Nutrition Evaluation of Indonesian Shortfin Eel (*Anguilla bicolor*) Meat for Functional Food Development

Harvey Febrianta* and Reynetha D.S. Rawendra

Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia

*harvey.febrianta@binus.ac.id, harvey606254@gmail.com

Abstract. This research presented that Shortfin Eel (*Anguilla bicolor*) has a high nutrient content that is useful as a component to maintain the body tissues and potential to be developed as functional food. This study aims to obtain scientific data about the nutritional value of fresh and boiled eel meat. The analysis included proximate and cholesterol content. Analysis performed on eel meat included proximate analysis (AOAC), and cholesterol level analysis (Liebermann-Buchard Color Reaction). The experimental design applied in this study was an independent T-Test analysis to compare the mean of the two groups unrelated to 5% significant level. The parameters measured were Proximate Composition (Moisture, Ash, Fat, Protein, Carbohydrate), and Cholesterol level. The results showed that boiling process of eel meat significantly affected the decrease in protein, fat, carbohydrate, and cholesterol levels. Decreased levels of fat due to the influence of heat during the boiling process followed by a decrease in cholesterol. Cholesterol levels of fresh eel meat was 68.96 ± 0.40 mg/100 g decreased to 65.06 ± 0.36 mg/100 g after boiling.

1. Introduction

Indonesia's marine resources have the potential as one of the largest sources of food in the world. But the potential is still not getting attention in proportion, one of which is the potential of shortfin eel (*Anguilla bicolor*). The demand for shortfin eel is quite high in international markets, especially in Japan, Hongkong, Germany, Italy, Taiwan, and Korea. Shortfin eel (*Anguilla bicolor*) has the potential as an export commodity [2]. This proves the interest of people to this aquatic biota is quite high and it can be developed as the functional food.

Shortfin eel is one of the aquatic biota that is expected for high nutrient content. Shortfin eel as a substance is suitable for consumption as food, it is an important source of nutrients for ensuring optimal healthy growth and maximum functional capacity. Nutrition interprets the interaction of nutrients and other substances in food in relation to maintenance, growth, health of an organism. Macronutrients are the chemical substances present in food that are use by living things. Nutrition is a chemical bond required by the body to perform its functions of generating energy, building, nurturing, and repairing tissues [11]. Nutrients can be grouped into six categories: carbohydrate, protein, lipid (fat), water, vitamins, and minerals. Nutrients in fish include protein, fat, carbohydrates, and cholesterol.

Shortfin eel (*Anguilla bicolor*) contain cholesterol as an essential element of cell membranes that provide structural support and serve as protective antioxidants. Cholesterol is an essential lipid constituent of cell membranes. Cholesterol is naturally produced in the body primarily by the liver, excessive cholesterol production can increase the risk of clogged arteries [5].
The purpose of this study was to determine the chemical composition of nutrients and cholesterol levels in fresh and boiled shortfin eel. The expected benefit of this research is to make the functional food product based on shortfin eel meat which contains low cholesterol, and safe for consumption.

2. Materials and methods
The study was conducted from February to July 2018. Materials used in this study was shortfin eel (Anguilla bicolor) consumption size or weight of about 300 g, originating from Cilacap, Central Java. The study was conducted in several stages including sampling of shortfin eel (Anguilla bicolor), sample preparation, boiling shortfin eel meat at 100°C for 20 minutes. Analysis performed on shortfin eel included proximate analysis (AOAC), and cholesterol level analysis (Liebermann-Buchard Color Reaction).

2.1. Moisture content (AOAC)
Determination of water content was based on samples weight before and after drying. An empty cup was dried in an oven for 1 hour at 105°C temperature, and then it was put in a desiccator for 15 minutes and afterward it was weighed. 1 gram sample was inserted into the cup and then it was dried in an oven at 105°C temperature until its weight was constant (Drying process was approximately done for 6 hours). Afterward, the cup was inserted into the desiccator for 30 minutes. Later, it was weighed again. The water content was determined by the formula of AOAC [4].

2.2. Ash content (AOAC)
Prepare the crucible and lid in the furnace at 600°C for 3 hour to ensure the impurities on the surface of crucible are burned off. Cool the crucible in the desiccator for 30 minutes and weighing. 5 grams of sample was then weighed and put in the crucible. The sample was then burned in the electric stove. When there was no longer smoke come out from the stove, the sample was put into the incinerating furnace with a temperature of 600°C. After 7 hour the crucible was inserted in a desiccators and then was weighed. The ash content was determined by the formula of AOAC [4].

2.3. Fat content (AOAC)
Two grams of sample (W1) were spread out over the cotton which was reposed on filter paper and then it was rolled up to be a thimble. Wrapped samples were inserted into a fat flask that had been weighed before (W2) and it was connected to Soxhlet tube. Later, sample was inserted into the tube Soxhlet extractor chamber and immerse with fat solvent (n-hexane). Then, reflux for 6 hours. Fat solvent in the flask was distilled until all it was evaporated. During distillation process, the solvent will be accommodated in an extractor chamber, and then it was discard so it did not enter anymore into the flask. Afterward, fat flask was dried in an oven at temperature of 105°C. Later, fat flask was put in a desiccator until reached constant weight (W3). The fat content was determined by the formula of AOAC [4].

2.4. Protein content (AOAC)
Protein content analysis was carried out by the Kjeldahl method [4]. The principle was the oxidation of carbonized materials and the conversion of nitrogen to ammonia by sulfuric acid, then ammonia reacts with excess acid form ammonium sulfate. The formed ammonium sulfate is described and the solution is made with NaOH. Evaporated ammonia will be bound with boric acid. Nitrogen contained in the solution is determined in number by titration using a standard acid solution. The samples were weighed as much as 0.1 to 0.5 g, put into a 100 mL in Kjeldahl flask, added with 1/4 tablet, then decrypted until the solution becomes green and SO₂ denatured. Put the samples to a 50 mL flask and diluted with distilled water until tera, put into a distillation flask, added with 5-10 mL of 30-33% NaOH and distillation. Destilat is accommodated in a solution of 10 ml of 3% boric acid and a few drops of indicator (solution 0.1% bromcresol green and 29 ml of 0.1% methyl red solution in 95% alcohol separately and
mixed with 10 ml bromcresol green with 2 mL methyl red) then titrated with 0.02 N HCl solution until the color of solution change to pink.

2.5. Cholesterol content (Liebermann Buchard)
Analysis of cholesterol levels was carried out using the Liebermann Buchard Color Reaction method. The sample was weighed as much as ±0.1 gram and put into a centrifuge tube, added with 8 mL ethanol solution and petroleum benzene in a ratio of 3:1, then stirred until homogeneous. The stirrer was rinsed with 2 mL of ethanol solution:petroleum benzene (3:1) later centrifuged used centrifuge (Eppendorf 5804r) for 10 minutes (3,000 rpm). The supernatant is poured into the beaker 100 mL glass and evaporated in a water bath. The residue is evaporated with chloroform (little by little), while poured into a scale tube (up to volume of 5 mL). The residue was then added to 2 mL of acetic anhydride and 0.2 mL Concentrated H$_2$SO$_4$ or 2 drops. Next mixed with vortex and left on dark place for 15 minutes. Then measured the absorbance used spectrophotometer (Uv-vis Genesys 10s) with a wavelength (λ) 420 nm and the standard used was 0.4 mg/ml.

Statistical analysis
The data obtained were analyzed using an independent T-Test analysis to compare the mean of the two groups unrelated to 5% significant level.

3. Results and discussion
3.1. Proximate Composition of Shortfin Eel (Anguilla bicolor)
The results of this study for average proximate and cholesterol of shortfin eel (Anguilla bicolor) are presented in Table 1. Based of the moisture content analysis result in fresh and boiled shortfin eel meat, the moisture content of the fresh shortfin eel meat from 70.81 ± 0.33% down to 69.90 ± 0.19% after boiling. Changes in moisture content are associated with volatile moisture properties when heated, and related to moisture type by location and its properties in a sample [13]. T-Test results showed that there were significant differences between the fresh and boiled shortfin eel.

| Proximate Composition | Fresh Meat        | Boiled Meat       |
|-----------------------|-------------------|-------------------|
| Moisture (%)          | 70.81±0.33        | 69.90±0.19        |
| Ash (%)               | 1.46±0.13         | 1.17±0.12         |
| Fat (%)               | 6.39±0.18         | 5.29±0.17         |
| Protein (%)           | 17.97±0.09        | 14.87±0.16        |
| Carbohydrate (%)      | 3.21±0.54         | 8.76±0.61         |
| Cholesterol (mg/100g) | 68.96±0.40        | 65.06±0.36        |

Based on Table 1, the test results show that the boiling process has an effect on the moisture content of the body due to the sig value. at 0.00 (0.00<0.05). The moisture content in the fish tend to have a pattern of inverted comparison with the levels of fat, which is when the water level is high then fat levels tend to be lower [11]. The result of ash content for fresh shortfin eel meat was 1.46 ± 0.13% and boiled meat was 1.17 ± 0.12%. Ash content is part of the mineral weight of the material based on its dry weight. Ash is an organic substance that does not evaporate, the rest of the combustion process or the oxidation results. The determination of ash content has something to do with the mineral of a material [10]. T-Test results show that there was a significant difference between ash content in fresh and boiled shortfin eel because of the sig value. at 0.02 (0.02<0.05). Differences in ash values due to some protein and fat components coming out of the tissue due to heating process, so that the change of ash content in the sample is more proportional. The mineral content who is found in the live habitat of shortfin eel may affects the ash content of the meat [12].
The results of protein content in fresh and boiled meat were presented in Table 1. The result showed that the protein content of fresh shortfin eel meat was 17.97 ± 0.09% and boiled shortfin eel meat was 14.87 ± 0.16%. The changes of protein content occur during the boiling process. Most of proteins are denatured when heated at 60ºC - 90ºC for one hour to decrease the protein content of the material. The amino acid is the precursor of most coenzymes, hormones, nucleic acids, and essential molecules [3]. T-Test results show that, boiling process affects the protein levels in the eel meat because the sig value at 0.00 (0.00<0.05). High protein content in shortfin eel meat indicates a difference values between each nutrient content of the 100% total nutrients.

The average of fat content in fresh and boiled Anguilla bicolor meat was shown in Table 1. Fat content in fresh shortfin eel meat was 6.39 ± 0.18% and boiled shortfin eel meat was 5.29 ± 0.17%. Fat is a component soluble in organic solvents such as hexane, chloroform, and ether. The fats contained in the fish are very easy to digest directly by the body and mostly consist of unsaturated fatty acids, it is needed for growth and decreased cholesterol levels [12]. Fats in shortfin eel meat can melt as a result of the heating process. T-Test results showed that the meat of fresh and boiled shortfin eel give a significant effect on fat content because of the sig value at 0.00 (0.00> 0.05). The melting of fats from boiling make aldehydes, alcohols, ketones, acids, and hydrocarbons evaporate during the heating [7].

Based on Table 1, it is known that carbohydrate content in fresh shortfin eel meat was 3.21 ± 0.54% and boiled shortfin eel meat was 8.76 ± 0.61%. Carbohydrate levels in food are always adjacent to other nutrients such as protein and fat [6]. T-Test results confirmed that the boiling treatment in the shortfin eel meat affects carbohydrate levels because of the sig value at 0.00 (0.00> 0.05). High carbohydrate content indicating a difference of values between each nutrient content of the 100% of total nutrients. The high carbohydrate value in shortfin eel meat is affected by the reduction of moisture content [1]. Carbohydrate content increased due to decreased levels of protein, fat, and ash in shortfin eel meat [14].

3.2. Cholesterol Levels of Shortfin Eel (Anguilla bicolor)

Table 1 showed the results of cholesterol content in fresh and boiled shortfin eel meat. Cholesterol content in fresh shortfin eel meat was 68.96 ± 0.40 mg/100g and boiled shortfin eel meat was 65.06 ± 0.36 mg/100 g. Cholesterol in large quantities in the blood form the deposits on blood vessel walls and make a narrowing called atherosclerosis [3]. Federal dietary guidelines recommend limiting cholesterol intake to less than 300 mg per day [9]. The cholesterol content in Japanese eel (Anguilla japonica) was 67.9 mg/100 g [6]. The method of boiling treatment has a significant effect on the decrease of shortfin eel cholesterol content.

![Figure 1. Correlation between Fat and Cholesterol](image-url)

Y = 1.7874X + 57.495

R² = 0.7189

P<0.05
T-Test results confirmed that the boiling treatment in shortfin eel meat had a significant effect on the decrease of cholesterol level because of the sig value at 0.00 (0.00>0.05). The decrease of cholesterol content can be caused by giving heat to shortfin eel which causes cholesterol dissolve along with the release of water from the material and the evaporation of volatile compounds, including alcohol and hydrocarbons [8].

Cholesterol and fat content in shortfin eel meat is an interrelated sequence. Regression analysis of correlation between cholesterol (Y) and fat (X) (Fig. 1) showed that the correlation between the increasing level of cholesterol by fat will form a linear line with equation $Y = 1.7874X + 57.495$ (R2 71.9%), meaning that 71.9% of fat is influenced by cholesterol. Cholesterol is a water insoluble substrate, and it is useful for the formation of some essential substances, namely bile acid synthesis, it is important for the absorption of fat [10]. The degradation of cholesterol levels due to the boiling process, will affect the decrease of fat content in shortfin eel meat.

4. Conclusions
Based on the research can be concluded that the boiling process of shortfin eel meat was significantly affect the decrease of protein, fat, carbohydrate, and cholesterol levels. The degradation levels of fat due to the influence of heat during the boiling process followed by the decrease in cholesterol levels.

Acknowledgement
We cordially thank to Bina Nusantara University for the support in facilitating and funding this research through the research grant 2018 managed by RTTO (Research and Technology Transfer Office).

References
[1] Aberoumand A 2012 Proximate composition of less known some processed and fresh fish species for determination of the nutritive values in Iran. J. of Agricultural Technology 8 917-922
[2] Affandi R 2005 Strategi pemanfaatan sumberdaya ikan sidat Anguilla spp. di Indonesia [in Bahasa] J. Iktiologi Indonesia 5 77–81
[3] Almatsier S 2006 Prinsip Dasar Ilmu Gizi [in Bahasa] (Jakarta: Gramedia Pustaka Utama)
[4] AOAC 2005 Official Method of Analysis of The Association of Official Analytical of Chemist (Virgini, USA: Association of Official Analytical Chemist, Inc)
[5] Colpo A 2005 LDL Cholesterol bad cholesterol science cholesterol J. of American Physiciansand Surgeons 10 83-89
[6] Oku T, Sugawara M, Choudhury M, Komatsu M, Yamada S and Ando S 2009 Lipid and fatty acid compositions differentiate between wild and cultured Japanese eel Food Chemistry 115 436-440
[7] Prabandari R, Mangalik A, Achmad J and Agustiana 2005 Pengaruh waktu perebusan dari dua jenis udang yang berbeda terhadap kualitas tepung limbah udang putih (Penaeus indicus) dan udang windu (Penaeus monodon) [in Bahasa] Enviro Scieniteae 1 24-28
[8] Riyanto R, Priyantono N and Siregar T 2007 Pengaruh perebusan, penggaraman dan penjemuran pada udang dan cumi terhadap pembentukan 7-ketokolesterol [in Bahasa] J. Pascapanen dan Bioteknologi Kelautan dan Perikanan 2 147-151
[9] Samaha FF 2003 A low carbohydrate as compared with a low fat diet in severe obesity J. Medicine 348 2074-2081
[10] Sampaio GR, Bastos D, Soares R, Queiroz Y and Torres E 2006 Fatty acid and cholesterol oxidation in salted and dried shrimp Food Chemistry 96 344 -351
[11] Sudarmadji S, Haryono B and Suhardi 2007 Analisa Bahan Makanan dan Pertanian [in Bahasa] (Yogyakarta: Liberty)
[12] Weber J, Bochi VC, Ribeiro C, Victorio AM and Emanuelli T 2007 Effect of different cooking methods on the oxidation proximate and fatty acid composition of silver catfish (Rhamdiaqueuen) fillets Food Chemistry 106 140-146
[13] Winarno FG 2008 Kimia Pangan dan Gizi [in Bahasa] (Bogor: M-Brio Press)
[14] Yulindra T, Dwi TS dan Suprayitno E 2013 Pengaruh konsentrasi residu daging ekstraksi albumin ikan gabus (Ophiocephalusstriatus) yang berbeda terhadap kualitas sosis ikan [in Bahasa] *THPi Student Journal* 1 51-60