C and 75% humidity. The chemical characteristics tested were water content and reducing sugar content, microbiological tests including TPC and molds-yeasts and sensory characteristics were tested every 3 days. The design of data analysis used linear regression. The results of the preliminary study showed that the best edible film formula was composed of 2% gelatin, 2% chitosan and 0.2% nanochitin, which resulted in a water vapor transmission rate of 15.25 g/m²/day and has an attractive appearance. Based on the standard SNI (2008), the moisture content of jelly candy packaged in edible film gelatin-chitosan-nanochitin can be maintained until the 36th day of storage, which is 19.25%, the reducing sugar content up to the 60th day of storage is 20.5%, TPC up to 20.5%. the 60th day of storage is log 3.5 CFU/g, and the sensory quality can be maintained until the 36th day. The molds and yeasts were not visible during storage.

Keyword: *composites edible film; climatic chamber; sensory characteristics*

INTRODUCTION

Packaging and storage of a food ingredient are two of the efforts made so that the product can be enjoyed for a longer time. Storage environmental conditions and type of packaging affect product quality. The packaging that is usually used to package food products is plastic material, as it is flexible, economical, strong, not easily broken and able to withstand the entry of oxygen, water vapor and carbon dioxide. However, plastic also has a weakness, namely that it is not easy to decompose, even though it has been stored for decades. As a result, there is a buildup of plastic waste which is the cause of environmental pollution (Setiani *et al.*, 2013). Therefore, to reduce plastic waste that pollutes the environment, edible films can be used as packaging materials for food products.

Edible film is a thin layer made of edible material that functions as a barrier against moisture, oxygen, light, lipids, solutes, and also as an inhibitor of bacteria during storage (Ali *et al.*, 2017). Edible film is a primary packaging that can be used to coat food products.

The edible film in this study was formulated with gelatin, glycerol, chitosan and nanochitin. Gelatin as an ingredient for making edible films is a hydrocolloid macro molecule that can form a thin elastic layer and form a transparent and strong film. Gelatin-based edible film formulas must be added with a plasticizer to obtain an elastic film, and not stiff. Krochta (2002), states that plasticizers made from protein raw materials commonly used in the manufacture of edible films are glycerol, sorbitol, triethylene glycol sucrose, and polyethylene glycol. However, gelatin-based edible film alone is less effective in extending the shelf life of the product because of its hydrophilic nature, which is able to absorb moisture from the environment so that it can affect the packaged product. In order to extend the shelf life of the product, gelatin-based edible films must be added with anti-bacterial components. The anti-bacteria used in the manufacture of this edible film are chitosan and nanochitin. Chitosan is a natural polysaccharide resulting from the deacetylation process (removal of the $-\text{COCH}_3$ group) of chitin. The best solvent used in the process of making chitosan-based polymer membranes is acetic acid solvent with a concentration of 1-2% (v/v) (Aryanto, 2002). Chitosan is used as an anti-bacterial as it will bind to cell membrane proteins, namely glutamate which is a component of cell membranes (Harianingsih, 2010). Cytoplasm can come out while carrying other metabolites, it is called lysis, which will inhibit cell division (regeneration) and cause cell death.

Nanochitin is the most attractive organic nanomaterial as it is a very small particle-sized chitin with high advantages such as low cost, anti-bacterial and non-toxicity (Rubentheren *et al.*, 2015). In addition, nanochitin can also reduce the ability of the film to absorb water (Sahraee *et al.*, 2016). (Naiu *et al.*, 2020) in their research revealed that nanochitin has a fairly high solubility level of 68.92% - 71.72% so that it can easily bring antimicrobial components into food, even though it is still hydrophobic because it has an amide group $-\text{NHCOCH}_3$).

Studies of the relationship between storage time and changes in the quality of *K. alvarezii* jelly candy packaged with gelatin-chitosan-nanochitin composite edible film has never been reported. Therefore, this research needs to be done to analyze and add references regarding quality changes that occur due to the storage period of products packaged in composite edible films. The product tested was candy packed with composite edible coating which had been stored at room temperature.

RESEARCH METHOD

Tools and Materials

The tools used in this research are hot plate (Thermo Scientific Cimarec), thermometer (110 °C), blender (Philips), digital scales (Excellent JCS B-30000), analytical balances (Fujitsu FSR-A), petri dishes, oven (Mettler UN 55 53L), desiccators (Dianrui), autoclaves (Tomy SX-500), test tubes, measuring cups, beakers, filter paper, WVTR glasses, incubators (Mettler), pipette.

The materials used in this research are seaweed of *Kappaphycus alvarezii*, sugar, water, fruit flesh of red dragon, chitosan (CV Bio-Chitosan), tuna bone gelatin which was processed with palm vinegar according to (Naiu & Yusuf, 2018), technical glycerol (PT. DwiLab Mandiri), beeswax (Rawhoney), and nanochitin referring to Naiu *et al.*, (2020), destilate water (BPPMDPP laboratory), acetic acid (15%), silica gel, and NA agar.

Research Methods

Research Procedures

This research consisting of two stages, namely the preliminary research which is to determine the best formula for edible film composed of gelatin, chitosan, nanochitin, and glycerol, and the main one is to analyze changes in the quality of *K. alvarezii* jelly candy coated with edible film based on gelatin-chitosan-nano chitin during storage. The observation method was carried out for 60 days which was converted to 15 days with accelerated storage conditions using a climatic chamber with a temperature of 45 °C and RH of 75% which refers to the study of Afifah *et al.*, (2017).

Making process of jelly candy following Murnaningsih *et al.*, (2020) and packaging process of jelly candies in edible film refers to Darmajana *et al.*, (2017) by placing the candies on the edible film then wrapped and cut them into smaller sizes. The step further is to place them in a closed container and then stored in the climatic chamber.

Analysis Procedur

Analysis of Water Vapor Transmission Rate (WVTR) (ASTM, 2000)

The rate of water vapor transmission was calculated by placing the edible film on a cup that had been added with silica gel with RH 0% then it tightened with a rubber band. The cup was weighed and placed in a desiccator that had been added with distilled water (RH 100% at 28°C). Every hour for ten hours the cup is weighed, and the water vapor transmission rate is calculated according to the equation:

$$WVTR = \frac{\text{silica gel weight gain (g)}}{\text{Edible film surface area(m}^2\text{) x storage time (hours)}}$$

Water Content Analysis (Oven Method) (BSN, 2008), Analysis of Reducing Sugar Content (calculated as inverted sugar) (BSN, 2008), Total Plate Count Analysis (BSN, 2009), Yeast and Mold Numbers (BSN, 2008), Organoleptic Analysis (BSN, 2006)

Experiment Design And Data Analysis

The WVTR (Water Vapor Transmission Rate) of edible film and presence of yeasts and molds on jelly candies were analyzed descriptively. Water content, sugar content, TPC, and sensory value were analyzed statistically by linear regression using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Water Vapor Transmission Rate

The average water vapor transmission of edible film from the research was 15.25 g/m²/day. The water vapor transmission in the edible film is thought to occur in the hydrophilic part of the film. In addition to gelatin and glycerol which are hydrophilic as components of the edible film in this study, the nanochitin used also contains hydrophilic parts. Naiu *et al.*, (2020), reported that nanochitin is hydrophobic because it has an amide group (–NHCOCH₃) which is insoluble in water, but still contains hydrophilic glucosamine. It was further explained that the solubility level of nanochitin particles was 68.92% -71.72% so that it had the potential to pass water vapor. Sahraee *et al.*, (2016) also stated that nanochitin naturally contains hydrophilic groups, although in edible films it can reduce the film's ability to absorb moisture.

This WVTR value is slightly higher than the WVTR of HDPE plastics as researched by (Yuliasih & Raynasari, 2014), which is 13.10 g/m²/24hours and much lower than that obtained by Tasha *et.al.*, (2016) who used the formulation of 2% sorghum starch, 2% CMC and 1% glycerol with a WVTR of 616,226 g/m²/24 hours. The high WVTR of edible film has the potential to pass more moisture into the product and also evaporate more water out of the product so that it will affect the taste and texture of the candy. Garcia *et al.*, (2000) reported that the smaller the migration of water vapor that occurs in the packaged product, the better the properties of the edible film in maintaining the shelf life of the packaged product.

Water content

Water content of jelly candy ranged from 16.75% to 21.75%. Linear regression graph shows the value of R^2 is 98.4%, which means that the water content of the jelly candy has a very strong correlation with the storage time that can be seen in Figure 1.

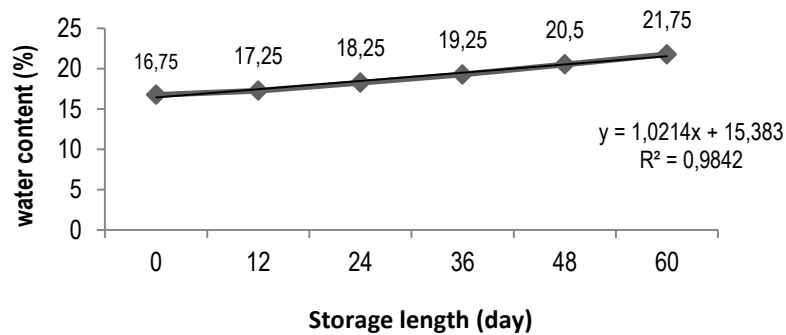


Figure 1. Changes in water content of jelly candy packaged in gelatin-chitosan-nanochitin-based edible film during storage

Figure 1 shows that the water content increases with the length of storage. The graph illustrates that the water content of jelly candy packaged with edible film still meets the SNI (2008) on storage until the 36th day, which is a maximum of 20%.

The water content tends to increase during storage is thought to be due to the relatively high humidity of the storage room, which is 75%, so that the jelly candy tends to absorb water from the environment. In addition, the edible film with a relatively high WVTR exceeding the JIS (1975) standard of 10 g/m²/day allows the transmission of water vapor into the jelly candy. According to Fehragucci (2012), chitosan is a good barrier to oxygen but not very good to water vapor. This is also supported by research conducted by Sahraee *et al.*, (2016) who found that nanochitin composite films still have natural hydrophilic properties. Meanwhile (Naiu *et al.*, 2020) stated that nanochitin has a fairly high level of solubility so that water vapor can still pass into edible film.

Reducing Sugar Level

The average sugar content ranged from 20.5%-15.1%. The graph of linear regression shows the value of R^2 is 92.2%, which means that the reducing sugar content has a very strong correlation with storage time. Changes in reducing sugar content of *Kappaphycus alvarezii* seaweed jelly candy packaged in gelatin chitosan-nanochitin-based edible film during storage can be seen in Figure 2.

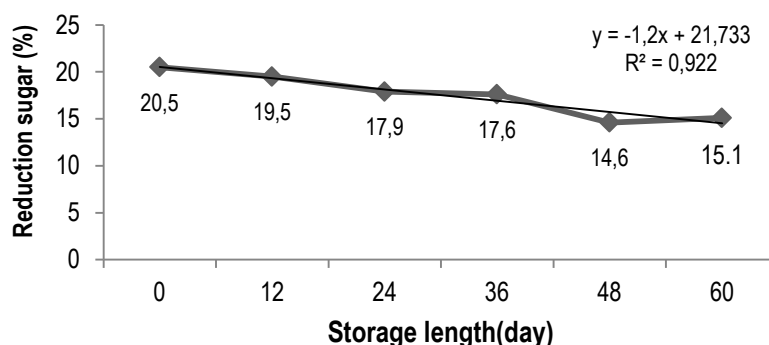


Figure 2. Changes in reducing sugar content of jelly candy packed with gelatin-chitosan-nanochitin-based edible film during storage

Figure 2 shows that reducing sugar content is highly correlated with storage time which is detected to continue to decrease during storage, although it still meets SNI (2008) standards, which is a maximum of 25.0%. This is presumably because the presence of chitosan and nanochitin in the gelatin edible film coating material is sufficient to inhibit the permeation of oxygen (O_2) from the environment into the candy. As a result, the respiration process that triggers the reduction of sugar into water molecules and carbon dioxide gas can be controlled. (Syarief & Halid, 1993) mention that oxygen is one component that can convert simple sugars into water molecules, carbon dioxide, and release heat through the respiration process.

According to Sahraee *et al.*, (2020) nanochitin in films can reduce the ability of oxygen to enter the product, because nanochitin is a good barrier to oxygen and carbon dioxide. (Harianingsih, 2010) explained in her research that chitosan as a coating material on strawberries can inhibit the outward respiration process because all the pores of the strawberry are closed. Decreases in reducing sugar levels can occur during storage because anaerobic respiration causes the breakdown of glucose into pyruvic acid causing reducing sugar levels to decrease.

Total Plate Count (TPC)

Total microbial count (TPC) averaged between log 1.40 cfu/g – log 3.50 cfu/g. The results of linear regression show the value of R^2 is 98.8%, which means that the TPC value is very strongly correlated with storage time. Changes in the value of TPC (Total Plate Count) jelly candy packaged with gelatin-chitosan-nano chitin-based edible film during storage can be seen in Figure 3.

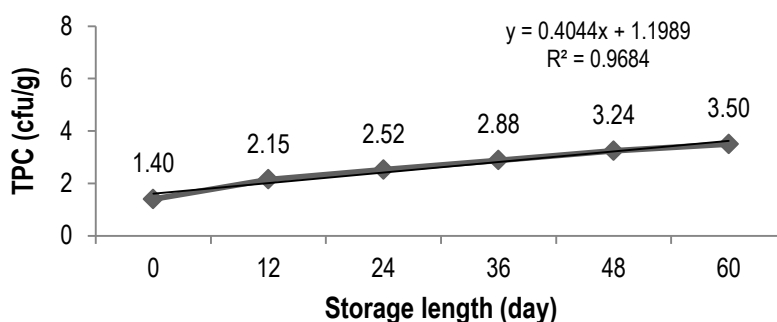


Figure 3. Changes in the TPC (Total Plate Count) of jelly candy packed with gelatin-chitosan-nanochitin-based edible film during storage

Figure 3 shows the increase in the TPC value of jelly candy with the length of storage. The TPC value on the last day of storage was recorded at log 3.50 cfu/g and is still within the maximum standard limit of SNI (2008), which is log 4.69 CFU/g. The slow growth of microorganisms in jelly candy is thought to be due to the edible film used to coat the candy contains chitosan and nanochitin which have anti-bacterial properties. Ridwan *et al.*, (2015), reported that edible film made from chitosan was able to provide an inhibitory effect on the rate of microbial increase of tilapia fillets stored at low temperatures (5°C). According to Krochta *et al.*, (1994) chitosan is one type of polysaccharide that has good barrier properties and also acts as an antimicrobial that can inhibit microbial growth so that it can protect packaged products from bacterial contamination .

Sahraee *et al.*, (2016) stated that nanochitin has good antimicrobial activity in biopolymers. Sahraee *et al.*, (2017) explained that nanochitin can penetrate microbial cell membranes and bind to their DNA causing inhibition of RNA and protein synthesis. Naiu *et al.*, (2020), reported that the high level of solubility of nanochitin (68.92% - 71.72%) made it easier to dissolve in the product carrying its anti-bacterial substances. In addition, the water content of jelly candy in this research increased during storage contributed to bringing the nanochitin component into the product causing it to be more protected from bacteria during storage because nanochitin could protect from inside the product.

Mold Numbers

Based on the results of the study, jelly candy packaged with edible film during storage did not show the presence of mold and yeast. This is presumably due to the effect of chitosan as one of the ingredients for edible films that can inhibit the growth of molds and yeasts. According to Misni *et al.*, (2017) chitosan has a positive charge from its amino acid group. Amino acid groups in the form of amino acyl (HCOCH₃)

and glucosamine (C₆H₉NH₂) in positively charged chitosan can bind to the negatively charged macromolecules on the surface of fungal cells (molds) causing an appresorium, which is the tip of the hyphae or sprout tube which is a place for attaching and penetrating the host by fungi will be inhibited (Restuati, 2008).

The absence of molds and yeasts in this study is different from that studied by (Wisudawaty *et al.*, 2016) who reported that yeasts can grow in products with high sugar content as they can be used as nutrients needed by yeasts to grow. In this study, molds and yeasts did not grow on the surface of the jelly candy packaged with edible film because the reducing sugar content was relatively low and tended to decrease during storage. Another factor is thought to be due to the storage temperature is quite high, that is 45 °C which is not suitable for the growth of molds and yeasts. According to Muchtar *et al.*, (2011) *Aspergillus niger* grows optimally at 35-37 °C and require sufficient oxygen (aerobic), while *A. fumigates* optimum growth at 37 °C. Badu *et al.*, (2013) stated that the mold is mesophilic, so it grows well at room temperature. The optimum growth temperature for most molds is around 25-30 °C.

Sensory Value

The sensory value of jelly candy which includes appearance, taste, aroma and texture can be seen in Table 1.

Table 1. The sensory value of jelly candy

Attributes	Sensory value					
Appereance	6.68 ± 1.44	6.36 ± 1.25	6.44 ± 1.04	5.76±0.88	5.28±1,31	4.76 ± 1.23
Taste	6.16 ± 1.43	6.24 ± 1.05	6.4 ± 0.96	5.8 ± 0.96	5.28 ± 1.43	4.76 ± 1.27
Flavour	6.16 ± 1.16	6.68 ± 1.18	6.52 ± 1.08	5.76 ± 0.78	5 ± 1.32	4.48 ± 1.23
Texture	6.16 ± 1.04	6.76 ± 1.05	5.24 ± 1.56	5.6 ± 1.32	5.4±1.35	4.84 ± 1.25

Table 1 shows the sensory properties of jelly candy which tend to decrease with storage time. The graph of linear regression shows that the appearance value was very strongly correlated with storage time with an R² value of 92.8%, the taste and texture values correlated quite strongly with the R² is 77.2% and 79% respectively. The aroma value was strongly correlated with the R² is 87.2%. The reduced appearance value is thought to come from the edible film layer which looks brownish yellow due to the presence of gelatin as a constituent of the edible film which gives a brownish yellow color due to the Maillard reaction.

Syahraeni *et al.*, (2017) stated that during heating in extraction process a non-enzymatic browning process or Maillard reaction occurs between amino groups in amino acids and the results of fat oxidation which are still quite high which causes brown pigment to occur.

The taste and aroma of jelly candy were not liked starting on day 48 because the sour taste began to be felt when eaten which was thought to be due to the breakdown of reducing sugar content into pyruvic acid during storage. According to Harianingsih, (2010) chitosan in edible film coatings as a barrier can inhibit aerobic respiration, and (Sahraee *et al.*, 2020) also stated that the properties of nanochitin which can reduce oxygen and carbon dioxide to enter the product Therefore, the rate of anaerobic respiration occurs in the product which breaks down the sugar content into pyruvic acid, causing a sour taste.

The jelly candy packed with edible film was still acceptable to the panelists until the 36th day of storage as it was still chewy when pressed, while entering the 48th day, the panelist's assessment became neutral. This is presumably because there are still parts of the edible film packaging that do not dissolve when eaten because chitosan and nanochitin still have components that are insoluble when eaten. According to (Rochima, 2007) the solubility level of chitosan reaches 79.39% (w/v) and according to Naiu *et al.*, (2020) the solubility level of nanochitin reaches 71.72% (w/v), which means the solubility level of chitosan and nanochitin does not reach 100% causing chitosan and nanochitin are still difficult to dissolve when eaten. This causes the texture of the jelly candy to feel rough on the tongue of the panelists, resulting in a reduced texture value. In general, the average value of all sensory attributes decreases at the end of storage until it is no longer acceptable to the panelists. The results of this study are in line with those reported by (Naiu *et al.*, 2020) that the sensory value of skin cream product using tuna bone gelatin decreases during storage with an average rate of decline of 7.04/day.

CONCULISON

Changes in the chemical and microbiological quality of jelly candy have a very strong correlation with storage time. Changes in sensory value are quite strongly related to storage time. Edible gelatin-chitosan-nanochitin composite film can inhibit microbial growth and reducing sugar content during storage and also can maintain the moisture content and sensory value of jelly candy according to SNI (2008) standards until the 36th day of storage.

ACKNOWLEDGMENTS

We would like to thanks the FPIK Leaders who have supported the implementation of collaborative research by students and lecturers in 2022.

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