Studies on Physicochemical and Sensory Characteristics of Overripe Tempeh Flour as Food Seasoning

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ABSTRACT

The process of making overripe tempeh flour is done with a variety of drying temperature that is at 55°C, 60°C, and 65°C. The flour is then analyzed the physical, chemical and sensory characteristics. This study also purpose to determine the effectiveness of drying temperature during process to produce the physicochemical and sensory characteristics are preferred. Overripe tempeh flour then analyzed the physical, chemical and sensory properties. Physical analysis was conducted on the yield), bulk density, starch solubility, and water absorption. Chemical analysis was conducted on the moisture content, ash content, fat content, dissolved protein content, reducing sugar content and antioxidant activity. Sensory analysis was conducted on the color, aroma, texture, and overall. Selected samples were analyzed further by HPLC and GC to determine the fraction of fat, protein and carbohydrates. The results showed that the overripe tempeh flour with the drying temperature of 60°C is more effective to produce the physical and chemical characteristics and preferred sensory. This process produces 28.10% yield, 0.53 g/cm^3 bulk density, 99.99% starch solubility, 1.96 ml/g water absorption, 8.45% water content, 4.03% ash content, 6.73% fat content, 4.55% soluble protein content, 2.07% reducing sugar content and 32.09% antioxidant activity This powder has a brown color, typical aroma is not too oppressive, and the texture is smooth and dry.

Keywords: Overripe tempeh, drying temperature, seasoning, physicochemical and sensory properties

INTRODUCTION

Tempeh is a traditional food that consumed by almost all people in Indonesia. It is made by natural culturing and controlled fermentation process. Tempeh can be made from various materials, but the popular is soybean tempeh. Although tempeh is made by the soy, it has a unique taste and is mildly flavor. Tempeh's fermentation process gives a higher content of protein, dietary fiber, and vitamins. It has a firm texture and an earthy flavor which becomes more pronounced as it ages. This process lasts for 24 to 36 hours at a temperature around 30^{o}C. If the process is carried out continuously it will produce overripe tempeh (Kiriakidis, 2005).

Overripe tempeh is made by extending the fermentation process. It usually lasts for 5 days (Andriani et al, 2010). An overpowering ammonia smell may accompany tempeh and gray or black patches of spores may form on the surface as it ferments. Overripe tempeh is popular on the island of java and used in small amounts as a flavoring/seasoning. Usually it used as a shrimp paste substitute. According to Shurtleff and Aoyagi (2001), 20% tempeh consumed in Central Java and East Java are overripe tempeh.

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During this time, overripe tempeh is used in a fresh form. In this form, overripe tempeh has a short shelf life and instability flavor. Therefore, it should be made an efforts so that seasoning of overripe tempeh can be easier to use and durable. One of these efforts is dry the overripe tempeh and made into flour.

Flouring process is divided into several stages that are drying, flouring, and sieving. This process will change the food properties. Drying process will reduce the moisture content so that the product becomes more durable. Drying process can affect the physical, chemical and sensory properties of the product. It really depends on the drying temperature. Drying temperature can affect the compounds in the product (Muchtadi, 2008). Therefore, the drying temperature should be appropriate to obtain a overripe tempeh flour with good quality.

MATERIAL AND METHODS

Sample Preparation

The raw material used in this study in the form of overripe tempeh 1. Overripe tempeh is made by using SOP from Andriani and Fauza (2011). Soybeans as a main ingredient is soybean import from the U.S. by brand GCU USA Soybeans no.1 and yeast starter by Brand RAPRIMA from Indonesian Institute of Science. The process is generally the same as making tempeh i.e. sorting, washing, and boiling the soybeans for 25 minutes. Soybeans then soaked for 15 hours. Soybean then peeled and boiled again for 25 minutes. Soybeans that have been cold and dry then added a yeast starter, wrapped in leaves and then incubated. Incubation carried out at a temperature of 30^{o}C for 120 hours (Andriani and Fauza, 2011). Overripe tempeh is already incubated then dried at 55^{o}C, 60^{o}C and 65^{o}C. Dry tempeh then milled using a 80 mesh sieve.

Type of Analysis

Overripe tempeh flour then analyzed the physical, chemical and sensory properties. Physical analysis was conducted on the yield (gravity), bulk density (Tien R M, 1992), starch solubility (Dedi F, 1992), and water absorption (Dedi F, 1992). Chemical analysis was conducted on the moisture content (Thermogravimetry), ash content, fat content (soxhlet), dissolved protein content (Lowry), reducing sugar content (Nelson Somogyi) and antioxidant activity (DPPH). Sensory analysis was conducted on the color, aroma, texture, and overall. Selected samples were analyzed further by HPLC and GC to determine the fraction of fat, protein and carbohydrates.

Statistical Analysis

Statistical analysis was conducted using a completely randomized design (CRD) with a single factor, drying temperature variations. The data then analyzed using Analysis of Variance (ANOVA) test and if there is a difference between the variation then proceed with the analysis of Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$ level of significance.

RESULTS AND DISCUSSION

Chemical Characteristics

Chemical analysis of overripe tempeh flour with the different drying temperatures can be seen in Table 1.

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Table 1. Chemical Characteristics of Overripe Tempeh Flour

Drying Temperature (°C)	*Analysis*					
	Moisture Content	*Ash Content*	*Fat Content*	*Dissolved Protein Content*	*Reducing Sugar Content*	*Antioxidant Activity*
55°C	10,42^{b}	2,58^{a}	8,20^{b}	4,25^{a}	1,79^{a}	27,77^{a}
60°C	8,45^{a}	4,03^{a}	6,73^{ab}	4,55^{b}	2,07^{b}	32,09^{b}
65°C	8,11^{a}	4,48^{a}	4,30^{a}	4,59^{b}	2,30^{b}	34,55^{b}

Numbers with similar alphabets showed no significant differences (P > 0.05) along the same column

Moisture Content

The moisture content can affect the appearance, texture, and flavor of the overripe tempeh flour. Moisture content is the amount of water contained in the material (Winarno, 2004). The moisture content of overripe tempeh flour is influenced by the drying temperature. The results show that the higher drying temperature then moisture content is getting down. The drying temperature of 60°C and 65°C did not show significant differences. The result is in standards by ISO Spices Flour (1998) for seasoning flour that is 12% (max).

Ash Content

The results of total ash content were not significantly different. Mineral content in the form of ash will increase with increasing temperature drying. Ash content of overripe tempeh flour that dried at a temperature of 50^{o}C is less than the other. This is because fewer components are decomposed as Darmajana statement (2007) that the higher of drying temperature will decompose the higher components and will affect the amount of ash content.

Fat Content

Fat content of overripe tempeh flour dried at a temperature of 60^{o}C is not significantly different from the flour that dried at a temperature of 55 and 60^{o}C. Fat content of overripe tempeh flour dried at 55°C significantly different with flour dried at 65°C. Drying at high temperatures can alter the composition of the fat organic molecules. According Muchtadi (1989), during heating and drying, fat can be broken so the fat content will decrease.

Dissolved Protein Content

Determination of dissolved protein content is using the Lowry method. Dissolved protein content of overripe tempeh flour with the drying temperature of 55°C is significantly different from 60°C and 65°C. The higher drying temperatures can increase the levels of dissolved protein content. Increasing of dissolved protein content of overripe tempeh flour significantly occurs at 55°C and 60°C. Increasing of dissolved protein content with temperature variations also occur in drying purple sweet potato flour (Apriliyanti, 2010). According to Utama (2010), protein content is influenced by moisture content. Increasing of dissolved protein content of flour that made with high temperature occur through the inhibition of hydrolysis of amino acids into volatile components with decreasing water content. The higher drying temperature then moisture content of overripe tempeh flour is getting down. The lower formation of ammonia, the nitrogen loss due to evaporation would be lower. Therefore, the dissolved protein content will increase with increasing drying temperature.

Reducing Sugar Levels

Determination of reducing sugar levels using Nelson-Somogyi method. Reducing sugar level of overripe tempeh flour with drying temperature of 55°C was significantly different with a drying temperature of 60 and 65°C. Reducing sugar levels will increase along with the increase in drying temperature. Increased sugar reduction reactions associated with Maillard Reaction. Maillard reaction is a reaction between reducing sugars with free amino acid groups contained in the soluble protein. According Mujianto (2009), an increase in Maillard products correlated with the levels of soluble protein. This means that the higher levels of soluble protein, the reducing sugar produced also increased. From the analysis it is known that increased levels of reducing sugars directly in line with the increased levels of dissolved protein levels.

Antioxidant Activity

Determine the antioxidant activity of overripe tempeh flour using DPPH method by absorbance at a wavelength of 517nm (Subagio *et al*, 2002). The result showed the antioxidant activity of overripe tempeh flour with different drying temperature varies between 27.77-34.55%. Antioxidant activity of overripe tempeh flour with the drying temperature of 55°C is significantly different from 60°C and 65°C. The higher drying temperatures obtained higher antioxidant activity. This is because the antioxidant compounds will not be damaged during the drying process (Pokorn'y and Jozef, 2001). This result was supported with Madrau (2009) which showed an increased antioxidant activity with increasing drying temperature during drying apricots and Utama (2010) which showed high antioxidant activity in dried spices Cabuk at higher temperatures.

Astawan (2003) stated that soybean as raw material tempeh contains isoflavones which are antioxidants. Isoflavones are the most prominent antioxidant that becomes dominant that can be measured in a test of antioxidant activity (Handajani, 2002). During the fermentation process, glucoside isoflavones (daidzin and genistin) hydrolyzed by glucosidase into aglycone forms (daidzein and genistein) were more active as an antioxidant. It also produced glisitein and factor II (6,7,4 tri - hidroksiisoflavon). The factor II has 3 times more antioxidant power than other aglycone. According to Wang and Murphy (1996) in Widoyo (2010), after 22 hours of fermentation, aglycone isoflavones contained in soybean increased 6.5 times from boiled soybeans.

Physical Characteristics

Physical characteristics of overripe tempeh flour are shown in Table 2. Different drying temperatures have varied effects on the physical characteristics of overripe tempeh flour.

Table 2. Physical characteristics of overripe tempeh flour at variation of drying temperature

Drying Temperature (°C)	*Analysis*			
	Yield (%)	*Bulk Density (g/cm³)*	*Solubility (%)*	*Water Absorption (ml/g)*
55°C	29,40[a]	0,59[b]	99,9956[a]	1,39[a]
60°C	28,10[a]	0,53[a]	99,9972[b]	1,96[ab]
65°C	26,46[a]	0,52[a]	99,9972[b]	2,35[b]

Numbers with similar alphabets showed no significant differences (P > 0.05) along the same row

Yield

The yield of overripe tempeh flour with the drying temperature of 55°C was significantly different with others. Yield of overripe tempeh flour with the treatment drying temperature of 55°C, 60°C, and 65°C respectively are 29.40%, 28.10%, 26.46%. If the drying temperature is higher, the yield will be lower. According to Utama (2010), yield is influenced by the moisture content.

Bulk Density

Bulk density is the physical properties of materials are influenced by the size of the material and moisture content. The bulk density of overripe tempeh flour with variant of drying temperature decreased with increasing drying temperature. Bulk density at a temperature of 55°C were significantly different from the bulk density at 60°C, but the bulk density at temperatures 60 ° C is not significantly different from 65°C. Bulk density of overripe tempeh flour with the drying temperature variations ranging from 0.59 g/cm^3- 0.52 g/cm^3. According Wirakartakusumah *et al* (1992), bulk density of powder generally range between 0 0.3 to 0, 8 g/cm^3. Decrease in bulk density of overripe tempeh flour due to moisture content in overripe tempeh flour during the drying process decreases with increasing temperature of drying. Low of moisture content cause the flour becomes increasingly lighter in the same container volume so that bulk density decreased with increasing drying temperature (Apriliyanti, 2010).

Solubility

Solubility is an important physical property in the seasoning. This is due to seasoning are added to food products and undesirable shape intact. Seasoning is expected to dissolve completely in the food product. The results show that the higher of temperature used for drying, the solubility would be higher. Solubility of the overripe tempeh flour that dried at a temperature of 55^{o}C is significantly different with the solubility of the starch that dried at 60^{o}C. However, the solubility of flour that dried at a temperature of 60^{o}C and 65^{o}C were not significantly different. High solubility indicates that flour more soluble in water. According to Winarno (2004), solubility of flour is influenced by hydrophobic and hydrophilic groups on the amino acid.

Water Absorption

Flour water absorption increased with increasing drying temperature. The highest water absorption obtained on overripe tempeh flour dried at a temperature of 65^{o}C is 2.35 ml/g. Drying temperature gives an influence to the water absorption of overripe tempeh flour. This is related to a moisture content of overripe tempeh flour. The lower the moisture content, the water absorption will be higher. This is consistent with research Agustina (2008) who also stated that the size of the water absorption in the instant corn flour is affected by the moisture content of the material.

Sensory Characteristics

Sensory evaluation is using 30 panelists. The panelists for this sensory test were seller of sambal tumpang in Surakarta. Sambal Tumpang is one of traditional food use overripe tempeh as a main ingredient. Sensory evaluation of overripe tempeh flour is based on the preference test as food seasoning. The test results of sensory characteristics of overripe tempeh flour can be seen in Table 3.

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Table 3. Sensory characteristics of overripe tempeh flour at variation of drying temperature

Drying Temperature	*Parameter*			
	Color	*Aroma*	*Texture*	*Overall*
55°C	2,87[a]	3,07[a]	3,80[a]	3,47[a]
60°C	3,77[b]	4,03[b]	4,07[b]	4,17[b]
65°C	3,47[b]	3,40[b]	3,67[ab]	3,53[b]

Numbers with similar alphabets showed no significant differences ($P > 0.05$) along the same row

Color

Sensory evaluation showed that the color of overripe tempeh flour dried at 55°C has a light brown color, overripe tempeh flour dried at a temperature of 60°C has a brown and overripe tempeh flour dried at a temperature of 65°C has a dark brown color. Data showed that the color of the most preferred by the panelists is overripe tempeh flour dried by using a temperature of 60°C because it have high consumer preferences. But this value is not statistically significantly different from overripe tempeh flour dried at a temperature of 65oC.

Aroma

Aroma is one of the important parameters in the seasoning. The sensory evaluation showed that the level of preference of overripe tempeh flour dried at a temperature of 55°C significantly different from the flour dried at 60 and 65°C. The most preferred by panelist is overripe tempeh flour dried at 60°C. Overripe tempeh flour has a distinctive aroma like overripe tempeh that is good and not too overpowering. At a temperature of 55°C, the flour is less preferred because it has an overpowering aroma. At a temperature of 60°C, the flour is the most preferred because the aroma is good (not too overpowering) and the flour that dried at a temperature of 65°C is less favored because of its aroma have started to decrease. This is due to the reduced ammonia / alcohol contained in soybean flour rotten because of the evaporation process.

Texture

Evaluation of overripe tempeh flour texture is using the sense of touch (finger). The result show that the most preferred texture is overripe tempeh flour dried by using a temperature of 60°C. Panelists stated that overripe tempeh flour texture is smooth and dry.

Overall

This tests aims to determine the level of preference to overall sensory characteristics. From the evaluation, it is known that the overripe tempeh flour drying with temperature 60°C has most preferred sensory characteristics. It because overripe tempeh flour drying with temperature of 60°C has a color, aroma, and texture of the most preferred by the panelists. However, its value is not statistically significantly different with overripe tempeh flour dried at 65°C.

Determination of Selected Overripe Tempeh Flour

Determination of overripe tempeh flour is selected using the selection matrix of temperature drying process that most effective and preferred by physicochemical and sensory characteristics. The chosen of overripe tempeh flour then analyzed characteristics of carbohydrates, fatty acids and amino acids. Overripe tempeh flour selected for further evaluation is overripe tempeh flour dried by using a temperature of 60^{o}C. This is due to the overripe tempeh flour dried at the temperature has a good physical and chemical characteristics and also sensory characteristics preferred by the panelists.

Carbohydrate Identification of Selected Overripe Tempeh Flour

Identification of selected overripe tempeh flour is using HPLC (High Performance Liquid Chromatography) method. HPLC is separation method of sample components based on polarity, meaning that the components in the sample will separated by polarity properties of each component. With a pump, a liquid mobile phase flows through the column detector. Sample is inserted into the mobile phase by injections. In the column occur separation components because of differences in the strength of solute-solute interactions between the stationary phases. Solute interaction that not strong with the stationary phase will be out longer. Each mixture component out is detected by a detector and recorded in the form of chromatograms. HPLC chromatogram is similar to gas chromatography chromatogram, where the number of peak states the number component, while the area of peak states the concentration of the components in the mixture. Computers are used to control the HPLC system, collect and process the measured data. (Hendayana, 2006). HPLC analysis results of overripe tempeh bosok dried at 60^{o}C can be seen in Table 4.

Table 4. Carbohydrate Identification of overripe tempeh flour dried at 60^{o}C

No	*Type of Carbohydrate*	*Results*
1	Galacturonic Acid	3,25 %
2	Stachyosa	1,02 ppm
3	Maltosa	< 5,61 ppm
4	Lactulosa	< 3,59 ppm
5	Glukosa	3473,64 ppm
6	Manosa	< 7,32 ppm
7	Arabinosa	< 8,78 ppm

HPLC results showed that most of the carbohydrates contained soybean flour is galacturonic acid. Galakturonic acid is one of the pectin components. Pectin is a soluble fiber in tempeh besides gum, hemisellulosa, and lignin. Pectin in tempeh comes from soybeans, which is the main ingredient of tempeh.

Fatty Acid Identification of Selected Overripe Tempeh Flour

Fatty acid analysis was performed using gas chromatography (GC). These methods require sample preparation prior to injection into chromatographic instruments. Chromatography involves two phases, namely the stationary phase and mobile phase. The stationary phase is usually a liquid that is bound to the surface, while a mobile phase is solvent or an inert carrier gas. Compared to other methods, the GC has the advantages that are simple implementation, a short time process, having high sensitivity and the ability to separate. This method can be used even if the sample size is very little or complex mixtures (Yazid, E, 2005). Results of fatty acid component in overripe tempeh flour dried at 60^{o}C with GC method can be seen in Table 5.

Table 5. Fatty Acid Identification of overripe tempeh flour dried at 60°C

No	*Type of Fatty Acid*	*Results (%)*
1	Kaproic Acid	0,031
2	Lauric Acid	0,02
3	Miristic Acid	0,086
4	Pentadekanoic Acid	0,154
5	Palmitoleic Acid	0,174
6	Palmitic Acid	14,122
7	Linoleic Acid	82,909
8	Arachidonic Acid	0,446
9	Linolenic Acid	0,797
10	Euric Acid	0,044
11	Eicosatrinoic Acid	0,688
12	Lignoceric Acid	0,082
13	Nervonic Acid	0,441
14	Teracosanoic Acid	0,006

During fermentation process, fatty acids in tempeh have tendency to an increase unsaturation degree of the fat. Thus, number of polyunsaturated fatty acids (PUFAs) increase. In the fermentation process, palmitic and linoleic acid decreased slightly but oleic and linolenic acids increased (Linolenic acid is not present in soybeans). In 100 g tempeh, it contained 220 mg of omega-3 and 3590 mg of omega-6. Unsaturated fatty acids have the effect of decreasing the content of serum cholesterol, so it can neutralize the negative effects of plant sterols in the body. Many types of fatty acids were detected from samples. There are about 14 kinds of fatty acids of the 24 tested in overripe tempeh flour dried at 60°C. Overripe tempeh flour does not contain caprylic acid, capric acid, heptadekanoic acid, stearic acid, arachidic acid, eicosenoic acid, behenic acid, docoheksanoic acid and oleic acid. The highest fatty acids in overripe tempeh flour are linoleic and palmitic acid.

Amino Acid Identification of Selected Overripe Tempeh Flour

Identification of amino acid using HPLC method. There are 9 kinds of amino acids contained in overripe tempeh flour. The results of the identification of amino acid in overripe tempeh flour can be seen in Table 6.

Table 6. Amino Acid Identification of overripe tempeh flour dried at 60°C

No	*Type of Amino Acid*	*Results (ppm)*
1	L-Histidine HCl	1149
2	L-Threonin	1432
3	L-Arginine HCl	3376
4	L-Methionine	401.7
5	L-Valine + L Thryptophan	3014
6	L-Phenylalanine	2271
7	L-Isoleucine	1841
8	L-Leucine	3177
9	L-Lycine HCl	3492

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Soybean as raw materials has high protein content. During the fermentation, the proteins are degraded into amino acids. Amino acids in soybean bosok include L-histidine HCl, L-Threonin, L-Arginine HCl, L-methionine, (L-Valine L+Thryptophan), L-Phenylalanine, L-Isoleucine, L-Leucine, L-Lycine HCl. Amino acids that many found in overripe tempeh flour are L-Phenylalanine, L-Lycine HCl, L-Arginine HCl, L-Leucine, L-Valine and L Thryptophan while the lowest content of amino acid is L-methionine. According to Liu (2008), Soy protein contains complete essential amino acids with methionine as a limiting amino acid. Leusin, isoleucine, lysine, and valine are the highest amino acid contained in soybeans. These results are same with the chromatographic analysis that showed L-Lycine HCl as the highest amino aci3.25d and L-methionine as the lowest. The values for the valine and leucine are also quite high. The analysis also detects that arginine detected in the sample is quite high at 3376 ppm

CONCLUSION

There are three types of flour were analyzed. These three types of flour have different drying temperatures of 55, 60 and 65°C. The analysis results of physical, chemical and sensory showed that overripe tempeh flour dried at 60 °C is selected flour. Chromatographic analysis on overripe tempeh flour showed that the highest carbohydrat content is galacturonic acid at 3.25%, the highest fatty acid is linoleic acid at 82.909 % and the highest amino acid is lysine at 3492 ppm

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