

Nutrient content and fatty acid profile of fermented shrimp (*Litopenaeus vannamei*) sausage

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Abstract

Cardiovascular disease affects the heart or blood vessels and is preventable by feeding on functional food containing unsaturated fatty acids, such as shrimp. Fermentation increases the functional food value, such as shrimp, by breaking down complex compounds in carbohydrates, proteins, and fats, with enzymes and microorganisms. Hence, this research was aimed to determine the differences in the fatty acid and nutritional profiles of shrimp sausages in different fermentation duration. This was an experimental research with a randomized design in the form of three levels of fermentation duration, consisting of 24, 48, and 72 hrs, and without fermentation as a control, with three repetitions. The fermentation was carried out spontaneously using a salt concentration of 1.2%, at 50°C for 3 hrs, and then reduced to 35°C. Parameters measured were the fatty acid profile, moisture, ash, fat, protein, and carbohydrate. The fatty acid profile was presented descriptively, while the statistical analysis of nutritional values used the One-Way ANOVA, Kruskal-Wallis test, and continued with the post hoc examination. There are fifteen types of fatty acids in fermented shrimp sausage, and their values change during the fermentation process. The highest fatty acid group in the fermented shrimp sausage was polyunsaturated (36.28% w/w). There was a significant difference in the mean water and fat content with diverse duration ($p \leq 0.05$). However, there were no significant differences in the ash, protein, and carbohydrate content with different duration ($p \geq 0.05$). The duration of the fermentation process affects the fatty acids level and nutritional value of fermented shrimp sausage. The longer the fermentation process duration, the higher the fatty acid levels and the lower the water content, ash, and protein.

1. Introduction

Cardiovascular Disease (CVD) is known to be a leading cause of death globally. Approximately 85% of the cases are caused by heart attacks and strokes (WHO, 2017). The Basic Health Research in 2018 stated that 1.5% or 15 out of 1000 Indonesians suffer from this disease (Ministry of Health of the Republic of Indonesia, 2018).

Risk factors include obesity, smoking, abnormalities in lipid profiles, inadequate physical activity, diet with high fat and cholesterol, and low fibre intake (Rafiony, 2013). CVD can be prevented by consuming foods that contain fatty acids, such as PUFA and MUFA, with a PUFA ratio higher than MUFA (Erwianto *et al.*, 2017).

The shrimp's fatty acid content is a high source of PUFAs, such as omega 3, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in the muscles (Li *et al.*, 2011; Michaelsen *et al.*, 2011; Simon *et al.*, 2012). Omega 3 intake is beneficial for heart disease and stroke by reducing cholesterol and triglyceride levels. Also, it increases blood vessels' elasticity and prevents the formation of harmful fats that stick to the arteries (Simopulus, 2016). EPA and DHA fatty acids are precursors of anti-inflammatory, antithrombotic, and vasodilatory activities, preventing dyslipidemia and hypertension (Food and Agriculture Organization, 2010; Simon *et al.*, 2012).

A food's functional value increases through the fermentation process, which breaks down complex

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compounds, such as carbohydrates, proteins, and fats with enzymes and microorganisms (Hutkins, 2006). During fermentation, proteolytic enzymes break down proteins to free amino acids and peptides having bioactive properties, such as antioxidants and antihypertensive (Lee *et al.*, 2008). Furthermore, the lipase enzyme and lactic acid bacteria hydrolyse fat resulting in much higher essential fatty acids (Magalhaes *et al.*, 2011). Fish paste fermentation takes 8 to 32 days, resulting in an increase in the fatty acids content, namely capric, stearic and myristic acid, EPA, and DHA (Anggo, Ma'ruf and Et, 2015). In the fermentation study of sorghum flour, a decrease in carbohydrate content occurs in small amounts. This may be caused by microorganisms, which use it as an energy source during fermentation (Setiarto *et al.*, 2016).

Seafood is rich in protein and is highly perishable, it has a short shelf life unless a preservation method is used. Preservation can take many forms. One of them is fermented sausage, a meat product mixed with spices and added to a starter culture to assist the fermentation process (Vural and Zvural, 2007). This is carried out uncontrollably by excluding microbes in a starter form. However, they play active roles by producing proteolytic enzymes that react with the product (Estiasih, 2009). Meanwhile, spontaneous fermentation is aided by adding 1.2% of salt (Honikel, 2008).

The fermentation process has a certain duration. Fermented catfish and tuna sausages have different pH values, whereas a longer fermentation duration has a lower pH level. Therefore, the longer the duration, the lower the pH value. Moreover, the fish protein undergoes hydrolysis into amino acids and peptides (Nursyam, 2011; Nisa and Wardani, 2016).

There is a dearth of information on fermented shrimp sausage. This research was conducted to determine the effect of duration variations of fermenting shrimp sausages on their fatty acid content and nutritional value.

2. Materials and methods

The research was conducted at the Food Laboratory of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, using fermented shrimp sausage products. The fatty acid content analysis in the fermented sausages was carried out at the Integrated Laboratory of Bogor Agricultural University, West Java. Meanwhile, the nutrient content analysis (carbohydrates, fats, proteins, water, and ash) was carried out at the Food Technology Laboratory, Semarang State University. This research was an experimental study adopting a randomized design with one factor.

2.1 Making fermented shrimp sausages

The tools used in making fermented shrimp sausage were digital scales, basins, blenders, spoons, jars, ovens, and aluminium foil. The materials used were headless shrimp, ice water, salt, corn oil, sugar, garlic, pepper, tapioca flour, egg white, and liquid smoke.

The head of the shrimp was removed, then washed and soaked with 2% lime juice. After grinding the shrimp, shrimp paste with 12% ice water, 9.3% egg white, 7.5% tapioca flour, 3.1% corn oil, 1.2% salt, 1.2% granulated sugar, 0.6% garlic, and 0.2% pepper were mixed until homogenized and inserted into the edible shell.

The shrimp sausage was soaked in 5% liquid smoke for 30 mins, then oven-dried at 50°C for 3 hrs. The cooked sausages were incubated at 35°C for 24, 48, and 72 hrs to produce fermented shrimp.

2.2 Analysis of fatty acids

Three grams of fermented shrimp sausage was mashed using a mortar. Methylation formation was carried out by inserting 0.2 g oil and 5 mL of 0.5 N NaOH-methanol into a test tube, then heated in a water bath for 20 mins at 80°C. When the mixture reached room temperature, 5 mL of BF₃ solution was added to the tube. The sample was reheated for another 20 mins in a water bath at 80°C, then it was let to rest. Subsequently, 2 mL of saturated NaCl and 5 mL of hexane were added to the sample, then the mixture was shaken. The hexane solution at the top was collected into a test tube. A total of 1 µL fat sample was injected into the gas chromatograph. The fatty acids were identified by a flame ionization detector and recorded with a chromatogram.

The fatty acid identification was carried out by balancing the sample retention time with the standard. The quantitative analysis was calculated using the following formula:

$$\left(\text{Fatty Acid Identification} = \frac{\text{fatty acid area A}}{\text{SI area}} \times \text{RF} \times \frac{\text{mg SI}}{\text{mg sample}} \right) \times 100$$

The RF (Respond Factor) value of each fatty acid was calculated from the FAME external standard chromatogram.

$$\text{RF of Fatty acid A} = \frac{\text{SI area}}{\text{SI concentration}} \times \text{RF} \times \frac{\text{Standardized fatty acid A concentration}}{\text{mg sample area of standardized fatty acid A}}$$

2.3 Analysis of nutritional content

2.3.1 Water content analysis

Water content analysis was carried out by AOAC 2005 two times using the oven method. The porcelain dish was placed in the oven for 30 mins at 100–105°C, then cooled in a desiccator and weighed. Approximately

2 g of the sample were dried in the oven for 6 hrs at 100° C. After the sample was cooled, the sample was repeatedly weighed until a constant weight was achieved.

Water content calculation:

$$\text{Water content (\%)} = \frac{\text{weight (g) of sample before drying} - \text{weight (g) of sample after drying}}{\text{weight (g) of sample before drying}}$$

2.3.2 Ash content analysis

The ash content analysis was carried out two times using the oven method. The porcelain dish was placed in the oven for 30 mins at 100–105°C, cooled, and weighed. Then, 2 g of the sample was weighed in the dish and burned over a flame until it did not smoke. Then, the ashing was carried out in a furnace at 550–600°C for approximately 1 hr. The sample in a dish was then cooled in a desiccator and weighed. The combustion stage was repeated in the furnace until a constant weight was obtained.

Ash content calculation:

$$\text{Ash Content} = \frac{(\text{A dish with ashed sample} - \text{initial dish weight})}{(\text{a dish with sample} - \text{initial dish weight})} \times 100 \%$$

2.3.3 Protein content analysis

Protein content analysis was carried out using the Kjeldahl method. Approximately 1 g of the sample was placed in the Kjeldahl flask. The sample was added with 7 g K₂SO₄, 0.8 g CuSO₄, and 200 mL H₂SO₄ then heated for 75 mins. The solution was allowed to rest until it reaches room temperature before transferring it into a round bottom flask. The solution was rinsed with 25 mL of distilled water, then 50 mL of 40% NaOH was added.

The solution was distilled in an Erlenmeyer containing 30 mL of H₃BO₃ and 3 drops of BCG-MR indicator. The result was titrated with 0.1N HCl till pink. Calculation:

$$N(\text{g}) = \frac{(A - B)}{1000} \times N_{\text{HCl}} \times 14.008 \times 100\%$$

$$N(\%) = \frac{N(\text{g})}{\text{sample weight (g)}} \times 100\%$$

$$\% \text{ Crude protein} = \% N \times \text{protein conversion factor}$$

Where A = Volume of HCl (mL) used in the sample titration, B = volume of HCl (mL) used in the blank titration and the protein conversion factor = 6.25.

2.3.4 Fat content analysis

The fat content was determined using the Soxhlet method. The flask with fat was dried in an oven at 105°C for 30 mins, then cooled for 15 mins in a desiccator. A total of 2–5 g of the sample was placed on a filter paper, then tied with fat-free cotton wool. The fat solvent was placed in the flask, then the sample was inserted into a soxhlet extraction device. The extraction was carried out for 3–4 hrs, then distilled and dried in an oven at 105°C

until it reached a constant weight, then cooled in a desiccator for 30 mins and weighed again. Calculation:

$$\text{Fat content} = \frac{W3 - W2}{W1} \times 100\%$$

Where W1 = sample weight (g), W2 = the weight of beaker without fat (g) and W3 = the weight of beaker with fat (g).

2.3.5 Carbohydrate content analysis

The carbohydrate content analysis was carried out using the difference method by subtracting 100% from the percentage of water, ash, protein, and fat content.

$$\text{Carbohydrate (\%)} = 100\% - (\% \text{ water} + \% \text{ ash} + \% \text{ protein} + \% \text{ fat})$$

2.4 Data analysis

The fatty acid data were obtained from the measurement results and presented descriptively, while data on nutritional value would be processed using a statistical software program. The data normality was tested using Shapiro-Wilk, since the data was <50. The water and fat content of fermented shrimp sausage were tested using ANOVA (Analysis of Variance) and continued with the post hoc Least Significance Different test. The ash, protein, and carbohydrate content of the fermented shrimp sausages were tested using Kruskal-Wallis. The effect of the independent variable on the dependent was considered significant when the p-value was ≤ 0.05.

3. Results

3.1 Fatty acid profile

There were 15 types of fatty acids obtained from the fermented shrimp sausages. The main fatty acids of fermented shrimp sausage in the Saturated Fatty Acid (SFA), MUFA, PUFA groups were palmitic acid (7.52% W/W), oleic acid (19.53% W/W), and linoleic acid (36.28% W/W), respectively in the 72 hrs of fermentation.

Total SFA increased at 24 hrs of fermentation, then decreased at 48 hrs and 72 hrs. Total MUFA decreased at 24 hrs and 48 hrs of fermentation, then increased at 72 hrs. PUFA group experienced a decrease in the total fatty acid content 24 hrs of fermentation, then increased at 48 hrs and 72 hrs. The changes in the fatty acid composition of shrimp sausage during the fermentation period were not the same, as shown in Table 1.

3.2 Nutrient content

There was a significant difference in the water content of fresh shrimp and fermented shrimp sausage

Table 1. Fatty acid composition in shrimp and fermented shrimp sausage (%W/W)

| Fatty acid | The Duration of Shrimp Sausage Fermentation | | | | |
|--|---|----------------------|-------------------|-------------------|-------------------|
| | Fresh Shrimp | Without Fermentation | 24 hrs | 48 hrs | 72 hrs |
| Saturated fatty acid (SFA) | | | | | |
| Myristic acid (C14:0) | 0.08 | 0.03 | 0.05 | 0.05 | 0.03 |
| Palmitic acid (C16:0) | 5.14 | 7.44 | 8.00 | 6.66 | 7.52 |
| Heptadecanoic acid (C17:0) | 0.22 | 0.06 | 0.08 | 0.09 | 0.05 |
| Stearic acid (C18:0) | 2.94 | 1.23 | 1.30 | 1.15 | 1.30 |
| Arachidic acid (C20:0) | 0.11 | 0.25 | 0.25 | 0.21 | 0.23 |
| Heneicosanoic acid (C21:0) | 0.03 | 0.02 | 0.03 | 0.02 | 0.02 |
| Behenic acid (C22:0) | 0.17 | 0.08 | 0.07 | 0.07 | 0.07 |
| Lignoceric acid (C24:0) | 0.10 | 0.12 | 0.17 | 0.11 | 0.12 |
| Total | 8.79 (37.30%) | 9.23 (12.40%) | 9.95 (16.70%) | 8.36 (13.60%) | 9.34 (14.10%) |
| Monounsaturated fatty acid (MUFA) | | | | | |
| Palmitoleic acid (C16:1) | 0.27 | 8.6 | 0.18 | 0.22 | 0.09 |
| Oleic acid (C18:1n9c) | 3.16 | 19.81 | 20.3 | 17.73 | 19.53 |
| Total | 3.43 (14.50%) | 28.41 (38.00%) | 20.48 (34.30%) | 17.95 (29.20%) | 19.62 (29.50%) |
| Polyunsaturated fatty acid (PUFA) | | | | | |
| Linoleic acid (C18:2n6c) | 5.97 | 36.06 | 28.78 | 34.00 | 36.28 |
| Linolenic acid (C18:3n3) | 0.34 | 0.50 | 0.31 | 0.51 | 0.57 |
| Arachidonic acid (C20:4n6) | 1.07 | 0.25 | 0.08 | 0.21 | 0.12 |
| Eicosapentanoic acid (C20:5n3) | 1.91 | 0.15 | 0.1 | 0.25 | 0.24 |
| Docosahexaenoic acid C22:6n3) | 2.07 | 0.08 | 0.04 | 0.14 | 0.21 |
| Total | 11.36 (48.20%) | 37.04 (49.60%) | 29.31 (49.10%) | 35.11 (57.20%) | 37.42 (56.40%) |
| Total fatty acid | 23.58 | 74.68 | 59.74 | 61.42 | 66.38 |

Shrimp sausage consisted of 62.3% fresh shrimp and 37.7% filler materials

with different durations of fermentation ($p = 0.043$). Table 2 showed that fresh shrimp had the highest water content (64.94%) compared to fermented shrimp sausage without fermentation, 24-, 48-, and 72-hrs fermentation. The shrimp sausage with the highest water content was without fermentation (51.44%), while the lowest was 72 hrs fermentation (44.02%). The difference in ash content of fresh shrimp and fermented shrimp sausage with different fermentation duration was not significant ($p = 0.068$). Fresh shrimp had the lowest water content (64.94%) compared to fermented shrimp sausage for 24, 48, and 72 hrs and without fermentation. The shrimp sausage with the highest water content was without fermentation (0.89%), while the lowest was 72 hrs fermentation (0.86%). There was a significant difference in the fat content of fresh shrimp and fermented shrimp sausage with different fermentation duration ($p < 0.001$). Fresh shrimp had the lowest fat content (7.45%) compared to fermented shrimp sausage for 24, 48, 72 hrs and without fermentation. The shrimp sausage with the highest fat content was fermented for 72 hrs (11.37%), while the lowest was without fermentation (9.01%). The difference in protein content of fresh shrimp and fermented shrimp sausage with different fermentation

duration was not significant ($p = 0.068$). Fresh shrimp had the highest protein content (18.99%) compared to fermented shrimp sausage for 24, 48, 72 hrs and without fermentation. The shrimp sausage with the highest protein content was without fermentation (12.93%), while the lowest was 72 hrs fermentation (9.21%). The difference in carbohydrate content of fresh shrimp and fermented shrimp sausage with different fermentation duration was not significant ($p = 0.068$). Fresh shrimp had the lowest carbohydrate content (64.94%) compared to fermented shrimp sausage for 24, 48, 72 hrs and without fermentation. The shrimp sausage with the highest carbohydrate content was fermented for 72 hrs (34.54%), while the lowest was without fermentation (25.72%).

4. Discussion

Shrimp contains nutrients rich in fat, protein, minerals, and vitamins (Larsen *et al.*, 2011). It is also a source of Polyunsaturated Fatty Acids (PUFA), especially Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) (Li *et al.*, 2011).

The major fatty acid compositions in shrimp are

Table 2. Nutrient analysis results of fermented shrimp sausage

| Fermentation Duration | Category | | | | |
|-----------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | Water (%) | Ash (%) | Fat (%) | Protein (%) | Carbohydrate (%) |
| Fresh Shrimp | 64.94±0.101* | 0.61±0.022 | 7.45±0.052* | 18.99±0.034 | 8.03±0.622 |
| Without Fermentation | 51.44±0.067* | 0.89±0.006 | 9.01±0.016* | 12.93±0.091 | 25.72±0.034 |
| 24 hrs | 49.91±0.065 | 0.88±0.006 | 9.96±0.017* | 11.94±0.084 | 27.31±0.304 |
| 48 hrs | 44.27±0.122* | 0.87±0.005 | 11.03±0.009* | 10.49±0.074 | 33.34±0.054 |
| 72 hrs | 44.02±15.434* | 0.86±0.006 | 11.37±0.010* | 9.21±0.065 | 34.54±0.190 |
| | p = 0.043 ^a | p = 0.068 ^b | p < 0.001 ^a | p = 0.068 ^b | p = 0.068 ^b |

*Values that are significantly different, ^aANOVA test, ^bKruskal Wallis test.

linoleic (5.97%), palmitic (5.14%), oleic (3.16%), stearic (2.94%), DHA (2.07%), EPA (1.91%), and arachidonic acid (1.07%). The variation of fatty acid composition was influenced by some factors such as species types, availability, and quality of feed, heat application, season changing, and environmental salinity (Simopoulos, 2016; Pires et al., 2018).

The total fatty acid without fermentation had the highest composition value (74.68%) due to the addition of corn oil as a source of linoleic (34–62%), oleic (19–49%), and palmitic acid (8–12%) (Dwiputra, 2015). The reduction of arachidonic acid, EPA, and DHA occurred in this study was inconsistent with the results of Mekarsari's research, which suggested that the increase in EPA was influenced by the increase in phenol having a role in inhibiting fat oxidation. However, the DHA content was undetected due to the nature of essential fatty acids; they are sensitive to temperature, light, and oxygen (Swastawanti and Sumardianto, 2004; Mekarsari et al., 2016).

Our results showed that PUFA and MUFA levels decreased at 24 hrs of fermentation. Decreased linoleic acid in soy sauce fermentation occurs on the 30th day. This is because fermentation accelerates the oxidation process (Haman et al., 2017; Zou et al., 2019). Meanwhile, the oxidation process was influenced by the meat quality, temperature, exogenous components (salt, nitrate, seasonings), and fumigation (Talon et al., 2000; Nassu et al., 2003).

The shrimp sausage fermentation at 48 hrs showed an increase in PUFA and MUFA. However, at 72 hrs, there was a decrease in EPA, arachidonic, palmitoleic, myristic and stearic acid. Visesangan's et al. (2006) study showed an increase in PUFA and MUFA fatty acids at the end of the 72 hrs fermentation of Nahm sausage. The lipolytic activity was influenced by enzymes from the muscle tissue or lipases from microorganisms that released fatty acids (Gambacorta et al., 2009). The Lactic Acid Bacteria (LAB) reduced the pH for lipolytic and proteolytic enzymes (Montel et al., 1998). The fermentation of fish paste decreased some of the essential fatty acids at 72 hrs. The lipolysis process

increases certain fatty acids and produces a distinctive flavour in fermented products (Murray et al., 2003).

The American Dietetic Association and Dietitians of Canada recommended the consumption of 500 mg/day of EPA + DHA for primary prevention of cardiovascular disease. Meanwhile, according to FAO/WHO, 250-2000 mg/day of EPA + DHA was recommended for the secondary prevention of cardiovascular disease. The recommended consumption of fermented shrimp sausages to carry out these needs was 50-100 g/day (Kris-Etherton et al., 2007; FAO/WHO, 2008).

The water content was a parameter related to the shelf life of a product. Our result shows that the highest water content was fresh shrimp. The lowest was fermented shrimp sausage with 72 hrs of fermentation, which was consistent with the study by Ferreira et al. (2006), who reported that water content decreased after fermentation (Ferreira et al., 2006). The research by Yanuar et al. (2015) and Swastawanti et al. (2004) showed a decrease in water content, which was influenced by temperature and smoking duration due to the evaporation in the smoked milkfish. Cellulose sleeves had pores that facilitated water expulsion from the sausages during the smoking process. The fermentation process decreased the pH value and increased the meat structure bonds. Therefore, the water-binding capacity decreased and was easily removed from the product (Soepomo, 2015). The addition of salt and sugar also decreased the water content due to the ion's hydration attracting water molecules in the food ingredient (Staf et al., 2014).

Ash is an inorganic residue from the combustion process of organic compounds showing the total minerals in the food ingredients (Andarwulan et al., 2011; Winarno, 2008). The results showed that the highest ash content was 0.86% in fermented shrimp sausage with 72 hrs of fermentation. This meets the Indonesian National Standard requirements, allowing ash content in seafood to be less than 3% (Badan Standarisasi Nasional (BSN), 1995). Mumpuni (2017) demonstrated no difference in the fermented sausage treatment since there was no variation in the

concentration of raw materials and seasonings. The ash content increased due to the addition of mineral substances to the spices. Liquid smoking lowered the ash content due to the loss of organic elements, such as carbon, sulfur, and phosphorus (Yanuar *et al.*, 2015). Nisa and Wardani's (2016) study on fermentation of catfish sausage showed a decrease in ash content due to minerals from microorganisms to sustain life. More than 19 minerals, dissolved by water and fat, were released during the process and it took a long period to ferment (Silfia *et al.*, 2017).

Fat is a lipid compound that produces more energy than protein or carbohydrate (Hutomo *et al.*, 2015). There was a difference in the mean of fat content ($p < 0.001$) in the shrimp sausages that fermented at 0 and 24 hrs, which means the fat content did not in accordance with the standard. The addition of corn oil increased fat since it contained 14 g of fat per one tablespoon. The research on smoking eels showed that the fat content increased, but was not significantly different. This indicated that using liquid smoke could maintain the raw material's quality without breaking down the fat composition (Hutomo *et al.*, 2015). Nisa and Wardani's (2016) research showed that the fat content of fermented catfish sausages was increased steadily. The Lactic Acid Bacteria (LAB) had a secondary lipolytic activity which broke down the fat into simple chemical compounds. The lipase enzyme controlled this activity by releasing fatty acids (Nisa and Wardani, 2016).

Protein is a molecule composed of amino acids as a tissue-building substance and a regulator of the digestion process (Winarno, 2008). The result of this research showed that the lowest protein content was 9.21% in fermented shrimp sausage with 72 hrs of fermentation. The Indonesian National Standard suggested that protein content in seafood was 6.9–15.5% (BSN, 1995). However, it decreased due to the addition of lime juice. The research by Asrullah *et al.* (2012) explained that adding 1 mL of lime juice caused a decrease in fish protein content due to amino acid racemization. The longer the protein reacted with the acid, the higher the peptide bonds were hydrolyzed. As a result, it damaged the protein's primary structure. Swastawanti *et al.* (2014) showed that the longer heating damaged the smoked fish protein, denatured its structure, coagulated, and became a simpler form. The protein content was decreased during the fermentation process. That result is consistent with previous studies where protein content in shrimp paste decreases during the fermentation process (Anggo *et al.*, 2014). Therefore, the protein compounds were broken down into their derivatives, such as proteolysis, peptone, peptides, and amino acids. (Nooryanti *et al.*, 2010).

Carbohydrates are the primary energy source consisting of carbon, hydrogen, and oxygen. There was no difference in the mean carbohydrate content ($p = 0.068$) for each treatment and it exceeded the standard between 10.2-20.9%. The addition of ingredients, such as tapioca flour and sugar increased the carbohydrate content of the raw material (shrimp). The smoking process increased the carbohydrates, which is consistent with Yanuar *et al.* (2015) that the longer and higher the temperature, the higher the carbohydrate content. However, in this study, the carbohydrate content results were obtained from the calculation formula and not based on laboratory analysis (Yanuar *et al.*, 2015). There was starch hydrolysis by enzymes into derivatives during the fermentation process, such as glucose and maltose (Rahayu and Puwoko, 2005). Moreover, decreased water content during the fermentation process will increase carbohydrate levels.

5. Conclusion

The fermentation process produced different types of fatty acids. In general, the shrimp sausage's fatty acid content of the shrimp sausage, so-called PUFA (37.42% w/w), increased at 72 hrs of the fermentation process. There was a significant difference between the mean of water and fat content of the shrimp sausage in different fermentation duration (24, 48, 72 hrs, and without fermentation), with $p < 0.05$. There was no significant difference between the mean of ash, protein, and carbohydrates content of shrimp sausage in different fermentation duration (0, 24, 48, and 72 hrs), with $p > 0.05$.

Conflict of interest

The authors declare there is no conflict of interest.

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