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Research Article
Microbiological Quality of Brown Seaweed (*Sargassum* Sp.) *Dodol* Packed with Carrageenan and Chitosan Coatings

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Abstract
Objective: The goal of this study was to determine the total microbial and mold counts from brown seaweed *Dodol* made with an edible coating of carrageenan and chitosan after storage at room temperature. **Materials and Methods:** A simple randomized complete design was used for edible packaging treatments of carrageenan and 5 levels of chitosan. **Results:** The total number of microbes that grew on brown seaweed *Dodol* packaged with treatment A on the 8th day was 4.0×10^{-3} CFU g^{-1}. While the E treatment exhibited 2.1×10^{-2} CFU g^{-1} on the 12th day. In contrast, *Dodol* that received treatment A was contaminated with 5.0×10^{-3} CFU g^{-1} mold on the 8th day. *Dodol* that received treatment K was contaminated with 6.0×10^{-2} CFU g^{-1} mold on the 4th day. With respect to the total plate count and mold test values, the seaweed *Dodol* products coated with the lowest to highest chitosan proportions maintained the quality of brown seaweed *Dodol* compared to that observed for the carrageenan coating. **Conclusion:** The number of microbial and mold colonies that grew on brown seaweed *Dodol* coated with an appropriate chitosan concentration meets the *Dodol* quality requirements according to the Indonesian National Standard (SNI 01-2986-1992) with a maximum limit of microbial and mold colonies of 5.0×10^{-2} CFU g^{-1}.

Key words: *Dodol*, edible coating, carrageenan, chitosan, brown seaweed

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food is a source of nutrition for both humans and microbes and is generally a good medium for microbial growth. The growth of microbes in food can cause beneficial changes, including improvements in nutrition and corner or storage life[1]. A food ingredients that is highly popular in Indonesia is seaweed, which is used in various food preparations, such as seaweed ice, jelly and puddings and can also be used in medicines and cosmetics. Furthermore, seaweed also has fairly high nutritional value, primarily in the form of carbohydrates, fats and proteins. However, not all types of seaweed are used in Indonesia. Where brown seaweed is regarded as waste and is only used as animal feed, while seaweed that has a commercial value includes *Gracilaria* sp. and *Eucheuma* sp.

Seaweed (*Sargassum* sp.) can be used as a superior commodity in a variety of healthy food preparations, such as brown seaweed *Dodol*, which is rich in antioxidants and other vitamin sources. Because brown seaweed contains various bioactive compounds, it has the potential to be developed as a nutraceutical ingredient. In addition to being a source of alginate and iodine, brown algae also contains pigments of brown algae, which is dominated by chlorophyll a and c, β-carotene, violaxanthin and fucoxanthin. Sources of metabolites such as carotenoids, laminaria, alginate, fucoidan, mannitol and phlorotannin have economic value[2]. *Dodol* is a processed agricultural products that has a slightly wet nature so, allowing, it to be eaten immediately without being moistened (rehydrated) but dry enough so that it can remain stable during storage[2].

Dodol is a type of semi-wet food (intermediate moisture food) that has a moisture content of 10-40%, an A_w value of 0.70-0.85, a soft texture, elastic properties, can be eaten immediately, does not require cooling and is durable during storage. *Dodol* is used to make many products in combination with other food ingredients such as seaweed. One of these products is brown seaweed (*Sargassum* sp.) *Dodol,* which consists of a mixture of glutinous rice flour, brown sugar, coconut milk and has a sweet, savory taste because brown seaweed contains alginate which has the ability to form a gel with the proportion of L-glucuronate[2].

A limitation of brown seaweed (*Sargassum* sp.) *Dodol* is its relatively short product life of approximately 4-5 days at room temperature which makes it susceptible to damage and its quality decreases as food is processed. Processing is also a concern in the production of healthy foods, including the packaging of brown seaweed *Dodol* such that it can last a long time and be safe for public consumption. If not packaged properly, brown seaweed (*Sargassum* sp.) *Dodol* can be affected by food spoilage microorganisms. In addition, the growth of microbes in food, especially those containing brown seaweed (*Sargassum* sp.), can also result in undesirable physical or chemical changes, making it unsuitable for consumption[2].

Packaging is used to maintain the quality of food and agricultural products, such fresh fruits, vegetables and processed products, serving as a barrier that controls the migration of moisture, odorants, gases and other ingredients from the materials to the environment or vice versa. Currently in Indonesia, there is still a reliance on plastic packaging made from petroleum raw materials, such as polystyrene and polyethylene and petroleum-based plastics, which are non-biodegradable and can cause environmental pollution. Therefore, it is necessary to develop other packaging materials with superior properties, such as plastics that can biodegraded and consumed by humans leading many researchers to develop a multi-functional packaging systems[3]. In principle edible films made from biodegradable biopolymers (natural polymers) polysaccharides, proteins, fats, or a combination of these compounds could be developed into packaging systems. Biopolymer-based films have been widely used as they function as a barrier to the transfer of fat, moisture, air and odor (taste) of processed products, such as fresh fruits and vegetables, frozen foods, meat and sweets[3,4].

To maintain the quality and minimize the degradation of brown seaweed *Dodol*, it is necessary to develop technology using safe and environmentally friendly packaging that utilizes waste from shrimp shells (chitosan) and carrageenan. Chitosan is a natural polymer and is one of the most important polysaccharides found in nature. For this reason, as a biomaterial with excellent biocompatibility and biodegradability, chitosan is widely used in various fields to make films, stabilizers, gel-forming agents, binders, etc[5,6]. Chitosan is also a potential food preservative due to its antimicrobial activity and also has antioxidant activities against foodborne microbes such as bacteria fungi[5,7]. Another polysaccharide that can be used to make edible coatings for raw materials is carrageenan.

According to Ahmadi *et al.*[8], carrageenan can be obtained from processed seaweed and can easily and inexpensively be made into edible coatings/films. Edible coatings are an environmentally friendly technology that can be applied to various products and function by maintaining and controlling moisture transfer, gas exchange or oxidation processes[8]. As an edible layer, edible coatings can provide an additional protective layer for products and have the same effect on internal gas composition as storage under a modified atmosphere. A primary benefit of using a polymer matrix is that it can be consumed together with food, increasing the safety, nutritional and sensory properties of food[9]. Edible coatings made from chitosan can inhibit the growth of bacteria and mold[10]. While alternative edible coatings can be made from carrageenan. Carrageenan is a hydrocolloid compound extracted from *K. alvarezii* red seaweed that can be used as a stabilizer, emulsifier, thickener. Carrageenan can be used as a stabilizer because it contains sulfate groups that are negatively charged along the polymer chain, making it hydrophilic and capable of binding water or other hydroxyl groups[11,12].

Due to the ability of carrageenan to function as an edible coating, its potential use for *Dodol* was assessed in this study. The goal of this study was to determine the total number of microbes contaminating brown seaweed (*Sargassum* sp.) *Dodol* packaged with an edible coating made from carrageenan and chitosan as a healthy processed food of Padang City.

MATERIALS AND METHODS

Material preparation: The chemicals used in this study included distilled water, physiological salts, alcohols, PP indicators, PCA media, $CaCl_2$, HCl, HgO, glycerol and acetic acid.

Tool preparation: The tools used in this study included knives, spoons, blenders, pans, stoves, basins, scales, 100 mL measuring cups (Pyrex), thermometers and plastics, autoclaves, as well as other tools in the laboratory used for chemical analysis and microbiology such as ovens, analytical scales, desiccators, Kjeldahl flasks, 250 mL Erlenmeyer flasks, crates, electric heaters, goblets, measuring cups, 1 and 10 mL volumetric pipettes, test tubes and an optical microscope equipped with a camera (Olympus). To test the microbiological quality of food, total plate count analysis and mold testing was performed according to SNI 01-2986-1992.

Experimental setup: The study began with the manufacture of *Dodol* with the addition of brown seaweed (*Sargassum* sp.) using 50 g seaweed. The procedure involved heating 250 mL of coconut milk (1 coconut: 1 L of water) with 250 g of brown sugar, 100 g of glutinous rice flour and 50 g of brown seaweed pulp, which was stirred constantly for 45 min until the mixture thickened and turned dark brown. Subsequently, the mixture was poured into a baking dish and allowed to cool, after which the cooled product was cut into rectangles (2×3 cm) and then dipped in an edible coating solutions comprising carrageenan and chitosan in the following proportions: A (100:0%), B (75:25%), C (50:50%), D (25:75%) and E (0:100%). The different treatments were assessed after storage periods of 0, 4, 8 and 12 days.

Generation of an edible coating solution from chitosan: Chitosan 1.5% (w/v) powder was added to a 100 mL measuring cup, after which a 1% acetic acid solution (v/v), 1% glycerol (v/v) and 0.8% $CaCl_2$ (w/v) was added to bring the volume to 100 mL. Subsequently, the mixture was stirred and heated using a magnetic hot plate stirrer to a temperature of 60°C and then stirred and heated to a temperature of 80°C, which was then maintained for 5 min.

Edible coating on brown seaweed *Dodol*: Edible coating solutions were made from carrageenan and chitosan mixed at proportions of (100:0%), (75:25%), (50:50%), (25:75%) and (0:100%), Subsequently, the brown seaweed *Dodol* was coated with one of the solutions and then stored in a closed container at room temperature (25°C) for 0, 4, 8 and 12 days. The relative humidity (RH) and ambient air temperature were measured using a hygrometer and a room thermometer and were maintained at 75% and 26°C, respectively.

Media preparation: Nutrient medium was prepared by dissolving 20 g of powdered medium in 1 L of distilled water, after which the mixture was boiled on a hot plate stirrer and then sterilized in an autoclave at 121°C for 15 min.

Microbial isolation: The isolation stage was carried out using the pour plate method, where 0.1 mL of each dilution was transferred to a tube before the addition of nutrient agar. Microbial isolation from seaweed *Dodol* samples coated with an edible coating was performed in duplicate with dilution factors of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. Subsequently, the samples were isolated and incubated at room temperature (25-27°C) for 24 h[13].

Microbial observations: Microbial colonies that grew in each sample plate were counted using a colony counter for plates harboring 30-300 CFU g^{-1} colonies[14]. If the number of colonies per sample was more than 300 CFU g^{-1}, it was classified as turbid.

Data analysis: The number of colony-forming units per gram for each sample was calculated using the following formula[15]:

$$\frac{\text{No. of colonies}}{\text{Dilution factor}}\times\frac{1}{\text{Dilution factor }(10^2)}$$

RESULTS AND DISCUSSION

Microbiological test results can determine whether a product is suitable for consumption by consumers with respect to food safety. Each food product exhibits differences in shelf life, since products may undergo changes in quality or become damaged due to microorganisms[16].

Total plate counts: The total plate count results for brown seaweed *Dodol* coated with carrageenan and chitosan are shown in Table 1. Total plate counts results for *Dodol* packed with an edible coating of carrageenan (A) on the 8th day indicated that it had been contaminated by microbes at a density of 4.1×10^{-3} CFU g^{-1} (Fig. 1) and increased on the 12th day to 1.7×10^{-5} CFU g^{-1}. In contrast, the brown seaweed *Dodol* packed with an edible coating made with chitosan (E) was not contaminated after the 12th day, with microbes observed at a density of 2.0×10^{-2} CFU g^{-1} (Fig. 2). Furthermore, brown seaweed *Dodol* that was not packaged with an edible coating (K) on the 4th day was contaminated at a density of 8.3×10^{-3} CFU g^{-1} (Fig. 3) and increased on the 8th and 12th days to densities of 8.8×10^{-5} and 2.0×10^{-5} CFU g^{-1}, respectively.

Total plate count analysis: The total plate count results indicated that seaweed *Dodol* products packaged using edible coatings made with chitosan, starting from the lowest to the highest percentage, can maintain the quality of brown seaweed *Dodol* compared to that observed for brown seaweed *Dodol* packaged using an edible coating made from carrageenan. The higher the concentration of chitosan added to the product of brown seaweed *Dodol*, the longer its shelf life was. The number of microbes observed in brown seaweed *Dodol* coated with chitosan at the highest concentration meets the quality requirements of *Dodol* according to the Indonesian National Standard SNI 01-2986-1992, with a maximum limit of microbial colonies of 5.0×10^{-2} CFU g^{-1}. The low total number of microbes that grew in brown seaweed *Dodol* products packaged with the chitosan solution was due to the antibacterial and antifungal properties of chitosan, which can effectively protect food[14].

Mold testing: The total mold results for brown seaweed (*Sargassum* sp.) *Dodol* packed with carrageenan and chitosan are shown in Table 2.

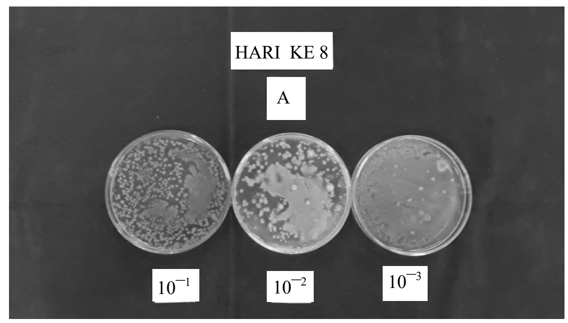

Fig. 1: The 8th day of storage (A)

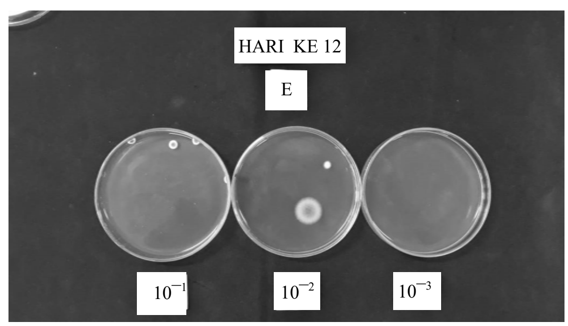

Fig. 2: The 12th day of storage (E)

Table 1: Total plate count analysis of brown seaweed (*Sargassum* sp.) *Dodol* packed with carrageenan and chitosan coating

	Total plate count (CFU g^{-1})			
	Storage period (day)			
Sample	0	4	8	12
A	2.1×10^{-1}	3.1×10^{-2}	4.1×10^{-3}	1.7×10^{-5}
B	2.1×10^{-1}	1.6×10^{-2}	4.8×10^{-2}	5.7×10^{-3}
C	2.6×10^{-1}	1.3×10^{-2}	3.2×10^{-2}	6.0×10^{-2}
D	2.4×10^{-1}	1.5×10^{-2}	2.3×10^{-2}	4.7×10^{-2}
E	2.0×10^{-1}	4.4×10^{-2}	1.7×10^{-2}	2.0×10^{-2}
K	3.0×10^{-1}	8.3×10^{-2}	9.0×10^{-4}	2.0×10^{-5}

Additional information; A-E: Carrageenan and chitosan at proportions of (100:0%), (75:25%), (50:50%), (25:75%) and (0:100%), respectively, K: Brown seaweed *Dodol*

Table 2: Total mold results for brown seaweed (*Sargassum* sp.) *Dodol* packed with carrageenan and chitosan coatings

	Total mold count (CFU g^{-1})			
	Storage period (day)			
Sample	0	4	8	12
A	1.5×10^{-1}	3.1×10^{-2}	5.0×10^{-3}	5.5×10^{-3}
B	3.0×10^{-1}	2.8×10^{-1}	3.2×10^{-2}	2.0×10^{-2}
C	1.5×10^{-1}	3.3×10^{-1}	4.3×10^{-1}	7.3×10^{-1}
D	1.3×10^{-1}	2.3×10^{-1}	3.7×10^{-2}	6.0×10^{-2}
E	1.5×10^{-1}	1.6×10^{-1}	2.0×10^{-1}	6.6×10^{-1}
K	1.5×10^{-1}	6.0×10^{-2}	5.3×10^{-6}	5.8×10^{-6}

Additional information; A: Carrageenan and chitosan at proportions of (100:0%), (75:25%), (50:50%), (25:75%) and (0:100%), respectively; K: Brown seaweed *Dodol*

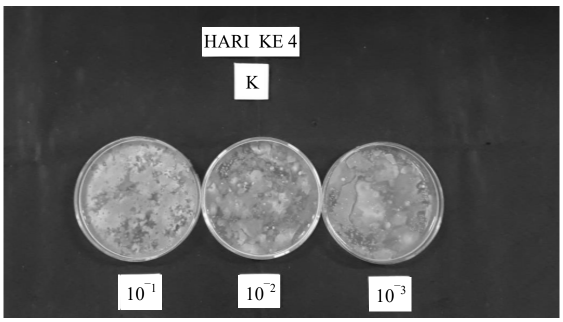

Fig. 3: The 4th day of storage (K)

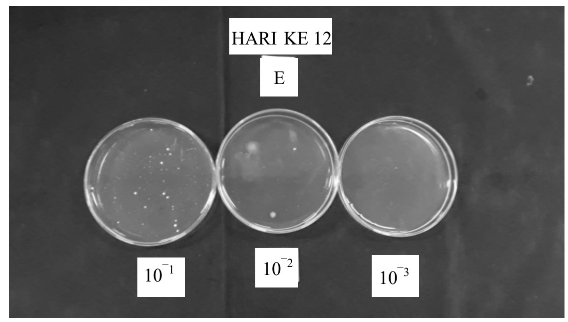

Fig. 5: The 12th day of storage (E)

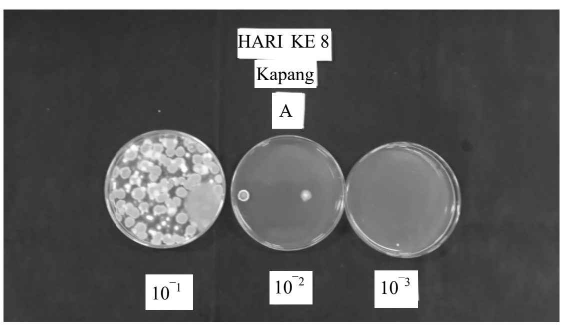

Fig. 4: The 8th day of storage (A)

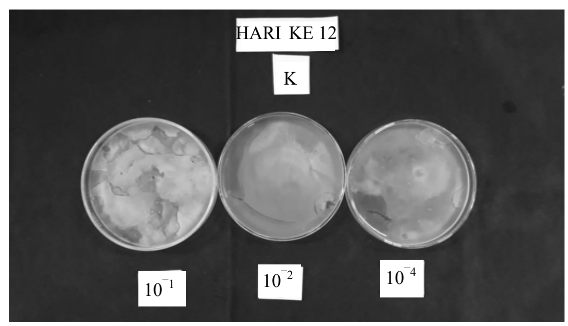

Fig. 6: The 12th day of storage (K)

Testing of total mold: The total mold results for *Dodol* packed with an edible coating made from carrageenan (A) on the 8th day showed that it was contaminated with 5.0×10^{-3} CFU g^{-1} (Fig. 4) and increased on the 12th day to 5.5×10^{-3} CFU g^{-1} (Table 2). In contrast, brown seaweed *Dodol* packed with an edible coating from chitosan shrimp (E) was not contaminated after the 12th day, showing a density of 2.0×10^{-2} CFU g^{-1} (Fig. 5). Furthermore, brown seaweed *Dodol* that was not packaged with an edible coating (K) on the 4th day was contaminated with 6.0×10^{-2} CFU g^{-1} mold (Fig. 6), which increased on the 8th and 12th days to 5.3×10^{-6} and 5.8×10^{-6} CFU g^{-1} mold, respectively. Compared with the total number count for the same E treatment, the total number of molds was higher because *Dodol* is a semi-wet food that is easily contaminated with mold. In contrast, the brown seaweed *Dodol* coated with the edible coating made from chitosan exhibited a longer shelf life than the brown seaweed *Dodol* coated with carrageenan. The number of molds in brown seaweed *Dodol* with a high chitosan concentration met the quality requirements for *Dodol* according to the Indonesian National Standard, SNI 01-2986-1992, with a maximum limit of microbial colonies of

5.0×10^{-2} CFU g^{-1}. The low total number of molds that grew in brown seaweed *Dodol* products packaged with the chitosan solution was due to its antimicrobial and antifungal activities.

According to Sugita *et al.*[14] chitosan can prevent the growth of *Candida albicans* and *Staphylococcus aureus*. In agriculture and food, chitosan is used, as an additive for animal feed rations, antimicrobials, antifungals, components of foodstuffs, stabilizers, gel and texture-forming materials, thickeners and emulsified food processed products, as carriers of food additives, flavors, nutrients, pesticides, herbicides, plant viruses and are also used in the deacidification of fruits, vegetables and fruit purifiers[14].

The potential of chitosan to be used as a natural food preservative has been widely reported based on in vitro trials as well as through its direct application in complex matrix foods[15,17-20]. Chitosan is useful as an excellent film-forming material[15,21]. Furthermore, chitosan films also have selective permeability to gases (CO_2 and O_2) and have good mechanical properties.

One solution to prevent *Dodol* damage and maintaining its quality during storage is the use of edible coatings as packaging. These materials come in direct contact with the product and can improve its quality and extend its shelf life while also functioning as a barrier to oxygen and water, slowing down oxidation and maintaining moisture levels so that it can be directly consumed[22].

Coating brown seaweed *Dodol* with shrimp chitosan at a concentration of 25% was able to maintain its quality at the best condition until the 12th day. In terms of food safety, chitosan can be used as a natural preservative that is safe for consumption due to it being a polysaccharide that is easily biodegradable[23].

Edible packaging can reduce moisture transfer, slow down and limit oxygen absorption, decrease respiration, inhibit ethylene production, cover fiber floors and carry additional functional ingredients such as antioxidants and antimicrobial agents that inhibit microbial growth and prevent discoloration. Many studies have investigated the use of a protective layer of chitosan due to its potential to improve quality and extend the shelf life of food products[16,24-28].

The total plate count results showed that edible coatings made from carrageenan did not last long, although the use of carrageenan as packaging aids in maintaining the quality of brown seaweed *Dodol* products even though the percentage is not significant. Carrageenan can be used to improve the stability of food in the form of suspensions (dispersion of solids in liquid) and emulsions (dispersion of gases in liquid). Furthermore, it can be used as a stabilizer because it contains sulfate groups that are negatively charged along the polymer chain, making it hydrophilic and capable of binding water or other hydroxyl groups[12].

Carrageenan can function as a bulking agent, carrier, emulsifier, gel-forming agent, glass agent, humectant, stabilizer, or thickener[7]. Carrageenan is a linear polysaccharide that is purified from red seaweed. There are three known types of carrageenan (kappa, iota and lambda carrageenan), which have different numbers and positions of functional groups in galactose dimers. Carrageenan is formed by gelation through a moderate drying process. After evaporation of the solvent, the double-helical polysaccharide will form a three-dimensional network followed by a solid film[29].

Coating sticky rice *Dodol* with chitosan extracted from waste at a concentration of 2% was shown to be able to maintain its quality until the 10th day, its color and texture parameters until the 15th day and its flavour and taste parameters above day-to-day storage levels until the 10th day. In addition to chitosan, alginate can also be used as an edible coating on *Dodol*. The use of 2.5% sodium alginate can maintain the quality of seaweed *Dodol* until the 8th day, as observed from the total microbial count of 5.0×10^{-4} CFU g^{-1}, the water content of 29.03%, an a_w value of 0.86, a pH of 6.21 and an organoleptic value of $7.00<\mu<7.47$[30]. The concentration of the coating component plays an important role in the process of controlling changes in quality and quantity loss.

CONCLUSION

Coating brown seaweed *Dodol* with chitosan can maintain its quality better than carrageenan as an edible coating.

SIGNIFICANCE STATEMENT

The results of this study showed that proper handling and packaging of brown seaweed *Dodol* using appropriate concentrations of edible coatings made from carrageenan and chitosan can maintain the quality of brown seaweed *Dodol*. Furthermore, the deterioration of the quality of seaweed *Dodol* products, such as with respect to microbial growth and mold, can be minimized during the shelf life of *Dodol*. The results of this study promotes a better understanding of microbial and mold growth on *Dodol* packaged using edible coatings made from carrageenan and chitosan. Thus, the new theory regarding the quality of brown seaweed *Dodol* packed with edible coating from carrageenan and chitosan can be accepted.

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