

Physical, chemical, microbiological and organoleptic properties of flavor seasoning combination of palm mushroom (*volvariella volvacea*) and snakehead fish (*channa striata*) with drying temperature variation

H. N. Ramdani¹, Y. Yuwana^{1*}, and Budiyanto Budiyanto¹

¹Bengkulu University, Department of Agricultural Technology, Faculty of Agriculture, Bengkulu, Indonesia

Abstract. The delicacy of food can be improved by employing flavor seasoning addition. The purpose of this study was to investigate the effect of the material combination of oil palm mushrooms and snakehead fish and the drying temperature variation on the flavor seasonings' physical, chemical, microbiological, and organoleptic properties. The experiment employed a Completely Randomized Design (CRD) consisting of two factors. The first factor was the ratio of oil palm mushrooms and snakehead fish of 25%: 75%, 50%: %, 75%: 25% and the second factor was drying temperatures of 50°C and 60°C. The ANOVA and DMRT (Duncan Multiple Range Test) tests were used to analyze the quantitative parameters while the Friedman test was utilized to analyze the qualitative parameters. The results of the research on the material comparison factor significantly affected the solubility, protein content, glutamic acid, and organoleptic. No significant effect on water and salt content. While the temperature factor significantly affects the solubility, protein, and organoleptic. No significant effect on water content, glutamic acid, and salt. The microbiological test has met the requirements of SNI. The panelists preferred the flavor seasonings of the material combination of 75% palm oil mushroom and 25% snakehead fish dried at a temperature of 60°C characterized by a water content of 2.71%, a solubility of 72.67%, protein of 22.47%, acid glutamic acid of 8.60%, salt content 2.72%, total plate number 0.81×10^3 colony/g, coliform <3 APM/g and yeast of 0.65×10^3 colony/g and the average panelists' preference's score of 3.64.

1 Introduction

Flavoring is a flavor enhancer that functions as an addition to the delicacy of food so that the food becomes sweeter, salty, and sour. Flavorings contain glutamic acid which can cause a distinctive taste called umami or savory. Synthetic flavorings harm health if consumed for a

* Corresponding author: yuwana@unib.ac.id

long time. Monosodium Glutamate (MSG) is a food additive used to enhance the taste [1]. Consumption of MSG of more than 0.5 – 2.5 grams per day will cause heart, neurological, respiratory, gastrointestinal, muscle, genital, and urinary tract, skin, and vision problems, the symptoms caused are referred to as MSG complex syndrome [2]. There is a need for innovations to reduce the consumption of synthetic flavorings by making flavorings with natural ingredients that are rich in benefits.

Natural flavoring ingredients are food additives obtained from animals or plants that are consumed directly or processed first through physical, microbiological, or enzymatic processes. Mushrooms contain glutamate which can be used as an ingredient to fabricate seasonings [3]. Palm mushroom (*Volvariella volvacea*) is one of the ingredients having the potential as a functional food. Oil palm fungus grows and develops on oil palm waste media in the form of empty oil palm fruit bunches (TKKS) which are abundant every year [4]. Oil palm mushrooms contain glutamic acid which is 4.0428g/100g [5]. Palm mushrooms contain a protein of 3.8g/100g [6]. The glutamic acid and protein content in oil palm mushrooms makes it possible to produce seasonings. To increase the savory taste and nutritional content of the seasoning, snakehead fish can be utilized as an additional ingredient for flavoring.

Glutamic acid is found in snakehead fish albumin. Snakehead fish contains 25.2 g/100 g protein and 15.0g/100 g glutamic acid [7]. Glutamic acid from the protein contained in palm mushrooms and snakehead fish plays a role in producing a savory taste so that it can be used as a flavoring. The fat content in snakehead fish can cause damage to the resulting product. Oil content with a fairly high proportion of unsaturated fatty acids in fishery products contributes to damage due to the oxidation process [8]. Steaming is one way to reduce the fat content of the fish produced so that it does not easily rancid. The processing method of snakehead fish meal by steaming with a mechanical drying temperature of 50°C for 9 hours resulted in a dissolved protein content of 7.75 %, a low water content of 8.22%, a yield of 16.47 %, and the products were organoleptically favored by the panelists [9].

One of the important steps in the process of producing mushroom-based flavorings is drying. Drying aims to reduce the water content to a certain extent so that the growth of microbes and the activity of enzymes that cause damage can be inhibited. The drying method must be paid attention to concerning the characteristics of the mushroom material used. The best temperature for mushroom drying was 40°C which resulted in a moisture content of 8.7% [10]. The best drying temperature and time in the manufacture of white oyster mushroom flour were 5.5 hours with a temperature of 65°C which produced the best oyster mushroom flour with a yield of 7.34%, water content of 4.30%, ash content of 4.75%, protein content of 4.75% [11].

The quality of flavoring needs to be considered for its quality requirements. According to INS 01-4273-1996, the quality requirements for flavoring powdered broth are maximum water content of 4%, a minimum protein content of 7%, a maximum NaCl of 65%, a maximum total plate number of 104 col/g, a maximum coliform 3 APM/g and mold and weir maximum 103 cabbage/g. There is no information about natural flavoring ingredients made from palm mushrooms combined with snakehead fish.

This study aimed to determine the effect of the ratio of ingredients and drying temperature on the physical, chemical, microbiological and organoleptic properties as well as to determine the appropriate ratio of composition and drying temperature in the manufacture of seasonings composed of oil palm mushrooms and snakehead fish.

2 Materials and methods

2.1 Materials and Instruments/tools

The experimental materials consisted of palm mushrooms (*Volvariella* sp), Snakehead fish (*Channa striata*), shallots, garlic, pepper, salt, and sugar. The chemicals included H2SO4, NaOH 30%, distilled water, ninhydrin, ethanol 80%, potassium chromate 5%, AgNO3, Whatman filter paper no. 42, PCA powder, brilliant green lactose bile broth (BGLB), buffered peptone water (BPW), Lauryl Sulphote Tryptose broth (LST), Potato Dextrose Agar (PDA).

The instruments/tools were a steamer, knife, basin, oven (summer), analytical scale (digipoud), sieve, 60 mesh sieve, baking dish, blender (Shaner), polyethylene plastic (zipper lock), and stove (rinnai), petri dish, desiccator (Pyrex Iwaki), water bath, Kjeldahl flask, Erlenmeyer, burette, clamps, 25 ml and 10 ml measuring cups, dropper, sung spoon, beaker, electric stove, test tube, pipette micro, measuring pipettes, incubators, Durham tubes, shaker platforms, colony counters, funnels, and UV-Visible spectrophotometers.

2.2 Experimental design

The experiment was arranged in a Completely Randomized Design (CRD) consisting of 2 factors, namely the substitution of oil palm mushroom (JS) and snakehead fish (IG), and the drying temperature (T). There were 6 levels of treatment and then 3 repetitions were carried out to produce 18 experimental units. The treatment table can be seen in Table 1, 2.

J1 = JS (25%): GI (75%)
J2 = JS (50%): IG (50%)
J3 = JS (75%): GI (25%)
T1 = 50 C
T2 = 60 C

Table 1. Table of action.

	J1	J2	J3
T1	J1T1	J2T1	J3T1
T2	J1T2	J2T2	J3T2

Table 2. Randomization of the order of experimental implementation.

Palm mushroom composition: Snakehead fish	Repetition	Temperature	
		50°C (T1)	60°C (T2)
J1	I	J1T1(1)	J1T2(4)
	II	J1T1(7)	J1T2(10)
	III	J1T1(13)	J1T2(16)
J2	I	J2T1(2)	J2T2(5)
	II	J2T1(8)	J2T2(11)
	III	J2T1(14)	J2T2(17)
J3	I	J3T1(3)	J3T2(6)
	II	J3T1(9)	J3T2(12)
	III	J3T1(15)	J3T2(18)

2.3 Experimental procedure

2.3.1 Preparation of materials for seasonings

Palm mushroom. Palm mushrooms were sorted, washed, and drained. The ingredients were weighed according to the treatment and then boiled. Furthermore, the mushrooms were dried using an oven at 50°C and 60°C separately until the moisture content reached 3%. After drying, the materials were mashed using a grinder and then filtered using a 60-mesh filter until all the ingredients were used up.

Snakehead. Snakehead fish were cleaned (weeded, and removed scales, gills, and entrails). The ingredients were weighed according to the treatment and then steamed for ± 50 minutes with a ratio of fish and steaming water 1:1/3. Furthermore, the fish were dried using an oven at a temperature of 50°C and 60°C separately until the water content reached 3%. After drying, the materials were mashed using a grinder and then filtered using a 60-mesh filter until all the ingredients were used up.

Red onion. The onion was cleaned from the skin and reduced in size to two parts and then boiled. Furthermore, the onions were dried in an oven at 50°C and 60°C separately until the water content reached 3%. After drying, the materials were mashed using a grinder and then filtered using a 60-mesh filter until all the ingredients were used up.

Garlic. The garlic was cleaned from the skin and reduced in size to two parts and then boiled. Furthermore, the onions were dried in an oven at 50°C and 60°C separately until the water content reached 3%. After drying, the materials were mashed using a grinder and then filtered using a 60-mesh filter until all the ingredients were used up.

2.4 Flavor-producing process

The ingredients that had been powdered were then mixed with all the ingredients (palm mushroom, snakehead fish, and additional spices) with the ratio of oil palm mushrooms and snakehead fish J1 = oil palm mushrooms (25%): snakehead fish (75%), J2 = oil palm mushrooms (50 %): snakehead fish (50%), J3 = palm mushroom (75%): snakehead fish (25%), and onion 25 grams, garlic 35 grams, pepper powder 1 gram, salt 10 grams, sugar 8 grams to be a natural seasoning. Then the natural seasonings were packaged in polyethylene plastic packaging (zipper-lock).

2.5 Observation of physical properties of flavors

2.5.1 Water content determination

The flavoring powder weighed as much as ± 2 grams in an empty cup whose weight was previously known and was then dried with each - each drying method. Drying was carried out using an oven at a temperature of 105°C for 5 hours. The sample was then cooled in a desiccator then weighed and heated again in the oven for 30 minutes, cooled in a desiccator, and then weighed, this treatment was repeated until a constant weight was obtained (the difference in weighing was less than 0.2 mg respectively). The water content of the flavoring was obtained through the calculation results on a wet basis [12].

$$\text{Water Content \%} = \frac{(\text{material weight} + \text{empty cup weight}) - \text{final weight}}{\text{starting material weight}} \times 100 \quad (1)$$

2.5.2 Solubility test

The flavoring powder was weighed as much as 1 gram (a) and dissolved in 20 ml of distilled water and then filtered through Whatman filter paper no. 42. Before use, the filter paper was dried in an oven at 105°C for 30 minutes and weighed (b). After filtering, the filter paper was dried again in the oven for 1 hour at 105°C. Next, the filter paper was cooled in a desiccator and then weighed until a constant weight was reached (c) [13].

$$\text{Solubility in water \%} = 1 - \frac{\text{Weight of filter paper} - \text{Initial weight of filter paper}}{\text{Weight of sample}} \times 100 \% \quad (2)$$

2.6 Observation of the chemical properties of flavoring

2.6.1 Protein level test

The sample was carefully weighed ± 0.5 grams, the sample was put into a Khjedhal flask, added ± 1 gram of a mixture of selenium and 25 ml of concentrated H₂SO₄, the Khjedhal flask, and its contents were shaken until all samples were moistened with H₂SO₄, crushed in a fume hood until clear and allowed to cool then poured into a 100 ml volumetric flask and rinsed with distilled water. The sample was allowed to cool and then squeezed until the line marks with distilled water and then shaken until homogeneous. A reservoir consisting of 10 ml of 2% H₃BO₃ was prepared, added 4 drops of indicator solution and mixed in an Erlenmeyer. The sample solution of 5 ml was pipetted into a distillation flask and added with 10 ml of 30% NaOH and 100 ml of distilled water. The solution was then distilled until the volume of the reservoir becomes ± 50 ml [14].

$$\text{Protein Content \%} = \frac{V \times N \times 14 \times 6,25 \times P}{\text{berat sampel (g)}} \times 100 \quad (3)$$

2.6.2 Glutamic acid test

Beginning with making standard solutions with concentrations of 0, 1, 2, 3, 4, and 5 ppm, 1 ml of the standard solution of glutamic acid was pipetted and added 1 ml of 0.1% ninhydrin which was then dissolved in ethanol solvent, and mixed until homogeneous. The solution was heated in a water bath for 5 minutes. It was then cooled to room temperature and the wavelength was measured at 570 nm using a UV-VIS spectrophotometer and then a standard curve was made.

The sample was weighed as much as 1 gram, then dissolved in 100 mL of distilled water, pipetted as much as 1 ml, added 1 ml of ninhydrin then heated in a water bath for 5 minutes. The sample was cooled at room temperature and then the absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 570 nm. The result obtained was used to determine glutamic acid in the sample formula $X = yb/a$ [15].

2.6.3 Salt (NaCl) test

The salt content test was carried out using the Argentometry method. The sample was weighed as much as 1 gram, then extracted using 15 ml of hot aqua dest (100°C) and waited for 15 minutes so that all the salt (NaCl) dissolves. The extraction was then repeated up to 8 times. The extracted liquid was collected in an Erlenmeyer and then added 3 ml of 5% potassium chromate and titrated with 0.05 N AgNO₃ slowly. The final result of the titration

was indicated by a change in the color of the solution from yellow to brick red and the formation of a precipitate. This happened because the NaCl solution reacted with AgNO₃ until the NaCl solution in the solution run out, then AgNO₃ reacted with the K₂CrO₄ indicator so that the color of the solution became brick red and a precipitate formed [16].

2.7 Organoleptic test of flavor seasoning

A total of 150 mg of natural flavoring was added to 50 ml of cup noodle water, then stirred [17]. Each treatment was given to 25 panelists and an organoleptic assessment of color, aroma, texture, taste, and overall (overall assessment) was carried out using a hedonic test of untrained panelists. The hedonic scale transformed into numbers can be seen in Table 3.

Table 3. Organoleptic Scale [18].

Hedonic Scale	Number
Very dislike	1
Do not like	2
Neutral	3
Like	4
Really like	5

2.8 Flavor seasoning microbiological test

2.8.1 Total plate number test

The sample of flavoring was weighed as much as 5 grams and then put into a sterile bottle by adding 45 mL of aqua dest solution, then homogenized for 2 minutes (10-1 dilution). Using a sterile pipette, 1 mL of the 10-1 dilution was taken and put into 9 mL of distilled water to obtain a 10-2 dilution. Subsequently, dilution (10-3) by taking 1 ml of the sample from the 10-2 dilution into 9 ml of aqua dest solution was made. One ml of each dilution was pipetted and put into sterile Petri dishes. A duplicate for each dilution was prepared. A total of 12-15 ml of PCA (Plate Count Agar) was added to each cup that already contained the sample. The plates were rotated so that the sample and PCA media were perfectly mixed. The plates were incubated in an inverted position at a temperature of 35°C ± 1°C for mesophilic bacteria or at a temperature of 45°C ± 1°C for thermophilic bacteria for 48 hours ± 2 hours. The dilution used was recorded and counted for the total number of colonies (INS, 2015). The calculation formula is:

$$N = \frac{\sum C}{[(1 \times n1) + (0,1 \times n2)] \times (d)}$$

(4)

where:

- N: the number of colonies of the product, expressed in colonies per ml or colonies per gram
- C: the number of colonies in all plates counted
- n1: number of cups in the first dilution calculated
- n2: number of cups in the second calculated dilution

2.8.2 Mold and kamir test

The sample of flavoring spices was weighed as much as 5 grams, then put in a sterile bottle by adding 45 ml of aqua dest solution, and then homogenized for 2 minutes (10-1) using a sterile pipette, 1 ml of 10-1 dilution was taken and put into 9 ml of solution. distilled water to get a dilution of 10-2. The next procedure was as follows: Prepare the next dilution (10-3)

by taking 1 ml of the sample from the 10-2 dilution into 9 ml of aqua dest solution. Pipette 1 ml of each dilution and put it into sterile Petri dishes. Add 12-15 mL of PDA (Potato Dextrose Agar) into each cup that already contains the sample. Rotate the cup back and forth and left-to-right so that the sample and PDA media were perfectly mixed. The plates were incubated in the dark and not upside down and arranged no more than 3 Petri dishes in an incubator at 25°C for 5 days. After an incubation period of 5 days, calculations were made, if after 5 days there was no growth of mold and kamir, the sample was re-incubated for 48 hours (INS, 2015).

2.8.3 Coliform test

The sample for flavoring was weighed as much as 25 grams and then put into a sterile plastic container and added 225 ml of Butterfield's phosphate buffer solution, which was then homogenized for 2 minutes. The next procedure was as follows: Prepare the 10-2 dilution by dissolving 1 ml of the 10-1 solution into Butterfield's phosphate buffer diluent. Shake at least 25 times for each dilution. Transfer using a sterile pipette as much as 1 ml of the solution from each dilution into 3 tubes of Laury trytose broth containing Durham tubes. Then the tubes were incubated at a temperature of 35°C ± 0.5°C. The gas formed after incubation is 24 hours ± 2 hours was noted. A positive tube was indicated by turbidity and gassing in the Durham tube. The negative tubes were re-incubated for 24 hours and the results were recorded at 48 hours ± 3 hours.

2.9 Data analysis

The experimental data were analyzed employing ANOVA and Duncan's Multiple Range Test (DMRT) available in the SPSS application with a significant level of 5% to determine the effect of treatment. The qualitative data of the preference test were analyzed by a non-parametric Friedman test.

3 Results and discussion

Observational parameters tested in this study consisted of physical properties (moisture content and solubility), chemical properties (protein content, glutamic acid, and salt), microbiological (TPC, coliform, mold, and yeast), and organoleptic properties of oil palm mushroom and snakehead fish flavorings based on the parameters of color, aroma, texture, taste, and overall. A summary of the results of the ANOVA and DMRT tests is presented in Table 4 and Table 5.

Table 4. ANOVA Test results.

Observation Parameter		Combintion of Oil Palm Mushrooms and Snakehead Fish (J)	Drying temperature (T) T1: 50°C, T2: 60°C	J*T
Physical Properties	Water Content	ns	ns	ns
	Solubility	*	*	ns
Chemical Properties	Protein Content	*	*	*
	Glutamic Acid	*	ns	*
	Salinity	ns	ns	ns

Note: Notation (*) states the treatment has a significant effect and ns (not significance) states that the treatment has no significant effect.

Table 5. DMRT test results.

Treatment		Water content	Solubility	Protein content	Glutamic acid	Salinity
Material Combination	J1 (25% :75%)	a	c	c	a	a
	J2 (50% :50%)	a	b	b	b	a
	J3 (75% :25%)	a	a	a	c	a
Drying Temperature	T1: 50°C	a	a	a	a	a
	T2 : 60°C	a	b	b	a	a

Description: The same symbol shows insignificantly different results, while different symbol notations show significantly different results.

3.1 Analysis of physical properties of the flavor seasoning

3.1.1 Water content

The results of the water content of the combination of oil palm mushrooms and snakehead fish with variations in drying temperature are shown in Figure 1 and Figure 2.

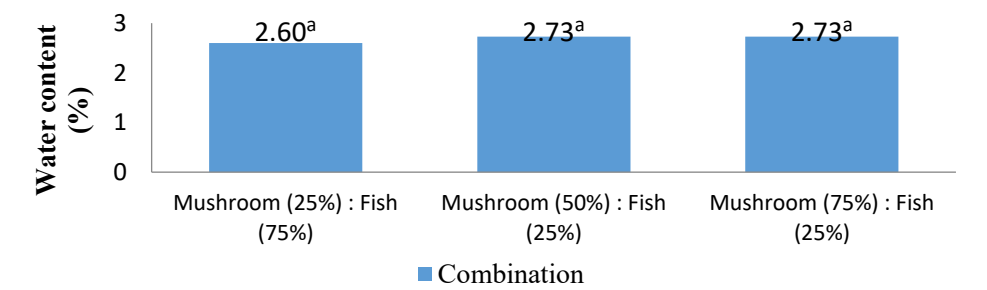


Fig. 1. The effect of the material combination on the water content of the flavor seasoning.

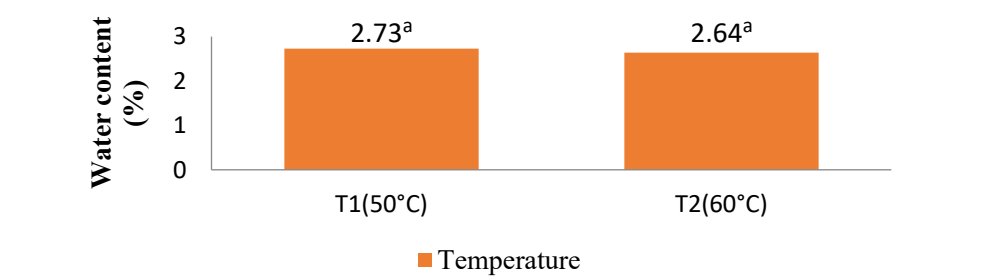


Fig. 2. Effect of drying temperature on the water content of flavor seasoning

The results of the water content test obtained in this study were smaller than 3% which ranged from 2% - 3% and had met the water content of INS 01-4273 (1996). Drying tomato-based flavorings with the addition of oyster mushrooms at a temperature of 60°C for 11 hours and 30 minutes obtained an average moisture content of 4.09% [19]. The higher water content can make it easy for bacteria, molds, and yeasts to breed so that changes can occur in food ingredients [20].

3.1.2 Solubility

The effects of the material combination and the drying variation on the flavor seasoning solubility are shown in Figure 3 and Figure 4.

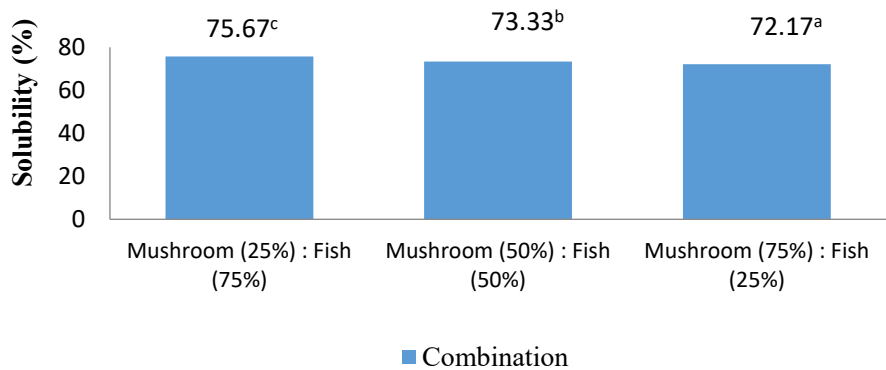


Fig. 3. The effect of the material comparison on the flavor seasoning solubility.

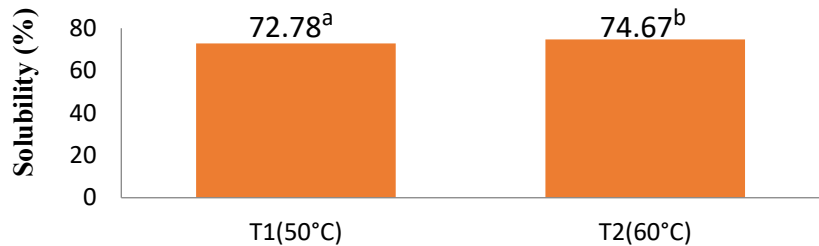


Fig. 4. Effect of drying temperature variation on the flavor seasoning solubility.

The ANOVA results showed that the material combination and drying temperature had a significant effect on the flavor seasoning solubility whereas the interaction between the two parameters had no significant effect. Figure 3 shows that with the increase in oil palm mushrooms, the flavor seasoning solubility decreased. This was because palm mushrooms have a higher fiber content compared to snakehead fish. Palm mushroom has a fiber content of 39.83% [21], on the other hand, snakehead fish has a fiber content of 21.83% [22]. Crude fiber is a plant fiber that cannot be dissolved in water [23]. So the addition of more snakehead fish will produce a higher solubility value. This is because snakehead fish contains albumin which is soluble in water [24]. Snakehead fish contains albumin of 4.71% [25].

Figure 4 indicates that the higher the temperature was the higher the flavor seasoning solubility. The solubility of starch will increase with the increase in temperature, and the rate of increase in solubility is typical for different starch [26]. Starch granules will absorb water, and hydrogen bonds in the starch structure are broken and replaced by hydrogen bonds in water so that over time, starch will expand and dissolve more easily [27].

3.2 Chemical Properties Analysis of Flavor Seasoning

3.2.1 Protein content

The effect of the interaction of the material combination and the temperature variation on the protein contents is shown in Figure 5.

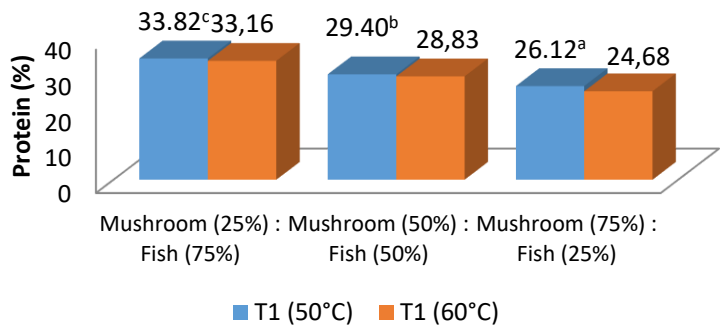


Fig. 5. The interaction effect of the material combination and the drying temperature variation on the protein content of flavor seasoning.

Figure 5 shows that the flavor seasoning protein content ranged from 24.68% - 33.82%. The highest protein content was found in the material combination of 25% palm mushroom and 75% snakehead fish with a drying temperature of 50°C which was 33.82%, while the lowest protein content was found in the material combination of 75% palm mushroom and 25 % of snakehead fish with a drying temperature of 60°C) which was 24.68%. The flavor seasoning protein content found in this study was much greater than the minimum protein content of above 7% and met the INS 01-4273 (1996). The ANOVA results showed that the material combination, the drying temperature variation, and the interaction between the parameters were significantly different. The higher the temperature used, the lower the protein content was produced. This is thought to be caused by the protein content in the material starting to denature due to increasing temperature. Heating for too long at high temperatures will cause the protein to be denatured [11]. Protein will experience denaturation if heated at a temperature of 50°C to 80°C [28]. The drying temperature also affects the protein content of taro tuber flour because the protein content in foodstuffs begins to denature due to the increase in drying temperature [29]. The Maillard reaction causes a loss of amino acids. The Maillard reaction does not require high temperatures, but the reaction rate will increase sharply with increasing temperature and drying time [30].

In this study, protein content increased along with the addition of snakehead fish [31]. reported that the concentration addition of snakehead fish increased the protein content of biscuits. In addition, snakehead fish has a higher protein content than oil palm mushrooms. Snakehead fish has a protein content of 25.2 g/100g [7], while oil palm mushrooms have a protein content of 3.8 grams [6].

3.2.2 Glutamic acid

The effect of the interaction between the material combination and the drying temperature variation on the flavor seasoning glutamic acid is presented in Figure 6.

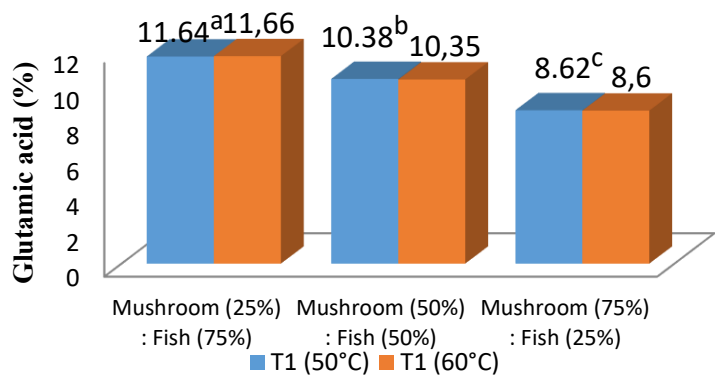


Fig. 6. The interaction effect of the material combination and drying temperature variation on the flavor seasoning glutamic acid.

The ANOVA results showed that the material combination and the interaction between the parameters affected significantly the flavor seasoning glutamic acid. Figure 6 shows that the highest levels of glutamic acid were found in the material combination of 25% palm mushroom and 75% snakehead fish with a drying temperature of 60°C) which was 11.66%, while the lowest levels of glutamic acid were found in the material combination of 75% palm mushrooms and 25% snakehead fish with a drying temperature of 60°C) which was 8.60%. The level of flavor seasoning glutamic acid increased with the addition of snakehead fish. Snakehead fish has a higher glutamic acid content than oil palm mushrooms. Snakehead fish contains 15.0 g/100g of glutamic acid [7] while palm mushrooms contain 4.0428 g/100g of glutamic acid [5]. Protein is an organic compound composed of amino acid monomers connected by peptide bonds, the greater the protein content, the more amino acid content it contains [32].

3.2.3 Salinity

The effects of the material combination and the drying variation on the flavor seasoning salinity are shown in Figure 7 and Figure 8.

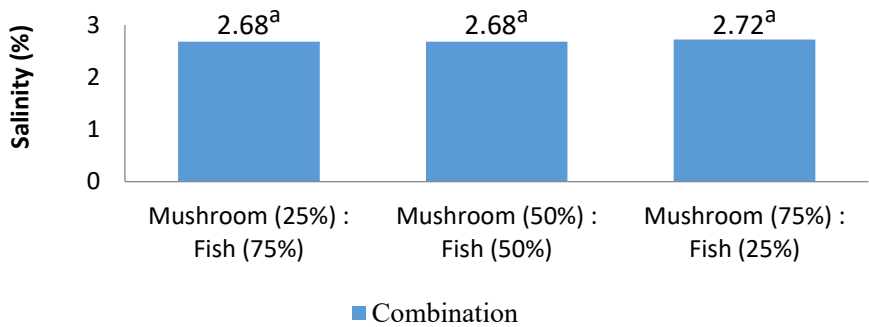


Fig. 7. The effect of the material combination on the flavor seasoning salt content.



Fig. 8. The effect of drying temperature variation on the flavor seasoning salt content.

The ANOVA results showed there was no effect of the material combination, the drying temperature variation, and the interaction of both parameters on the flavor seasoning salt content. The flavor seasoning salt content found in this study meets the requirements for the quality of salt content in INS 01-4273 (1996) so that it is still safe for consumption. INS 01-4273 (1996) requires that the salt content in the powdered broth flavoring is not more than 65% because high salt levels can trigger hypertension [33] There was no significant effect of the temperature factor because the addition of salt was done after all the ingredients were in the oven. Salt is preservative and not toxic [33]. The low salt content can affect the shelf life of flavoring spices. Salt can act as a preservative, and selective inhibitor of certain polluting microorganisms, and salt can affect the water activity of a substrate so that it can control microbial growth [34].

3.3 Microbiological analysis of flavoring seasonings

3.3.1 ALT (total plate number)

The total microbial analysis aimed to determine the number of microbes contained in the combination of palm mushroom and snakehead fish flavoring. The microbiological quality of a food product is determined by the number and types of microorganisms present in the food [33]. Testing other pathogenic bacteria is an indicator to evaluate the quality or safety of processed food products with INS standards. This microbiological quality will determine the shelf life and safety of food products. The results of the microbiological test for the total plate number (ALT) for the flavor seasoning combinations of palm mushrooms and snakehead fish expressed in colonies/g are presented in Table 6.

Table 6. Total Plate Number (ALT) for the flavor seasoning combination of oil palm mushrooms and snakehead fish with the drying temperature variation

NO	Sample	Temperature °C (T1:50)(T2:60)	ALT Flavoring (colony/g) INS 01-4273 (1996) Max 104 colony/g
1	J1	T1	0.95 x 10 ³
2	J2		0.90 x 10 ³
3	J3		0.89x 10 ³
4	J1	T2	0.90 x 10 ³
5	J2		0.82 x 10 ³
6	J3		0.81 x 10 ³

Table 5 shows the highest flavor seasoning ALT was found in the material combination of 25% mushrooms and 75% snakehead fish with a drying temperature of 50°C whereas the

lowest flavor seasoning ALT was found in the combination of 75% mushroom and 25% snakehead fish with a drying temperature of 60°C. The flavor seasoning ALTs test found in this study still meets the quality of microbial contamination prescribed by INS 01-4273 (1996). This was influenced by extrinsic factors (environmental conditions, handling, and storage methods) and intrinsic factors during the processing of flavoring spices. Products made from fish and seasonings that are processed by heating can reduce the number of microbes [35]. Drying in the process of making seasonings produces a low product water content, and the high water content can make it easy for bacteria, molds, and yeasts to breed so that changes can occur in food ingredients [14].

3.3.2 *Coliform content*

The microbial analysis is used as an indicator of the bacterial population, including the presence of coliform bacteria, which is a type of pathogenic bacteria. The group of coliform bacteria consists of the genus and species of bacteria, namely *Enterobacter*, *Klebsiella*, *Aeromonas*, and *Escherichia coli*, all of which belong to the family *Enterobacteriaceae*. The results of the flavor seasoning coliform test are shown in Table 7.

Table 7. The results of the coliform test for the flavor seasonings with the drying temperature variation.

NO	Sample	Temperature °C (T1:50) (T2:60)	MPN/100ml index results INS 01-4273 (1996) Max 3 APM/g
1	J1	T1	3
2	J2		<3
3	J3		3
4	J1	T2	3
5	J2		3
6	J3		<3

The average number of coliforms found in this study was 3 APM/gram and then still meets the quality requirements for coliform microbial contamination prescribed by INS 01-4273 (1996). According to Zelpina [36] coliform count is used as an indicator to determine the quality of food sanitation concerning the presence of other pathogenic organisms such as bacteria, viruses, or protozoa. Although the flavor seasonings produced are still suitable for consumption and meet the INS criteria for flavoring (INS 01-4273:1996), there is a need for better handling, processing, and storage of products to improve the quality of the flavoring quality. Arif [37], said that the application of an INS-standardized food safety system and the application of a Hazard Analytical Critical Control Point (HACCP) in the food safety assurance process requires product safety from physical, chemical, and biological contamination.

3.3.3 *Mold and kamir*

Fungal contamination of flavorings can be harmful to humans if the amount exceeds the requirements set by (INS 01-4273:1996) which does not exceed 104 colonies/gram. Microbial contamination can reduce the efficacy of the product due to impaired formula stability, and changes in appearance, and causes inactivation of the active ingredients in these ingredients [38]. The results of the flavor seasoning mold and yeast are shown in Table 8.

Table 8. The flavor seasoning mold and kamir with drying temperature variation.

NO	Sample	Temperature °C (T1:50)(T2:60)	Molds and fungi (colony/g) INS 01-4273 (1996) Max 103 colonies/g
1	J1	T1	0.92 x 10 ³
2	J2		0.86 x 10 ³
3	J3		0.82x 10 ³
4	J1	T2	0.89 x 10 ³
5	J2		0.79 x 10 ³
6	J3		0.65 x 10 ³

The highest mold and kamir contamination was observed in the material combination of 25% mushroom and 75% snakehead fish with a drying temperature of 50°C whereas the lowest one was found in the material combination of 75% mushroom and 25% snakehead fish with a drying temperature of 60°C. The high number of yeast colonies was thought to be due to the presence of oxygen and water activity, thus supporting microbial growth. The lack of good packaging and storage of flavoring products is thought to affect the increase in water content in the flavor seasoning produced [39]. said that poor packaging will reduce resistance to water, oxygen, or other odors. The long storage can increase the water content and the availability of water will affect the growth of fungi, the high water content will make microorganisms in the mushrooms grow faster. The high water content can make it easy for bacteria, molds, and yeasts to breed so that changes can occur in food ingredients [20]. Table 7 shows that the number of molds/kamir of all samples met the INS criteria for flavoring powdered broth (INS 01-4273:1996) and leads the produced flavor seasoning safe for consumption.

3.4 Organoleptic

3.4.1 Color

The score of panelists' preference for the flavor seasoning color is presented in Figure 9.

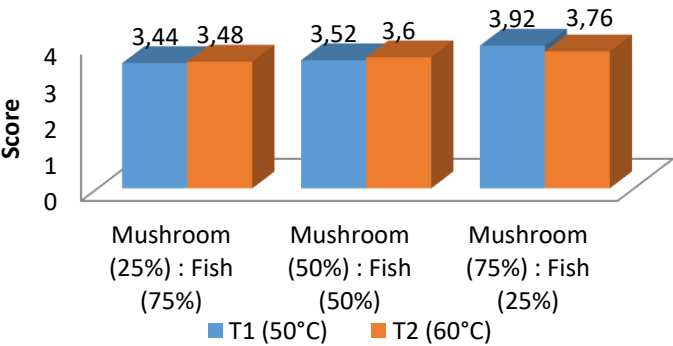


Fig. 9. The score of panelists' preference for the flavor seasoning color.

The panelists' preference level for the flavor seasoning color ranged from 3.44 - 3.92 which was in the range of quite like-like. The highest score was given for the material combination of 75% mushroom and 25% fish at a drying temperature of 50°C with a value of 3.92 (brown) whereas the lowest score was the material combination of 25% mushroom and 75% fish at drying temperature 50°C) with a value of 3.44. The Friedman test indicated that the material combination and the drying temperature affected significantly the flavor

seasoning color. The panelists preference increased with the increase of mushrooms percentage. The brown color revealed the domination of the mushroom color [40]. reported that the higher the addition of edible mushrooms, the brown the resulting color. The protein content derived from snakehead fish and palm mushrooms plays a role in the Maillard reaction and the drying process is thought to cause the brown color of the flavoring. The brown color of the flavoring is due to the Maillard reaction. The Maillard reaction is a reaction between carbohydrates, from protein to produce hydroxymethylfurfural compounds which continue to become furfural [41]. The furfural formed leads to generating a brown melanoidin compound.

3.4.2 Smell

The score of panelists' preference for the flavor seasoning smell is given in Figure 10.

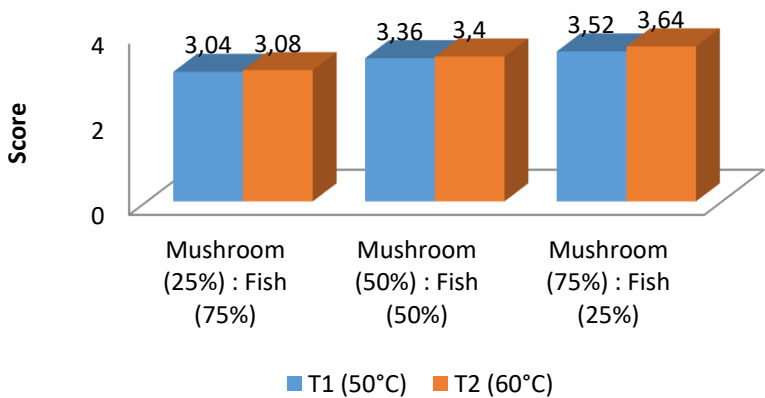


Fig. 10. The score of panelists' preference for the flavor seasoning smell.

The panelists' preference level for the flavor seasoning smell was in the range of 3.04 - 3.64 which was quite like-like. The highest score was given to the material combination of 75% mushroom and 25% fish at a drying temperature of 60°C with a value of 3.64 whereas the lowest score was addressed to the material combination of 25% mushroom and 75%) fish at drying temperature of 50°C with a value of 3.44. The Friedman test showed that the material combination and the drying temperature had a significant effect on the flavor seasoning smell. The higher the ratio of snakehead fish was the lower the score for the smell. This revealed a slightly fishy smell. This agrees with the increase in the level of substitution of snakehead fish is the cause of the decreased level of preference for the smell of the resulting cracker products [42].

The drying temperature affects the flavor seasoning smell. The higher temperatures will increase the nutritional content of flavorings, especially amino acids in the protein [43]. It is also stated that the smell of a food ingredient greatly affects the reaction to the level of preference because the smell of food containing glutamic acid will trick the brain as if it has tasted something delicious [44]. In addition, the smell of the spices is also influenced by the spices used. The ingredients in spices have a strong smell and taste so the use of small amounts of these materials can generate a taste effect on food [45].

3.4.3 Texture

The score of panelists' preference for the flavor seasoning texture is shown in Figure 11.

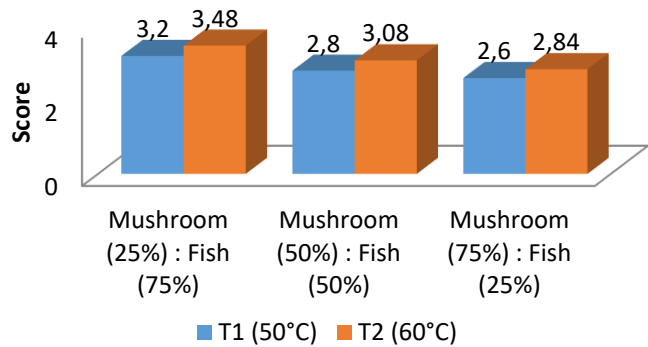


Fig. 11. The level of preference for the flavor seasoning texture.

The panelists' preference level for the flavor seasoning texture was in the range of 2.60 to 3.48 which was quite like-like. The highest score for texture was given to the material combination of 25% mushroom and 75% fish with a drying temperature of 60°C with a value of 3.48 in contrast the lowest score was for the material combination of 75% mushrooms and 25% fish with a drying temperature of 50°C with a value of 2.26. The Friedman test showed that the material combination and the drying temperature had a significant effect on the flavor seasoning texture. The higher the addition of mushrooms, the panelists' preference for the texture decreased. This is because the substitution of more mushrooms can still be felt in the granules of flavoring. This was in line with the results of the solubility test where the higher the substitution of mushrooms was the lower the solubility value because the more mushrooms added was more difficult the flavor seasoning to dissolve. Edible mushroom flour has a distinctive amylose structure, which results in a solubility value [26]. The high water content will produce large particles and can form clumps resulting in the texture of the flavoring being less smooth [32]. The processing of foodstuffs containing carbohydrates, proteins, and fats affects the appearance (smell, taste, mouthfeel, aftertaste, and texture (consistency, softness, elasticity, and crispness) [46].

3.4.4 Flavor

The score of panelists' preference for the flavor seasoning flavor is indicated in Figure 12.

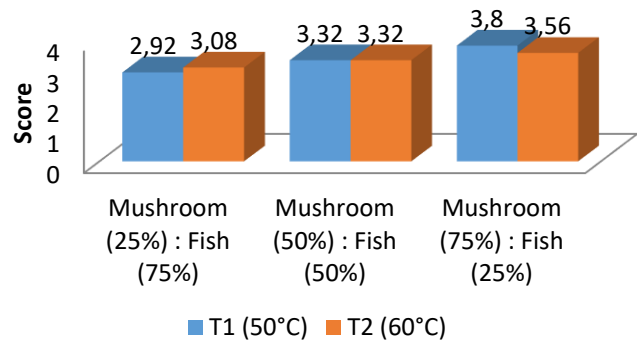


Fig. 12. The level of preference for the flavor seasoning flavor.

The panelists' preference level for the flavor seasoning flavor was in the range of 2.92 - 3.80 which was quite like-like. The highest score for flavor was given to the material combination of 75% mushroom and 25% fish with a drying temperature of 60°C with a value of 3.80 in contrast the lowest score was for the material combination of 25% mushroom and 75% fish with a drying temperature of 50°C with a value of 2.92. The Friedman test showed that the material combination and the drying temperature had a significant effect on the flavor seasoning flavor. The higher the addition of mushrooms, the level of preference of the panelists for the flavor seasoning increased. The flavoring of the straw mushroom dominates because it has a high savory value [47]. The drying of fish at a temperature of 50°C underwent fat oxidation resulting in a lack of taste [48]. Glutamic acid has a very important role because it can cause a delicious taste and increase the desired taste [41].

3.4.5 Overall

The score of panelists' preference for the flavor seasoning overall level is presented in Figure 13.

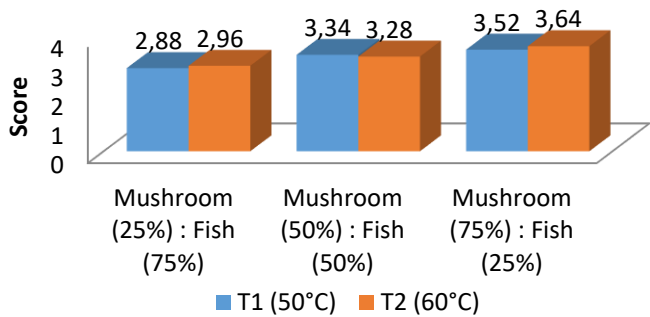


Fig. 13. The level of preference for the flavor seasoning overall score.

The panelists' preference level for the flavor seasoning overall score was in the range of 2.88 - 3.64 which was quite like-like. The highest score overall was given to the material combination of 75% mushroom and 25% fish with a drying temperature of 60°C with a value of 3.64 whereas the lowest score was given for the material combination of 25% mushroom and 75% fish with a drying temperature of 50°C with a value of 2.88.

The Friedman test showed that the material combination and the drying temperature had a significant effect on the flavor seasoning overall score. The overall preference for the flavor flavoring was influenced by all attributes that are tested for color, aroma, taste, and texture. As the addition of fish decreased, a higher level of preference overall was given. This was because the snakehead fish produced a slightly fishy smell.

In general, the panelists still liked the flavor seasoning composed of oil palm mushrooms and snakehead fish, but from Figure 13 it can be seen that the panelists preferred the flavor seasoning with the addition of more palm mushrooms.

4 Conclusion

The material combination of palm oil mushrooms and snakehead fish had a significant effect on the flavor seasonings solubility, protein content, and glutamic acid, the drying temperature affected significantly the flavor seasonings solubility and protein content, and the interaction of the material combination and the drying temperature gave significant effect on the flavor seasonings protein content and glutamic acid. The material combination as well as the drying

temperature had a significant effect on the panelists' organoleptic acceptance of the flavor seasonings' color, smell, texture, flavor, and overall. The higher snakehead fish produced the higher solubility value, protein content, and glutamic acid of the flavor seasonings but reduced the level of panelists' preference for flavor, smell, color, and overall category. The higher the temperature used resulted in the higher solubility level, and panelists' preference for color, smell, texture, flavor, and overall. The flavor seasonings' ALT microbiological level, coliform level, and molds/kamir level met the requirements prescribed by (INS 01-4273 1996). The panelists preferred the flavor seasonings of the material combination of 75% palm oil mushroom and 25% snakehead fish dried at a temperature of 60°C.

Acknowledgment is delivered to the Faculty of Agriculture and Agricultural Technology for financial assistance.

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