ISSN 2087-3336 (Print) | 2721-4729 (Online)

TEKNOSAINS: Jurnal Sains, Teknologi dan Informatika
Vol. 11, No. 1, January 2024, page. 21-32
http://jurnal.sttmcileungsi.ac.id/index.php/tekno
DOI: 10.37373

Analysis of milkfish meatballs with the addition of legume protein isolate

Siti Munawaroh[1*], Bayu Kanetro[1], Agus Slamet[1]

[1*]Departement of Agricultural Product Technology, Faculty of Agro-industry, University of Mercu Buana Yogyakarta, Indonesia, Jl. Wates km 10, Argomulyo, Bantul, Yogyakarta, Indonesia 55753

*✉ ptkmunawaroh@gmail.com

Submitted: 15/05/2023 Revised: 22/05/2023 Accepted: 24/05/2023

ABSTRACT

A popular and nutrient-rich fish jelly product called milkfish meatballs also includes saturated fatty acids and amino acids that are linked to an increased risk of cardiovascular disease. The goal of this study was to create milkfish meatballs with the best and desired chemical, physical, amino acid, fatty acid, and cholesterol profiles by adding legume protein isolate. The study's nine treatments included milkfish meatballs with soy protein isolate, sword kara protein isolate, and cowpea protein isolate added in amounts of 0%, 15%, and 30%, respectively. The produced meatballs underwent physical (texture, folding test, color test), chemical (proximate), and preference analysis. After analyzing the fatty acid profile, amino acid profile, and cholesterol before and after in vitro digestion, the two most popular treatments (milk meatballs with the addition of sword kara protein isolate/cowpea protein isolate or milkfish meatballs with the inclusion of sword kara protein isolate) were chosen. The milkfish meatballs that received the best treatment were those with the addition of 15% soy protein isolate and 30% swordfish protein isolate, with ratios of omega-6 to omega-3 of 2.176 and 1.811, respectively, and ratios of arginine to lysine of 1.49 and 0.99, respectively. This study's findings suggest, milkfish meatballs with the addition of 30% karapel protein isolate have the best preference value and the ability to serve as a functional diet to prevent hypercholesterolemia.

Keywords: Cholesterol; isolate from beans; amino acids; milkfish meatballs.

1. INTRODUCTION

A typical Indonesian dish is called bakso [1], circular or various forms made from tapioca and beef or surimi that have been mashed together [2]. Fish balls, as defined by SNI 7266: 2014, are processed fishery products that include at least 40% minced fish meat or surimi mixed with flour and other ingredients as needed. They also go through formation and cooking, which is the process of ripening the product by putting it in hot water and heating it in two stages over the course of the time and temperature specified.

Fish meatballs are processed fish products created by mixing ground fish with flour and seasonings. A circle is imprinted on the dough. Meatballs can be made manually by hand or with a meatball printing machine, which prints the dough before the meatballs are boiled. Fish balls that have been cooked have the ability to float when they are boiled. Fish balls have a savory flavor, a chewy consistency, and a distinct scent [3]. The type and degree of freshness of the fish, the quantity and type of tapioca used as a filler, the use of binder, the temperature and length of stirring, and the heating technique all affect the texture of fish balls [4]. Clean white meatballs that are free of contaminants and aren't combined with other ingredients are required for fish meatballs to meet quality standards [5].

In Southeast Asia, particularly along Indonesia's coast, milkfish are commonly cultivated. Particularly along Java's North Coast, most specifically in the Pati and Gresik regions [6]. A brackish water fish species with considerable economic importance is the milkfish. Because it is a source of animal protein with a high nutritional value, a delectable flavor, and a low cholesterol content, this type of fish has gained recognition from the larger community [7].

One of the most well-liked fish jelly items in the area is milkfish meatballs. This product is simple to find and offered in a variety of markets or supermarkets. Because they are chewy, white, relatively affordable, very healthy, and have a pleasant scent, milkfish meatballs are popular with consumers. Due to its excellent nutritional value, milkfish meatballs, which employ milkfish as their primary source of animal protein, are a great choice for kids to eat. Milkfish has a yield of about 40% for freshwater milkfish and 50% for brackish water milkfish, making it a rich source of protein (20–24%), fat, amino acids, fatty acids, minerals, and vitamins. In milkfish meat, the macrominerals are Ca, Mg, Na, and K, while the microminerals are Fe, Zn, Cu, and Mn. Glutamate has the highest amino acid composition, and oleic has the highest amount of unsaturated fatty acids (31-32%). The vitamins A, B1, and B12 are present in milkfish meat [8]. 129 kcal of energy, 20 g of protein, 4.8 g of fat, 150 mg of phosphorus, 20 mg of calcium, 2 mg of iron, 150 SI of vitamin A, and 0.05 mg of vitamin B1 are all present in 100 g of milkfish [9].

Previous research has indicated that milkfish have a high amino acid content, particularly lysine (0.67% in freshwater and 0.53% in brackish water milkfish), and a low arginine amino acid level (0.25% in freshwater and 0.29% in brackish water milkfish). With a low level of stearic fatty acids (3.26% in freshwater milkfish and 4.01% in brackish water milkfish), palmitic acid has the greatest saturated fatty acid content in milkfish meat (39.37% in freshwater milkfish and 41.48% in brackish water milkfish) [10]. Lysine has a tendency to raise cholesterol levels, whereas arginine has the reverse effect [11]. Myristic and palmitic acids are among the saturated fatty acids that are most hypercholesterolemic, but stearic acid is regarded as neutral or even hypocholesterolemic. Products made from milkfish are not advised for those who have high cholesterol [12]. Therefore, it is required to add legume protein isolate to milkfish meatballs in order to lessen the hypocholesterolemic effect. Increasing anti-nutritional factors, enhancing digestibility, and increasing the bioavailability of legume amino acids can be accomplished through protein isolation [13]. Pure peptides that are cardioprotective can be found in nut protein isolate.

Local Indonesian beans called sword kara and cowpea have the potential to be turned into a nutritious source of vegetable protein. Cowpea and the sword bean are both cheap and simple to grow. Nuts are an essential functional food because they are high in quality protein, complex carbohydrates, dietary fiber, and have a comparatively low-fat level. Consuming nuts is linked to the prevention and management of cardiovascular disease (CVD), among other health advantages.

This study aims to combine vegetable protein with animal protein, specifically milkfish protein, to create functional food products in the form of meatballs that have a more complete amino acid profile, are hypocholesterolemic, and have the preferred organoleptic qualities by consumers.

2. METHOD

Research was conducted in the Laboratory of Chemistry and Agricultural Products Processing, Mercu Buana University, Yogyakarta, Jl. Yogya-Wates Km.10, Yogyakarta, namely in vitro digestion testing and meatball color testing. At CV. Chem Mix Pratama Yogyakarta, proximate testing was conducted. The APHP Laboratory at SMKN 1 Pandak, which is located at Kadekrowo, Gilangharjo, Pandak, Bantul, Yogyakarta 55761, conducted the sample preparation procedure and preference test. At the Saraswanti Laboratory (SIG) Bogor, tests for cholesterol, amino acid profiles, and fatty acid profiles were performed. At the UGM FTP Laboratory, milkfish meatballs underwent texture testing. When the study was carried out between September and November 2021.

The primary tools used in this study were trays, cloth, ovens (Fomac 2 deck 6 tray BOV-ARF60H), disk mills (FFC 23+Engine Honda GC160 5.5PK), magnetic stirrers (IKA-Combimag RCT), and heaters. The tools used to make fish balls included a bone and bone separator machine (FMBBS09S Brand Fomac), a food processor (Phillips HR7627), knives, basins, scales (ACS). Centrifugation, analytical balance (Ohaus), kjeldhal flask (Iwaki), distillation apparatus (Pyrex), soxhlet flask (Pyrex), pH meter (Istek), oven (Memmert UN55), desiccator (Duran 30cm), Erlenmeyer flask (Pyrex), beaker (Iwaki), glass funnel (Pyrex), test tube (Pyrex), measuring stages of research include:

a. Sword kara and cowpea protein isolate preparation

The process for making protein isolate was adjusted at the protein precipitation stage, where the protein was precipitated at an isoelectric pH of cowpea and sword kara [14]. The extraction stage of the isolation process involved precipitating at a pH of 4 after extracting at an alkaline pH of 9 first. Milkfish meatballs were made using the finished cowpea protein isolate and cowpea protein isolate.

b. The process of making milkfish meatballs

The two stages of this research—preliminary research and main research—were each completed separately. For the manufacturing of milkfish meatballs, preliminary research was done to achieve a variety of concentrations of soy protein isolate, sword kara protein isolate, and cowpea protein isolate. While the primary goal of the research was to determine the ideal ratio of the three types of protein isolates (cowpea, sword kara, and soybean) to use in the production of milkfish meatballs, other tests were also undertaken to determine which meatballs would perform best. a flowchart for making milkfish meatballs with soy protein, sword kara protein, and cowpea protein isolate added.

In this study, analyses were done on samples of meatballs that had soy protein isolate, sword kara protein isolate, and cowpea protein isolate added as well as proximate measurements of moisture content, total protein content, total fat content, ash content, and calculated carbohydrate content.

A completely randomized design (CRD) was employed as the experimental strategy in this investigation. The kind of protein isolate (soy protein isolate, sword kara protein isolate, and cowpea protein isolate) and the proportion of addition of protein isolate (0%, 15%, and 30%) were the two treatment components in the treatment design. In this study, there were two types of controls: control 1 consisted of milkfish meatballs without any protein isolate added as a negative control, and control 2 consisted of milkfish meatballs with the best treatment's concentration of soy protein isolate as a positive control. Additionally, the statistics of the collected data were examined using ANOVA and the DMRT (Duncan Multiple Range Test) significant difference test [15]. The fatty acid profile, amino acid profile, and cholesterol were tested in vitro before and after the two best treatments had been determined, namely one best treatment for milkfish meatballs with the addition of soy protein isolate and one best treatment for milkfish meatballs with the addition of sword kara protein isolate or milkfish meatballs with the addition of cowpea protein isolate. The two datasets were then examined using ANOVA statistics and the Tukey test for significant differences.

3. RESULTS AND DISCUSSION

3.1 Amino acid profile

Table 1 shows the amino acid composition of milkfish meatballs before and after in vitro digestion with the addition of 15% soy protein isolate or 30% karaword protein isolate. **Table 1** demonstrates that the amino acid profile of milkfish meatballs with the addition of 15% soy protein isolate and 30% kara sword protein isolate differed significantly from one other. In this study, the amino acid profiles of milkfish meatballs with additions of 15% soy protein isolate and 30% karaword protein isolate were determined because these two samples had the highest overall preference values, or in other words, the meatballs with these two treatments were still well-liked by consumers.

Table 1. Amino acid profile of milkfish meatballs added with 15% soy protein isolate or 30% karapel protein isolate before and after in vitro digestion

Types of amino acids *	Amino acid content (% bk material)			
	Before in vitro digestion		After in vitro digestion	
	Milkfish meatballs with the addition of ISP 15%	Milkfish meatballs with the addition of 30% Karasword protein isolate	Milkfish meatballs with the addition of ISP 15% (percentage decrease)	Milkfish meatballs with the addition of 30% Karasword protein isolate (percentage decrease)
Non-essential amino acids				
L-Alanine	1,97 ± 0,002 [a]	1,86 ± 0,01 [b]	0,12 ± 0,01 [a] (93,81 ± 0,26 [a])	0,12 ± 0,00 [a] (93,59 ± 0,05 [a])
L-Arginine ***	3,61 ± 0,00 [a]	2,68 ± 0,01 [b]	< 0,12 ± 0,00 [a] (96,74 ± 0,00 [a])	< 0,12 ± 0,00 [a] (95,61 ± 0,01 [b])
L-Aspartic Acid	3,06 ± 0,01 [a]	3,04 ± 0,02 [a]	0,11 ± 0,01 [a] (96,54 ± 0,17 [a])	0,09 ± 0,00 [a] (96,98 ± 0,02 [a])
L-Glutamic Acid	5,14 ± 0,01 [a]	5,54 ± 0,02 [b]	0,30 ± 0,01 [a] (94,22 ± 0,28 [a])	0,24 ± 0,00 [b] (95,71 ± 0,02 [b])
Glycine ***	2,18 ± 0,00 [a]	1,75 ± 0,01 [b]	0,10 ± 0,00 [a]	0,10 ± 0,00 [a]

Types of amino acids *	Amino acid content (% bk material)			
	Before in vitro digestion		After in vitro digestion	
	Milkfish meatballs with the addition of ISP 15%	Milkfish meatballs with the addition of 30% Karasword protein isolate	Milkfish meatballs with the addition of ISP 15% (percentage decrease)	Milkfish meatballs with the addition of 30% Karasword protein isolate (percentage decrease)
			(95,38 ± 0,23 ^{a})	(94,38 ± 0,04 ^{b})
L-Proline	1,57 ± 0,00 ^{a}	1,48 ± 0,01 ^{b}	0,05 ± 0,00 ^{a} (96,77 ± 0,17 ^{a})	0,04 ± 0,00 ^{a} (97,05 ± 0,02 ^{a})
L-Serine	2,61 ± 0,01 ^{a}	2,09 ± 0,00 ^{b}	0,07 ± 0,01 ^{a} (97,26 ± 0,19 ^{a})	0,06 ± 0,00 ^{a} (96,99 ± 0,02 ^{a})
Essential Amino Acids				
L-Histidine	1,66 ± 0,00 ^{a}	1,05 ± 0,00 ^{b}	< 0,09 (94,56 ± 0,00 ^{a})	< 0,09 (91,42 ± 0,00 ^{b})
L-Isoleucine **	1,85 ± 0,01 ^{a}	1,65 ± 0,00 ^{b}	0,06 ± 0,00 ^{a} (96,94 ± 0,17 ^{a})	0,055 ± 0,00 ^{a} (96,66 ± 0,07 ^{a})
L-Leucine **	3,36 ± 0,01 ^{a}	2,96 ± 0,01 ^{b}	0,11 ± 0,00 ^{a} (96,61 ± 0,18 ^{a})	0,108 ± 0,00 ^{a} (96,34 ± 0,03 ^{a})
L-Lysine **	2,43 ± 0,01 ^{a}	2,70 ± 0,17 ^{b}	0,11 ± 0,00 ^{a} (95,52 ± 0,21 ^{a})	0,098 ± 0,00 ^{a} (96,38 ± 0,03 ^{b})
L-Phenylalanine	2,47 ± 0,00 ^{a}	1,75 ± 0,00 ^{b}	< 0,15 (94,13 ± 0,01 ^{a})	< 0,145 (91,70 ± 0,01 ^{b})
L-Threonine	2,29 ± 0,00 ^{a}	1,73 ± 0,00 ^{b}	0,06 ± 0,00 ^{a} (97,55 ± 0,12 ^{a})	0,06 ± 0,00 ^{a} (96,64 ± 0,01 ^{b})
L-Valin	2,02 ± 0,01 ^{a}	1,79 ± 0,00 ^{b}	0,07 ± 0,00 ^{a} (96,57 ± 0,17 ^{a})	0,07 ± 0,00 ^{a} (96,26 ± 0,00 ^{a})
Arginine:Lysine ratio	1,49	0,99	1,08	1,20

Information:

*value is based on the statistical Tukey test (T Test) and is the average of two independent analyses. At the 95% level of confidence, numbers with the same letter in a row are not statistically different.

** amino acids linked to elevated amounts of cholesterol in the blood circulation

*** amino acids that are connected to decreased blood cholesterol levels

The order of the essential amino acids in milkfish meatballs with the addition of 15% soy protein isolate is L-glutamic acid, L-arginine, L-aspartic acid, L-serine, glycine, L-alanine, L-proline, and L-tyrosine, from highest to lowest amount. While L-glutamic acid, L-aspartic acid, L-serine, L-alanine, glycine, L-proline, and L-tyrosine were present in milkfish meatballs with the addition of 30% karaword protein isolate. When soy protein isolate was added, milkfish meatballs' amino acid profile displayed a greater value and was noticeably different from milkfish meatballs that had 30% carapace protein isolate added. This occurred because soy protein isolate had a higher concentration of the necessary amino acids than did kara protein isolate. For instance, the glutamic acid concentration of swordfish protein isolate was 5.03% (ww), compared to 12.52% (wk) in soy protein isolate [16].

Table 2 lists the amino acid profiles of milkfish meat, soy protein isolate, and swordfish protein isolate. L-glutamic acid, L-acid aspartate, L-arginine, and L-serine are the four non-essential amino acids that predominate in milkfish, soy protein isolate, and swordfish protein isolate. It is well recognized that each of these amino acids contributes to a product's fundamental flavor. Food products have an umami flavor because they contain glutamic acid that is highly abundant and well above the threshold of taste [17]. Along with glucose, glutamic acid can serve as the primary fuel for brain cells. The salt monosodium glutamate (MSG), a derivative of glutamic acid, is typically encountered in daily life and is frequently employed as a flavor enhancer [18]. Due to its function in transamination reactions, glutamic acid plays a role in the metabolism of amino acids. It is also necessary for the creation of molecules like glutathione, which is needed to remove highly harmful peroxides, and the polyglutamate folate cofactor [19]. Aspartic acid is another non-essential amino acid that predominates in the study's sample. Aspartic acid and glutamic acid are two amino acids associated with aspects of fish flavor [20]. As a precursor to amino acids and for the regulation of hormones, aspartic acid is crucial [21].

Table 2. The amino acid contents in milkfish meat, soy protein isolate, and kara word protein isolate

Types of amino acids *	Amino acid content (% bk material)		
	Milkfish	**Soy protein isolate**	**Sword kara protein isolate**
Non-essential amino acids			
L-Alanine	1,22 ± 0,00	3,05 ± 0,02 [a]	1,67 ± 0,01 [b]
L-Arginine ***	1,40 ± 0,00	8,30 ± 0,05 [a]	3,07 ± 0,00 [b]
L-Aspartic Acid	1,77 ± 0,01	6,76 ± 0,04 [a]	3,50 ± 0,02 [b]
L-Glutamic Acid	2,93 ± 0,01	12,52 ± 0,09 [a]	5,03 ± 0,02 [b]
Glycine ***	0,94 ± 0,00	3,90 ± 0,02 [a]	1,88 ± 0,00 [b]
L-Prolin	0,73 ± 0,00	4,19 ± 0,00 [a]	1,90 ± 0,00 [b]
L-Serine	1,20 ± 0,00	5,36 ± 0,03 [a]	2,86 ± 0,01 [b]
L-Tyrosine	0,71 ± 0,00	3,56 ± 0,02 [a]	1,28 ± 0,00 [b]
Essential amino acids			
L-Histidine	0,50 ± 0,00	2,71 ± 0,01 [a]	1,23 ± 0,01 [b]
L-Isoleucine **	0,94 ± 0,00	3,87 ± 0,02 [a]	1,83 ± 0,01 [b]
L-Leucine **	1,72 ± 0,00	6,87 ± 0,04 [a]	3,42 ± 0,01 [b]
L-Lysine **	1,89 ± 0,01	4,28 ± 0,02 [a]	1,94 ± 0,01 [b]
L-Fenilalanin	0,86 ± 0,00	5,82 ± 0,04 [a]	2,43 ± 0,01 [b]
L-Threonin	1.05 ± 0,00	3,85 ± 0,02 [a]	2,09 ± 0,01 [b]
L-Valin	1,03 ± 0,00	3,79 ± 0,02 [a]	1,97 ± 0,00 [b]

Information:

* data generated from two replicate analyzes and in units of dry weight of material.

** amino acids associated with high cholesterol levels in the blood circulation

*** amino acids associated with low cholesterol levels in the blood circulation

L-leucine, L-fenilalanin, L-lysine, L-treonin, L-isoleucine, L-histidine, and L-valin, with respective amounts of 3.35% (bk), 2.47% (bk), 2.29% (bk), 1.85% (bk), 1.66% (bk), and 1.02% (bk), dominate the essential amino profile in milkfish meatballs with the addition of 15% soy protein isolate. With the addition of 30% karaword protein isolate, this type of amino acid also predominates in milkfish meatballs, which have the following amino acid contents: L-leucine 2.96% (bk), L-lysine 2.70% (bk), L-valine 1.79% (bk), L-fenilalanin 1.75 (bk), L-threonine 1.73% (bk), and L-isoleucine 1.65% (bk). Lysine is an amino acid necessary for healthy growth, and its lack results in immunodeficiency [22].

When 15% soy protein isolate was added to milkfish meatballs, the arginine/lysine ratio was 1.49 before in vitro digestion, and it was reduced to 1.08 following in vitro digestion. The ratio of arginine/lysine in milkfish meatballs increased from 0.99 before in vitro digestion to 1.20 following the addition of 30% karaword protein isolate. Over 90% of amino acids substantially decreased after in vitro digestion. L-serine, which was reduced by 97.26% after the addition of 15% soy protein isolate, and L-proline, which was reduced by 97.05% after the addition of 30% karaword protein isolate, were the two non-essential amino acids that showed the largest reduction. L-threonine (97.55%) and L-isoleucine (96.66%) were the essential amino acids that decreased the most after in vitro digestion in milkfish meatballs with the addition of 15% soy protein isolate and karaword protein isolate, respectively.

3.2 Cholesterol

Table 3 shows the cholesterol values of milkfish meatballs with 15% soy protein isolate or kara sword protein isolate added.

Table 3. Cholesterol levels of milkfish meat and milkfish meatballs at the beginning and conclusion of in vitro digestion when combined with 15% soy protein isolate or 30% swordfish protein isolate

Cholesterol (mg/100g)	Milkfish	Milkfish meatballs with the addition of 15% soy protein isolate	Milkfish meatballs with the addition of 30% Karasword protein isolate
Initial cholesterol	46,18 ± 0,19	24,11 ± 0,11 [a]	24,68 ± 0,11 [b]
Final cholesterol after in vitro digestion		6,74 ± 0,13 [a]	6,27 ± 0,14 [a]
% decrease during in vitro digestion		72,11 ± 0,43 [a]	74,54 ± 0,49 [b]

* a number followed by the same letter in one column shows no significant difference at the 95% confidence level

According to **Table 3**, the cholesterol levels of milkfish meatballs with a 15% soy protein isolate addition and those with a 30% karaword protein isolate addition were considerably different. Milkfish meatballs with 15% soy protein isolate added had a cholesterol content of 24.11 mg/100g, whereas milkfish meatballs with 30% karaword protein isolate added had a cholesterol value of 24.68 mg/100g. Due to the cholesterol level of the ingredient, specifically swordfish protein isolate, milkfish meatballs with the addition of 30% swordfish protein isolate have a greater cholesterol load. **Table 4** displays the concentrations of sword kara protein isolate and soy protein isolate.

Table 4. Cholesterol levels of milkfish meat, soy protein isolate, and swordfish protein isolate

Cholesterol (mg/100g)	Milkfish	Soy protein isolate	Sword kara protein isolate
Cholesterol	46,18 ± 0,19	0,41 ± 0,07 [a]	1,00 ± 0,14 [b]

* a number followed by the same letter in one column shows no significant difference at the 95% confidence level.

Sword kara protein isolate cholesterol values in **Table 4** differed significantly from soy protein isolate. Sword kara protein isolate has greater cholesterol values than soy protein isolates.

A drop in cholesterol levels occurred during in vitro digestion. Following in vitro digestion, the cholesterol levels of milkfish meatballs with 15% soy protein isolate and 30% karapel protein isolate were 6.74 mg/100g and 6.27 mg/100g, respectively. The milkfish meatballs with the addition of 30% karaword protein isolate had the largest percentage reduction in cholesterol levels (74.54%), whereas the milkfish meatballs with the addition of 15% soy protein isolate had the lowest percentage reduction (72.11%). This is believed to be closely related to the amount of omega-6 fatty acids and arachidonic acid. Milkfish meatballs with the addition of sword kara protein isolate had a 30% (0.08%) higher arachidonic acid (AA) content than milkfish meatballs with the addition of 15% (0.07%) soy protein isolate. In terms of omega-6 content, milkfish meatballs with a 30% karaword protein isolate addition have 1.03%, whereas milkfish meatballs with a 15% soy protein isolate addition have 0.99%. Arachidonic acid and omega-6 were found to have a significant impact on blood plasma HDL and LDL cholesterol levels [23]. In addition to arachidonic acid and omega-6, omega-3 is also known to have an impact on lowering cholesterol levels. Karaword protein isolate increased the omega-3 content of milkfish meatballs by 30% (or 0.47%) compared to milkfish meatballs with 15% soy protein isolate (or 0.45%). The rate at which LDL is removed from the circulation is increased by unsaturated fatty acids because they increase the expression of LDL receptors [24].

The efficiency of lowering cholesterol levels in animal protein due to the possible presence of isoflavone antioxidant chemicals found in karaword seeds is demonstrated by the reduction of cholesterol levels in milkfish meatballs after the addition of 30% of swordfish protein isolate. The highest antioxidative activity as an antioxidant and an above average cholesterolemic impact were demonstrated by the isoflavone genistein. Carolyn also looked into the isoflavone genistein from soybean seeds' ability to lower overall cholesterol levels. Her research's findings demonstrated that protein, mono- and polyunsaturated fatty acids, anthocyanins, and isoflavones all had an impact on the lowering of cholesterol molecules [25]. A possible source of hypocholesterolemic peptides is legume protein. For instance, the parent protein of the LPYPR hypocholesterolemic peptide, glycinin, is found in soybeans. This peptide shares structural similarities with enterostantin, a peptide that lowers cholesterol and is naturally released by the mucosa of the stomach and intestine [26]. Together with another glycinin-derived peptide, IAVPGEVA, LPYPR is a structural homologue of enterostatin and a competitive inhibitor of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, an important enzyme in the control of cholesterol production. By causing the conversion of cholesterol to extra bile salts that lower LDL cholesterol, HMG CoA reductase inhibition and the capacity to bind bile acids can help prevent hypercholesterolemia. Other bioactive peptides can similarly lower plasma triglycerides (TAG) via lowering the production of very low-density lipoprotein (VLDL) in the liver. Therefore, a diet rich in legumes may reduce the risk of cardiovascular disease (CVD). Concanavalin A, which is present in sword kara, has also been proven to drastically lower serum TC by 81%, liver cholesterol and TAG levels, while also raising HDL-C by 87% in comparison to the control group. Concanavalin A's (conA) lectin activity is directly correlated with its capacity to decrease cholesterol [27]. ConA alters the gastrointestinal environment in a variety of ways and is moderately resistant to degradation in the gut. These modifications may result in an increase in neutral sterol and triglyceride excretion through the feces, which inhibits the absorption of dietary cholesterol and lowers serum triglyceride levels.

3.3 Fatty acid profile

Important nutritional components called fatty acids have the potential to impact human health. To ascertain the potential of soy protein isolate or sword kara protein isolate in milkfish meatballs in avoiding cardiovascular disease, the full fatty acid profile was tested in this study. **Table 5** shows the outcomes of analyzing the fatty acid profile of milkfish meatballs before and after in vitro digestion with the addition of 15% soy protein isolate or 30% swordfish protein isolate.

Table 5. Fatty acid profile of milkfish meatballs with the addition of 15% soy protein isolate or 30% of karaword protein isolate before and after in vitro digestion

Fatty acid	**Fatty acid levels before in vitro (% bk)**		**Fatty acid levels after in vitro (%bk) (Decrease to baseline (%))**	
	Milkfish meatballs + 15% soy protein isolate	**Milkfish meatballs + 30% swordfinch protein isolate**	**Milkfish meatballs + 15% soy protein isolate**	**Milkfish meatballs + 30% swordfinch protein isolate**
C 12:0 (Laurat)	0,13 ± 0,00 a	0,15 ± 0,00 b	0,07 ± 0,01 a (47,41 ± 4,28a)	0,07 ± 0,00 a (55,46 ± 0,45a)
C 14:0 (Miristat)	0,33 ± 0,00 a	0,36 ± 0,00 b	0,03 ± 0,00 a (89,65 ± 0,72a)	0,04 ± 0,00 a (89,92 ± 0,11a)
C 15:0 (Pentadecanoate)	0,08 ± 0,00 a	0,09 ± 0,00 b	tt (100 ± 0,00a)	tt (100 ± 0,00a)
C 16:0 (Palmitate)	1,92 ± 0,02 a	2,37 ± 0,00b	0,16 ± 0,01 a (91,55 ± 0,56a)	0,03 ± 0,00 b (99,79 ± 0,01b)
C 17:0 (Heptadecanoate)	0,03 ± 0,00 a	0,03 ± 0,00 a	tt (100 ± 0,00a)	tt (100 ± 0,00a)
C 18:0 (Stearate)	0,39 ± 0,00 a	0,42 ± 0,00 b	0,05 ± 0,00 a (86,32 ± 0,83a)	0,04 ± 0,00 b (91,05 ± 0,13b)
C 24:0 (Lignofiber)	0,13 ± 0,00 a	0,13 ± 0,00 a	tt (100 ± 0,00a)	tt (100 ± 0,00a)
∑ Saturated Fatty Acid (SFA)	3,03 ± 0,02 a	3,57 ± 0,00 b	0,32 ± 0,02 a (89,56 ± 0,72a)	0,37 ± 0,00 a (89,52 ± 0,05a)
C 16:1 (Palmitoleate)	0,28 ± 0,00 a	0,30 ± 0,00 b	0,02 ± 0,00 a (93,23 ± 0,52a)	0,02 ± 0,00 a (94,58 ± 0,19a)
C 18:1 (Oleate)	1,83 ± 0,00 a	2,94 ± 0,04 b	0,14 ± 0,01 a (92,39 ± 0,26a)	0,20 ± 0,01 b (93,31 ± 0,07b)

Fatty acid	Fatty acid levels before in vitro (% bk)		Fatty acid levels after in vitro (%bk) (Decrease to baseline (%))	
	Milkfish meatballs + 15% soy protein isolate	Milkfish meatballs + 30% swordfinch protein isolate	Milkfish meatballs + 15% soy protein isolate	Milkfish meatballs + 30% swordfinch protein isolate
C 20:1 (eicocyanates)	0,06 ± 0,00 a	0,06 ± 0,00	Tt (100 ± 0,00[a])	tt (100 ± 0,00[a])
∑ Mono Unsaturated Fatty Acid (MUFA)	2,17 ± 0,00 [a]	3,37 ± 0,04 [b]	0,16 ± 0,01 [a] (92,70 ± 0,29[a])	0,21 ± 0,01 [b] (93,67 ± 0,08[a])
C 18:2 (Linoleate)	0,88 ± 0,00 [a]	0,96 ± 0,01 [b]	0,13 ± 0,00 [a] (85,38 ± 0,45[a])	0,12 ± 0,00 [a] (87,76 ± 0,02[b])
C 18:3 (Linolenic)	0,07 ± 0,00 [a]	0,06 ± 0,00 [a]	tt (100 ± 0,00[a])	0,01 ± 0,00 (88,13 ± 0,26[b])
C 20:2 (Eicosadienoice)	0,05 ± 0,00 [a]	0,04 ± 0,00 [b]	tt (100 ± 0,00[a])	tt (100 ± 0,00[a])
C 20:4 (Arachidonate/AA)	0,07 ± 0,00 [a]	0,08 ± 0,00 [b]	0,03 ± 0,00 [a] (55,47 ± 3,70[a])	0,03 ± 0,00 [a] (65,61 ± 0,09[a])
C 20:5 (eikosapentaenoate/EPA)	0,16 ± 0,00 [a]	0,28 ± 0,00 [b]	0,01 ± 0,00 [a] (93,24 ± 0,01 [a])	0,01 ± 0,00 [b] (97,71 ± 0,00 [b])
C22:6 (docosahexaenoate/DHA)	0,23 ± 0,00 [a]	0,21 ± 0,00 [a]	0,02 ± 0,00 [a] (92,85 ± 0,51[a])	0,01 ± 0,00 [b] (94,59 ± 0,03[b])
∑ Polyunsaturated Fatty Acid (PUFA)	1,49 ± 0,00 [a]	1,63 ± 0,01 [b]	0,19 ± 0,01 [a] (87,52 ± 0,46[a])	0,17 ± 0,00 [a] (89,61 ± 0,01[a])
∑ Unsaturated Fatty Acid	3,66 ± 0,00 [a]	2,07 ± 0,01 [b]	0,34 ± 0,01 [a] (90,59 ± 0,36[a])	0,38 ± 0,01 [a] (81,52 ± 0,28[b])
PUFA:SFA ratio	0,49 ± 0,00 [a]	0,46 ± 0,00 [b]		
Omega-3 totals	0,45 ± 0,00 [a]	0,57 ± 0,00 [b]	0,03 ± 0,00 [a] (94,05 ± 0,25[a])	0,03 ± 0,00 [a] (95,57 ± 0,04[b])
Total omega-6	0,99 ± 0,00 [a]	1,03 ± 0,01 [b]	0,16 ± 0,00 [a] (83,89 ± 0,59[a])	0,14 ± 0,00 [a] (86,09 ± 0,03[b])
Omega-6:omega-3 ratio	2,18 ± 0,01 [a]	1,81 ± 0,00 [b]		
Omega 9	1,83 ± 0,00 [a]	2,94 ± 0,04 [b]	0,14 ± 0,00 [a] (92,39 ± 0,26[a])	0,20 ± 0,01 [b] (93,31 ± 0,07[b])
EPA + DHA	0,39 ± 0,00 [a]	0,49 ± 0,00 [b]		

tt = not detected

* a number followed by the same letter in one line shows no significant difference at the 95% level of confidence.

According to **Table 5**, milkfish meatballs with the addition of 30% more types of protein isolate (more diverse) contained 23 types of saturated fatty acids and unsaturated fatty acids, while milkfish meatballs with the addition of 15% soy protein isolate contained 21 types of saturated and unsaturated fatty acids. Milkfish meatballs with the addition of 30% karaword protein isolate before in vitro had a greater concentration of saturated fatty acids (SFA) than milkfish meatballs with the addition of 15% soy protein isolate. When 15% soy protein isolate was added to milkfish meatballs, the SFA level was 3.029% (bk), but it was 3.572 (bk) when kara sword protein isolate was added. Because saturated fatty acids and unsaturated fatty acids have different chemical bonds, they have different chemical and physical properties. Saturated fatty acids, for example, can raise blood cholesterol levels. The tendency to reduce blood cholesterol levels is greater the longer the carbon chain and the more double bonds there are [28]. Consumption of saturated fatty acids is directly associated with plasma cholesterol levels and mortality from coronary heart disease [29]. The main dietary component responsible for the rise in

ISSN 2087-3336 (Print) | 2721-4729 (Online)
DOI 10.37373/tekno.v11i1.579

plasma cholesterol brought on by an increase in LDL cholesterol is saturated fatty acids. Because they have an impact on modulating the expression of the LDL receptor on the hepatic cell membrane, saturated fatty acids have the capacity to raise LDL cholesterol levels. When these fatty acid concentrations in the hepatic cell membrane are high, the LDL receptor protein undergoes down-regulation, which slows the clearance of LDL from the blood plasma. Myristic acid (C14:0) and palmitic acid (C16:0) are the most hypercholesterolemic saturated fatty acids, but stearic acid (C18:0) is neutral or even hypocholesterolemic. Due to the high rate of conversion of stearic acid to 20:1 monounsaturated fatty acids, stearic acid has a neutral or even hypocholesterolemic impact.

At the 95% confidence level, the amount of monounsaturated fatty acids and polyunsaturated fatty acids in milkfish meatballs with a 15% soy protein isolate addition before in vitro was substantially different from milkfish meatballs with a 30% carapace protein isolate addition. Oleate, in successive proportions of 1.83% (bk) and 2.94% (bk), was the most prevalent form of monounsaturated fatty acid in meatballs with the addition of 15% soy protein isolate and milkfish meatballs with the addition of 30% karaword protein isolate before in vitro. With a subsequent value of 1.03% (bk) and 0.99% (bk), milkfish meatballs with the addition of 30% karapel protein isolate had a higher and significantly different content of polyunsaturated fatty acids, particularly omega-6, than milkfish meatballs with the addition of 15% soy protein isolate. However, milkfish meatballs with the addition of 15% soy protein isolate had a higher total concentration of polyunsaturated fatty acids than milkfish meatballs with the addition of 30% karaword protein isolate.

Table 6. Fatty acid profile of milkfish meat, soy protein isolate, and karaword protein isolate

Fatty acid	Milkfish (% bk)	Soy protein isolate (% bk)	Sword kara protein isolate (% bk)
C 12:0 (Laurat)	0,05 ± 0,00	-	0,01 ± 0,00
C 14:0 (Miristat)	0,13 ± 0,00	-	0,02 ± 0,00
15:0 (Pentadecanoate)	0,04 ± 0,00	-	0,01 ± 0,00
C 16:0 (Palmitate)	1,06 ± 0,01	0,84 ± 0,01 [a]	0,77± 0,01 [b]
C 17:0 (Heptadecanoate)	0,02 ± 0,00	-	-
C 18:0 (Stearate)	0,19 ± 0,00	0,16 ± 0,01 [a]	0,10 ± 0,00 [b]
C 20:0 (arachid)	0,02 ± 0,00	-	0,04 ± 0,00
C 24:0 (Lignofiber)	0,07 ± 0,00	-	0,10 ± 0,00
∑ Saturated Fatty Acid (SFA)	1,57 ± 0,01	1,01 ± 0,01 [a]	1,48 ± 0,64 [a]
C 16:1 (Palmitoleate)	0,12 ± 0,00	-	0,04 ± 0,00
C 17:1 (Heptadecenoat)	0,01 ± 0,00	-	-
C 18:1 (Oleate)	1,34 ± 0,02	0,47 ± 0,01 [a]	2,57 ± 0,00 [b]
C 20:1 (eicocyanates)	0,04 ± 0,00	-	0,13 ± 0,00
∑ Mono Unsaturated Fatty Acid (MUFA)	1,51 ± 0,02	0,47 ± 0,01 [a]	2,75 ± 0,01 [b]
C 18:2 (Linoleate)	0,41 ± 0,01	1,33 ± 0,00 [a]	0,36 ± 0,00 [b]
C 18:3 (Linolenic)	0,07 ± 0,00	0,18 ± 0,00 [a]	0,39 ± 0,00 [b]
C 20:2 (Eicosadienoic)	0,02 ± 0,00	-	-
C 20:3 (Eicosatrienoate)	0,02 ± 0,00	-	-
C 20:4 (Arachidonate/AA)	0,03 ± 0,00	-	-
C 20:5 (eikosapentaenoate/EPA)	0,06 ± 0,00	-	0,03 ± 0,00
C22:6 (docosahexaenoate/DHA)	0,08 ± 0,00	-	-
∑ Polyunsaturated Fatty Acid (PUFA)	0,70 ± 0,01	1,50 ± 0,01 [a]	0,78 ± 0,00 [b]
∑ Unsaturated Fatty Acid	2,21 ± 0,03	1,97 ± 0,00 [a]	3,53 ± 0,01 [b]

Fatty acid	Milkfish (% bk)	Soy protein isolate (% bk)	Sword kara protein isolate (% bk)
PUFA:SFA ratio	0,44 ± 0,01	0,14 ± 0,00 [a]	0,08 ± 0,00 [b]
Omega-3 totals	0,22 ± 0,01	0,18 ± 0,00 [a]	0,42 ± 0,00 [b]
Total omega-6	0,46 ± 0,01	1,33 ± 0,00 [a]	0,36 ± 0,00 [b]
Omega-6:omega-3 ratio	2,06 ± 0,02	7,56 ± 0,11 [a]	0,87 ± 0,00 [b]
Omega 9	1,34 ± 0,02	0,47 ± 0,01 [a]	2,57 ± 0,00 [b]
EPA + DHA	0,15 ± 0,00		

* a number followed by the same letter in one line shows no significant difference at the 95% level of confidence.

According to the procedure described in the scientific journal Minekus, in vitro digestion was performed in this study to simulate digestion in the mouth, stomach, and intestine [30]. **Table 6** provides information on the fatty acid profile of milkfish meatballs after in vitro addition of either 15% soy protein isolate or 30% swordfish protein isolate. The table shows that the amount of fatty acids reduced during in vitro digestion. Omega-3 decreased by the greatest proportion in milkfish meatballs with soy protein isolate, by 94.05%, whereas EPA decreased by the greatest percentage in milkfish meatballs containing kara sword protein isolate, by 97.71%. In order for humans to have a physiologically balanced state, an omega-6 to omega-3 fatty acid ratio that is balanced is required. It is well recognized that omega-3 fatty acids can delay the beginning of cardiovascular disease (CvD). The following describes how omega-3 prevents CvD: Omega-3 lowers blood triglyceride levels through lowering triglyceride-rich very low-density lipoproteins (VLDLs) and reducing hepatic triglyceride production. Heart disease risk is indicated by high blood plasma triglyceride levels. Another consequence of heart disease is hypertension, which can be treated with high omega-3 fatty acids. This promotes membrane fluidity and the balance of prostanoids, which in turn regulates the state of arteries and arterioles that are too narrow [31]. Omega-3 fatty acids are helpful for persons with hypertension and hypercholesterolemia because they lower triglyceride levels, which can prevent atherosclerosis [32].

4. CONCLUSION

The 30% carapace protein isolate addiction in milkfish meatballs has the potential to be a beneficial diet for those with hypercholesterolemia. The physical, chemical, and culinary characteristics of the meatballs were altered by the addition of bean protein isolate. With the addition of protein isolate, milkfish meatballs' protein and ash contents rise while their water, fat, and carbohydrate contents fall. The most preferred way of therapy for milkfish meatballs is the addition of 30% karaword protein isolate, according to in vitro hypocholesterolemic testing based on their amino acid content and fatty acid profile. The arginine:lysine ratio was 0.99 when 30% karapel protein isolate was added to milkfish meatballs.

REFERENCE

[1] Sari, H. A., S. B. W. (2015). Karakteristik Kimia Bakso Sapi (Kajian Proporsi Tepung Tapioka: Tepung Porang dan Penambahan NaCl) Chemical Characteristic Beef Meatballs (Proportion of Tapioca Flour : Porang Flour And Addition Of Salt). Jurnal Pangan Dan Agroindustri, 3(3), 784–792.

[2] Suryaningrum, D., E. Hastarini, D. Ikasari, I. M. (2016). Pedoman Usaha Industri Rumah Tangga untuk Pengolahan Produk Bernilai Tambah Hasil Perikanan. Penebar Swadaya, Jakarta.

[3] Munawaroh, S. (2018). Diversifikasi Produk Perikanan Berbasis Ikan. Pustaka Widyatama.

[4] Suryaningrum, D., E. Hastarini, D. Ikasari, I. M. (2016). Pedoman Usaha Industri Rumah Tangga untuk Pengolahan Produk Bernilai Tambah Hasil Perikanan. Penebar Swadaya, Jakarta.

[5] Mulyadi, N. D. (2015). Bahan Ajar Diklat Peningkatan Kompetensi Guru Produktif Level Lanjut Angkatan 1. Kementerian Pendidikan dan Kebudayaan, Pusat Pengembangan dan Pemberdayaan Pendidik dan Tenaga Kependidikan Pertanian.

[6] Mulyawan, I., Zamroni, A., & Priyatna, F. N. (2017). Kajian keberlanjutan pengelolaan budidaya ikan bandeng di Gresik. Jurnal Kebijakan Sosial Ekonomi Kelautan dan Perikanan, 6(1), 25-35.
[7] Dewi, E. N., Purnamayati, L., & Kurniasih, R. A. (2019). Karakteristik mutu ikan bandeng (Chanos chanos Forsk.) dengan Berbagai Pengolahan. Jurnal Pengolahan Hasil Perikananan Indonesia, 22(1), 41-49.
[8] Astuti, K. D., Ningsih, N. W., Hidayatullah, R., Azizah, S. N., Soviana, R., Depari, Z. M. P., ... & Astuti, D. W. (2021). Pelatihan Pembuatan Abon Bandeng Bagi Masyarakat Desa Tenjoayu Kecamatan Tanara Provinsi Banten. Jurnal Abdimas Indonesia, 1(3), 61-65.
[9] Umam, M. K., Qozaemah, S., Yusak, A., Rahim, A. R., Sukaris, S., & Fauziyah, N. (2021). Upaya Meningkatkan Penghasilan Rumah Tangga Dengan Pelatihan Bandeng Cabut Duri Di Desa Gosari Kecamatan Ujung Pangkah Kabupaten Gresik. DedikasiMU: Journal of Community Service, 3(1), 705-712.
[10] Hafiludin, H. (2015). Analisis Kandungan Gizi Pada Ikan Bandeng Yang Berasal Dari Habitat Yang Berbeda. Jurnal Kelautan: Indonesian Journal of Marine Science and Technology, 8(1), 37-43.
[11] Utari, D. M., Rimbawan, R., Riyadi, H., Muhilal, M., & Purwantyastuti, P. (2011). Potensi asam amino pada tempe untuk memperbaiki profil lipid dan diabetes mellitus. Kesmas: Jurnal Kesehatan Masyarakat Nasional (National Public Health Journal), 5(4), 166-170.
[12] Riccardi, G., Angela A. Rivellese, C. M. W. (2011). Metabolisme Zat Gizi. EGC.
[13] Frota, K. D. M. G., Lopes, L. A. R., Silva, I. C. V., and Arêas, J. A. G. (2017). Nutritional quality of the protein of Vigna unguiculata L. Walp and its protein isolate. Revista Ciencia Agronomica, 48(5), 792–798.
[14] Kanetro, B. (2018). Amino acid profile of soybean (Glicine max) sprout protein for determining insulin stimulation amino acids. International Food Research Journal, 25(6), 2497-2502.
[15] Adiluhung, W. D., & Sutrisno, A. (2018). Pengaruh Konsentrasi Glukomannan Dan Waktu Proofing Terhadap Karakteristik Tekstur Dan Organoleptik Roti Tawar Beras (Oryza sativa) Bebas Gluten. Jurnal Pangan dan Agroindustri, 6(4).
[16] Kusnandar, F., Karisma, V. W., Firlieyanti, A. S., & Purnomo, E. H. (2020). Perubahan Komposisi Kimia Tempe Kacang Merah (Phaseolus vulgaris L.) Selama Pengolahan. Jurnal Teknologi Pangan, 14(1)..
[17] Zhao, C. J., Schieber, A., & Gänzle, M. G. (2016). Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations–A review. Food Research International, 89, 39-47.
[18] Swastawati, F., Wijayanti, I., & Prasetyo, S. D. Y. B. (2018). Profil nutrisi dan kualitas galantin bandeng dengan penambahan jenis dan konsentrasi asap cair yang berbeda. JPHPI, 21(3), 433-42.
[19] Anita, S., HARYONO, H., & WAHYUDEWANTORO, G. (2019). Nutritional component of Barbonymus balleroides: A wild fresh water fish from Indonesia. Biodiversitas Journal of Biological Diversity, 20(2), 581-588.
[20] Pratama, R. I., Rostini, I., & Rochima, E. (2018). Profil asam amino, asam lemak dan komponen volatil ikan gurame segar (Osphronemus gouramy) dan kukus. Jurnal Pengolahan Hasil Perikanan Indonesia, 21(2), 218-231.
[21] Chasanah, E., Nurilmala, M., Purnamasari, A. R., & Fithriani, D. (2015). Komposisi kimia, kadar albumin dan bioaktivitas ekstrak protein ikan gabus (Channa striata) alam dan hasil budidaya. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan, 10(2), 123-132.
[22] Nuban, N. S., Wijaya, S. M., Rahmat, A. N., & Yuniarti, W. (2020). Makanan Tradisional dari Ulat Sagu sebagai Upaya Mengatasi Malnutrisi pada Anak. Indonesian Journal of Nursing and Health Sciences, 1(1), 25-36.

[23] Demetz, E., Schroll, A., Auer, K., Heim, C., Patsch, J. R., Eller, P., ... & Tancevski, I. (2014). The arachidonic acid metabolome serves as a conserved regulator of cholesterol metabolism. Cell metabolism, 20(5), 787-798.

[24] Riccardi, G., Angela A. Rivellese, C. M. W. (2011). Metabolisme Zat Gizi. EGC.

[25] Carolyn, A., Farishal, A., & Berawi, K. N. (2019). Potensi Pemberian Isoflavon Kedelai Terhadap Kadar Kolesterol Total dan LDL pada Penderita Obesitas. MEDULA, medicalprofession journal of lampung university.

[26] Angeles, J. G. C., Villanueva, J. C., Uy, L. Y. C., Mercado, S. M. Q., Tsuchiya, M. C. L., Lado, J. P., ... & Torio, M. A. O. (2021). Legumes as functional food for cardiovascular disease. Applied Sciences, 11(12), 5475.

[27] Kayashima, T., Okazaki, Y., Katayama, T., & Hori, K. (2005). Dietary lectin lowers serum cholesterol and raises fecal neutral sterols in cholesterol-fed rats. Journal of nutritional science and vitaminology, 51(5), 343-348.

[28] Ngadiarti, I., Kusharto, C. M., Briawan, D., Marliyati, S. A., & Sayuthi, D. (2013). Kandungan Asam Lemak Dan Karakteristik Fisiko-kimia Minyak Ikan Lele Dan Minyak Ikan Lele Terfermentasi (Fatty Acid Contents and Physico-chemical Characteristics of Catfish Oil and Fermented Catfish Oil). Nutrition and Food Research, 36(1), 82-90.

[29] Yuliantini, E., Sari, A. P., & Nur, E. (2015). Hubungan asupan energi, lemak dan serat dengan rasio kadar kolesterol total-HDL. Nutrition and Food Research, 38(2), 139-147.

[30] Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T. O. R. S. T. E. N., Bourlieu, C., ... & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. Food & function, 5(6), 1113-1124.

[31] Susanto, E., & Fahmi, A. S. (2012). Senyawa fungsional dari ikan: aplikasinya dalam pangan. Jurnal Aplikasi Teknologi Pangan, 1(4).

[32] Salsabilla, B. H. (2019). Efektivitas pemberian extra virgin olive oil (EVOO) untuk menurunkan kolesterol pada pasien hiperkolesterol (Doctoral dissertation, Tugas Akhir, Universitas Muhammadiyah Magelang).