FORTIFICATION OF SQUID INK AS A SOURCE OF PROTEIN
to the level of preference for biscuit

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ABSTRACT

Squid has ink bags with a fairly good nutritional value with protein content, melanin in the form of melanoprotein, glutamic acid and aspartic acid which gives a savory taste. Ink can be used as a natural black dye and includes untapped waste. Research to determine the highest level of ink in squid on the biscuits so as to obtain the product that panelists receive most. This research was conducted at the Laboratory of Fisheries Product Processing at the Faculty of Fisheries and Marine Sciences, Padjadjaran University, Pasundan University Food Engineering Laboratory in March to October 2019. The research method used was an experimental method with five studies of squid assistance, namely 0%, 0.5%, 1%, 1.5% and 2% based on the weight of wheat flour. The parameters discussed in the study were squid ink yield, air content, ash content, lipid content, blood protein and trials and hedonic tests which included appearance, aroma, texture and taste of biscuits. Based on the results of research on the favorite level of squid ink biscuits can be denied from what is meant by squid ink for all that is needed Other concentrations with an air content of 2.64%, ash content of 1.92%, lipid content of 3.86%, protein content 8.26% and defense value of 1,448.16 gF/cm².

Keywords: Biscuits, Fortification, Levels of Preference, Squid Ink

INTRODUCTION

Squid is a fairly abundant sea product in Indonesia and is in great demand by the community of seafood and Chinese food enthusiasts. The export of squid in the January 2015-April 2019 period increased by 17.72%. In 2017 the export of squid was 32.77 thousand tons and increased in 2018 to 45.55 thousand tons and in 2019 amounted to 47.03 thousand tons (DJPDSPKP 2019).

Squid has a characteristic in the difference that is the existence of an ink bag. Squid ink contains protein of 10.88% consisting of essential and non essential amino acids (Mukholik 1995). The dominant non essential amino acid levels are glutamic acid and aspartic acid. The content of glutamic acid which causes a savory taste in squid ink (Okozumi and Fujii 2000). So far, many people consider squid ink to be of no use, even though squid ink has many benefits and benefits for bodily health (Agusandi 2013). Squid ink is used as a flavor enhancer and natural coloring agent, besides that squid ink has health benefits (Sasaki et al. 1997).
Dyes are widely used in food products, one of which is biscuits. (Mudjajanto 2007). One alternative that is safe for consumption and can improve the quality of nutrition in biscuits is by increasing squid ink as a natural coloring and high nutritious snack. The use of squid ink as an additive to the product is also one of the opportunities that has the potential to reduce squid ink waste wasted. Squid ink (Loligo sp.) The black one used for natural dyes also enhances flavor and taste (Astawan 2008).

Fortification is one or several nutrients (nutrients) into food with the aim to increase consumption of nutrients so that nutritional status increases. (BPTP 2016). Squid ink can be made as an additive to food products that can be used as a source of protein and also as a natural coloring agent. One product that can be fortified is biscuits. Biscuits are products obtained by baking dough from wheat flour with other food ingredients and without food additives allowed (BSN 1992).

Biscuits were chosen as alternative products fortified with squid ink because biscuits are a product that is very popular with the people of Indonesia, both children, adults and adults (Wijaya 2010). Biscuits are also a bakery product more often than other bakery products (Dogan 2006). Biscuits have a low moisture content so they have a long shelf life (Kramer and Twigg 1973).

Fortification of biscuits can affect chemical characteristics and organoleptic characteristics. Based on this background, it is necessary to discuss the squid ink in the biscuits most approved by the panelists by conducting research on squid inks on the chemical and organoleptic characteristics of biscuits.

MATERIALS AND METHODS

MATERIALS AND TOOLS
The ingredients used are squid ink obtained from Ciawitali Market, Kunci Biru flour (Bogasari), eggs, margarine (Blue Band), vanilla, baking powder (Koepoe-koepoe), milk powder (Dancow) and granulated sugar (Gulaku). The tools used in the digital scales are 0.01 gram accuracy, electric ovens, mixers, trays, spoons, knives, basin containers, cutting boards, plastic plates, cups and biscuit molds.

RESEARCH METHODS
The method used is an experimental method with five ways to use squid ink 0%, 0.5%, 1%, 1.5% and 2% based on the weight of wheat flour. The first step is to take squid ink and calculate the yield of squid ink, then proceed with making biscuits and do each approval on organoleptic complexity based on preference level (hedonic test) using 20 semi-finger panelists and proximate test based on water yield, content ash, lipid content and protein content as well as the hardness test in the best control and help with approval of squid ink.

PREPARATION AND CALCULATION OF SQUID INK RENDEemen
The squid to be used is then stored with clean water, drained and weighed. The ink bag is taken from the squid's body, then taken in a clean container. The ink in the squid bag is taken by means of a full pressure, then the ink that comes out is collected in a clean container. The yield is a substitute for the dry weight of the product produced by the weight of the raw material (Yuniarifin et al 2006). Yield uses percent (%) units. This yield value is useful for learning a lot of material that can be used. If the value of the product or material is higher, then more can be used (Hiswaty 2002). The results of calculating squid ink with an average weight of 44.55 g / head produce squid ink with an average weight of 0.3 g / head with a resulting body length between 12 cm to 19 cm. The yield of squid ink produced is 6.38 grams from the weight of squid 3,100 grams resulting in squid ink yield of 0.21%.

MAKING BISCUITS
The making of biscuits was approved by Soenaryo. The process of making biscuits according to Sunaryo (1985). Eggs, sugar, butter, beaten until fluffy for 15 minutes, until homogeneous, then, added liquid ink, squid, until the dough, biscuits, according to, and beaten, then flour, vanilla, baking powder, milk mixed into the dough, stirring, and stirring. Bake in 150 0C oven for 17 minutes until cooked.

**ORGANOLEPTIC TEST**
Organoleptic test to determine the nature or factors of taste and acceptability of food. Organoleptic tests carried out are tests of preference, aroma, texture, and appearance. Organoleptic tests are carried out by giving examples of each different aid presented by giving examples, then panelists to give an impression on the hedonic scale.

**CHEMICAL TEST**
The chemical characteristics discussed were the most understood by panelists and controls (0%). Chemical characteristics include air content, ash content, protein content and lipid content according to AOAC (2005).

**DATA ANALYSIS**
Proximate test data were analyzed descriptively and compared with the value of the Indonesian National Standard for the quality of SNI 01-2973-2011 biscuits. Organoleptic test data were analyzed using Friedman's two-way analysis of variance with the Chi-Square test (Siegel 1991).

**RESULTS AND DISCUSSION**

**BISCUITS' FAVORITE LEVEL**
The results of observations of favored tests conducted on organoleptic characteristics are the appearance, aroma, texture and taste of the biscuits.

<table>
<thead>
<tr>
<th>Squid Ink Concentration (%)</th>
<th>Appearance Value</th>
<th>Aroma Value</th>
<th>Texture Value</th>
<th>Taste Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.2 bc</td>
<td>7.5 a</td>
<td>5.7 a</td>
<td>6.3 a</td>
</tr>
<tr>
<td>0.5</td>
<td>5.6 a</td>
<td>7.2 a</td>
<td>6.2 ab</td>
<td>6.2 a</td>
</tr>
<tr>
<td>1</td>
<td>5.8 ab</td>
<td>7.3 a</td>
<td>6.5 ab</td>
<td>6.4 ab</td>
</tr>
<tr>
<td>1.5</td>
<td>6.9 bc</td>
<td>7.8 a</td>
<td>7.4 b</td>
<td>7.8 b</td>
</tr>
<tr>
<td>2</td>
<td>7.8 c</td>
<td>6.7 a</td>
<td>6.6 ab</td>
<td>6.0 a</td>
</tr>
</tbody>
</table>

The results of observations of hedonic tests on the appearance of biscuits with the addition of squid ink are presented in Table 1. Based on panelist ratings of the appearance of biscuits it was found that the addition of squid ink to biscuits had an average range of appearance of 5.6 to 7.8. The appearance of squid ink biscuits at 2% treatment has the highest average value of 7.8 (preferred), with the appearance of biscuit color being darker or darker in color compared to other treatments. The 0.5% treatment biscuit appearance had the lowest average value of 5.6 (neutral), with the color appearance of light gray biscuits.

This is due to the addition of squid ink causing the resulting color to be darker or to a darker black color because squid ink contains natural melanin so the color is blacker. The results obtained show that panelists prefer 2% treatment with clearer or blacker color than 0.5% treatment which results in light gray, faded and less attractive colors. The appearance of the biscuits is presented in Figure 1.
Figure 1. Biscuit Appearance Based on Treatment Addition of Squid Ink

This is supported by Agusandi’s research (2013), the value of panelist preferences towards the appearance of poor noodles increases for each payment of squid ink, this causes 0.5% and 1% of the black color of the noodles to still look faded so that the panelists are less favored, more panelists like Settings 1.5% considering the clear black color is not yet thick.

The brownish-yellow color of the biscuits without the approval of the squid ink was requested by Maillard’s reaction. Maillard reactions often occur in food products during heating. Maillard reaction is the reaction between an amino group of a free amino acid peptide or protein chain residue with a carbonyl group of any well heated lipid (Sarastuti et al. 2015). Black color on biscuits, using squid ink with a concentration of 0.5%, 1%, 1.5% and 2% due to differences in the natural melanin pigment contained in squid ink

Based on statistical tests on 0.5% squid ink approval training showed significantly different appearance with 0% and 2% training. The 1.5% treatment is not significantly different from all exercises. The appearance of biscuits at all meetings still requires panelists because it is still in the average range of 5.6 to 7.8 (participation).

Statistical test results show that the squid test results provide real results on the level of panelist acceptance on the appearance of the biscuits produced. Based on preference level, all assistance is still issued by panelists because of the average value of appearance, all assistance is still within the limits of the product value.

AROMA

The observations of the hedonic test on the aroma of the biscuits by approving the squid ink are presented in Table 1. Based on the panelist’s assessment of the aroma of the biscuits obtained in accordance with the requested squid ink on the biscuits, increase the average aroma of 6.7 to 7.8. The aroma of squid ink biscuits at the 1.5% consultation has the highest average value of 7.8 (accepted), with the aroma of biscuits rather the distinctive aroma of squid ink. The aroma of biscuits in favor of 2% has the lowest average value of 6.7 (agree), with the biscuit’s aroma more distinctive than the squid ink but not overpowering.

Because this is because the ink increases squid causes the resulting aroma to smell fishy because squid ink has a fishy aroma. The results obtained indicate that the panelists preferred 1.5% consultation with the aroma of biscuits rather typical smell of the squid ink compared to...
2% consultation which had the aroma of biscuits more typical aroma of squid ink but not stinging. The smell of biscuits that arises at each agreed coverage at an approved concentration of squid ink. The higher the concentration that is given, the distinctive aroma of squid ink will increasingly smell, but increasing 2% squid ink aroma still requires the lowest average value. According to Subandono et al. (2013), the aroma arising on biscuits can also be caused by various other ingredients in the dough ingredients such as margarine and sugar.

Based on statistical tests on training without approval of squid ink (0%) to approval of 2% squid ink permits showed no significant difference in aroma. Without the approval of squid ink (0%) to approval of squid ink 0.5%, 1%, 1.5% and 2% are still needed by panelists. The addition of squid ink does not affect the taste level of the biscuit's aroma because in each coverage only different amounts of squid ink are added, besides all the compositions of all ingredients added the same.

Statistical test results showed that squid ink did not give real results on the level of panelist acceptance of the aroma of biscuits produced. Based on the level of preference in all needs is still taken by the panelists because the average value of the scent of all aid is still within the upper limits of the product value.

TEXTURE

Based on the panelist's assessment of the texture of the biscuits it was found that the addition of squid ink to the biscuits had an average texture range of 5.7 to 7.4. The texture of squid ink biscuits at 1.5% treatment has the highest average value of 7.4 (preferred), with a crisper and denser texture. texture of 0% biscuits treatment has the lowest average value of 5.7 (neutral), with crispy and solid biscuits texture. The results obtained showed that the panelists preferred the 1.5% treatment with a more crispy and dense biscuit texture than the 0% treatment which had a crispy and dense biscuit texture.

According to Mukholik (1995), the texture in the hedonic test is influenced by several factors, including the raw material for squid ink which is alkaline which will produce a product with a good texture when gluten in flour interacts with squid ink which is alkaline. According to Apriyanto et al. (1989) biscuit crispness is influenced by protein, amylose and amylopectin content. Protein has hydrophilic properties which has a high water absorption, so the higher the protein content in biscuits, the texture tends to be harder or less crispy. The crispness of the biscuits is also influenced by the use of margarine and egg yolks which has a role in improving the texture. According to Syahrul et al. (2010) the crispness factor (texture) determines whether a dry product is preferred or not. The texture of food products depends on the ingredients used, especially the protein content.

Based on statistical tests that the treatment without the addition of 0% squid ink with the treatment of adding 1.5% squid ink showed a significantly different texture. The treatment of adding squid ink 0.5%, 1% and 2% was not significantly different from the treatment of 0% and 1.5%. The treatment without addition of squid ink (0%) to the treatment of adding squid ink 0.5%, 1%, 1.5% and 2% is still preferred by panelists.

Statistical test results show that Squid Ink has a significant influence on the level of panelist acceptance on the texture of the resulting biscuits. Based on the level of preference in all treatments is still preferred by panelists because the average texture value of all treatments is still above the product rejection limit.

TASTE

The observation results of the hedonic test on the taste of biscuits with the addition of squid ink are presented in Table 1. The taste of squid ink biscuits at 1.5% treatment has the highest average value of 7.8 (preferred), with sweet biscuit flavor a little savory and a bit like a squid ink. The taste of the 2% biscuits treatment has the lowest average value of 6 (preferred),
with the dominant taste of squid ink. The results obtained showed that the panelists preferred the 1.5% treatment with slightly savory sweet taste and slightly squid ink compared to the 2% treatment which had the dominant taste of squid ink. The savory taste caused by biscuits is derived from squid ink. According to Okozumi and Fuji (2000), squid ink contains glutamic acid which causes a savory taste to squid ink.

The taste of the biscuits that arise in each treatment depends on the concentration of the addition of squid ink. The higher the concentration given, the special taste of squid ink will be dominant, but until treatment 2% the taste of biscuits is still preferred. This is in accordance with the statement of Winarno (2004), that several factors can affect taste, among other chemical compounds, temperature, concentration and other taste interactions. According to DeMan (1997), that the taste caused by food products can come from the food itself also comes from substances that are added from outside during the process, so that it can cause a sharp taste or vice versa to be reduced. According to Hastuti (2012) in Herni (2018), the taste caused by biscuits is also influenced by the addition of raw materials such as sugar, margarine and egg yolks, because sugar tends to give a distinctive flavor by the caramelization during the oven process.

Based on statistical tests that the treatment without the addition of squid ink (0%) with the treatment of adding squid ink 0.5% and 2% showed a taste that was not significantly different, but significantly different from the 1.5% treatment. The treatment of adding squid ink 1% was not significantly different from the treatment of 0%, 0.5%, 1.5% and 2%. The taste of biscuits in all treatments is still preferred by panelists because it is still in the average range of 6 to 7.8 (preferred).

Statistical test results show that the addition of squid ink has a significant influence on the level of panelist acceptance on the taste of the biscuits produced. Based on the level of preference, all treatments are still preferred by panelists because the average value of the appearance of all treatments is still above the value limit of product rejection.

### Table 2. Chemical Composition of the Best Treatment Biscuits

<table>
<thead>
<tr>
<th>No.</th>
<th>Chemical Composition of Biscuits</th>
<th>Proximate Test Results (%)</th>
<th>SNI Biscuits (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Kadar Air*)</td>
<td>3,1936</td>
<td>2,6381</td>
</tr>
<tr>
<td>2.</td>
<td>Kadar Abu**)</td>
<td>1,7495</td>
<td>1,9213</td>
</tr>
<tr>
<td>3.</td>
<td>Kadar Lemak***)</td>
<td>4,1261</td>
<td>3,8638</td>
</tr>
<tr>
<td>4.</td>
<td>Kadar Protein**)</td>
<td>7,8266</td>
<td>8,2625</td>
</tr>
</tbody>
</table>

Note: *) SNI 2973-2011  
**) SNI 2973-1992

### CHEMICAL TEST

The results of observations of chemical tests consisting of water content, ash content, lipid content and protein content were carried out on the control treatment (0%) and treatment with the addition of the most preferred squid ink.

### WATER CONTENT

Based on the results of the administration obtained biscuit water content in the treatment without the addition of squid ink 0% that is equal to 3.1936% and biscuit water content in the treatment of adding squid ink 1.5% which is equal to 2.6381%, it shows that with addition of squid ink the water content decreased by 0.5%. The more squid ink that is added, the lower the water content contained, even though squid ink has a fairly high water content. According to Agusandi et al. (2013), the squid ink moisture content was an average of 78.46%. Moisture content of biscuits without the addition of squid ink and with the addition of squid ink is still...
below the required value range SNI 2973-2011 which is a maximum of 5%, so it can be said that the water content of biscuits with the addition of squid ink meets the quality requirements of biscuits based on SNI.

The low or decreased water content in biscuits can also be affected by the roasting process due to the simultaneous heat transfer and mass transfer. Heat transfer occurs from the heating source to the heating media (hot surface and hot air) to the baked material. The period of movement occurs is the movement of water from material to air in the form of steam (Muchtadi 2013). The value of water content that is too low causes the biscuits to have a burnt taste and the color will be too dark, if it is too high then the structure will not become crunchy and may break and taste changes during storage will occur more quickly (Manley 2000).

**ASH CONTENT**

Based on the results of the administration obtained the content of biscuit ash in the treatment without the addition of squid ink 0% that is equal to 1.7495% and the content of biscuit ash in the treatment of adding squid ink 1.5% that is equal to 1.9213%, it shows that with the addition of squid ink to ash content increased by 0.2%. The increase in ash content in the addition of squid ink biscuits is due to the ash content contained in squid ink. According to Agusandi et al. (2013), squid ink contained ash content of 2.74%. Ash content determined by SNI 01-2973-1992 is a maximum of 1.5%. The level of biscuit ash without addition or addition of squid ink has a higher value than the specified standard. The results of the ash content in the biscuits indicate that the addition of squid ink tends to increase but the resulting ash content does not show a significant increase.

According Tahar et al (2017), ash content has something to do with minerals of food. Ash content also serves to know that the higher the ash content, the worse the quality of the food. According to Akbar (2016), ash content can be used to determine whether or not a processing is good, know the types of ingredients used and determine the nutritional value parameters of a food ingredient. According to Styawati et al (2013), a decrease in ash content is expected, because the decreasing ash content means that the organic matter content will increase. Organic material contains food substances that are quite important, namely protein, lipid, and carbohydrates and vitamins.

**LIPID CONTENT**

Lipid is a source of energy for the body that can provide energy values greater than carbohydrates and protein which is 9 kcal / g. The function of lipid in the body, among others, is as an energy source, part of the cell membrane, a mediator of inter-cell biological activity, an insulator in maintaining body temperature balance, protective body organs and solvents of vitamins A, D, E, and K (Sartika 2008). Lipid also functions as a source of flavor and gives the product a soft texture (Winarno 2004). Lipid is the third most important component in the biscuit industry (Manley 1998).

Based on the results of the administration obtained biscuit lipid content in the treatment without the addition of squid ink 0% which is equal to 4.1261% and biscuit lipid content in the treatment of adding squid ink 1.5% which is equal to 3.8638%, it shows that with addition of squid ink lipid content decreased by 0.3%. The results of the lipid content in the biscuits indicate that the addition of squid ink tends to decrease but not significantly. This decrease in lipid content is due to the low lipid content of squid ink. According to Gonzalez et.al. (2003), squid ink contained a lipid content of 0.2% whereas according to Prabawati (2005) squid body lipid content was 0.2% -1.4% derived from monounsaturated lipidty acids and polyunsaturated lipidty acids.

Biscuit lipid content determined by SNI 01-2973-1992 is a minimum of 9.5%. The lipid content of biscuits without addition or with the addition of squid ink has a lower value than the
specified standard. According to Irmayanti (2017), the low lipid content of biscuits is influenced by the lack of use of additives such as margarine during the biscuit dough process. According to Lingga (2012) in Irmayanti (2017), margarine is in the form of bound as lipoprotein, where margarine when added to the dough, then the dough will have high lipid content as well. According to Windsor (2001) in Pratama (2014), lipid content is also influenced by the cooking process when baking. Protein will coagulate if the material is heated so that a lot of water and lipid comes out.

PROTEIN CONTENT

Based on the results of the administration obtained biscuit protein levels in the treatment without the addition of squid ink 0% which is 7.8266% and biscuit protein levels in the treatment of adding squid ink 1.5% which is equal to 8.2625%, it shows that with addition of squid ink protein content increased by 0.4%. The increase in protein content is due to the high levels of protein in squid ink. The higher the concentration of squid ink given the value of the protein content will be higher. According to Mukholik (1995), squid ink contains protein of 10.88% consisting of essential and non essential amino acids. According to Okozumi and Fujii (2000), squid ink melanoprotein contains dominant amino acids which are dominant in the form of lysine, leucine, arginine and phenylalanine. The dominant non essential amino acid levels are glutamic acid and aspartic acid. According to Almatsier (2006) in Hidayati (2016), the quality of a protein is determined by the type and proportion of amino acids it contains, high-quality protein is a protein that contains all types of essential amino acids in proportions suitable for growth. All animal proteins are good proteins because they have more complete amino acids.

The protein content of biscuits determined by SNI 2973-2011 is a minimum of 5%, so it can be said that the protein content of biscuits without addition or addition of squid ink meets the quality requirements of biscuits based on SNI which is more than 5%. The results of protein content in biscuits indicate that with the addition of squid ink it tends to increase.

According to Pratama et al. (2014), the high and low measured values of protein can be influenced by the amount of water content lost (dehydration) from the material. The measured protein value will be even greater if the amount of water lost is greater. According to Sebranek (2009), the measured protein content depends on the amount of ingredients added and is largely influenced by the water content. Buckle et al. (1987) added that protein content is influenced by water content and fat content. The higher the protein content, the lower the water content.

HARDNESS TEST

Hardness is an important indicator in analyzing food textures, especially in baked products such as bread and biscuits that show resistance to breaking due to the applied force (Wenzhao et al. 2013 in Istinganah et al. 2017).

Based on the results of the administration obtained the value of biscuits hardness in the treatment without the addition of squid ink 0% that is equal to 1007.45 gF/cm² and the protein content of biscuits in the treatment of adding squid ink 1.5% that is 1448.16 gF/cm². The level of hardness is influenced by the degree of gelatinization, degree of development, water solubility index and water absorption index. The higher degree of starch gelatinization will cause a higher degree of development, so that the value of violence decreases (Muchtdi et al 1998 in Pitrawati 2008). Increasing value of violence shows a harder and less crispy texture compared to products that have a lower hardness value (Pratama 2014). According to Apriyanto et al. (1989) hardness is also influenced by protein content where the higher the protein content in biscuits, the texture tends to be tougher or less crispy because the protein has hydrophilic properties which has a high water absorption.
The value of the hardness level of the resulting biscuit product is also influenced by several factors, namely the formulation of biscuits and the thickness of the biscuits. The temperature and the baking time also affect the hardness value of the biscuits produced. Rapid heating at high temperatures causes denatured proteins and loses the ability to bind water, melts fat and disperses throughout food so that changes in the surface become dry and the texture becomes more crispy. Changes in texture due to baking are determined by the nature of the food, temperature, and duration of heating (Pratama et al. 2014).

CONCLUSION
Based on the results of biscuit research with the addition of squid ink, all treatments were still favored by panelists. The treatment of adding 1.5% squid ink to the biscuits was the panelists’ most preferred treatment based on organoleptic tests. Chemical characteristics of biscuits with the addition of squid ink produced were 2.64% water content, 1.92% ash content, 3.86% content content, 8.26% protein content and hardness value of 1,448.16 gF / cm².

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