Increasing the quality of anchovy (Stelophorus sp.) with additional concentration of chitosan

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Abstract. Anchovy is a main capture fishery commodity in North Maluku. Currently, the processing activity of anchovy is relatively simple and traditional, consequently, causing anchovy quality to be easily degraded. One solution to overcome the problem is through the addition of chitosan concentration. This study aimed to determine effect of concentration addition of chitosan on quality of water content, protein, fat, carbohydrates, organoleptic and Total Plate Count (TPC) test. Method used in this research was the analysis of water quality test, protein content, fat content, carbohydrate content, calorie content, organoleptic test and TPC test. The study results showed that the highest water content value was 13.65% at 0.1% concentration, the highest protein value was 61.16% at 0.5% concentration, the highest ash content value was 16.5% at 1% concentration, the highest fat value was 3.99% at 1% concentration, and the highest carbohydrate content found was 11.94% at 0.1% concentration, and the highest calorific value was 2.51% at 1% concentration. Meanwhile, average organoleptic value of the highest appearance was 6.87% at a 0.3% concentration, the highest odor was 7.55% at a 0.3% concentration, the highest texture was 7.45% at a 0.1% concentration and color was 6.25% at a 0.3% concentration. Results of the TPC test showed that anchovy with chitosan treatment and without chitosan during storage changed for all treatments. The average TPC value at all concentrations (0.1%, 0.3% and 0.5%) had the lowest TPC value compared to the control.

1. Introduction
Fish is an important source of food needed by human body for the reason that it has high nutritional value. The chemical composition of fish consists of 65-80% water content, 17-22% protein content, 0.5-2% fat content and 1-2% ash. The chemical composition shows that fish is a highly perishable food. Fish have potential to experience three types of quality degradation, namely microorganism damage, chemical damage, and physical damage [1], [2], [3]. The factors that determine the quality of fish are handling and processing. Quality of handling and processing greatly determines the quality of processed products because quality influences their selling price [4], [2], [3].

Fish processing is a solution to maintain nutritional value. Quality of processed products can be seen from their appearance, taste, smell, and texture which are attractive factors for consumers [1], [4], [2], [3]. Method of processing anchovy at Toniku Village begins with anchovy fishing using a fish chart and followed by fish landing. Subsequently, quality of the anchovy will be sorted by size and type, and then proceeds to drying process and ends with packing stage. In the processing carried out, several important stages are ignored, these include sorting anchovy based on level of freshness, washing with fresh water, and or draining fish before drying. These issues contribute to the relatively low quality of anchovy [2], [3].
One solution to improve the quality of anchovy is by adding chitosan. Chitosan is an organic chemical compound that is safe and has anti-bacterial and fungal properties, accordingly it is safe to be used for improving quality of fish processing products. Chitosan is a chitin derivative product with chemical formula N-acetyl-D-glucosamine, and is a cationic polymer having 2000-3000 monomers, non-toxic, and has a molecular weight of about 800 kD or kilo Dalton (g/mol) [5], [6], [7]. This study aimed to determine effects of increasing chitosan concentration on quality of water content, protein, fat, carbohydrates, calories, organoleptic and Total Plate Count (TPC) in anchovy processing.

2. Research methods
Sampling collection was carried out at Toniku Village, West Halmahera District. Chemical and microbiological analysis was carried out at the Fisheries Product Microbiology Laboratory in Gadjah Mada University, Yogyakarta. This research was conducted from September 2020 to May 2021.

2.1 Research procedure
2.1.1. Anchovy dipping in chitosan solution
First step was preparation of chitosan solution using concentrations of 0.1%, 0.3%, 0.5% and 1%. Anchovies were dipped in a 1% solution of chitosan and acetic acid which had been prepared with an immersion time of 0.6 – 1.5 minutes. After that, the anchovies were dried.

2.1.2. Drying after dipping process
After the dipping process, anchovies were dried in the sun for approximately 8 hours to 1 day long. After drying, the fish were packed in sample bags made of Polyethylene with a thickness of 0.025 mm.

2.1.3. Chemical test
a. Water activity (a_w) analysis
The moisture content test was carried out by weighing 2 grams of sample, which was put it in a porcelain cup and then dried in an oven at a temperature of 95-100ºC for 5 hours. The results of the water content test were calculated by the formula.

\[
\text{Water Content} = \frac{(B-C)}{(B-A)} \times 100 \%
\]

Where: A = Weight of the cup; B = Weight of the cup + initial sample; C = Weight of cup + dry sample

b. Ash content analysis
A cup was dried in the oven for 24 hours, weigh the cup (A). Then the sample weighed 3 kg and put into a cup (B). The cup was heated at 600ºC for 4 hours. The cup was cooled in a desiccator for 1 hour and then the cup was weighed (C). The results of the ash content test were calculated by the formula.

\[
\% \text{ Ash Content} = \frac{C-A}{B-A} \times 100\%
\]

Where: A = weight of empty cup (g); B = weight of the cup that already contains the sample; (g); C = weight of the cup containing the ashes sample (g)

c. Protein analysis
Working procedure of the fat content test was carried out using the Soxhlet method. After weighing 2 grams of the sample into a beaker, 30 ml of 10 N HCl and 20 ml of water was added. After boiled for 2 minutes, the sample was filtered in a hot state. The data was calculated using the formula:

\[
\%N = \frac{(A-B)}{NHCLx14} \times 100\%
\]

Where: A= Sample titration; B= Blank titration; Conversion Factor = 6.25
d. Fat analysis

The sample was then mixed with 50 ml of hexane extract and sterile water for 2-3 hours at a temperature of 80°C. Hexane extract was distilled and fat extract was dried at a temperature of 100-1050°C, followed by cooling and weighing processes of the sample. The data was calculated using the formula:

\[ \text{Fat level} = \frac{W_1 - W_x}{W_2} \times 100\% \]

Where: \( W \) = weight of empty flask (g); \( W_1 \) = pumpkin weight + fat extract (g); \( W_2 \) = sample weight (g)

c. Carbohydrate and calorie analysis

Carbohydrate test was calculated using the following formula:

\[ \text{Carbohydrate level (\% KH)} = 100\% - (A + B + C + D + E) \]

Where: \( A \) = Water content; \( B \) = Ash content; \( C \) = Protein content; \( D \) = Calorie level

2.1.4. Organoleptic test analysis

The organoleptic test parameters carried out for this study were aroma, taste, texture, and color. The test results were calculated by the following formula:

\[ P \left( X - 1.95 \times \frac{S}{\sqrt{n}} - \mu - X + 1.95 \times \frac{S}{\sqrt{n}} \right) = 95\% \]

Where: \( P \) = Average quality value interval; \( X \) = Average quality value; \( S \) = Standard deviation of quality values; \( N \) = Number of panelists; and 1.95 = Coefficient of standard deviation at 95% level.

2.1.5 Total plate count test (TPC)

TPC method was used to determine the total number of aerobic and anaerobic microorganisms (psychrophilic, mesophilic and thermophilic) in fishery products (SNI 01-2332.3-2006). Isolation stage was carried out using the pour method, in which 0.1 ml for each dilution was poured into a cup before being given gel nutrient media. Isolation of microbes from samples was carried out in duplicate with dilution factors of 10-1, 10-3 and 10-5. Subsequently, the samples were isolated and incubated at room temperature 25-27°C for 24 hours. The observation stage of microbial colonies growing in each sample plate was calculated using a colony counter, the number of microbial colonies analyzed was in the range of 30-300 colonies CFU/g [2], [3]. If the number of colonies per sample is more than 300 CFU/g, it is categorized as turbid metric (TBUD). The number of CFU/gram for each sample was analyzed or calculated using the formula [2]:

\[ CFU = \frac{\text{Number of Colonies}}{\text{Diluent Factor}} \times \frac{1}{\text{Diluent Factor}} \]

3. Data analysis

The calculated data were analyzed in a completely randomized design (CRD), with 4 variations in the concentration of chitosan solution consisting of A (control), B (0.1%), C (0.3%), D (0.5%) and E (1%). The research data was processed using analysis of variance (ANOVA) and if the results were found to be significantly different, a further test (Duncan) would be conducted.

4. Result and discussion

4.1. Anchovy chemical analysis results

The results of chemical analysis of anchovy for water content showed that the highest value was 13.9% at a concentration of 0.5%, and the lowest was 1.50% at a concentration of 1%. The results of ANOVA showed that the use of chitosan in dried anchovies had a significant effect on the average...
The value of water content. This can be seen from the value of $F_{\text{count}} (663.365) > F_{\text{table}} (5.192186)$. The average results of the proximate test on anchovies can be seen in Table 1.

The results of water content analysis in anchovies at concentrations of 0.1%, 0.3%, 0.5% and 1% showed a decrease, when compared to the control. The results indicated that there was an effect of water content on the addition of chitosan. According to [8], [9], the use of chitosan can reduce water content faster than without chitosan, water content is a factor that has an influence on the durability of a food. The results of the analysis of the highest ash content was 16.48% at a concentration of 1%, and the lowest was 15.5% at a concentration of 0.5% chitosan, which decreased by 15.545. These results indicated that the average value of each treatment had increased. In other words, the use of chitosan in anchovies had an effect on the average value of ash content. This result can be seen from the $F_{\text{count}}$ value which is greater than $F_{\text{table}} (19.579) > (51.92186)$.

### Table 1. Chemical test of anchovy with concentration of chitosan

| Concentration | Chemical variables |
|---------------|--------------------|
|               | Water (%) | Ash (%) | Protein (%) | Fat (%) | Carbohydrate (%) | Calorie (%) |
| Control       | 16.84*    | 14.38*  | 52.24*      | 3.09*   | 13.46*           | 26.07a      |
| 0.1           | 13.66b    | 15.9b   | 55.19b      | 3.32b   | 11.94b           | 25.79b      |
| 0.3           | 13.51b    | 16.22c  | 53.96c      | 3.58b   | 6.38b            | 25.69c      |
| 0.5           | 13.89c    | 15.55b  | 61.16c      | 3.59b   | 5.82c            | 25.07d      |
| 1             | 13.50c    | 16.84c  | 59.57d      | 3.99c   | 6.47d            | 25.06b      |

Note: The data was the average of two repetitions of numbers followed by the same letter showing no significant effect and significant effect on other letters.

The use of chitosan in anchovies increased value of ash content faster than without chitosan, besides that value of ash content with chitosan was probably due to the chitosan solution used still has a fairly high mineral content because the demineralization process was not perfect [10], [8]. According to [11], [12] ash/mineral content in fish also depends on ecological factors such as season, culture or growing location, and amount of available nutrients, explaining that the main constituent components of bone are minerals.

Data from analysis of protein content showed that the highest value was 61.16% at a concentration of 0.5% and the lowest value was 55.19% at a concentration of 0.1%. These results indicated that the average total protein value of each treatment had increased. This increase occurred since amino acids in anchovies such as isoleucine, leucine and valine are not soluble in water, so that when water content decreases the protein does not decrease.

In addition, the protein content will increase along with reduced water content which will result in an increase in protein content in a material [9], [13], [14]. According to [15], the higher percentage of protein value, the better quality of the fish for consumption.

The analysis results also showed that the highest fat content was 3.98% at a concentration of 1% and the lowest was 3.32% at a concentration of 0.1%. The results of ANOVA showed that the average value of fat content in each treatment of 0.1%, 0.3%, 0.5% and 1% increased, which can be seen from the value of $F_{\text{count}} (24.427) > F_{\text{table}} (5.192186)$. According to [16], [17], [18] this is due to coating with chitosan solution can increase the value of fat content in fish.

Carbohydrates are a source of calories for humans. Carbohydrates can be obtained from animal and vegetable sources. Carbohydrates obtained from animal sources consist of glycogen [6]. The amount of carbohydrates in fish meat is very small, ranging from 0.05-0.86% [17], [17]. Data resulted from analysis showed that the highest carbohydrate content was 6.7% at a concentration of 0.3%, and the lowest was 5.82% at a concentration of 0.5%. The results of ANOVA showed that the average value
of carbohydrates in each treatment was 0.1%, 0.3%, 0.5% and 1% decreased. This can be seen from the value of $F_{count}$ carbohydrates ($64.823 > F_{table}$ (5.192186)). The decrease in carbohydrate value is due to the increase in protein value in anchovies, when chitosan was added, besides the use of chitosan could reduce carbohydrates compared to no chitosan [19], [20], [21], [22].

The highest calorie analysis was 251.78% at 0.1% concentration, and the lowest was 2.49% at 0.3% concentration. These results indicated that the caloric value in all treatments 0.1%, 0.3% and 0.5%, increased while the 1% treatment decreased. This can be seen from the value of $F_{count}$ (10.740) > $F_{table}$ (5.192186). According to [19], [20], [8] the use of chitosan can reduce calories compared to without chitosan.

4.2. Results of organoleptic quality analysis

The results of the organoleptic test showed that, the highest appearance value was 6.87 at a concentration of 0.3%, and the lowest was 6.0 at a concentration of 0.5%, the highest odor value was 7.55 at a concentration of 0.3%, and the lowest was 7.3 at a concentration of 0.5%, the highest texture value was 7.49 at a concentration of 0.5%, concentration of 0.1%, and the lowest was 6.61% at a concentration of 0.5%. Meanwhile, the value of the highest level of color preference is 6.29 at a concentration of 0.3%, and the lowest is 5.61% at a concentration of 0.1%. The results of this organoleptic test indicated that the level of preference for the appearance is like, the level of liking for smell is very like, the result for texture is very like, and the result of the color test on the average level of liking is rather like. The results of the organoleptic test are simple and fast, because the results can be known immediately so it is very popular to be used to test the deterioration of fish quality [21], [22], [31]. The organoleptic values of anchovy with different concentrations of chitosan can be seen in Table 2.

![Table 2](image)

| Concentration (%) | Appearance | Smell | Texture | Color |
|-------------------|------------|-------|---------|-------|
| 0.1               | 6.13       | 7.52  | 7.45    | 5.61  |
| 0.3               | 6.87       | 7.55  | 7.39    | 6.29  |
| 0.5               | 6.03       | 7.32  | 6.61    | 5.71  |
| 1                 | 5.89       | 7.22  | 6.57    | 5.45  |

4.3. TPC value

The results of the TPC test showed that anchovy with chitosan treatment and without chitosan during storage changed for all treatments. The average TPC value at all concentrations (0.1%, 0.3% and 0.5%) had the lowest TPC value compared to the control. The TPC value in the control treatment was $8.4 \times 10^2$ CFU/g. While the TPC value at 0.1% concentration is $1.5 \times 10^3$ CFU/g, 0.3% concentration is $1.8 \times 10^3$ CFU/g, 0.5% and 1% concentration is $1.9 \times 10^3$ CFU/g. The low value of TPC is because chitosan has antibacterial power, specifically, it can inhibit microbial growth [28], [29], [30], [31].

![Table 3](image)

| Concentration (%) | Average TPC |
|-------------------|-------------|
|                   | $10^{-1}$  | $10^{-2}$  | $10^{-3}$ |
| 0.1%              | 2.2x10^3   | 1.3x10^3   | 1x10^3    | 1.5x10^3    |
| 0.3%              | 3x10^3     | 1.2x10^3   | 1.2x10^3  | 1.8x10^3    |
| 0.5%              | 2.4x10^3   | 2.2x10^3   | 1.2x10^3  | 1.9x10^3    |
| 1%                | 2.4x10^3   | 2.2x10^3   | 1.2x10^3  | 1.9x10^3    |
| control           | 1.3x10^2   | 1.2x10^3   | 1.2x10^3  | 8.4 x10^2   |
A thin layer (edible coating) of chitosan that covers the entire surface of the fish will inhibit the entry of $O_2$ and water through the surface of the fish's body and can make it difficult for microbes to grow [23]. The total number of bacteria is highly dependent on high value of water activity, besides fish meat is a product that is easily and quickly decomposed if it is not directly processed [28], [29], [30]. The results of the TPC test of freshness can be seen from the number of bacteria that developed in fish. The value of the TPC calculation results can be seen in Table 3.

5. Conclusion
The study concluded that the addition of chitosan could improve quality of anchovy, which could be seen in the test values. Study results showed that best chemical values found were achieved at a concentration of 0.5%, the highest organoleptic values were at concentrations of 0.3% and 0.5%, and TPC values at all concentrations were lower than control.

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