The Effect of Storage Time and Number of Layers on The Physicochemical Properties of Beef Sausage Coated with Gelatin-Carragenan

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Abstract. Edible coating is a type of primary packaging that has been widely used in processed meat products. Several previous studies have reported that edible packaging can extend the shelf life of a product. However, studies regarding the effect of the number of layers on shelf life have not been thoroughly researched. This research aims to determine the effect of the number of layers and storage time on the quality of beef sausages with a gelatin-carrageenan coating. A completely randomized factorial design was used as the research design, with the first factor being the number of layers (L0, L1, L2, L3) and the second factor being the storage time (W0, W1, W2, W3, W4, W5). This research used 3 replications, so that in total there were 72 research samples. The research data were analyzed using analysis of variance (ANOVA). The research results showed that differences in the number of layers, storage time, and the interaction of the two treatments had a significant effect ($P<0.05$) on reducing water content, weight loss, and increasing the water holding capacity and pH value of beef sausage. The best treatment was the L3W5 treatment, with a water content value of 65.49%, weight loss of 4.11%, water holding capacity of 47.48%, and a pH value of 5.86. All of these values still meet Indonesian national standards after 35 days of storage.

1 Introduction

Sausage is a type of processed meat whose production continues to increase throughout the year. Statistical data records that worldwide sausage production in 2023 will reach 14.01 million metric tons with per capita consumption of around 1.6-1.8 kg per year [1]. The primary and secondary packaging used to wrap sausages is plastic packaging. Plastic is packaging that is practical, cheap, and easy to apply but has the weakness of being difficult to destroy and is detrimental to the environment. BPS data records that plastic waste in 2023 will reach 69 thousand tons [2]. Several alternatives that have been made to reduce the use of plastic as primary packaging for sausages are using edible coating packaging. Edible coating is a thin layer made from natural materials. Edible coating is made from composite

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materials and is biodegradable [3]. Several studies have reported that edible coatings have a positive impact on food products.

Edible coating made from starch has been able to maintain the quality of crispy pink apples in chilled storage [4]. Edible coatings made from canna starch, potato starch, and sweet potato starch can maintain the brightness level of tomatoes for 6 days of storage [5]. Edible coating has also been widely applied as primary packaging to replace plastic for sausage products. Hydrocoloids from seaweed extracts such as agar have also been reported to be used as ingredients for making edible films and can be used as sausage deterioration bio- indicators [6]. A gelatin-based edible coating incorporated with aloe vera and green tea extract is able to inhibit bacterial growth and maintain the freshness of fresh-cut apples [7]. Fish bone gelatin-based edible coating is able to maintain the texture and sensory value of fish sausage until 21 days of storage [8]. A bovine split-hide gelatin-based edible coating incorporated with soy protein isolate and transglutaminase enzyme is also reported to maintain the quality of fish sausages for 15 days of chilled storage [9]. Apart from cattle and fish, gelatin can also be produced from poultry bones, as reported by [10]. Duck bone gelatin has the potential to be used as a raw material for making edible coatings.

The method for making edible coatings is divided into 5 types, including dipping, foaming, spraying, casting, and controlled dripping. The dipping method is the most widely used method because it is practical, able to cover the entire surface evenly and in a controlled manner [11]. The dipping method has been used to make edible coatings from arrowroot flour [12], casein-chitosan [13], agar [6], and gelatin [8]. The number of dipping used still varies, and each research has different conditions. There has been no further study regarding the influence of the number of layers on sausage quality. The difference in the number of layers of coating is thought to strongly influence the quality of sausages during storage. Based on this background, it is necessary to conduct research on the effect of the number of coatings and storage time on the quality of sausages. The aim of this research is to explore how effectively the number of layers can influence the quality of sausages during storage.

2 Materials and Method

2.1 Materials

Bones from duck were obtained from poultry slaughterhouse, carragenan, aquadest, glycerol, meat, corn starch, cassava starch, and seasoning powder. Chemical reagent for the analysis include hydrochloric acid, sodium hydroxide, buffer standard, and others reagents were analytical grade, purchased from PT Merck TBK (Jakarta, Indonesia).

2.2 Gelatin and Edible Coating Manufacture

Gelatin extraction from duck bone was based on the [10] methods with slight modifications. The bones are cut into small pieces and then soaked in an aqueous solution for 30 minutes with continuous stirring. The bones were soaked in a 1.75 M NaOH solution for 2 hours, then the bones were soaked in distilled water to neutralize the pH. Next, we soaked the bones in 1 M HCl for 12 hours. The soft bone (ossein) that has been formed is soaked in distilled water for 1 hour. Ossein is heated at 75 °C for 2 hours, followed by heating at 85 °C for 2 hours, then filtered with Whatman paper. The filtrate was centrifuged at 7000 rpm for 15 minutes. The pellets that have been obtained are then dried in a vacuum oven until gelatin powder is formed. Making edible coatings is based on the method of [8] with several modifications. 2% gelatin was dissolved in 500 mL distilled water (solution A), and then 1% carrageenan was dissolved in 500 mL distilled water (solution B). The two solutions were mixed, then added with 1% glycerol, and then heated at 90 °C for 5 minutes. The edible coating solution that has been formed is ready to be used.

2.3 Sausage Manufacture and Edible Coating Application

The sausage-making procedure is based on the method of [14]. One kg of beef is crushed using a grinder and then put into a coper machine. The supporting ingredients and spices are put into a cupper and stirred until homogeneous. Next, the sausage is molded and then boiled at 90°C for 20 minutes. The application of edible coating from gelatin-carrageenan to sausages was carried out based on the method of [15]. Sausages are dipped in an edible coating solution containing a mixture of gelatin, carrageenan, and glycerol for 5 minutes. Sausages are dipped 3 times (L1), 4 times (L2), 5 times (L3), and without dipping (L0). Next, the sausage is dried using a food dehydrator at a temperature of 40^0C. Sausages were stored at 6°C with 75% humidity and observed for 0 weeks (W0), 1 weeks (W1), 2 weeks (W2), 3 weeks (W3), 4 weeks (W4), and 5 weeks (W5).

2.4 Analysis of Sample

2.4.1 Water Content Assay

The water content test was carried out using the method of [16]. The sterile petri dish was weighed using an analytical balance, then 2 grams of sample were added and calculated as the initial weight. The petri dish was placed in an oven at 105^0C for 18 hours. The petri dish was cooled in a desiccator and then weighed 3 times to obtain a stable final weight. The water content value is expressed in percentage.

2.4.2 Water Holding Capacity

The water holding capacity (WHC) test is carried out to determine the ability of sausages to bind water. WHC test based on the method of [17]. The sample was weighed as much as 10 grams and then ground using a mortar. The sample was put into a centrifuge tube, and 10 ml of distilled water was added. The centrifuge was run for 20 minutes at a speed of 4000 rpm. The supernatant is separated from the pellet formed. The WHC value is a comparison between the volume of the supernatant and the weight of the material. WHC is expressed as a percentage.

2.4.3 pH

pH testing is carried out using a pH meter. The electrodes were washed first using distilled water. The pH meter is standardized using a buffer solution of pH 4 and pH 7. The electrode needle is inserted into the sample for 10 seconds, and the value can be seen on the pH meter screen.

2.4.4 Weight Loss

Weight loss testing is carried out gravimetrically [16], namely comparing the difference in weight of sausages before storage and after storage according to the predetermined treatment. The weight loss value is expressed as a percentage.

2.5 Statistical Analysis

This research used a completely randomized factorial design with 2 factors, namely the first factor was the number of coating layers while the second factor was storage time. All the test were conducted in triplicate. The separations of mean was accomplished using one-way analysis of variance (ANOVA) for p <0.05. Post-hoc analysis for significance was obtain using Duncan's Multiple Range Test (DMRT).

3 Result and discussion

3.1 Water Content

The water content test was carried out to determine whether differences in storage time and number of coating layers affected the quality of the sausage. Water content values are presented in Table 1.

Table 1. Average value of water content (%) of beef sausages with different amounts of storage time and number of coating layers

Storage time	Number of coating layers				Average
	L0	L1	L2	L3	
W0	65.19±0.06^{i}	64.72±0.04^{k}	64.48±0.05^{l}	63.76±0.03^{o}	64.54±0.03^{f}
W1	65.91±0.04^{f}	64.96±0.04^{j}	64.65±0.04^{k}	64.04±0.03^{n}	64.89±0.02^{e}
W2	66.05±0.02d^{de}	65.19±0.06^{h}	64.94±0.04^{i}	64.34±0.04^{m}	65.14±0.03^{d}
W3	66.38±0.02^{c}	65.53±0.04^{h}	65.16±0.03^{h}	64.90±0.06^{i}	65.49±0.04^{c}
W4	66.74±0.04^{b}	65.94±0.02^{ef}	65.65±0.07^{g}	65.10±0.04^{i}	65.86±0.04^{b}
W5	67.22±0.08^{a}	66.66±0.04^{b}	66.13±0.04^{d}	65.49±0.04^{h}	66.38±0.03^{a}
Average	66.24±0.03^{w}	65.51±0.03^{x}	65.16±0.04^{y}	64.60±0.03^{z}	

Values are presented as means ± standard deviation of triplicate.
L0, non layers ; L1, 3 layers ; L2, 4 layers ; L3, 5 layers
W0, 0 days ; W1, 7 days ; W2, 14 days ; W3, 21 days ; W4, 28 days ; W5, 35 days
$^{a\text{-}o}$ figures with diferent rows and coloumn differ significantly (p<0.05)

Based on the ANOVA test results in Table 1. It can be seen that the length of storage time has a significantly different effect (p≤0.05) on water content. The highest average water content was found in treatment W5 (66.38%), while the lowest water content value was found in treatment W0 (64.54%). The longer the storage time, the water content of beef sausages will increase; this is thought to be because beef sausages absorb water vapor in the surrounding environment so that bacteria can grow. The sausages in this study were stored at 6°C with 57% humidity. The texture of the sausage changed from chewy to soft, and there was mold on the outer layer of the sausage. According to [8], the temperature and humidity of the environment around the product can affect the quality of the sausage being stored. [18], stated that high temperatures in the surrounding environment will cause high humidity. High humidity during product storage will experience physical, chemical, and organoleptic changes due to the activity of microorganisms, resulting in increased water content. [19], added that long storage time can increase the water content because there is protease enzyme activity and pathogenic microorganisms that cause protein degradation in sausages so that the protein cannot bind water.

Data in table 1. showed that the difference in the number of coatings had a significantly different effect ($p \leq 0.05$) on the water content of beef sausage. The highest average water content value was found in treatment L0 (66.24%), while the lowest water content value was found in treatment L3 (64.60%). The difference in the amount of edible coating on beef sausages affects the increase in the resulting water content. Sausages without edible coating have a higher water content value, while sausages coated with edible coating have a water content value that tends to be lower. This is thought to be because the thicker the edible coating layer is, the better it is able to maintain the water balance in the product so that water does not easily come out because the edible coating functions as a barrier or barrier to the entry and exit of water and oxygen. [20], stated that the thicker the edible coating layer, the denser the layer will be to prevent oxygen and water from entering, thus increasing the shelf life of the product. Products coated with multi-layered edible coatings also have low water vapor transmission values, so bacteria can't grow them, and the quality of the product will increase.

Sausages coated with 5 layers of edible coating and stored for 35 days showed an increase in water content that was not too high compared to 0 days of storage. The entire surface of the product coated with edible coating will make it difficult for microbes to develop because the edible coating will inhibit the entry of oxygen and water vapor through the product surface [21]. The edible coating materials used, namely gelatin and carrageenan, also support protecting the product. This is because gelatin in making edible film has transparent, easily soluble, strong, and flexible properties, capable of inhibiting the transfer of oxygen and carbon dioxide [22]. The thicker the edible coating layer, the tighter the product's pores will be, thus preventing water from escaping. The use of carrageenan as an edible coating has the ability to bind large amounts of water because carrageenan has hydrophilic properties. Carrageenan has free hydroxyl ions (OH-) and sulfate ester content [23]. The combination of gelatin and carrageenan produces a complex matrix that is able to retain water from the environment into the sausage.

The interaction between the number of coatings and the length of storage time had a significantly different effect ($P \leq 0.05$) on the water content of beef sausages. The highest average value for the entire sample was in the L0W5 treatment at 67.22%, while the lowest average value was in the L3W0 treatment, which had a value of 63.76%. Duncan's further test results showed that each treatment had a different notation from notation a to notation o. This shows that differences in the number of coatings and storage time have an influence on the water content value. [24] stated that the edible coating layer is able to add moisture to the product and act as an intermediary for the gas exchange process between the inside and outside of the product, thereby increasing the shelf life of the product. Oxygen and water vapor entering through the surface of the product during storage will be inhibited by the edible coating layer that covers the entire surface of the product so that it is difficult for microbes to develop, causing a decrease in product quality. [25] added that beef sausages that are coated with edible coating have a relatively low water content compared to beef sausages without coating because edible coating has properties as a barrier to water vapor, oxygen, fat, and other volatile compounds from food during storage time.

Water is the most important component in influencing the texture and taste of food products. Increasing water content in beef sausages can cause a change in texture from chewy to soft [26]. Increasing water content causes reduced nutritional content, browning, and rancidity, accelerates microbial growth, and accelerates the rate of deterioration of the material [27]. Food products that have high water content make the product more fragile and have a relatively short shelf life [28]. The moisture content value of sausages in this research has increased. Sausages treated with 5 layers of edible coating experienced an increase in value from 63.76% to 65.49%, or around 1.73%. Sausages without edible coating experienced an increase in value from 65.19% to 67.22%, or around 2.03%. This data provides evidence that applying an edible coating to sausages is able to withstand the rate of increase in water content. This happens because edible coating is able to prevent the entry of free water from the surrounding environment so that the water content does not increase. [20] in their research, reported that kurisi fish sausages coated with edible coating experienced an increase in water content from 32.70% to 34.70%, while sausages without edible coating experienced an increase in water content from 34.32% to 35.19%. The maximum limit for water content in sausages is 67% [29]. All samples still meet SNI standards up to 35 days of storage except sample L0.

3.2 Water Holding Capacity

Water holding capacity is the ability of protein to hold or bind water in the meat structure. Water holding capacity values are presented in Table 2.

Table 2. Average value of water holding capacity (%) of beef sausages with different amounts of storage time and number of coating layers

Storage time	Number of coating layers				Average
	L0	L1	L2	L3	
W0	78.35±1.18^{c}	81.95±1.17^{b}	84.00±1.12^{b}	88.77±1.18^{a}	83,27±0.56^{a}
W1	27.98±1.13^{jk}	40.46±1.18^{h}	43.30±1.12^{h}	61.77±1.18^{d}	43,38±0.59^{b}
W2	25.50±1.13^{klm}	34.50±1.15^{i}	41.50±1.14^{h}	57.50±1.14^{e}	39,75±0.57^{c}
W3	23.90±1.16^{lm}	33.00±1.15^{i}	40.00±1.18^{h}	56.00±1.18^{e}	38,23±0.57^{c}
W4	22.49±1.18^{m}	29.50±1.18^{j}	35.97±1.13^{i}	51.97±1.14^{f}	34,99±0.55^{d}
W5	18.98±1.13^{n}	26.49±1.18^{jkl}	33.99±1.13^{i}	47.48±1.15^{g}	31,74±0.59^{e}
Average	32.86±0.46^{z}	40.98±0.47^{y}	46.46±0.45^{x}	60.58±0.47^{w}	

Values are presented as means ± standard deviation of triplicate.
L0, non layers ; L1, 3 layers ; L2, 4 layers ; L3, 5 layers
W0, 0 days ; W1, 7 days ; W2, 14 days ; W3, 21 days ; W4, 28 days ; W5, 35 days
$^{a\text{-}n}$ figures with diferent rows and coloumn differ significantly (p<0.05)

Analysis of variance in Table 2 shows that the difference in the number of coatings has a significant influence ($p \leq 0.05$) on the value of water holding capacity. The water holding capacity value tends to increase with the number of layers of edible coating. The lowest value is found in L0, namely 32.86% while the highest is in L3, namely 60.58% The edible coating layer is thought to function as a barrier for the water contained in the sausage from coming out and remaining bound to the meat tissue matrix. This is what causes the water-holding capacity of sausages coated with edible coating to be higher than the control. According to [30], products coated with edible coating have a slow decrease in water holding capacity.

This shows that edible coating is able to inhibit changes in chemical processes in the product during low-temperature storage so that the water holding capacity value can be well maintained. [31] added that layer thickness affects water holding capacity. The thicker the edible coating layer, the more complex the constituent molecules will be, so the edible coating pores will be smaller.

The water vapor transfer rate of edible coating is related to the thickness of the edible coating, the thicker the edible coating, the lower the water vapor transfer rate. This is because the polymer content increases so that the bonds between molecules become more complex [32]. The thicker the layer will inhibit the amount of water vapor released from the product into the environment so that the product does not dry quickly [31]. The hydrophilic nature of carrageenan causes it to have the ability to bind large amounts of water. This is because carrageenan has free hydroxyl ions (OH-) and sulfate ester content. Gelatin also has hydrophilic properties. The hydroxy groups in the amino acid sequence of gelatin are able to bind large amounts of water [23]. According to [33], carrageenan is a polysaccharide that can provide effective protection against fat oxidation and oxidation of other food components. Apart from preventing moisture loss, the thin polysaccharide layer of carrageenan is also less permeable to oxygen so the rate of water evaporation is low.

The difference in storage time had a significantly different effect ($P \leq 0.05$) on water holding capacity. The highest water holding capacity value was found in the 0 day storage treatment, namely 83.26%, while the lowest value was found in the 35 day storage treatment, amounting to 31.74%. The water holding capacity did not decrease significantly when stored for 14 days and 21 days, namely 39.75% and 38.23%. However, at 35 days of storage, there was a significant decrease of up to 31.74%. The value of water holding capacity continues to decrease with increasing storage time. This is thought to be due to microbial activity. Microbes use the protein in sausages to grow by breaking it down into simpler components with the help of free water. This causes protein denaturation and a decrease in WHC values. The texture of sausages stored for 35 days also turns mushy, and there are signs of mold growth. According to [34], the decrease in water holding capacity is caused by the activity of spoilage microbes, which causes protein denaturation. [35] stated that storage temperature can cause denaturation of sarcoplasmic proteins, which results in coagulation of myofibril proteins. This affects the interaction between the protein surface and the surrounding water phase, thereby reducing the water holding capacity. [36] added that during storage the bound water content will change to free water so that the ability of meat to bind water decreases. This is caused by microbial activity and loss of myofibrillar protein integrity.

The interaction between storage time treatment and the number of layers were found to have a significant effect ($p \leq 0.05$) in water holding capacity. Further tests using Duncan showed that all treatments had different values from each other. This is shown in the notation a to n. The highest WHC value was found in the L3W0 treatment with a value of 88.77%, while the lowest was in the L0W5 treatment with a value of 18.98%. All samples experienced a decrease in water holding capacity as storage time increased. However, sausages without edible coating experienced the largest decrease, namely from 78.35% to 18.98%, or around 59.37%. Sausages without edible coating lose the ability to hold water by more than half the total weight of the sausage. Sausages with 3 layers, 4 layers, and 5 layers of edible coating experienced a decrease of 55.46%, 50.01%, and 41.29%, respectively. Increasing the thickness of the edible coating layer used on beef sausages shows the ability to maintain the water holding capacity value. According to [34], during storage, the product will experience protein denaturation. Protein is the main raw material for bacterial growth. The breakdown of protein by bacteria causes a denaturation reaction that is accompanied by an increase in water content and a decrease in the WHC value. The use of coating materials can inhibit the entry of microbes into food products. [37] added that the edible coating layer made from gelatin contains protein so that the protein can bind free water contained in sausages during storage.

The water holding capacity value is inversely related to the water content value. The higher the water content of the sausage, the lower the water holding capacity value. This can be seen in the L0W5 treatment, which has a water holding capacity value of 18.98% with a water content of 67.22%. Meanwhile, the L3W5 treatment has a water holding capacity value of 47.48% with a water content of 65.49%. The ability of meat to hold water is an important characteristic because with high water holding capacity, the meat has good quality [38]. Food damage caused by microorganisms occurs because these microorganisms utilize the components in the food to reproduce and metabolize, so that the food experiences changes in smell and taste that indicate spoilage [39]. Similar research as reported by [38], stated that beef sausage with the addition of gelatin as a binding agent produced a water holding capacity of 55.32%, while the control treatment produced a water holding capacity of 44.54%. Beef sausages coated with an edible coating made from gelatin-carrageenan were able to produce a higher water holding capacity of 88.77% at 0 days of storage. The WHC value continues to decrease with increasing storage time. However, the edible coating treatment was able to prevent a decrease in the WHC value. Sausages with edible coating had better WHC values than the control ones.

3.3 pH

pH shows the acidity or alkalinity value of a food ingredient. pH values are presented in Table 3.

Table 3. pH value of beef sausages with different amounts of storage time and number of coating layers

Storage time	Number of coating layers				Average
	L0	L1	L2	L3	
W0	5.81±0.02^{gh}	5.89±0.02^{de}	5.97±0.02^{c}	6.27±0.02^{a}	5.98±0.01^{a}
W1	5.76±0.01^{ij}	5.77±0.02^{hi}	5.87±0.01^{de}	6.046±0.02^{b}	5.86±0.01^{b}
W2	5.71±0.01^{k}	5.70±0.04^{k}	5.82±0.03^{jk}	5.98±0.02^{c}	5.80±0.02^{c}
W3	5.59±0.02^{n}	5.69±0.04^{kl}	5.72±0.03^{g}	5.91±0.03^{d}	5.73±0.01^{d}
W4	5.53±0.02^{o}	5.65±0.02^{lm}	5.66±0.02^{lm}	5.88±0.01^{de}	5.68±0.02^{e}
W5	5.47±0.03^{p}	5.63±0.03^{mn}	5.64±0.01^{lm}	5.86±0.02^{ef}	5.65±0.01^{f}
Average	5.64±0.02^{z}	5.72±0.03^{y}	5.78±0.02^{x}	5.99±0.01^{w}	

Values are presented as means ± standard deviation of triplicate.
L0, non layers ; L1, 3 layers ; L2, 4 layers ; L3, 5 layers
W0, 0 days ; W1, 7 days ; W2, 14 days ; W3, 21 days ; W4, 28 days ; W5, 35 days
$^{a\text{-}p}$ figures with diferent rows and coloumn differ significantly (p<0.05)

The result of the variance analysis in Table 3 showed that the interaction between storage time and number of coating layers had a significant effect ($p < 0.05$) on pH. The highest pH value was found in the L3W0 treatment, namely 6.27. Meanwhile the lowest pH value was found in the L0W5 treatment, namely 5.47. Duncan's further test showed that all treatments had different values from each other. This is shown in the notation a to p. Sausages without edible coating experienced a decrease in pH from 0 days to 35 days by 0.34. Sausages with 3 layers, 4 layers, and 5 layers of edible coating experienced a decrease in pH, namely 0.26, 0.33, and 0.41. Even though in terms of ratio, the L3 treatment experienced the largest decrease in pH, but in terms of data, the pH value of the L3 treatment remained the highest among all treatments. A decrease in pH value indicates microbial activity. According to [40], oxygen and water vapor entering through the surface of the product during storage will be inhibited by the edible coating layer that covers the entire surface of the product so that it is difficult for microbes to develop, causing a decrease in product quality. [41] stated that the edible coating layer on beef sausages during storage functions as a barrier to mass transfer such as moisture, oxygen, lipids, and dissolved substances to improve the subsequent handling system for these food products. [37] added that the use of an edible coating made from gelatin on beef sausages stored in the refrigerator has a better ability to protect the product against oxygen, has the desired mechanical properties, and increases the structural unity of the product. A decrease in pH value is related to a decrease in water holding capacity. A pH that tends to be acidic will increase the contraction of actin and myosin to actomyosin and result in a decrease in water holding capacity. This causes rapid ATP breakdown and increases the protein denaturation process [42].

Different amounts of coating had a significantly different effect ($p \leq 0.05$) on pH. Treatment L0 has the lowest pH value, namely 5.64. Treatments L1 and L2 have similar pH values, namely 5.72 and 5.78, while treatment L3 has the highest pH value, namely 5.99. Based on this data, we can get an idea that the use of an edible coating layer is able to maintain the pH value of the sausage. A low pH indicates damage to the sausage because the pH of fresh sausage ranges from 6.3 to 6.5. According to [43], the density of a coating material depends on the number of layers used. The thicker the layer, the more complex the tissue matrix produced. This causes the pores of the coating to become smaller and prevents oxygen from entering the packaged product. [44] stated that microbial contamination can be detected from an increase in the total acid or pH of a food ingredient. The application of coating materials such as thick edible coatings will have a low water vapor transmission value so that it can protect the product from microbial contamination.

The data in table 3 shows that differences in storage time have a significantly different effect ($P \leq 0.05$) on pH. The pH value decreased from 0 days to 35 days of storage, namely 5.98 and 5.65 respectively. The decrease in pH value is closely related to microbial activity. Product damage will continue to occur as storage time increases. However, the use of an edible coating is thought to be able to slow down the decline in pH values. [45] stated that microbes break down proteins and carbohydrates into simpler molecules, namely in the form of organic acids such as lactic acid. This breakdown causes a decrease in the pH value, and the product also experiences a change in texture. The solid texture changes to soft. According to [39], microbial activity causes the glycolysis process to occur. Glycogen in sausages is broken down through the process of anaerobic glycolysis when there is no oxygen. This breakdown produces lactic acid. This acid affects the pH value. Increased microbial activity causes an acceleration in the decrease in pH values. [46], in his research, reported that beef sausages added with dragon fruit skin experienced a decrease in pH value during storage time. The pH values from 0 days to 20 days of storage were 5.86 and 5.67, respectively. The edible coating layer made from gelatin-carrageenan has been proven to be more capable of preventing a decrease in pH values. This is because the pH value of 5.68 was achieved longer, namely at a storage time of 28 days.

3.4 Weight loss

Weight loss is the amount of weight loss of a stored food product. Weight loss values are presented in Table 4. Based on the ANOVA test results in Table 4. It can be seen that the length of storage time has a significantly different effect ($p \leq 0.05$) on weight loss. The highest average weight loss was found in treatment W5 (5.24%), while the lowest weight loss value was found in treatment W0 (1.08%). The longer the storage time, the weight loss of beef sausages will increase. The sausages in this study were stored at 6°C with 75% humidity. The increase in weight loss value is thought to be due to the evaporation of oxygen gas from the sausage. Gas evaporation is a natural process for a product to reach equilibrium with the surrounding environment. According to [47], weight loss is a decrease in the weight of a material after undergoing the storage process. The longer the storage, the greater the product weight loss. [48] stated that unpackaged meat generally experiences surface evaporation. The use of packaging is expected to avoid excessive evaporation of the product.

Table 4. Weight loss of beef sausages with different amounts of storage time and number of coating layers

Storage time	**Number of coating layers**				**Average**
	L0	**L1**	**L2**	**L3**	
W0	1.33 ± 0.02^{p}	1.06 ± 0.05^{q}	0.91 ± 0.02^{r}	0.82 ± 0.03^{s}	1.08 ± 0.03^{f}
W1	3.07 ± 0.04^{jk}	2.82 ± 0.05^{l}	2.08 ± 0.02^{o}	1.71 ± 0.01^{p}	2.42 ± 0.03^{e}
W2	3.32 ± 0.04^{i}	3.08 ± 0.02^{j}	2.78 ± 0.02^{l}	2.23 ± 0.04^{n}	2.85 ± 0.02^{d}
W3	3.94 ± 0.02^{e}	3.43 ± 0.03^{h}	3.00 ± 0.04^{k}	2.56 ± 0.02^{m}	3.24 ± 0.01^{c}
W4	4.09 ± 0.03^{d}	3.79 ± 0.04^{f}	3.56 ± 0.04^{g}	3.12 ± 0.02^{j}	3.63 ± 0.03^{b}
W5	6.83 ± 0.02^{a}	5.51 ± 0.04^{b}	4.54 ± 0.03^{c}	4.11 ± 0.03^{d}	5.24 ± 0.02^{a}
Average	3.77 ± 0.02^{w}	3.28 ± 0.03^{x}	2.82 ± 0.02^{y}	2.42 ± 0.03^{z}	

Values are presented as means ± standard deviation of triplicate.
L0, non layers ; L1, 3 layers ; L2, 4 layers ; L3, 5 layers
W0, 0 days ; W1, 7 days ; W2, 14 days ; W3, 21 days ; W4, 28 days ; W5, 35 days
$^{a\text{-}s}$ figures with diferent rows and coloumn differ significantly ($p<0.05$)

Data in table 4. showed that the difference in the number of coatings had a significantly different effect ($p \leq 0.05$) on the weight loss of beef sausage. The highest average weight loss value was found in treatment L0 (3.77%), while the lowest weight loss value was found in treatment L3 (2.42%). Sausages with 3 layers of edible coating (L1) had a lower weight loss of 0.49% than the control. Sausages with 4 layers of edible coating (L2) had a lower weight loss of 0.95% than the control. Meanwhile, sausages with 5 layers of edible coating (L3) had a lower weight loss of 1.35% than the control. Edible coating has been proven to be able to withstand increasing amounts of weight loss. The thicker the edible film used, the lower the weight loss value. [49] state that edible coating inhibits the migration of compounds found in food ingredients so that it can reduce the rate of increase in weight loss. The weight loss value depends on the density of the packaging contents and the permeability of the packaging. Edible coating is permeable to water vapor, so it can prevent an increase in weight loss. According to [50], a thick layer has a low water vapor transmission value, so it can protect the product from damage caused by microbes. The thicker edible coating shows the denser resulting layer. Edible coating functions as a barrier for oxygen and water to enter food products. Low weight loss indicates low microbial growth.

The interaction between the number of coatings and the length of storage time had a significantly different effect (P≤0.05) on the weight loss of beef sausages. The highest average value for the entire sample was in the L0W5 treatment at 6.83%, while the lowest average value was in the L3W0 treatment, which had a value of 0.82%. Duncan's further test results showed that each treatment had a different notation from notation a to notation s. All treatments had different weight loss percentages. Sausages without coating (L0) experienced an increase in weight loss from 0 days of storage time to 35 days of storage time of 5.5%. Sausages with 3 layers (L1), 4 layers (L2), and 5 layers (L3) experienced an increase in weight loss from 0 day storage time to 35 day storage time by 4.45%, 3.63%, and 3.29%, respectively. Edible coatings are able to withstand the rate of increase in weight loss because they act as a barrier against oxygen, air, and microbial contamination. An increase in respiration rate will cause the breakdown of compounds such as carbohydrates in sausages. This reaction produces CO_2, energy, and water, which evaporate through the surface of the product. It causes weight loss in the product [51]. Edible coating from chitosan is able to inhibit the increase in water content of fish sausages. Edible coating can be a good barrier to oxygen and water so that it can inhibit the product's respiration rate [52].

The weight loss value is closely related to the water holding capacity. High water holding capacity indicates low weight loss values. This can be seen in the L3 treatment, which has a water holding capacity value of 47.48% with a weight loss of 4.11%. Meanwhile, the L0 treatment had a water holding capacity value of 18.98% with a weight loss of 6.83%. Similar research as reported by [4], apples that were coated with edible coating and without edible coating (control) for 5 weeks of storage had weight loss values of 1.0% and 1.85%, respectively. [53] also reported something similar. Tomatoes coated with potato starch and without a layer of potato starch (control) had weight loss values of 1.55% and 2.41%, respectively. Edible coating is a good barrier against water and oxygen. Edible coating can also control the respiration rate, so it is widely used to package frozen meat and chicken products, sausages, seafood products, fruit, and intermediate moisture food [54]. Control sausages and sausages with edible coating are presented in Figure 1.

Fig. 1. a). non-coated sausage with 0 day storage, b). sausage with 3 layers, 4 layers, and 5 layers coating with 0 day storage, c). non-coated sausage with 35 day storage, d). sausage with 3 layers, 4 layers, and 5 layers coating with 35 day storage

4 Conclusion

The present result confirm that the number of coatings and the length of storage time have a significant effect ($p \leq 0.05$) on the water content, water holding capacity, pH, and weight loss of beef sausage coated with gelatin-carrageenan. The application of 5 layers (L3) of edible coating was proven to be able to maintain the quality of sausages for up to 35 days (W5) of storage with water content, WHC, pH, and weight loss values of 65.49%, 47.48%, 5.86%, and 4.11%, respectively.

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