

# Organoleptic, chemical composition, and histology of fresh vs. fried Indonesian anchovies (*Stolephorus* sp.)

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## Abstract

Fried anchovy (*Stolephorus* sp.) is a popular food in Indonesia due to its good taste and ample availability and affordability. Yet, the actual nutritional aspects of this local dish remain uninvestigated. This study aimed to determine the best temperature and time for frying anchovies and to compare the histology and fatty acid and cholesterol profiles of fresh and fried anchovy meat. Anchovies were fried at 160 °C or 180 °C for 5, 10, 15, and 20 minutes, and the best frying conditions, namely at 180°C for 15 minutes, were established from the highest organoleptic test scores from a tasting panel assessment. Histologically, fried anchovy meat became shrunk, compact, and brittle, as explained by identified myomere changes. The fatty acid profile of fresh vs. fried anchovies revealed a marked reversal in omega-6: omega-3 ratios, and fried anchovies contained more cholesterol than fresh ones (0.825 mg/100 g vs. 0.270 mg/100 g, respectively). These changes were triggered by the use of oil and the temperature-driven chemical changes inherent to the frying process. Having investigated the organoleptic and nutritional aspects of frying anchovy meat, we propose the results of this investigation as a guide for dietary decisions and as a reference for further investigation on the subject.

**Keywords:** anchovy (*Stolephorus* sp.); chemical analysis; fried fish; histology; nutrition; food processing.

## 1. Introduction

Anchovy (*Stolephorus* sp.) is an abundant fish in various Indonesian waters and a locally high valued food. Anchovy's high gastronomic value, relies on its small size, broad availability, relatively convenient price, and its straightforward preparation. Furthermore, studies have determined that a single anchovy fish is an excellent calcium source, which is found in the bones of the fish that are eaten along with the flesh, for people with osteoporosis.[1]

Overall fish production in Indonesian seas increased sharply from 2020 (21,834,105.35 tons) to 2021 (21,872,810.30), this growth was driven by an increase in anchovy catches passing from 234,562.05 tons in 2020 to 259,883.80 tons in 2021 [2]. Anchovies are abundant in all Indonesian territorial waters, including Southeast Sulawesi, West Sumatra, the Madura Strait, and the Tomini Bay area. Anchovies are gregarious fish, living in schools of up to thousand individuals; occurring seasonally, from February to August. The highest catches usually occur in July and August [3].

In Indonesia anchovies are preferably eaten fried. Food frying is one of the oldest cooking methods known to mankind. Fried foods entail unique sensory properties, which increase their appeal to consumers; however, constant frying oil reuse and the generally reported high frying temperatures (170-200 °C) [4] likely affect food's nutritional value. The effect of frying has been investigated

in various fish meats, including skipjack (*Katsuwonus pelamis*) [5], rice field eel (*Monopterus albus*) [6], fish fin [7, 8, 9] frying sardines (*Sardina pilchardus*) [10], seabass (*Dicentrarchus labrax*) [11], red snapper (*Lutjanus campechanus*) [12], and freshwater fish fry [13]. Regarding anchovy meat, information about its chemical composition, fatty acid and cholesterol content, and tissue structure remain uninvestigated. The present study aims to determine the optimal frying temperature and time for anchovy meat, as revealed by the change in proximate characteristics, fatty acids, cholesterol, and tissue structure between fresh and fried anchovy meat. Anchovy meat assessments were made at frying conditions of 160 °C and 180 °C for 5, 10, 15, and 20 minutes.

## 2. Materials and methods

### 2.1. Materials and tools

Anchovy meat constituted the investigation subject in this study. The employed anchovies in this study were on average  $8.29 \pm 0.47$  cm long and  $1.94 \pm 0.46$  high, and weighed  $0.25 \pm 0.02$  g. The fish specimens were obtained from Pelabuhan Ratu by ordering one day in advance, and delivered using a closed tub car, which contains anchovies that have been packed in a cool box filled with ice to maintain freshness and commercial palm oil.

The materials used for fish meat proximate analysis include distilled water, 0.1 N HCl, 40 % NaOH, selenium catalyst,  $\text{H}_2\text{SO}_4$ , 2 %  $\text{H}_3\text{BO}_3$ , filter paper, fat-free cotton, hexane solvent, 0.1 % bromocresol green, and methyl red 0, 1 %. The materials used for fish fatty acid analysis were 0.5 N NaOH in methanol,  $\text{BF}_3$ , saturated NaCl, n-hexane, and  $\text{Na}_2\text{SO}_4$ . The materials used for fish cholesterol analysis are ethanol, petroleum benzene, alcohol, acetic anhydride, and concentrated  $\text{H}_2\text{SO}_4$  (Merck). The materials used for meat tissue analysis were BNF solution, alcohol, xylol, hematoxylin and eosin dyes, and distilled water. For morphometric assessments a caliper and an analytical balance were used.

Anchovies were fried in a deep fryer FRY-EZL1 (Roller and Grill) and spinner. The equipment employed for fish meat proximate analysis included an analytical balance, tweezers, porcelain dishes, a stove, a bulb, pipettes, test tubes, Erlenmeyers, Kjeldahl tubes, Soxhlet tubes, fat flask, a oven, a desiccator, a furnace, and a burette (Iwaki Pyrex, USA). Fatty acid analysis required a gas chromatograph Shimadzu GC 2010 Plus with standard Supelco<sup>TM</sup> 37 Component FAME Mix, homogenizer, evaporator, erlenmeyer (for fatty acid extraction), a separating funnel and vials (for methylation). Cholesterol analysis required a UV-200-RS Single Beam spectrophotometer, test tubes, vortex, pipettes, a centrifuge, and an evaporator. Meat tissue was inspected under an Olympus CX41 microscope, involving an Olympus DP21 camera, stream start software, sample storage glass, printing table, printed carton, oven, Yamato RV-240 microtome, a heating table, object glasses, and a coloring rack.

### 2.2. Research methods

Around 150 g of fish were fried, placing the specimens into a deep-frying pan containing 4 L of commercial palm oil heated at 160 °C or 180 °C. Experimental frying times were 5, 10, 15, and 20 minutes. Subsequently the fried fish were cooled at room temperature and then organoleptic tests were carried out with 30 panelists. Each panelist assessed twice anchovy meat fried under each of the combined frying temperature and time conditions. Before and after the frying, anchovies were weighed to determine changes in meat mass. Samples of fresh fish and fried fish were mashed,

respectively. The crushed anchovy samples were put into aluminum foil and then put in plastic, tightly closed, and coded. Fresh and fried anchovy samples are ready for analysis, which include organoleptic, proximate, fatty acid, cholesterol, and meat tissue structure.

### **2.2.1. Organoleptic test**

Organoleptic tests were conducted following SNI instructions for the quality assessment of fishery products (SNI 2346-2015) [14]. The organoleptic test carried out in this study included several parameters, namely appearance, color, texture, aroma, and taste. The organoleptic test in this study involved 30 semi-trained panelists who were randomly selected. Parameter were graded following the hedonic test score sheet SNI-01-2346-2011 with values of 1-9, where 1 = dislike extremely, 2 = very dislike, 3 = dislike, 4 = dislike slightly, 5 = neutral, 6 = somewhat like, 7 = like, 8 = like very much, 9 = like extremely. The samples tested were anchovies fried at temperatures of 160 °C and 180 °C for 5, 10, 15, and 20 minutes.

### **2.2.2. Anchovy meat proximate analysis**

An anchovy meat proximate analysis was conducted to determine its chemical composition, including moisture, ash, protein, fat, and carbohydrate content assessments using the by-difference method [14].

### **2.2.3. Cholesterol content**

Fresh and fried anchovy samples of 0.1 g were placed into centrifuge tubes, with 8 mL of ethanol and petroleum benzene solution in a 3:1 ratio, then stirred until homogeneous. Samples were rinsed with 2 mL of ethanol: petroleum benzene (3:1) solution and then centrifuged at 3 000 rpm for 10 minutes. The supernatant was poured into a 100 mL beaker glass and evaporated in a water bath. The residue was evaporated with chloroform (bit by bit) while being poured into a graduated tube (up to a volume of 5 mL). A volume of 2 mL of acetic anhydride and 0.2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> were then added to the sample. The material was then homogenized with a vortex and left in a dark place for 15 minutes. The absorbance was then read on a spectrophotometer with a wavelength ( $\lambda$ ) of 420 nm. [15]

### **2.2.4. Fatty acid profiling**

The fatty acid analysis of fresh and fried anchovies followed the principle of converting fat into its derivatives, namely in the form of methyl esters so that they can be detected through gas chromatography (GC), whose working principle is the separation between gas and thin films of liquid based on differences in material type and its temperature [15]. Fatty acids, in the form of oil, were extracted from anchovy samples, weighing between 7 g and 10 g, using a non-polar solvent (petroleum ether) according to the Soxhlet method. The methylation step, to form derivative compounds from fat into methyl esters, was performed by placing of 0.02 g of the extracted oils with 0.5 N NaOH-methanol, boron trifluoride (BF<sub>3</sub>), and isooctane in a water bath for 20 minutes at 80 °C and then cooling at room temperature. Next, a total of 2 mL of BF<sub>3</sub> were added to the samples and then reheated in a water bath at 80 °C, for 20 minutes, and then cooled at room temperature. A total of 2 mL of saturated NaCl and 1 mL of isooctane were then added and shaken. The isooctane solution in the upper phase of the mixture was transferred with the help of a dropper into a 2 mL glass vial containing Na<sub>2</sub>SO<sub>4</sub>. A total of 1 mL of the sample was injected into the gas chromatograph column. The fatty acids in the methyl ester were identified using

a flame ionization detector (FID) or a flame ionization detector and the response was recorded and visualized as a chromatogram (peak). Identification of fatty acids is done by matching the retention time of the sample with the retention time of a known standard.

#### 2.2.5. Fish tissue structure

The histological preparations of fresh and fried anchovies involved three major steps, namely tissue fixation with BNF, dehydration with one alcohol series, and tissue purification with Xylol solution. For the histologic preparations, anchovy tissue was fixed in a 10 % formalin (BNF) solution, then sliced and arranged onto tissue cassettes, dehydrated, dried using a vacuum machine, and blocked with liquid paraffin. The blocks were cut every 3-5 mm with a microtome and the pieces were attached to a slide. Subsequently, the slides were stained manually with hematoxylin and eosin. The staining provided a clear balance of blue and red colors in the tissue, so that cell components can be clearly identified. Data were obtained through qualitative descriptions of changes in histologic preparations under a light microscope. [16]

### 2.3. Data Analysis

Panelists' preference scores for fresh and fried anchovies were analyzed via a nonparametric test with a hedonic scale using the Kruskal Wallis test.

## 3. Results and Discussion

### 3.1. Anchovy (*Stolephorus* sp.) morphometrics

The anchovy specimens in this study were, on average,  $8.29 \pm 0.47$  cm long and  $1.94 \pm 0.46$  in height, weighing  $0.25 \pm 0.02$  g. According to [17], anchovies have an elongated (fusiform) or slightly flat (compressed) body shape, as was observed in this study. We also noted a distinctive silvery-white sash extending from head to tail with small, thin scales that are easy to remove, and the maxillary bones extended to reach the gill slits [18]. The specimens used in this study were of expected size; according to a previous study [19], the first anchovies collected from fisherman's catches around Pelabuhan Ratu Bay had a modal length of 5.89 cm, in the second catch the average length was 5.13 cm, in the third sampling length was 5.65 cm, and in the fourth sampling was 4.34 cm and 7.84 cm. Anchovy body size variation results from intrinsic sea-water environmental factors, including resource availability, competition, predation, fishing, etc.

### 3.2. Organoleptic assessment

#### 3.2.1. Appearance

Fish meat appearance is the main parameter defining consumer's acceptance. Kruskal-Wallis test results on meat organoleptic variables showed that the different tested frying temperatures and times did not significantly affect (at a 95 % confidence level) the visual appeal of fried anchovies among panelists. Overall, panelists' appearance assessment parameters ranged from 6.53 (slightly like) to 7.43 (like); however, frying changed the appearance of the anchovy to a brown color [20]. We were able to determine that frying at a temperature of 180 °C for 6 minutes produces a yellowish appearance and shrinks the anchovy's meat.

### 3.2.2. Color

Color was one of tested organoleptic variables on anchovy meat. The results of the Kruskal Wallis test revealed (at a 95 % confidence level) that none of the tested frying temperatures and times significantly affected panel preference for fried anchovy color. Panelists' assessment of color parameters ranged from 6.83 (slightly like) to 7.53 (like). Another study conducted by [21] showed that frying time (1 to 3 minutes) resulted in significant catfish meat color differences and preferences. We observed that frying changes anchovy meat color to yellowish brown. The surface or outer layer of fried foods change color to brown due to the Maillard reaction [22], and the intensity of this color depends on frying length and temperature, as well as on the chemical composition of the food's outer surface.

### 3.2.3. Odor

Regarding fried anchovy aroma, the Kruskal Wallis test results, at a 95 % confidence level, indicated that the different tested frying temperatures and times did not significantly affect panelists' preferences for fried anchovy aroma. Aroma scores ranged from 6.60 (slightly like) to 7.37 (liked) among panelists. Fat and protein content determine cooked fish meat quality, because they trigger distinctive aroma and taste profiles [23], namely fried anchovies have a specific fried fish aroma. Moreover, the amount of volatile components released by a product is influenced by temperature and natural components [24].

### 3.2.4. Texture

Food texture is a key variable within organoleptic tests, since it explains a large fraction of food acceptance in tasting panels. The Kruskal Wallis test, with a 95 % confidence level, showed that frying temperatures and times had a significantly different effect on fried anchovy preference levels among panelists. Fried anchovies achieve variable textures; the higher the frying temperature and the longer the frying time, the crunchier the meat texture. Zzaman *et al.* [25] determined that at lower water content in hamburger meat, the heat generated from frying will be able to evaporate a larger amount of water to produce a crisper and crunchier texture. Furthermore, the results of our study are in accordance with other observations on fried fish meat texture [26], namely frying time exerts a significant difference in the texture of the material being processed, foods that are fried at a temperature of 190 °C for 1.5 minutes reach the highest level of hardness (texture) when compared to foods that are fried at the same temperature (190 °C) for less time (0.5 minutes). Panelists' assessment of texture parameters ranged from 3.50 (dislike) to 7.03 (like).

### 3.2.5. Taste

Taste is one of the most important assessment parameters for organoleptic tests in determining food preference levels of tasting panelists. The taste parameter influenced the acceptance of anchovy meat. The results of the Kruskal Wallis test, at a 95 % confidence level, showed that different frying temperatures and times had significant effects on the panelists' taste preferences of fried anchovies. Panel scores on taste varied broadly from 3.63 (dislike) to 7.30 (like). Fried anchovies have a savory taste which is thought to come from their fat content. Frying temperature and time affects food composition, the higher the temperature and the longer time of the cooking procedure, the higher the average number of water molecules that undergoes evaporation, thus concentrating flavor components (*e.g.* fat) [18]. Moreover, research conducted by [26] on shredded snakehead fish showed that the use of oil results in a savory taste and added calories.

In the present study, the organoleptic aspects of appearance, color, and aroma of frying temperature and time did not significantly affect tasting panel preference for anchovies, but the same frying protocol led to texture and taste outcomes with significantly different preferences. Fried anchovies achieve different textures; crunchier meat textures are caused by higher and longer frying temperatures and times. A study on the organoleptic effects of different cooking methods on freshwater fish [27] revealed that frying with oil or fat media at high temperatures can cause the water contained in foodstuffs to evaporate, resulting in weight loss and also lead to reactions among food components that build textures. Furthermore, our results agree with the statement of [17] that frying time leads to a significant difference in the texture of the material being processed; certain foodstuffs that are fried at a temperature of 190 °C for 1.5 minutes have the highest level of hardness (texture) when compared to foods that are fried at a temperature of 190 °C for 0.5 minutes.

Texture and taste were the main factors in this study defining panel preference for fried anchovies. Optimal frying temperature and length were obtained from the treatments with the highest organoleptic scores, namely 180 °C for 15 minutes. According to [28], for baking and frying, temperatures between 180 °C and 190 °C are recommended. Subsequent analyses were conducted with anchovies fried under the best performing organoleptic conditions.

### 3.3. Chemical composition analyses

The chemical composition of foodstuffs can be investigated by a proximate analysis. The chemical composition variables analyzed for anchovies included water content, ash, protein, and fat. The results of the analysis of the chemical composition of anchovy are shown in **Table 1**.

**Table 1.** Chemical composition anchovy

Proximate	Share (mg/100g) in fresh anchovy	Share (mg/100g) in fried anchovy	Share as reported by Palani <i>et al.</i> [29]
Moisture (BB)	78.68 ± 0.26 <sup>a</sup>	7.71 ± 0.07 <sup>a</sup>	80
Ash (BK)	13.14 ± 0.11 <sup>a</sup>	6.82 ± 0.14 <sup>a</sup>	16.86
Fat (BK)	3.76 ± 0.07 <sup>a</sup>	41.06 ± 0.66 <sup>a</sup>	3.03
Protein (BK)	76.76 ± 0.50 <sup>a</sup>	52.45 ± 0.05 <sup>a</sup>	74.08
Carbohydrate (BK)	6.34 ± 0.06	0	6.13

Notes: (BK): dry basis; (BB) wet basis; <sup>a</sup> Significant pairwise differences in proximate variable values between fresh and fried anchovies. Anchovies were fried at 180 °C for 15 minutes.

Table 1 reveals the chemical composition differences of fresh and fried anchovies, which were also statistically tested. Moreover, aligned with [29], fresh anchovies entailed a higher protein content with lesser ash and fat than fried anchovies. T-test results revealed significant differences in water, protein, ash, and fat content between fresh and fried anchovies at 180 °C for 15 minutes. According to [28], the variable frying temperatures (150 °C, 170 °C, and 190 °C) have a significant effect on the moisture content of the processed material but not on fat, protein, and ash content.



### 3.4. Anchovy cholesterol content

Cholesterol is a chemical compound that the body needs to build cell membranes and hormones such as estrogen and testosterone. Bloodstream cholesterol is regulated by the liver. Frying food increases its cholesterol levels. We observed that fried anchovies have increased almost three times their cholesterol levels, as shown in **Table 2**.

**Table 2.** Anchovy cholesterol content analysis results

Sample	Cholesterol content (mg/100g)
Fresh anchovy	$0.27 \pm 0.014$
Fried anchovy (at 180 °C for 15 minutes)	$0.82 \pm 0.07$
Silver carp nuggets (Ojagh <i>et al.</i> [28])	50

We detected an average of 0.82 mg of cholesterol per 100 g of fried anchovy meat, whereas fresh anchovies had a lower cholesterol content. This is in accordance with results obtained by [29] in which the frying process increased the cholesterol content of fresh eel from 30.15 mg/100 g to 170.44 mg/100 g. Also, Nurjanah and colleagues, [5] observed that the cholesterol content of skipjack tuna increased from 49.12 mg/100 g to 173.92 mg/100 g due to frying. It is well known that such a foodstuff cooking method leads to cholesterol increases [30], particularly in anchovies. [13] stated that changes in food's cholesterol contents are driven by the type of oil used, the intensity of frying, and the length of the frying process. This is reinforced by the statement of [31] that due to its fatty acid content, coconut oil can increase cholesterol in fried foods when used to fry. The content of saturated fatty acids in coconut oil is 59.24 %.

### 3.5. Anchovy fatty acid profile

The anchovy fatty acid content analysis (see **Table 3**) revealed an enrichment of palmitic acid ( $17.96 \% \pm 0.622 \%$ ) in the fresh fish sample, whereas palmitic and oleic acid levels were highest in the fried fish samples. The increase in fatty acids is relevant to the study of [31] where linoleic, oleic, and palmitic acid shares in cooking oil of 9.23 %, 30.91 %, and 42.60 %, respectively, leading to an increase in these fatty acids after frying. The increase in palmitic acid content after frying can be likely due to the integration of palmitic acid from the frying oil and the decrease in water content.

The results of the comparison of fatty acids in **Table 4** show that the ratio of omega-6 / omega-3 in fried anchovies (at 180 °C for 15 minutes) is 31.03. [32] stated that palm oil has a high content of linoleic acid, causing a large omega-6 / omega-3 ratio. This is reinforced by the statement of [33] that the difference in the omega-6 / omega-3 ratio in fried fish samples can be driven by the fatty acid composition in the fresh ingredients and the type of oil used. Fried anchovy is thought to be harmful for health if consumed in excess; an omega-6 / omega-3 ratio above 2.07 [5] can trigger cardiovascular disease [33]. The results of the comparative analysis of fatty acids can be seen in Table 4.

Total omega-3 fatty acids in fresh fish were higher than omega-6 fatty acids, but ratios reversed after frying. According to [34], the omega-6 to omega-3 ratios in eight seawater fish species (*Epinephelus aeneus*, *Trigla lucerna*, *Merlangius merlangus*, *Scomber scombrus*, *Pomatomus saltator*, *Sparus auratus*, *Dicentrarchus labrax*, and *Siganus rivulatus*) and in six freshwater fish species (*Clarias gariepinus*, *Cyprinus carpio*, *Siluris glanis*, *Tina tinca*, *Rutilus frisii*, *Sander lucioperca*) of commercial importance are no roughly 1:1.

**Table 3.** Results of fatty acid analysis in fresh and fried anchovies

Fatty acids	Share (mg/100 g) in fresh anchovy	Share (mg/100 g) in fried* anchovy	Share (mg/100 g) in palm oil
<b>Saturated fatty acids</b>			
Laurat (C12:0)	0.12±0.07	0.13±0	-
Tridekanoat (C13:0)	0.04±0	0.00±0.00	-
Miristat (C14:0)	2.21±2.41	0.75±0.02	0.88
Pentadekanoat (C15:0)	0.63±0.03	0.03±0.07	-
Palmitat (C16:0)	17.96±0.62	31.1±0	42.6
Heptadekanoat (C17:0)	0.84±0.04	0.08±0.07	-
Stearat (C18:0)	5.31±0.33	2.87±0.01	8.13
Arakidat (C20:0)	0.35±0.01	0.23±0.07	0.27
Behenat (C22:0)	0.24±0.07	0.05±0	-
Lignoserat (C24:0)	0.97±0.77	0.06±0	-
<b>Total SAFA</b>	<b>28.69±0.42</b>	<b>35.32±0.07</b>	-
<b>Monounsaturated fatty acids</b>			
Palmitoleat (C16:1)	2.90±0.17	0.16±0.07	-
Oleat (C18:1n9c)	8.82±0.41	38.1±0.25	30.91
Cis-11-Eikosenoat (C20:1)	0.26±0	0.12±0.00	0.35
<b>Total MUFA</b>	<b>11.9±0.19</b>	<b>38.38±0.08</b>	-
<b>Polyunsaturated fatty acids</b>			
γ-Linolenat (C18:3n6)	0.07±0	0.00±0.00	-
Cis-11,14-Eikosadinoat (C20:2)	0.15±0.07	0.04±0.00	0.26
Linoleat (C18:2n6c)	1.54±0.10	9.13±0.04	9.23
Arakidonat (C20:4n6)	1.50±0.02	0.02±0.07	-
EPA (C20:5n3)	3.29±0.07	0.06±0.00	-
DHA (C22:6n3)	11.41±0.03	0.23±0.02	-
<b>Total PUFA</b>	<b>17.98±0.18</b>	<b>9.49±0.01</b>	-
<b>Total</b>	<b>59.14±0.94</b>	<b>83.21±0.24</b>	-
<b>Not detection</b>	<b>40.86±0.94</b>	<b>16.78±0.24</b>	-

**Table 4.** Fatty acid groups and ratios of anchovy meat

Fatty acid group	Fresh anchovy (mg/100 g)	Fried anchovy (mg/100 g)*	Fried cakalang (mg/100 g)**
SAFA	28.69	35.32	36.55
MUFA	11.9	38.38	34.22
PUFA	17.98	9.49	12.81
EPA	3.29	0.06	0.52
DHA	11.41	0.23	3.44
PUFA/SAFA	0.62	0.26	0.35
Σomega-3	14.71	0.29	4.15
Σomega-6	3.12	9.15	8.59
omega-6/omega-3	0.21	31.03	2.07



\*Anchovies were fried at 180 °C for 15 minutes; \*\*Nurjanah *et al.* [5];

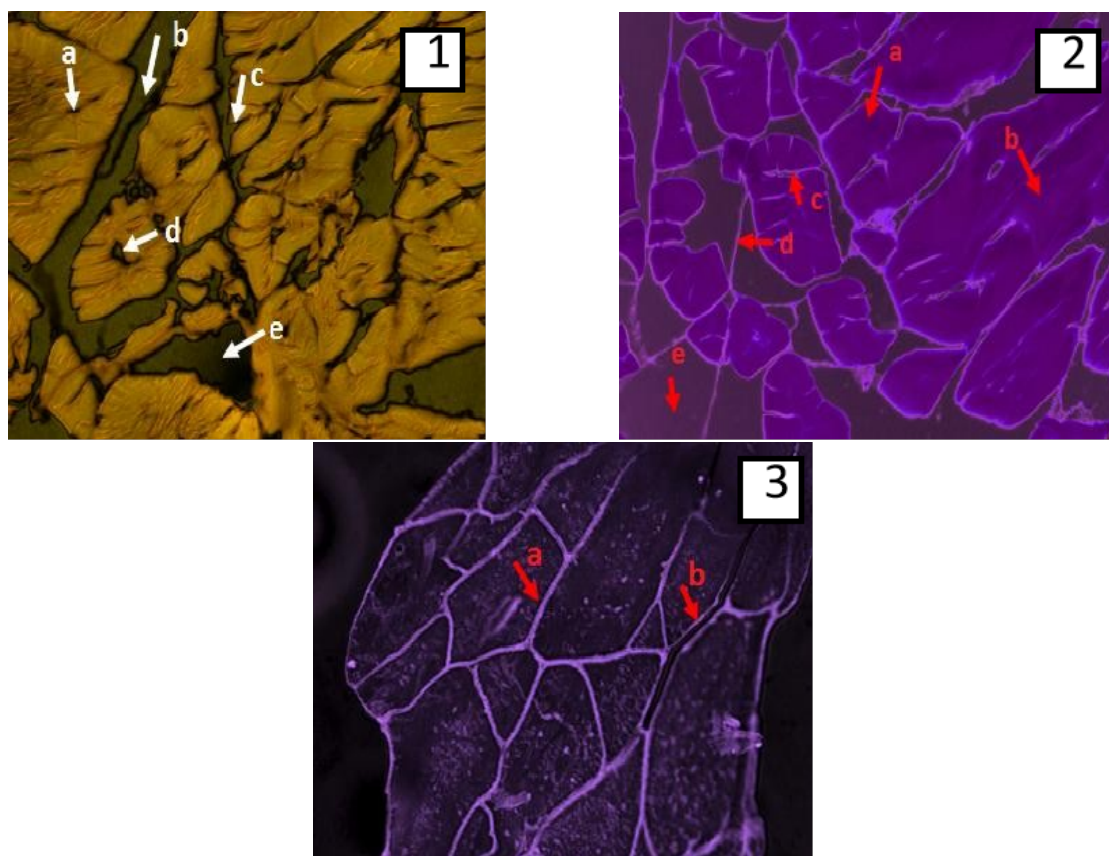
Table 4 shows an increase in the omega-6:omega-3 ratio after frying, from 0.21 to 31.03; the recommendation from [35] regarding the ratio of omega-6:omega-3 for consumption is 0.6:1.7. Our results showed that the ratio obtained exceeded the recommended ratio, and that only fresh anchovies were in accordance with the predetermined limits. According to [36] the amount and ratio of omega-6 and omega-3 fatty acids are central to determine the amount of consumption needed so as not to have a negative impact on health. Similarly, EPA and DHA contents decreased due to the frying process. [25] stated that the decrease in EPA and DHA was due to the sensitive nature of EPA and DHA to light, temperature, and oxygen. This is reinforced by the statement by [36] that the frying effect on seabass (*Dicentrarchus labrax*) meat, can reduce its relative EPA 3.6 levels by 30 % and the relative DHA levels by 28 %. Finally, [37] also stated that meat DHA content decreases after heat treatment.

### 3.6. Anchovy muscle tissue analysis

Tissue preparations and observations were done to understand the structural differences between fresh and fried anchovy meat. The structure of fresh anchovy meat fried at 180 °C for 15 minutes can be seen in **Fig. 1**. Anchovy meat structure changed due to frying. The process of fixation and dehydration shrunk the myomere, resulting in a wide gap between the myoseptum and myomere. Treatments lead to myomere damage, as revealed by cracking and a porous structure. Myomere cracking can start from the edge of the myomere or from within it. Fresh myomere cracks tend to form parallel windings. Although myomere space is often found in fresh anchovy meat, the dissolved material of the myomere is not found in that space. The spaces between the myomeres are likely due to the dehydration, as part of the fixation and sample preparation process [38]. Stated that dehydration causes the ability to bind water by myofibril proteins to be reduced.

Frying shrunk anchovy meat, so that the myoseptum disappeared, enlarging the space between myomeres. Hassaballa *et al.* [39] observed that the heating process causes myofibril protein denaturation and shrinkage, forcing the meat to release its liquids, and Ayala *et al.* [40] heat-driven fish meat cooking alter its structure because of protein coagulation and shrinking during the cooking process. We observed that (i) frying at 180 °C for 15 minutes compacted myomeres without leaving visible cracks within them, (ii) myomeres tended to touch each other, blurring the borders between them, and (iii) myomere boundaries tended to be brittle, so each myomere was easily separated. Frying also led to water loss, thus fried anchovy meat became more compact than fresh anchovy meat.

Anchovies are rich in essential amino acids, such as leucine, valine, and threonine, and contain substantial shares of non-essential amino acids, such as glutamic acid, aspartic acid, and glycine [41]. The overall amino acid profile of meat influences its taste characteristics [42]. The amino acid content in fresh anchovies increases after being processed into fried or crispy [43]. This enhancement is due to added ingredients and Maillard (MRPs) reactions involved in crispy anchovy production [44]. Heat causes protein degradation leading to a decreased water holding capacity within myofibers [45].



**Figure 1.** Fresh and fried anchovy meat structures under 40 x10 magnification. (1) Fresh anchovy meat slide 1. a=cracked myomere , b=remaining myoseptum, c=myomere cracked from its edge, d=porous myomere, e=inter myomere. (2) Fresh anchovy meat slide 2. a=intact transversal myomere, b=intact longitudinal myomere, c=fractured transversal myomere, d=myoseptum, e=interstitial space filled with material. (3) Fried anchovy meat slide; fried at 180 °C for 15 minutes: a=intact myoseptum, b=disintegrated myoseptum.

#### 4. conclusions

From the organoleptic assessment perspective, in this study, we determined that the best temperature and time for frying anchovies is 180 °C for 15 minutes. A higher frying temperature negatively affected the texture and taste of anchovy meat, decreasing its palatability and acceptability. Likewise, a high frying temperature reduces anchovy meat water share and increases fat content. Frying alters anchovy meat's omega-6:omega-3 ratio, raising its cholesterol levels. Structurally, a frying temperature of 180 °C causes the myomere to shrink and compact, and the remaining myoseptum becomes brittle.

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## 6. Conflict of interest

The authors declare no personal and/or financial conflict of interests.

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### Comparación de la composición organoléptica y química, así como de la histología de las anchoas indonesias (*Stolephorus* sp.) frescas y fritas.

**Resumen:** La anchoa frita (*Stolephorus* sp.) es un alimento popular en Indonesia debido a su buen sabor, amplia disponibilidad y asequibilidad. Sin embargo, los aspectos nutricionales reales de este plato local no han sido investigados. Este estudio tuvo como objetivo determinar la mejor temperatura y tiempo para freír anchoas, así como comparar la histología y los perfiles de ácidos grasos y colesterol de la carne de anchoa fresca y frita. Las anchoas se frieron a 160 °C o 180 °C durante 5, 10, 15 y 20 minutos. Determinamos como mejor condición de fritura aquella que obtuvo la puntuación más alta en las pruebas organolépticas realizadas por un panel de degustación: 180 °C durante 15 minutos. Histológicamente, la carne de anchoa frita se encogió, volviéndose compacta y quebradiza, lo cual se explica por los cambios observados en los miomeros. El perfil de ácidos grasos de las anchoas frescas versus fritas reveló una marcada inversión en las proporciones omega-6, y omega-3. Las anchoas fritas contenían más colesterol que las frescas (0.825 mg/100 g frente a 0.270 mg/100 g, respectivamente). Estos cambios fueron provocados por el uso de aceite y los cambios químicos inducidos por la temperatura inherentes al proceso de fritura. Tras investigar los aspectos organolépticos y nutricionales asociados a la fritura de la carne de anchoa, proponemos que los resultados de esta investigación sirvan como guía para decisiones dietéticas y como referencia para futuras investigaciones sobre el tema.

**Palabras Clave:** Análisis químico; Anchoa (*Stolephorus* sp.); Histología; Nutrición; Procesamiento de alimentos; Pescado frito.

### **Comparação da composição organoléptica e química, assim como da histologia das anchovas indonésias (*Stolephorus* sp.) frescas e fritas.**

**Resumo:** A anchova frita (*Stolephorus* sp.) é um alimento popular na Indonésia devido ao seu bom sabor, ampla disponibilidade e acessibilidade. No entanto, os aspectos nutricionais reais deste prato local não foram investigados. Este estudo teve como objetivo determinar a melhor temperatura e tempo para fritar anchovas, assim como comparar a histologia e os perfis de ácidos graxos e colesterol da carne de anchova fresca e frita. As anchovas foram fritas a 160 °C ou 180 °C durante 5, 10, 15 e 20 minutos. Determinamos como melhor condição de fritura aquela que obteve a pontuação mais alta nos testes organolépticos realizados por um painel de degustação: 180 °C durante 15 minutos. Histologicamente, a carne de anchova frita encolheu, tornando-se compacta e quebradiça, o que é explicado pelas mudanças observadas nos miómeros. O perfil de ácidos graxos das anchovas frescas versus fritas revelou uma inversão acentuada nas proporções de ômega-6 e ômega-3. As anchovas fritas continham mais colesterol do que as frescas (0,825 mg/100 g em comparação com 0,270 mg/100 g, respectivamente). Essas mudanças foram desencadeadas pelo uso de óleo e pelas alterações químicas induzidas pela temperatura inerentes ao processo de fritura. Após investigar os aspectos organolépticos e nutricionais associados à fritura da carne de anchova, sugerimos que os resultados desta investigação sirvam como um guia para decisões alimentares e como referência para futuras pesquisas sobre o tema.

**Palavras-chave:** Análise química; Anchova (*Stolephorus* sp.); Histologia; Nutrição; Peixe frito; Processamento de alimentos.

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