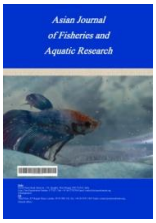

*Asian Journal of Fisheries and Aquatic Research*

***13(4): 10-17, 2021; Article no.AJFAR.70799***
***ISSN: 2582-3760***

# Study of Preferred Level of Biscuits Fortified With Nilem Fish Egg Protein Concentrate

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author USM designed the study, performed the statistical analysis, and wrote the original draft of paper, which was revised by authors EL and J approved by all authors. Authors EL, ER and J managed the analyses of the study. All authors have read and agreed to the published version of the manuscript*

***Article Information***

DOI: 10.9734/AJFAR/2021/v13i430270
Editor(s):
(1) Dr. Rakpong Petka. Khon Kaen University, Thailand.
Reviewers:
(1) Elisa Julianti, Universitas Sumatera Utara, Indonesia.
(2) Dyna Theng, Royal University of Agriculture, Cambodia.
Complete Peer review History: https://www.sdiarticle4.com/review-history/70799

***Original Research Article***

***Received 10 May 2021***
***Accepted 16 July 2021***
***Published 24 July 2021***

## ABSTRACT

Fish eggs are an essential source of protein to increase nutritional value because of their excellent quality protein content but have not been widely used, especially in food processing. Biscuits are one of the most consumed products globally, made from cereal flour such as wheat flour or other flour. Four different formulations containing nilem fish egg protein concentrate (NFEPC) were used in this study to produce biscuit samples with better sensory characteristics and higher protein content. This study aimed to determine the level of addition of nilem fish egg protein concentrate in the biscuits making, which panelists preferred based on their organoleptic assessment. In this study, the method used was an experimental method with four levels of treatment of nilem fish egg protein concentrate with concentrations of 0%, 2.5%, 5.0%, and 7.5% (w/w) carried out on the organoleptic characteristics, which include the appearance, aroma, texture, and taste of biscuits. The results showed that the optimum addition of nilem FEPC was at a 2.5% level preferred by consumers regarding all characteristics (appearance, aroma, texture, and flavor), with the average score ranging from 5.7 to 6.2 on average.

*Keywords: Biscuit; protein concentrate; nilem fish egg; organoleptic.*

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## 1. INTRODUCTION

Biscuits are one of the most popular and favorable pastries, made from wheat flour with other food ingredients, by heating and shaping processes [1]. Therefore, biscuits were included in the snack group that is very popular both for parents and children [2]. Biscuits consist of a lot of carbohydrate content but a small degree of protein content. Therefore it is crucial to add protein sources in the formulation. Biscuit products can be easily enriched with protein, vitamins, and minerals to meet the specific needs of particular target groups of the populations [3]. One of the protein sources that can be used is fish egg protein concentrate.

The utilization of protein-based fishery products continues to develop, and fish egg protein concentrate is one of them. Fish Egg Protein Concentrate (FEPC) is a fishery product produced by removing water and oil from fish eggs, increasing the concentration of protein, which is an excellent source of highly digestible amino acids other than other nutrient materials [4,5]. Generally, this product is applied to high-carbohydrate foods [6].

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Nilem fish (Osteochilus hasselti C.V) is a freshwater fish commodity that has a lot of potential that can be developed. One of the fishery products that have the potential as a source of an eggs is nilem fish (*Osteochilus hasselti*). The proportion of nilem fish eggs in adult fish is about 18-26% of fish body weight [7]. According to research by Rostika et al [8], fecundity is the number of eggs per unit weight or length. The observed nilem fish has a weight of about 108-418 g and a fecundity of 26,200-123,880 eggs. So that the potential for nilem fish eggs is about 75-108 g of its body weight. Nilem fish eggs are of very good quality, so they can be exported as a substitute for caviar [9].

Nilem fish (Osteochilus hasselti C.V) is freshwater fish commodity which has many potential that can be developed. One fishery product with potency as a source of an egg is nilem fish (*Osteochillus hasselti*). The proportion of nilem fish eggs in adult fish is about 18-26 % of the fish's body weight [7]. Fecundity is the number of eggs per unit of weight or length The observed Nilem fish weigh is approximately 108-418 g and the fecundity is 26,200-123,880 eggs. Nilem fish egg can be exported as caviar substitute Nilem fish is an endemic Indonesian fish that is easy to cultivate and environmentally friendly because this fish eats phytoplankton [8;9].

The fortification of nilem fish egg protein concentrate on biscuits is expected to produce biscuits with higher protein content but will impact the level of preference. Therefore, this research was carried out to determine the appropriate level of addition of nilem fish egg protein concentrate in the production of biscuits to obtain the most preferred product by panelists.

## 2. MATERIALS AND METHODS

Biscuits ingredients (wheat flour - salt - sugar - shortening fat - vanilla - baking powder - eggs - milk) were purchased from the local market, Jatinangor Bandung, Indonesia. Egg fish protein concentrate was prepared from nilem fish egg. Fig. 1 presented the flow diagram of Nilem fish protein concentrate preparation at the Laboratory of Fishery Products Processing Technology, Faculty of Fisheries and Marine Sciences, Padjadjaran University.

### 2.1 Preparation of Biscuits

The ingredients were weighed at the formula: 200 g of wheat flour and NFEPC fortification at levels (0, 2.5, 5.0, and 7.5%); 40.0 g of refined sugar; 4.0 egg yolks ( 60.0 g); 50.0 g of margarine; 0.08 g of baking powder; 0.4 g of vanilla and 60 ml skim milk. The eggs, sugar, and margarine were initially homogenized with a hand blender for 15 minutes. Then, add half of the required flour and all other ingredients and mixed again. The remaining flour was added and mixed for 140 seconds to form a solid texture. The dough was sheeted for 3 mm thickening and was cut to the desired shape. Biscuits were created manually and baked at 150°C for 20 minutes in a thermostatically controlled oven with an air fan. The biscuits cooled at room temperature for 75 minutes.

### 2.2 Organoleptic Analysis

The method used is an experimental method consisting of 4 treatments, namely the addition of nilem fish egg protein concentrate, namely 0%, 2.5%, 5%, and 7.5% of the weight of the wheat flour used. Observations were made on the organoleptic preferences of biscuits, including the appearance, aroma, texture, and taste of biscuits. Organoleptic properties of the biscuits were evaluated in Padjadjaran University, Department of Fisheries by 20 panelists.

Four coded samples of biscuits were presented to each of the panelists. They were asked to assess the biscuits for color, flavor, taste, texture, appearance and overall acceptability using the 5-point hedonic scale (1 = Dislike very much, 2 = Dislike moderately, 3 = Neither like nor dislike (Neutral), 4 = Like moderately, 5 = Like very much) for the assessment.

### 2.3 Statistical Analysis

The experimental data were subjected to analysis of variance, at the confidence level of a p=<0.05, using IBM SPSS Statistic 23 software (IBM, Inc., USA). The tests of Friedman were used to determine statistically significant differences and continued with the multiple comparison test to determine the differences between parameters. Furthermore, the Bayes method was used to determine the decision to produce the most organoleptically preferred biscuit product used.

## 3. RESULTS AND DISCUSSION

### 3.1 Appearance

The appearance of a product is the first thing the panelists assess whether the product is acceptable or not. Consumers tend to choose foods that have an attractive appearance [10]. The average level of preference for the appearance of biscuits from different levels of Nilem FEPC addition is shown in Table 1.

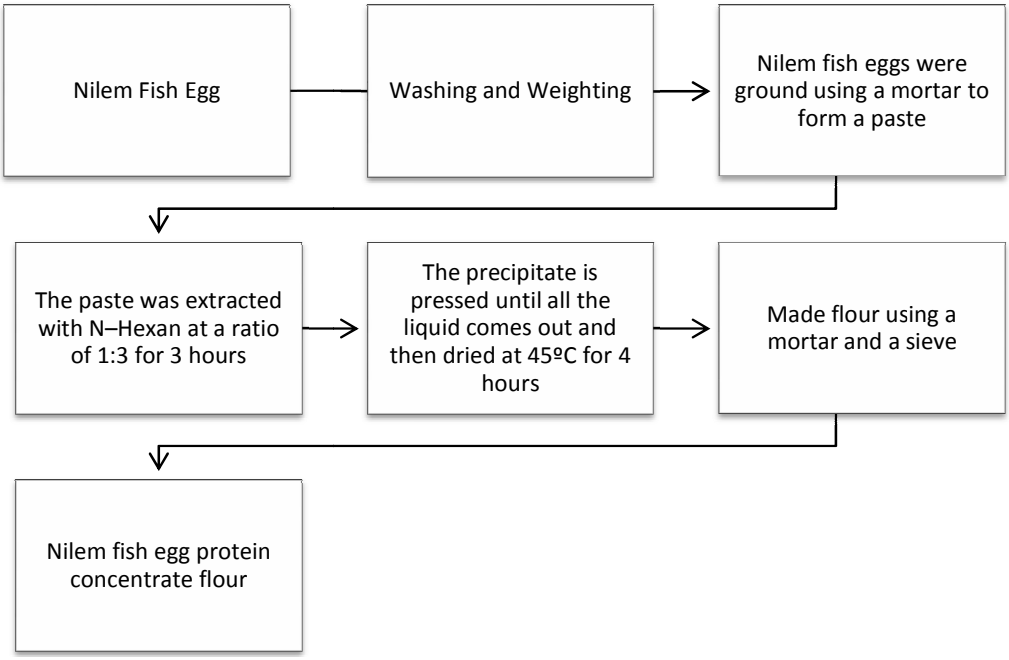


**Fig. 1. Flow diagram of the preparation of Nilem fish eggs protein concentrate**

**Table 1. The appearance quality of biscuits from different levels of fortification of Nilem FEPC**

| Fortification Treatment | Median for the appearance of preferred biscuit | The average level of preference for appearance |
|---|---|---|
| 0 | 4 | 4.15 [b] |
| 2.5 | 4 | 3.6 [ab] |
| 5.0 | 4 | 3.85 [ab] |
| 7.5 | 3 | 2.85 [a] |

** [a,b] Mean values with the same superscript letter in same column are not significantly different at p< 0.05*

Analyzing the appearance of biscuits (Table 1), it can be seen that biscuits without the addition of NFECP had the highest average value of 4.15 (like moderately to like very much). The appearance of the biscuits is bright yellow. The biscuits produced from the addition of 5% nilem FEPC were bright yellow in color appearance and had an average value of preference 3.85 (like moderate); there were brown spots, compact and neat shape. Biscuits with the highest preference score obtained from treatment 2.5% have an average value of 3.6 (medium likes) with bright yellow color, few brown spots, compact and neat shape. The appearance of biscuits with the addition of nilem FEPC 7.5% has the lowest average value of 2.85 (neutral) with a bright yellow color, and there is many brown spots, compact and neat shape. This result was in agreement with the study done by Fajrianti *et al.* [11]. Photos of biscuits obtained from various treatments for adding nilem FEPC are shown in Fig. 2.

The fortification of more protein concentrate of nilem fish eggs in making biscuits can affect the appearance of the resulting biscuits. One of the factors that contribute to the appearance of biscuits was caused by the Maillard reaction. During the baking process, with the addition of nilem FEPC, there would be a reaction between reducing sugars and amino acids produced in a brownish color in the final product of biscuit making [12]. Although, for the appearance parameter, the differences were generally not statistically significant between control and addition NFEPC 2.5-5.0%, while within control and addition NFEPC 7.5%, the difference proved to be statistically significant.

The increase in the addition of nilem FEPC to the biscuits correlated with the rise in brown spots, making the biscuits appear darker. This agrees with a study conducted by Sari [13], that the addition of sepat siam fish flour to the characteristics of biscuits. The addition of Sepat Siam fish meal resulted in more biscuits with a darker appearance. Sari [13] showed that biscuits with the addition of 10% Sepat Siam fish meal were the most preferred by panelists because they produced a color that was not too dark.

## 3.2 Aroma

Aroma is an assessment of organoleptic characteristics that affect the level of preference of a product. A product can be known the quality by the aroma produced. In general, food products with good quality have a distinctive aroma that is still delicious, while foods with a rotten smell indicate a decrease in the quality of the product. The results of the average preference level of biscuit aromas from a different concentration of nilem FEPC are shown in Table 2.

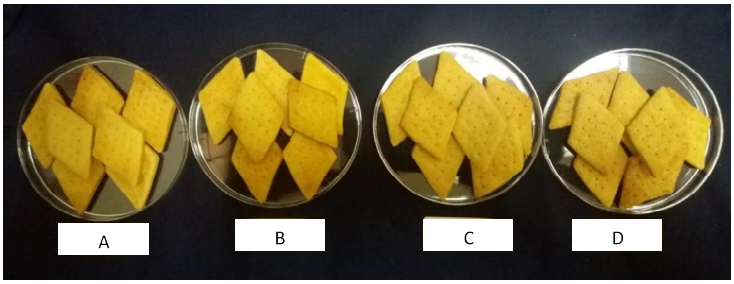


**Fig. 2. The appearance of biscuits with various treatments: A. Addition of 0% nilem fish egg protein concentrate (NFEPC) (Control), B. NFEPC 2.5%, C. NFEPC 5%, and D. NFEPC 7.5%**

**Table 2. preference level of biscuit aroma from different level of Nilem FEPC Addition**

| Treatment of addition of Nilem Fish Egg Protein Concentrate (%) | Median Aroma preference level | Average of aroma preference level |
|---|---|---|
| 0 | 3.5 | 3.45 [b] |
| 2.5 | 3 | 3.5 [b] |
| 5.0 | 3 | 3.25 [b] |
| 7.5 | 2 | 2.35 [a] |

** [a,b] Mean values with the same superscript letter in same column are not significantly different at p< 0.05*

The aroma assessment is shown in Table 2. According to the data, those biscuits supplemented with nilem FEPC 2.5% obtained a higher total score. Addition at a level of 2.5% kept the aroma parameter close to the control sample. Table 2 showed that the biscuits without addition nilem FEPC had an average preference level of 3.45 (neutral to like moderately). Biscuits with the addition of 2.5% nilem FEPC had an average preference level of 3.5 (like moderately). Both treatments produced the same aroma as control biscuits. The 5.0% treatment had an average preference level of 3.25 (neutral to like moderately), the aroma of biscuits with a slight aroma of fish eggs, which the panelists still favored. Biscuits with the addition of 7.5% nilem FEPC obtained the lowest level of aroma preference with a score. 2.35 (moderately dislike). The specific aroma of nilem fish eggs on these biscuits is stronger, so it is less favored by the panelists. This result was in accordance with research conducted by Vellomena *et al.* [14], which stated that the addition of 7.5% nilem protein had a strong fish aroma. When comparing the aroma characteristics between parameter NFEPC concentration, no statistically significant differences were observed between control and addition NFEPC 2.5-5.0%, while within control and addition NFEPC 7.5%, the difference proved to be statistically significant

The best formula that the panelists can receive were biscuits with the addition of 2.5% nilem FEPC. The biscuits were produced with a distinctive aroma of nilem eggs but not too strong. This is in line with Vellomena [14] and Iqbal [15] regarding the addition of nilem fish eggs to the processed products of sticks and biscuits, which shows that the processed sticks and biscuits smelled of nilem fish, which got stronger with the addition of more nilem fish eggs.

## 3.3 Texture

The texture is an assessment of organoleptic characteristics by giving a touch to the surface of a product. Assessing the texture of a food product is done by using the fingertips [16]. The average level of preference for biscuit texture from different levels of addition of nilem FEPC is shown in Table 3.

Table 3 showed that the level of preference for biscuit texture with the addition of nilem FEPC. Regarding texture, biscuits supplemented with 2.5 and 5.0% were most liked by the panelists and had the same average level of preference, with a score of 3.35 (like moderately). The preference for biscuit texture without addition NFEPC had the highest average level of preference, with a score of 3.6 (like moderately). The level of preference for biscuit texture obtained from the addition of 7.5% nilem FEPC has the lowest average value of 3.05 (neutral). This study confirms results reported by Vellomena *et al.* [14], flour with a protein content of nilem fish product, 0-5.0%, tends to yield a neat and fragile product.

Assessment based on texture obtained results that were not significantly different between each treatment. The addition of nilem fish egg protein concentrate in various treatments did not significantly differ ($p < 0.05$) to the texture of the resulting biscuit.

## 3.4 Taste

Taste is the main assessment in testing the organoleptic characteristics of a food product. Taste assessment is one of an important references to determine the level of preference in acceptance of a food product. Factors that determine the taste of a food ingredient include chemical compounds, temperature, and interactions with other flavor components [17]. The taste of a food ingredient is divided into four, namely salty, sweet, sour, and bitter; the formulations used in processing foodstuffs affect the taste attributes of these foodstuffs [18]. The average level of preference for biscuit flavors from different levels of addition of nilem FEPC is shown in Table 4.

The level of preference for biscuits resulting from no addition of nilem fish egg protein concentrate treatment had the highest average value of 3.8 (like moderately). Biscuits obtained from the addition of nilem FEPC 2.5% had an average level of preference with a score of 3.35 (neutral). The biscuit taste from both treatments is slightly sweet from sugar and savory taste from egg yolk and butter. The 5.0% treatment had an average value of 3.3 (neutral), the biscuit taste was more savory than the two previous treatments (0% and 2.5%). The preference for biscuit taste from the nilem FEPC addition level of 7.5% had the lowest average preference level of 2.45 (moderately dislike). The biscuit taste was savory but had a strong fish egg taste. The panelists did not like it. The taste assessment obtained results that were not significantly different ($p < 0.05$) from the 0%, 2.5% and 5.0% treatments, but significantly different ($p < 0.05$) from the 7.5% treatment.

**Table 3. Average Preference Level of Biscuit Texture from Different Level of Nilem FEPC Addition**

| Treatment of addition of Nilem Fish Egg Protein Concentrate (%) | Median of texture preference level | Average of texture preference level |
|---|---|---|
| 0 | 4 | 3.6 [a] |
| 2.5 | 3 | 3.35 [a] |
| 5.0 | 4 | 3.35 [a] |
| 7.5 | 3 | 3.05 [a] |

** [a] Mean values with the same superscript letter in same column are not significantly different at p< 0.05*

**Table 4. average preference level of biscuit flavor from different level of nilem FEPC Addition**

| Addition of Nilem Fish Egg Protein Concentrate (%) | Median | Average of Taste preference level |
|---|---|---|
| 0 | 4 | 3.8 [b] |
| 2.5 | 3,5 | 3.35 [b] |
| 5.0 | 3 | 3.3 [b] |
| 7.5 | 2 | 2.45 [a] |

** [a,b] Mean values with the same superscript letter in same column are not significantly different at p< 0.05*

**Table 5. Decision Matrix for the rate of panelists acceptance of biscuits fortified nilem fish protein concentrate with Bayes Method**

| Treatment | Criteria | | | | Alternative Value |
|---|---|---|---|---|---|
| | Appearance | Aroma | Texture | Taste | |
| 0 | 4.15 | 3.45 | 3.60 | 3.80 | 3.75 |
| 2.5 | 3.60 | 3.50 | 3.35 | 3.35 | **3.42** |
| 5.0 | 3.85 | 3.25 | 3.35 | 3.30 | 3.39 |
| 7.5 | 2.85 | 2.35 | 3.05 | 2.45 | 2.60 |
| **Criteria Value** | 0.16 | 0.20 | 0.17 | **0.46** | 13.16 |

## 3.5 Decision-Making

Decision-making was made based on the Bayes method, based on the alternative weight values of 4 criteria, i.e., the appearance, aroma, texture, and taste of biscuits. The results of the calculation of the decision-making in determining the best treatment are presented in Table 5.

Among the obtained judging results and the panelist's evaluation for overall acceptability, it could be observed that the fortification of nilem FEPC at levels 2.5 and 5.0% into wheat flour blend used in biscuit making did not cause any significant (at p≤ 0.05) effect on overall acceptability of produced biscuits and better acceptability. This is in agreement with those reported by Mohamed *et al.* [5] and Vellomela [14], which stated that the addition of less fish protein concentrate was more acceptable to the panelists.

Other decision-making using the Bayes method found that biscuits with the addition of 2.5% nilem fish egg protein concentrate got the highest value with an alternative value of 3.42 (neutral to moderate). In comparison, biscuits with the addition of 7.5% nilem fish egg protein concentrate got the lowest alternative value with a score of 2.60 (moderately dislike) of all experimental parameters. Thus, sensory data indicated that biscuit supplemented with 2.5% Nilem FEPC did not affect all the produced biscuit sensorial assessments. Moreover, the biscuit was appreciated by panelists for its pleasant taste and good texture. Therefore, it can be concluded that the most preferred biscuit with the addition of treated fish egg protein concentrate was 2.5%.

## 4. CONCLUSION

This study concludes that nilem fish eggs are an excellent source to produce FEPC. FEPC made from nilem fish has a high nutritional value, and it was more effective for fortifying biscuits to increase the protein content. Moreover, the level

used 2.5% did not affect the appearance and other sensory characteristics. Therefore, it can be concluded that the most preferred biscuit based on the criteria appearance, aroma, texture, and taste obtained from the addition of 2.5% nilem FEPC from wheat flour used. The average value of preference level for appearance, aroma, texture, and taste obtained from the treatment was 3.60 (like moderately), 3.50 (like moderately), 3.35 (like moderately) and 3.35 (like moderately), respectively. Therefore, making biscuits with this formula is possible to improve the nutritional characteristics of the biscuits produced.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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