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Abstract. The prevalence of hypertension in South Kalimantan based on the health development program in 2018 reached 44.1%, which means South Kalimantan is the province with the highest prevalence of hypertension in Indonesia. Excessive consumption of salt (sodium) can cause a direct impact on blood pressure: people with high levels of salt consumption have an increase in blood pressure. Efforts to reduce salt consumption must also pay attention to the level of acceptance by consumers. Seasoning reformulation should maintain the product taste. Among various seasoning reformulation technologies are reducing salt particle size and blending salt with other ingredients as a flavor enhancer. This study aimed to determine the roles of salt (NaCl) size and the addition of the Nagara bean tempeh flour and oyster mushroom formulation in enhancing the umami taste to reduce salt use. Reducing the salt particle size down to 100 mesh had not given any significant effect yet on the strengthening of the perception of salty and umami taste. On the other hand, the formulation of Nagara bean tempeh flour and white oyster mushroom in a ratio of 100:0 could give a strong perception of umami taste to the seasoning powder, which was no different from the formulation in a ratio of 90:10. In conclusion, the glutamate component contained in Nagara bean tempeh flour can be an alternative source of good umami taste.

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

1. Introduction

According to the statement of the World Health Organization (WHO) in 2018, 1.13 billion people in the world had hypertension, meaning that 1 in 3 people in the world was diagnosed with hypertension. The prevalence of hypertension in the population older than 18 years old was 34.1%, and the highest proportion was found in South Kalimantan (44.1%) [1]. Previous studies stated that high sodium consumption affected the incidence of hypertension [2][3] and that the consumption of salty foods was correlated with the incidence of hypertension, with the risk of suffering from hypertension being higher [4]. Reduction of dietary sodium reduced not only blood pressure and the incidence of hypertension, but also morbidity and mortality from cardiovascular disease [2].

The challenge faced by the food industry in reducing salt use lies in the fact that salt is readily usable and that it has functional value in flavor and texture development [5][6]. Efforts to reduce salt consumption must also pay attention to the level of acceptance by consumers. Seasoning reformulation with reduced salt must maintain the taste of the product. Among seasoning reformulation technologies are reducing the salt size and blending salt with other ingredients as a flavor enhancer.

Vegetable ingredients that contain glutamic acid can be used as flavor enhancers. The glutamic acid contained provides an enhanced taste and thus will reduce the use of MSG and salt. Glutamic acid plays a role in producing a distinctive umami taste and becomes a raw material for flavor enhancers. According to [7], umami is a distinctive taste that is influenced by glutamate and nucleotide compounds such as inosinate and guanylate in many food products that play a role in the palatability and acceptance of a food product. The characteristics of umami play a role in increasing the flavor in food by providing meaty and savory flavors.

[8] formulated a flavor enhancer as a substitute for MSG by using natural ingredients from several mushrooms, including oyster mushrooms, shiitake, and straw mushrooms. The results showed that mushrooms can serve as ingredients for natural flavoring because apart from being tasty and delicious, they are also safe for health. Oyster mushroom has the most prominent aroma and appearance and is the most promising because it is relatively cheaper than other mushrooms. Oyster mushroom is a consumption mushroom of the wood mushroom type which has a more complete nutritional content when compared to other vegetable commodities. It contains high proteins, vitamins, minerals, and fibers [9]. According to [8], oyster mushroom can be used as a flavor enhancer. This is due to the presence of glutamic acid in oyster mushroom at 21.70 mg/g.

Likewise, Nagara beans have high protein content and fairly complete amino acid composition. Nagara beans are a type of cowpea that grows endemic in the Hulu Sungai Selatan district of South Kalimantan. According to [10], Nagara beans in a tempeh fermentation process demonstrate umami characteristics, which come from the presence of glutamic acid in the amount 2.183%.

Research on the impact of reducing sodium chloride in a product on the sensory characteristics of the product and its acceptance by consumers is still scant. Some research suggests that a simple reduction in sodium chloride particle size can provide a way to reduce sodium overall. Optimizing the reduction of salt use can be done by reducing the size of the salt. Finer salt sizes will provide purity and faster dissolution. According to [11], partially dissolved salt crystals in the mouth are swallowed without giving a salty taste. Improving salt solubility can increase the salty taste, and it is conducted by reducing the salt particle size to increase the surface of the salt in contact with the solvent.

This research aimed to examine the formulation and quality of seasoning powder from blending Nagara bean tempeh flour, oyster mushroom powder, and microparticulate salt that could enhance the umami taste together.

2. Methodology

2.1. Materials

The materials used in this research included Nagara beans from Hulu Sungai Selatan, South Kalimantan, Indonesia, tempeh starter (Raprima), white oyster mushroom, commercial salt under the brand Kapal (100 mesh), sugar (100 mesh), chemicals for analysis, including $AgNO_3$ (Merck), K_2CrO_4 (Merck), and NaOH (Merck), phenophtalein indicator (Merck), 96% alcohol (Merck), Folin-Ciocâlteu reagent (Merck), NaOH (Merck), $NaCO_3$ (Merck), $CuSO_4$ (Merck), Natrium K tartrate (Merck), oven (Memmert), furnace (Memmert), filter paper, and glassware for analysis.

2.2. Methods

2.2.1. Production of tempeh

Nagara beans were soaked in water in a ratio of 1:4 for 5 hours, then the skin was peeled and cleaned. The Nagara beans were steamed for 10 minutes, and then they were drained and cooled, added with tempeh starter at 0.2% and wrapped in plastic, and fermented for 42 hours. The tempeh produced was sliced and dried at 60 °C for 48 hours, then grounded and filtered at 100 mesh.

2.2.2 Production of oyster mushroom flour

An amount of mushrooms was washed and blanched for 10 minutes, then dried at 60 °C for 48 hours. Crushed dried mushrooms were filtered at 100 mesh.

2.2.3. Formulation of seasoning powder

Seasoning powder was formulated using salt, Nagara bean tempeh flour, white oyster mushroom flour, and sugar. Seasoning formulations were carried out with variations in salt size treatment and in the ratio of nagara bean tempeh powder to oyster mushrooms. Fifty percent salt was formulated with 50%

composite of Nagara bean tempeh powder and oyster mushroom, and then 30% sugar of 100 mesh was added out of 100% of the entire formulation. The salt particle sizes used were 60 mesh, 80 mesh, and 100 mesh. The proportions for tempeh flour to oyster mushroom flour used were 100:0, 90:10, 70:30, 50:50, and 0:100.

2.2.4. Parameters of analysis

Each formula of seasoning powder was tested for chemical analysis, including water content, tritatable acid, ash content, NaCl content (Mohr's method), and soluble protein (Lowry's method). The sensory analysis applied a scoring test for salty taste, umami taste flavor, and preferences.

2.2.5. Analysis of data

The chemical data obtained were subjected to Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT), while the sensory data obtained were subjected to Kruskal-Wallis test and Multiple Comparison.

3. Results and Discussion

3.1. Chemical properties

The water content of the seasoning powder from Nagara bean tempeh and oyster mushroom powder refers to the Indonesian National Standard (SNI) for flavor enhancers. The water content of seasoning powder according to SNI 01-4273-1996[12] is a maximum of 4%. The moisture content of the seasoning formulated of Nagara bean tempeh and oyster mushroom powder ranged from 2.53% to 3.80%. This shows that the water content of the seasoning powder product was below the maximum limit of the SNI standard. An analysis of variance shows that the proportion of Nagara bean tempeh flour and mushroom had a significant effect ($p < 0.05$) on the water content of the seasoning powder. Duncan's test results are presented in Table 1.

Table 1. Duncan's Multiple Range Test (DMRT) for water content data (%)

Salt particle size	Proportion of Nagara bean tempeh flour to oyster mushroom					Mean
	100:0	90:10	70:30	50:50	0:100	
60 mesh	2.86 ±0.98	2.76±0.77	2.91±0.65	3.21±0.71	3.73±0.30	3.09±0.40
80 mesh	2.65±0.71	2.87±0.79	3.01±0.85	3.31±1.09	3.58±0.23	3.08±0.37
100 mesh	2.53±0.78	2.71±0.86	3.12±0.33	3.20±0.22	3.80±0.17	3.07±0.50
Mean	2.68±0.17[a]	2.78±0.09	3.01±0.11a[b]	3.24±0.06[b]	3.70±0.12[c]	

Note: The same letters indicate that the Duncan's Multiple Range Test (DMRT) results are not significantly different

The higher the proportion of oyster mushroom flour the higher the water content in the seasoning powder formulation. The difference in water content was affected by the water content of the materials used; the water content of oyster mushrooms is higher than that of Nagara bean tempeh. [13] stated that the water content of oyster mushrooms is 88.75%, while the water content of Nagara bean tempeh is 65.11%[10]

Besides the water content, we need to pay attention to the mineral and salt content in the seasoning powder. Ash content is closely related to inorganic or mineral components contained in a material. The analysis of variance (ANOVA) shows that salt particle size and the proportions of Nagara bean tempeh to oyster mushroom flour had significant effect ($p < 0.05$) on the ash content of the seasoning powder. The Duncan's test results on ash content of it are presented in Table 2.

Table 2. Duncan's Multiple Range Test (DMRT) for ash content data (%)

Salt particle size	Proportion of Nagara bean tempeh flour:oyster mushroom					Mean
	100:0	90:10	70:30	50:50	0:100	
60 mesh	39.78±1.90	39.44±1.28	39.96±1.00	40.76±0.86	41.10±0.82	40.21±0.70^{b}
80 mesh	39.61±2.05	38.42±0.35	39.31±0.65	40.14±1.40	40.71±0.85	39.64±0.86^{a}
100 mesh	39.29±1.56	38.65±0.50	39.33±0.79	39.71±0.63	40.25±0.45	39.45±0.59^{a}
Mean	39.56±0.25^{b}	38.84±0.53^{a}	39.53±0.37^{b}	40.20±0.53^{bc}	40.69±0.43^{c}	

Note: The same letters indicate that Duncan's Multiple Range Test (DMRT) results are not significantly different

The finer the salt particle size the smaller the ash content produced. Salt is a crystalline mineral composed of sodium and chloride. Excessive sodium intake can cause hypertension, cardiovascular problems, and stroke. Therefore, there is a lot of consumer concern on these health risks, and the food industry is trying to minimize the salt content of food products [14].

The more proportion of oyster mushroom flour the higher the ash content produced. Oyster mushrooms contain relatively more mineral than Nagara bean tempeh. [15] stated that the nutritional content of white oyster mushrooms is 26.28–29.91% protein, 86.90–89.60% moisture, 0.48–0.91% fat, 19.64–22.82% fiber, 31.37–38.17% carbohydrate, and 5.18–6.39% ash. On a dry basis, according to Tolera and Abera, the mineral content of white oyster mushrooms is 9.76%. The mineral contents of oyster mushroom are 342–410 mg/100 g calcium, 1009–1133 mg/100 g phosphorus, 17–21 mg/100 g iron, 277–359 mg/100 g sodium, and 2088–2281 mg/100 g potassium.

The NaCl content of seasoning powder products ranged from 29.91% to 34.20% (Table 3). The analysis of variance (ANOVA) shows that the particle size of salt and the proportion of Nagara bean tempeh flour to oyster mushroom flour had no significant effect ($p < 0.05$). NaCl content according to SNI 01-4273-1996 is a maximum of 65%. This shows that the NaCl content of the flavor enhancer product is below the maximum limit of the SNI standard.

Table 3. NaCl content in the seasoning powder formulation (%)

Salt particle size	Proportion Nagara bean tempeh flour:oyster mushroom					Mean
	100:0	90:10	70:30	50:50	0:100	
60 mesh	32.07±1.85	31.01±0.89	31.83±0.93	32.06±0.67	29.91±0.33	31.38±0.93
80 mesh	32.56±1.34	31.05±3.21	31.70±3.22	31.82±2.22	30.74±2.07	31.57±0.71
100 mesh	33.08±1.71	32.02±2.61	32.34±1.74	34.20±1.95	31.88±0.76	32.70±0.96
Mean	32.57±0.50	31.36±0.57	31.96±0.34	32.69±1.31	30.84±0.99	

Note: The same letters indicate that Duncan's Multiple Range Test (DMRT) results are not significantly different

Salt particle size and proportion of Nagara bean tempeh flour to mushroom flour had no significant effect on NaCl content ($p > 0.05$). This suggests that the salt added to each formulation is the same even though the size of the particulates is different. This is in line with a previous study which stated that finer salt substitution at the same weight of micronized salt only gave a slight reduction in salt content but did not cause any significant changes in sodium content [16].

To describe the free amino acid content, soluble protein was measured. Protein solubility is interpreted as the proportion of the value of food protein that is soluble under certain conditions. According to [17], protein solubility is influenced by amino acid composition, molecular weight, and amino acid polarity. The environmental factors that affect protein solubility are pH, temperature, and processing conditions. The analysis of variance (ANOVA) shows that the proportion of tempeh flour to oyster mushroom flour had a significant effect ($p < 0.05$) on soluble protein levels. The Duncan's test results on soluble protein are presented in Table 4.

Table 4. *Duncan's Multiple Range Test* (DMRT) of soluble protein data (mg/g)

Salt particle size	Proportion Nagara bean tempeh flour:oyster mushroom					Mean
	100:0	90:10	70:30	50:50	0:100	
60 mesh	121.83±3.16	97.29±6.21	108.64±11.57	95.62±8.57	83.94±5.35	101.46±8.98
80 mesh	100.13±4.25	106.47±8.97	115.99±8.57	109.48±10.86	96.29±7.15	105.67±4.84
100 mesh	101.30±5.90	111.65±2.98	107.31±3.87	106.64±6.21	108.81±3.61	107.14±2.37
Mean	$107.15±7.63^{b}$	$105.14±4.54^{a}$	$110.64±2.92^{b}$	$103.91±4.57^{ab}$	$96.35±7.77^{b}$	

Note: The same letters indicate that Duncan's Multiple Range Test (DMRT) results are not significantly different

Soluble protein is obtained from the hydrolysis of proteins into simple molecules, both peptides and amino acids. In a tempeh fermentation process, protein hydrolysis occurs into peptides and amino acids. Nagara beans contain glutamate and aspartate amino acids which are quite dominant. If both amino acids are increased in free conditions, the umami taste of the seasoning will be improved. Aspartic acid and glutamic acid play a very important role in providing umami flavor when in free amino acid conditions. Nagara bean tempeh contains soluble protein of 22.30 mg/g, with aspartic acid content of 0.663% and glutamic acid of 1.369% [10]. [18] stated that the dominant amino acids in oyster mushrooms include asparagine at 2.15 mg/kg, aspartic acid at 2.02 mg/kg, and glutamic acid at 3.06 mg/kg, while [19] stated that the contents of aspartic acid and glutamic acid are 2.04 mg/g and 5.01 mg/g, respectively.

3.2. Sensory properties

Evaluation of food quality can be done by relying on sensory quality. The sense of taste in the oral cavity can evaluate the caloric content of the food consumed, detect the presence of salt, and protect us from ingesting toxic molecules [20]. Umami is a distinct taste, arising from the perception of amino acids, such as l-glutamate, and 5′-ribonucleotide. Likewise, there are other taste qualities such as fat taste, according to fatty acid taste [21], metallic taste, and kokumi taste, which are associated with long-lasting elasticity, thickness, and savory taste sensation [22].

Table 5. Umami scoring test of the seasoning powder formulation

Treatments		Score
Salt Particle Size	Proportion of Nagara bean tempeh:oyster mushroom	
60 mesh	100:0	3.25 ± 0.14^{a}
	90:10	3.38 ± 0.18^{ab}
	70:30	3.48 ± 0.04^{ab}
	50:50	3.60 ± 0.14^{ab}
	0:100	3.63 ± 0.04^{ab}
80 mesh	100:0	3.35 ± 0.14^{ab}
	90:10	3.65 ± 0.14^{ab}
	70:30	3.33 ± 0.11^{a}
	50:50	3.43 ± 0.04^{ab}
	0:100	3.38 ± 0.11^{ab}
100 mesh	100:0	3.80 ± 0.00^{b}
	90:10	3.55 ± 0.00^{ab}
	70:30	3.63 ± 0.04^{ab}
	50:50	3.50 ± 0.14^{ab}
	0:100	3.55 ± 0.14^{ab}

Note: The same letters indicate Multiple Comparison (Post Hoc Test) results that are not significantly different

The saltiness scoring scale shows values in the range 3.60–3.90. This indicates that flavor enhancer formulation was salty. Kruskal-Wallis analysis results show that the salt particle size and the proportion of Nagara bean tempeh flour to mushroom flour had no significant effect ($p < 0.05$) on the saltiness of the flavor enhancer formulation. According to [23], the salty taste sensation is caused by table salt or sodium chloride (NaCl). Sodium ions enter through ion channels in the apical microvilli, or through channels on the side of the taste cell, which will awaken the taste cell. Finer salt sizes can also increase the salty taste.

The results of the analysis of the umami taste scoring scale show values in the range 3.25–3.80. This indicates that the umami taste produced by the flavor enhancer formulation was quite strong. The Kruskal-Wallis analysis results show that the particle size of salt and the proportion of Nagara bean tempeh flour to mushroom flour had significant effects on the umami taste of the seasoning powder formulation. The umami taste characteristics of the seasoning formulation can be seen in Table 5.

The Multiple Comparison (Pos Hoc Test) test results show that the highest umami score was at 100 mesh salt size and the composite of Nagara bean flour and white oyster mushroom of 100: 0. The term *umami* comes from the Japanese language, meaning meaty or savory [24]. Protein is hydrolyzed during the fermentation process that liberates glutamate into free glutamate and gives an umami taste [25]–[27]. In addition, umami taste also interacts very strongly when mixed with sweet and salty taste [28].

The panelists assessed the savory taste at the level of 3.25–3.60, which means that the seasoning powder product was slightly likable in terms of the umami taste resulted. Smaller sodium chloride particles can promote higher levels of saltiness because they dissolve more quickly in the mouth. [29] evaluated the effect of different sizes and varieties of sodium crystals in potato chips on the perception of trained panelists, and the results showed that smaller crystals caused a faster, but less intense, perception of salty taste compared to larger crystals. Meanwhile, in this study, reducing the size of salt to 100 mesh had not been able to significantly increase the perception of saltiness. However, in combination with the composite of Nagara bean tempeh powder, it provided good strengthening of the umami taste.

4. Conclusion

Reducing the salt particle size down to 100 mesh had not given any significant effect yet on the strengthening of the perception of saltiness and umami taste. The highest soluble protein was found in the proportion of Nagara bean tempeh flour and oyster mushroom of 70:30, but insignificant difference was demonstrated by the proportion of 100:0. Meanwhile, the formulation of Nagara bean tempeh flour and white oyster mushroom at 100:0 could give a strong perception of umami taste to the seasoning powder.

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