

Carboxymethyl Chitosan as A Homemade Sausage Preservative

Mardiyah Kurniasih^{1*}, Purwati¹, Ratna Stia Dewi², Dadan Hermawan¹, Eva Vaulina¹

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Jenderal Soedirman University, Jl. Dr. Soeparno, Purwokerto 53123, Indonesia.

²Mycology & Phytopathology Laboratory, Faculty of Biology, Jenderal Soedirman University, Jl. Dr. Soeparno, Purwokerto 53123, Indonesia

*Corresponding email: m_kurniasih@yahoo.com

Received 30 December 2018; Accepted 23 April 2019

ABSTRACT

Carboxymethyl chitosan has antimicrobial activity. The solubility of carboxymethyl chitosan makes it easy to apply as a food preservative. Sausage is a processed product of meat, and it is classified as perishable food. The purpose of this study was to synthesize carboxymethyl chitosan, investigate the microbiological quality and shelf-life of homemade sausage treated with carboxymethyl chitosan. Carboxymethyl chitosan was obtained through the process of carboxymethylation of alkaline chitosan with monochloroacetic acid. Chitosan in the study was synthesized from shrimp skin. Sausages treated with carboxymethyl chitosan then measured water content, ash content, TPC (Total Plate Count) and organoleptic values for four consecutive days. The results showed that carboxymethyl chitosan could extend the shelf life of sausages both stored at room temperature or cold temperatures.

Keyword: carboxymethyl chitosan, preservative, homemade sausages, TPC value, organoleptic value

INTRODUCTION

Food decay is mostly caused by a microbial activity which uses the food for their metabolism. Therefore, preservatives are needed to prolong shelf life. The purpose of the preservation is to inhibit or to avoid damage to the food, to keep the quality, to avoid toxicity as well as to make it easy to handle and store. Beef is one of the foodstuffs which is very susceptible to microorganism contamination because its high nutrient content is the microorganism source of food. Some processed beef products are meatballs and sausages. Sausage is one of the foods that are easily made and liked by children. Nitrite is one of the preservatives used in preserving meat to keep flavor and color, to inhibit the microbial growth and lipid oxidation [1]. On the other hand, nitrite can promote the formation of N-nitroso compounds (NOCs), which is carcinogenic [2]. Therefore the meat industry is currently focusing on developing nitrite alternatives [1]. Chitosan is a biopolymer which is likely to be a substitute for nitrate in preserving meat. Chitosan is produced from chitin deacetylation. Chitin is found in the shells of organisms such as crustaceans, insects, mollusks and shrimp [3].

Chitosan is biomaterials widely used because of their biocompatibility and biodegradability [4]. The presence of free amino groups along the chitosan chains enables this macromolecule to dissolve in a dilute acid solvent because protonation of these groups

The journal homepage www.jpacr.ub.ac.id

p-ISSN : 2302 – 4690 | e-ISSN : 2541 – 0733

takes place [5]. Chitosan shows high antimicrobial activity towards various microorganisms, including fungi and Gram-positive and Gram-negative bacteria [6], [7]. A cationic group in chitosan that binds sialic acid in the phospholipid which can inhibit microbe growth [8]. The unique characteristic of chitosan, the excellent ability to form a film, antibacterial and anti-fungi ability in the food system have been confirmed [6]. Chitosan can be applied to prolong the shelf life of foodstuffs [6], fruits [9]. According to [8] 2.5% chitosan coating can prolong the shelf life of tofu and meatballs, with a value of TPC (Total Plate Count) on three days (room temperature) were 1.56×10^5 dan 2.2×10^5 .

Amino groups in chitosan are protonated at pH less than 6.5, so they can only dissolve in an acid solution with the low solubility at pH of more than 6.5. The chitosan modification is suggested because of the limitation of chitosan's solubility in water. Carboxymethyl chitosan is one of the modifications to increase its solubility [10] because hydrogen bonds between carboxymethyl chitosan and water are more than hydrogen bonds between chitosan and water. The characteristics of carboxymethyl chitosan are low toxicity, good biocompatibility and the ability to form a film. O-carboxymethyl chitosan can increase the antimicrobial activity and can be more potential compared to chitosan [11] because the number of cationic groups in carboxymethyl chitosan is more than chitosan. High solubility so that it is easy to apply and large antimicrobial activity makes carboxymethyl chitosan potentially be used as a preservative. The application of chitosan as a preservative is limited due to its low solubility. The purpose of this study is to synthesize carboxymethyl chitosan and use it as a homemade sausage preservative.

EXPERIMENT

Chemicals and instrumentation

The materials used in this research were shrimp skin, acetate acid (Merck), methanol (Merck), monochloroacetic acid (Merck), sodium hydroxide (Merck), HCl (Merck), NaOCl (technical grade), nutrient agar (NA) (Merck), vinegar (Sukasari), tapioca flour, salt, garlic, white eggs and pepper. Instrumentation used in this research was oven, desiccator, Whatman filter paper, FTIR spectrophotometer (Shimadzu 8400s).

Procedure reaction

Synthesizing chitosan

Shrimp skin powder was deproteinized using 4% NaOH (1:10) at 80 °C for one hour. The deposit obtained was then washed using distilled water and dried at 60 °C. The process was continued with demineralization by reacting the deposit with 1 M HCl (1:15) at room temperature for three hours. The sediment was then washed using distilled water and dried again. The resulted deposit was depigmented using 4% NaOCl (1:10) for one hour. The deposit was strained, washed using distilled water, and dried at 60 °C. The resulted deposit was chitin. The chitin was deacetylated using 60% NaOH at 120 °C for three hours. The sediment was washed again using distilled water and dried. The deposit obtained was now chitosan [7]. Chitosan was analyzed using *Fourier Transform Infrared* (FTIR) spectroscopy. Determination of the degree of deacetylation (DD) chitosan was carried out using an IR spectrum based on the ratio of A_{1655} / A_{3450} using the baseline b proposed by Baxter [12].

Synthesizing carboxymethyl chitosan

Chitosan (1.0 g) was alkalinized using 20 mL 40% NaOH (b/v) for 15 min. It was then added with 7.0 g of monochloroacetic acid into the chitosan solution and stirred for four hours at 80 °C. The mixed solution was then neutralized using 10% acetic acid (v/v) and

added with 70% methanol (v/v). The mixture was strained and washed using methanol. The suspended deposit on the Whatman filter paper was modified chitosan [10]. The deposit was dried at 55 °C. Carboxymethyl chitosan was then analyzed using FITR spectroscopy.

Carboxymethyl chitosan addition in home-made sausage

Five hundred grams of beef was ground along with 25 g of ice. The ground beef was mixed with 50 g of tapioca flour, 2.0 g of salt, 5.0 g of garlic, 20 mL of white eggs and 2.0 g of pepper. The beef and the ingredients were mixed well. They were called the mixture. The mixture was added with carboxymethyl chitosan solution (solvent using vinegar 0.5%).

The mixture was put into the sausage casings. There were knots between one end of the sausage and the next sausage as a place to push the casing or separate sausages. The sausages were steamed for 30 minutes at moderate heat, while the lid was slightly open to avoid the sausage casings from breaking. There was a quality test for the sausages for four consecutive days. The sausages were stored at the room temperature (25 °C) and in a freezer (-16 °C).

Carboxymethyl chitosan added into the sausage production was meant to prolong the shelf life of the sausages. The addition was conducted in various concentrations of 0.5, 1, 1.5% (v/v). The ratio between the beef weight and the carboxymethyl chitosan solution was 1:1, 1:5, 1:3 (g/mL). The optimization trial scheme can be seen in Table 1. The control was sausages containing no carboxymethyl chitosan (sample 10, 11 and 12).

Table 1. The optimization trial scheme of carboxymethyl chitosan as a preservative for sausages

Concentration	The ratio (R) between the beef weight and carboxymethyl chitosan solution (g/mL)		
	1:1	1:3	1:5
0.5 %	Sample 1	Sample 2	Sample 3
1 %	Sample 4	Sample 5	Sample 6
1.5 %	Sample 7	Sample 8	Sample 9
Vinegar 0.5%	Sample 10	Sample 11	Sample 12

Sausage Quality Test

The water content in the sausages

The cup to be used was heated in an oven first for 60 minutes at 125 ± 1 °C, and then it was cooled inside a desiccator for 20 minutes to remove the water vapor and weighed (W_0). Samples were weighed as much as 1-2 g in the dried cup (W_1) and then heated in the oven for 2-4 hours after the oven temperature reached 125 ± 1 °C and cooled inside the desiccator for 30 minutes and weighed (W_2) [13]. The water content was calculated as follows: % of water content = $\frac{W_1 - W_2}{W_1 - W_0} \times 100\%$.

The ash contents

The cup to be used was heated in an oven first for 60 minutes at 550 ± 5 °C, and then it was cooled inside a desiccator and weighed (W_0). Samples were weighed as much as 1-2 g in the dried cup (W_1) and then burned into ashes inside a furnace at 550 ± 5 °C until it became white ashes. The samples were then cooled in the desiccator and weighed (W_2) [13]. The step was repeated until a constant weight was reached. The ash content was calculated as follows: % of ash content = $\frac{W_1 - W_2}{W_1 - W_0} \times 100\%$.

The sausage pH

As many as 5 g of the sausage was added with 45 mL distilled water and crushed well in a mortar. The mixture was then strained using filter paper until the filtrate was obtained. The filtrate was calculated for is pH using pH meter.

Total Plate Count

As many as 1 g of the sausage was weighed and crushed aseptically in the mortar and put into a test tube containing nine mL sterile distilled water and shaken. The sample was taken using a sterile pipette as many as one mL for the next dilution. The solution which was diluted for 10^4 and 10^5 times was taken 0.1 mL using a sterile pipette aseptically, moved into a Petri dish containing NA as the media and flattened. This was then incubated for 48 hours. The total microbes according to *total plate count* method were calculated as follows:

$$\text{Total colony} = \sum \text{Colony} \times \frac{1}{\text{Dilution factor}} \times \frac{1}{10^{-1}}$$

Organoleptic test

The scoring used the scoring test system of sausage organoleptic. The scoring tests range 1-5 in which score five means “like it very much” while score one means “dislike it very much.”

Statistics

Sausage quality test data obtained then statistically tested using analysis of variance for factors concentrations carboxymethyl chitosan, the ratio between carboxymethyl chitosan solution and the weight of the meat, the storage time to determine the effect. The next test used Duncan to know the best value, which was a value showing the lowest at a subset.

RESULT AND DISCUSSION

Chitosan and carboxymethyl chitosan

Synthesizing chitosan began by isolating chitin. Shrimp skin was the basic material for isolating chitin. Deproteinization, demineralization, decolorization were stepped in isolating chitin. The deproteinization step aims to break the chemical bonds between chitin and protein [14]. Demineralization and deproteinization were two specific steps in isolating chitin [15]. The minerals in the shrimp skin were calcium carbonate and calcium phosphate [3]. Calcium compounds reacted with hydrochloric acid and formed calcium chloride and phosphoric acid dissolved in water. Depigmentation was a process to remove the dye pigments in chitin. The pigments were carotenoids, like β -carotene and astaxanthin.

Deacetylation process was then carried out toward the obtained chitin to get chitosan. Deacetylation was a process to remove acetyl groups contained in chitin. Deacetylation was conducted using a high concentration of NaOH solution. Concentrated NaOH is one of the important factors that can influence the deacetylation process of chitin, and the quality of chitosan obtained [3]. During the process, NaOH would break down into Na^+ and OH^- Ions. Hydroxyl ion would charge carbonyl carbon which was electropositive. The breaking of acetyl groups in chitin resulted in chitosan to have positive charges and to be able to dissolve in organic acid solution. The final product from this reaction was chitosan, and the byproduct was sodium acetate. The deacetylation reaction mechanism is shown in Figure 1.

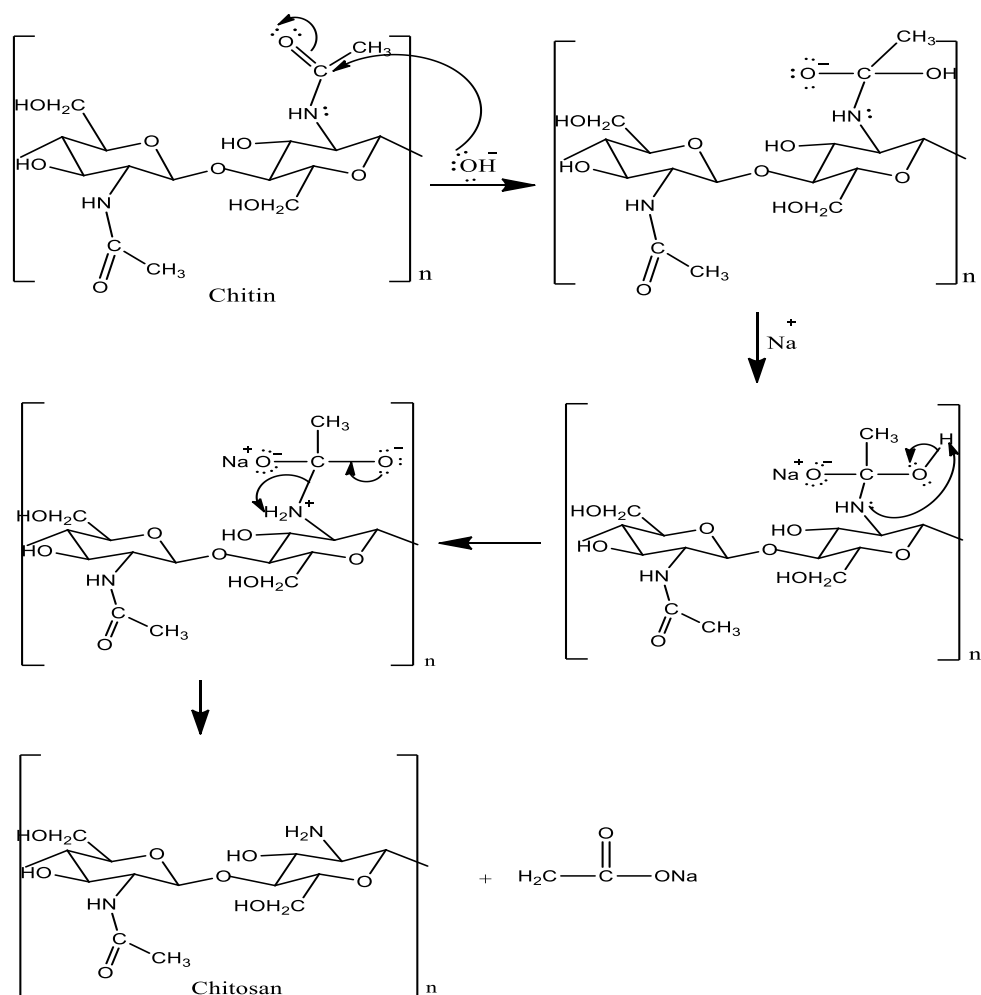


Figure 1. Reaction scheme of chitin deacetylation

Carboxymethyl chitosan was obtained through the process of carboxymethylation of alkaline chitosan with monochloroacetic acid. First, chitosan was alkalinized using NaOH. Chitosan would bind Na^+ from NaOH to create chitosan-Na. When monochloroacetic acid was added, ions exchanging took place. The Na^+ ion would bind Cl^- ion (released by monochloroacetic acid) creating sodium chloride, while chitosan, having released Na^+ ion would be reactive against carboxymethyl groups from monochloroacetic acid, resulting in carboxymethyl chitosan [10]. According to [11], the use of highly concentrated NaOH (>25%) caused carboxymethyl groups (CH_2COOH) from monochloroacetic acid to substitute hydroxyl group (OH^-) and amino group (NH_2) in chitosan.

The characteristics of chitosan and carboxymethyl chitosan

FTIR spectra from chitosan and carboxymethyl chitosan are shown in Figure 2. Figure 2 shows that in the spectrum, chitosan IR showed there was a wave number of 3448 cm^{-1} which was overlapping absorption between $-\text{OH}$ and N-H . At a wave number of 2885 cm^{-1} , there was the stretch vibration of C-H at $-\text{CH}_2$ and $-\text{CH}_3$ [3]. The peak appeared at 1597 cm^{-1} corresponds to an amide II ($-\text{NH}_2$ bending) [3]. The absorption peak at 1087 cm^{-1} showed the vibration range C-O coming from C-O-H or C-O-C . Carboxymethyl chitosan's IR spectrum showing the widened spectrum at the wave of 3425 cm^{-1} was the stretch vibration of $-\text{OH}$.

The widening occurred because there was a carboxymethyl group that formed a dimer with hydrogen binding. Chitosan's IR spectrum was then used in the calculation using *baseline b* to determine the degree of deacetylation (DD) chitosan. Upon the calculation, the value of DD chitosan was 77.03%. The DD of chitosan was influenced by the concentration of NaOH [16].

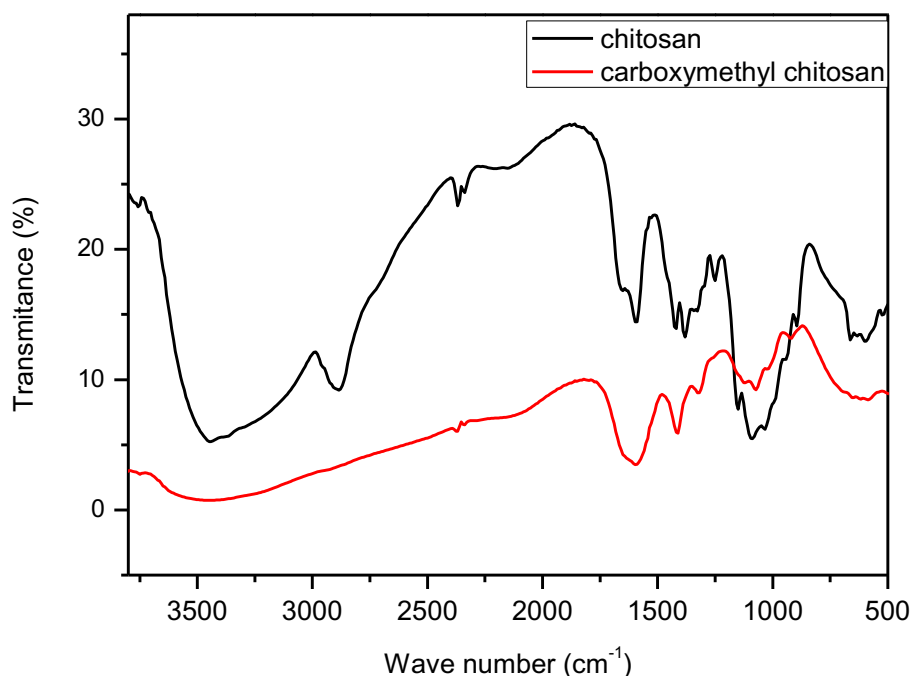


Figure 2. IR spectra from chitosan and carboxymethyl chitosan

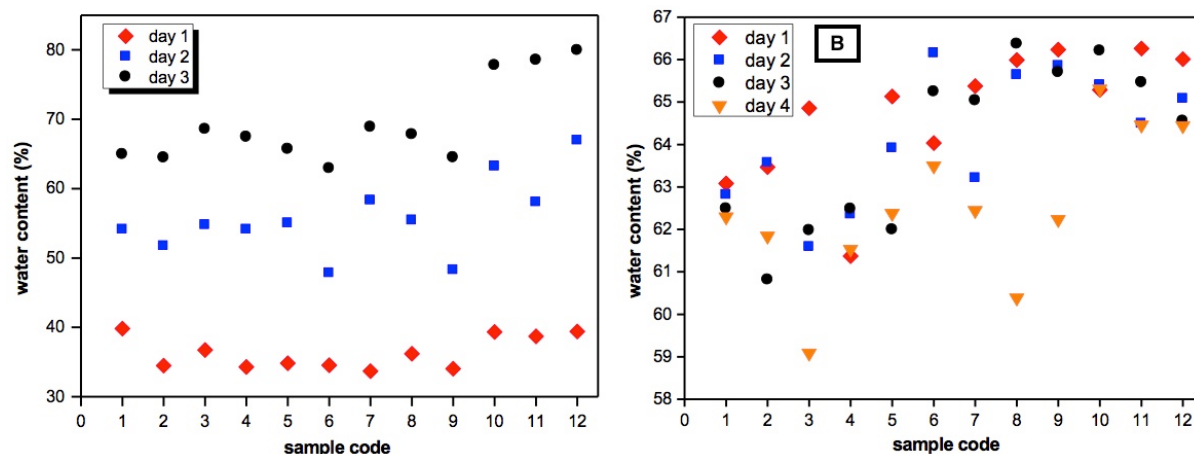
The quality of Sausages Made from Carboxymethyl Chitosan Preservatives

The water content in sausages

The weight loss during heating is used to calculate water content. In principle, the free molecules of water in the samples were vaporized. The samples were then weighed until constant weight reached with the assumption that all water in the samples had been vaporized. The difference in weight between before and after the drying process is the amount of water evaporated. The water content was very significant in determining the foodstuffs' durability because it affected the physical characteristics, chemical changes, enzymatic and microbiological food. The average water content at the room temperature and cold temperature with the addition of carboxymethyl chitosan can be seen in Figure 3.

Figure 3A shows the average of water content in sausages at the room temperature storing from day one until day 3. The water content on day 1 ranged 33.67% - 39.89%, on day 2 was 47.84% - 58.07%, and on day 3 was 62.91% - 68.90%. This value suggested that the water content at the room temperature increased following the length of storing. Sausages added with carboxymethyl chitosan could last until day 2 with the lowest water content which was 47.84%. Those without the addition of carboxymethyl chitosan (sample 10, 11, and 12) had more water content compared to those with the addition. The water content of controlling sausage on day 2 reached 66.94%. The free or available water in food supports microbial growth, and participates in and supports chemical and enzymatic reactions and spoilage processes. The water content in sausages with carboxymethyl chitosan addition had less

water content compared to those without carboxymethyl chitosan addition. This was caused by the thin layer (edible coating) of carboxymethyl chitosan covering the surface of the sausages. This caused microbes difficult to grow. This caused microbes difficult to grow in sausage coated carboxymethyl chitosan. Chitosan could interact with compounds in bacteria's cellular surface, and it was adsorbed to form the layer which inhibited cell transportation, so the cells lacked substances to grow and finally lead them to their death.



Figures 3. The water content in sausages at the room temperature (A) and cold temperature (B)

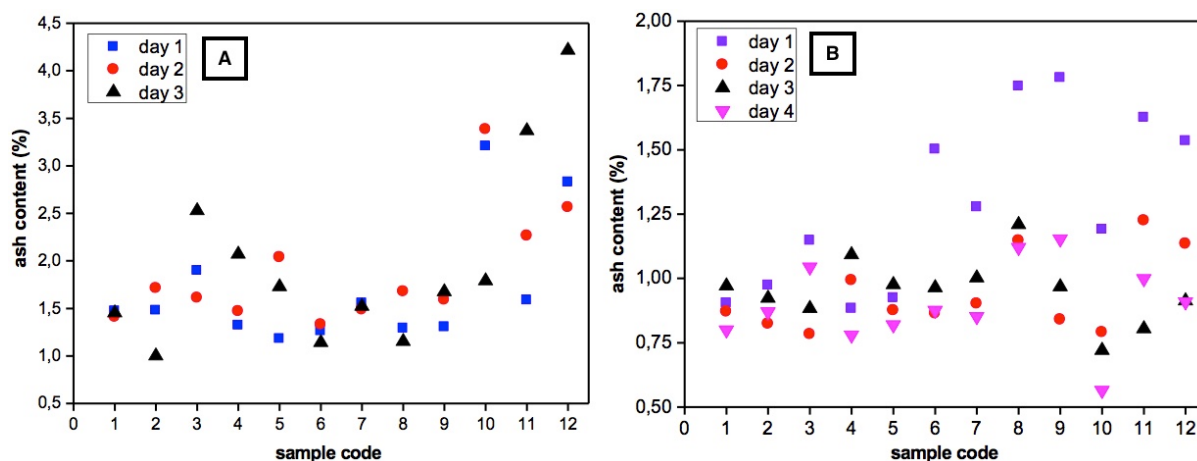
Figure 3B shows the average value of water content in sausages at cold temperature storing. The water content at the cold temperature was lower than that at room temperature. Until day four water content in both sausages with treatment and controlling sausage was still in standard, which was 59.08% - 66.37%. The water content in sausages stored at cold temperature was more durable compared to that stored at room temperature. Storing at cold temperature could inhibit microorganism growth that could damage and increase the water content. The changing in water content occurred because there was a balance between the relative humidity of the material and that of the air, in which the material was stored in the condition with higher air humidity than the material's relative humidity. Thus, the material would absorb the water in the air.

The result of statistical analysis showed that the various concentrations addition, the ratio between carboxymethyl chitosan solution and the meat and storage day as well as the combination directly affected the water content. The next test used Duncan to know the best value. The best value from the room temperature storing was shown by 1% carboxymethyl chitosan with the ratio between the weight of the meat, and the carboxymethyl chitosan solution was 1:3, stored for two days. While at the cold temperature, the best result was shown by 1% carboxymethyl chitosan and the ratio was 1:3, stored for four days.

Sausages' ash content

Ash content was calculated based on the weight of the ash formed during the burning in the furnace until white ashes formed. According to [13], the maximum limit for ash content in meat was 3% (b/b). The average value of ash content at the room temperature and cold temperature can be seen in Figure 4. Figure 4A shows the average values of ash content in sausages at the room temperature on day one until day 3. None of those values exceeded the standard which was 0.99% - 2.53%. The controlling sausages had the increasing ash content

each day until day 3 and exceeded the standard. The ash content on day 3 was 4.22%. This suggested that sausages made from carboxymethyl chitosan preservatives still met the standard [13] until day 2. Figure 4B shows the average values of ash content in sausages at cold storing for four days. The ash in sausages was the minerals contained in meat. Most meat products were rich sources of iron.



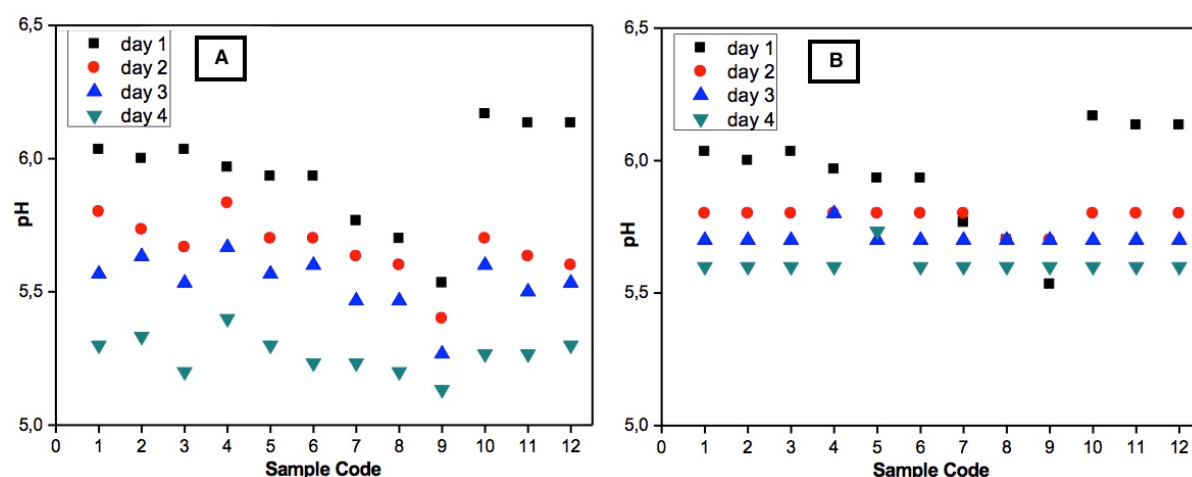
Figures 4. The ash content at the room temperature (A) and cold temperature (B)

The statistical tests showed that sausages stored at room temperature with 1% carboxymethyl chitosan and the ratio between the weight of the meat and carboxymethyl chitosan solution 1:3 was the most optimum in increasing the durability of sausages. This was because it has a low mean value and the ash content which was below the standard [13]. The optimum condition to increase the durability at a cold temperature according to statistical tests was 0.5% concentration of carboxymethyl chitosan solution and the ratio 1:3 and stored for four days. This could be proven by ANOVA test that interaction between various concentrations of carboxymethyl chitosan and the ratio as well as the storing duration directly affected the value of ash content. This was shown by a significant number which was less than 0.05. Duncan tests for sausages at room temperature suggested that the various concentrations did not give a real difference against the ash content because they were at the same subset. However, the storing duration, the ratio and the interaction of various concentrations, the ratio between the weight of the meat and carboxymethyl chitosan solution, and the storing length suggested the other way around.

Sausages' pH

The pH value or the acidity level of the meat would affect the quality of processed meat. The high pH could improve the water holding capacity. The results of 4-day observation are shown in Figures 5. Figures 5 suggested that sausages' pH were getting low day by day. The pH of sausages with treatment was better than those without treatment. Based on the ANOVA test, the storing duration suggested a real difference in sausages' pH. The various concentrations suggested the same thing, while the ratio between the weight of the meat and carboxymethyl chitosan solution 1:1 and 1:5 did not show a real difference against sausages' pH, while the ratio 1:3 suggested the other way around. The next test using Duncan showed the same results; there were differences in the storing duration, the various concentrations. Analytical statistic suggested that the optimum condition to maintain the pH at room

temperature was carboxymethyl chitosan solution at 1% concentration with the ratio 1:3 and the storing duration was two days.



Figures 5. pH of sausages at the room temperature (A) and cold temperature (B)

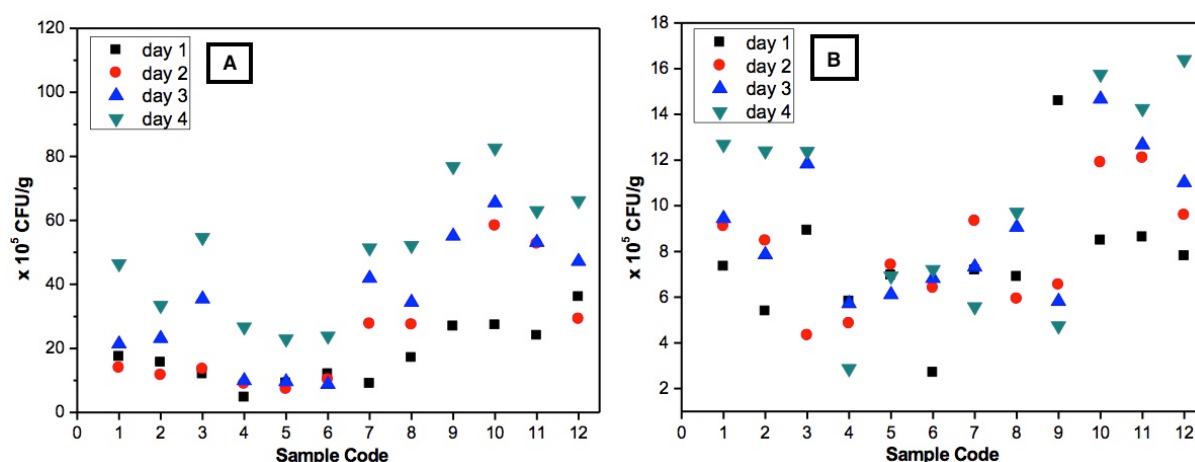
Microbes' total value

TPC method was used to know the number of microbes existing in sausages after carboxymethyl chitosan with different treatment was added. This method used solid media while the final result was a visually observable colony in the form of a number in the colony (CFU) per gram. The result can be seen in Figures 6. Figures 6 suggested that the TPC values were increasing day by day. The TPC values at room temperature were increasing every day. The TPC values sausages stored at cold temperature were increasing every day but not significant compared to those stored at room temperature. The increasing number of microbes during the storing was caused by the high water content and the availability of nutrients for the microbes' growth as well as the environment temperature. The temperature was one of the most critical environmental factors affecting the lives and growth of microorganisms. This was because the cold temperature could inhibit the growth and activities of destructive organisms.

The colony total shown in Figures 6 was then analyzed using ANOVA test, in which the storing duration, concentration, and ratio between the weight of the meat: carboxymethyl chitosan had a direct effect towards microbes' total with a significant number less than 0.05. Duncan test was then conducted to show that there was a real difference. Duncan test suggested that microbes total stored at room temperature was different compared to storing duration, carboxymethyl chitosan concentration, as well as the ratio between the weight of the meat and carboxymethyl chitosan solution. This was shown in their presence at different subsets. The results suggested that the total microbes values of sausages stored at cold temperature were not different on the 3rd and 4th store, but they were different from those stored on the 1st and 2nd. Concentrations at 0.5% and 1% were not different from microbes' total, but they were different from microbes' concentration. The ratios between the weight of the meat with carboxymethyl chitosan were 1:1, 1:2, 1:3 suggested different result with the microbes' total values.

Based on the analytical statistic, the optimum condition to prolong the shelf life of sausages was at room temperature and cold temperature. The optimum condition at room temperature was at 1% concentration of carboxymethyl chitosan, the ratio between the weight

of the meat with carboxymethyl chitosan 1:3 and stored for two days. The analysis data for those stored at cold temperature could be observed at 0.5% concentration, ratio 1:3 and four days storing. This result suggested that sausages added with carboxymethyl chitosan and stored at cold temperature lasted longer than those stored at room temperature.



Figures 6. TPC value at the room temperature (A) and cold temperature (B)

Carboxymethyl chitosan could be used as preservatives because it had antimicrobial characteristics that could inhibit microorganism growth with its two active groups: $-\text{NH}_3^+$ group and $-\text{COO}^-$ group. The $-\text{NH}_3^+$ polycationic groups would interact with negative charges on the microbes' surface causing cells' surface permeability to change, composing cells like protein, amino acid, and glucose to disappear which later would inhibit cells' metabolism growth and lead to the cells' death [7]. Carboxymethyl chitosan could have direct interaction with the cell membrane and could cause cells' protein leakage. In addition to that, carboxymethyl chitosan acted as a chelating agent [11] that would bind essential nutrition so it would inhibit the fungi growth.

Organoleptic values

Sausages' organoleptic values were measured for four consecutive days. The average score for texture, odor, mucus, and the color of sausages stored at room temperature was presented in Table 2. These results were then proven by a statistical test. Based on the results, it was known that various concentrations of carboxymethyl chitosan, the ratio between the weight of the meat and carboxymethyl chitosan solution, and storing duration affected the texture, odor, mucus and color change of sausages. The interaction of various concentrations of carboxymethyl chitosan, the ratio between the weight of the meat and carboxymethyl chitosan solution, and storing duration affected the texture, odor, mucus and color changes of sausages.

Duncan test was conducted to show that there was a real difference. Based on the test, there was an effect of various concentrations of carboxymethyl chitosan, the ratio between the weight of the meat and carboxymethyl chitosan solution, and storing duration made a real difference against the texture, odor, mucus and color changes. The optimum condition to maintain the texture, odor, mucus, and color at room temperature was for sausages with 1% carboxymethyl chitosan solution, the ratio 1:3.

The next organoleptic test was conducted towards sausages stored at cold temperature. The value for four days could be seen from the average scoring for texture, odor, mucus, and color of sausages stored at cold temperature was presented in Table 3.

Table 2. Sausages' organoleptic values at room temperature.

Concent ration	Organ oleptic	The ratio between the weight of the meat and carboxymethyl chitosan											
		(1 : 1)				(1 : 3)				(1 : 5)			
		Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
Carboxy methyl chitosan 0.5%	Texture	4,31	3,133	2,14	1,580	4,400	3,200	2,227	1,640	4,27	3,080	2,213	1,560
	Odor	4,067	2,88	1,56	1,040	4,120	2,880	1,400	1,080	4,133	3,080	1,080	1,000
	Mucus	4,147	3,130	1,800	1,000	4,227	3,133	1,867	1,200	4,270	3,250	1,520	1,120
	Color	4,120	3,027	2,170	1,147	4,167	3,027	1,767	1,120	4,370	3,160	1,933	1,000
Carboxy methyl chitosan 1.0 %	Texture	4,507	3,193	2,387	1,600	4,493	3,213	2,227	1,593	4,480	3,267	2,200	1,613
	Odor	4,053	3,027	1,133	1,000	4,067	3,027	1,940	1,080	4,040	3,053	1,940	1,000
	Mucus	4,147	3,107	2,027	1,333	4,227	3,227	1,667	1,250	4,400	3,080	1,200	1,040
	Color	4,327	3,173	2,073	1,133	4,307	3,787	1,747	1,160	4,293	3,853	1,680	1,120
Carboxy methyl chitosan 1.5%	Texture	4,440	3,120	2,280	1,600	4,400	3,147	2,293	1,580	4,520	3,280	2,213	1,593
	Odor	4,080	3,080	1,480	1,040	4,040	2,800	1,280	1,080	4,160	3,027	1,213	1,013
	Mucus	4,320	3,120	2,080	1,120	4,240	3,160	3,040	1,240	4,160	3,040	2,000	1,080
	Color	4,253	3,133	2,067	1,373	4,320	3,253	1,893	1,267	4,227	2,947	1,737	1,120
Vinegar 0.5%	Texture	4,080	2,253	1,513	1,200	4,240	2,160	1,893	1,200	4,173	2,120	1,960	1,240
	Odor	4,067	3,053	1,347	1,013	4,093	2,733	1,307	1,040	4,133	3,013	1,227	1,013
	Mucus	4,227	2,867	1,213	1,013	4,227	2,853	1,240	1,013	4,240	2,760	1,120	1,000
	Color	4,213	3,187	1,800	1,080	4,200	3,120	2,080	1,080	4,160	3,147	1,560	1,067

This showed that until day 4, the public still liked the sausages' condition. These results were then proven using a statistical test. The analysis suggested that various concentrations of carboxymethyl chitosan, the ratio between the weight of the meat and carboxymethyl chitosan solution, and storing duration made a real difference against the mucus and color changes of sausages, but they did not affect against the texture and aroma directly. The interaction of various concentrations of carboxymethyl chitosan, the ratio between the weight of the meat and carboxymethyl chitosan solution, and storing duration also gave a real effect against the mucus and color changes in sausages. The next Duncan test was to show the presence of a real difference. The test suggested that various concentrations of carboxymethyl chitosan, the ratio between the weight of the meat and carboxymethyl chitosan solution, and storing duration made a real difference against the mucus and color changes of sausages because they were at a different subset. The optimum condition to maintain the texture, odor, mucus, and color at cold temperature was by adding 0.5% carboxymethyl chitosan solution with the ratio at 1:3.

The organoleptic values of sausages at room temperature suggested that the longer the storing duration, the lower the values of texture, odor, mucus, and sausages. The low values mean low quality of sausages. On day 1, the public still liked the texture, odor, mucus, and color of the sausages. This was different from sausages stored at room temperature in which the organoleptic value was decreasing although it was still likable until day 4. The appearance was affected by several issues such as storing duration, water content, protein content, and fat level. The high level of water content would cause microbes to grow quickly on the products thus affecting the sausages' appearance. Bacteria growth in food could destroy compounds composing texture (like carbohydrate and protein) and cause products to be soft and decrease nutrition. The presence of microbes on food could cause food damage, change the aroma, color, taste, and reduce nutrition. Sausages stored at cold temperature were

likely to have more durable texture than those kept at room temperature. This was because there was not much increase in water content in sausages stored at a cold temperature, so that microorganism growth was not as much as those stored at room temperature.

Table 3. Sausages' organoleptic values at cold temperature.

Concentration	Organoleptic	The ratio between the weight of the meat and carboxymethyl chitosan											
		(1 : 1)				(1 : 3)				(1 : 5)			
		Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
Carboxy methyl chitosan 0.5%	Texture	4,347	4,227	4,240	4,187	4,347	4,347	3,973	4,00	4,253	4,227	4,000	4,00
	Odor	4,600	4,600	4,480	4,400	4,613	4,600	4,520	4,360	4,573	4,56	4,480	4,440
	Mucus	4,320	4,387	4,440	4,400	4,36	4,440	4,440	4,120	4,320	4,347	4,360	4,333
	Color	4,200	4,120	4,080	3,973	4,173	4,133	4,147	4,147	4,360	4,400	4,280	4,307
Carboxy methyl chitosan 1 %	Texture	4,267	4,280	4,280	4,247	4,267	4,240	4,253	4,173	4,240	4,240	4,200	4,20
	Odor	4,533	4,560	4,520	4,533	4,600	4,573	4,533	4,547	4,560	4,547	4,507	4,520
	Mucus	4,333	4,400	4,413	4,387	4,400	4,440	4,440	4,400	4,360	4,373	4,400	4,393
	Color	4,320	4,373	4,373	4,333	4,227	4,267	4,293	4,320	4,267	4,293	4,280	4,320
Carboxy methyl chitosan 1.5%	Texture	4,340	4,340	4,327	4,280	4,280	4,240	4,267	4,240	4,213	4,240	4,280	4,267
	Odor	4,600	4,600	4,52	4,520	4,560	4,547	4,507	4,480	3,187	4,520	4,480	4,480
	Mucus	4,360	4,347	4,387	4,373	4,360	4,400	4,400	4,387	4,320	4,347	4,360	4,320
	Color	4,200	4,227	4,280	4,267	4,120	4,147	4,200	4,173	4,200	4,240	4,280	4,280
Vinegar 0.5%	Texture	4,253	4,200	4,227	4,133	4,293	4,240	4,213	4,160	4,200	4,200	4,080	4,080
	Odor	4,600	4,560	4,480	4,44	4,600	4,560	4,480	4,440	4,600	4,560	4,480	4,440
	Mucus	4,320	4,360	4,373	4,333	4,373	4,427	4,440	4,373	4,320	4,413	4,427	4,320
	Color	4,173	4,187	4,147	4,133	4,240	4,213	4,240	4,200	4,200	4,213	4,280	4,280

CONCLUSION

Carboxymethyl chitosan is a biodegradable polymer that has great potential as a food preservative due to its antimicrobial activity and non-toxicity. It has been proven that the addition of carboxymethyl chitosan can also help extend the life of sausages. Organoleptic test results showed that the panelists could accept sausages made from preservatives carboxymethyl chitosan in various textures, odors, mucus, and colors. The values of water content, ash content, pH and TPC from sausages made from preservatives carboxymethyl chitosan were varied.

ACKNOWLEDGMENT

The authors gratefully acknowledge financial support from the Ministry of Research, Technology and Higher Education (Indonesia).

REFERENCES

- [1] Alahakoon, A. U., Jayasena, D. D., Ramachandra, S., and Jo, C., *Trends Food Sci. Technol.*, **2015**, 45(1), 37–49.
- [2] Cantwell, M. and Elliott, C., *J. Clin. Nutr. Diet.*, **2017**, 3(4), 1–4.
- [3] Ben Seghir, B. and Benhamza, M. H., *J. Food Meas. Charact.*, **2017**, 11(3), 1137–1147.
- [4] Younes, I., Sellimi, S., Rinaudo, M., Jellouli, K., and Nasri, M., *Int. J. Food Microbiol.*, **2014**, 185, 57–63.
- [5] Aranaz, I., Mengibar, M., Harris, R., Panos, I., Miralles, B., Acosta, N., Galed, G. and Heras, A., *Curr. Chem. Biol.*, **2009**, 3(2), 203–230.
- [6] F. N. Hafdani and N. Sadeghinia, *Int. J. Pharmacol. Pharm. Sci.*, **2011**, 5(2), 46–50.
- [7] M. Kurniasih, Purwati, and R. S. Dewi, "Toxicity tests, antioxidant activity, and

- antimicrobial activity of chitosan,” in *IOP Conference Series: Materials Science and Engineering*, **2018**, 349, 1.
- [8] R. Purba, S. H. Suseno, A. F. Izaki, and S. Muttaqin, *Int. J. Appl. Sci. Technol.*, **2014**, 4(2), 212–217.
- [9] N. Van Toan, T. T. Hanh, P. Vo, and M. Thien, *TOBIOMTJ*, **2013**, 4, 1–5.
- [10] M. Kurniasih, Purwati, D. Hermawan, and M. Zaki, *Malaysian J. Fundam. Appl. Sci.*, **2014**, 10(4), 189–194.
- [11] V. K. Mourya, N. N. Inamdar, and A. Tiwari, *Adv. Mater. Lett.*, **2010**, 1(1), 11–33.
- [12] T. A. Khan, K. K. Peh, and H. S. Ch'ng, *J. Pharm. Pharm. Sci.*, **2002**, 5(3), 205–212.
- [13] Badan Standarisasi Nasional Indonesia, SNI 3820:2015, *Sosis daging*. Indonesia: Badan Standardisasi Nasional, 1995.
- [14] I. Younes and M. Rinaudo, *Mar. Drugs*, **2015**, 13(3), 1133–1174.
- [15] S. O. Majekodunmi, E. O. Olorunsola, and C. C. Uzoaganobi, *Am. J. Polym. Sci.*, **2017**, 7(1), 15–22.
- [16] M. S. Hossain and A. Iqbal, *J. Bangladesh Agril. Univ.*, **2014**, 12(1), 153–160.