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The Optimization of Chitosan Coating and Drying Temperature on the Quality of Sumbawanese dried fish using Response Surface Methodology

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Abstract—Sumbawanese dried fish is a traditional tamarind-salted dried fish derived from *Sardinella lemuru*. This study aimed to determine the chemical properties (moisture and crude protein content), microbial properties, and the organoleptic properties of the product by optimizing its chitosan coating and drying temperature. The analysis using Response Surface Methodology showed that the optimum chitosan concentration and temperature was acquired at 3.51% and 60.93˚C, respectively. These optimum conditions resulted in moisture content of 23.47% and protein content of 49.91% of the product. The number of microbial colonies in all treatments complied with The National Standard of Indonesia (SNI 8273 – 2016), however, the fungal growth was found on the samples dried at 53˚C and 55˚C on the 20th day. The organoleptic test showed that fish dried at 60°C with the addition of 4.62% chitosan was the most prefered based on the taste and aroma properties, whereas the best texture was obtained by drying the fish at 53˚C with the addition of 4.62% chitosan.

***Keywords*— Sumbawanese dried fish, *Sardinella lemuru*, chitosan, drying temperature, Response Surface Methodology.**

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INTRODUCTION

Sumbawa Island, located in West Nusa Tenggara province, has an abundant sources of fisheries products which produced 665.030,35 tons of fish in 2021, including *Sardinella lemuru* (Sumbawa Regional Development Agency 2021). The production of this fish in Sumbawa Regency has increased from 55,443.80 tons in 2017 to 57,051.45 tons in 2019 (Central Bureau of Statistics 2019). *Sardinella lemuru* (hereinafter lemuru), a type of small pelagic fish-eating plankton, is an important fish in Sumbawanese traditional cuisine. Lemuru has been used to produce "ikan bage", dried fish made by marinating the fish in salt and tamarind paste mixture as preservatives and dried for further storage (Sawaluddin 2016). The fish is dried for only 5 hours which is shorter compared to the drying time of the regular salted fish in the market. The short drying time is required to maintain the light and soft texture of the fresh fish (Nahardiansyah 2018).

Nahardiansyah (2018) also reported that the temperature and drying time of "ikan bage" affected the protein content, moisture content, and fat content of the product. The study also concluded that the best "ikan bage" is generated by dried the fish at 60˚C for 4 hours which resulted in 41.71%, 38.54%, and 5.72% of moisture content, protein content, and fat content, respectively. However, due to its moisten texture this fish product has a short-time shelf life. Therefore, a new method is required to extend the shelf life while maintaining the moist characteristic of the product. Thus, one of the preservation methods that can be explored is by using chitosan as a coating media.

Chitosan is an organic compound derived from chitin that is commonly used as a coating material for food products to inhibit the growth of destructive microorganisms like pathogenic bacteria and putrefactive microorganisms including fungi, gram-positive bacteria, and gram-negative bacteria by minimizing the reaction between the product and the environment through the coating (Holipah et al. 2010; Arifin and Nugroho 2016; Darmayanti et al. 2016).

According to the research conducted by Gafur et al. (2017), the addition of chitosan affects the moisture, protein, and fat content of salted fish. The best treatment was obtained from the addition of chitosan 4% in *Rastrelliger* sp which resulted in 9.545%, 48.905%, and 1.543% of moisture content, protein content, and fat content of the fish.

The use of chitosan will only protect the material physically and chemically by creating thin film on the surface of the fish to prevent gaseous and moisture circulation into the fish and microbial disturbances (Pamekas 2007). Thus, a combination between the chitosan coating and drying are required to decrease the mositure content of the fish which will eventually prolong the fish shelf life. Therefore this study aimed to determine the optimum drying temperature and chitosan concentration to produced "ikan bage"

MATERIALS AND METHODS

Materials

This study used lemuru fish with an average weight of 25-grams each collected from Ai' Bari Hamlet, Kukin Village, North Moyo District, and chitosan (Bio Chitosan). Other ingredients used are fine table salt (Refina), local seedless tamarind (bage), aquadest, K_2SO_4 (Merck), $CuSO_4$ (Merck), H_2SO_4 (Merck), H_3BO_3 3% (Merck), NaOH 40% (Merck), H_2SO_4 0.1 N (Merck), HCl (Sigma Aldrich) and chloroform (Merck).

Research Methods

This research was conducted from February to June 2019 at the Integrated Food Laboratory, Faculty of Agricultural Technology, Sumbawa University of Technology (sample preparation and sensory analysis), the Microbiology Laboratory (microbial analysis), and the Laboratory of Nutrition and Animal Feed Sciences, Faculty of Animal Husbandry, University of Mataram (chemical analysis).

This study was designed using the Randomized Group Design (RGD) consisted of two factors, drying temperatures (55-65°C) and chitosan concentrations (1%-4%) with the total of 13 experimental units. The data then analyzed by the Response Surface Methodology optimization technique using Minitab 21 to further determine the optimal point (Box and Draper 2007).

Sample Preparation

Lemuru fish (300 g) was cleaned and gutted then soaked in 150 g of salt dissolved in 500 ml of water for 15 minutes After that, the fish then soaked in chitosan and tamarind solution obtained by extracting 150 g tamarind in 500 ml water and adding chitosan with different concentration. The fish then dried using an oven at 55°C to 65°C for 4 hours.

Sample Analysis

Moisture content

Measurement of moisture content was conducted using the gravimetric method. A total of 1 g of the "ikan bage" flesh was dried in the oven at 105°C for 8-12 hours until constant weight was obtained (AOAC, 2010). Moisture content of the sample is calculated by the following formula:

$$\text{Moisture content (\%)} = \frac{\textit{Initial Weight - Final weight}}{\textit{Initial Sample Weight}}\ x\ 100\% \qquad (1)$$

Protein Content

Protein content was measured using the Kjeldahl method. The fish was weighed for 0.25 g and added into Kjeldahl flask then 1.5 g of $CuSO_4$: K_2SO_4 (1:7) and concentrated H_2SO_4 (7.5 ml) were added, concecutively. The sample then destructed in a fume hood around 45 minutes until the solution emmit clear and smokeless appearance. The solution was diluted in 100 ml of aquadest and 50 ml NaOH 40% then distilled with 25 ml of H_3BO_3 3% in 250 ml erlenmeyer. The distillation process was dismissed when the solution has reduced to 100 ml. The distillate was immediately titrated with a standard solution of H_2SO_4 0.1 N, and the titration finalized when the colour of the solution turned pink (AOAC, 2010). The value of crude protein (%) is calculated using the formula:

$$\frac{\text{ml titration x 0,1 x 0,014 x 6,25}}{\text{Sample Weight}} \text{x } 100\% \qquad (2)$$

Where:
0.1 = Normality H_2SO_4
0.014 = Atomic weights of Nitrogen
6.25 = Protein conversion factor

Total Plate Count (TPC)

Microbiological testing of "ikan bage" was carried out using the TPC (Total Plate Count) method. The fish sample (1 g) was crushed in stomacher and diluted into 9 ml of NaCl (10^1 dilution). Then 1 ml of the diluted solution was poured into the petri dish contained 15 ml of diluted plate count agar and hardened. After that, the dish was incubated at 35 °C for 24 - 48 hours. The total of microbial growth were measured using hand tally counter with the count range from 25 to 250 colonies (Sukmawati 2018; Madina 2018) using formula as follow:

$$\text{NoC} = \text{Number of captive colonies x} \frac{1}{\text{Dilution factor}} \qquad (3)$$

Sensory Evaluation

Sensory evaluation was carried out using hedonic test by 25 untrained panelists (Meilgaard et al. 2000; Tarwendah 2017). This test determined the preference of consumers to the color, aroma, taste, and texture of the final product using 1-5 hedonic scale, where 1=dislike extremely, 2=dislike, 3= neither like nor dislike, 4=like, 5=like extremely.

RESULTS AND DISCUSSIONS

The analysis of the data from this study showed that the temperatures and chitosan concentrations discussed bellow were the most optimum in this study. Therefore, only these optimum temperatures and concentrations will be dicussed further in this study.

Moisture Content

Moisture content indicates the amount of bound water in the food that can be evaporated (Yuarni, et al. 2015). The moisture content of "ikan bage" in this study complied with the moisture content of salted fish set by the Indonesian National Standards Agency (SNI) 8273 -2016 which is a maximum of 40%.

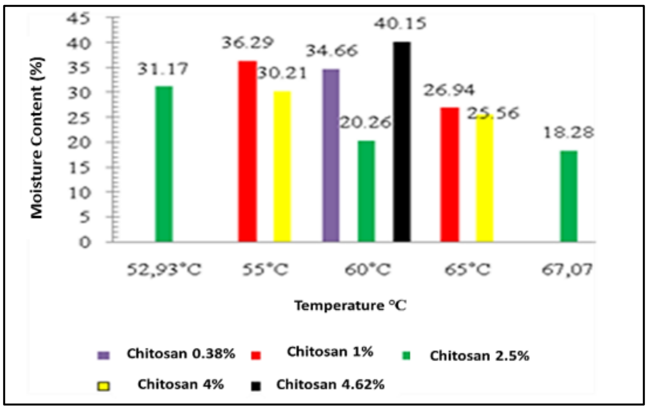
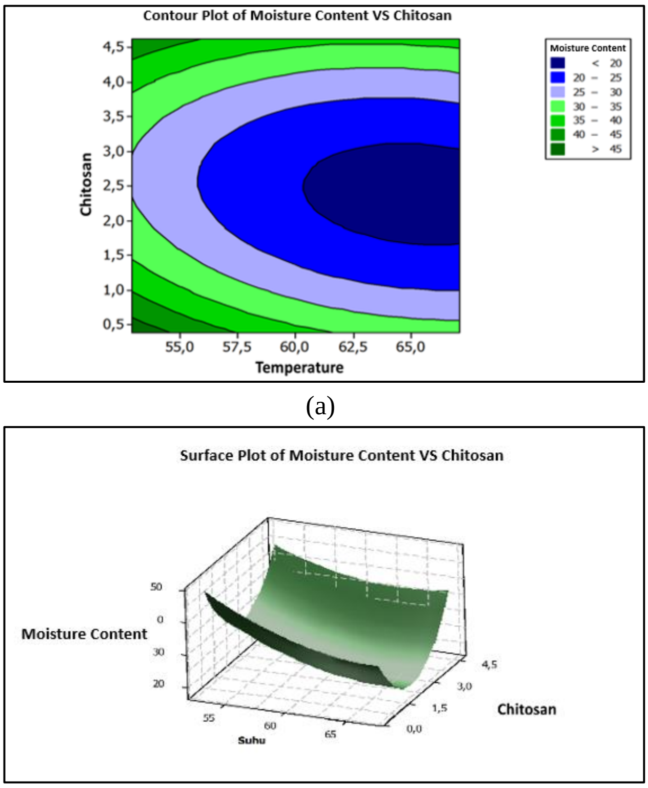


Fig. 1 The moisture content of "ikan bage" analyzed using RSM

The lowest moisture content (18.28%) was measured from the fish dried at 67.07°C and addition 2.5% of chitosan, whereas, the highest moisture (40.15%) content was obtained from the fish dried at 60 °C and the addition of chitosan 4.62% as shown in Figure 1. These results showed that the increased of the temperature can gradually decreased the moisture content of the fish. According to Winarno (2002), the higher the drying temperature, the faster evaporation occurs, thus moisture content in the material tend to be depleted. This is because the increase in drying temperature increased the speed of the air flow then increased the heat energy and eventually a faster water evaporation transferred from the material to the atmosphere (Rachmawan 2001; Albert 2013).

The fish dried at 60°C with the addition of 1% chitosan is lower than that of fish with the addition of chitosan 0.38% at the same temperature (Figure 1). This is due to the higher concentration of chitosan, the higher the amount of bound water in the fish. According to Sitindaon (2007), chitosan has a polar group (H +) that can bind water which decreased the amount of free water in the fish. However, Figure 1 also shows the increased of moisture content when 4.62% of chitosan was added to the fish. This might be caused by the saturated chitosan during the process which then increased the free water instead of the bound water. However, the differences of moisture content between the fish treated with different chitosan concentrations was not statistically significant which might be caused by the accumulation of moisture in the fish due to the formed film that trapped the moisture and oxygen inside the fish. This result was similar to the study conducted by Agustini (2007) and Wittriansyah et al. (2019) where the use of 0.5% and 1.0% of chitosan did not significantly affect the moisture content of salted anchovies (*Stolephorus heterolobus*).

The optimum temperature and chitosan concentration which affect the moisture content of the fish derived from a response contour and surface plot (Figure 2). The plot shows the maximum and minimum conditions obtained from 45% and 20% of moisture content, respectively. Thus, the minimum curve point also can be seen, where the lowest moisture content obtained at >60°C with the addition of chitosan 1.5%-3%. This raw estimation point needs to be optimized by optimization plot which resulted in the optimum temperature of 65.35 °C and 2.39286% of chitosan resulted in a moisture content of 18.0760%.



(b)

Fig. 2 (a) Contour graph and (b) Surface graph of temperature and chitosan concentration effect on the moisture content of "ikan bage"

Protein Content

Protein content in this study expressed as the crude protein content contained in "ikan bage".

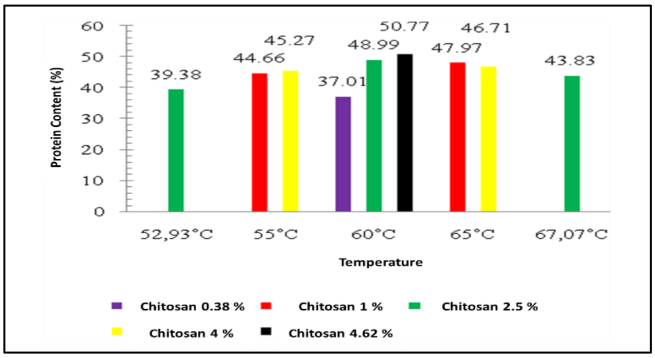
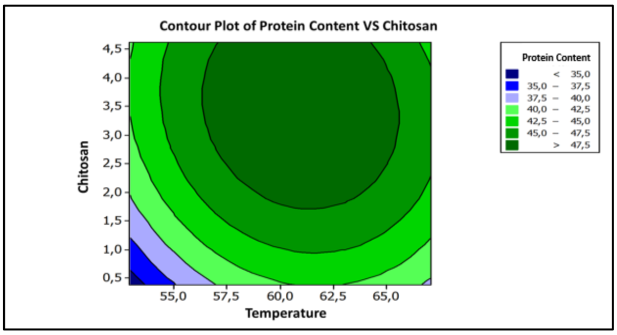
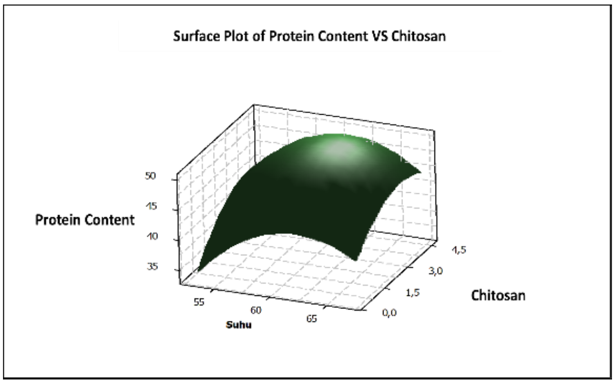


Fig.3 The Protein content of "ikan bage" analyzed using RSM

The highest protein content (50.77%) was measured in the fish that was dried at 60 °C with 4.62% while the lowest protein content (37.01%) was obtained from the fish which dried at 60°C and 0.38% of chitosan. The increased of protein content was observed in the increase of temperature from 52.93°C to 65°C which might be caused by the inhibition of microbial growth by the chitosan layer which means the less amount of protein used. However, there was a decreased protein content of the fish that was dried at 67.07°C which might be due to the denaturation of the protein at high temperatures (Fellows 2000; Yuarni et al. 2015). Denaturation occurs because of the modification of protein structure without breaking the covalent bond where the heat will penetrate into the fish flesh and damaging the protein (Yuniarti et al. 2013).



(a)



(b)

Fig. 4 (a) Contour graph and (b) Surface graph of temperature and chitosan concentration effect on the protein content of "ikan bage"

Figure 4 shows the maximum and minimum protein content obtained from 60°C-65°C and 3.5% - 4.5% of moisture content, respectively. These raw estimation point needs to be optimized by optimization plot which resulted in the optimum temperature of 60.93°C and 3.51% of chitosan resulted in a protein content of 49.91%.

Optimization of Moisture content and Protein Content

The optimization of drying temperature and chitosan concentration based on the moisture and protein content can be seen in Table 1. According to the highest desirability value (0.86%), this study concluded that the optimum temperature was 60.93°C and optimum chitosan addition was 3.51% resulted in a moisture content of 23.47% and a protein content of 49.91%.

Table 1 Desirability values for various process conditions

No	T (°C)	Chitosan (%)	Moisture content (%)	Protein Content (%)	Desirability
1	60.93	3.51	23.47	49.91	0.86
2	60.39	4.51	35.00	49.13	0.83
3	59.40	0.54	35.00	42.73	0.59
4	67.07	0.38	32.47	39.47	0.43
5	52.93	4.29	39.55	42.53	0.17

Total Plate Count

Table 2 showed that less microbial colonies was formed as the chitosan concentration was increased which illustrated by the decreased of microbial colonies from 3 x 101 CFU/gr to 20 x 101 CFU/gr in the fish treated with 4% and 1% chitosan, respectively. Also, the drying temperature affects the number of microbes measured in the fish. The mold began to appear at the 20th day on the fish that was dried at 53°C and 55°C which might be caused by the high moisture content at these temperatures compared to that of higher temperatures. This study concluded that the microbial colonies detected was complied to the Indonesia National Standard which is 1 x 10^5 CFU/gr.

Table 2 Average number of microbial colonies in bage fish

T (°C)	Chitosan (%)	TPC (CFU/gr) 4 Days	10 Days	20 Days
55	1	0	1 x 10^1	fungus
65	1	0	0	20 x 10^1
55	4	0	1 x 10^1	fungus
65	4	0	0	3 x 10^1
53	2.5	0	1 x 10^1	fungus
67	2.5	0	1 x 10^1	17 x 10^1
60	0.38	0	0	2 x 10^1
60	4.62	0	0	46 x 10^1
60	2.5	0	0	1.5 x 10^1

Moisture content in food can enhance the growth of microorganisms which allows more microbes and fungi to grow and develop within the material (Fraizer 2000). Table 1 showed that although no mould was developed on the fish that was dried at 67°C, there was a growth of 17 x 101 CFU/gr of microbial colonies. This was because chitosan can inhibit the growth of these microorganisms by covering the entire surface of the fish through thin film layer which will inhibit the penetration of oxygen and water through the surface of the fish body, resulting in the inhibition of aerobic microbes development (Hui 2007; Sarwono 2010; Wittriansyah et al. 2019).

The antimicrobial property of chitosan is due to the positive charge of amine in its functional group. The membrane cells of microbes are negatively charged which will electrostatically attracted to the positive charge of chitosan which impaired the balance of the osmotic pressure inside the microbial cells (Elmoslemanya et al. 2010; Holipah et al. 2010). Anugrah (2016) explained that the treatment using chitosan as a coating in dried salted fish decrease the number of microbial colonies compare to that of without chitosan coating.

Organoleptic test: Texture preference

The texture preference values range between 2.80 and 3.84. The highest preference was obtained from the fish that was dried at 60 °C with 2.5% of chitosan which means that the panellists preferences range from like to neither dislike nor like the fish.

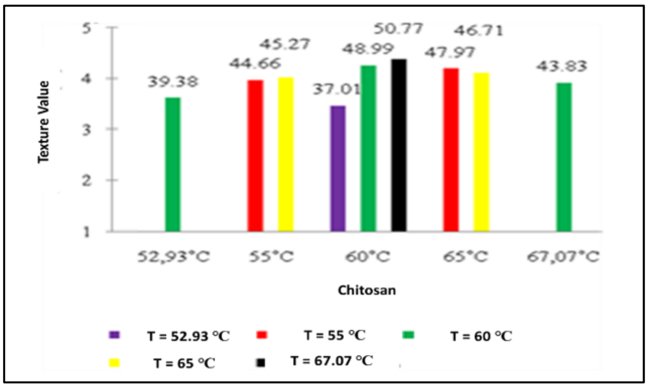


Fig. 5 The effect of chitosan coating and drying temperature on texture preference of "ikan bage"

The decreased of panellists preference for the fish texture dried at 67.07℃ was likely due to the firm texture of the fish. This is in accordance with Purnomo (1995) and Riansyah (2013) who explained that moisture content and water activities in food plays an important role in determining the texture of food ingredients which means the decreasing of moisture content is perpendicular to the increasing of the stiffness of the fish flesh.

Food products may undergo changes in texture due to the growth of microorganisms which degraded cellulose, pectin, or collagen in food into smaller units through enzyme activity then resulted in the textures softening (Bell et al. 2005). Therefore, adding chitosan to "ikan bage" can inhibit microbial growth. However, there was no significant differences of the texture preferences based on various chitosan concentrations and drying temperatures. This is in accordance with the research of Jo et al. (2001) and Dompeipen et al. (2016) which stated that the addition of chitosan did not affect food texture.

Organoleptic test: aroma preference

The aroma preference value ranges from 2.84 to 3.32 as shown in Figure 6.

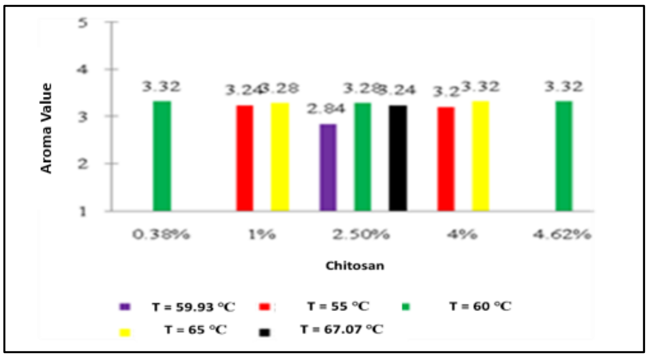


Fig. 6 The effect of chitosan coating and drying temperature on aroma preference of "ikan bage"

The highest preferences were obtained from the fish dried at 60°C with 0.38% and 4.62% of chitosan which means that the panellists preferences range from like to neither dislike nor like the fish. Figure 6 also showed the decreased in aroma preferences in fish dried at 52.93 °C which suspected to be caused by the fish being undercooked at the respective temperature. similar to that, panellist also has low preference for the fish dried at 67.07°C which might be caused by the burnt aroma due to the high drying temperature. The "ikan bage" has a very distinctive aroma when dried, thus if the fish is dried at a temperature that is too high for a long time, the distinctive aroma will disappear. This caused the panellists to prefer the fish dried at 60 °C. The concentration of chitosan in this study was insignificantly affected the fish aroma. According to Cahyadi (2009) and Isnawati et al. (2015), chitosan act as a coating to block the aroma from coming out of the fish. In addition, the edible coating properties of chitosan will also prevent oxidation so that it does not cause rancid aromas in the fish.

Organoleptic test: taste preference

According to the hedonic scale, the taste preference value ranges from 3.28 to 3.60 as shown in Figure 7.

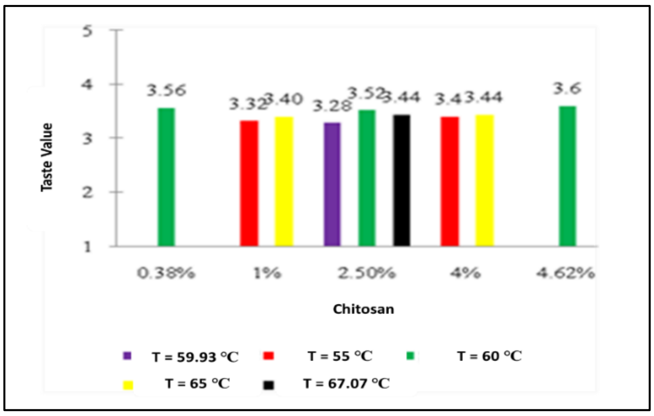


Fig. 7 The effect of chitosan coating and drying temperature on taste preference of "ikan bage"

The highest taste preference was obtained from the fish dried at 60°C with 4.62% of chitosan, which means that the panellists neither like nor dislike the taste of the fish. The addition of various chitosan concentrations was not significantly affecting the taste preference. This is because chitosan is a polysaccharide which does not exhibit flavour, thus adding chitosan does not affect the taste of the fish.

This study concluded that "ikan bage" in this study generally has an acceptable organoleptic characteristic proven by the organoleptic value which averaged at 3 (neither like nor dislike) in texture, aroma, and taste of the fish.

CONCLUSIONS

The optimization of drying temperature and chitosan concentration for "ikan bage" production using response surface methodology showed that 60.93°C and 3.51% was the optimum combination to produce 23.47% and 49.91% of moisture content and protein content, respectively. Microbial growth was observed on the 20th day on the fish that dried at 53 and 55°C with 1%, 2.5%, and 4% of chitosan. In addition, hedonic test also indicates that the taste, texture, and aroma were generally acceptable to the panellists.

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