The effectiveness of chitosan from shrimp skin as a natural preservative for meatball

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Abstract. Meatball is one of the most favorite processed food in Indonesia. It has delicious taste, tasty gravy, and affordable price. It is also easy to find a meatball stall in Indonesia, including Jember. Preservatives are usually used to maintain the condition of a meatball. Some preservatives, however, contain harmful substances, like formalin and borax. Both formalin and borax are not allowable for consumption since it may harm human’s health. The present study aimed to investigate a main ingredients for natural food preservative, to be further recommended as a preservative for meatball. The food preservative to be investigated in this study is chitosan made from shrimps shell wastes. To answer the research objective, a method used was an experiment of making chitosan solution. The concentration of chitosan created in the study is 1,5%. To test preservative efficacy of using chitosan, three treatments were given, (1) meatballs are coated with 1,5% of chitosan; (2) meatballs are mixed with 1,5% chitosan solution; and (3) meatballs without chitosan. These three treatments were observed and tested using organoleptic testing based on the length of storage, on day 1, 2, 3, and 4 in a room with a certain temperature. The parameters used for testing the preservatives included taste, color, odor, and texture. The study indicated that the 1,5% of chitosan solution was found effective for preserving meatballs naturally when used as coating.

1. Introduction
Meatball is a popular food in Indonesia. It can be found in almost every part of the country. Meatball is usually served with noodle, gravy, added with ketchup, soy sauce, chili sauce, and seasonings. It is favorite due to its delicious and mouthwatering taste, even best when served hot. It also becomes
people’s favorite because of its affordable price. Eating meatball is recommended when it is still in good shape and not stale.

When stored in a long period, meatball’s quality may be reduced, affecting its taste, odor, color, and texture. In order to avoid quality loss when stored, meatballs are usually added with preservatives. The preservatives should not contain non-food safe materials, like formalin and borax which are harmful for health. Therefore, it calls for a need for natural preservatives for meatballs which are unlikely to cause side effects on health.

One of natural preservatives which are food safe is chitosan. It is produced from processing chitin contained in shrimps shell. Shrimps shells contain protein, calcium, and chitin, thus have advantages for health. Shrimp belongs to Crustaceans whose shells contain 25-40% protein, 45-50% calcium carbonate, and 15-30%chitin. Chitosanis derived from chitin polysaccharides, which makes it a natural product [1].

Chitosan is biodegradable and non-toxic [2], thus recommended for preserving food. The reactive amino and hydroxyl groups contained in chitosan are powerful substance for preservative and color stabilizer. Moreover, chitosan can be used as preservative because of its ability to inhibit the growth of putrefactive microorganisms. Chitosan can also function as coating on preserved products, thus reduce contacts with the environment [3]. Furthermore, it also works as an anti-bacterial material for food products [4]. With its anti-microbial characteristic, chitosan is capable of preventing the pathogen bacteria and putrefactive microorganisms, which includes fungi [5].

There are a number of advantages from using chitosan as natural preservative, such us this it has a structure looking like cellulose-tissue as found in fruit and vegetables. Several hypotheses regarding the working mechanism of chitosan as an anti-bacterial states are within the notion that its affinity attribute is very powerful when working with microbe DNA, thus they can be bonded and distract mRNA and protein synthesis [4]. Therefore, it is important to conduct a deeper study regarding the use of chitosan as natural food preservatives. The study aimed to investigate the process of creating chitosan from shrimps shell wastes and its effectiveness as natural preservatives for meatballs.

2. Research method

This is an experimental research was conducted by producing chitosan from chitin contained in shrimps shell wastes. The procedure began with washing the shrimps shell wastes in flowing water. After washed, the clean shrimps shell was drained by drying it under the sun or microwaved until it was totally dry. Then, the dry shrimps shell was is ground using a blender into powder. The shell powder was further processed to demineralize. Demineralization was done to separate the mineral content of the shell, like CaCO₃. This process was done by heating HCl 1 M solution for an hour at 90°C. Once this process was complete, the procedure was continues to deproteinization process by deluting NaOH 3,5% for an hour at 90°C. The next process was deacetylation, the process to remove an acetyl group, using NaOH solution by 50% for an hour at 120 – 140°C. This process later produce chitosan [6],[7],[8].

The effectiveness testing of chitosan as preservatives for meatballs was done at the 1,5% concentration of chitosan solution [9]. Technically, this particular testing was done in three treatments: first, meatballs are mixed with 1,5% of chitosan solution; second, meatballs are coated by 1,5% chitosan; and third, meatballs are not added with chitosan. Furthermore, observation was done to all treatments for four consecutive days. The data obtained from this observation was documented, and organoleptic test was conducted. The documentation was done by observing and capturing every treatment during testing. Then, the organoleptic test was done to get the data if there were any change to taste, color, odor, and texture of meatballs under the three treatments. The whole procedure of producing chitosan can be seen in Figure 1, while the procedure of chitosan testing on meatballs can be observed by Table 1.
Figure 1. The process of making chitosan from shrimp shells

Table 1. Procedure of chitosan testing on meatballs

| Treatment | Description                                                                 | Observation (Days) | Parameter              | Score/Criteria                  |
|-----------|-----------------------------------------------------------------------------|--------------------|------------------------|---------------------------------|
| 1         | Before cooking the meatball dough is mixed with 20 ml of 1.5% chitosan solution, then cooked, drained, and stored at room temperature. | D1,D2,D3,D4        | Taste, color, aroma, texture | 5 (Very good); 4 (Good); 3 (Not good); 2 (Bad); 1 (Very bad) |
| 2         | Meatballs are cooked, drained, and stored at room temperature, without being given chitosan. | D1,D2,D3,D4        | Taste, color, aroma, texture | 5 (Very good); 4 (Good); 3 (Not good); 2 (Bad); 1 (Very bad) |
| 3         | Meatballs are cooked, drained, and stored at room temperature, without being given chitosan. | D1,D2,D3,D4        | Taste, color, aroma, texture | 5 (Very good); 4 (Good); 3 (Not good); 2 (Bad); 1 (Very bad) |

3. Results

In this section, we will describe our findings the effectiveness of the use 1.5% chitosan solution. The study was carried out by doing three treatments firstly, the meatball with no chitosan, secondly the chitosan solution is mixed with meatball, and the last the chitosan solution is coated to the meatball surface. The meatballs were stored in a place with room temperature, and we analyse the change of four indicators, namely taste, color, odor and texture. For detail description of results findings of chitosan preservative on meatballs are presented below.
3.1 First treatment: without chitosan

The first treatment was to observe meatballs without adding chitosan into the dough. The results of the four-day observation can be seen in Table 2, Figure 2, and Figure 3.

Table 2. Result of organoleptic test on meatballs without chitosan

| Days | Meatballs | Taste | Color | Odor | Texture |
|------|-----------|-------|-------|------|---------|
|      |           | 1     | 2     | 3    | 4       | 5     | 1     | 2     | 3    | 4    | 5     |
| D-1  | M1        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M2        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M3        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M4        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M5        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
| D-2  | M1        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M2        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M3        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M4        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M5        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
| D-3  | M1        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M2        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M3        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M4        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M5        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
| D-4  | M1        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M2        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M3        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M4        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |
|      | M5        | ✓     | ✓     | ✓    | ✓      | ✓     | ✓     | ✓     | ✓    | ✓    | ✓     |

Figure 2. Bar chart showing organoleptic test result on meatballs without chitosan
Figure 3. Observation documentation of meatballs without chitosan

3.2 Second treatment: mixed with 1.5% chitosan solution

The second treatment is to test the effectiveness of chitosan on meatballs by mixing 20 ml chitosan solution at 1.5% concentration into meatballs dough. After four consecutive days of observation, the data were obtained, as presented in Table 3, Figure 4, and Figure 5 in the following.

Table 3. Result of organoleptic test on meatballs mixed with 1.5% chitosan solution

| Days | Meatballs | Taste | Color | Odor | Texture |
|------|-----------|-------|-------|------|---------|
| D-1  |           |       |       |      |         |
| M1   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M2   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M3   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M4   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M5   | ✓         | ✓     | ✓     | ✓    | ✓       |
| D-2  |           |       |       |      |         |
| M1   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M2   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M3   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M4   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M5   | ✓         | ✓     | ✓     | ✓    | ✓       |
| D-3  |           |       |       |      |         |
| M1   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M2   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M3   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M4   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M5   | ✓         | ✓     | ✓     | ✓    | ✓       |
| D-4  |           |       |       |      |         |
| M1   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M2   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M3   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M4   | ✓         | ✓     | ✓     | ✓    | ✓       |
| M5   | ✓         | ✓     | ✓     | ✓    | ✓       |
3.3 Third treatment: coated with 1,5% chitosan solution

The third treatment is to test chitosan effectiveness on meatballs coated chitosan solution with 1,5% concentration into meatballs dough. The result of four consecutive days observation can be seen in Table 4, Figure 6 and Figure 7.

**Table 4.** The result of organoleptic test on meatballs coated with 1,5% chitosan solution

| Days | Meatballs | Taste | Color | Odor | Texture |
|------|-----------|-------|-------|------|---------|
|      |           | 1 2   | 3 4   | 5    | 1 2     |
| D-1  | M1        | ✓ ✓   | ✓     | ✓ ✓  | ✓ ✓     |
|      | M2        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
|      | M3        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
|      | M4        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
|      | M5        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
| D-2  | M1        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
|      | M2        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
|      | M3        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
|      | M4        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
|      | M5        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
| D-3  | M1        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
|      | M2        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
|      | M3        | ✓ ✓   | ✓ ✓   | ✓ ✓  | ✓ ✓     |
4. Discussion

Now, we will show the analysis of the effectiveness of the use 1.5% chitosan solution on meatball respected to our results findings. The results show that third treatment of citosan solution is was significantly effective for being preservative for meatballs, namely when we coated the citosan solution on the meatball surface. The followings are the details of discussion.

4.1 First treatment: without chitosan

Based on the observation, meatballs without chitosan started to change in color on day 2, from lighter color on day 1 to darker on day 2. This implies that meatballs started to lose its quality due to change in color, indicating that they got oxidized and probably contaminated by damaging microbes. Afterwards, on day 3, changes were more significant in taste, color, odor,
and texture. Finally, on day 4 changes became more obvious. The meatballs did not taste good, the color turned dark, while their odor smelled like rotted, and the texture became a little stiff. These happened because chitosan as preservatives became ineffective on day 4, therefore more microbes contaminated the meatballs [9].

4.2 Second treatment: mixed with 1.5% chitosan solution
The result of observation on day 1 and 2 indicates no significant change in taste, color, odor, or texture of meatballs. This finding implies that meatballs had not been contaminated by microbes. Further, it implies that the 1.5% chitosan solution added to meatball dough works well [9]. On day 3, changes were identified in color and texture; its light color turned , and its chewy texture turned tender on the third day. This change indicates the process of meatballs being oxidized. Finally on day 4, changes were observed in all parameters, taste, color, odor, and texture of the meatballs. The meatballs tasted less delicious and a little sour, looked darker, smelled bad, and the texture became mushy. These changes implied that chitosan as preservatives became even less effective, therefore more putrefactive microbes came and contaminated the meatballs.

4.3 Third treatment: coated with 1.5% chitosan solution
Based on the result identified on day 1, 2, and 3, there were no significant changes in taste, color, odor, and texture of meatballs coated with 1.5% chitosan solution. This result indicates that meatballs remained in the same quality as no damaging microbes contaminated them. Furthermore, the meatballs remained good until three days because the 1.5% chitosan solution used as coating worked well. Such chitosan coating on meatballs were found more effective in preventing microbes’ contamination[7]. Then, on day 4, the quality started to decrease, like there were changes in taste, color, odor, and texture. The taste of the meatballs became less delicious and a little sour. Their color turned darker, the meatballs started to smell bad, and the texture became tender even mushy. These changes emerged due to the ineffective preservatives on day 4, thus damaging microbes started to contaminate the meatballs.

5. Conclusion
The study is briefly concluded in three points, as follows: 1) Chitosan can be produced by processing shrimps shell through demineralization, deproteinization, and deacetylation. 2) Chitosan can be made as natural preservatives for meatballs. It has been proven through the study that meatballs when added with chitosan with 1.5% concentration can maintain the quality longer than when not added with it. 3) Chitosan is more effective as natural preservatives for meatballs when it is as a coating.

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