Quality Profile and Antioxidant Activity of Milkfish (*Chanos chanos*) Sausage with the Addition of Red Bean Flour (*Phaseolus vulgaris* L.)

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ABSTRACT

Red bean are renowned for high antioxidant capable of preventing the Reactive Oxygen Species (ROS) formation that triggers the formation of free radicals. Meanwhile, milkfish (*Chanos chanos)* has a high protein content and low fat compared to other brackish water fish. Therefore, this study aimed to determine the chemical quality, microbial, and sensory acceptance of milkfish sausage with red bean flour. A completely randomized design (CRD) was used with one treatment factor namely variations in the ratio of milkfish to red bean flour at F0 (100%:0), F1 (75%:25%), F2 (50%:50%), and F3 (25%:75%). Parameters analyzed include moisture, ash, fat, total protein, carbohydrate, dietary fiber, antioxidant, microbiological total plate count (TPC), as well as organoleptic features namely color, taste, aroma, texture, and elasticity. Data were analyzed using SPSS version 23.0 which was tested with One Way ANOVA followed by Duncan Multiple Range Test (DMRT) at the 5% significance level when significant differences were found. The results showed that red bean flour in milkfish sausage had a significant effect on all treatments. The higher the amount of red bean flour added, the greater the value of ash, carbohydrate, and dietary fiber content, while the value of water, fat, protein, and TPC decreased. The strongest antioxidant activity indicated by IC_{50} was found in F3 at 83.21 ppm. TPC testing showed that all formulations meet the quality requirements of SNI fish sausage. The level of consumer acceptance significantly influenced the acceptance of color, taste, texture, chewiness, aftertaste, and overall. Milkfish sausage added with red bean flour had good chemical quality, microbial, and antioxidant activity.

Keywords: Antioxidant; milkfish; red bean flour; sausage

INTRODUCTION

Chronic non-communicable disorders are a degenerative disease caused by a decrease in body organ performance with age (Handajani et al., 2012). In 2018, there was an increase in the number of degenerative diseases in Indonesia, including cases of diabetes mellitus (0.5%), stroke (3.9%), hypertension (8.13%), and cancer (0.39%) (Riskesdas, 2018). Additionally, lack of fiber intake in the long term can increase the risk of degenerative diseases (Handajani et al., 2012). The primary causes of the diseases include irregular diet, lifestyle factors, and environmental changes that lead to the formation of free radicals (Fridalni et al., 2019). Antioxidant are essential for the body to neutralize free radicals in preventing additional processes that can cause oxidative (Kikuzaki et al., 2002).

Food from plants is a source of antioxidant due to the flavonoid and polyphenol content which act as exogenous antioxidant (Annidara, 2021). The isoflavone compounds contain one of the antioxidant found in flavonoids (Parwata, 2016). Isoflavone in plants is

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abundantly found in nuts, including red bean (Mada et al., 2017).

Red bean contains antioxidant polyphenolic compounds in the form of procyanidin about 7-9% of which are found in the shells (Pratiwi & Panunggal, 2016). Antioxidant activity has an important role in counteracting free radicals, especially in polyphenolic compounds (Ganesan & Xu, 2017). In 100 g of red bean ingredients, there are 22.3 g of protein which is known as a source of vegetable protein with vitamins B, folasin, calcium, phosphorus, fiber, thiamine, complex carbohydrates, and iron (Andari, 2021). Red bean have very low-fat levels and contain almost no saturated fat (Ekasari, 2010). It is also a good source of fiber, with 100 g producing 26.3 g of fiber which consists of water-soluble and water-insoluble (Rusilati et al., 2007).

According to a previous report, red bean flour are suitable for use as a binder for processed foods including sausages, nuggets, and meatballs, because it has a fairly high vegetable protein content (Grahito, 2018). Processed foods are usually prepared using animal meat such as chicken, beef, and fish including milkfish. Based on the nutritional composition, milkfish are low in fat and high in protein. About 100 g of milkfish has 129 kcal of energy, 20 g of protein, 4.8 g of fat, 20 mg of calcium, 150 mg of phosphor, 2 mg of iron, 150 SI of vitamin A, and 0.05 mg of vitamin B1 (Akbar et al., 2021)

Sausages are food products made from ground beef that are then restructured and given additives (BTP) to help maintain the shape. Fish sausage is a cylindrical chewy food made from ground beef with a special wrapper (casing). Basically, sausages can be made from almost any type of fish, such as tuna, lemuru fish, and swordfish (Cahyani, 2011).

Starch must be used in the fillers used in sausages, for example, tapioca flour can be used as a filler because the main content is starch, which can bind water from the material, giving it a sturdy and compact texture (Kusnandar, 2019). In addition, fillers generally consist of carbohydrates that have little influence on emulsification. The binder used is red bean flour because it contains high protein (Rompis & Londok, 2022). Fillers and binders in sausages are added to improve slicing characteristics, shrinkage of cooking time, and emulsion stability (Rompis & Londok, 2022).

This study aimed to determine the chemical quality, microbial, and sensory acceptance of milkfish sausage added with red bean flour. The product obtained was high in protein, low in fat, and contained sufficient antioxidant that can prevent free radicals from the normal oxidation process.

METHODS

Materials

The materials used in this study include red bean purchased from Giwangan Market (Bantul, Yogyakarta, Indonesia), and milkfish from Prawirotaman Market (Mergangsan, Yogyakarta City, Indonesia). The milkfish was characterized by a fresh smell and transparent appearance. Other materials used include tapioca flour, salt, ground pepper, garlic powder, shallots, sugar, ice water, hexan, NaCI (0.85%), Plate Count Agar (PCA),

Table 1. Milkfish sausage formulation with the addition of red bean flour

Ingredients	F0 (100% milkfish)	F1 (75% milkfish: 25% red bean flour)	F2 (50% milkfish: 50% red bean flour)	F3 (25% milkfish: 75% red bean flour)
Milkfish	500 g	375 g	250 g	125 g
Red bean flour	-	125 g	250 g	375 g
Tapioca flour	75 g	75 g	75 g	75 g
Salt	10 g	10 g	10 g	10 g
Sugar	10 g	10 g	10 g	10 g
Pepper	0,8 g	0,8 g	0,8 g	0,8 g
Red onion	5 g	5 g	5 g	5 g
Garlic powder	5 g	5 g	5 g	5 g
Egg white	20 g	20 g	20 g	20 g
Ice water	400 mL	400 mL	400 mL	400 mL

Methanol PA, 2,2-diphenyl- 1picrylhydrazyl (DPPH), Na_2SO_4, $CuSO_4$, TiO2, NaOH 40%, Na2S2O3 5%, H3BO4, MR-BCG indicator, and HCI 0.02 N.

The equipment used includes gas stove, 80 mesh sieve, steamer or boiler, petri dish (Normax and Anumbra), oven (Memmert UN-55/UN-30), incubator (Memmert), Muffle Furnace (B-One), Soxhlet (Iwaki) 100 mL, UV-Vis spectrophotometer (B-One), autoclave all American (B-One), vortex (Thermo Scientific), laminar airflow messagrate, centrifuge (Oregon), and cabinet dryer.

Methods

Red bean flour Preparation

Red bean selected were of good quality and characterized by kidney shape, soft texture, and large size. The samples were washed thoroughly, soaked in water for 12 hours, and then boiled for 10 minutes. This was followed by drying for 24 hours at a temperature of 60–70 °C in a cabinet dryer. Smooth red bean flour was produced by mashing using a grinder, then sifted with an 80-mesh sieve (Cahyani, 2011). The product was packed in air-resistant plastic, stored in a closed container, and placed at room temperature to avoid sunlight.

Milkfish sausage formulation with the addition of red bean flour

Milkfish sausage was prepared by adding red bean flour (binder), tapioca flour (filler), and other spices. The variation in the ratio of milkfish and red bean flour was 75%, 50%, and 25% as shown in Table 1. This ratio and formulation were based on a reference (Cahyani, 2011) with slight modifications.

Milkfish sausage Preparation

Milkfish were selected with good quality, namely fresh with large scales, intact, hard texture, and healthy physical condition as indicated by the color of the eyes. The samples were cleaned of scales, the thorns were removed, leaving only the meat. This was followed by grinding or mashing using a blender until smooth, then spices were prepared and mixed until evenly distributed. Subsequently, the sausage dough was mixed evenly into the sleeve or wrapper casing and tied with a rope. The sample was steamed for up to 20 minutes at a temperature of 45 °C, cool at room temperature for 10 minutes then put into an air-resistant plastic and stored in a refrigerator (Cahyani, 2011).

Water content analysis

Moisture content analysis was performed using the oven method based on AOAC (2005). Empty weighing bottles were dried for 1 hour at 105 °C in the oven, then cooled for 15 minutes in a desiccator. The bottles were weighed, and the results were recorded, then a sample of 1-2 g was placed in each. The contents of the bottle were dried in a 105 °C oven, and drying continued until the desired weight was reached. After drying, the bottle and the contents were cooled in a desiccator, weighed, and the moisture content determined. The water content value was calculated using Equation 1.

$$\% \text{ Water content} = \frac{w1-w2}{w1-w0} \times 100\% \quad (1)$$

Where W0 = empty bottle weight (g), W1 = weight of bottle and sample before drying, and W2 = weight of bottle and sample after drying.

Ash content analysis

Ash content analysis was carried out using the oven or kiln method based on AOAC (2005). The cup is pre-oven at 100-105 °C for 30 minutes. The cup is cooled in a desiccator to remove moisture and weighed (A). The sample was weighed at a weight of 3 g per cup (B), then burned on a burner flame until it was smokeless and continued by ashing in a furnace for 5 hours at a temperature of 550–600 °C until whitish ash was obtained. The sample that has been opened is cooled in a desiccator and weighed (C). The ash content was calculated using Equation 2.

$$\% \text{ Ash content} = \frac{C-A}{B-A} \times 100\% \quad (2)$$

Where A = weight of empty cup (g), B = cup weight + initial sample (g), and C = cup weight + dry sample (g).

Fat content analysis

Analysis of fat content was carried out using the Soxhlet method based on AOAC (2005). The mashed sample is weighed in a cotton filter paper weighing 2 g. Then dried for about 1 hour in an oven set at a temperature of less than 80 °C. Then installed with a series of soxhlet tools such as a thimble, boiling flask, condenser, and cooler. After that, extract the sample for approximately 6 hours using other fat solvents. Hexan is distilled and the fat extract is dried in an oven at 105 °C. After cooling, it is weighed until its weight is constant. The following formula calculates the fat content value (Equation 3).

$$\% \text{ Fat content} = \frac{w2-w1}{w} \times 100\% \quad (3)$$

Where W = sample weight (g), w1 = weight of fat gourd before extraction (g), and w2 = weight of fat gourd after extraction (g).

Protein content analysis

Protein content analysis was carried out using the Kjeldhal method based on AOAC (2005). The sample was mashed, weighed to 0.2 g, and placed into a Kjedhal flask. About 4 mL of concentrated H_2SO_4 was added along with 0.7 g of N catalyst (250 g of Na_2SO_4 + 5 g of $CuSO_4$ + 0.7 g of TiO_2). The mixture was placed in a fume hood until the color of the sample changed to clear green, followed by cooling and mixing with 10 cc of pure water. About 20 mL of NaOH-TiO (40% NaOH + $Na_2S_2O_2$ 5%) was added during distillation, and the distillate was collected using 4% H_3BO_3 mixed with the MR-BCG indicator. The mixture was distilled to contain 60 mL, with the distillate color changing from red to blue. Distillation was stopped once the volume reached 60 mL, then followed by titration with a standard 0.02 N HCI solution until the color changed from blue to pink. The protein content value was calculated using Equation 4.

$$N\ \% = \frac{VT \times N\ HCI\ (0{,}02\ N) \times BAN\ (14{,}008)}{Sample\ weight\ (mg)} \times 100\% \quad (4)$$

Where VT = titration volume, BAN = atomic weight of nitrogen, N = normality HCI, and FK = conversion factor.

Analysis of carbohydrate content

Carbohydrate content was determined using calculations by difference (Winarno, 1986), which refers to a reduction of 100% from water, ash, fat, and protein. This implies that carbohydrate levels depend on the reduction factor due to the influence of other nutrients. The carbohydrate content value was determined using Equation 5.

Carbohydrate content (%) = 100% - (water + protein + fat + ash) (5)

Analysis of dietary fiber

Analysis of dietary fiber was carried out using a method based on AOAC (2005). Initially, a sample of 0.5 g was inserted into an Erlenmeyer glass. To make the suspension, 25 mL of 0.1 M phosphate buffer solution with pH 7 was added and mixed. About 0.1 mL of alpha-amylase enzyme was added then the Erlenmeyer glass was covered with aluminum foil and incubated in a water bath for 15 minutes at 100°C while occasionally stirring. A 20 mL of aquadest and 5 mL of 1N HCI were added after the sample was removed and cooled. The Erlenmeyer glass containing the sample was added with 1 mL of 1% pepsin enzyme then tightly closed and incubated in a water bath for 1 hour at 40 °C. Distilled water and 5 mL of NaOH 1N were added along with the enzyme beta-amylase 0.1 mL. This was followed by filtration using filter paper with a constant weight. About 2x10 mL of ethanol and acetone were used to wash the sample. Insoluble dietary fiber was determined by weighing the sample after drying in the oven for one night at 105 °C. Filtrate was added with 400 mL of 95% warm ethanol after sufficient volume up to 100 mL and allowed to stand for an hour. Filtration was carried out using ash-free filter paper, washed with 10 mL of ethanol and acetone, dried overnight at 105 °C in an oven and the final weight (dissolved dietary fiber) was calculated. The dietary fiber value was calculated using Equation 6.

Total dietary fiber % = insoluble dietary fiber + dissolved dietary fiber (6)

Antioxidant analysis

Antioxidant activity analysis was carried out using the DPPH method (Ulfah, 2016). A 50 mL measuring flask was filled with 0.00197 g (1.97 mg) of DPPH powder that had been dissolved in 50 mL of pro-analysis methanol. The flask was filled until the limit mark, then covered with aluminum foil (DPPH 0.1 mM). All sample formulations were weighed up to 10 mg, and dissolved in 10 mL (1000 ppm) of pro-analysis methanol. About 5 mL of 0.1 mM DPPH stock solution was taken along with 5 mL of pro-analysis methanol, covered with aluminum foil, and inserted into a test tube. UV-Vis spectrophotometer was used to measure the absorbance of blank solutions at a wavelength of 517 nm. Furthermore, test tubes were filled with 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1 mL, and a total of 1000 ppm of the sample mother solution and obtained solution concentrations of 10 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. Each concentration was added with methanol pro analysis and sufficiency up to 10 mL. About 5 mL of DPPH 0.1 solution was added to the sample test solution, which had been pipetted up to 5 mL of each concentration, in a test tube, covered with aluminum foil, and then vortexed until homogenous. Subsequently, incubation was carried out for 30 minutes and absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The antioxidant value was calculated using Equation 7.

$$\%\ RSA = \frac{(A\ blanko - A\ sample)}{A\ sample} \times 100\% \quad (7)$$

The Activity Antioxidant Index (AAI) has a function to classify the antioxidant properties of the sample

(Sawiji, 2022). The antioxidant activity was considered weak, moderate, strong, and very strong when the AAI value was < 0.5, 0.5-1.0, 1.0-2.0, and > 2.0 respectively.

Microbial test total plate count (TPC)

The ALT microbial test was conducted using the SNI 01-2332.3 (Badan Standarisasi Nasional, 2006). A total of 200 mL PCA media was made, then the sample was mashed and weighed up to 1 g. The mashed sample was mixed with 9 mL of 0.85% NaCI solution and homogenized for 2 minutes (dilution 10^{-1}). The sample was homogenized after adding a dilution of 10^{-1} in a volume of 1 mL to a dilution factor of 10^{-2}. A similar procedure was carried was for dilution factors of 10^{-3} and others. Each dilution factor of 10^{-1} to 10^{-5} consisted of 9 mL 0.85% NaCI solution. The pour method was used for the isolation stage by pipetting 1 mL of 10^{-1}, 10^{-2}, 10^{-3}, 10,$^{-4}$ and 10^{-5} dilutions respectively into sterile petridishes. About $\pm$ 15 mL of sterile PCA was added, and the solution was shaken evenly (triplo for each dilution). The cup was incubated at a temperature of 36–27 °C for 48 hours in an inverted position after the agar media solidified. Too much to count (TMTC) occurs when there are more than 250 colonies per cup in all dilutions. Dilutions of 10^{-3}, 10,$^{-4}$ and 10^{-5} were used to calculate the number of growing colonies. The antioxidant value was calculated using Equation 8.

$$\Sigma = E \times \frac{1}{FP} \quad (8)$$

Where E = enumeration and FP = dilution factor.

Organoleptic test

Organoleptic properties were assessed through hedonic or preference tests which include parameters of color, taste, aroma, texture, chewiness, aftertaste, and overalls. A total of 32 untrained panelists of the Departement of Food Technology, Faculty of Industrial Technology, Ahmad Dahlan University. Five hedonic scales were used including 1 (very dislike), 2 (dislike), 3 (somewhat liking), 4 (like), and 5 (very like) (Setyaningsih et al., 2010).

Statistical Test

The data obtained were statistically analyzed using the SPSS software version 23 for Windows. One-way ANOVA was conducted on water content, ash content, fat content, protein content, analysis carbohydrate content, analysis of dietary fiber, antioxidant activity test was measured using Duncan's multiple range test ($p < 0.05$). Micobial test total plate count (TPC) was conducted using the SNI 01-2332.3 (Badan Standarisasi Nasional, 2006). Organoleptic test are assessed through hedonic or preference tests which include parameters of color, taste, aroma, texture, chewiness, aftertaste, and overalls.

RESULTS AND DISCUSSION

Water Content

The water content value of milkfish sausage added with red bean flour is shown in Table 2. The higher the amount of red bean flour added, the lower the water content produced. The water content for F0 and F1 exceeded the maximum limit based on the SNI quality requirements of fish sausages at 68% (SNI, 2013). Additional testing conducted by Duncan showed that the water content of milkfish sausage was significantly affected by the addition of red bean flour. The average water content produced was lower compared to another study (Natasia, 2022) which used carrot paste and obtained a value of 59.96%.

According to Hafiludin (2015), the water content in brackish water milkfish is relatively high at 70.78%, compared to red bean flour at 5.70% (Grahito, 2018). The use of tapioca flour also causes an increase

Table 2. Result of chemical properties analysis

Formulation	Parameter				
	Water	Ash	Fat	Protein	Carbohydrate
F0 (100% milkfish)	78,85 ± 0,49^{d}	1,68± 0,02^{a}	4,41 ± 1,30^{ab}	12,44± 0,04^{d}	2,72 ± 1,00^{a}
F1 (75% milkfish: 25% RBF)	71,41 ± 0,43^{c}	2,15 ± 0,55^{b}	5,19 ± 0,17^{b}	10,60 ± 0,75^{c}	10,63± 0,57^{b}
F2 (50% milkfish: 50% RBF)	62,69 ± 0,92^{b}	2,01 ± 0,44^{ab}	3,27 ± 1,02^{a}	8,74 ± 0,12^{b}	23,28± 0,69^{c}
F3 (25% milkfish: 75% RBF)	59,76 ± 1,04^{a}	2,29 ± 0,04^{b}	3,13 ± 0,71^{a}	8,20 ± 0,12^{a}	26,61± 0,59^{d}

Notes: a, b, c, d, e = letter notation is not similar, meaning that there is no real effect on the level Duncan test has a value of 5%
RBF = red bean flour

in the water content due to the starch content which binds water (Umanahu et al., 2023). The steaming process also affects the increase in water content. The main ingredients used in the steaming process affect the water content of foodstuffs due to the hot steam produced (Natasia, 2022).

Ash Content

Table 2 shows the ash content of milkfish sausage produced with the addition of red bean flour. The addition of red bean flour increased the ash content but was still within the limit for SNI. Based on the quality requirements of fish sausages, the ash content should not exceed 2.5% (SNI, 2013). Additional testing conducted by Duncan showed that the ash content of milkfish sausages was not significantly affected by the inclusion of red bean flour. The average ash content produced was lower compared to another study (Sudi et al., 2018) of skipjack tuna with carrots and sago starch, which obtained a value of 1.86%.

The increase in ash content was due to the high amount of red bean flour added. In general, the ash content can be determined by the minerals present in fish sausage products. About 100 g of red bean contains a high mineral of approximately 3.7% which can increase the ash content in the product (Cahyani, 2011). In addition, smears on milkfish meat up to 1.7% have the potential to increase ash content in sausages produced (Kencana & Darmanto, 2018). Ash content is also influenced by the supporting ingredients used in the process of making fish sausage including tapioca flour, eggs, and salt (Hati et al., 2020).

According to Wibowo (2018), several factors affect the ash content of food, namely the method of ashing, the type of food, temperature, and drying time. In the drying process, the longer the time and the higher the temperature used, the greater the ash content. Furthermore, Wahyuni (2009) stated that the processing of food ingredients causes a decrease in mineral content due to various factors such as heat, pH, oxygen, and combination.

Fat Content

Table 2 shows the fat content value of milkfish sausage added with red bean flour. The greater the amount of red bean flour added, the lower the fat content produced. Based on the SNI quality requirements of fish sausages, the fat content should not exceed 7% (SNI, 2013). This implies that the fat content produced meets the SNI quality requirements. Further testing conducted through Duncan showed that the fat level of milkfish sausage was not significantly affected by the inclusion of red bean flour. The average fat content produced was lower compared to another study (Natasia, 2022) of milkfish sausage added with carrot paste which obtained a value of 7.09%.

The different basic ingredients used to make sausages have varying fat values. According to Akbat et al. (2021), the fat content of milkfish per 100 g is 4.8 g. Hafiludin (2015) also stated that the fat content of brackish water milkfish is 0.853%. Red bean flour has a lower fat content of 2.21 g (Perwita et al., 2021) compared to milkfish. Therefore, the amount of red bean flour added determines the amount of fat produced. This indicates that reducing milkfish meat added to sausages has an impact on decreasing the fat content. Milkfish contains 32.11% monounsaturated fatty acid (Malle et all. 2019), in addition to 4.63% milkfish oil with 0.36% EPA and 1.17% DHA (poly-unsaturated fatty acid omega-3) (Sugata et al., 2019).

Protein Content

Table 2 shows the protein content of milkfish sausage added with red bean flour. The greater the amount of red bean flour added, the lower the protein content. The results indicated that F0 and F1 produced protein levels above the SNI quality requirements of fish sausages, namely 9% (SNI, 2013). Additional testing conducted through Duncan showed that the protein content of milkfish sausage was significantly impacted by the inclusion of red bean flour. The average protein content produced was lower compared to another study (Grahito, 2018) of fish cork sausage added with red bean flour which obtained a value of 23.52%.

High levels of protein are caused by the significant amount of milkfish added to the treatment. This is because milkfish protein is estimated at 20 g while that of red bean flour is 17.24 g (Perwita et al., 2021). The structure of proteins can change during processing at high temperatures (steaming), resulting in denaturation. In general, denaturation caused by the same heating and an increase in the concentration of red bean flour led to a decrease in the protein content of fish sausages. Dewi et al. (2019) stated that during processing, the quality of milkfish will decrease especially the protein content. Milkfish has 20-24% protein consisting of amino acid lysine 2.25% and glutamate 1.23% as well as omega-3 fatty acids accounting for 14.2% of total fat (Dewi et al., 2019).

Carbohydrate Content

Table 2 shows the carbohydrate content of milkfish sausage prepared with the addition of red bean flour. The higher the amount of red bean flour added, the greater the carbohydrate level produced. Additional testing conducted through Duncan showed that the inclusion

Table 3. Results of dietary fiber content analysis

Formulation	Insoluble fiber %	Soluble fiber %	Total fiber %
F0 (100% milkfish)	2,31 ± 0,95[a]	0,25 ± 0,25[a]	2,57 ± 0,11[a]
F1 (75% milkfish: 25% RBF)	6,17 ± 0,87[b]	0,44 ± 0,36[b]	6,61 ± 0,05[b]
F2 (50% milkfish: 50% RBF)	8,38 ± 0,79[c]	0,61 ± 0,05[c]	9,01 ± 0,87[c]
F3 (25% milkfish: 75% RBF)	9,25 ± 0,57[d]	0,67 ± 0,05[d]	9,60 ± 0,11[d]

Notes: a, b, c, d, e = letter notation is not similar, meaning that there is no significant effect on the level Duncan test has a value of 5%
RBF = red bean flour

of red bean flour significantly affected the amount of carbohydrates in the milkfish sausage. The average carbohydrate level produced was lower compared to another study (Grahito, 2018) of fish cork sausage added with red bean flour, which obtained a value of 15.9%.

The addition of red bean flour caused milkfish sausages to have a high carbohydrate content. Perwita et al. (2021) stated that 100 g of red bean flour contains 12.83 g of carbohydrate content, while milkfish has less amount. Moreover, Hafiludin (2015) stated that the carbohydrate content of brackish water milkfish is 2.780%. Nutritional factor also affects carbohydrate content, the lower the nutritional factor, the higher the carbohydrate content (Palijama et al., 2020). Water, ash, fat, and protein content are nutritional factors that affect the amount of carbohydrates. According to Rais et al. (2019), ingredients that contain carbohydrates when added to a product increase the level during the cooking process. The use of tapioca flour and eggs in sausages also causes the carbohydrate content to increase.

Dietary Fiber Content

Table 3 shows the dietary fiber content of milkfish sausage added with red bean flour. The greater the amount of red bean flour added, the higher the dietary fiber content produced. Duncan's further test showed that the addition of red bean flour had a significant effect on the dietary fiber.

The addition of red bean flour caused milkfish sausages to have a high content of dietary fiber. This is because red bean has a fairly high fiber of about 26.3 g per 100 g (Rusilati et al., 2007). The amount used to make fish sausages affects the level of dietary fiber.

Antioxidant Activity

The DPPH method was used to determine the ability to capture free radicals formed. Antioxidant activity is influenced by phenol group compounds, the higher the number of phenol group compounds found in a food, the stronger the antioxidant activity (Pratiwi & Panunggal, 2016). The results of antioxidant activity are presented in Table 4. Duncan further tests showed that the addition of red bean flour had a significant effect on milkfish sausage.

IC_{50} value is the amount of antioxidant concentration in the sample that can reduce DPPH free radicals by 50%. The smaller the value, the stronger the antioxidant produced in warding off free radicals (Wassalwa, 2016). Based on the IC_{50} value, the highest antioxidant activity was 219.14 ppm (moderate) while

Table 4. Antioxidant activity

Sample	IC_{50} (ppm)	Antioxidant power (Syaifuddin, 2015)	AAI	Antioxidant properties (Scherer & Godoy, 2009)
F0 (100% milkfish)	141,11 ± 2,95[c]	Medium	1,40 ± 0,40[b]	Strong
F1 (75% milkfish : 25% RBF)	219,14 ± 1,69[e]	Medium	0,91 ± 0,05[a]	Medium
F2 (50% milkfish : 50% RBF)	149,55 ± 8,85[d]	Medium	1,34 ± 0,07[b]	Strong
F3 (25% milkfish : 75% RBF)	83,21 ± 0,20[b]	Strong	2,40 ± 0,00[c]	Very strong
Vitamin C	7,06 ± 0,07[a]	Very strong	28,31 ± 0,30[d]	Very strong

Notes: a, b, c, d, e = letter notation is not similar, meaning that there is no significant effect on the level Duncan test has a value of 5%
RBF = red bean flour

the lowest value was 83.21 ppm (strong). The higher the amount of red bean flour added, the greater the IC_{50}.

Red bean flour contributes high antioxidant activity to milkfish sausage. It contains polyphenol compounds in the form of prosianidins estimated at 7-8% which are found in the coat (Pratiwi & Panunggal, 2016). Other content includes flavonoids, tannins, and phenolic acid, with significant antioxidant properties. According to a previous study, antioxidant activity has an important role in eliminating free radicals, especially polyphenols (Ganesan & Xu, 2017). F1 treatment had moderate antioxidant activity strength compared to F0. This is presumably because, during F1 treatment testing, the sample mixed with DPPH solution was not tightly covered using aluminum foil or not exposed to sufficient light. DPPH is very sensitive to light and can reduce the accuracy of the antioxidant activity examination process (Prastiyani, 2021).

The AAI has a function to classify antioxidant properties (Sawiji, 2022). Based on AAI, the highest average value of 2.40 indicates strong antioxidant properties while 0.91 represents moderate.

High and low antioxidant activity is also influenced by the heating process (steaming). Antioxidant components are easily damaged when exposed to heat. Furthermore, the oxidation reaction of the compound can be accelerated by heat. An increase in the rate of oxidation processes caused by heat can lead to breakdown processes. An oxidated antioxidant will experience damage and lose the ability to provide electrons for the neutralization of free radicals (Ratna et al., 2022).

Microbial Total Plate Count (TPC)

Results from the microbial Total Plate Numbers (ALT) are shown in Table 5. The highest average score was 11.6 x 10^3 CFU/mL and the lowest was 8 x 10^3 CFU/mL. The observation results showed that the greater the amount of red bean flour added, the fewer the colonies produced. Red bean contains polyphenolic bioactive compounds as antibacterial. It also has polyphenolic compounds in the form of procyanidin with levels of about 7-9%. The antibacterial properties of polyphenols can inhibit the spread of pathogenic bacteria (Hijriyanti et al., 2020).

Workers, tools, materials used, environmental hygiene, and other sources can contribute to bacterial contamination. Although fish sausages have been heated through the steaming process, food-related microorganisms may still thrive. Bacteria resistant to heating include *Clostridium* and *Bacillus* (its spores), while those often contaminated during handling comprise *Y.* e*nterocolytic* and *I. monocytogenes*. Considering both bacteria can thrive in low temperatures (cold), it is important to consider the shelf life of products, temperature control, and sanitation during processing when producing ready-to-eat food stored cold (Fauzi et al., 2017).

Organoleptic Test

Organoleptic assessment of food products is very important to improve the color, taste, aroma, texture, and other aspects. Hedonic tests were selected to determine organoleptic acceptance of milkfish sausages and the results are presented in Table 6.

Color

In the color assessment of milkfish sausage, the highest average value was 4.09% falling in the like category, while the lowest was 3.25 in the somewhat like category. Duncan further tests showed that the addition of red bean flour had a significant effect on milkfish sausage.

The color of milkfish sausage depended only on the amount of red bean flour used, as no dye was added. Milkfish sausage will become darker (brownish) when more red bean flour is added, and this can be attributed to the anthocyanin content (Cahyani, 2011). Red bean produces a red-brown color during processing due to a Maillard reaction. This enzymatic reaction between reducing sugars

Table 5. Total plate count (TPC) microbial test results

Treatment	TPC results	SNI
F0 (100%)	3,46 x 10^5 CFU/mL	
F1 (75% milkfish: 25% RBF)	9,6 x 10^3 CFU/mL	
F2 (50% milkfish: 50% RBF)	8 x 10^3 CFU/mL	5x10^4 CFU/mL
F3 (25% milkfish: 75% RBF)	11,6 x 10^3 CFU/mL	

Notes: RBF = red bean flour

(A) F0 (B) F1 (C) F2 (D) F3

Figure 1. Milkfish sausage with variations of (A) F0 100% milkfish, (B) F1 75% milkfish: 25% red bean flour, (C) F2 50% milkfish: 50% red bean flour, (D) F3 25% milkfish: 75% red bean flour

Table 6. Organoleptic test results

Test Parameter	F0 (100% milkfish)	F1 (75% milkfish: 25% RBF)	F2 (50% milkfish: 50% RBF)	F3 (25% milkfish: 75% RBF)
Color	$3,25 \pm 1,19^{a}$	$3,88 \pm 0,90^{b}$	$4,09 \pm 0,77^{b}$	$3,78 \pm 0,87^{b}$
Taste	$3,69 \pm 1,35^{b}$	$4,13 \pm 0,97^{b}$	$3,66 \pm 0,78^{b}$	$2,72 \pm 0,81^{a}$
Aroma	$3,56 \pm 1,24^{ab}$	$3,88 \pm 1,18^{b}$	$3,78 \pm 0,83^{ab}$	$3,25 \pm 0,88^{a}$
Chewiness	$3,56 \pm 1,26^{b}$	$3,88 \pm 1,00^{b}$	$3,41 \pm 0,97^{b}$	$2,69 \pm 0,78^{a}$
Texture	$3,16 \pm 1,27^{ab}$	$3,84 \pm 0,95^{c}$	$3,41 \pm 0,97^{bc}$	$2,69 \pm 0,82^{a}$
Aftertaste	$3,50 \pm 1,27^{b}$	$3,94 \pm 0,91^{b}$	$3,59 \pm 0,75^{b}$	$2,75 \pm 0,67^{a}$
Overall	$3,56 \pm 1,16^{b}$	$4,06 \pm 0,80^{c}$	$3,63 \pm 0,75^{bc}$	$2,88 \pm 0,94^{a}$

Notes: a, b, c, d, e = letter notation is not similar, meaning that there is no significant effect on the level Duncan test has a value of 5%
RBF = red bean flour

from starch (polysaccharide) with the free amino acids causes the color of the sausage to become darker (Agusta, 2020).

Taste

In the taste assessment of milkfish sausage, the highest average value obtained was 4.13% in the like category, while the lowest was 2.72% in the dislike category. Duncan further tests showed that the addition of red bean flour had a significant effect on the taste of milkfish sausage.

The resulting taste between treatments is due to components present in the formulation such as fish meat, kidney bean, and marinades. Garlic components containing *Alicin* compounds in the formulation can contribute flavor to sausage (Siwi, 2015). Red bean contains lipoxygenase enzyme that hydrolyses fat and produces methanal compounds attributed to the unpleasant smell (Agusta, 2020). Therefore, the resulting sausage tasted similar to red bean and was less liked by the panelists.

Aroma

In the aroma assessment of milkfish sausage, the highest average value obtained was 3.88% in the like category, while the lowest was 3.25 in the somewhat like category. Duncan test showed that the addition of red bean flour had no significant effect on the aroma of milkfish sausage.

The aroma of the sausage is attributed to the components of milkfish, red bean, and spices. Components such as garlic contain *Alicin* which contributes aroma to sausages (Siwi, 2015). Adding a large proportion of red bean to the sausage produce an aroma of unpleasant or beany flavor due to the presence of lipoxygenase enzymes (Silaban et al., 2023).

Chewiness

In the chewiness assessment of milkfish sausage, the highest average value obtained was 3.88% in the like category, while the lowest was in the dislike category. Duncan test showed that the addition of red bean flour has a significant effect on the chewiness of milkfish sausage.

The chewiness of milkfish sausage can be affected by the addition of ingredients and the resulting water content. It may increase or become denser when more red bean flour is added to the treatment.

Texture

In the texture assessment of milkfish sausage, the highest average value was 3.84% in the like category while the lowest was 2.69% in the dislike category. Duncan test showed that the addition of red bean flour had a significant effect on the texture of milkfish sausage.

The texture of the sausage is influenced by the water content. With higher water content, the texture becomes mushy or soft, while lower water content makes the texture hard or dense (Laki & Ilminingtyas, 2022). This implies that in producing sausages, the water content must be in line with SNI standards. Adding an excessive amount of red bean flour changes the texture of the sausage due to the starch and amylopectin content. In addition, the high protein content of red bean inhibits the process of gelatinization (Debora et al., 2023).

Aftertaste

In the aftertaste assessment of milkfish sausage, the highest average value obtained was 3.94% in the somewhat like category, while the lowest was 2.75% in the dislike category. Duncan showed that the addition of red bean flour had a significant effect on the aftertaste of milkfish sausage. Aftertaste which tastes a bit bitter is caused by the components of red bean specifically acrylamide (Kifayah & Basori, 2015).

Overall

In the overall assessment of milkfish sausage, the highest average score was 4.06 in the like category, while the lowest was 2.88% in the dislike category. Duncan test showed that the addition of red bean flour had a significant effect on milkfish sausage.

CONCLUSION

In conclusion, the addition of red bean flour had a significant effect on the chemical properties of water, protein, carbohydrates, dietary fiber, and antioxidant content. However, no significant effect was observed on the parameters of ash and fat content. The treatment also affected microbial properties because the greater the amount added, the fewer the colonies produced. The addition of red bean flour had a significant effect on organoleptic acceptance on the parameters of color, taste, texture, chewiness, aftertaste, and overall but no significant effect was observed on the parameter of aroma.

CONFLICT OF INTEREST

This article titled "Quality Profile and Antioxidant Activity of Milkfish Sausage (*Chanos chanos*) with the Addition of Red Bean Flour (*Phaseolus vulgaris* L.)" is free from any conflict of interest.

REFERENCES

Akbar, A., Nurmiah, S., & Susanti, G. (2021). Proportion of use of Banana Peel (Musa paradisiaca L) and Milkfish Meat (*Chanos Chanos*) in the Manufacture of Abon. *Jurnal Lutjanus*, *26*(1), 20–28.

AOAC. (2005). *Official Methods of Analysis of AOAC International*. AOAC International.

Badan Standarisasi Nasional. (2006). SNI 01-2332.3 Microbiological Test Method-Part 3: Determination of Total Plate Count (TPC) in Fishery Products.

Cahyani, K. (2011). *Kajian Kacang Merah (Pheseolus vulgaris) Sebagai Bahan Pengikat Dan Pengisi Pada Sosis Ikan Lele*. [Skripsi] Universitas Sebelas Maret.

Dewi, E., Purnamayati, L., & Kurniasih, R. (2019). Karakteristik Mutu Ikan Bandeng (*Chanos chanos Forsk*.) Dengan Berbagai Pengolahan. *JPHPI*, *22*(1).

Fridalni, N., Minropa, A., & Febriyanti. (2019). Pengenalan Dini Penyakit Degeneratif. *Jurnal Abdimas Saintika*, *1*(1). https://dx.doi.org/10/30633/jas.v1i1.483

Ganesan, K., & Xu, B. (2017). Polyphenol-rich dry common beans (*Phaseolus vulgaris L.)* and their health benefits. *International Journal of Molecular Sciences*, *18*(11). https://dx.doi.org/10/30633/jas.v1i1.483

Grahito, A. (2018). *Pengaruh Substitusi Tepung Biji Kacang Merah (Pheseolus vulgaris) Pada Pembuatan Sosis Ikan Gabus (Ophiocephalus striatus) Terhadap Sifat Fisika Kimia Dan Organoleptik*. [Skripsi] Universitas Brawijaya.

Hafiludin. (2015). Analisis Kandungan Gizi Pada Ikan Bandeng Yang Berasal Dari Habitat Yang Berbeda. *Jurnal Kelautan*, *8*(1). https://doi.org/10.21107/jk.v8i1.811

Handajani, A., Roosihermiatie, B., & Maryani, H. (2010). Faktor-faktor Yang Berhubungan Dengan Pola Kematian Pada Penyakit Defeneratif Di Indonesia. *Buletin Penelitian Sistem Kesehatan*, *13*(1), 42–53. https://doi.org/10.22435/bpsk.v13i1 Jan.2755

Hijriyanti, H., Pestariati, & Sasongkowati. (2020). Perbandingan Media Alternatif Kacang Merah Dengan Median Mannitol Salt Agar Terhadap Pertumbuhan Bakteri Staphylococcus aureus. *Jurnal Analisis Kesehatan Sains*, *9*(1).

Kifayah, R., & Basori. (2015). *Cookies Berbasis Pati Garut (Marantha arundinaceae L.) Dengan Tepung Bekatul Dan Tepung Whole Wheat Sebagai Sumber Serat*. *12*(1). https://doi.org/10.21070/nabatia.v12i1.1597

Laki, L., & Ilminingtyas, D. (2022). Pengolahan Sosis Ikan Bandeng (Chanos chanos) dengan Penambahan Serbuk Daun Kelor (Moringa oleifera Lamk) untuk Meningkatkan Kandungan Serat. *Jurnal Agrifoodtech*, *1*(2), 50. https://doi.org/10.56444/agrifoodtech/vli2.307

Natasia, E. M. (2022). *Karakteristik Fisikokimia Sosis Ikan Bandeng (Chanos chanos) Dengan Penambahan Pasta Wortel (Daucud carota)*. [Skripsi] Universitas Hasanuddin.

Palijama, S., Breemer, R., & Topurmera, M. (2020). Karakteristik Kimia dan Fisik Bubur Instan Berbahan Dasar Tepung Jagung Pulut dan Tepung Kacang Merah. *AGRITEKNO: Jurnal Teknologi Pertanian*, *9*(1), 20–27.

Perwita, E., Tri, P, L., & Anna, C. (2021). Proporsi Tepung Kacang Merah (*Phaseolus vulgaris L.)* Dan Bubuk Daun Kelor (*Moringa oleifera L.)* Terhadap Sifat Organoleptik Snack Bar Labu Kuning. *Jurnal Tata Boga*, *10*(2), 303–313.

Prastiyani, H. (2021). *Uji Kadar Flavonoid Total Dan Aktivitas Antioksidan Ekstrak Kulit Buah Naga Merah (Hylocereus polyrhizus)*. [Skripsi] Universitas dr. Soebandi.

Pratiwi, H., & Panunggal, B. (2016). Analisis Total Fenol Dan Aktivitas Antioksidan Pada Yoghurt Ganyong (Canna edulis) Sinbiotik Dengan Substitusi Kacang Merah Phaseolus vulgaris L). In *Journal of Nutrition College* *5*(1). https://doi.org/10.14710/jnc.v5i1.16358

Rais, M., & Ratnawaty, F. (2019). Analisis Kandungan Gizi Dan Uji Organoleptik Pada Bakso Tempe Dengan Penambahan Daun Kelor (*Moringa oleifera*). *Jurnal Pendidikan Teknologi Pertanian*, *5*, 189–200. https://doi.org/10.26858/JPTP.V5I0.9080

Ratna, W. O., Muhammad, D., & Sadimantara, S. (2022). The Effect of Peanut Flour (*Arachis hypogaea L.*) and Purple Sweet Patio (*Ipomoea batatas L. Poiret*) Formulation on the Organoleptical Assessment and Nutritional Value of Cookies. *Journal of Agricultural Sciences*, *02*(03), 189–196.

RISKESDAS. (2018). *Laporan Nasional*.

Rompis, J. E., & Londok, J. J. (2022). Bahan Pengikat Dan Bahan Pengisi Sosis Daging Sapi. In *e-book*. Cv. Patra Media Grafindo. Bandung.

Rusilati, Kusharto, Clara, M. (2007). *Sehat Dengan Makanan Berserat*. Agromedia Pustaka. Jakarta.

Scherer, R., & Godoy, H. T. (2009). Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. *Food Chemistry*, *112*(3), 654–658. Elsevier. https://doi.org/10.1016.j.foodchem.2008.06026

Setyaningsih et all. (2010). Analisis Sensoris untuk Industri Pangan dan Agro. IPB Press. Bogor.

Siwi, R. (2015). *Perbedaan Kualitas Dan Nilai Gizi Sosis Jamur Tiram Substitusi Daging Ayam*. [Skripsi] Universitas Negeri Semarang.

Syaifuddin. (2015). *Uji Aktivitas Antioksidan Bayam Merah (Alternanthera amoena Voss.) Segar Dan Rebus Dengan Metode DPPH (1,1-diphenyl-2-picylhydrazyl)*. [Skripsi] Universitas Islam Negeri Walisongo.

Ulfah, S. (2016). *Uji Aktivitas Antioksidan Ekstrak Daun Rambutan (Nephelium lappaceum Linn) Dengan Metode DPPH (2,2-Difenil-1-Pikrilhidrazil)*. [Skripsi] Uin Syarif Hidayatullah.

Wassalwa, M. (2016). Pengaruh Waktu Infusa dan Suhu Air yang Berbeda Terhadap Aktivitas Antioksidan dan Vitamin C pada Infused Water Kulit Pisang. In *Jurnal Ilmiah Mahasiswa Pendidikan Biologi* (Vol. 1, Issue 1).