



Characterization and Identification of Chemical Functional Groups in Oyster Mushroom (*Pleurotus Ostreatus*) Broth added with Vegetables and Spices

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Abstract

Adding the nutritional content of broth powder without destroying the distinctive taste of the broth can be done using pure mushroom extract and additional vegetables and spices. This study aims to determine the organoleptic characteristics and identify the chemical functional groups of glutamic acid in the best samples of oyster mushroom broth powder added with vegetables and spices. The results of the organoleptic test were tabulated in a table and then analyzed using a descriptive test. The organoleptic characteristics of the best color parameters were in treatment B1 which had a pale white color. The best treatment of aroma, texture, and taste parameters were found in the B2 treatment with a characteristic aroma of typical broth, fine powder texture, and has a distinctive taste of broth. The identification of the functional groups of secondary metabolites using the Fourier Transform Infrared (FTIR) instrumentation. Identification results with FTIR showed absorption at wave number (cm^{-1}): 3419.74, 2934.62, 1639.56, 1409.70, 1055.55. There is N-H, O-H, C-H, C=O carbonyl group and carboxylic C-O, which identify the presence of amide compound, phenol acid, alkane, aldehydes, amid I and carboxylic acid, which are known as glutamic acid in this sample.

Keywords: Organoleptic, Functional groups identification, Oyster Mushroom broth powder.

INTRODUCTION

Flavoring is a food additive that gives flavor to ingredients, so that a food can be sweeter, sourer, saltier, and more savory. Flavoring consists of 2 types, namely natural flavoring and synthetic flavoring. Natural flavor enhancers are made from plants and animals directly or through physical, microbiological or enzymatic processes. Meanwhile, synthetic flavorings do not exist directly in nature but are produced from chemical processes (Tamaya *et al.*, 2020), such as the production of broth powder which is a type of flavor enhancer. There are several types of ingredients used in powdered broth mixtures that are commonly consumed by the public, depending on the raw materials used. For example, the basic ingredients of beef, chicken or seafood are processed like making pure broth and then dried through a drum drying or spray drying process to form a powder (Swasono, 2011).

Commercial instant broth that is sold freely in the market is mostly added with substances of Monosodium glutamate (MSG) (Octaviyanti, 2017). According to (Innayah, 2019), MSG is a sodium salt related to an amino acid in the form of glutamic acid. It looks like white crystals which are stable but subject to degradation by strong oxidizing agents. Even though the use of MSG is permitted, excessive use of MSG can be toxic to health. Consuming MSG excessively can cause Chinese restaurant syndrome with symptoms of dizziness and

nausea (Butnariu & Sarac, 2019). Broth needs the addition of other ingredients to increase the nutritional content, especially natural fiber which is much needed to help digestive metabolism. Crude fiber is a component of vegetables efficacious to help digestion metabolism. The instant broth currently circulating in the market lacks of natural fiber. This can be seen from the composition of the ingredients and the nutritional content, which only features flavor enhancers (MSG and salt) which have the potential to cause hypertension due to the high salt and saturated fat cholesterol content (Swasono, 2011).

The use of mushrooms as a raw material for making broth can be used as solution for healthier life choices (Widyastuti, 2013). The content of glutamic acid in oyster mushrooms can act as a source of delicious and savory taste in food (Hidayah, 2019). And in terms benefits, according to (Ningsih, 2018), oyster mushrooms can prevent hypertension and heart disease, reduce weight and diabetes, act as antitumor agent, cure anemia, prevent and treat malnutrition, and also treat iron deficiency.

Making non-MSG powdered broth can be a solution for food that tastes good but also healthy. Apart from mushrooms, there are also some vegetables and rhubarb which also contain glutamic acid. For example, carrots, shallots, and pepper which sequentially have glutamic acid levels of 33g/100g, 18g/ 100g (Karjadidjaja & Idawati, 2009), and 0.012g/100g (Lisastiwanti & Haryanto, 2017). The use of additional ingredients such as vegetables and spices in making broth powder is done to add health benefits. This study aims to determine the organoleptic characteristic and to identify the chemical functional groups of glutamic acid in the best samples of oyster mushroom broth powder added with vegetables and spices.

RESEARCH METHODS

The materials used in this study including oyster mushrooms, carrots, celery leaves, onions, garlic, shallots, sugar, salts, and ground white pepper. The tools used in this study including spoons, knives, cutting boards, basins, blenders, dehydrators, dry spice grinders, organoleptic test questionnaires, and FT-IR machine. The design of this study was modified from the research methods of (Widyastuty & Atmaranti, 2020) and (Abidin *et al.*, 2020), then followed by a pre-research test. This type of research was a randomized block design (RBD), which consisted of 4 treatments and 3 replications to obtain 12 treatment combinations. Different treatments were found in the number of carrots, onions, shallots and garlic for each sample as novelty in this study, a comparison was made sequentially on samples B1 100:40:30:60, B2 80:60:40:50, B3 60, 80, 50, 40, B4 40:100:60:30. Each repetition amounted to 550 grams of sample so that the total treatment reached 1650 grams of sample.

Preparation of oyster mushroom broth powder is conducted by preparing all the ingredients needed for the broth seasoning, make sure that the vegetables used are fresh and have a good quality, clean all the ingredients, cut all the ingredients, then wash thoroughly. Furthermore, the material is weighed according to the composition starting sequentially of mushrooms, carrots, onions, garlic, shallots, and celery leaves as listed on research design. The next process is drying the materials using a dehydrator machine at 55°C for 8-10 hours. The drying process is carried out until the material texture looks like a crackers that is easy to break. After that, the dry ingredients are mixed using a blender until it becomes powder and then sieved.

Characteristics of feasibility / sensory acceptability of the sample can be known through the assessment given by several panelists. Sensory attributes such as color, aroma, texture, and taste. The organoleptic test is part of the product development stage, in which all panelists are untrained panelists. There are 25 examiners or panelists with different backgrounds of gender, ethnicity, social level, and education. The results of this assessment are subjective and relative according to each panelist.

Identification of functional groups was carried out using the FT-IR (Fourier Transform Infrared) instrumentation. FT-IR is a type of instruments that is widely used to determine the molecular vibration spectrum of the structure of chemical compounds. The vibration of a specific group shows certain results. Right at a wavelength of 2.5-50µm or at 4000-200cm⁻¹ is known as the mid-infrared type. The vibrations used for identification are bending vibrations, especially rocking, which is in the wave number region of 2000-400cm⁻¹. Each organic compound has a unique absorption, so that this is often referred to as the fingerprint region

RESULTS AND DISCUSSION

Sensory characteristics of oyster mushroom broth powder were analyzed using the analysis of organoleptic test results with 4 types of parameters, namely color, aroma, texture and taste. The results of the acceptability or organoleptic test are tabulated in a table and then analyzed using a descriptive test, as can be seen in Table 1.

Table 1. Results of The Organoleptic Test Analysis

Treatment	Organoleptic Parameters			
	Color Hedonic (Mean)	Aroma Hedonic (Mean)	Texture Hedonic (Mean)	Taste Hedonic (Mean)
B1	4.00	3.99	3.61	3.75
B2	3.88	4.01	4.01	3.71
B3	3.89	3.68	3.75	3.33
B4	3.72	3.64	3.68	3.13

Source : Modified from the research method by Abidin (2019)

Information:

Descriptive score of color characteristics: 1 = yellow-brown; 2 = yellow; 3 = white-yellow; 4 = off white; 5 = white.

Descriptive score of aroma characteristics: 1 = very typical of broth; 2 = not typical of broth; 3 = fairly typical of broth; 4 = typical of broth; 5 = very typical of broth.

Descriptive score of powder texture characteristics: 1 = very not smooth; 2 = not smooth; 3 = moderately smooth; 4 = smooth; 5 = very fine.

Descriptive score of taste characteristics: 1 = very not savory; 2 = not savory; 3 = quite savory; 4 = savory; 5 = very savory

Color Characteristics

The assessment of the organoleptic test results showed the highest average hedonic color, namely 4.00. In treatment B1, this score value lies in the pale white level. Treatments B2 and B3 have an average value of 3.88 and 3.89, this means that treatments B2 and B3 have a yellowish-white color. The lowest rating is in the B4 treatment with a value of 3.72, a white-yellow color characteristic which tends to be dark. Elements of a person's initial assessment of a food product can be seen in terms of color. Before other factors are considered, the color factor visually plays a very important role. Therefore, consumer acceptance of a food product often begins by looking at its color appearance.

The average results of color evaluation of the broth powder added with vegetables and spices can be seen in Table 1. Based on the results of the analysis of variance, the panelists' ratings of color ranged from 3.72 to 4.00, which is a level that tends to be 'liked'. The average broth color produced in this study was white-dark yellow. The result of maxing the white basic ingredients of powdered oyster moshrooms, then combined with the colors of other ingredients

such as shallots and carrots makes the oyster mushroom broth powder form an attractive yellowish-white color.

The difference in color of each treatment was influenced by different variations of giving carrots and shallots. According to (Rahmayani *et al.*, n.d.), carrots contain b-carotene and anthocyanin compounds. The formation of color variations in the broth powder occurred due to the percentage of added carrots and shallots which was different for each treatment. The color results are different from the results of Octaviyanti research (2017) which has a very green chicken broth powder, thus it is less attractive to consumers. The average results of all treatments were at a sufficient level to be liked by the panelists, namely 3-4. If it is pursued, the superior value is in treatment B1 in terms of color, which means that the panelists are more dominant in liking pale white broth powder. As for the B2 and B3 treatments, which if interpreted as an average panelist gave a white-yellowish color characteristic. And the value of treatment B4 which has the lowest value compared to the other treatments. It means that on average the panelists do not like white-yellowish broth powder. Instead, they like it to be dark in color.

Aroma Characteristics

The results of the organoleptic test showed that the hedonic value of the aroma parameter was the highest, namely 4.01 in treatment B2. In the score list, this value is at the typical broth category. On average, the results of the B1 aroma assessment have quite distinctive aroma characteristics of broth. Characteristics that are quite typical for broth are also present in treatment B3 and B4 which respectively have values of 3.58 and 3.64. The average results of the aroma assessment of oyster mushroom broth powder added with vegetables and spices can be seen in Table 1. The highest average value of the aroma parameter was found in treatment B2 with a characteristic aroma of broth. As for treatments B1, B3 and B4, in terms of the aroma of the treatment, it means that it has a fairly distinctive aroma of broth.

The organoleptic test results showed that the optimal treatment in terms of aroma was the B2 treatment. In a more detail, it had characteristics that were quite typical of broth but not strong. This treatment is in line with the percentage of vegetables and spices used so that the aroma produced is at an optimal level. Aroma is a chemical substance that is mixed in the generally at a very low concentration, with which humans perceive through the process of smelling. Aroma can be perceived as pleasant or unpleasant odors, with relative sizes for each person. The term fragrance or aroma is commonly used especially in the food and cosmetic industries. Aroma is one of the parameters that determines the good taste of a food (Tamaya *et al.*, 2020).

The spices used in this study consisted of garlic, shallots, onions and pepper, while the dominant vegetable was the addition of carrots. The aroma that arises is usually the result of a mixture of different, sometimes many smelling compounds. Materials or compounds added to food to improve taste are usually substances that contain volatile compounds. According to (Rasman *et al.*, 2018), the volatile compounds used are generally obtained from herbs and spices. The pungent aroma of this broth powder is due to the presence of piperine and piperanine substances in pepper (Abidin *et al.*, 2020). The aroma that arises from this broth powder is from a high proportion of garlic (Srihari *et al.*, 2015). Treatment with a high proportion of garlic causes the aroma of the broth to be strong to slightly overpowering. The role of oyster mushrooms in this product in terms of aroma can be analogous to the smell of meat. Like the research conducted by (Wardani & Widjanarko, 2013) who made high-fiber imitation meat based on oyster mushrooms, due to the characteristics of the aroma, texture and taste of oyster mushrooms that resemble beef.

Texture Characteristics

The highest average texture parameter value, namely 4.01, was in treatment B2. This score value indicated the characteristics of a fine powder texture. Meanwhile, the average value of treatment B1 and B3 has a fairly smooth texture characteristic. The relatively smooth texture characteristic also exist in the B4 treatment, but a more detail observation showed that the broth powder tends to be granular. Texture is an interaction caused by a food ingredient that can be distinguished by the sense of touch. One of the factors that determines the quality of food is texture. Hence, it also influences a person's interest to food (Rasman *et al.*, 2018). The texture of the broth powder added with vegetables and spices can be seen in Table 1. The types of texture sensors that can be assessed by fingertips include wetness, dryness, hardness, smoothness, roughness, and oiliness.

The highest texture parameter assessment result was the B2 treatment which was at the level preferred by the panelists. This means that the average panelist like the smooth texture of the broth powder. While the average texture score of treatment B1 and B3 showed that the broth powder has a fairly smooth texture. Furthermore, for the average score of treatment B4 showed that the broth powder texture is quite smooth but slightly granular. The difference in the texture characteristics values obtained might occur due to suboptimal sieving process and differences in the percentage of vegetables and spices used. The properties of food products or objects which include crispness, hardness, elasticity and smoothness greatly determine the level of panelist acceptance of the product (Kereh *et al.*, 2022). Based on this assessment value, it showed that the panelists preferred fine-texture broth powder, similar to the texture characteristics of the B2 treatment. The use of oyster mushroom broth powder as a basic ingredient helps to get the perfect texture, which is crunchy. Meanwhile, onions are used for functional ingredients such as thicker, stabilizers and gelling agents. The addition of high levels of onions in the B4 treatment made the characteristics of the broth powder less refined.

Taste Characteristics

The assessment of the organoleptic test results showed the highest average hedonic taste, namely 3.75 in treatment B1. This score value shows the characteristics of a savory taste. Treatments B2 and B3 have an average score of 3.71 and 3.33, which means that the two treatments have quite savory taste characteristics. The lowest rating is in the B4 treatment with a value of 3.13 which has quite savory taste characteristics. Taste is the most important part of a food product as it is a determining factor for consumer acceptance. In assessing the food taste, the sense of taste is used more. Taste sensing is divided into 4 factors, namely salty, sour, sweet and bitter (Rasman *et al.*, 2018). The term savory/umami is caused by the presence of glutamate compounds and certain peptide compounds (Nadia, 2004).

The taste parameter in all treatments means that the taste of the oyster mushroom broth powder is at a fairly savory taste level. The difference in the value of the taste characteristics obtained might occur due to the difference in the percentage of vegetables and spices used in the broth powder. The highest concentrations of added dried vegetables and spices sequentially were in B1 (74.8g), B2 (73g), B3 (70.55g), and B4 (69.3g). The results of subjective observations by researchers indicate that the characteristics of B2 are the optimal mixture composition. Base on the evaluation of 25 panelists, on average, they chose treatment B2 as the best treatment. One of the panelists wrote comments and descriptions on the questionnaire that *'the taste of B2 which has a savory and salty taste is more palatable'*.

The taste of a food can come from the food itself and if it is treated or processed, the taste can be affected by the ingredients added during processing. The spicy taste in the sample comes from shallots, which are added because they are identical to the spicy taste sensation caused by contained capsaicin compound. The savory taste in the sample comes from white

oyster mushrooms, carrots, garlic, shallots, and white pepper because they contain glutamate amino acids. Naturally, the glutamate content is 33g/100g in carrots, 18g/100g in shallots, and 0.012g/100g in white pepper. According to Nadia (2004), savory taste can be caused by the presence of glutamate compounds that form umami flavors which are used as a delicious taste enhancer.

Chemical Functional Group Identification

Results of identification of functional groups of secondary metabolites of the best samples of oyster mushroom broth powder *Pleurotus ostreatus* (The B2 Treatment) using FT-IR can be observed in Figure 1.

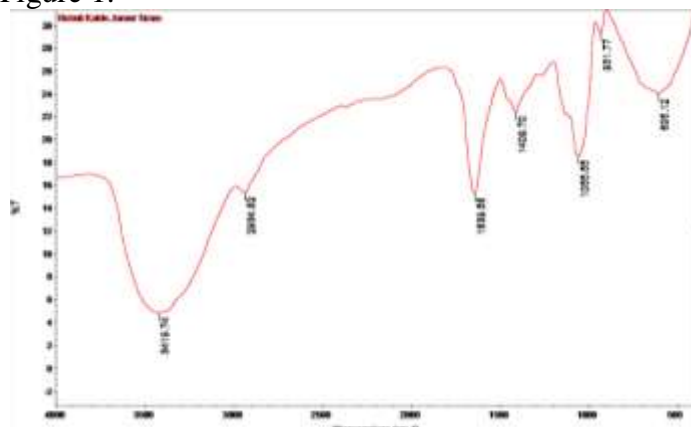


Figure 1. IR spectrum of the best sample of oyster mushroom broth powder

Based on the analysis of the infrared (IR) spectrum from the best sample of oyster mushroom broth powder *Pleurotus ostreatus*, there are several functional groups that show the characteristic of amides, acids, aldehydes, alkanes, types of amide I and carboxylic acid, as shown in Table 2.

Table 2. Specific functional groups at certain wavenumbers

Number wave (cm ⁻¹)	Wavenumbers (cm ⁻¹)	Intensity	Group Function	Compound Type
341	3500-	Sharp	N-	Amidies
9.74	3300	Strong	H	
293	2000-	Currentl	O-	Acid Compound / Phenol Alcohol (H Bonds)
4.62	3600	y	H	
293	2850-	Currentl	C-	Alkane
4.62	2970	y	H	
140	1340-	Weak	C-	Alkane
9.70	1470	Sharp	H	
163	1690-	Sharp	C	Aldehydes, Ketones, Carboxyl Acids, Esters
9.56	1760	Strong	=O	
163	1600-	Sharp	C	Amid I
9.56	1690	Strong	=O	
105	1030-	Sharp	C-	Alcohols, Eters, Carboxyl Acids, Esters
5	1050	Strong	O	

In the process of infrared spectroscopy, infrared light is passed through the sample and then the fraction that is absorbed in the wavelength range is measure to produce a spectrum that shows the qualitative information of the protein. The spectrum formed is used to see the intensity of the functional groups of glutamic acid compounds as derivatives of non-essential amino acid proteins. Based on the data in Table 2, wavenumber 3500-3300cm⁻¹ and 2934.62

cm^{-1} signifies the presence of acid/alcohol compounds and amide compounds sequentially having a strong and moderate sharp intensity. Alkane functional group at wavenumber 2934.62 cm^{-1} and 1409.70 cm^{-1} has a specific vibration for C-H. The alkane compound formed is at medium intensity and weakly sharp.

Aldehyde compounds from oyster mushroom broth powder *Pleurotus ostreatus* shows absorption at wavenumber 1639.56 cm^{-1} . Absorption numbers on these waves also indicate the presence of amid I (C=O) which is at a strong sharp intensity. In this case it shows the existence of glutamic band at a wavelength of 1055 cm^{-1} indicating the presence of a C-O group. The C-O functional group is included in a carboxylic acid (COOH) bond which is at a strong sharp intensity.

CONCLUSION

Based on the results of research that has been carried out on the organoleptic characteristics, the color parameters were in treatment B1 which had a pale white color. The best treatment of aroma, texture and taste parameters were found in the B2 treatment with a characteristic of typical of broth aroma, fine powder texture, and a distinctive taste of broth. The results of the identification of chemical functional groups of glutamic acid in the best sample showed the characteristics of the N-H group which means an amide compound, the O-H group which means an amide compound, the O-H group which means an acidic compound in H bonds, two C-H groups which mean alkanes, two C=O groups which mean aldehydes/ ketones/ carboxyl acids/ ester/ amide I and the C-O group which means alcohol/ ether/ carboxylic acid/ ester compounds.

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