Effect of Fermentation Time on Nutrition Content, Physical Properties, pH, Amino Acids, Fatty Acids Composition and Organoleptics on Fermented Mackerel Sausage (*Rastrelliger kanagurta* Cuvier) Characteristics

Diana Nur Afifah[a,b*], Intan Ratna Sari[a], Nanda Trisna Prastifani[a], Faizah Fulyani[c], Gemala Anjani[a,b], Nurmasari Widyastuti[a,b], and Vivilia Niken Hastuti [a,b]

[a] Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, Jl. Prof. H. Soedarto, SH, Tembalang, Semarang 1269, Indonesia
[b] Center of Nutrition Research (CENURE) Jl. Prof. H. Soedarto, SH, Tembalang, Semarang 1269, Indonesia
[c] Department of Medicine Biology and Biochemistry, Faculty of Medicine, Universitas Diponegoro, Jl. Prof. H. Soedarto, SH, Tembalang, Semarang 1269, Indonesia
*Corresponding author
d.nurafifah.dna@fk.undip.ac.id
Tel: +62 877-7038-0468

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Abstract

Fermentation increases the functional value of food. During fermentation, chemical changes occur in organic substrates, such as carbohydrates, proteins, and fats due to enzyme activities of microorganisms. Functional foods containing unsaturated fatty acids are an alternative for preventing cardiovascular disease. The Indian mackerel (*Rastrelliger kanagurta* Cuvier) is rich in protein, polyunsaturated fatty acids, and non-essential and essential amino acids. Fish that are processed into sausage and fermented can be used as an alternative functional food to prevent cardiovascular disease. This study analysed the effect of fermentation time on nutritional content (carbohydrate, protein, fat, water, ash, amino acid, and fatty acid contents), physical properties, pH, and organoleptic properties in fermented mackerel sausage. This was a completely randomized experimental study with three fermentation times of 1, 2, and 3 days, and 0 days as a control. Fermentation was carried out spontaneously with 1.9% salt and sugar without adding a bacterial culture. The drying temperature was 50°C for 3 hours, and the fermentation temperature was 35°C. The fermentation duration of mackerel sausage affected the nutritional contents (carbohydrates, protein, fat, water, ash, amino acids, and fatty acids), physical properties (hardness and chewiness), pH, and organoleptic properties (colour, aroma, taste, and texture). Overall, the longer the fermentation time, the higher the carbohydrate, protein, fat, total ash content, total amino acid, total fatty acid, hardness and decreased organoleptic (colour, aroma, taste, texture), elasticity, and water contents. The best formulation for fermented mackerel sausage was 1 day of fermentation time.

Keywords: Indian mackerel (*Rastrelliger kanagurta* Cuvier); Sausage; Nutritional content; Physical properties; Organoleptic; Fermentation time

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1 Introduction

Cardiovascular disease (CVD) is a major contributor to global morbidity and mortality (Murray et al., 2012). CVD describes a combination of several diseases that attack the blood vessels and heart. One of the manifestations of CVD is coronary heart disease (CHD), which occurs due to hardening or atherosclerosis of the coronary arteries resulting in a decrease of blood supply to the heart muscle (Frak et al., 2022). In 2016, nearly 30% of the world's population or about 17.9 million people died from CVD; 85% of these deaths were due to heart attacks and strokes. According to the results of Basic Health Research in 2018, almost 1.5% of Indonesia's population was diagnosed with heart disease, and Central Java Province was fourth overall with a prevalence of 1.4% (Indonesia Basic Health Research, 2018).

Diet plays an important role in reducing the prevalence of CVD. Consumption of cardioprotective food groups and adequate and regular exercise help prevent heart disease (Casas et al., 2018; Jung et al., 2018). Cardioprotective foods included those high in fibre, antioxidants, vitamins, minerals, polyphenols, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) (Casas et al., 2018; Jung et al., 2018). One food alternative that contains low saturated fat and high protein is found in locally based foods, such as fish. Indian mackerel (*Rastrelliger kanagurta* Cuvier) is an easily available food source that is priced relatively inexpensively compared to other marine fish. Mackerel is usually prepared by frying. However, this is considered a poor method for CHD sufferers because frying forms trans-fats, which can increase the risk of CHD.

Fish is a perishable food. Thus, an alternative to processing mackerel is to prepare sausage. Sausages are made from processed meat that is crushed and preserved by salting (Mohan, 2014). Processed red meat in sausage contains high levels of saturated fatty acids (SFA) that can cause blockage of blood vessels or atherosclerosis (Ragino et al., 2019). Mackerel is rich in PUFA contents, such as omega-3 fatty acids, eicosapentaenoic acid, and docosahexaenoic acid (Marichamy et al., 2009; Sonavane et al., 2017). Regular consumption of these fats can reduce the progression of plaque formation in the arteries (Bäck, 2017). In addition, fish protein is composed of complete types of non-essential and essential amino acids. Amino acids repair damaged tissue, protect the liver from toxic substances, lower blood pressure, and regulate cholesterol metabolism (Hoffer, 2016; Nurjanah et al., 2015). One of the traditional methods of preserving meat is fermentation of meat products that used for improving safety, shelf life, organoleptic, and nutritional attributes (Lazic et al., 2019). Fermented sausage is smoked with liquid smoke before being incubated or fermented at 35^{o}C. This smoking method helps the sausage to become drier, thereby extending shelf life. In addition, fermentation increases the functional value of the food. During fermentation, proteolytic enzymes degrade proteins into amino acids and peptides, while lipolytic enzymes hydrolyse fats to become free fatty acids (Bou et al., 2017; Juturu & Wu, 2016). According to previous research, free amino acids, such as leucine, valine, and lysine, increase during fermentation (Bou et al., 2017). In addition, the lactic acid produced during fermentation sharpens and strengthens the taste of the sausage so that it forms a sour taste and a decrease in pH, which causes protein coagulation and a hard texture as a characteristic of fermented sausage (Mohan, 2014). Therefore, fermented sausage is a source of processed meat that has the potential to become a functional food. Functional foods contain unsaturated fatty acids (UFA), such as PUFAs and MUFAs, and are an alternative food that prevents CVD (Damanik et al., 2018). The fermented fish and their health benefit, as well as the contribution of microorganism that produces many nutrition element with higher bioavailability linked with several beneficial properties (Zang et al., 2020).

Fermentation time affects the quality of the product because fermentation changes the chemical composition and physical properties of the product. In this study, the selected fermentation times were 0, 1, 2, and 3 days. The choice of fermentation times was based on a study reporting that 1 day of fermentation is the optimal duration. Thus, we were interested in conducting research on the nutritional content, physical properties, pH, and organoleptic properties of fermented mackerel sausage as a functional food product that can be used as an alternative to prevent CHD based on variations in fermentation time (Xiong & Mikel, 2001).

2 Materials and Methods

A completely randomized experimental study was performed using four fermentation times, consisting of control (0 day), 1, 2, and 3 days for the fermented mackerel sausage. The variables were tested at the Diponegoro University Nutrition Science Laboratory, UPT Integrated Laboratory of Diponegoro University, Bogor Saraswanti Laboratory, and the Fishery Product Quality Testing and Application Center (BP2MHP/Badan Pengujian dan Penerapan Mutu Hasil Perikanan).

2.1 Preparation of the Fermented Mackerel Sausage Samples

The fresh mackerel flesh was purchased from Rejomulyo fish market, Semarang City, Central Java, Indonesia. The raw materials for preparing the fermented mackerel sausage consisted of 48% of the total weight of mackerel, 1.9% salt and sugar, 14.6% tapioca flour, 9.7% corn oil, 1.5% garlic and onion, 14.6% ice, 2% lime, 3.8% egg white, 15% carrageenan flour, liquid smoke, and sausage sleeve. Commercial salt, sugar, tapioca flour, corn oil, garlic, onion, lime, egg white, and carrageenan flour were obtained from TBK Fortune, Semarang City. The liquid smoke was produced by Fronthea from Diponegoro University (Swastawati et al., 2019). The sausage casing was purchased from Harmony mart, Semarang City. The mackerel sausage was prepared in three stages. Stage 1 included the manufacturing of the fermented mackerel sausage, mixing all raw material sequentially and pouring into an edible sausage casing; stage 2 was smoking the mackerel sausage by soaking it in a 5% liquid smoke solution for 30 min at a set temperature of ± 10^{o}C. The mackerel sausage was subsequently placed in an oven at 50^{o}C for 3 hours; and stage 3 was the fermentation process from day 0 to day 3 in an incubator at 35^{o}C. Fermentation was carried out spontaneously with no bacterial culture.

2.2 Proximate Analysis, Physical Properties, and pH

Testing of the physical properties and pH was carried out at UPT Integrated Laboratory of Diponegoro University. Testing of fat, protein, water, and ash contents was carried out at the Fishery Product Quality Testing and Application Center (BP2MHP/Badan Pengujian dan Penerapan Mutu Hasil Perikanan) Semarang. All analyses were performed in triplicate. Protein content was analysed by the Kjeldahl method (Magomya et al., 2014). Fat content was determined using the Soxhlet method (Zarnowski & Suzuki, 2004). Analysis of water content used the oven-drying method. Ash content was determined by heating samples of sausage at 550 ^{o}C for 5 hours in a muffle furnace and weighing the resultant ash. The carbohydrate content analysis was determined by difference (AOAC International., 2006). The pH was measured using a digital pH meter (pH meter TPX-90i Chemical Laboratories Co., Ltd). The physical properties were determined using a Brookfield CT3^{TM} texture analyser (Brookfield Inc, USA). The optimal treatment formulation was decided using the de Garmo method (DeGarmo et al., 1993).

2.3 Analysis of Amino Acid and Fatty Acid Composition

The analysis of the fatty acid composition was carried out using gas chromatography (GC) in duplicate at the Saraswanti Laboratory, Bogor. The GC analyses were performed on 7890A Gas Chromatography System (Agilent Technologies, California, US) equipped with flame ionization detector and splitless injector (1 μL). Injector and detector temperature were set at 270 ^{o}C and 280 ^{o}C, respectively. The column used was a DB-23 (60 m × 0.25 mm, with film thickness of 0.25 μm)(J and W Scientific, Folsom, CA). The GC oven program was as follows: 130 ^{o}C (hold 2 min), to 170 ^{o}C at 6.5 ^{o}C /min (hold 5 min), to 215 ^{o}C at 2.75 ^{o}C/min (hold 12 min), to 230 ^{o}C at 30 ^{o}C/min (hold 30 min). Helium and nitrogen of ultrahigh purity grade were used as carrier gases at flow rates of 11.07 and 31.24 mL/min. Fatty acid identification was determined by comparing retention time of the peaks with the respective external standards. The concentration of fatty acids was calculated with the following equation from AOAC 996.06 (AOAC International, 2002). Five fatty acids were analysed based on the double-bond group, such as saturated fatty acids (SFA), polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), unsaturated fatty acids (UFA), and omega-3 fatty acids. The percentages of saturated, unsaturated, monounsaturated, and polyunsaturated fatty acid were calculated as follows using Eqs.1-4:

$$SFA(\%) = \sum \text{SFA injection}(\%) \times \text{fatty acid}(\%) \quad (1)$$

$$UFA(\%) = \sum \text{UFA injection}(\%) \times \text{fatty acid}(\%) \quad (2)$$

$$MUFA(\%) = \sum \text{MUFA injection}(\%) \times \text{fatty acid}(\%) \quad (3)$$

$$PUFA(\%) = \sum \text{PUFA injection}(\%) \times \text{fatty acid}(\%) \quad (4)$$

The amino acid composition was determined by high performance liquid chromatography (HPLC) in duplicate at the Saraswanti Laboratory Bogor, using a Shimadzu CBM 20A chromatograph. Fifty milligrams of dried sample were placed in a test tube with ground glass stopper and mixed with 5 mL of citrate phosphate buffer (pH 4.6) containing 10 mg β-glucosidase (37 units). The sample was hydrolysed at 37^{o}C for 4 h, and 5 mL of ethanol was added. After centrifugation for 10 min, 8 mL of the supernatant was dried in a vacuum evaporator. The compounds were identified by retention times and spectra in comparison with standards and quantified by the peak area. Fifteen amino acids were analysed; 8 of 15 were the essential amino acids histidine, threonine, leucine, lysine, arginine, valine, isoleucine, and phenylalanine, whereas the remaining seven were non-essential amino acids proline, tyrosine, glycine, alanine, aspartic acid, glutamic acid, and serine. Amino acid requirements were calculated based on the recommendations of the World Health Organization (WHO) for each group, as shown in Table 1 (World Health Organization et al., 2007).

2.4 Organoleptic Testing

Organoleptic testing was carried out by semi-trained panellists consisting of 30 students from the Department of Nutrition Science Diponegoro University, who agreed and signed informed consent. The organoleptic testing was conducted compliance with all regulations and confirmation that informed consent was obtained. Attributes tested were colour, taste, aroma, and texture. The evaluation of the hedonic test was categorized into a scale of 1 to 4 (1= really dislike, 2 = dislike, 3 = like, 4 = really like).

2.5 Data Analysis

The data analysis was performed using SPSS statistics software 25.0 for Windows (IBM, Chicago, IL, USA). The normality of the data was tested using the Shapiro-Wilk test because the data were $<$50. Analysis of the proximate data, physical properties, pH, and organoleptic properties was by analysis of variance (ANOVA) and the Kruskal-Wallis test followed by the Bonferroni and Mann-Whitney tests. ANOVA and the Kruskal-Wallis test were performed for the amino acid and fatty acid analyses followed by the Games-Howell post hoc test. A P-value $\leq$ 0.05 was considered significant.

3 Results and Discussion

3.1 Proximate Composition

Mackerel sausage fermented for 3 days had the highest fat, protein, ash, and carbohydrate contents compared to the other treatments. In contrast, mackerel sausage fermented for 3 days had the lowest water content compared to the other treatments. Significant differences in the contents of fat, protein, water, ash, and carbohydrate were observed in mackerel sausage fermented for different durations, as shown in Table 2.

A significant difference in fat content was observed corresponding to the number of days of fermentation. The fish sausage contained 3.37% fat on day 0. Fat content increased to 4.41% after 3 days of fermentation. This was in accor-

Table 1: Amino Acid Requirements

Amino Acid	Requirements	
	mg/g protein/day	mg/kg weight/day
Histidine	15	10
Isoleucine	30	20
Leucine	59	39
Lysine	45	30
Methionine	16	10
Phenylalanine + Tyrosine	38	25
Threonine	23	15
Valine	39	26

dance with SNI 7755: 2013, which states that the maximum fat content in fish sausage should be 7.0%. The increase in fatty acids was due to secondary lipolytic activity of lactic acid bacteria (LAB), which actively release fatty acids from fat molecules during fermentation (Raveschot et al., 2018).

The fermented mackerel sausages contained 15.34% protein before fermentation (control day/day 0), and increased to 33.91% after 3 days of fermentation. This was in accordance with SNI 7755: 2013, which states that fish sausage should contain at least 9.0% protein. This increase in protein was due to the increased level of total nitrogen in the fermented mackerel sausage and an increase in the population of LAB. The increase in nitrogen was caused by LAB proteases, which actively breakdown protein into amino acids and peptides, resulting in an increase in nitrogen and protein (Raveschot et al., 2018).

The water content in the fermented mackerel sausage varied depending on the fermentation duration. The water content, which ranged from 65.32 to 22.49% of the total nutrition component, gradually decreased from days 0 to 3 of fermentation. This result was due to the raw materials added as well as evaporation during the smoking and fermentation processes, which dried out the surface of the sausage (Savijoki et al., 2006). The maximum water content of fish sausage should be 68.0% according to SNI 7755: 2013. The results of this study were in accordance with this value, as total water content in mackerel sausage prior to fermentation (0 day/control) was 65.32%. Before fermentation, some water molecules form hydrates with other molecules (oxygen, nitrogen, carbohydrates, protein, and other organic compounds) so that the water turns into free water. The free water will evaporate significantly due to enzyme activity breaking bonds of water into free water during the fermentation processes.

The ash content of the mackerel sausage was significantly different during the 3 days of fermentation. Ash content indicates the presence of minerals in food; the higher the ash content, the higher the level of minerals in the food. Ash content increased after the third day of fermentation compared to day 0 due to the accumulation of minerals in the product during fermentation. Not all minerals are burned during the ashing process; thus, ash accumulates in the product (De Vuyst & Vandamme, 1994). The maximum ash content of fish sausage should be 2.5%, according to SNI 7755:2013. This percentage is in accordance with the results of this study, as total ash content in mackerel sausage prior to fermentation (0 day/control) was 2.05%.

3.2 Amino Acid Composition

The main amino acids in mackerel were glutamic acid, aspartic acid, and lysine. Glutamic acid was the most plentiful non-essential amino acid in mackerel at 3,583.73 mg/100 g, while the es-

Table 2: Proximate analysis of the fermented mackerel sausages

Formula	(Mean ± Standard Deviation) (%) n = 3				
	Fat Content	Protein Content	Water Content	Ash Content	Carbohydrate
F0	3.37 ± 0.03	15.34 ± 0.30	65.32 ± 0.14	2.05 ± 0.02	13.91 ± 0.48
F1	3.55 ± 0.02	20.62 ± 0.28	53.79 ± 0.50	2.84 ± 0.02	19.18 ± 0.81
F2	4.20 ± 0.03	31.97 ± 0.60	34.83 ± 0.09	4.10 ± 0.02	24.87 ± 0.68
F3	4.41 ± 0.02	33.91 ± 0.32	22.49 ± 0.10	4.64 ± 0.03	34.54 ± 0.40
p	0.015*	0.001**	0.015*	0.015*	0.001**

** One-way ANOVA; *Kruskal-Wallis test.

sential amino acid lysine was 2,023.94 mg/100 g (Table 3).

Threonine, leucine, valine, isoleucine, and serine levels were not significantly different between the fermentation treatments ($p > 0.05$). However, significant differences in the levels of histidine, lysine, phenylalanine, proline, tyrosine, aspartic acid, glycine, alanine, arginine, and glutamic acid were observed between the fermentation treatments ($p < 0.05$). The highest total amino acid content was detected in mackerel sausage fermented for 3 days at 10,940.85 mg/100 g.

Total amino acid content in the fermenting and control sausage was 10,935.52 mg/100 g on day 0. Total amino acid content decreased to 9,656.64 mg/100 g on day 1 of fermentation. Total amino acid content increased from day 1 to 10,364.19 mg/100 g on day 2 of fermentation. The levels of some amino acids and total amino acid content increased on day 3 of fermentation. However, the levels of proline, aspartic acid, glycine, and glutamic acid decreased. The main amino acid in mackerel was glutamic acid (3,583.73 mg/100 g), which agreed with previous studies conducted by Nurjanah et al. (2015) and Oluwaniyi et al. (2010) that mackerel is rich in glutamic acid. The high glutamic acid content in mackerel is caused by deamination of glutamine (Nurjanah et al., 2015).

Total amino acid content in the fermented sausages increased to 10,940.85 mg/100 g on day 3, which was higher than the total amino acid content in mackerel, the control sausage, or the fermented sausage on days 1 and 2. Changes in the amino acid levels in fermented mackerel sausage differed among the amino acid groups (Table 4).

Total amino acid content decreased on day 1 of fermentation compared to the unfermented mackerel sausage because of the high level of amino acid anabolism needed for LAB to adapt and grow (Nursyam, 2011; Sulaiman et al., 2016). Marathe and Ghosh (2009) showed that LAB require peptides, vitamins, and protein catabolic compounds, such as nitrogen and amino acids, to grow. Total amino acid content increased on days 2 and 3 of fermentation. The increase in total amino acid content during fermentation was caused by LAB hydrolysing the protein components into amino acids (Nursyam, 2011).

The WHO suggests daily lysine intake of 45 mg/g protein (World Health Organization et al., 2007) or 30 mg/kg body weight (World Health Organization et al., 2007) (Table 1). According to the Nutritional Adequacy Rate 2019 (Republic of Indonesia, 2019), adult males must consume 65 g protein/day and adult females must consume 60 g/day. An average 100 g fermented mackerel sausage contained 1.066,58 mg lysine, which equalled 36% of the recommended daily nutrient intake of lysine for an adult male and 40% for an adult female.

3.3 Fatty Acid Composition

The major fatty acids detected in mackerel were UFA, including PUFAs of 60 mg/100 g, MUFAs of 25 mg/100 g, SFAs of 60 mg/100 mg, and omega-3 fatty acids of 42.25 mg/100 g (Table 5). Unsaturated fatty acids, PUFAs, and MUFAs did not differ after fermentation ($p > 0.05$), whereas

Table 3: Amino acid composition of mackerel (mg/100 g)

Amino Acid (AA)	Amino Acid Levels
Essential Amino Acids	
Histidine	1,094.68 ± 1.38
Threonine	1,149.92 ± 0.69
Leucine	1,731.73 ± 2.62
Lysine	2,023.94 ± 2.97
Valine	1,147.63 ± 0.11
Isoleucine	1,040.33 ± 0.82
Phenylalanine	970.86 ± 1.25
Arginine	1,355.77 ± 1.39
Total of Essential Amino Acids	10,514.86
Non-Essential Amino Acids	
Proline	633.81 ± 0.34
Tyrosine	760.84 ± 43.92
Aspartic Acid	2,311.91 ± 5.32
Glycine	931.22 ± 1.51
Alanine	1,238.97 ± 2.45
Glutamic Acid	3,583.73 ± 5.40
Serine	902.36 ± 2.33
Total of Non-Essential Amino Acids	10,362.84
Total of AA	20,877.70

SFAs and omega-3 fatty acid levels were significantly different after fermentation ($p < 0.05$). The third day of fermentation recorded the highest total fatty acid content of 11,428.73 mg/100 g. Total fatty acid content was 11,130.60 mg/100 g on day 0 of fermentation (control). The PUFA and MUFA levels in the control sausage were higher than the SFA levels. Total fatty acid content decreased to 6.921,9 mg/100 g on day 1 of fermentation, whereas fatty acid levels increased on days 2 and 3 of fermentation (Table 6).

The results of this study showed that mackerel flesh contained high unsaturated fatty acid contents, namely PUFAs of 60 mg/100 g and MUFAs of 25 mg/100 g. Mackerel flesh also contained SFAs of 60 mg/100 mg and omega-3 fatty acids of 42.25 mg/100 g. This composition differed from those reported by Marichamy et al. (2009), Nurjanah et al. (2015), and Sonavane et al. (2017) because the species, season, environment, and quality of feed influence the composition of fatty acids in mackerel (Özogul & Özogul, 2007; Sonavane et al., 2017).

Adding corn oil as a substitute for animal fat during fermentation increased the levels of SFAs, MUFAs, and PUFAs in the fermented sausage, compared to raw mackerel flesh because corn oil naturally contains PUFAs (54.7% of total fatty acids), MUFAs (27.6% of total fatty acids), and SFAs (13% of total fatty acids) (St-Onge & Travers, 2016). The levels of SFAs, PUFAs, MUFAs, and omega-3 fatty acids decreased on day 1 of fermentation because the oxidation process was faster due to high unsaturation. The oxidation rate is determined by the quality of the flesh, temperature, exogenous components (seasoning, nitrate, and salt), and the antioxidant protective effect of the food ingredients (Nassu et al., 2003; Talon et al., 2000; Visessanguan et al., 2006). Total fatty acids increased gradually on days 2 and 3 of fermentation. The increase in fatty acids indicates lipolysis by LAB (Visessanguan et al., 2006), as LAB secrete lipolytic enzymes to breakdown fat into free fatty acids

Table 4: Amino acid composition of mackerel (mg/100 g)

Amino Acids (AA)	Duration of Mackerel Sausage Fermentation					p-Value
	Fresh mackerel (48%)[c]	Day 0	Day 1	Day 2	Day 3	
Essential Amino Acids						
Histidine	52.,44 ± 0.66*	611.82 ± 3.44*	289.79 ± 0.49*	331.05 ± 0.07*	491.60 ± 5.47*	$p < 0.001^a$
Threonine	551.96 ± 0.33	662.22 ± 1.61	541.71 ± 1.96	555.83 ± 2.15	651.01 ± 6.09	$p = 0.068^b$
Leucine	831.23 ± 1.26	885.17 ± 10.13	856.32 ± 2.04	865.86 ± 1.71	966.45 ± 8.50	$p = 0.068^b$
Lysine	971.49 ± 1.43*	1,112.45 ± 5.61*	1,038.63 ± 5.41*	1,093.18 ± 3.95*	1,067.94 ± 7.38	$p < 0.001^a$
Valine	550.86 ± 0.57	617.94 ± 1.90	551.75 ± 1.41	575.68 ± 1.29	671.10 ± 4.46	$p = 0.081^b$
Isoleucine	499.36 ± 0.40	517.64 ± 12.38	508.93 ± 0.80	517.57 ± 0.24	598.37 ± 8.05	$p = 0.101^b$
Phenylalanine	466.01 ± 0.60*	510.66 ± 13.71	410.96 ± 0.68*	416.47 ± 0.74*	530.09 ± 6.30*	$p < 0.001^a$
Arginine	650.77 ± 0.66*	557.08 ± 5.22*	554.14 ± 2.36*	603.68 ± 0.78*	676.60 ± 6.38*	$p < 0.001^a$
Total Essential AA	5,047.13	5,474.98	4,752.23	4,962.32	5,653.16	
Non-Essential Amino Acids						
Proline	304.23 ± 0.16*	356.50 ± 3.20*	329.52 ± 0.74*	422.45 ± 0.09*	385.68 ± 5.40	$p < 0.001^a$
Tyrosine	365.20 ± 21.08	310.06 ± 0.69*	272.56 ± 0.60*	268.15 ± 0.30*	341.72 ± 3.28*	$p = 0.001^a$
Aspartic Acid	1,109.71 ± 2.56*	1,175.20 ± 1.42*	1,061.64 ± 2.98*	1,122.92 ± 1.32*	1,082.61 ± 9.98	$p < 0.001^a$
Glycine	446.98 ± 0.73*	524.43 ± 0.98*	440.00 ± 1.73*	603.00 ± 1.68*	550.13 ± 5.35*	$p < 0.001^a$
Alanine	594.70 ± 1.18*	722.24 ± 3.10*	644.61 ± 2.73*	732.06 ± 2.50*	700.94 ± 5.90*	$p < 0.001^a$
Glutamic Acid	1,720.19 ± 2.60*	1,834.40 ± 5.30*	1,709.17 ± 8.41*	1,794.31 ± 5.96*	1,681.46 ± 11.16*	$p < 0.001^a$
Serine	433.13 ± 1.12	537.71 ± 8.99	446.91 ± 1.11	461.98 ± 0.75	545.15 ± 6.70	$p = 0.078^b$
Total Non-Essential AA	4,974.16	5,460.54	4,904.41	5,401.87	5,287.69	
Total AA	10,021.30	10,935.52	9,656.64	1,364.19	10,940.85	

Note: * significant difference in mean value. a = ANOVA; b = Kruskal-Wallis test; c, indicates the amount of mackerel in the fermented sausage.

during fermentation.
The American Heart Association (AHA) recommends consuming 1 g of omega-3 PUFAs/day (Cao et al., 2015). An average 100-g serving of fermented mackerel sausage contained 416.5 mg of omega-3 fatty acids, which is equal to 42% of the omega-3 daily intake recommended by the AHA. Therefore, it is suggested to consume at least two or three fermented mackerel sausages per day to meet the recommendation.

3.4 Physical Characteristics (Hardness and Springiness)

The 3-day fermentation treatment produced the hardest texture of mackerel sausage compared to the other treatments, whereas the 1-day fermentation produced sausage with the best springiness compared to the other treatments. This is consistent with a previous study on fermented catfish sausage, in which the levels of hardness and springiness were not much different (Nisa & Wardani, 2016). Significant differences in the hardness and springiness levels were observed in fermented mackerel sausage compared to the control (Table 7).

3.5 Acidity Level (pH)

The 3-day fermented mackerel sausage had the lowest pH compared to the other treatments. In this study, fermented mackerel sausage experienced a mild decrease in pH. This result agreed with a study on fermented catfish sausage (Nisa & Wardani, 2016), in which a relatively small pH decrease was reported. Significant differences in pH were observed on the different days of fermentation (Table 8).

3.6 Organoleptic Test (Acceptability)

A hedonic test was used to rate how panellists assessed mackerel sausage after the different fermentation times. The panellists were asked to evaluate the colour, aroma, taste, and texture

Table 5: Fatty acid composition of mackerel (mg /100 g)

Fatty Acids	Level of Fatty Acids
Saturated fatty acid (SFA)	60.00 ± 0.00
Monounsaturated fatty acid (MUFA)	25.00 ± 7.07
Polyunsaturated fatty acid (PUFA)	60.00 ± 0.00
Unsaturated fatty acid	80.00 ± 0.00
Omega-3 fatty acid	42.25 ± 1.06
Total Fatty Acids	187.25

Table 6: Fatty acid composition in mackerel (48%) and fermented sausage (mg/100 g)

Fatty Acids	1	Duration of Mackerel Sausage Fermentation				*P-Value*
	Fresh mackerel (48%)[c]	Day 0	Day 1	Day 2	Day 3	
Saturated fatty acid (SFA)	28.80 ± 0.00*	2,255.00 ± 7.07*	1,130.00 ± 14.14*	1,445.00 ± 21.21*	2,335.00 ± 7.07*	$p < 0.001^a$
Monounsaturated fatty acid (MUFA)	12.00 ± 3.39	3,030.00 ± 0.00	2,020.00 ± 14.14	2,110.00 ± 0.00	3,175.00 ± 7.07	$p = 0.065^b$
Polyunsaturated fatty acid (PUFA)	28.8 ± 0.00	5,210.00 ± 14.14	3,535.00 ± 21.21	3,695.00 ± 7.07	5,300.00 ± 14.14	$p = 0.067^b$
Unsaturated fatty acid	38.40 ± 0.00	8,250.00 ± 14.14	5,555.00 ± 35.36	5,805.00 ± 7.07	8,480.00 ± 28.28	$p = 0.067^b$
Omega 3 fatty	20.28 ± 0.51*	625.60 ± 3.54*	236.90 ± 1.56*	398.85 ± 7.00*	613.73 ± 0.46*	$p < 0.001^a$
Total	89.88	11,130.60	6,921.90	7,648.85	11,428.73	

Note: * indicates a significant difference in mean value. [a] ANOVA. [b] = Kruskal-Wallis test. [c] indicates the amount of mackerel in a fermented sausage.

Table 7: Average physical characteristics (hardness and springiness) of fermented mackerel sausage

Formula	(Mean ± Standard Deviation)	
	Hardness	Springiness
F0	762.16 ± 93.65	7.3 ± 2.51
F1	708.33 ± 86.61	9.5 ± 0.36
F2	3,030.00 ± 316.82	9.4 ± 0.11
F3	4,781.33 ± 332.88	9.2 ± 0.05
P	<0.001**	0.047*

** One-way ANOVA; * Kruskal-Wallis test.

Table 8: Average pH level of fermented mackerel sausage

Formula	pH (Mean ± Standard Deviation)
F0	5.69 ± 0.02
F1	5.62 ± 0.01
F2	5.59 ± 0.02
F3	5.46 ± 0.04
P	<0.001**

** One-way ANOVA; * Kruskal-Wallis test.

of the sausage. Significant differences in the acceptability of colour, aroma, taste, and texture of mackerel fermented sausage were observed depending on the day of fermentation. The most preferred type was F0 (day 0), while the second most favourable was F1 (day 1). This result is similar to fermented catfish sausage, of which the day 0 and day 1 fermentation types are favoured (Table 9).

The colour parameter is the first consideration when consumers choose a food product. If the colour of the food is not attractive, it will reduce consumer acceptance even though nutritional content is complete. There was a marked difference in colour acceptability between F0 and F1. The colour of the control sausage (F0) tended to

Table 9: Acceptability of fermented mackerel sausage

Formula	(Mean ± Standard Deviation)			
	Colour	Aroma	Taste	Texture
F0	3.80 ± 0.55 (Really like)	3.77 ± 0.72 (Really like)	3.70 ± 0.70 (Really like)	3.63 ± 0.76 (Really like)
F1	3.23 ± 0.56 (Like)	3.20 ± 0.61 (Like)	3.30 ± 0.59 (Like)	3.37 ± 0.55 (Like)
F2	2.10 ± 0.48 (Dislike)	2.20 ± 0.76 (Dislike)	2.53 ± 0.62 (Dislike)	2.17 ± 0.79 (Dislike)
F3	2.03 ± 0.80 (Dislike)	2.03 ± 0.85 (Dislike)	2.07 ± 0.94 (Dislike)	1.67 ± 0.54 (Really dislike)
P	<0.001*	<0.001*	<0.001*	<0.001*

** One-way ANOVA; * Kruskal-Wallis test.

Table 10: Deviation values of nutritional quality, physical texture, pH, and acceptability formulation of fermented mackerel sausage

Variable	NP				Nba	Nbu	S
	F0	F1	F2	F3			
Carbohydrate	13.91	19.18	24.87	34.54	13.91	34.54	-20.63
Protein	15.34	20.62	31.97	33.91	33.91	15.34	18.57
Fat	3.37	3.55	4.20	4.41	3.37	4.41	-1.04
Water	65.32	53.79	34.83	22.49	22.49	65.32	-42.83
Ash	2.05	2.84	4.10	4.64	2.05	4.64	-2.59
Accepted Formulation	14.9	13.1	9.0	7.8	14.9	7.8	7.1
Elasticity	7.3	9.5	9.4	9.2	9.5	7.3	2.2
Hardness	762.16	708.33	3,030.00	4,781.33	708.33	4,781.33	-4,073
pH	5.69	5.62	5.59	5.46	5.46	5.69	-0.23

Note: treatment value (NP), the best treatment value (Nba), the worst treatment value (Nbu), Δ Nba and Nbu (S).

be clean brownish-white and lighter, while the F1 colour was brownish-white and slightly darker. The colour became darker during fermentation due to the Maillard reaction. The Maillard reaction occurs when the carbonyl compounds from the smoking process react with amino acids in the food. The colour formed on smoked food products is related to temperature, humidity, and protein content. Generally, the longer the fermentation, the darker the colour, so that fermentation further reduces the colour acceptability of fermented mackerel sausage (Rozum, 2009).

Aroma is important because it determines the quality of a food product. Aroma arises from volatile substances that are soluble in water and fat (Zhang et al., 2010). The longer the fermentation, the lower the aroma acceptance of fermented mackerel sausage. The acceptance test results indicated this for the F0 and F1 fermented mackerel sausage. The least preferred were the F2 (day 2) and F3 (day 3) fermented sausages.

Taste is an organoleptic property that greatly affects preference for a product and determines the decision to reject or accept the final product. The most preferred and preferred fermented mackerel sausages were the F0 and F1, sausages,

Table 11: Weights and scores for the best fermented mackerel sausage formula

Variable	BV	BN	F0		F1		F2		F3	
			Ne	Nh	Ne	Nh	Ne	Nh	Ne	Nh
Carbohydrate	0.8	0.10	1	0.1	0.71	0.071	0.46	0.046	0	0
Protein	1	0.13	0	0	0.28	0.036	0.89	0.115	1	0.13
Fat	1	0.13	1	0.13	0.82	0.106	0.20	0.026	0	0
Water	0.5	0.06	0	0	0.26	0.015	0.71	0.042	1	0.06
Ash	0.5	0.06	1	0.06	0.69	0.041	0.20	0.012	0	0
Accepted Formulation	1	0.13	1	0.13	0.74	0.096	0.16	0.020	0	0
Elasticity	0.8	0.10	0	0	1	0.10	0.95	0.095	0.86	0.086
Hardness	0.8	0.10	0.98	0.098	1	0.10	0.42	0.042	0	0
pH	1	0.13	0	0	0.30	0.039	0.43	0.055	1	0.13
Total	7.4	0.94	4.98	0.518	5.8	0.604	4.42	0.453	3.86	0.406

Note: variable weight (BV), normal weight (BN), effectiveness value (Ne), and yield value (Nh).

respectively. The longer the fermentation, the sourer the sausage will taste. The sour taste occurs because lactic acid is derived from low molecular weight compounds, such as peptides and free amino acids, aldehydes, organic acids, and amines produced by proteolysis (Zhang et al., 2010). The fermented mackerel sausage produced lactic acid and produced a small amount of acetic acid, ethanol, acetoin, carbon dioxide, and pyruvic acid, which imparted a sour taste to the sausage (Lazic et al., 2019; Zhang et al., 2010).

The texture of sausage is chewy. In this study, the most preferred textures of the fermented mackerel sausage were the F0 and F1. The components of fermented mackerel sausage that contributed to the texture were tapioca flour, carrageenan, and skim milk. The difference in the degree of chewiness is due to the amount of myofibril proteins (actin and myosin) that determine the gel properties of the sausage. Adding carrageenan helps in the formation of the elastic gel, which increases elasticity (Goff & Guo, 2019). Furthermore, the interaction between proteins and polysaccharides in foodstuffs plays an important role in the structure and stability of processed products (Ayadi et al., 2009).

3.7 Selected Formula Weight

Results of the analysis based on the de Garmo method showed that the 1-day fermented mackerel sausage (F1) had the best formula (Table 10 and Table 11). It scored the highest yield value (Nh) of 0.604 with 19.18% carbohydrate, 20.62% protein, 3.55 % fat, 53.79% water, 2.84% ash, 9.5 mm thickness, 708.33 g hardness, 5.62 pH, 3.23 colour, 3.20 aroma, 3.30 taste, and 3.37 texture. The best formulation was determined based on the de Garmo analysis. The assessment of the fermented mackerel sausage considered all variables that played a role in determining product quality, including nutritional quality, physical properties, pH, and organoleptic properties. The variables of nutrition (fat, protein), pH, and organoleptic quality were given the same weight because they were considered equally important in determining consumer interest in a new food product. The pH value indicates whether LAB are growing in the fermented product (Lazic et al., 2019; Nisa & Wardani, 2016). Fermented mackerel sausage with a fermentation time of 1 day was chosen as the best formulation.

4 Conclusions

A longer fermentation time resulted in higher nutritional quality (protein, fat, carbohydrates, and ash), and the hardness level increased. However, the acceptance variable (colour, aroma, taste, and texture), pH, and the degree of elasticity decreased as fermentation progressed. Different results for each type of amino acid and fatty acid were detected for the different fermentation durations. Overall, fermentation played a role increasing amino acid and fatty acid composition. The best formulation for fermented mackerel sausage was a fermentation time of 1 day. It scored the highest yield value (Nh) of 0.604 with 19.18% carbohydrate, 20.62% protein, 3.55 % fat, 53.79% water, 2.84% ash, 9.5 mm thickness, 708.33 g hardness, 5.62 pH, 3.23 colour, 3.20 aroma, 3.30 taste, and 3.37 texture.

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