

## Ingredient modification to improve nutrition of Indonesian *Koya* made of Nile and soy as a source of protein

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### Article history:

Received: 9 September 2020  
Received in revised form: 18 October 2020  
Accepted: 17 December 2020  
Available Online: 11 April 2021

### Keywords:

*Koya*,  
Nile  
Tilapia,  
Protein,  
Soy,  
Tempeh

### DOI:

[https://doi.org/10.26656/fr.2017.5\(2\).498](https://doi.org/10.26656/fr.2017.5(2).498)

### Abstract

*Koya* is an Indonesian food often used as a seasoning topping. *Koya* is made from prawn crackers and fried onions. It is popular and can be used as an alternative to improve human nutrition, primarily to fulfill the protein needs in children. One of the high-protein sources is the Nile tilapia which is easily cultured in Indonesia. Tilapia can be combined with soy, a high protein local food. The aim of this study was to determine the characteristics of *Koya* made from Tilapia and combined with either soy or fermented soy (tempeh). *Koya* was made from a combination of the main ingredients, such as Nile tilapia-soy flour (NS) and Nile tilapia-tempeh flour (NT) with a ratio of tilapia: soy flour/tempeh flour 40:60, 50:50, and 60:40, respectively. Each *Koya* was tested for its chemical composition and sensory evaluation. The results indicated that the combination of Nile tilapia-soy flour and Nile tilapia-tempeh had a significant effect on the chemical and sensory characteristics. With the higher concentration of tilapia; the moisture, ash, and protein composition increased, but the fat content decreases. *Koya* with 60% of tilapia either combined with 40% soy (NS3) or 40% tempeh (NT3), was the most preferred by panelists. *Koya* NS3 contained moisture, ash, fat, protein, and carbohydrates of 13.06%, 5.15%, 19.59%, 54.19%, and 21.50%; respectively while NT3 of 13.32%, 3.89%, 19.28%, 48.72%, and 28.06%; respectively. *Koya* NS3 and NT3 contained linoleic and linolenic fatty acids and higher essential and non-essential amino acids than commercial *Koya*.

## 1. Introduction

Indonesia is a maritime country, and its marine production is continuously rising from year to year. One of the marine products in Indonesia that keep increasing is Nile tilapia. Total production of Nile tilapia has increased from 914 tons in 2013 to 992 tons in 2015 (Sulistiyo, 2017). Its production continues to increase and able to encourage the level of public consumption. Besides its good tastes, tilapia also has a high nutritional content. Nile tilapia contains a high protein level of 14-18% and a low of fat 2-3% (Desta *et al.*, 2019). Protein on tilapia contains complete essential amino acids that are beneficial to health (Yarnpakdee *et al.*, 2014). Tilapia is consumed by processing it into food products such as

roll (Chambo *et al.*, 2017) and fish nuggets (Lima *et al.*, 2015). The high protein content in tilapia can be used as a source of protein in fast food such as *Koya*.

*Koya* is a native Indonesian food in the form of powder. *Koya* is usually added as a seasoning topping. Other seasoning toppings which commonly used are shredded, both shredded fish and meat, also coconut flakes which are used as a flavor enhancer. *Koya* is preferred because of its distinctive odor and savory taste, thus increasing appetite. Nowadays, the community put more attention to practical food with high nutrition for a healthy lifestyle (Ngozi *et al.*, 2017). Fish *koya* is a practical seasoning powder which high in nutrition. Regina *et al.* (2012) produced mackerel fish *koya* with

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protein 28.14%, but the aroma was less preferred by panelists. Anandito *et al.* (2019) produced snakehead *koya* fish with the taste parameters were on neutral level for panelists. Snakehead fish production in Indonesia yet considered low and not a main commodities of freshwater fish (Indonesia Statistics, 2017). Therefore, *koya* fish from tilapia as main commodities of freshwater fish in Indonesia were developed due to the availability of raw material. Fish *koya* from tilapia presumably contains high nutrition particularly protein. Tilapia contains an amino acid, such as lysine and arginine that is beneficial for kids in the growth and development stage (Yarnpakdee *et al.*, 2014; Uauy *et al.*, 2015). Tilapia can be combined with vegetable protein sources such as soy. In order to increase its protein content, soy must contain unsaturated fatty acids and other nutritional content.

Soy has become a source of vegetable protein. Soy contains isoflavones that are beneficial to health, including reducing the risk of coronary heart disease and cancer (Messina, 2016). Soybean seeds contain 42% protein, 19% fat, and 19% carbohydrates. Soybean processing cause changes in nutritional content. Soybeans processing can increase its protein content (Sharma *et al.*, 2014). Soy in Indonesia has been processed into various products, such as tempeh, tofu, sweet soy sauce, tauco, and soy powder. Soy powder is also commonly used as a topping for native cuisine. The overripe tempeh also could be processed into seasoning powder (Gunawan-Puteri *et al.*, 2015). The fermentation process can increase the protein content compared to unfermented soybean seeds. Bavia *et al.* (2012) stated that protein content in tempeh increase 41% compared to soybean seeds, while fat content in tempeh is not significantly different from soybean seeds. It depends on the variety used. Soybean has a good beany flavor (Ravi *et al.*, 2019), which can cover the fishy smell from Nile tilapia; the combination of animal and vegetable protein is expected to produce complete nutrition content.

Recently, research on *Koya* is still rarely conducted. This seasoning topping is a typical Indonesian food that is very popular and liked. *Koya* can be used as an alternative to solve the problem of public malnutrition. The combination of tilapia and soy as a source of protein is expected to be able to produce healthy *Koya* and rich in protein. Besides, the combination of tilapia and tempeh in *Koya* was also examined. The aim of this study was to determine the characteristics of *Koya* made from tilapia combined with either soy or fermented soy (tempeh).

## 2. Materials and methods

### 2.1 Materials

The material used in this study was fresh red tilapia weighed 250 g per fish obtained from a local market in Surakarta, Central Java, Indonesia. Yellowish white soybean weighed 16-20 g per 100 pieces obtained from soybean farmers in Grobogan, Central Java, Indonesia. While tempeh obtained from a Tempeh producer in Kampung Krajan, Mojosongo, Central Java, Indonesia. The tempeh used was 48 hours fermented soybean. Moreover, spices were used for making *Koya* (shallots, garlic, galangal, ginger, lemongrass, bay leaves, lime leaves, coriander, coconut milk, candlenut, palm sugar, salt, cooking oil, and "Sun Kara" instant coconut milk) and "2 Gajah" commercial *Koya* obtained from the local market in Surakarta, Central Java, Indonesia.

### 2.2 Koya making

The making of *Koya* was conducted based on Regina *et al.* (2012) with modifications. The spices showed in Table 1 were sautéed with cooking oil until fragrant. After the spices fragrant, instant coconut milk was added and heated until boiling. Ginger, galangal, lemongrass, bay leaves, lime leaves, palm sugar, and salt were added. Then steamed tilapia was added and stirred until homogeneous. Soybeans that have been floured was added and stirred until homogeneous and brownish. The *Koya* mixture then blended and sieved with a sixty-mesh sized sieve. For *Koya* made from tilapia and tempeh, *Koya* was made with the same steps, but the soy was replaced with tempeh that had been dried at 70°C for 6 hours then floured.

### 2.3 Proximate analysis

Proximate analysis including moisture, protein, fat, ash, and carbohydrate by difference (AOAC, 2005).

### 2.4 Sensory analysis

Sensory analysis was performed by a hedonic test using five scales consisting of 7: very likes, 6: likes, 5: somewhat likes, 4: neutral, 3: somewhat dislikes, 2: dislikes and 1: very dislikes. Hedonic tests were performed for color, taste, aroma, texture, and overall. The panelists were 40 untrained panelists (Huda *et al.*, 2012).

### 2.5 Amino acid profiles analysis

The amino acid analysis was performed using HPLC (Shimadzu, Japan) with a C18 4.6 × 250 mm column. The eluent was methanol: acetate buffer = 80: 20. The 5 g of sample was acid hydrolyzed with 20 mL of 6 N HCl which had been vortexed then heated in an oven at 110°C

Table 1. Formulation of *Koya* from Nile Tilapia and Soy (NS) and Nile Tilapia and Tempeh (NT)

Materials	NS1/NT1	NS2/NT2	NS3/NT3
Nile Tilapia (g) / (%) (N)	108 (40%)	135 (50%)	162 (60%)
Soy (S) / tempeh flour (g) / (%) (T)	162 (60%)	135 (50%)	108 (40%)
Garlic (g)	55	55	55
Shallot (g)	40	40	40
Candlenut (g)	5	5	5
Coriander (g)	2	2	2
Coconut milk (ml)	200	200	200
Ginger (g)	3	3	3
Galangal (g)	6	6	6
Lemongrass (g)	8	8	8
Bay leaves (sheet)	2	2	2
Orange leaves (sheet)	4	4	4
Palm sugar (g)	25	25	25
Salt (g)	3	3	3
Total	423	423	423

NS1 (Nile: soybean 40:60); NS2 (Nile: soybean 50:50); NS3 (Nile: soybean 60:40); NT1 (Nile: tempeh 40:60); NT2 (Nile: tempeh 50:50) and NT3 (Nile: tempeh 60:40)

for 12 hrs. Heating was performed to accelerate the hydrolysis reaction and remove gases in the sample which able to interfere with the chromatogram result. The heated sample then cooled to room temperature, then neutralized with 6 N NaOH. After that, it was clarified with 5 mL Pb-Acetate 40% and 2 mL of 15% oxalic acid then adjusted to 50 mL using distilled water. Approximately, 3 mL of the sample was taken then filtered with 0.45  $\mu\text{m}$  millex. Next, 20  $\mu\text{L}$  from the extraction result was taken and added 980  $\mu\text{L}$  of 0.1 N formic acid. Taking 50  $\mu\text{L}$  of sample to be added with 450  $\mu\text{L}$  of OPA solution, then vortex and reacted for 3 minutes. The last step was injecting 40  $\mu\text{L}$  into the HPLC. The separation of all amino acids until finished. The calculation of the amino acid concentration present in the material was done by making a standard chromatogram using ready-made amino acids that undergo the same treatment as the sample (AOAC, 2005).

### 2.6 Fatty acid profiles analysis

Fatty acid profile analysis was performed using gas chromatography (Shimadzu, Japan). The first stage was the extraction process using the Soxhlet method, then 20 g of fat was weighed in the form of oil. The next stage was the methylation process; this process aimed to form methyl ester, a fatty acid derivative compound. The methylation process was performed by refluxing the fatty acids on a water bath using the solvent NaOH-methanol, isooctane, and  $\text{BF}_3$ . Around 20 mg of the sample was put into a test tube, and 1 mL of 0.5 N NaOH-methanol was added, then heated for 20 minutes, then the sample was cooled. The 2 mL of 20%  $\text{BF}_3$  solution and 5 mg/mL of internal standard, then the sample was reheated for 20

minutes and cooled. The cooled mixture then added with 2 mL saturated NaCl and 1 mL isooctane, then the mixture was shaken carefully. The isooctane solution formed transferred into a tube which had been mixed with 0.1 g anhydrous  $\text{Na}_2\text{SO}_4$  using a spotting pipette and left for 15 minutes, then an injection of 1  $\mu\text{L}$  FAME standard mixture (Supelco 37 component fatty acid methyl ester mix). Around 1  $\mu\text{L}$  sample was injected into Gas Chromatography (GC). The retention and peak time of each fatty acid was measured and compared with standard retention times (AOAC, 2005).

### 2.7 Statistical Analysis

This research was conducted in triplication. Data were analyzed using ANOVA. Then Duncan was performed as the post hoc test.

## 3. Results and discussion

### 3.1 Chemical composition of raw materials

The primary raw materials of *Koya* such as tilapia, soy, and tempeh were analyzed its chemical composition, including moisture, ash, fat, protein, and carbohydrate content. The results are presented in Table 2. The results showed that the highest composition on Nile tilapia was protein 15.97%. Different results showed by Desta *et al.* (2019) tilapia 14.77% protein. It indicated that protein was the main content in tilapia — the other raw ingredients such as soy and tempeh flour. Protein was also the highest component in soy and tempeh flour, 55.93% and 42.33%; respectively. This result was higher compared to Uwem *et al.* (2017) the protein content in soy flour is 35.60%, while Syida *et al.* (2018) indicated that tempeh flour contained 36.86%

Table 2. Chemical Composition of Nile Tilapia, Soy, and Tempeh

Parameters	Nile Tilapia ( <i>Oreochromis niloticus</i> )	Soy Flour	Tempeh flour
Moisture (%)	80.01±0.04	6.31±0.00	4.76±0.24
Ash (%)	0.98±0.06	4.60±0.00	2.53±0.13
Fat (%)	0.51±0.11	19.25±0.00	22.93±0.35
Protein	15.97±0.03	55.93±0.02	42.33±0.19
Carbohydrates by diff. (%)	2.45±0.05	13.92±0.01	27.15±0.28

Values are expressed as mean data (% wet basis) ± standard deviation

protein.

### 3.2 Chemical characteristics of Koya

Examining the chemical characteristics of *Koya* aimed to determine the chemical content and the change in chemical composition during the processing. Instead of the three *Koya* formulas in the study, the proximate analysis was also performed on commercial *Koya*. It aimed to compare the nutritional value such as the proximate value of *Koya* from this study and the commercial one. The proximate analysis consisted of moisture, ash, fat, protein, and carbohydrate content. The results of the chemical analysis of *Koya* are presented in Table 3.

#### 3.2.1 Moisture

Moisture content in ingredients could affect the quality of the food products. Dry products would decrease their quality if they contained high moisture content. Table 3 shows the moisture content of *Koya* NS ranged from 12.31-13.06% and NT ranged from 8.37-13.32%, while the commercial was 8.70%. The results by Huda *et al.* (2012) produces coconut flakes with moisture content ranging from 8-13%.

The moisture content in *Koya* NS and NT increased along with the addition of Nile tilapia and soy flour or tempeh flour as the concentration decreased. Tilapia contained 80.01% of moisture which higher than soy and tempeh. The same result showed by Farzana and Mohajan (2015) that the addition of soy flour could reduce the moisture content in biscuits. It is due to the high levels of solids in soy compared to other ingredients in the formula. The solid content in tilapia, soy and tempeh flour (Table 2) showed that the solid content of tempeh flour and soy flour were higher compared to tilapia. Yulianti *et al.* (2019) stated that the addition of tempeh flour could reduce the moisture content of ingredients as indicated by a decrease in the moisture content of pasta with the addition of tempeh flour compare to control.

The moisture content of *Koya* NS and NT was higher than commercial *Koya*. It was because of the shrimp crackers which used as the raw materials in commercial *Koya*. The shrimp crackers frying process could reduce

the moisture content, which was why the commercial *Koya* had a low moisture content. Zhang *et al.* (2015) stated that fried potato chips could be reduced their moisture content from 86% (wb) to 1.2% (wb). It occurred because the water in the material had evaporated due to temperatures that exceed the water boiling point during the frying process.

#### 3.2.2 Ash

The concentration comparison of Nile tilapia-soy flour (NS) and Nile tilapia-tempeh (NT) affected the ash content of *Koya*. The ash content of *Koya* NS ranged from 4.88-5.15% and *Koya* NT 2.68-3.89%. Huda *et al.* (2012) the ash content of serunding was 4%. The ash content of *Koya* NS and NT were higher than commercial *Koya*. This result is related to the soy ash content is greater than tempeh. The tempeh making process causes a decrease in ash (Bavia *et al.*, 2012). Commercial *Koya* had the lowest ash content at 0.55%. Its constituent materials influenced it.

#### 3.2.3 Fat

According to Table 3, the fat content of *Koya* NS ranged between 19.59-21.70% and *Koya* NT about 19.28-20.82%. The higher the addition of tilapia and the lower the addition of soy or tempeh flour, the fat content on *Koya* decreased. Huda *et al.*, (2012) produce shredded fish containing 18-31% fat. The fat content on *Koya* NS and NT were not different, and it was influenced by the fat content on its constituent ingredients, especially soy. During the fermentation process of soybeans into tempeh, the fat content did not change much as indicated by the results of fat content in soybeans and tempeh that were not significantly different (Bavia *et al.*, 2012). When compared to the fat content in commercial *Koya*, the fat content of *Koya* NS and NT were lower. The fat content on commercial *Koya* was 30.06%. It was because the raw material in commercial *Koya* was shrimp crackers. A frying process on shrimp crackers contributed to its fat content. During frying, the pores of the material will open due to water in the material that evaporates quickly. Cooking oil will enter the material from the open pores and replace the water (Zhang *et al.*, 2015).

Table 3. Chemical Characteristics of *Koya*

Sample	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrates by diff. (%)
NS1	12.31±0.00 <sup>a</sup>	4.88±0.00 <sup>a</sup>	21.70±0.02 <sup>c</sup>	49.68±0.04 <sup>a</sup>	23.51±0.01 <sup>c</sup>
NS2	12.44±0.01 <sup>b</sup>	5.08±0.00 <sup>b</sup>	20.65±0.01 <sup>b</sup>	51.25±0.00 <sup>b</sup>	22.89±0.01 <sup>b</sup>
NS3	13.06±0.01 <sup>c</sup>	5.15±0.00 <sup>c</sup>	19.59±0.00 <sup>a</sup>	54.19±0.03 <sup>c</sup>	21.50±0.02 <sup>a</sup>
NT1	8.37±0.28 <sup>a</sup>	2.68±0.09 <sup>a</sup>	20.82±0.02 <sup>c</sup>	47.91±0.09 <sup>a</sup>	28.63±0.07 <sup>b</sup>
NT2	12.03±0.14 <sup>b</sup>	2.97±0.18 <sup>b</sup>	20.43±0.09 <sup>b</sup>	48.30±0.15 <sup>b</sup>	28.43±0.22 <sup>b</sup>
NT3	13.32±0.18 <sup>c</sup>	3.89±0.04 <sup>c</sup>	19.28±0.06 <sup>a</sup>	48.72±0.15 <sup>c</sup>	28.06±0.18 <sup>a</sup>
Commercial	8.70±0.26	0.55±0.02	30.06±0.39	6.88±0.14	62.52±0.37

Values are expressed as mean±standard deviation. Values with different superscript within the column are significantly different ( $\alpha = 0.05$ ).

NS1 (Nile: soybean 40:60); NS2 (Nile: soybean 50:50); NS3 (Nile: soybean 60:40); NT1 (Nile: tempeh 40:60); NT2 (Nile: tempeh 50:50) and NT3 (Nile: tempeh 60:40)

### 3.2.4 Protein

Protein in *Koya* NS ranged from 49.68-54.19% and NT 47.91-48.72%. Protein content in *Koya* was influenced by raw materials, supporting materials, and processing. The raw materials such as tilapia fillets content 15.97% protein in wet weight or equivalent to 79.97% in dry weight. The protein content of soy flour was 55.93% wet weight, or 59.70% dry weight and tempeh flour was 42.33% wet weight or equivalent to 44.54% dry weight. There was a process that will make the raw material drier. According to its dry weight, the protein content on tilapia fillets was higher than soy and tempeh flour, the more tilapia fillets added, the higher the protein content. The protein level of *Koya* in this study was higher than the shredded fish, around 27-28% (Huda et al., 2012). *Koya* NS and NT were high in protein. It showed that the constituent ingredients (tilapia, soy, and tempeh) contributed to the high levels of protein in *Koya*.

Table 3 shows that *Koya* NS and NT *Koya* had much higher protein content than commercial *Koya* made from shrimp and garlic crackers. Commercial *Koya* contained 6.88% protein. Raw materials and processing process could be the factors of lower protein in commercial *Koya*. The frying process can denature proteins in ingredients. Protein denaturation can occur because of the processing, especially with heat treatment.

### 3.2.5 Carbohydrates

The carbohydrate levels are analyzed using rough calculations or called carbohydrate by difference. The carbohydrate levels calculated by difference were influenced by other nutritional components, such as moisture, ash, protein, and fat content. The lower the other nutritional components, the higher the carbohydrate content and vice versa. Carbohydrate *Koya* NS ranged from 21.50–23.51% and NT 28.06–28.63%. The more tilapia fillets added, the lower the carbohydrate content. It was because the raw material of *Koya*, the

tilapia fillets had lower carbohydrate content compared to soy and tempeh flour. These results by Chambo et al. (2017) the carbohydrates in roll decreases with the increasing of tilapia concentration.

The commercial *Koya* had a much higher carbohydrate content than *Koya* NS and NT, around 62.52%. According to Nguyen et al. (2013), the primary ingredient of shrimp crackers is tapioca flour. Tapioca flour contains starch, and it has a high carbohydrate content. The high levels of carbohydrates in shrimp crackers produce *Koya* with high carbohydrate levels.

## 3.2 Organoleptic characteristics of *Koya*

*Koya* was analyzed using sensory tests to determine the level of panelist preference on the color, aroma, taste, texture, and overall. The results of the sensory analysis are presented in Table 4.

### 3.2.1 Color

Table 4 shows that the *Koya* NS and NT in the colour parameters had a significant effect on each formula. Colour had an essential role because it could attract the characteristics of *Koya*. In this preference test, *Koya* NS1 and NT3 were the most preferred by panelist with values of 5.35 and 5.83. It showed that NS1 and NT3 were the best in color. *Koya* NS1 was light brown, and NT3 was a slightly darker brown. The brown color was the result of a Maillard reaction that occurred during processing. Maillard reaction is a non-enzymatic browning reaction between reducing sugars and amino acids during the heating process. This reaction produces Maillard Reaction Products that gives a brown colour to the product. The difference of brown colour in *Koya* NS1 and NT3 were influenced by the availability of amino acids and reducing sugars in the ingredients. Rannou et al. (2016) stated that the Maillard speed reaction was influenced by several factors, such as the reactant concentration, in this case, reducing sugars and amino acids. Tempeh in *Koya* NT3 produced a slightly darker

Table 4. Organoleptic Characteristics of *Koya*

Sample	Parameters				
	Color	Aroma	Taste	Texture	Overall
NS1	5.35±1.05 <sup>c</sup>	4.40±1.46 <sup>a</sup>	3.80±1.31 <sup>a</sup>	5.95±1.26 <sup>c</sup>	3.63±1.51 <sup>a</sup>
NS2	4.45±1.43 <sup>b</sup>	5.30±1.07 <sup>b</sup>	4.40±1.01 <sup>b</sup>	4.83±1.26 <sup>b</sup>	4.58±1.22 <sup>b</sup>
NS3	3.38±1.63 <sup>a</sup>	6.00±0.88 <sup>c</sup>	5.45±1.28 <sup>c</sup>	4.13±1.81 <sup>a</sup>	5.38±0.91 <sup>c</sup>
NT1	4.48±1.09 <sup>a</sup>	4.35±0.95 <sup>a</sup>	4.35±1.01 <sup>a</sup>	5.30±0.91 <sup>c</sup>	4.23±0.77 <sup>a</sup>
NT2	5.03±1.03 <sup>b</sup>	4.88±1.14 <sup>b</sup>	5.00±0.88 <sup>b</sup>	4.63±0.90 <sup>b</sup>	4.65±0.77 <sup>b</sup>
NT3	5.83±0.81 <sup>c</sup>	5.60±0.91 <sup>c</sup>	5.58±0.91 <sup>c</sup>	4.08±0.97 <sup>a</sup>	5.13±0.97 <sup>c</sup>

Values are expressed as mean±standard deviation. Values with different superscript within the column are significantly different ( $\alpha = 0.05$ ).

NS1 (Nile: soybean 40:60); NS2 (Nile: soybean 50:50); NS3 (Nile: soybean 60:40); NT1 (Nile: tempeh 40:60); NT2 (Nile: tempeh 50:50) and NT3 (Nile: tempeh 60:40)

brown colour. The amino acid content in tempeh was higher than soy due to the fermentation process. Bujang and Taib (2014) stated that the amino acid in tempeh is higher than soybeans by 24 hours fermentation process. Fish *koya* from this study was better than Regina *et al.* (2012) which produced a darker appearance. This was due to the natural color of mackerel fish.

### 3.2.2 Aroma

Sensory analysis of the aroma parameters showed that panelists could accept *Koya* with a neutral to the like range. *Koya* NS3 and NT3 were the most preferred by panelists with scores of 6.00 and 5.60, respectively. Based on the aroma value, the *Koya* with the most addition of tilapia 60%, was the most preferred by the panelist. The aroma that arose from the *Koya* NS and NT were the fragrant aroma from the combination of spices, tilapia, soy (NS), and tempeh (NT). The more tilapia added, the stronger the fish smell. Pratama *et al.* (2018) stated that the volatile component in tilapia comes from the hydrocarbon, alcohol, aldehyde, and ketone groups. The volatile component was detected due to environmental influences, like the processing process. Chukeatirote *et al.* (2017) stated that the main aroma components in soybean are alcohol, acids and esters, ketones, aldehydes, and furans. Tempe contains nineteen compounds that form its distinctive aroma and increases significantly to twenty-one aroma-forming compounds when tempeh fried. The main volatile compounds come from aldehydes and ketones, hydrocarbons, mono and sesquiterpenes, sulfur-containing compounds, nitrogen-containing compounds, alcohol, and furan (Jelen *et al.*, 2013).

Ginger, galangal, bay leaves, garlic, shallot, lime leaves, and lemongrass was added as the spices. There was also the aroma of coconut milk and palm sugar. The aroma came out due to the presence of volatile substances in the spice. Spices contain volatile essential oils. These volatile compounds are responsible for the

aroma formation in *Koya*. During the process, heat energy destructs the spice cell wall and the release of volatile compounds (An *et al.*, 2015). According to Rannou *et al.* (2016), Maillard reactions that occur during *Koya* processing also play a role in the formation of aromas. During the Maillard reaction, an intermediate compound like the dehydro-reductor from sugar dehydration and fission products (volatile compounds 2,3-butadiene, 2,3-pentadiene or volatile precursors) from sugar fragmentation causes aldehyde formation through Strecker degradation. This aldehyde plays a role in flavor formation. This result was better than Regina *et al.* (2012) with mackerel fish *koya* aroma that was less preferred by panelists. This was due to the strong fishy odour of seafood than freshwater fish.

### 3.2.3 Taste

Based on the taste parameters, the *Koya* can be accepted by panelists with a somewhat dislike to like range. *Koya* NS3 and NT3 were the most preferred by panelists with scores of 5.45 and 5.58, respectively. The higher the tilapia added, the more preferred by panelists. *Koya* had a savory taste derived from the combination of tilapia, tempeh or soy flour, and seasonings. Tilapia contributes a savory taste to *Koya* fish because it contains glutamic acid. Yarnpakdee *et al.*, (2014) showed that protein hydrolysate on tilapia contains dominant amino acids like glutamate acid, lysine, and aspartate acid. Soy flour has beany, greasy, pointy and bitter flavors, so it is less preferred (Damodaran and Arora, 2013), while raw tempeh which modified with the addition of 2% *S. cerevisiae* has a dominant taste of astringent, bitter, savory, and sour (Kustyawati *et al.*, 2017). It was the reason why *Koya* with the highest percentage of tilapia was the most preferred. The taste parameter of *koya* in this study was better than Anandito *et al.* (2019), which on neutral level for high concentration of snakehead fish *koya* preferred by panelists.

### 3.2.4 Texture

Texture analysis showed that panelists could accept *Koya* with a neutral to the like range. *Koya* NS1 and NT1 were the most preferred by panelists with scores of 5.45 and 5.58, respectively. It showed that the amount of tilapia added gave different texture value. These results by Cortez Netto *et al.* (2014), the amount of tilapia produces different texture values. The higher the tilapia added, the texture value decreased. *Koya* NS1 and NT1 had a rough and dry texture. It was also related to its moisture content. *Koya* NS1 and NT1 had the lowest moisture content among all sample. Low moisture content resulting in a dry texture.

### 3.2.5 Overall

Overall testing was intended to determine the panelist acceptance level, including color, aroma, taste, and texture. The overall parameters could be a whole, which panelists preferred the most. Table 4 shows that the composition of tilapia and soy or tempeh flour was significantly different against the overall value. The preference of *Koya* NS ranged from somewhat disliked to somewhat like, while *Koya* NT got neutral to somewhat like range. *Koya* NS3 and NT3 were the most favored by panelists with a value of 5.38 and 5.13, respectively.

### 3.3 Fatty acids and amino acids profile

*Koya* NS3 and NT3 as the most preferred by panelist were tested for fatty acid and amino acid profiles. The results are presented in Tables 5 and 6.

Table 5 shows that *Koya* NS and NT contained eleven types of fatty acids that had been successfully identified. These fatty acids were divided into three types based on their chemical structure. The first category was

saturated fatty acids (SFA), including caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, and arachidic acid. While monounsaturated fatty acids (MUFA) in *Koya* were palmitoleic acid and oleic acid, the polyunsaturated fatty acids (PUFA) in *Koya* were linoleic acid and linolenic acid.

The fatty acids in *Koya* fish came from the primary and supporting ingredients. Nile tilapia contains saturated fatty acids (caprylic acid, capric acid, lauric acid, heptadecanoic acid, myristic acid, palmitic acid, stearic acid, arachidic acid, and behenic acid), and unsaturated fatty acids (myristoleic acid, palmitoleic acid, oleic acid, linoleic acid, arachidonic acid, erucic acid, arachidonic acid, and  $\alpha$ -linolenic acid) (Navarro *et al.*, 2012). The fatty acid in soy and tempeh flour consists of palmitic acid, stearic acid, oleic acid, linoleic acid, and eicosanoic acid (Kanghae *et al.*, 2017). The saturated fatty acids (SFA) in commercial *Koya* was higher than NS3 and NT3 with a value of 42.13%. The identified saturated fatty acids consist of seven fatty acids. The most SFA found in commercial *Koya* was palmitic acid. Saturated fatty acids in *Koya* NS3 and NT3 were dominated by lauric acid, about 15.49% and 15.86%, respectively. The addition of coconut milk caused high lauric acid. Coconut milk is a coconut oil source, with 38.40% of lauric acid (Azevedo *et al.*, 2020). Lauric acid in *Koya* also came from tilapia. Chen *et al.* (2013) stated that lauric acid is one of the fatty acids in tilapia was 1.41%. Lauric acid is beneficial for the human body. Shah and Limketkai (2017) stated that lauric acid is a medium fatty acid easily absorbed by the digestion system. Also, it is the potential to reduce obesity and neurological disorders.

Table 5 shows that palmitoleic and oleic fatty acids were monounsaturated fatty acids (MUFA) found in

Table 5. Fatty acids profile

No	Fatty acid	NS3	NT3	Commercial
1	Caprylic acid (C8:0)	2.29	2.39	0.03
2	Capric Acid (C10:0)	1.78	1.93	0.03
3	Lauric acid (C12:0)	15.49	15.86	0.24
4	Myristic acid (C14:0)	6.08	5.57	0.87
5	Palmitic acid (C16:0)	10.81	9.75	37
6	Stearic acid (C18:0)	2.43	3.37	3.84
7	Arachidic Acid (C20:0)	0.27	0.17	0.12
<b>Total SFA</b>		<b>39.15</b>	<b>39.04</b>	<b>42.13</b>
8	Palmitoleic acid (C16:1)	0.91	0.82	0.21
9	Oleic Acid (C18:1)	21.82	14.96	42.87
<b>Total MUFA</b>		<b>22.73</b>	<b>15.78</b>	<b>43.08</b>
10	Linoleic acid (C18:2)	20.51	27.9	10.98
11	Linolenic acid (C18:3)	3.57	3.68	0.18
<b>Total PUFA</b>		<b>24.08</b>	<b>31.58</b>	<b>11.16</b>

NS3 (Nile: soybean 60:40) and NT3 (Nile: tempeh 60:40)

Table 6. Amino Acids Profile

No	Amino acids	NS3	NT3	Commercial
<b>Non-essential amino acids</b>				
1	L-Alanine	1.2	1.23	0.74
2	L-Arginine	0.7	1.08	0.52
3	L-asparagine	0.02	<0.01%	0.09
4	L-aspartic acid	1.37	1.6	1.31
5	L-glutamate acid	2.17	2.38	2.23
6	L-Glutamine	<0.01	<0.01%	<0.01%
7	L-Glycine	1.35	1.74	0.74
8	L-Tyrosine	0.47	0.74	0.26
<b>Total Non-essential amino acids</b>		<b>7.28</b>	<b>8.77</b>	<b>5.89</b>
<b>Essential amino acids</b>				
1	L-histidine + L-serine	1.17	3.61	1.14
2	L-Isoleucine	0.6	1.03	0.5
3	L-Leucine	1.12	1.77	0.97
4	L-Lysine	1.37	0.61	1.14
5	L-Phenylalanine	0.67	1.08	0.59
6	L-Threonine	1.27	1.79	0.92
7	L-Tryptophan + L-Methionine	0.6	0.32	0.16
8	L-Valin	0.72	1.42	0.59
<b>Total Essential amino acids</b>		<b>7.52</b>	<b>11.63</b>	<b>6.01</b>

NS3 (Nile: soybean 60:40) and NT3 (Nile: tempeh 60:40)

*Koya* NS3, NT3, and commercial. Palmitoleic and oleic in *Koya* F3 were from tilapia, soy, and tempeh flour. According to Navarro *et al.* (2012), tilapia contains palmitoleic and oleic acid. Kanghae *et al.* (2017), soy and tempeh contain high amounts of oleic acid. Compared to commercial *Koya*, *Koya* NS3 and NT3 *Koya* had higher levels of polyunsaturated fatty acids (PUFA). The total PUFA in the *Koya* NS3 and NT3 was 24.08% and 31.58%, respectively while in the commercial *Koya* was 11.16%. The identified PUFA in NS3, NT3, and commercial *Koya* were linoleic acid and linolenic acid. Linoleic acid could reduce the risk of cardiovascular symptoms (Marangoni *et al.*, 2020). Linolenic fatty acids can be precursors of other omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In contrast to arachidonic acid (ARA) which formed proinflammatory eicosanoids, EPA and DHA formed anti-inflammatory eicosanoids. Linolenic fatty acids help resolve the inflammation and alter the vascular biomarkers and carcinogens function, moreover, reducing the risk of cancer and providing substantial protection against other chronic and metabolic diseases such as diabetes, obesity, osteoporosis, neurological degeneration, and fractures (Saini and Keum, 2018). Based on this research, NS3 and NT3 are beneficial in terms of fatty acid content compared to commercial *Koya*.

The amino acid in *Koya* NT3 came from its constituent ingredients, tilapia, and soy tempeh flour. According to Yarnpakdee *et al.* (2014), Nile tilapia

contains essential amino acids such as histidine, isoleucine, threonine, methionine, leucine, phenylalanine, and lysine. Non-essential amino acids of tilapia were alanine, aspartic acid, glutamic acid, and serine — also, the conditional amino acids such as arginine, glycine, and tyrosine. Tempe contains amino acids histidine, serine, arginine, glycine, aspartate, glutamate, threonine, alanine, proline, lysine, tyrosine, methionine, valine, isoleucine, leucine, and phenylalanine (Syida *et al.*, 2018). All these amino acids play a role in the formation of the amino acids in *Koya*.

Essential amino acids that could be found in *Koya* consisted of eight types; one of them was histidine. However, in this study, histidine was detected together with one of the non-essential amino acids, serine. Histidine and serine in *Koya* NT3 were higher than in commercial *Koya* with 3.61% and 1.14%, respectively. Both *Koya* contains histidine because the primary raw materials, tilapia and tempeh flour in NT3 *Koya* and shrimp in commercial *Koya* also contain histidine (Yarnpakdee *et al.*, 2014; Priyadarshini *et al.*, 2015; Syida *et al.*, 2018).

#### 4. Conclusion

The combination of Nile tilapia-soy flour (NS) and Nile tilapia-tempeh (NT) had a significant effect on the chemical and organoleptic of *Koya*. *Koya* NS3 (tilapia : soy flour = 60% : 40%) and NT3 (tilapia : tempeh flour = 60% : 40%) were the most preferred by the panelists.

*Koya* NS3 contained moisture, ash, fat, protein, and carbohydrates of 13.06%, 5.15%, 19.59%, 54.19%, and 21.50%; respectively while NT3 contained moisture, ash, fat, protein, and carbohydrates of 13.32%, 3.89%, 19.28%, 48.72%, and 28.06%; respectively. *Koya* NS3 and NT3 contained linoleic and linolenic fatty acids and higher essential and non-essential amino acids than commercial *Koya*.

### Conflict of interest

The authors declare no conflict of interest.

### Acknowledgments

This research was funded by Universitas Sebelas Maret (Grant No. 516/UN27.21/PP/2019).

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