Fatty acid profiles of Paper Squid (*Loligo edulis*, Hoyle)

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**Abstract.** Squid is one of the fisheries products with high production volume. It has the same characteristics as other fishery products, which are highly perishable in nature. Therefore it is important to optimize squid utilization. Diversification of squid products can be done into value-added products such as paper squid. The aim of this study was to investigate the fatty acids profile contained in paper squid (*Loligo edulis*, Hoyle). Analysis of fatty acids composition in squid was done by the gas chromatography method. The results showed that the fatty acids detected in paper squid consisted of 10 types of saturated fatty acids, 7 types of monounsaturated fatty acids, and 8 types of polyunsaturated fatty acids. The highest content of saturated fatty acids is palmitic acid, which is 7.45%. The highest content of monounsaturated fatty acid is oleic acid, which is 0.79%. The highest content of polyunsaturated fatty acids is docosahexaenoic acid, which is 22.57%.

1. **Introduction**

Squids are one of the most economically valuable fishery products included in class Cephalopod. The potential of squid in Indonesia is found in almost surface waters, such as waters of the Malacca Strait, the South China Sea, the Java Sea, the Makassar Strait, the Morowali waters in Central Sulawesi, the Banda Sea, the Seram Sea, the Arafuru Sea, and the Flores Sea [1]. Squid production in Indonesia has increased steadily to 140,499 tons in 2013, 150,623 tons in 2014, and increased again to 197,000 tons in 2015 [2].

Squid has the same characteristics as other fishery products. The qualities of squid deteriorate rapidly after death. Therefore, it is important to optimize squid utilization. In Maluku, the utilization of squid just developed into traditional processed food and primarily consumed in the fried and dried form. The exploration of value-added squid products is highly important to be developed. Paper squid is one of the diversified squid products in the form of thin sheets, and its processing combines several methods such as drying and pressing. Paper squid are also considered ready-to-eat (RTE) foods as a snack with a crispy texture.

Recent studies have shown that the organoleptic score of paper squid (*Loligo sp.*) ranging between 4.5 to 4.8. While the color, texture, and taste had a score of 4.5, the appearance had a score of 4.7, and the odor had a score of 4.8 [3]. Tahalea (2019) also reported that the paper squid contains 10.26% of water, 79.69% of protein, 3.50% of fat, 3.04% of ash, and 3.51% of carbohydrate. The amino acid composition of paper squid has been observed that detected 9 essential amino acids such as threonine, arginine, methionine, valine, phenylalanine, isoleucine, leucine, lysine, histidine, and 6 types of non-essential amino acids such as aspartic acid, glutamic acid, serine, glycine, alanine, and tyrosine [4]. The primary focus of this research was to investigate the fatty acids profile of paper squid (*Loligo edulis*, Hoyle).
2. Materials and methods

2.1. Materials
Fresh squid (Loligo edulis) were obtained from Nolloth, Central Maluku, Maluku, Indonesia. Materials used for the analysis of fatty acid profiles are 0.5 N NaOH-methanol, BF$_3$ 20%, NaCl, n-hexane, and Na$_2$SO$_4$ anhydrous.

2.2. Sample processing
Fresh squid was weeded to remove bowels, ink, and other impurities. After weeding, the fresh squid was immediately washed under running water. These samples were dried under the sun for 1-2 days. Furthermore, the samples were oven-dried for 15 minutes at 70°C to flex the squid texture. The dried squid was pressed using a molen, in order to obtain thin forms and dried in the oven for 45 minutes at 100°C. Hereinafter, the paper squid was vacuum packed until it is used for analysis (Figure 1).

![Research flowchart.](image)

2.3. Fatty acids analysis
The fatty acid analysis consisted of three stages; lipid extraction, methylation, and chromatographic analysis. The squid was extracted using the soxhlet method. Samples were weighed using analytic weights and wrapped in filter paper. Furthermore, it was covered with fat-free cotton and put into the soxhlet, which connected to a weighed flask. The oil is then extracted for 5-6 hours using n-hexane solvent and dried in the oven for 1 hour at 100-105°C. The oil extract was cooled in a desiccator and weighed.
The extraction method was performed by AOAC 969.33 [5]. 20 mg of extracted oil was weighed in a tube sealed with a Teflon-lined cap, and 1 mL of 0.5 N NaOH in methanol was added to prepare the FAMEs. The mixture was then pushed with nitrogen and heated in a water bath for 20 minutes. 2 mL of BF₃ 20% was added to the mixture, heated for 20 minutes, and then cooled. After cooling, 2 mL of saturated NaCl solution and 1 mL of hexane were added and shook for 15 seconds. The hexane layer was pipetted into a tube containing about 0.1 g of Na₂SO₄ anhydrous and allowed to stand at room temperature for 15 minutes. The organic phase was filtered and injected into gas chromatography.

3. Results and discussion
Fatty acids are long-chain organic acids, commonly having 4-24 carbon atoms in chain length, which have a carboxyl group (COOH) with a long aliphatic chain [6]. Analysis of fatty acid composition included three steps; oil extraction, methylation, and Gas Chromatography analysis. The principle behind the analysis of fatty acid profiles is to change fatty acids into derivatives using chromatographic techniques [7]. The result of fatty acids analysis of *L. edulis* showed that fresh squid contained 28 types of fatty acids, while dried squid contained 23 types of fatty acids, and paper squid contained 25 types of fatty acids. The fatty acid composition of *L. edulis* presented in table 1.

| Fatty Acid                   | Fresh Squid     | Dried Squid    | Paper Squid  |
|------------------------------|-----------------|----------------|--------------|
| **Saturated**                |                 |                |              |
| Caprilic Acid (C8:0)         | 0.31 ± 0.08     | 0.67 ± 0.09    | 0.13 ± 0.06  |
| Capric Acid (C10:0)          | 0.24 ± 0.06     | 0.50 ± 0.04    | 0.09 ± 0.03  |
| Lauric Acid (C12:0)          | 1.41 ± 0.11     | 3.25 ± 0.23    | 0.42 ± 0.09  |
| Myristic Acid (C14:0)        | 0.80 ± 0.13     | 1.57 ± 0.13    | 0.40 ± 0.05  |
| Pentadecanoic Acid (C15:0)   | 0.27 ± 0.08     | 0.29 ± 0.11    | 0.19 ± 0.06  |
| Palmitic Acid (C16:0)        | 10.67 ± 0.66    | 11.68 ± 0.62   | 7.45 ± 0.57  |
| Palmitoleic Acid (C16:1)      | 0.34 ± 0.06     | 0.71 ± 0.07    | 0.30 ± 0.01  |
| Stearic Acid (C18:0)         | 2.96 ± 0.41     | 2.77 ± 0.31    | 3.02 ± 0.52  |
| Arachidic Acid (C20:0)       | 0.11 ± 0.03     | 0.09 ± 0.02    | 0.10 ± 0.04  |
| Heneicosanoic Acid (C21:0)   | 0.06 ± 0.04     | -              | -            |
| Behenic Acid (C22:0)         | 0.09 ± 0.02     | 0.07 ± 0.01    | 0.06 ± 0.01  |
| Tricosanoic Acid (C23:0)     | 0.02 ± 0.01     | -              | -            |
| Lignoceric Acid (C24:0)      | 0.07 ± 0.03     | -              | -            |
| **Total**                    | 17.69 ± 2.91    | 21.52 ± 3.20   | 12.48 ± 2.11 |
| **Monounsaturated**          |                 |                |              |
| Myristoleic Acid (C14:1)     | 0.03 ± 0.01     | -              | 0.04 ± 0.01  |
| Palmitoleic Acid (C16:1)      | 0.29 ± 0.08     | 0.31 ± 0.08    | 0.16 ± 0.06  |
| Heptadecanoid Acid (C17:1)   | 0.05 ± 0.03     | 0.07 ± 0.04    | 0.05 ± 0.03  |
| Elaidic Acid (C18:1; n-9)     | 0.03 ± 0.01     | 0.03 ± 0.01    | 0.09 ± 0.02  |
| Oleic Acid (C18:1; n-9c)      | 1.11 ± 0.37     | 1.98 ± 0.32    | 0.79 ± 0.12  |
| Eicosenoic Acid (C20:1)       | 0.03 ± 0.00     | 0.03 ± 0.01    | 0.09 ± 0.02  |
| Nervonic Acid (C24:1)         | 0.12 ± 0.05     | 0.11 ± 0.06    | 0.11 ± 0.05  |
| **Total**                    | 1.65 ± 0.40     | 2.52 ± 0.72    | 1.31 ± 0.27  |
| **Polyunsaturated**          |                 |                |              |
| Linoleic Acid (C18:2; n-6c)   | 0.43 ± 0.10     | 0.63 ± 0.12    | 0.33 ± 0.18  |
| γ-Linolenic Acid (C18:3; n-6c)| 0.21 ± 0.11     | -              | 0.15 ± 0.03  |
| Linolenic Acid (C18:3; n-3)   | 2.48 ± 0.47     | 2.29 ± 0.35    | 2.47 ± 0.25  |
| Eicosadienoic Acid (C20:2)    | 0.14 ± 0.04     | 0.16 ± 0.04    | 0.19 ± 0.04  |
| Arachidonic Acid (C20:4; n-6) | 1.62 ± 0.10     | 1.47 ± 0.23    | 1.48 ± 0.23  |
| Eicosapentaenoic Acid (C20:5; n-3) | 6.93 ± 0.95 | 8.82 ± 1.03    | 7.75 ± 1.05  |
| Docosadienoic Acid (C22:2)    | 0.08 ± 0.01     | 0.10 ± 0.01    | 0.07 ± 0.03  |
| Docosahexaenoic Acid (C22:6; n-3) | 26.84 ± 0.33 | 2.45 ± 0.64    | 22.57 ± 0.66 |
| **Total**                    | 38.71 ± 9.18    | 39.90 ± 9.15   | 35.00 ± 7.79 |
In this study, the total content of fatty acids was 58.05% in the fresh squid, 63.94% in the dried squid, and 48.78% in the paper squid. The increase of fatty acid total in the dried squid might be the effect of drying methods, which reduced the moisture content. Morris et al. (2004) reported that changes in the nutrition content of food were affected by the heating process [8]. The result showed a further decrease in total fatty acid when the temperature was increased. However, at the high temperature, fatty acids component can produce volatile compounds such as aldehydes, ketones, acids, and hydrocarbons.

Figure 2. Histogram of palmitic acid in squid.

Saturated fatty acids (SFA) are fatty acid which does not contain double bonds [6]. According to the result, palmitic acid showed the highest saturated fatty acid in squid. Winarno (2008) sated that palmitic acid contained 15-50% of the total fatty acid in foods [9]. Usmaawati (2012) reported that palmitic acid content in dried squid (Loligo pealii) was 20.75% [10]. However, the result showed that in various species, age, size, and environmental conditions had affected the fatty acid content. French et al. (2002) reported that palmitic acid significantly increased total cholesterol levels [11]. German et al. (2004) also reported an increase in cholesterol could increase the incidence of atherosclerosis and subsequently increased the risk of coronary artery disease [12].

Figure 3. Histogram of oleic acid in squid.

The result showed that the monounsaturated fatty acids (MUFA) with the highest content were oleic acid (Figure 3). Oleic acid is the most monounsaturated fatty acid found in nature. Özogul (2007) reported that the oleic acid content of commercially important fish species from the Mediterranean, Aegean, and Black Seas ranged from 52-79% of total MUFAs [13]. Oleic acid is also a metabolic precursor for producing most PUFAs [14]. According to Peddyawati (2008), oleic acid intake was related to brain cell growth in infants [15]. Muchtadi et al. (1993) also stated that oleic acid considerably increased HDL cholesterol levels and decreased LDL cholesterol levels [16].

Polyunsaturated fatty acids (PUFA) are fatty acids that contain two or more double bonds [6]. The result showed that the polyunsaturated fatty acids in squid were dominated by eicosapentanoic acid.
acid (EPA) and docosahexaenoic acid (DHA). Both EPA and DHA have gained great importance of health in the human body. Chapkin et al. (2008) reported that EPA and DHA were the major structural components in phospholipid membranes, which have importance in ion transport and membrane fluidity [17]. In this study, the EPA content was 6.93%, 8.82%, and 7.75% in the fresh squid, the dried squid, and the paper squid, respectively (Figure 4).

In general, the double bonds of fatty acids of triglyceride structure changes as the temperature rises. The result showed a decrease in the EPA content when the paper squid was produced using high temperature. Morris et al. (2004) stated that rapid autoxidation of PUFA in foods without antioxidants addition and oxygen reduction strongly affected an increase in the rate of oxidation during heating [8]. However, EPA plays an important role in biological processes, including the reproductive system, immunity, and brain function. The main advantage of EPA is the production of prostaglandins, which regulate the heart rate, blood pressure, the formation of blood clots, fertilization, and immune function [16].

![Figure 4](image)

**Figure 4.** Histogram of polyunsaturated fatty acids in squid; (a) EPA, (b) DHA.

Figure 4 shows the docosahexaenoic acid content in fresh squid, dried squid, and paper squid were 26.84%, 26.45%, and 22.57%, respectively. Mahaffey (2004) reported that DHA is a structural component of lipid that enriches the phospholipid component of the retina and nonmyelin membrane of the nervous system. The effects of oxygen, temperature, and light on the docosahexaenoic acids were investigated. It decreased the docosahexaenoic acid content of the paper squid [18]. Mulyaningtyas (2011) studied the changes in fatty acids due to different processing methods of *Corbicula javanica* and concluded that the DHA content had significant decreases by the heating process [19].

4. Conclusion

Fatty acid profiles of paper squid (*Loligo edulis*, Hoyle) consist of 10 types of saturated fatty acids (SFA), 7 types of monounsaturated fatty acids (MUFA), and 8 types of polyunsaturated fatty acids (PUFA). The highest content of saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid is palmitic acid (7.45%), oleic acid (0.79%), and docosahexaenoic acid (22.57%), respectively. Paper squid shows a high content of unsaturated fatty acids and a decrease in the saturated fatty acids content, which have a negative health impact.

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