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Comparison of Macro Nutritional Value, Dissolved Protein, Amino Acids and Minerals of Fresh and Crispy-Product of Anchovy *(Stolephorus Commersonnii)*

Fronthea Swastawati[1*], Putut Har Riyadi[1], Hersanti Sulistyaningrum[2], Sepsina Resky[2], Slamet Suharto[1]

[1]Department of Fish Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang 50275, Central Java, Indonesia
[2]Department of Nutrition Master of Science, Faculty of Medicine, Diponegoro University, Semarang 50275, Central Java, Indonesia

***Correspondence:** Fronthea Swastawati
Email: fronthea_thp@yahoo.co.id; fronthea.swastawati@live.undip.ac.id

ABSTRACT
This study aimed to determine the acceptability and the comparison of the nutritional composition of crispy and fresh anchovies. Organoleptic test and acceptability of fresh and crispy anchovies were done according to Indonesian National Standards; macronutrient analysis was done by proximate test; dissolved protein level was analyzed by lowry method; amino acids level were analyzed by HPLC and mineral content was analyzed by XRF (X-Ray Fluorescence) Spectrometer WDXRF Rigaku Supermini 200. This study showed that the taste of crispy anchovies is favoured, and the nutritional value of crispy anchovies was higher than fresh anchovies. The crispy anchovies were rich in fat while fresh anchovies rich in dissolved protein. The most elevated amino acid contained in crispy and fresh anchovies was glutamic. The calcium content of fresh anchovies was higher than crispy anchovies. This study revealed that the consumption of crispy anchovies could meet the daily needs of minerals as long as according to the presentation recommendations.

Keywords: Nutritional value, crispy-product, anchovy, *Stolephorus commersonnii.*

Correspondence:
Fronthea Swastawati
Department of Fish Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang 50275, Central Java, Indonesia
Telp: +62 856-2681-613; Fax: (024) 7460017
Email: fronthea_thp@yahoo.co.id; fronthea.swastawati@live.undip.ac.id

INTRODUCTION
Anchovy is the highest marine fishery commodity of Central Java in 2017, which is Rp 142.528.825.[1] It is easy to be obtained in the market and has an affordable price. Anchovy has a potential nutritional value for human health[2] and all parts of the body, including the bone, can be consumed. This fish can be processed into various dishes such as *pepes* (grill in banana leaves), *rempeyek* (deep-fried cracker), and crispy (fried dry). The crispy anchovy is processed by frying the fresh anchovy after adding by floor.[3] The fried process of anchovies crispy can improve the taste, texture, and eliminate microbes so it can be stored longer.[4] Quality assessment of fresh anchovies that used for crispy production was done through organoleptic tests based on INS (Indonesian National Standards). The use of raw material of fresh anchovy influence the quality of the crispy product. Acceptability of crispy products was done through INS hedonic test, such as appearance, taste, odor and texture test.[5,6] The nutritional content of fresh anchovy is presented in Tabel Komposisi Pangan Indonesia while anchovies crispy products are unknown, so it needs to be tested. The test result obtained in the form of information of anchovies crispy nutritional value such as energy, fat, carbohydrate, protein, dissolved proteins, amino acids, and minerals. This information can be used to determine the comparison of fresh and crispy anchovies nutritional value. This study aimed to determine the acceptability and the comparison of the nutrient composition of crispy and fresh anchovies.

MATERIALS AND METHODS
Organoleptic test
Anchovy fish (*Stolephorus commersonii*) was obtained from Jepara Fish Market/Fishing Port. Anchovy was observed by standard panellists who have high ability and sensitivity in evaluating organoleptic of product quality. The test of quality fresh anchovies relied on human sense. Characteristics were tested, including eyes and gills appearance, body surface mucus, odor, and texture. Panellists stayed in a calm place, free from things that can interfere with the objectivity of the assessment, with uniform lighting in all directions so that it did not affect the appearance of fresh anchovy.[5] The test was done at 09.00-10.00 am when the panellists were not starving or full. The ten panellists were lecturers of the Nutrition Department of Medical Faculty of Diponegoro University. Each panellist observed 5 fresh anchovies that were placed in a container on the organoleptic test table. The quality criteria of organoleptic assessment shown in Table 1.

Table 1: Quality criteria for organoleptic assessment

Score	Criteria
7.42-9.00	Very ideal
5.82-7.41	Ideal
4.22-5.81	Ideal enough
2.61-4.21	Less than Ideal
1.00-2.60	Not Ideal

Source: SNI.01-2723-2000

Hedonic test
The level of preference for crispy anchovies was tested through the assessment sheet of the hedonic test. Appearance, odor, taste, and texture were tested. Preference level specification varies, from dislike extremely, dislike very much, dislike moderately, dislike slightly, neither like or dislike, like slightly, like

moderately, like very much, like extremely range 1 to 9. The hedonic test was done based on the INS. Assessment in the form of number, which then analyzed statistically to be categorized.[5]

Proximate test

Crispy anchovies production was made by Small and Medium Enterprises (SME) guided by Diponegoro University, Jepara. The proximate test was done in the Laboratory of Food Technology Unika Soegijopranoto Semarang by using the AOAC method procedure for fat analysis, *micro-Kjeldahl* for protein analysis and by difference method to test carbohydrate.[7]

Dissolved protein test

Dissolved protein test of fresh and crispy anchovies was done in the Laboratory of Center for Food and Nutrition Studies Gajahmada University, Yogyakarta, by using Lowry method in duplicate terms. Lowry method is more sensitive and accurate compared with other methods, so the sample number that needs is smaller. Moreover, the time required for the Lowry method is shorter.[8]

Amino acids profile test

Profile amino acids test of fresh and crispy anchovies was done in the Laboratory of Saraswanti Bogor with HPLC. Amino acid standards used for calibration were from Thermo Scientific. Fluorescence detection was used to read a sample at 37°C, and a flow rate of 1.0 mL per minute. Each sample was injected as much 5µL.[9,10]

Mineral content test

The mineral contents were done in the integrated laboratory of Diponegoro University with Spectrometer XRF *(X-Ray Fluorescence)* WDXRF Rigaku Supermini 200. The advantage of this method is a smaller amount of sample that need (1 gram), high accuracy and precision and can determine all mineral content directly.[11,12]

Data analysis

The data was analyzed by ANOVA test by using SPSS for Windows v.22. The experiment was done in triplicate.

RESULTS AND DISCUSSIONS

Organoleptic test

The organoleptic test was done according to the Indonesian National Standards (INS) Criteria. The score given was started from 1 to 9 based on INS with average worthiness 7 (Indonesian National Standards, 2010). Organoleptic tests relied on human sense, including a sense of sight, hear, taste, smell, and touch. Senses can know, distinguish, detect, compare, and express likes or dislikes.[13] Result of organoleptic test shown in Table 2.

Table 2: Organoleptic test result of fresh anchovies

Panellist Code	A	B	C	D	E	F	G	H	I	J	Total Score	Mean
Appearance	7.6	8	8.3	8	8	8.6	8	8.6	7.3	7.6	80	8.0
Odor	8	8	9	8	9	8	8	9	9	9	85	8.5
Texture	9	8	9	9	9	8	8	9	9	9	87	8.7

	N	Minimum	Maximum	Mean	Std. Deviation
Appearance	10	7.3	8.6	8.000	.4243
Odor	10	8.0	9.0	8.500	.5270
Texture	10	8.0	9.0	8.700	.4830
Valid N (listwise)	10				

Appearance test of fresh anchovies that used as raw material for crispy anchovies production has a higher average of INS that was 8. The average of odor test result of fresh anchovies was 8.5, and the texture was on average 8.7 (Table 2). Table 3 showed the raw materials of fresh anchovies had fulfilled INS eligibility standard that was an ideal category.

Table 3: Organoleptic analysis results of fresh anchovies according to INS criteria

	N	Minimum	Maximum	Mean	Std. Deviation
Appearance	10	4.00	5.00	4.9000	.31623
Odor	10	5.00	5.00	5.0000	.00000
Texture	10	5.00	5.00	5.0000	.00000
Valid N (listwise)	10				

Factors influence the quality of fresh anchovies, such as time and temperature storage. Fish that late to be handled and low-temperature storage can cause the reduction of fresh fish quality.[14] The use of quality raw materials affected the quality of the product. The high quality of the fresh fish makes high quality of the fish product.[15]

Hedonic test

The hedonic test was done according to preference criteria of INS. The score is given from 1 to 9 according to INS with average worthiness 7 (like). Score 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely (Indonesian National Standards, 2010).

Table 4: Hedonic test result of crispy anchovies

Panellist code	A	B	C	D	E	F	G	H	I	J	Total Score	Mean
Appearance	7	7	7	7	5	9	6	9	7	7	71	7.1
Odor	8	7	8	8	5	8	6	6	7	7	70	7.0
Taste	8	7	7	9	8	9	7	7	9	8	79	7.9
Texture	8	8	8	7	8	9	9	9	7	7	80	8.0

Table 5: Hedonic test result according to INS criteria

	N	Minimum	Maximum	Mean	Std. Deviation
Appearance	10	5.00	9.00	7.1000	1.19722
Odor	10	5.00	8.00	7.0000	1.05409
Taste	10	7.00	9.00	7.9000	.87560
Texture	10	7.00	9.00	8.0000	.81650
Valid N (listwise)	10				

Hedonic test results of crispy anchovies according to the appearance have an average of 7.1 that showed the panellists like the product's appearance. Crispy anchovies odor has an average on 7,0, taste 7.9, and texture 8.0 mean like very much. The highest average was about the texture of crispy, that was 8.0, and the lowest was 7.0 from the odor (Table 4).

Proximate test

This study compared the nutritional value of fresh anchovies and the fish that processed into a crispy product. Nutrient value information of 100gram fresh *Stolephorus commersonii* was obtained from the Indonesian Food Material Composition Table, while the information of the nutrient value of 100 gram crispy anchovies was obtained from the proximate test.[6] Nutritional value modification is shown in Table 6.

Table 6: Macronutrient content of 100 g fresh and crispy anchovies

No	Sample	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
1.	Fresh Anchovies	74	10.3	1.4	4.1
2.	Crispy Anchovies	508	24	28	40
Δ	Nutritional value modification	434	13.7	26.6	35.9

The highest nutrient modification value based on Table 6 was energy content 434 kcal, and the lowest was protein content 13.7 gram. The modification of nutrient content caused by the addition of the floor has a high carbohydrate because of the frying process used vegetable oil. The protein content of fish showed that fish are a good source of protein. The level of protein contained in fish influenced by consumption and digestibility of fish against their nutritional feed.[16] Fresh anchovies contained 10.3-gram protein and increased to 24 gram after processed into crispy. The fat content of fresh anchovies increased from 1.4 gram to 28 gram after processed. Fat content in fish is different because it was affected by some factors, including water temperature, food type, water salinity, fish age, and fish species.[17]

Dissolved protein test

The digestive system quickly absorbs dissolved protein. These characteristics influenced by some factors, such as source, processing condition, temperature, pH, ionic

strength, and the presence of other ingredients.[18-20] Dissolved protein in fish is the result of hydrolysis of proteins into peptides, dipeptides and free amino acids with a small molecular weight that easily dissolved in water through enzymatic process or microbes activity.[21] The level of dissolved protein in fresh or crispy anchovies was measured by the Lowry method using a spectrometer. The result is shown in Table 7.

Table 7: Dissolved protein per 100 g materials

Sample	Level (%)
Fresh Anchovies	2.140 ± 0.070
Crispy Anchovies	0.605 ± 0.007
Δ Modification	1.535

The dissolved protein analysis result showed fresh anchovies dissolved protein was higher (2.14%) than crispy anchovies (0.60%). There was a reduction of dissolved protein in crispy anchovies about 72% (1.535). It was due to the frying process with high temperature denatured dissolved protein of crispy anchovies. It has been known that the denaturation process will affect the solubility of protein.[22] Heating with temperature >85 ºC caused unfolded protein, so it has an impact on interior amino acid opening and increased the interaction of other proteins that reduced the solubility.[23]

Amino acid profile

The analysis of amino acid essential and nonessential was need to be done to know the composition of amino acids contained in fresh and crispy anchovies. The overall amino acid can affect the taste characteristics of the sample. The amino acid content in fresh anchovies increased after being processed into crispy. The enhancement was due to additional materials and Maillard (MRPs) reaction that occurred in the process of crispy anchovy's production. Maillard (MRPs) reaction also caused the modification of color to be brown and fluorescence intensity.[10] The heating process caused protein degradation that leads to decreased water holding capacity of the myofibrillar protein fraction. Enhancement of amino acid profile of crispy anchovies caused by water content reduction in the process of heating, it was lined with Biji *et al*.[24]

Table 8: The amino acid profile of 100g fresh and crispy anchovies

Amino Acid Type	Fresh Anchovies	Crispy Anchovies	Δ Modification
L Histidine*	2676.65 ± 3.39	7590.05 ± 32.95	4913.4
L Threonine*	6312.11 ± 21	13224.38 ± 15.88	6912.27
L Prolin	6628.93 ± 15.17	11188.83 ± 26.44	4559.9
L Tirosin	3454.88 ± 13.2	8753.47 ± 22.46	5298.59
L Leusin*	10311.25 ± 47	18888.55 ± 15.40	8577.3
L Aspartic Acid	12368.97 ± 58.15	22048.18 ± 57.18	9679.21
L Lisin*	11549.44 ± 54.03	17315.27 ± 24.70	5765.83
Glycine	12659.85 ± 41.21	16741.66 ± 49.86	4081.81
L Arginine*	8971.11 ± 35.98	17800.10 ± 19.66	8828.99
L Alanin	10914.87 ± 65.75	15528.28 ± 34.19	4613.41
L Valin*	6927.89 ± 22.97	14665.73 ± 18.27	7737.84
L Isoleusin*	5675.15 ± 17.05	10775.125± 7.60	5100.125
L Serin	5909.19 ± 27.76	14528.25 ± 24.45	8619.06
L Glutamic Acid	20881.05 ± 85.99	39608.53 ± 40.13	18727.48
L Fenilalanin*	5732.19 ±32.78	12322.455± 4.06	6590.265

Note: *Amino acid essential

Based on Table 8, the lowest amino acid of fresh anchovies was histidine (2,676.65) and then increased after processed into crispy (7,590.05). In contrast, the highest amino acid content of fresh anchovies was glutamic acid (20,881.05) and raised in crispy anchovies (39,608.53). The enhancement of amino acid content of glutamate in crispy anchovies was 18,727.48. It was in line with the research of Adu *et al*.[25]. Glutamic acid has an essential role in taste and odor modification,[10] also in the metabolism of glutathione molecule synthesis that removes peroxide and polyglutamate cofactor.[26] Modification of aspartic acid was quite high, that was 9,679.21. Aspartic acid plays an active role in the regulation of hormone secretion in the body, such as the precursor of glycine, cysteine, and tryptophan in the process of signalling. Aspartic acid amount in anchovies fish was including the highest compared to other fish.[26]

Minerals content

Anchovy is a type of small fish (Figure 1) that has high economic value and contain many nutrients than can be utilized by a human. A hundred grams of fresh anchovies contained 74 kcal energy, 10.3 gram protein, 1.4 grams fat,

4.1 gram carbohydrate, 972 mg calcium, 126.1 mg potassium and 3.9 mg iron.[6]

Figure 1: Anchovy fish *(Stolephorus commersonii)* morphology.[27]

The analysis of the mineral content of both fresh and crispy anchovies (*Stolephorus commersonii*) can be determined by XRF (*X-Ray Fluorescence*) spectrometer. XRF Spectrometer was chosen because it can describe the type and level of almost all mineral content in the fish.[12] XRF analysis showed the percentage (%) of elements contained in the anchovies. This percentage is a calculation of the overall peak area that appears on the chart pattern. The resulting data was the number of moles of each element which was then converted to mass per cent (multiplying the relative atomic mass of the component that appears). Minerals content percentage obtained from the distribution of mass per cent by sample weight multiplied by 100%. Anchovies sample that has been analyzed in the study was 310.8544 mg/cm^2. The result of the final analysis of type and mineral level was shown in Table 9.

Table 9: Mineral content analysis of anchovies

No.	Mineral	Level of minerals (%)	
		Fresh Anchovies	Crispy Anchovies
1.	P (Phosphor)	2.3	2.3
2.	S (Sulfur)	2.4	2.3
3.	Cl (Chloride)	3.9	8.5
4.	K (Potassium)	4.6	8.7
5.	Ca (Calcium)	20	9.6
6.	Zn (Zink)	0.56	0.2
7.	Br (Bromine)	-	0.12
8.	Fe (Iron)	0.24	0.22
9.	Si (Silicon)	1.75	-

The recommended amount per serving of crispy anchovies is 20 grams. Macrominerals contained in crispy anchovies were phosphor, sulfur, calcium, and potassium. Macrominerals are a type of mineral required in the body in the amount of > 100mg/day and used for the formation of various organ components.[28] The phosphor content of crispy anchovies was 2.3% and 460mg phosphor if 20mg crispy anchovies were consumed. So it able to meet the daily needs of phosphorus that is 250-400 mg/day.[28] The role of phosphor in the body is bone and teeth formation, regulate body base balance, helps absorption and transportation of nutrients through the process of phosphorylation.[29] Crispy anchovies content sulfur about 2.3%. It is required in the process of amino acid biosynthesis, cell, and tissue homeostasis maintenance. Research of Palego *et al.*[30] showed the importance of sulfur balance in patients with heart disease, diabetes (insulin formation) and Alzheimer's disease.

The highest mineral content in crispy anchovies was calcium (9.6%). Twenty-gram crispy anchovies contained 1,920 mg calcium. Calcium daily needs are 1,300 mg, and it is required for bone and tooth formation (99% in the form of calcium), regulate blood clotting, biological reaction catalysator, play a role in muscle contraction, nerve cell sensor signalling, thyroid gland maintenance, and prevent osteoporosis and rickettsia.[31,32] Vitamin D is known to increase the effectiveness of calcium absorption. The potassium level of crispy anchovies was 8.7% and equal to 1740 mg per 20 gram. Daily needs of potassium are about 2000 mg. Potassium has a vital role in strengthening the heart muscle, maintain the balance of electrolyte fluid and gastric acid-base. Less potassium consumption can cause weak, lethargic, decreased appetite and the ability of the heart to pump blood. Potassium absorption occurs in the small intestine and is excreted through faeces, sweat and stomach fluid.[28,32] Potassium intake can reduce blood pressure in hypertensive patients.[33] Crispy anchovies also contain microminerals such as chloride, bromine, zinc, and iron. Microminerals daily needs are < 100 mg/day. The amount of microminerals content in the body is less than 0.01% body weight.[28]

Zinc content in crispy anchovies was 0.2% and equal to 40 mg/20 gram of crispy anchovies. Daily needs of zinc are 133 µg/kg body weight. Zinc is very needed in the defence system mechanism and homeostasis process. The excretion of zinc occurs in the kidney, skin, and intestine. In malnourished children, a lot of zinc is lost through urine.[31,34] Children are a population that vulnerable to zinc deficiency, and it can cause infection easily, disruption of child development and delay in the maturity of sexual organs.[31] Consumption of 20 grams of crispy anchovies can meet the daily needs of zinc.

Iron is a type of micro minerals contained in crispy anchovies as much as 0.22% equal to 44 mg in 20-gram crispy. Daily needs of iron are 26 mg. Iron is required for energy metabolism, defence system mechanism, and nerve cell signalling. Iron deficiency can lead to anaemia, susceptible to infectious diseases and decreased individual productivity.[35] Iron absorption is more effective if it consumed together with Vitamin C, A, and protein. At the same time, the consumption of foods rich in phytate and fibre (> 35 mg/day) can inhibit iron absorption.[36]

CONCLUSION

In summary, the taste of crispy anchovies is favoured, and the nutritional value of crispy anchovies was higher than fresh anchovies. The crispy anchovies were rich in fat while fresh anchovies rich in dissolved protein. The most elevated amino acid contained in crispy and fresh anchovies was glutamic. The calcium content of fresh anchovies was higher than crispy anchovies. This study revealed that the consumption of crispy anchovies could meet the daily needs of minerals as long as according to the presentation recommendations.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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