



# Determination of Fatty Acid (Epa, Dha, Cholesterol) and Phenols in Squid (*Loligo*Sp.) with Addition Different Liquid Smoke Concentrations

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## ABSTRACT

Liquid smoke is the result of pyrolysis and distillation with the removal of carcinogenic polyaromatic hydrocarbons which affect taste, and color, and act as antioxidants in the product. Antioxidants in liquid smoke are obtained from phenol compounds in liquid smoke. Antioxidants in liquid smoke can inhibit oxidation reactions by binding to free radicals, thus avoiding damage to the fatty acids in the product. Liquid smoked squid is a fishery product that utilizes processing in the form of smoking and salting. Liquid smoked squid is processed by soaking it in a salt solution added with liquid smoke and then smoking it in an oven using gradual temperatures, namely 45°C, 70°C, and 90°C. This study aims to determine the best concentration of the addition of liquid smoke in the manufacture of liquid smoke squid and to determine the moisture content, fat content, EPA, DHA, cholesterol, phenol content, and the preference of panelists for liquid smoke squid. The research method used a completely randomized design (CRD) with treatment by adding liquid smoke concentrations of 0%, 1%, 3%, and 5%. The data obtained were then tested with the normality test, homogeneity test, ANOVA test, and honestly significant difference follow-up test to know the differences in each treatment. The results of data analysis showed that the use of different concentrations of liquid smoke in squid caused significantly different effects on water content, fat content, EPA, DHA, cholesterol, and phenol content of liquid smoke squid. Liquid smoke squid with a concentration of 5% has the best value with a water content of 33.48%, a fat content of 4.03%, an EPA content of 272.50 mg/100 gram, DHA of 1078.57 mg/100 gram, cholesterol 489.29 mg/ 100 grams, phenol content 0.17%, and hedonic test with a confidence interval of  $4.06 \leq \mu \leq 4.25$  indicating that the product was highly liked by the panelists..

Keywords: *cholesterol, DHA, EPA, liquid smoke, squid*

## 1. Introduction

Squid is a commodity of fishery that people like because it has chewy meat and a savory taste. Squid is categorized as a commercial commodity because 75% of the number of squid in the world is a fishery product that can be consumed. Squid is one of the fishery products that is widely consumed because it contains nutrients in the form of selenium, riboflavin, and vitamin B12. According to Nurulludin *et al.* (2021), squid resources (*Loligo*sp.) are one of the leading export commodities for Indonesian fishery products after shrimp, tuna, and seaweed. The potential for squid in WPPNRI 573 in 2011 was 2,100 tons, and the potential for squid in 2016 and 2017 was the same, namely 8,195 tons (KP Ministerial Decree No. 45/MEN/2016 and KP Ministerial Decree No. 50/MEN/217). Utilization of squid resources has reached full utilization level (*fully exploited*).

Squid contains 90% of the body's daily cholesterol requirements, so consumers need to be careful when consuming this product. Apart from containing bad fat in the form of cholesterol, squid also contains unsaturated fatty acids as compounds that suppress cholesterol in the squid itself. The cholesterol content in squid reaches 150mg/100 grams. According to Mekarsari *et al.* (2016), squid has high nutritional content, so it is needed by the body. Apart from having high nutrition which is good for health, squid also has a high risk to health because it contains high cholesterol of 159 mg/100 grams, making it dangerous for health.

Fishery products are rich in benefits for the body, one of which is fatty acids. Fish oil contains omega-3s such as docohexaenoic acid (DHA) and eicosapentanoic acid (EPA). Squid contains good fats in the form of EPA and DHA which can reduce cholesterol. Squid contains 13.4-17.4% EPA and 12.8-15.6% DHA. According to Widiastutiet *et al.* (2019), compound unsaturated fatty acids (PUFA) belonging to the omega-3 group were detected in liquid smoked squid, namely, linoleic, arachidonic, linolenic, EPA and DHA acids with an amount of 17.37%. Omega-3 fatty acids, such as EPA and DHA, can naturally be obtained from fatty fish, especially sea fish.

Fishery products can be processed into smoked products. Using liquid smoke requires a shorter time compared to traditional smoking methods. The use of liquid smoke can also reduce cholesterol levels in the products because it contains aldehyde compounds, carboxylic acids, and phenols. Additionally,

liquid smoke is more environmentally friendly as it reduces air pollution. The innovation of liquid smoke does not compromise the purpose of fumigation. Smoking with liquid smoke still acts as a preservative and enhances the taste, aroma, and texture of the fish.

According to Hutomoet al. (2015), one of the widely developed methods of processing fishery products is fumigation with liquid smoke. Liquid smoke is a liquid created by dispersing smoke vapor in water or liquid condensation resulting from the pyrolysis of wood, coconut shells, or similar materials. Liquid smoke has antioxidative properties and is classified as natural.

## 2. Materials and tools

The ingredients used in making liquid smoked squid products are fresh squid (*Loligo* sp.) in medium size, liquid smoke grade 1, table salt, and clean water. The tools used in testing are *scoresheet* hedonic and organoleptic, oven, analytical balance, porcelain dish, desiccator, burette, test tube, Soxhlet, electric heater, vial, pipette, gas chromatograph, centrifuge tube, centrifuge, stirrer, *beaker glass*, water bath, UV-Vis spectrophotometer, pipette, and *vortex shaker*.

### 2.1 Methods

#### 2.1.1 Sensory (SNI 2731.2:2010)

Sensory testing uses *score sheet* squid after melting (*thawing*), namely SNI 2731.2:2010. The research data was then tested using the Kolmogorov-Smirnov test to determine the normality of the data. The data will then be tested by ANOVA using a 95% confidence interval ( $\alpha=0.05$ ).

#### 2.1.2. Water Content (BSN, 2013)

The water content test in the sample can be calculated based on the weight lost during heating in the oven. Water content testing according to BSN, SNI No. 2908: 2013 The water content obtained can be calculated using the following calculations:

$$\text{Water content (\%)} = \left( \frac{W1 - W2}{W1 - W0} \right) \times 100\%$$

#### 2.1.3. Fat Content (AOAC, 2005)

Fat content was tested using the Soxhlet method. It is calculated using the following formula:

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

Calculation of fat content based on dry weight (%). It is calculated using the following formula:

$$\text{Dry weight} = \text{fat content} \times \left( \frac{100}{100 - \text{water content}} \right)$$

#### 2.1.4. EPA and DHA

EPA and DHA testing was carried out in three stages: derivatization (Christie, 1989), gas chromatography analysis (Pontoh, 2016), and determination of fatty acid content.

#### 2.1.5 Cholesterol (Budiarti et al., 2016)

Cholesterol in samples can be tested using the Liebermann-Burchard Color Reaction method. It is calculated using the following formula:

$$\text{Cholesterol} = (\text{Sample Absorbance}) / (\text{Standard Absorbance}) \times (\text{Standard Concentration}) / (\text{Sampel Weight})$$

#### 2.1.6 Phenol (Princesset al., 2018)

The determination of phenol levels in samples is carried out by measuring the maximum wavelength using UV-Vis spectrophotometry. Absorbance is measured in the 600-800 nm wavelength range, which corresponds to the complementary color of the solution, blue-green.

### 2.1.7 Hedonic (BSN, 2006)

Hedonic tests on samples can be carried out with non-standard panelists, specifically 30 people. The assessments in the hedonic test are based on the panelists' level of liking. The number of favorability levels varies depending on the specified quality range. Assessments can be converted into numerical values and analyzed statistically to draw conclusions. The data is obtained from tabulated assessment sheets, and the quality value is determined by calculating the average results for each panelist at a 95% confidence level.

### 2.2 Making Liquid Smoke Squid

The process of making liquid smoked squid begins with cleaning the squid from dirt. Then soak the squid in water mixed with salt and liquid smoke with each concentration liquid smoke, namely 0%, 1%, 3%, and 5% for 30 minutes. The next step is to drain the squid which has been soaked for 30 minutes. Squid smoking is carried out in stages, namely 45°C, 70°C and 90°C with a time of one hour each. The aim of smoking in stages is so that the texture and nutritional content of the squid are not damaged.

### 2.2 Data analysis

The research was conducted using a Completely Randomized Design (CRD). The treatments applied were different concentrations of liquid smoke used, namely 0%, 1%, 3%, and 5%. The tests carried out in this research were sensory tests, EPA and DHA tests, cholesterol, water content, fat content, phenol, and hedonic. The data obtained will be analyzed using analysis of variance (ANOVA). Results that show real differences will be continued with the BNJ Test because they have small variability values ( $P < 5\%$ ). Non-parametric tests are carried out using Test *Kruskall Wallis*. The purpose of the Test *Kruskall Wallis* is to determine whether there are significant differences between variables using numerical and ordinal data scales. Processing data from hedonic testing using Test *Kruskall Wallis*, If the results show significantly different ( $P < 5\%$ ), then continue with the test *multiple comparisons*.

## 3. Result and Discussions

### 3.1 Water Content

Table 1. Characteristics of Liquid Smoke Squid

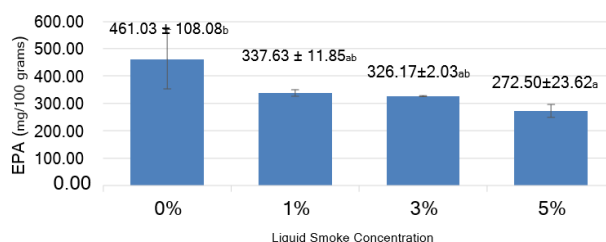
Characteristics	Treatment			
	0%	1%	3%	5%
Water (%)	51.93±7.21 <sup>b</sup>	44.39±3.33 <sup>ab</sup>	40.30±2.19 <sup>ab</sup>	33.48±7.26 <sup>a</sup>
Fat (%)	1.37 ± 0.22 <sup>a</sup>	3.02 ± 0.18 <sup>b</sup>	2.97 ± 0.18 <sup>b</sup>	4.03 ± 0.55 <sup>c</sup>
EPA (mg/100g)	461.03 ± 108.08 <sup>b</sup>	337.63 ± 11.85 <sup>ab</sup>	326.17±2.03 <sup>ab</sup>	272.50±23.62 <sup>a</sup>
DHA (mg/100g)	1764.70 ± 410.06 <sup>b</sup>	1313.17±32.70 <sup>ab</sup>	1216.70 ± 4.78 <sup>ab</sup>	1078.57 ± 124.42 <sup>a</sup>
Cholesterol (mg/100g)	763.90 ± 153.61 <sup>b</sup>	649.43 ± 55.90 <sup>ab</sup>	615.62±60.63 <sup>ab</sup>	489.29±58.54 <sup>a</sup>
Phenol (%)	0.12±0.01 <sup>a</sup>	0.12±0.02 <sup>a</sup>	0.14±0.01 <sup>a</sup>	0.17±0.01 <sup>b</sup>

The water content obtained from the research ranged from 33.48% to 51.93%. Water content testing data shows a significant decrease in water content when the liquid smoke concentration is increased to 1%. This indicates that the addition of liquid smoke is effective in reducing the product's water content. The results obtained from the research on the water content of liquid smoke-treated squid are still within the Indonesian National Standard (SNI) limit of 60%. The water content obtained from testing in each treatment was influenced by several factors: the addition of liquid smoke, the addition of salt, and the smoking process. Liquid smoke forces water out of the material, reducing the free water content. The addition of salt to the soaking water also contributes to the decrease in water content because salt is hygroscopic and draws fluid out of the squid, replacing it with salt. Fumigation affects the water content as well, causing the water to evaporate from the material. According to Jakunget al. (2020), liquid smoke that penetrates the material can force the water out, thereby decreasing the water content. The oven temperature further aids in reducing water content; the higher the oven temperature, the lower the water content.

### 3.2 Fat Content

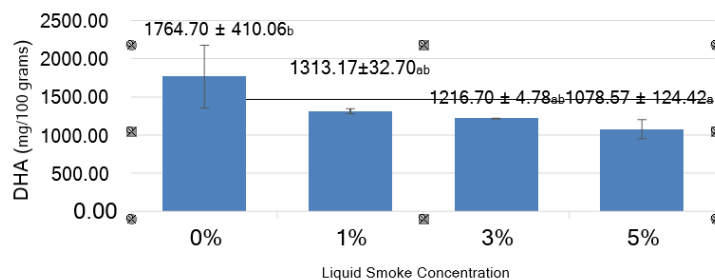
The fat content obtained from the research ranged from 1.37% to 4.03%. Fat content testing data shows a significant increase in fat content when the liquid smoke concentration is increased to 5%. This indicates that water content is inversely proportional to fat content due to the presence of phenol compounds, which inhibit oxidation reactions. The results obtained from the research on the fat content of liquid smoked squid are still within the Indonesian National Standard (SNI) limit of 20%, demonstrating an increase in the product's fat content. This increase is attributed to the addition of salt and liquid smoke.

Phenolic compounds are antioxidants, which can prevent fat oxidation reactions in products, thus avoiding damage such as rancidity. Adding salt and liquid smoke to fishery products helps protect the fat. This is because while salt and hemoprotein have a prooxidant effect, the phenolic substances in smoke exhibit antioxidant activity, which protects the fat in the ingredients. Phenolic compounds from liquid smoke are antioxidant agents that inhibit oxidation reactions and maintain product quality. According to Erdiman *et al.* (2022), an increase in fat content indicates that increasing the concentration of liquid smoke has a significant effect. This increase in fat content is due to the phenol components present in liquid smoke. Consequently, the fat treated with liquid smoke will have a higher phenol content, thereby preventing dangerous reactions within the fat.



The EPA content obtained from research ranged from 272.50 to 461.03 mg/100g. The results from the EPA research indicate a decrease in EPA levels. Omega-3 fatty acids in fishery products, such as EPA and DHA, are easily damaged when exposed to high temperatures during heating. Therefore, careful attention must be given to processing and cooking methods to prevent reducing or eliminating the potential of these fatty acids in the product. Adding liquid smoke to products can mitigate the reduction of EPA content because it contains phenols, which have antioxidant properties and protect the EPA in the product, preventing fat oxidation reactions. According to Private *et al.* (2018), the antioxidant compounds in liquid smoke can effectively protect fats from oxidation, thus preserving the EPA and DHA content from high temperatures or oxygen exposure during processing. The phenol components and their derivatives in liquid smoke act as antioxidants, inhibiting the fat oxidation process in smoked fish.

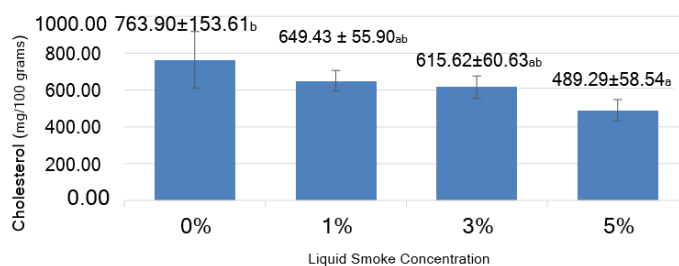
EPA is a type of beneficial fat that can balance and even reduce levels of harmful fats, such as cholesterol, in the blood. In addition to reducing the risk of cardiovascular disease, EPA can lower the risk of depression and enhance eye and brain performance. EPA and DHA can improve cognitive function and overall brain development, as well as enhance visual acuity. According to Kjerstad *et al.* (2020), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are important for brain development and the prevention of cardiovascular disease due to their anti-inflammatory properties. Consuming EPA and DHA can help maintain health in both children and adults.



The DHA content obtained from research ranged from 1078.57-1764.70mg/ 100g. Liquid smoke helps maintain DHA levels because liquid smoke contains phenolic compounds with antioxidant properties, so good fats are not oxidized. According to Amahorseja and Noya (2019), the high EPA and DHA content in smoked fish is due to the smoke components contained in liquid smoke, namely phenol, carbonyl, and total smoke which play a role in inhibiting fat oxidation, so the omega-3 fatty acid content is not oxidized. One of the unsaturated fatty acids contained in liquid smoked squid is DHA which is rich in benefits for the human body. Unsaturated fatty acids in the form of DHA can improve cognitive function, and brain formation and help eye sharpness because DHA is the main fat structure in the formation of the retina of the eye, and helps improve memory. The role of DHA in the human body is as an anti-inflammatory, so it can reduce inflammation in the body and anti-cholesterol because it can overcome high cholesterol which causes blockages or cardiovascular disease by fat accumulation. According to Sprague *et al.* (2020), unsaturated fatty acids, EPA and DHA, are often found in fishery products. Unsaturated fatty acids are beneficial for human health, especially in neurological development and in reducing chronic diseases such as cardiovascular disease and other inflammation.

### 3.3 Cholesterol

Total cholesterol obtained from research ranged from 489.29 to 763.90 mg/100g. Cholesterol testing based on the graph shows a significant decrease in cholesterol when the liquid smoke concentration is increased to 5%. This indicates that the addition of liquid smoke is effective in reducing cholesterol in the product. Squid contains high cholesterol, necessitating limited consumption due to its potential to cause cardiovascular disease. According to Zhang *et al.* (2016), squid has high cholesterol, which is associated with cardiovascular damage. Squid is a major contributor to cholesterol in food. Cholesterol levels can be reduced by balancing with EPA and DHA. Daily cholesterol consumption should not exceed 300 mg/day. This limit suggests that the liquid-smoked squid product still meets the normal consumption limits. According to Lopiet *et al.* (2014), it is recommended that cholesterol consumption be no more than 300 mg/day because the liver produces approximately 1,000 mg of cholesterol per day.



### 3.4 Total Phenol

The total phenol obtained from the research ranged from 0.12-0.17%. One of the reasons for the increase in phenol levels in liquid smoke squid is the addition of liquid smoke to the ingredients. Liquid smoke contains phenol compounds which when used in raw materials, the phenol will be absorbed into the product. Phenol acts as an antioxidant, which makes this compound become a preservative. Phenol in the product can give the product a smoky aroma. According to Choiriyahet *et al.* (2021), phenolic compounds in liquid smoke are produced by the disintegration of lignin content in the raw material. The higher the lignin content, the higher the phenol content. Phenolic compounds provide the aromatic, antioxidant, and antimicrobial characteristics of liquid smoke.

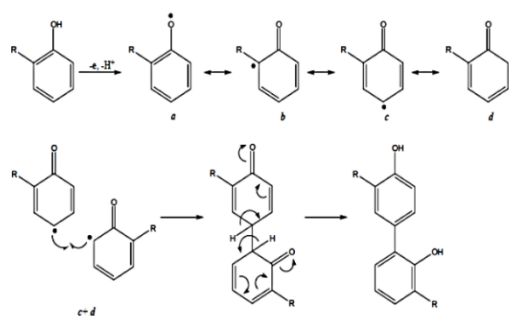


Figure 1. Formation Reaction and

### 3.4 Phenoxyl Radical Incorporation (Asihet *et al.*, 2022)

Figure one explains reaction formation and incorporation of phenoxyl radicals. Phenolic compounds capture free radicals directly through the donation of hydrogen atoms. Radicals are made inactive, where R is a free radical and FI-O is a phenoxyl radical. Antioxidant activity depends on the order of functional groups in the core structure. The antioxidant mechanism of phenol begins with the ability of the phenol group to bind free radicals

The phenolic compounds contained in the product can give the product a distinctive taste and color. The product will have a longer shelf life because phenol acts as an antibacterial and antioxidant. The antibacterial properties of phenol can inhibit the growth of bacteria in the product and the antioxidant properties of phenol can inhibit the oxidation reaction of fat in the product. The antioxidants and antibacterials in phenol can act as product preservatives. According to Permanasari *et al.* (2020), the compounds from liquid smoke are a combination of phenolic compounds and acids that play a role in donating an H atom through electron transfer, so that phenol becomes a free radical, a stable compound or called a phenoxyl radical. The self-stabilization of phenoxyl radicals is carried out by phenol compounds through the resonance effect of the phenol reaction with free radicals. This shows that phenol derivatives are good hydrogen donors in the reaction inhibition process due to the presence of radical compounds, so phenol compounds are called radical inhibitors. According to Asihet *et al.* (2022), flavonoids are oxidized by radicals, resulting in more stable and less reactive radicals. These polyphenolic compounds can directly capture superoxide, while other flavonoids can capture highly reactive oxygen radical derivatives called peroxynitrites as antimicrobial and antioxidant. The advantage of using liquid smoke is as a preservative for products.

### 3.5 Hedonic Quality Analysis

Hedonic testing carried out on liquid smoked squid with various concentrations looked at appearance, smell or aroma, taste and texture using a scale to determine consumer preferences for the product. The scale given is in the form of numbers 1-5 with information in the form of number 1 indicating very dislike, number 2 indicating dislike, number 3 indicating neutral, number 4 indicating like, and number 5 indicating very like.

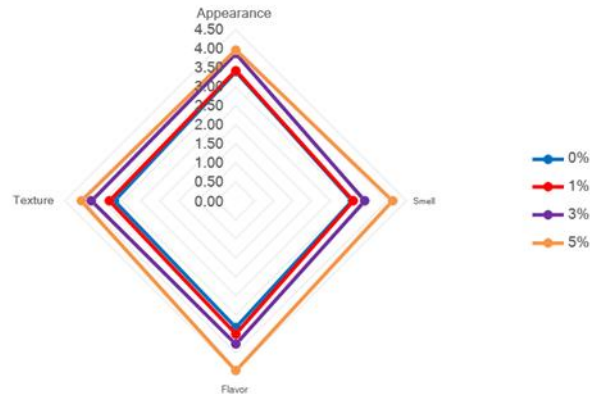


Figure 4. Hedonic Test Results

### 3.6 Appearance

The change in appearance of squid is caused by the smoking process. Smoking using high temperatures in the oven causes the fat in the ingredients to melt and penetrate the surface of the meat. The addition of liquid smoke has an effect because the liquid smoke content will react with the chemical components in the fish. The content of liquid smoke that plays the most role in changing the color appearance of the product is carbonyl compounds due to interactions with the amino groups in the material. The golden white color of the product is caused by the chemical reaction of phenol, acid, and oxygen from the air. This change is caused by a reaction between the chemical sugars and amino acids from proteins with the aldehyde or ketone groups from liquid smoke. The chemical reaction between sugar and compounds from liquid smoke is called a reaction Maillard. According to Saraswati (2018), a reaction occurs Maillard due to the presence of reducing sugars containing free carbonyl ends in the form of aldehydes and ketones which react with free amine groups in amino acids. The presence of sugar, amino acids, pH, temperature, catalyst, and water content in the material will cause the formation of melanoidin or brown pigment. Liquid smoke contains acids, phenol derivatives, and carbonyls which play a role in forming product color. The heating or smoking process using an oven can affect the appearance of the product due to the use of high temperatures in the process. The appearance of the product is identical to the color of the product. The more attractive the product color, the higher the panelists' assessment of the product. According to Maligan *et al.* (2018), appearance is a parameter that can be seen visually which causes panelists to be interested and like the product. The appearance of a product is the main attractive factor before panelists like quality characteristics, such as taste, aroma, color, and texture. In general, consumers choose food that has an attractive appearance.

### 3.7 Smell

The change in the aroma is caused by the addition of liquid smoke containing polycyclic aromatic hydrocarbon compounds in the form of phenol. The aroma produced by liquid smoke depends on the raw materials for making liquid smoke. The raw materials used in making liquid smoke are usually softwood and hardwood which can produce a smoke aroma. The aromatic compounds from the liquid smoke will be absorbed by the product, thereby increasing the panelist's assessment of the product. According to Tense *et al.* (2020), wood commonly used in smoking will produce good smoke and provide an attractive aroma to the product. The attractive aroma is because liquid smoke contains polycyclic aromatic hydrocarbons. The compound that plays a role in providing aroma to products is phenol.

### 3.8 Flavor

The change in taste is caused by the addition of liquid smoke, which contains phenol compounds that penetrate the surface of the product, resulting in a delicious, distinctive smoky flavor. Smoke aerosol that adheres to the surface of the fish will penetrate the fish tissue, so the concentration of liquid smoke will affect the amount of smoke sticking and penetrating the squid. The higher the concentration of liquid smoke added to the squid and the longer the smoking period, the more it will cause an aftertaste or a bitter aftertaste. This can diminish the deliciousness of the fish due to the loss of water content and reduce the panelists' evaluation of the sample. The taste produced in the sample is a crucial factor for the panelists because, despite an attractive appearance, if the taste is unappealing, the panelists' assessment of the product will be low. According to Misbahul *et al.* (2022), panelists' acceptance of food products is strongly influenced by taste, even if other parameters are good. If consumers do not like the taste of the product, it will be rejected.

### 3.9 Texture

The addition of liquid smoke causes a reaction between the smoke components and the proteins on the surface of the product. This is supported by the heating process during smoking. The addition of liquid smoke to the product can result in an increase in the stromal fraction and a decrease in the nitrogen fraction of myofibrillar proteins and free sulphhydryl groups. This will be related to the formation of protein cross-links on the surface which can produce a hard and stable outer crust so that the resulting product is ready to undergo degradation by heat, microbes, and water. The cross-links that form will get in the way of penetration of smoke into the product so that the subsurface area is not exposed to fire and becomes soft. According to Saraswati (2018), changes in the texture of products occur due to smoking which causes a reaction of smoke components with surface proteins. This reaction produces cross-linking which inhibits the penetration of smoke compounds. Changes in the texture of liquid smoked squid are caused by the heating process of smoking squid. The raw material in the form of fresh squid has a dense, compact, elastic texture. The process of smoking squid using an oven causes changes in the texture of the squid because the smoking uses high temperatures. Change in texture from dense, compact, elastic to chewy but easy to chew. According to Ikrawan *et al.* (2019), texture is an important aspect of assessing the quality of food products. Texture is one of the factors that influences consumer acceptance of food products. Texture measurement is very important because it can affect the product image.

## 4. Conclusions

Addition smoke liquid with various concentrations influence reducing water content, fat content and cholesterol in squid. The use of liquid smoke is considered effective in maintaining the quality of squid. Hedonic testing showed that the liquid smoked squid that was much sought after by panelists was the 5% concentration which was superior in terms of appearance, aroma, taste and texture.

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