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Determination of the best cooking time and the characteristics of Nile tilapia *pepes* (an Indonesian traditional fish product) processed by microwave oven

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Abstract— *The present study aimed to examine the best cooking time of Nile tilapia pepes processed by the microwave oven and to observe its chemical characteristics. The research was conducted in two stages. The treatment in the first stage was pepes cooking time which consists of four levels. The observed parameter was pepes sensory using multiple comparison tests. The experimental design to determine the chemical characteristics of pepes in the second stage was Completely Randomized Design with three levels namely raw, steamed, and microwave oven pepes in three replications. The observed parameters were moisture content, protein, amino acids, and free fatty acid levels. The result shows that the best cooking time of pepes with a microwave oven was five minutes. Analysis of variance showed that cooking affected the total selected essential amino acid and 14 kinds of amino acids content that were analyzed, namely aspartic acid, glutamic acid, serine, histidine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine content of pepes but not significantly affected on the moisture content, protein, glycine and free fatty acids content of pepes. The total selected essential amino acid and 14 kinds of amino acids content of raw pepes were significantly different to cooked pepes (microwave oven and steamed pepes), but the total selected essential amino acid and 14 kinds of amino acids content of microwave oven pepes were not significantly different to steamed pepes. The chemical characteristics of microwave oven pepes were 73.04% WB of moisture, 67.95% DB of protein, 28.48% DB of total selected essential amino acids, and 7.22% in oil of free fatty acids content.*

Keywords— ***Nile Tilapia, Microwave Oven, Steaming, Pepes, Amino Acid.***

I. INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is a cultivable fish whose availability is not affected by the season. Nile tilapia is a potential local foodstuff in Indonesia. Based on the Data, Statistics, and Information Center of the Secretariat General of the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia in 2022, tilapia is a fish commodity with the highest production in aquaculture in Indonesia in the first quarter of 2022, namely 258 thousand tons (Kementerian Kelautan dan Perikanan, 2022).

Nuryanto *et al.* (2022) wrote that Nile tilapia has a high nutrient content, especially protein, calcium, and monounsaturated fatty acids. High nutrient content in Nile tilapia is proteins, calcium, oleic fatty acids, palmitic,

linoleic, and stearic acids, with 18.46 g, 74.38 g, 8.13%, 7.87%, 3.67%, and 4.30%, respectively. The most considerable amino acid content is aspartic acid, glutamic acid, lysine, arginine, and leucine, namely 2.16%, 3.45%, 1.84%, 1.88%, and 1.69%, respectively. Nile tilapia can be processed into various products and dishes such as fish *pepes. Pepes* is an Indonesian traditional fish product made of fish and spices, wrapped with banana leaves and then cooked by steaming for 30 minutes (Liuhartana, Priyanto and Hamzah, 2013).

Microwaves are a form of non-ionizing electromagnetic radiation with a frequency higher than regular radio waves but lower than infrared light. Microwaves relate to electromagnetic waves in the 300 to 300,000 MHz of frequency range. The microwave oven heats food by passing microwave radiation through it. The penetration depth of microwaves is dependent on food composition. Lower microwave frequencies with longer wavelengths have a more penetrating effect. The microwave oven may use less energy for cooking or reheating small amounts of food than a cook stove. Although microwave ovens are touted as the most efficient appliance, the energy savings are primarily due to the reduced heat mass of the food container (Bordoloi and Ganguly, 2014). In general, microwave processing is time-saving, energy-efficient, and yields good quality fish products with high nutritional value. Microwave cooking or blanching does not change the nutritional composition of fish (Viji *et al.*, 2022).

This study investigated the best cooking time and the influence of microwave processing on the chemical characteristics of *pepes* (moisture content, protein, amino acids, and free fatty acid levels). Cooking *pepes* with microwave oven was presumed to decrease the heating time, therefore diminishing the nutritional content decline. The previous research (Liuhartana, Priyanto and Hamzah, 2013) concluded that the minimal cooking time of Nile tilapia *pepes* processed by a microwave oven is 5 min based on microbial total plate count (TPC), internal temperature, and visual observation.

II. MATERIALS AND METHODS

Raw Material

Fresh Nile tilapia (*Oreochromis niloticus*) with 250g ± 20% in weight, tamarind, shallot, garlic, red chili, salt, sugar, ginger, candlenut, turmeric, galangal, lemon grass, and banana leaves were purchased from the traditional market in Palembang, South Sumatera. The materials were brought to the WSTPHP Laboratory (Workshop Teknologi Pengolahan Hasil Perikanan) of The Fisheries and Marine Faculty of PGRI Palembang University in Palembang, South Sumatra. Fresh fish was eviscerated and descaled, then washed with tap water. Raw *pepes* were prepared according to a method of Liuhartana, Priyanto and Hamzah (2013).

Equipment

The equipment used in this study included scales, knives, commercial microwave oven (Sharp model R-2491N(W), 2450 MHz, 800 W), steamer pot, gas stove, digital thermometer (Krisbow KW06-308 digital thermometer, range temperature -40°C to 250°C), a set of laboratory equipment for chemical analysis including pipettes, Petri dishes, dishes, Kjeldahl flasks, volumetric flasks, distillation apparatus, HPLC (Shimadzu model 20A with ODS-2 Hypersil column), oven and desiccator.

Research Procedure

In the first stage of the research, one factor was studied, namely cooking time by the microwave oven, and consisted of three levels: 5 (T1), 6 (T2), and 7 minutes (T3) with three replications. The 5 min as the first level was determined based on the minimal cooking time found by the previous study (Liuhartana, Priyanto and Hamzah, 2013). In addition, the *pepes* were kept in a microwave oven for another 3 minutes before being removed to spread the heat in the *pepes* evenly. The observed parameter was pepes sensory using multiple comparison tests. The sensory test was conducted at the Sensory Laboratory, Faculty of Agriculture, Sriwijaya University in Palembang, South Sumatra, Indonesia.

The experimental design to determine the chemical characteristics of *pepes* in the second stage was Completely Randomized Design with three levels namely raw, steamed, and microwave oven *pepes* in three replications. The observed parameters were moisture content, protein, amino acids, and free fatty acid levels. The chemical tests were conducted at Integrated Laboratory, IPB Bogor University in Bogor West Java, Indonesia.

Chemicals and reagents

NaOH, Brij-30 solution, H_2SO_4, H_3BO_3, and selenium mixture were purchased from Merck. Amino acid standard solution, ortophthaaldehyde (OPA), was purchased from Sigma. All chemicals and reagents used in the present work were of high purity and analytical grade.

Samples Analysis

Multiple Comparison Test

The Multiple Comparison Test was done according to Setyaningsih, Apriyantono, and Sari (2010), with some modifications. The 25 trained panelists were involved in the sensory test. The standard sample is steamed *pepes* for 30 minutes, coded 'B'. The four samples that will be compared with the standard sample are steamed *pepes* for 30 minutes,

microwaved *pepes* for 5 minutes, 6 minutes, and 7 minutes. The four types of samples are coded using a random 3-digit number. Panelists were first asked to identify standard samples and compare the samples presented with standard samples. Then, the assessment was carried out sequentially on the parameters of color, aroma, texture, and taste using five comparison scales, namely significantly better than the standard sample (score = 5), better than the standard sample (score = 4), as good as the standard sample (score = 3), worse than the standard sample (score = 2), significantly worse than the standard sample (score = 1). Finally, the panelists wrote the assessment results on the prepared questionnaire sheet.

The data obtained from the multiple comparison sensory tests were converted into a score and tabulated. First, an Analysis of Variance (ANOVA) was carried out, followed by Duncan's Multiple Range Test for significantly different samples.

Water Content

Water content determination is based on the difference in sample weight before and after drying. An empty porcelain cup is heated in the oven for 1 hour at 105 °C. The cup is cooled in a desiccator and then weighed after it cools down. First, the empty weight of the cup is weighed. As much as 2-10 g of sample is weighed into the cup. The cup is heated in the oven at 105 °C for 4 hours, cooled in a desiccator, and the cup and sample are weighed. Next, the cup and sample are heated for 2 hours at 105 °C, cooled in a desiccator, and the cup and sample are weighed. Heating and weighing were done until the weight remained (AOAC, 2005).

The water content is calculated by the formula:

Water content (%)=$(W_1-W_2)/W_1 \times 100\%$

W_1 = initial sample weight (g)

W_2 = Sample weight after drying (g)

Protein Content

Protein levels were measured using the Kjeldahl method. First, as much as 0.1 g of the sample was weighed, then put into a 100 mL Kjeldahl flask, then 2 g of selen mixture and 10 mL of concentrated H_2SO_4 were added. The sample is heated over an electric heater or Bunsen burner until it boils and the solution becomes clear greenish and then left to cool. The 10 mL of sample was then put into the distillation apparatus, and added 150 mL of distilled water and 50 mL of 40% NaOH, and then distilled. The distillation results were collected in an Erlenmeyer containing 10 mL of 2% H_2BO_3 solution, then titrated with 0.1 N HCl. The blank solution was analyzed like the sample (AOAC, 2005).

Calculation of protein levels is calculated by the formula:

Protein content (%) = $((V_1-V_2)\times N\times 0.014\times fk)/W\times 100\%$

W = sample weight (g)

V_1 = volume of 0.1 N HCl used in sample titration (mL)

V_2 = volume of 0.1 N HCl used in titration blank (mL)

N = normality HCl (0.1 N)

fk = conversion factor for protein from food in general = 6.25, for *pepes* = 6.25

Amino Acid Content

Amino acid analysis was carried out in the following stages. First, the hydrolyzed sample was dissolved in 5 mL of 0.01 N HCl and then filtered through millipore paper. Next, potassium Borate Buffer pH 10.4 was added in a 1:1 ratio. Next, a total of 10 µL of the sample was put into a clean empty vial, and added 25 µL of OPA reagent was left for 1 minute to complete the derivatization. Finally, as much as 5 µL of the 3rd step sample was injected into the HPLC column and then waited until all amino acids were separated. The time required is about 25 minutes (AOAC, 2005).

The percentage of amino acids in the sample can be calculated by the formula:

AA (%) = (µmol AA × Mr AA)/(µg sampel) × 100%

AA = Amino acids

Mr AA = Molecular weight of amino acids: Asp = 133.1; Glu = 147.1; Ser = 105.1; His = 155.2; Gli = 75.1; Thr = 119.1; Arg = 174.2; Ala = 89.1; Tyr = 181.2; Met = 149.2; Val = 117.1; Phe = 165.2; Ile = 131.2; Leu = 131.2; Lys = 146.2.

Free Fatty Acid Content

The level of free fatty acids in fat is determined by the following steps. First, the sample in the form of fat to be tested was weighed as much as ± 1 g in a 125 mL Erlenmeyer. Next, the sample was added with 50 mL of PA ethanol solvent containing pp indicator and shaken until the oil or fat dissolved. Finally, the solution was titrated with 0.1 N NaOH solution from a 10 mL burette with a calibration of 0.05 mL, and a blank was performed with every two or three titrations. The endpoint's color is a light pink that does not fade after 5-10 minutes (SNI 01-2352-1998).

Free fatty acid levels are calculated by the formula:

Free fatty acid content (% in oil) = $(V\times N\times M)/W\times 100\%$

V = Volume of NaOH for sample titration (sample - blank) (ml)

N = Normality of NaOH solution (0.1 N)

M = Molecular weight of dominant acid (oleic acid 282)

W = Sample weight (mg)

Statistical analysis

1. Parametric Statistical Analysis of Sensory Test Data

The data from the multiple comparisons sensory tests were converted into a score and tabulated. An analysis of variance or ANOVA (Analysis of Variance) was carried out, followed by Duncan's Multiple Range Test or Duncan's Significant Distance Difference for significantly different examples.

2. Parametric Statistical Analysis

The data was calculated using a completely randomized design (CRD) with the treatment of *pepes* cooking methods consisting of 3 levels (raw *pepes*, steamed *pepes*, and microwave *pepes*) with 3 replications. The data is further processed using analysis of variance or ANOVA (Analysis of Variance). Significance in the analysis of variance was carried out by comparing the F-table at 5% and 1% tests. Duncan Multiple Range Test (DMRT) was carried out on significantly different samples to determine the effect of each treatment.

III. RESULTS AND DISCUSSION

Determination The Best Cooking Time of Nile Tilapia *Pepes* Processed by Microwave Oven

Through the multiple comparison tests, panelists were asked to assess the differences in color, aroma, texture, and taste between each *pepes* served with standard *pepes*. Standard *pepes* are *pepes* that are steamed for 30 minutes, while other pepes are *pepes* that are microwaved for 5 minutes, 6 minutes, and 7 minutes (Figure 1.)

1. Color

The results of the multiple comparison tests on the color attribute of *pepes* in general panelists gave the lowest score in treatment B_0 (*pepes* cooked by steaming for 30 minutes) with an average rating of 2.88 while the highest score was in treatment B_1 (*pepes* cooked with a microwave oven for 5 minutes) is 3.12. The average panelist assessment of *pepes* color is shown in Figure 2. The analysis of variance showed that cooking *pepes* in a microwave oven had no significant effect on the color of *pepes* produced.

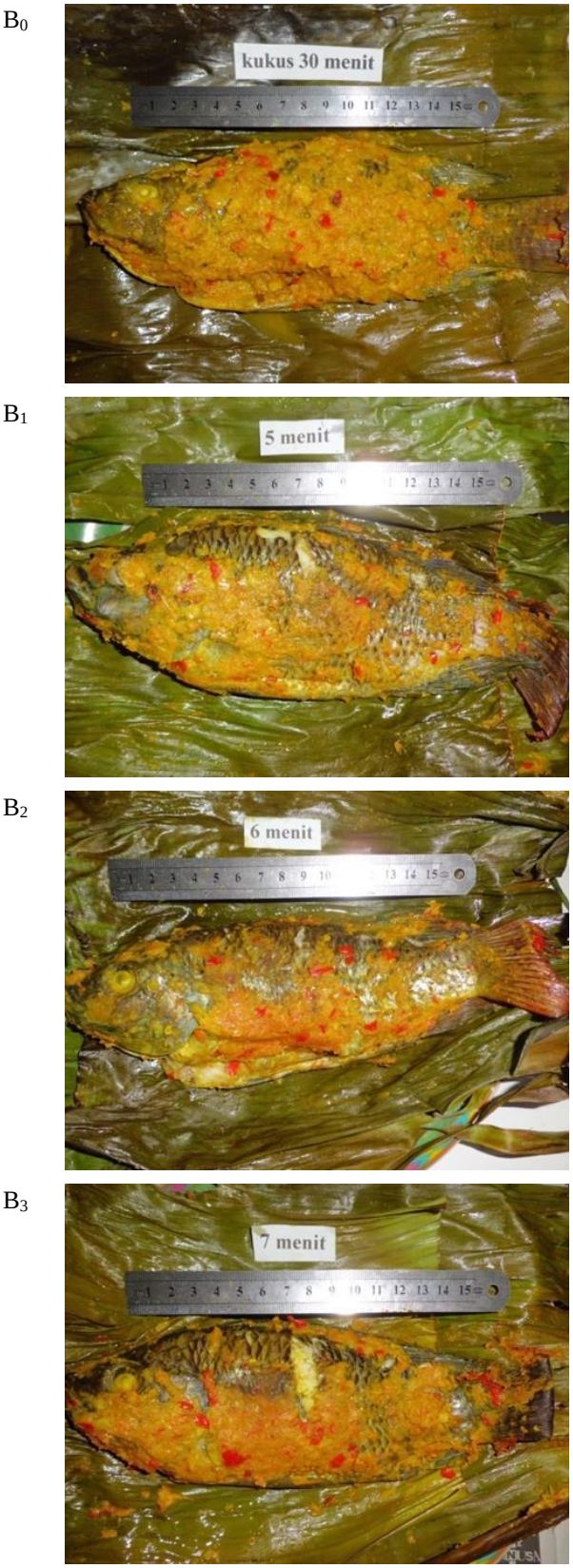

Fig.1. Nile tilapia pepes. B_0 = steamed pepes for 30 minutes, B_1 = pepes in the microwave for 5 minutes, B_2 = pepes in the microwave for 6 minutes, B_3 = pepes in the microwave for 7 minutes. The picture above with a ruler length comparison of 15 cm.

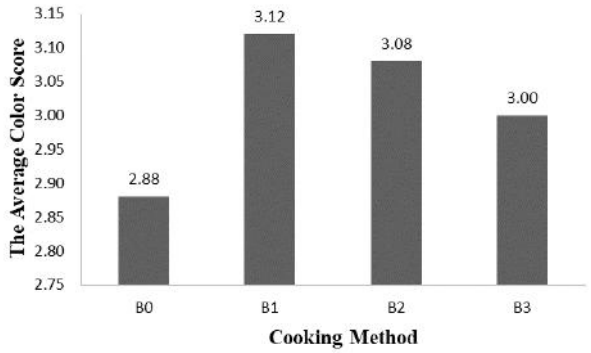

Figure 2. Multiple comparison test of pepes color. B_0 = steamed pepes for 30 minutes, B_1 = pepes in the microwave for 5 minutes, B_2 = pepes in the microwave for 6 minutes, B_3 = pepes in the microwave for 7 minutes. Analysis of Variance (ANOVA) showed no significant differences between samples.

2. Aroma

The results of the multiple comparison tests on the aroma attribute of *pepes* in general, the panelists gave the lowest score in treatment B_2 (*pepes* cooked in a microwave oven for 6 minutes) with an average rating of 2.88 while the highest score was in treatment B_1 (pepes cooked in an oven microwave for 5 minutes) is 3.24. The average panelist's assessment of the aroma of *pepes* is presented in Figure 3. The analysis of diversity showed that cooking *pepes* in a microwave oven had no significant effect on the aroma of *pepes*.

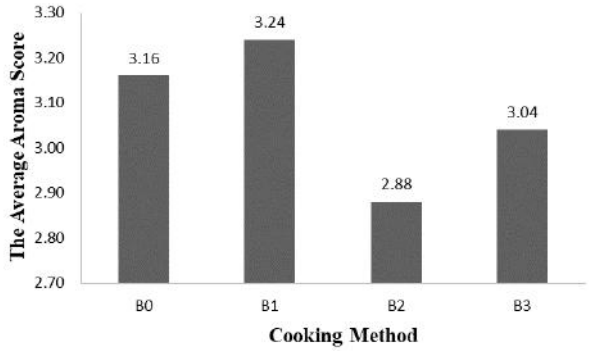

Fig.3. Multiple comparison test of pepes aroma. B_0 = steamed pepes for 30 minutes, B_1 = pepes in the microwave for 5 minutes, B_2 = pepes in the microwave for 6 minutes, B_3 = pepes in the microwave for 7 minutes. Analysis of Variance (ANOVA) showed no significant differences between samples.

3. Texture

The results of the multiple comparison test for the texture attribute on *pepes* in general, the panelists gave the lowest score in treatment B_2 (*pepes* cooked in a microwave oven for 6 minutes) with an average rating of 2.92 while the highest score was in treatment B_3 (*pepes* cooked in an oven microwave for 7 minutes) is 3.40. The average panelist assessment of the color of the *pepes* is presented in Figure 4. The results of the analysis of diversity showed that cooking *pepes* in a microwave oven had no significant effect on the texture of *pepes* produced.

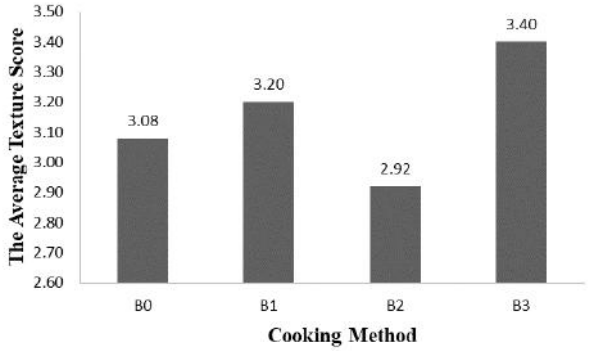

Fig.4. Multiple comparison test of pepes texture. B_0 = steamed pepes for 30 minutes, B_1 = pepes in the microwave for 5 minutes, B_2 = pepes in the microwave for 6 minutes, B_3 = pepes in the microwave for 7 minutes. Analysis of Variance (ANOVA) showed no significant differences between samples.

4. Taste

The results of the multiple comparison test for the taste attribute of *pepes* in general, the panelists gave the lowest score in treatment B_0 (steamed *pepes* for 30 minutes) and B_2 (*pepes* cooked in a microwave oven for 6 minutes) with an average rating of 3.04 while the value the highest was in treatment B_1 (*pepes* cooked in a microwave oven for 5 minutes) which was 3.56. The average panelist's assessment of the taste of *pepes* is presented in Figure 5. The analysis of variance showed that cooking *pepes* in a microwave oven had no significant effect on the color of *pepes* produced.

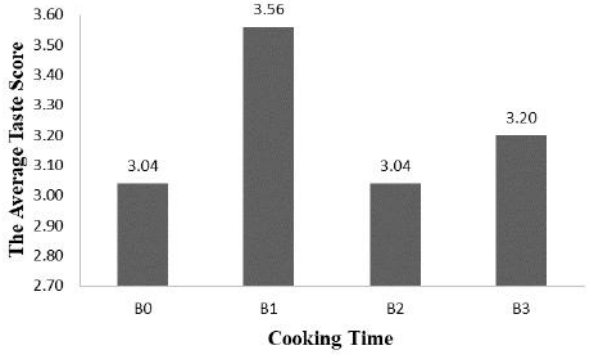

Fig.5. Multiple comparison test of pepes taste. B_0 = steamed pepes for 30 minutes, B_1 = pepes in the microwave for 5 minutes, B_2 = pepes in the microwave for 6 minutes, B_3 = pepes in the microwave for 7 minutes. Analysis of Variance (ANOVA) showed no significant differences between samples.

Cooking *pepes* in a microwave oven had no significant effect on the color, aroma, texture, and taste of *pepes*, which is supposed to be caused by the similarity in the conditions of products cooked in a microwave oven and steaming. However, the short duration of cooking in a microwave oven and the low air temperature on the product's surface cause the product to remain soggy (Yaylayan and Roberts, 2001). Cooking by steaming also causes the surface of the product to get wet due to moisture.

The conclusion from the first stage of the study is that the best time to cook *pepes* in a microwave oven is 5 minutes which is the shortest cooking time because the results of the analysis of variances show that cooking *pepes* in a microwave oven for 5 minutes, 6 minutes and 7 minutes has no significant effect on color, aroma, texture, and taste of *pepes* produced. The results of the first research stage were then used for the second research stage, namely determining the chemical characteristics of *pepes*.

Determination The Chemical Characteristics of Nile Tilapia *Pepes* Processed by Microwave Oven

The results of the second research stage, namely the best cooking time for *pepes* in a microwave oven for 5 minutes, were then used for the second research stage. Raw *pepes* and steamed *pepes* were also analyzed for comparison.

1. Water Content

Pepes water content ranges from 72.14% to 73.75%. The highest water content was in treatment C_0 (raw *pepes*), and the lowest concentration was in treatment C_1 (*pepes* steamed for 30 minutes). Cooking (steaming and microwaving) causes a decrease in the moisture content of *pepes*. The decrease in the water content of C_1 was more significant than that of C_2 (*pepes* cooked in a microwave for 5 minutes). The average moisture content of *pepes* from all treatments is presented in Figure 6. The analysis of variance showed that the cooking method had no significant effect on the moisture content of the *pepes* produced.

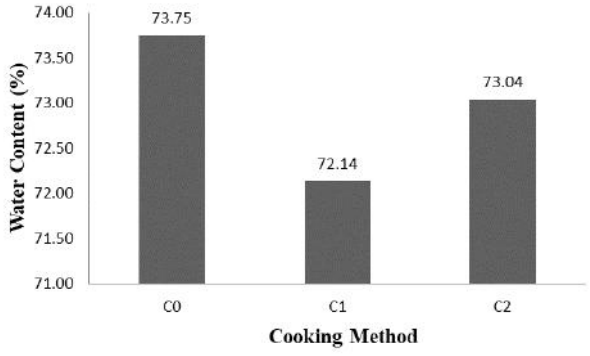

Fig.6. Multiple comparison test of pepes water content. C_0 = raw pepes, C_1 = pepes cooking by conventional method (steamed pepes for 30 minutes), C_2 = pepes in the microwave for 5 minutes. Analysis of Variance (ANOVA) showed no significant differences between samples.

A decrease in water content in steamed fish was also found in the study results of Ghelichpour and Shabanpour (2011) on golden gray mullet (*Liza aurata*) fillet, Dhanapal *et al.* (2012) on tilapia steaks (*Oreochromis mossambicus*), Devi and Sarojnalini (2012) on *Amblypharyngodon mola* and Aisyah (2012) on cobia fish (*Rachycentron canadum*) meat. In addition, a decrease in water content in fish cooked in a microwave oven was also shown by the results of research by Unusan (2007) and Asghari, Zeynali, and Sahari (2013) on rainbow trout fish fillets (*Oncorhynchus mykiss*) Weber *et al.* (2008) on silver catfish fillet (*Rhamdia quelen*).

Kusnandar (2011) explains that besides functioning as a source of nutrition, protein also has specific functional properties that can affect the characteristics of food products. Among these properties is its function for the absorption and binding of water. The heating process can cause protein denaturation, resulting in food ingredients losing their water-holding capacity. The higher the heating temperature, the lower the amount of bound water. The water content of *pepes* C_1 (steamed for 30 minutes), which was lower than the moisture content of *pepes* C_2 (microwave oven 5 minutes), was supposed to be caused by the heat received by *pepes* C_1, which was greater than that of *pepes* C_2. This causes *pepes* C_1 to lose more water content. Oduro, Choi and Ryu (2011) also showed a similar result on chub macherel (*Scomber japonicus*).

2. Protein Content

Pepe's protein content ranges from 65.55% (DB) to 70.05% (DB). The highest protein content was in treatment C_0 (raw *pepes*), and the lowest concentration was in treatment C_1 (*pepes* steamed for 30 minutes). Cooking causes a decrease in *pepes* protein levels. The decrease in protein content of C_1 was more significant than that of C_2 (*pepes* cooked in the microwave for 5 minutes). The average protein content of *pepes* from all treatments is shown in Figure 7.

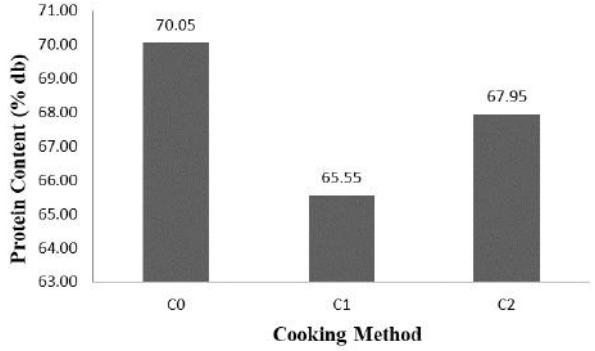

Fig.7. Multiple comparison test of pepes protein content. C_0 = raw pepes, C_1 = pepes cooking by conventional method (steamed pepes for 30 minutes), C_2 = pepes in the microwave for 5 minutes. Analysis of Variance (ANOVA) showed no significant differences between samples.

The analysis of variance showed that the cooking method had no significant effect on the protein content of the *pepes*. There was a decrease in the protein content of *pepes* from raw to cooked (steamed and microwaved), but the difference was not significant. The results of the research also show a decrease in protein content (% DB) due to steaming by Aisyah (2012) on cobia fish (*Rachycentron canadum*) meat. A decrease in protein content (% DB) in fish cooked in a microwave oven was also shown by the results of Asghari, Zeynali, and Sahari's (2013) research on rainbow throut (*Oncorhynchus mykiss*) filets.

The protein content of *pepes* C_1 (steamed for 30 minutes) was lower than the protein content of *pepes* C_2 (microwave oven 5 minutes), thought to be caused by the heating process for *pepes* C_1 which took longer than *pepes* C_2 (the steaming time for *pepes* was much longer than the cooking time for *pepes* in a microwave oven). The results of the research by Tapotubun, Nanlohy, and Louhenapessy (2008) showed that the protein content of fish decreased with increasing heating time. The longer the heating time, the smaller the water-soluble protein is lost along with the water that comes from the fish meat.

3. Amino Acid Content

The total levels of selected essential amino acids contained in *pepes* ranged from 19.05% DB to 28.48% DB. The highest concentration of selected total essential amino acids was in treatment C_2 (microwave oven 5 minutes), and the lowest concentration was in treatment C_0 (raw *pepes*). The highest amino acid in *pepes* was glutamic acid, and the lowest was histidine of the 15 amino acids analyzed. The amino acid content increases from raw *pepes* to cooked *pepes* (steamed and microwaved). The average levels of selected amino acids and essential amino acids in total *pepes* from all treatments are presented in Table 1.

The analysis of diversity showed that the cooking method had no significant effect on the glycine content of *pepes*. However, the diversity analysis showed that the cooking treatment method significantly affected the levels of the total selected essential amino acids and 14 other types of amino acids, which were analyzed in *pepes*.

The results of the Duncan Multiple Range Test (DMRT) showed that the total selected essential amino acids and 14 types of amino acids were analyzed, namely aspartic acid, glutamic acid, serine, histidine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine and lysine in treatment C_0 were significantly different from treatments C_1 and C_2. However, treatment C_1 was not significantly different from treatment C_2. In addition, the levels of total selected essential amino acids and 14 types of amino acids in raw *pepes* were significantly different from those of cooked *pepes* (microwave oven and steamed). However, the levels of total selected essential amino acids and 14 types of amino acids of *pepes* cooked in the microwave were not significantly different from steamed *pepes*.

The following studies also show the same results. Unusan (2007) stated that the content of essential amino acids and non-essential amino acids in rainbow throut (*Oncorhynchus mykiss*) meat increased significantly from raw to cooked condition but was not significantly different between fish meat cooked in a microwave oven and with a conventional oven. Research by Oduro, Choi, and Ryu (2011) on chub macherel (*Scomber japonicus*) soaked in salt solution concluded that there was a significant increase in the essential amino acid content of fish meat from raw to cooked conditions cooked by steaming, microwave ovens, frying, and roasting. The study also showed that glutamic acid is an amino acid with the highest levels in chub mackerel. Erkan, Ozden, and Selcuk (2010) stated that cooking by frying, roasting, and steaming causes a marked increase in the amino acid content of several marine fish. Apriyana (2011), who studied the long-pong snail (*Fasciolaria Salmo*), and Insanabella (2012), who studied the red-eyed snail (*Cerithidea obtusa*) stated that steaming causes an increase in amino acids.

Table 1. Amino acid profile of raw pepes, steamed pepes and microwave pepes

Amino Acid	C_0 (%DB)[2,3]		C_1 (%DB)[2,3]		C_2 (%DB)[2,3]	
Aspartic acid	4.61	a	5.73	b	5.75	b
Glutamic acid	7.37	a	10.57	b	11.42	b
Serine	1.78	a	2.55	b	2.74	b
Histidine*	1.12	a	1.62	b	1.73	b
Glycine	2.46		3.02		3.24	
Threonine*	1.94	a	2.90	b	3.06	b
Arginine*	2.97	a	4.20	b	4.47	b
Alanine	2.91	a	4.06	b	4.37	b
Tyrosine	1.42	a	2.08	b	2.18	b
Methionine*	1.35	a	1.90	b	2.06	b
Valine*	2.34	a	3.45	b	3.61	b
Phenylalanine*	1.98	a	2.98	b	3.13	b
Isoleucine*	2.22	a	3.30	b	3.47	b
Leucine*	3.57	a	5.05	b	4.98	b
Lysine*	3.81	a	5.65	b	5.90	b
Total selected essential amino acids[1]	19.05	a	27.22	b	28.48	b

C_0 = raw *pepes*

C_1 = konventional (steamed 30 minutes)

C_2 = microwave oven 5 menit

* = essential amino acids

[1] Sum of histidine, threonine, arginine, methionine, valine, phenylalanine, isoleucine, leucine and lysine

[2]Average of three replicates

[3]Every two means in one row that have the same letter are stated to be not significantly different at the 5% level.

The cooking process causes an increase in *pepes* amino acid levels. Fellows (2000) suggests that heat treatment is a significant cause of changes in the nutritional properties of foods. The heating process causes protein denaturation (Kusnandar, 2011), which results in the unfolding of protein coils so that peptide bonds can be broken (Almatsier, 2009). The heating process in food cooking creates conditions that make it easier for proteins to be hydrolyzed into free amino acids. Damodaran (1996) suggested that most food proteins are denatured when exposed to moderate heat treatment (60-90°C, 1 hour or less). Extensive denaturation of proteins often results in insolubility, which can impair the functional properties of proteins depending on their solubility. From a nutritional point of view, protein denaturation can increase the digestibility and bioavailability of essential amino acids. Moderate heating of purified vegetable protein and egg protein preparations improves their digestibility without creating toxic derivatives.

The levels of 14 types of amino acids in C_2 (*pepes* cooked in the microwave for 5 minutes) were higher than in C_1 (*pepes* cooked for 30 minutes), presumably because protein hydrolysis was more effective due to the influence of microwaves. Margolis, Jassie, and Kingston (1991) stated that amino acids, peptides, and proteins are charged molecules with dipolar properties. Proteins are composed of several dipolar moieties that contribute to the net dipole moment of the total protein, further modified by hydration shell molecules. Electromagnetic energy can increase the rotational energy of bonds connecting the dipolar moieties to nearby atoms, thereby reducing the energy required to break bonds and increasing the likelihood of hydrolysis processes. The combination of heating and possibly increasing the rotational energy of the peptide bonds can catalyze more effective peptide bond hydrolysis and maintain the integrity of the less stable amino acids. Chiou and Wang (1989) reported an improved method for analyzing amino acids using microwaves with a new and faster HPLC. Microwaves are used to hydrolyze proteins at the protein and peptide hydrolysate preparation stage. The research results of Margolis, Jassie, and Kingston (1991) showed that complete hydrolysis of proteins could be achieved by exposure to microwaves under controlled conditions. Hydrolysis can be achieved in a short time at relatively low temperatures. Besides providing a thermal effect, microwave radiation can also cause or facilitate the breaking of peptide bonds by local interactions with each polarized amide group of the peptide chain. The highest levels of glutamic acid, among other amino acids, cause a savory taste in *pepes*. Glutamic acid is an amino acid that can give a savory taste (Kusnandar, 2011).

4. Free Fatty Acid Content

Pepes-free fatty acid levels range from 7.25% in oil to 7.22% in oil. The highest levels of free fatty acids were in treatment C_0 (raw *pepes*), and the lowest was in treatment C_1 (*pepes* steamed for 30 minutes). The average levels of pipes-free fatty acids from all treatments are shown in Figure 8.

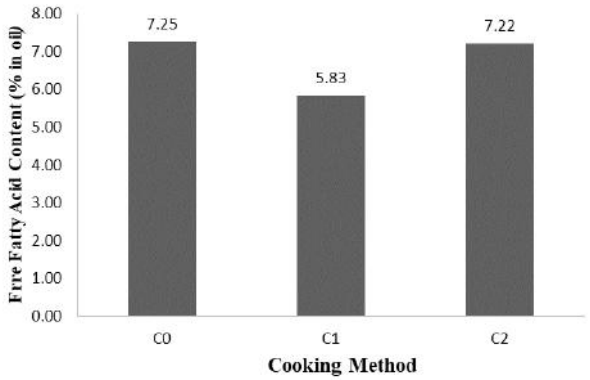

Fig.8. Multiple comparison test of pepes free fatty acid content. C_0 = raw pepes, C_1 = pepes cooking by conventional method (steamed pepes for 30 minutes), C_2 = pepes in the microwave for 5 minutes. Analysis of Variance (ANOVA) showed no significant differences between samples.

The analysis of variance showed that the cooking method had no significant effect on the levels of *pipes* free fatty acids produced. There was a decrease in free fatty acids from raw *pepes* to cooked (steamed and microwaved) *pepes*, but the difference was insignificant.

A decrease in free fatty acid levels due to cooking was also shown in a study by Weber *et al*. (2008) on silver catfish filet (*Rhamdia quelen*). Cooking with boiling, conventional baking, microwave baking, grilling, and deep frying with soybean oil, canola oil, and partially hydrogenated vegetable oil causes a decrease in free fatty acid levels in silver catfish filets. Weber *et al*. (2008) suggested that it was caused by the loss of volatile free fatty acids during cooking, or it could also be due to the deactivation of the lipase enzyme due to heating, thereby preventing the formation of free fatty acids in cooked fish.

The reaction of releasing free fatty acids from glycerin in the molecular structure of fat or the reaction of hydrolysis of fats or lipolysis can be triggered by the activity of lipase enzymes or heating, which causes the breaking of ester

bonds and the release of free fatty acids. Each release of one free fatty acid molecule requires one water molecule so that the hydrolysis reaction of fat can occur if there is water and heating (Kusnandar, 2011). The water in *pepes* comes from fish and spices.

The free fatty acid content of *pepes* C_1 (conventional, steamed 30 minutes), which is lower than the free fatty acid content of *pepes* C_2 (microwave oven 5 minutes), is thought to be caused by the heating received by *pepes* C_1, which is greater than *pepes* C_2 so that there are more volatile free fatty acids lost. More and more lipase enzymes are inactive. Deactivation of the lipase enzyme due to heating prevents the formation of free fatty acids.

Conclusions

Based on the research results obtained, conclusions can be drawn, namely (1) The best time for cooking *pepes* with a microwave oven is five minutes, (2) The cooking method has a significant effect on the total levels of selected essential amino acids and 14 types of amino acids analyzed, namely aspartic acid, glutamic acid, serine, histidine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine and lysine *pepes* but had no significant effect on water content, protein, glycine and free fatty acids *pepes*, (3) The levels of total selected essential amino acids and 14 types of amino acids for *pepes* would be significantly different from cooked *pepes* (microwave oven and steamed) but the levels of total selected essential amino acids and 14 types of amino acids for *pepes* cooked in microwave were not significantly different from steamed *pepes*, (4) The chemical characteristics of *pepes* are water content 73.75% DB, protein 70.05% DB, selected essential amino acids total 19.05% DB and content of free fatty acid 7.25% in oil. The chemical characteristics of the steamed *pepes* are 72.14% WB of water content, 65.55% DB of protein, 27.22% DB of total amino acids, and 5.83% free fatty acids in the oil. On the contrary, the chemical characteristics of *pepes* cooked in the microwave were 73.04% DB of water, 67.95% DB of protein, 28.48% DB of selected amino acids, and 7.22% in the oil of free fatty acids.

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