



Characteristics of Seasoning Powders from Nagara Bean Tempeh and Oyster Mushroom for Umami Enhancer to Reduce Salt-use

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Authors' contributions

This work was carried out in collaboration among all authors. Author SS designed the research of the paper. Authors SS and SG conducted the experimental work. Authors SS and HMAH analyzed the data and wrote the manuscript. Authors SS, SG and HMAH revised and approved the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Nagara bean tempeh and oyster mushroom have high protein, and free glutamate can increase the umami effect. The formulation is needed to obtain the desired umami level. The research aimed to formulate seasoning powders of Nagara bean tempeh flour, white oyster mushroom flour, salt, and sugarto enhance the umami taste.

Study Design: This research formulated seasoning powder using a randomized block design involving two treatment factors: 1) the concentration of salt, Nagarabean tempeh flour, and white oyster mushroom flour, and 2) the percentage of sugar.

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Place and Duration of Study: Laboratory of analytical chemistry and industrial environment, Department of Agroindustrial Technology, Faculty of Agriculture, LambungMangkura University, between June and November 2021.

Methodology: The seasoning flavor formulation includes the proportion of salt 50% to 20 %, and the remaining composition is the ratio of Nagara bean tempeh flour and oyster mushroom flour 50: 50. The addition of sugar 0%, 25%, and 30% is also studied in this formulation to determine the effect of strengthening the umami taste. The seasoning powder was analyzed by chemical and sensory analysis.

Results: Data analysis showed that the NaCl content in the seasoning powder decreased according to the reduced salt concentration in the seasoning formula. The proportions of salt: Nagara bean tempeh flour and oyster mushroom flour 20: 40: 40 produced the highest soluble protein content, total acid, and insoluble solids. Adding 25-30% sugar strengthened umami sensory test results more than the others. The results of salty taste scoring correlated with the proportion of salt in the formulation, where the salt ratio of 40%-50% was significantly not different.

Conclusion: Seasoning powder formulation using salt, Nagara bean tempeh flour, and oyster mushroom flour did not yet provide a strong umami effect. The umami effect should be enhanced by added sugar. Seasoning powder with slightly strong umami was obtained in the formulation of salt: Nagara bean tempeh flour: oyster mushroom flour by 20: 40: 40 with the sugar addition of 25%. According to the formula, salt consumption can be reduced by adding Nagara bean tempeh flour and white oyster mushroom flour in seasoning powder.

Keywords: Nagara bean; oyster mushroom; salt; sugar; seasoning powder; tempeh; umami.

1. INTRODUCTION

The challenges in the food industry to reduce salt use include the common use of NaCl salt, low price, and functional value in flavor and texture development [1,2]. The main effect of sodium is "palatability," which causes fewer food preferences due to less sodium, texture characteristics, and durability. Several strategies to reduce salt use were using less salt on food, mixing salt with other ingredients, applying finer salt and preservatives, using flavor enhancers, and spicing or adding commercial salt [3].

Flavor enhancers are essential in less flavorful food by providing the required appeal [4]. A flavor enhancer is a substance increasing the taste of other substances without adding its distinctive taste or new taste [5], and a flavor potentiator is a substance increasing the "perceived intensity" taste of another substance [6].

Food and food ingredients containing high-free amino acids or protein hydrolysates have been used in cooking for centuries to improve the sensory qualities of various food [7]. Umami flavors help boost food flavors by providing a savory taste. Glutamate is a flavor enhancer to rich a savory taste [8]. Monosodium glutamate, disodium 5'-inosinate, and 5'-guanylate are the most commonly used to provide umami components commercially available worldwide [9].

Inosinate is solely found in animal food products, while guanylate is only found in plants, especially mushrooms, with 10 and 150 mg/100 g concentrations. The most important thing to have a strong umami taste is the synergy of glutamate and nucleotides [5,10]. Fuke and Konosu [11] studied that the taste of crab can be duplicated with a solution containing specific amino acids (glycine, alanine, arginine), umami substances (glutamate and inosinate), and salts (NaCl and K₂HPO₄). However, if umami substances are removed, the crab taste will disappear.

Glutamate, one of the common amino acids found naturally, is present in many proteins and peptides. Glutamate is also produced in the human body and bound to other amino acids to form structural proteins [6]. When glutamate is tied to a protein molecule, it does tasteless and does not impart an umami taste to food. However, protein hydrolysis during fermentation, ripening, and heat of the cooking process will release free glutamate [12]. Food containing large amounts of free glutamates, such as tomatoes, mushrooms, and cheese, traditionally make savory dishes [13–15]. Several essential things need to be emphasized regarding consumer acceptance of products related to reducing salt; therefore, there is an urgent reformulation of seasoning with salt reduction, but it maintains product qualities. One way reformulation can be done is by reducing the salt's size and blending it with other ingredients

with flavor enhancer capabilities. Susi [16] stated that fermented Nagara beans with forging provided umami characteristics because they contained glutamic acid of 2.183% of their material weight. The presence of glutamic acid in Nagara beans was expected to be a flavor enhancer in the suitable formulation. Due to the increase in taste, the salt content and flavor enhancer will reduce the original salt content, and thus the sodium content will decrease.

The flavor enhancer formulation combines Nagara bean and white oyster mushroom flour. Mushrooms have a high glutamic acid content, which plays a role in causing the umami taste; therefore, it can be used as a flavoring agent. Glutamate is a non-essential amino acid that the body can synthesize independently. Glutamate in the free form will give an umami flavor; the higher the free glutamate content, the stronger the umami taste. During fungal development, the complete range of free amino acids and components of monosodium glutamate (including aspartic acid and glutamate) increased significantly from 36.11 to 60.18 mg/g of dry weight and between 11.20 and 26.21 mg/g, respectively [17].

Mushrooms contain high-free amino acids, which will affect the taste of food with a delicate taste, softening the sharp taste of some components. Likewise, combining amino acids will create a unique natural flavor combination. Mau [18] stated that the monosodium glutamate (MSG)-like component was produced by aspartate and glutamate components, while the content of dissolved sugars, polyols, and sweet ingredients could suppress the bitter taste. The research aimed to formulate seasoning powder by adding Nagara bean tempeh flour and oyster mushroom flour to salt, which can enhance the umami taste to reduce salt use.

2. MATERIALS AND METHODS

2.1 Materials

Nagara beans from Hulu Sungai Selatan, South Kalimantan, Indonesia, tempeh starter (Raprima), white oyster mushroom, commercial salt with brand Kapal (100 mesh), sugar (100 mesh), chemicals for analysis are AgNO₃

(Merck), K₂CrO₄ (Merck), NaOH (Merck), Phenolphthalein indicator (Merck), alcohol 96% (Merck), Folin-ciocalteu reagent (Merck), NaOH (Merck), NaCO₃ (Merck), CuSO₄ (Merck), Natrium K tartrate (Merck), oven Memmert, furnace Memmert, filter paper and glassware for analysis.

2.2 Methods

2.2.1 Production of tempeh (Rewritethis section with proper English and using short sentence)

Nagara beans were soaked in water at 25°C 1: 4 for 5 hours; then, the skin was peeled and cleaned. Cleaned beans were steamed for 10 minutes, drained, and cooled until 25°C. The beans were added tempeh starter of 0.2% and wrapped in plastic, and then fermented for 42 hours. The tempeh was sliced into 2 mm thickness and dried at 60°C for 48 hours. Using a dry grinder (Mitochiba CH-200, China), dried tempeh was crushed and filtered through a 100 mesh screen. The fine powder was sealed and packaged in polyethylene plastic before being kept dry and at room temperature (20°C) in a covered container.

2.2.2 Production of oyster mushroom flour

Oyster mushrooms were cut into 3 cm sizes and washed with clean water twice to remove dirt and foreign material. Oyster mushrooms were drained to reduce the remaining water. Mushrooms were treated by steam blanching at 100 C for 10 minutes, then drained for 30 minutes. The mushrooms were dried at 60°C for 24 hours, then dried mushrooms were grounded using a grinder (Mitochiba CH-200) and sieved through a 100 mesh. Fine mushroom powder was sealed in polyethylene plastic and kept dry, at room temperature (20 C), in a covered container.

2.2.3 Formulation of seasoning powder

The seasoning powder was formulated using salt, Nagara bean tempeh flour, white oyster mushroom flour, and sugar. The comparison of the proportions of salt: Nagara bean tempeh flour: and white oyster mushroom flour is as follows in Table 1.

Table 1. Formulation seasoning powder based Nagara bean tempeh flour and white oyster mushroom flour (rewrite this table, I didn't understand anything from it, Make a table with 4 column)

Salt: Nagara bean tempeh flour: white oyster mushroom flour	Nagara bean tempeh flour	White oyster mushroom flour	Sugar (%)
50	25	25	0
40	30	30	0
30	35	35	0
20	40	40	0
50	25	25	25
40	30	30	25
30	35	35	25
20	40	40	25
50	25	25	30
40	30	30	30
30	35	35	30
20	40	40	30

2.2.4 Characteristics of seasoning powder

Seasoning powder was tested including water content, ash content, total insoluble solids, total acid (titration method), NaCl content (Mohr method), soluble protein (Lowry method), and reducing sugar (DNS method). The sensory analysis applied a scoring test for salty taste, umami taste, and preference.

2.2.4.1 Water Content [18]

Weigh the empty cup (A) and add the sample as much as ± 2 g into the cup (B). Place the cup filled with the sample into the vacuum oven at 95°C-100°C, with an air pressure of not more than 100 mmHg for 5 hours, or put it into the non-vacuum oven at 105°C for 16 – 24 hours. Transfer the cup using a clamp into the desiccator for ± 30 minutes and then weigh it (C). Do a minimum duplo test (twice). Calculate the water content in seasoning as formula:

$$\% \text{ water content} = \frac{B-C}{B-A} \times 100(\%) \dots \dots \dots (1)$$

Where:

- A = is the weight of the empty cup (g);
- B = is the weight of the cup + initial sample (g);
- C = is the weight of the cup + dry sample (g)

2.2.4.2 Ash Content [18]

Weigh a sample of 3 to 5 g. The samples were drizzled for 5 to 8 hours at 550°C in a furnace

until white or gray. 30 minutes of cooling in a desiccator before weighing. Determine the sample's ash content.

$$\% \text{ ash content} = \frac{W2-W1}{W} \times 100(\%) \dots \dots \dots (2)$$

Where:

- W is sample weight (g),
- W1 is the weight of the empty cup (g),
- W2 is the weight of the empty cup and ash (g).

2.2.4.3 Titratable Acidity [18]

The sample is weighed between 2 and 5 grams, placed in an Erlenmeyer flask, and 100 ml of distilled water is added. Stir until evenly distributed and filtered with filter paper. 10 ml of the filtrate should be placed in an Erlenmeyer, and 2–3 drops of 1% phenolphthalein should then be added. 0.1 N NaOH was then used to titrate it. When a consistent pink color emerged, the titration was terminated. Determine the % titratable acidity.

$$\% \text{ Titratable acidity} = \frac{mL \text{ NaOH} \times N \text{ NaOH} \times MW \text{ dominant acid} \times DF}{\text{sample weight (g)} \times 1000 \times \text{valence}} \times 100 (\%) (3)$$

Where:

- DF = Dilution Factor
- Dominant Acid = Citric Acid (C₆H₈O₇) MW = 192, valence = 3

2.2.4.4 Total Insoluble Solids [19]

Solubility (dispersibility) was measured to measure the level of solubility in the resulting seasoning. The first step was to weigh the powder samples up to 2 grams (A), after which they were dissolved in 100 ml of distilled water and filtered through filter paper Whatmann no. 42. The filter paper was weighed after being dried at 105°C in the oven for 30 minutes prior to use. Following filtering, filter paper and leftovers are dried for three hours at 105°C in an oven before being weighed. Sample residual as total insoluble solids after drying (B). Calculate the percentage of the total insoluble solid:

$$\% \text{ total insoluble solid} = \frac{W2}{W1} \times 100 (\%) \dots\dots\dots(4)$$

Where:

W2 = residue after drying (g)
 W1 = initial sample weight (g)

2.2.4.5 Analysis of NaCl (Mohr method) [20]

Determination of sodium chloride base Mohr method. In principle, dry ash from furnace samples can be directly titrated with silver nitrate. The silver ions precipitate as silver chloride until the chloride ions are depleted, and the excess silver is measured with potassium chromate. The sample is weighed as much as 5 g and furnace to determine the ash content. The ash was placed to a 250 ml Erlenmeyer after being rinsed with the least amount of water feasible. Titrate with 0.1 M silver nitrate solution after adding 1 ml of a 5% potassium chromate solution. When the initial shade of hazy red appears, the titration has achieved its endpoint. The equation can determine the salt content is:

$$\% \text{ NaCl} = \frac{T \times M \times 5.84}{W} \dots\dots\dots (5)$$

Where,

T = titer
 M = molarity of silver nitrate
 W = sample weight (g)

2.2.4.6 Analysis of soluble protein (Lowry methods) [21]

Bovine Serum Albumin was prepared as a standard solution with a concentration of 300

g/mL. The standard BSA solution was then placed in a test tube to get the levels of 30 to 300 g/mL. 8 mL of Lowry B reagent was added to each tube, which was then vortexed and left to stand for 10 minutes. The absorbance was then measured at 750 nm after 1 mL of Lowry A reagent had been added, vortexed, and left to stand for at least 20 minutes. Weighing 1 gram sample, dissolving it in 50 mL of distilled water, vortexing it, and then centrifuging it at 3500 rpm for 10 minutes to separate the filtrate from the precipitate were the steps used to make the sample. A sample of 1 mL of the filtrate was used in the measuring procedure.

2.2.4.7 Analysis of reducing sugar (Dinitrosalicylic acid method) [22]

An amount of 0.2 mL sample was prepared and added with 0.2 mL of DNS solution, then boiled at 100°C for 10 minutes, cooled and added 5 mL of distilled water, vortexed, and then the absorbance was measured at 540 nm. A standard glucose solution was prepared at 1 mg/mL and varied at a 0.1 to 1.0 mg/mL concentration to obtain a standard curve.

2.2.4.8 Scoring test of sensory

Sensory testing was carried out using a scoring test on seasoning products. Semi-trained panelists of twenty people were asked to provide an assessment by giving a score on salty taste, umami taste, and preference for seasoning products. Panelists were asked to taste the seasoning 1 weak, 2 less strong, 3 sufficiently strong, 4 strong, and 5 very strong

2.3 Statistical Analysis

The experiment was conducted using a Randomized Block Design, and the quantitative data obtained were analyzed for variance (ANOVA); if there was a significant effect, the treatment was continued with the Duncan Multiple Range Test ($\alpha= 0.05$) and the qualitative sensory data was tested by Kruskal Wallis if it had a significant effect, then continued with the multiple comparison test.

3. RESULTS AND DISCUSSION

The formulated seasoning powder has a relatively low moisture content of less than 10%, and this is a consideration for predicting the potential for seasoning agglomeration because salt and sugar are hygroscopic. Table 2 shows

the moisture content of seasoning powder based on Nagara bean tempeh flour and white oyster mushroom flour within the range of 3.83–7.68% (wet basis). The water content of seasoning tends to be lower with increasing sugar concentration than when no additional sugar is applied. The water content significantly increases while the salt level decreases in the Nagara bean tempeh and oyster mushroom flour.

The interaction of the proportion of salt: Nagarabean tempeh flour: and mushroom flour with the ratio of added sugar significantly affected water content, ash content, and NaCl

content ($p < 0.05$). The measured ash and NaCl content correlated with the amount of salt added. Adding sugar will decrease the estimated NaCl content due to the increase in solids in the formulation. The single factor of salt proportion: Nagara bean tempeh flour: mushroom flour, and sugar proportion factor only affected total insoluble solids, titratable acidity, soluble protein, and reducing sugar ($p < 0.05$). The higher the proportion of Nagara bean tempeh flour and white oyster mushroom flour to salt, the soluble protein increases as well as in total insoluble solids and titratable acidity (Table 3).

Table 2. Duncan test results ($p < 0.05$) on water content, ash content, and salt content (NaCl) in the formulation of seasoning powder based on Nagara bean tempeh flour and oyster mushroom flour

Salt: Nagara bean tempeh flour: mushroom flour	Sugar (%)	Water content (%)	Ash content (%)	NaCl (%)
50:25:25	0	4.27±0.41 ^{ab}	47.90±0.97 ^g	44.00±1.94 ^g
40:30:30		6.05±0.88 ^c	38.87±0.70 ^f	36.53±0.91 ^f
30:35:35		6.24±0.37 ^c	29.99±0.00 ^d	26.44±0.27 ^d
20:40:40		7.68±0.68 ^d	20.92±0.02 ^b	18.14±1.91 ^b
50:25:25	25	4.08±1.07 ^{ab}	38.43±1.45 ^f	34.00±1.47 ^{ef}
40:30:30		4.37±0.07 ^{ab}	30.57±0.81 ^d	28.52±0.84 ^d
30:35:35		4.45±0.41 ^{ab}	23.89±0.31 ^c	21.44±0.54 ^c
20:40:40		6.02±0.74 ^c	16.27±0.42 ^a	14.55±0.14 ^a
50:25:25	30	3.83±1.29 ^a	36.14±0.15 ^e	33.19±0.37 ^e
40:30:30		4.15±0.91 ^{ab}	29.52±0.65 ^d	28.32±2.25 ^d
30:35:35		4.49±1.08 ^{ab}	23.11±0.56 ^c	20.63±0.23 ^{bc}
20:40:40		4.98±1.07 ^b	15.13±0.35 ^a	13.11±0.12 ^a

Data with different letters in the same column indicated different Duncan test results ($p < 0.05$)

Table 3. Duncan test results ($p < 0.05$) on the parameter of undissolved solid, titratable acidity, soluble protein, and reducing sugar in the formulation of seasoning powder based on Nagara bean tempeh flour, and oyster mushroom flour

Treatments		Undissolved solid (%)	Titratable acidity (%)	Soluble protein (mg/g)	Reducing sugar (mg/mL)
Proportion salt:tempeh flour:mushroom flour	50:25:25	34.31±7.26 ^a	0.59±0.13 ^a	96.06±9.74 ^a	6.44±1.64 ^a
	40:30:30	40.73±7.57 ^b	0.78±0.16 ^b	113.19±10.06 ^b	7.34±1.95a ^b
	30:35:35	44.59±6.48 ^c	0.83±0.14 ^b	129.24±12.76 ^c	8.88±3.29b ^c
	20:40:40	50.31±6.55 ^d	1.01±0.21 ^c	143.42±11.59 ^d	9.77±2.72 ^c
Sugar ¹	0	51.16±6.01 ^{b1}	0.96±0.24 ^{b1}	131.06±20.06 ^{c1}	6.48±1.32a ¹
	25	38.77±6.96 ^{a1}	0.74±0.16 ^{a1}	111.99±16.31 ^{a1}	7.09±1.01a ¹
	30	37.53±6.41 ^{a1}	0.71±0.17 ^{a1}	118.39±23.28 ^{b1}	10.75±2.78 ^{b1}

Data with different letters in the same column at the same treatment row indicated different Duncan test results ($p < 0.05$)

Table 4. Sensory characteristics data of seasoning powder formulated salt: Nagara bean tempeh flour: mushroom flour: sugar

Salt: Nagara bean tempeh flour: mushroom flour	Sugar (%)	Umami taste	Salty taste	Preference (likely)
50:25:25	0	2.50±0.14	4.00±0.13	1.67±0.02
40:30:30		2.67±0.12	3.83±0.18	1.33±0.05
30:35:35		2.50±0.15	3.00±0.10	2.33±0.17
20:40:40		2.83±0.18	3.00±0.16	1.83±0.07
50:25:25	25	3.50±0.25	3.83±0.18	1.67±0.05
40:30:30		3.67±0.22	2.83±0.17	2.00±0.09
30:35:35		3.17±0.18	3.00±0.16	2.33±0.03
20:40:40		3.17±0.13	2.33±0.15	3.00±0.16
50:25:25	30	2.67±0.12	3.83±0.17	2.33±0.06
40:30:30		3.00±0.13	3.50±0.18	2.83±0.17
30:35:35		3.50±0.24	2.50±0.18	2.67±0.08
20:40:40		3.83±0.28	2.17±0.14	2.83±0.17

Note: 1= weak, 2= less strong, 3=strong enough, 4=strong, 5 = very strong

Sensory evaluation on the seasoning powder formulation was tested, including saltiness, umami taste, and preference level. The results of the score for saltiness correlated with the amount of salt in the formulation; the salt ratios of 40%–50% were significantly similar, and the salt proportion of 30% was only slightly different by 20%. The sweetness of the sugar disguises the salty taste of the formulation, hence the saltiness decreased with the addition of sugar. The average salty taste of overall seasoning powder formulations scored 3.15 (sufficient), with the lowest score of 2.17 (less) in the formulation of salt: tempeh flour: oyster mushroom of 20: 40: 40 with 30% sugar, while the salty score on strong taste level (score 4) in the composition of salt: tempeh flour: oyster mushroom at 50: 25: 25 without sugar. Table 4 presents the sensory characteristics of seasoning powder.

Better to present the result in a table form rather than figure. The table will be easy for readers to understand.

3.1 Chemical Characteristics

Moisture content is a crucial factor in seasoning powder products since, at high water concentrations, the seasoning would readily agglomerate and lose its flowability. The particle size's salt and sugar components will rapidly absorb water, which will produce clumping and shorten the shelf life of seasoning. Without additional sugar, the ratio of salt, tempeh, and mushroom flour has a greater impact on the seasoning's water content. Because the water content of oyster mushrooms was lower

(11.33%) and tempeh flour had a greater water content of 15.32%, there was a correlation between the proportion of tempeh and oyster mushrooms and the water content of the seasoning powder. Increasing the proportion of tempeh and oyster mushrooms was correlated to an increase in seasoning powder's water content because tempeh flour's water content was higher at 15.32%, and the water content of mushrooms was 11.33%.

The formulation of 50% NaCl with no added sugar had the highest ash concentration at 47.90%, whereas the formulation with sugar addition had a significantly lower ash content (Table 2) as well as a lower amount of salt due to the addition of tempeh and oyster mushrooms. Nagara beans have a low mineral level (2.68%), whereas mushroom flour often has a greater mineral content (5.68%). According to Chirang & Intarapichet [23], oyster mushrooms have an ash concentration of 5.81%. Copper, iron, and selenium are also present, along with calcium (20 mg/100 g), potassium (2700 mg/100 g), sodium (48 mg/100 g), and potassium [24]. The salt content in the formulated seasoning powder is still lower than the commercial seasoning salt content. Tahmaz et al. [25] examined seven distinct commercial seasonings. The results revealed that the salt concentration of the seasonings ranged from 41.89 to 59.43% and that the ash content ranged from 58.77 to 67.27%. SNI 01-4273-1996 specifies that a seasoning powder's maximum salt level is 65%, therefore the seasoning powder made with Nagara bean tempeh flour and white oyster mushroom flour still complies with the regulations.

The solubility of seasoning powder indicates some components of seasoning that can dissolve and correlate to the taste and umami flavor produced. The insoluble solids increased with the increasing proportion of tempeh and mushroom to salt. At the salt proportion of 20% with no added sugar, the number of insoluble solids is 58%, and 22% of the components of tempeh flour and mushrooms are soluble. The solubility of NaCl is 360 g/L while sugar is 2000 g/L at 25°C, which means the composition of tempeh and white oyster mushrooms mainly causes the insoluble solids. Tempeh flour and oyster mushroom have insoluble fiber components, so not all can be dissolved in water at 25°C. According to Stamets [24], oyster mushrooms contain fiber of 33.44 g/100 g with a total mass of carbohydrates of 56.33 g/100 g, while Oyetayo et al. [26] stated that dietary fiber in oyster mushrooms is 45.50%. The crude fiber content in Nagara bean tempeh is 12.09%, and carbohydrate (*by difference*) is 59.20%.

The titratable acidity in the seasoning powder formula was 0.48-1.18%, meaning it has a low edge. Organic acids come from tempeh flour and white oyster mushrooms. The increasing proportion of tempeh flour and oyster mushrooms can increase total acid. The overall amount of acids is noticeably reduced with the addition of sugar. Fresh Nagara bean tempeh has a comparatively high intensity of the taste sensations of bitterness, saltiness, umami, and sweetness. Their intensity of them may lessen as a result of the drying process used to make the tempeh. Fernandes et al. [27] detected some organic acid content in oyster mushrooms (*Pleurotus ostreatus*), including oxalic acid, quinic acid, citric acid, and fumaric acid, with the dominant content of citric acid 2.25 g/100 g.

Organic acids in tempeh are produced at the soaking process stage because it allows the growth of lactic acid bacteria-producing acid and fermentation. The pH of tempeh tends to increase, and it is caused by the development of molds in tempeh that hydrolyzed protein into peptides, amino acids, and even into ammonia which tends to neutralize acids on tempeh. Barus et al. [28] mentioned that the acidification process of tempeh occurred at the 24-hour immersion stage until there was a decrease in pH to 4.4-4.9, while lactic bacteria were found in tempeh. In the mold fermentation process by *Rhizopus* and *Aspergillus*, tempeh's pH increased with the fermentation process's length, thereby reducing the total acid in tempeh was needed [29,30].

The gravimetric method was used to determine the NaCl content, which positively correlated with the ash or mineral content. The amount of sodium chloride (NaCl) utilized is reflected in the salt content; at the most incredible salt composition of 50%, the NaCl content is 44.0%. The NaCl concentration declined by adding less salt to the tempeh and oyster mushroom flour. NaCl content ranges from 14.55 to 13.11% when added sugar is between 25 and 30%. The formulation has a 20% salt level and no added sugar, only having a NaCl content of 18.14%. Foods can have their salt level decreased to 30–40% by adding glutamate without losing their flavorful flavor. It has been suggested that by including mushrooms in food, salt intake can be decreased, or even eliminated, and the flavor of the mushrooms can serve as the seasoning [31]. In this study, Nagara bean tempeh flour was used instead of mushroom flour in seasoning powder to better enhance the umami taste of seasoning powder.

Soluble protein was obtained from the hydrolysis of proteins into simple molecules, peptides, and amino acids. Protein hydrolysis occurs in peptides and amino acids in the tempeh fermentation process. Free glutamate amino acid was expected to increase the umami flavor in the seasoning. Soluble protein in the seasoning formulation of no added sugar tends to be measured higher, soluble protein in the proportion of salt: tempeh flour: oyster mushroom flour 20:40:40 of 153.39 mg/g.

Nagara bean tempeh contains a soluble protein of 22.30 mg/g with an aspartic acid content of 0.663% and glutamic acid of 1.369% [16]. Similarly, according to Abeer et al. [32], the dominant amino acids in oyster mushrooms include asparagine 2.15 mg/kg, aspartic acid 2.02 mg/kg, and glutamic acid 3.06 mg/kg. At the same time, Chirinang & Intarapichet [23] stated that aspartic acid and glutamic acid content were 2.04 mg/g and 5.01 mg/g, respectively. Aspartic and glutamic acids provide umami flavor when in free amino acid conditions. The solid fermentation process will cause the hydrolysis of proteins to become peptides and amino acids, especially the release of glutamate and aspartate amino acids, making the taste of Nagara bean tempeh savorier. However, the reduced effect of umami in the seasoning powder formulation is thought to be due to the drying process in producing powder. According to Nur et al. [33], 4 peptides in soy tempeh had more than two glutamic acid residues. Glutamate residues show

that the peptide encourages tempeh's umami flavor. The molds in fermented soybeans may produce a variety of enzymes, including proteases, lipases, α -amylases, and glutaminases, and they may simultaneously degrade soy proteins to create peptides.

The sugar components in the ingredients, significantly reducing sugar and sucrose sugar, will give a sweet taste effect and, when combined with a salty taste, could strengthen the umami taste. Table 3 shows that increasing the tempeh and oyster mushroom flour ratio increased the reduced sugar content and positively correlated with the increasing proportion of sucrose. Table 2 illustrates that the increase in reducing sugar was high due to the effect of sucrose sugar, where sucrose was composed of glucose and fructose. Nagara beans contained several simple sugars, which include glucose in the range of 3.62-4.83 g/100g, fructose of 0.41-0.52 g/100g, and sucrose of 0.54-0.70 g/100g [34], while Stamets [24] stated that the sugar content in white oyster mushroom was 18.10 g/100g, while the glucose content in oyster mushroom (*pleuratusastreatus*) was 15.01g/100g [35].

3.2 Sensory Characteristics

The evaluation of the umami taste in seasoning powder formulations showed that formulas with tempeh flour and oyster mushroom proportions did not significantly affect the umami taste in seasoning powder, which will further strengthen the umami flavor by the presence of sugar. In the salt proportion of 40 and 50%, the umami taste was quite strong with the sugar addition of 25%, while at the salt proportion of 30 and 20%, it means that the ratio of Nagara tempeh flour: oyster mushroom flour is 35:35 and 40:40, the umami flavor strengthened with the addition of 30% sugar. Therefore, some efforts can be made to reduce salt levels with seasoning powder formulation at a salt ratio of 30 or 20%. According to Rangan & Barceloux [8], monosodium glutamate is a commonly used flavor enhancer combining sodium salt and glutamic acid, which will amplify the salty sensation of salt. Adding 0.38% monosodium glutamate to 0.4% salt can provide the same saltiness level as 0.8% NaCl.

The umami taste was mainly obtained due to the presence of glutamic acid and 5' nucleotides, as well as 5-nucleotides combined with L glutamate and L aspartate have a synergistic effect on the perception of umami taste [14,36]. Currently,

several peptides such as Gly-Cys-Gly (GCG), Glu-Pro-Glu (EPE), and Cys-Met (CM) also exert umami-strengthening effects [37]. It is known that Nagara bean tempeh contains the amino acids glycine 0.175%, cysteine 0.280%, proline 0.135%, and methionine 0.513% [16]. Oyster mushroom (*Pleurotusastreatus*) has monosodium glutamate (MSG) characteristics like 41.26 mg/g and EUC (equivalent Umami Concentration) value of 150.55 mg/g, and this is relatively lower than the type of *Baletusedulis* mushroom (EUC 1186.45 mg/g). The fermentation of tempeh increased the number of peptides that were identical to monosodium glutamate (MSG)-like. It has been demonstrated that seasoning from overripe tempeh, whether it is dried or made from fresh tempeh flour, has a higher glutamate content (14.5% and 15.9%) than fresh tempeh (12.6%) [38].

The preference test resulted in a balance of saltiness and umami taste. The average preference score was 2.24, which was instead like, with a tendency to a high score in the ratio of salt: tempeh flour: oyster mushrooms of 20: 40: 40, both with the sugar addition of 25 and 30%. The formulation of no added sugar was relatively low at the preference level because the relative saltiness was still quite intense, and the umami taste from the proportion of Nagara bean tempeh flour: oyster mushroom flour was not strong enough.

Umami's flavoring agent combined with a salt (sodium chloride), which increases the acceptability of many foods, can solve the problem of less palatable food with reduced salt content. Palatability can be maintained when reducing NaCl by adding glutamate [15]. Yamaguchi [14] stated that adding umami flavoring substances can increase the taste and reduce the desired salty taste. Glutamate contains less sodium than NaCl which allows for lower salt intake. The seasoning powder's salty flavor can be maintained and the amount of salt used can be decreased without affecting the powder's acceptability by using a 40:40 ratio of Nagara bean tempeh flour and oyster mushroom flour. Manabe [39-41], the flavor attributes of dried bonito stock (*Katsuwonuspelamis*) could improve saltiness and minimize the loss of palatability that would otherwise comply with from reducing the salt concentration.

4. CONCLUSION

The umami taste of the seasoning powder resulting from the formulation of Nagara bean

tempeh flour, white oyster mushroom flour, and salt is not strong enough. The enhancement of umami taste is obtained by adding an amount of sugar to the formulation. Drying on tempeh flour and mushrooms can reduce the umami taste of the material. The taste of umami is strong enough to be obtained from the salt: Nagara bean tempeh flour and mushroom powder at 20:40:40 with the addition of 25% sugar. The resulting umami flavor is correlated with the soluble protein content, which rises at a ratio of 40:40 between tempeh flour and oyster mushroom flour and is linear with its free glutamate concentration. According to the formula, using less salt in seasoning powder can be accomplished by including Nagara bean tempeh flour and white oyster mushroom flour.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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