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Sensory Characteristics of Seasoning Powders from Overripe Tempeh, a Solid State Fermented Soybean

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Abstract

Fresh tempeh and overripe tempeh were oven–dried and freeze–dried to produce tempeh powders. Freeze–dried tempeh powders maintained aroma while oven–drying of the same product increased color intensity of the samples. Overripe tempeh powders had higher levels of sourness, umami, bitterness, saltiness, and pungent aroma compared to fresh tempeh. Oven–dried overripe and fresh tempeh powders showed higher glutamic acid content (14.5 %, 15.9 %) compared to freeze–dried tempeh powders (13.9 %; 13.9 %) and the original tempeh cakes (12.8 %, 12.6 %), respectively. The results indicated potencies of fermented soybean for seasoning development.

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Keywords: Fermented soybean; overripe tempeh; seasoning; solid state fermentation.

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Nomenclature

SSF	Solid state fermentation
MSG	Mono Sodium Glutamate
S60	Oven-dried overripe tempeh powder
F60	Fresh tempeh powder
SFD	Freeze dried overripe tempeh powder
FFD	Fresh tempeh powder
d	day
h	hour
min	minute

1. Introduction

Production of flavor using solid-state fermentation (SSF) is a feasible option and also includes production, but not limited to food secondary metabolites, amino acids, organic acid, ethanol, single cell protein and enzymes[1]. SSF has generated scientific interest because the bioprocess has potential to successfully convert inexpensive agro-industrial residues, as well as plants, to the production of several valuable secondary metabolites, including compounds that influence food flavor[2,3]. Fermentation of tempeh, an Indonesian traditional food is a typical example of the application of SSF technology. Soy–derived products are historically and currently some of the most important foods in the Asian region where diets remain predominantly plant–based. Fermented soybeans have good potential to provide nutrients to humans. The fermentation of soybeans by *Rhizopus* moulds in tempeh production increases the vitamin content, reduces non–nutritional metabolites[4], serves as good source of protein, calcium, low saturated fat, folic acid and is low in sodium[5]. Soybean substrates have been used for the production of aroma compounds using SSF at laboratory scale. Table 1 shows a list of microorganisms involved in flavor compounds produced in the SSF using soybean as substrate.

Table 1. Production of aroma compounds at laboratory scale using solid-state fermentation[6-11]

Microorganism	Substrate	Flavour compound	Family compound
Zygosaccharomyces rouxii	Miso, soybean, rice koji.	HEMF (soy sauce flavour)	Furanone
Aspergillus oryzae	steam rice (koji)	1-octen-3-ol (flavour compound of mushroom and fungal flavours)	Alcohols, aldehydes, ketones
Ceratocystis fimbriata	Cassava bagasse, apple pomace, amaranth, soybean	(fruity aroma such as banana, pear, melon, apple, and lemon). Mainly ethyl acetate and ethanol. 16 different components	Alcohol, aldehydes, ester, ketones
Rhizopus oryzae	Tropical agro-industrial substrates (*Cassava bagasse*, apple pomace, amaranth, soybean)	Mainly ethanol (80 %). Others acetaldehyde, 1-propanol, ethyl acetate, ethyl propionate and 3-methyl butanol (3-MB)	Alcohol, aldehydes, ester
Bacillus subtillis (Natto)	Soybean	2,5-Dimethylpyrazine (2,5-DMP) and tetra-methylpyrazine (TTMP)	Pyrazines (nutty and roasty flavour),
Bacillus subtilis	Soybean	2,5-Dimethylpyrazine (2,5-DMP) and tetra-methylpyrazine (TTMP)	Pyrazines (nutty and roasty flavour),

Despite the popularity of tempeh due to its superior nutritional and functional properties, scientific data on overripe tempeh is scanty. Overripe tempeh (*tempe semangit*) is a term used for over–fermented tempeh with pungent odour and dark appearance; the fermented Indonesian condiment is commonly used in Javanese cuisine[12]. To consumers who may not be familiar with overripe tempeh, the characteristics of the product may be perceived as spoilage leading to loss of appetite (Figure 1). However, overripe tempeh is commonly consumed in Javanese households and the product is valued similar to fresh mature tempeh. Overripe tempeh is often added to Javanese traditional cuisines using chilli and/or coconut milk as basic flavor such as *oseng–oseng, sambel goreng, lodeh, gudeg*, and other[12]. Tempeh semangit is also commonly used as food ingredient in Javanese cuisine such as *sambal tumpang*, *sambel tempe bosok, bergedel, oseng–oseng tempe bosok*, and a snack known as *menjeng*. Most important roles of overripe tempeh in food are to enhance the flavor of the dishes and impacting a distinct, favourable odour[12]. Unique taste and odour of overripe tempeh has lead to the study of its potencies as condiment, which may add nutritional, safety and economic values of tempeh.

a. b. c.

Figure 1. Left to right: (a) Dry soybean, (b) Fresh tempeh (3 d mould fermentation), (c) Overripe tempeh (5 d mould fermentation)

2. Materials and methods

2.1. Materials

Soybeans were obtained from Ciputat, Tangerang, Indonesia; tempeh starter Raprima® was obtained from Rumah Tempe Indonesia, Bogor. Other reagents used were sucrose, caffeine, NaCl, monosodium glutamate (MSG), citric acid, hydrochloric acid (HCl), and acetonitrile. The equipment used were: conventional oven (Ariston, Australia), freeze dryer (Heto PowerDry PL9000)**,** moisture analyzer (Sartorius), High Performance Liquid Chromatography type Breeze 2 (Waters, United States of America), AccQtag column 18 (3.9 mm × 150 mm) and various standard glassware.

2.2. Fermentation of tempeh

The soybeans were soaked overnight to assist the removal hulls, and then boiled until cooked. The cooked beans were further soaked for 24 h to allow lactic acid fermentation to take place. Following fermentation, the beans were washed to remove the acid and hulls. The beans were then inoculated with mixed starter culture (Raprima® commercial tempeh starter) to allow mold fermentation to take place. Soybeans inoculated with starter mixture were packed into small perforated plastic bags with. The inoculated soybeans were placed at ambient temperature for three days to produce fresh tempeh as control and five days to ferment overripe tempeh. After fermentation, the products were stored at refrigeration temperature until required for use.

2.3. Drying of tempeh powders

Fresh tempeh was cut into thin slices (2 mm to 3 mm) for facilitate efficient drying. The tempeh slices were dried by the oven– and freeze–drying methods. Oven–drying was conducted at 60 °C[12] and freeze–drying was conducted at -80 °C. Drying temperatures were selected based on recommended soybean drying and vegetable dehydration[13]. Oven–dried overripe tempeh powder (S60) and fresh tempeh powder (F60), and also freeze–dried overripe tempeh powder (SFD) and fresh tempeh powder (FFD) were prepared by grinding the dried materials using a hammer mill.

2.4. Sensory evaluation

Sensory evaluation was done to describe the flavor of tempeh. Two sensory evaluation methods were employed, attribute difference tests and descriptive analysis[14]. In the attribute difference test, thirteen trained sensory panelists rated the intensity of basic tastes of tempeh powders (sweet, salty, bitter, sour) including umami. The results were plotted in a spider web diagram to compare the taste of each sample. In a separate sensory evaluation test, 36 common sensory panelists were conducted descriptive analysis using the free–choice–profiling test. The panelists evaluated the flavor of the samples using their own words. Each attribute described by the panelists was listed and the frequencies of the flavor perceived or detected by the subjects were plotted in bar chart.

2.5. Amino acid analysis

Hydrolysis of sample was done by measuring 0.1 g of sample into 5 mL of 6 M HCl and kept at 110 ºC for 24 h to break the protein into constituent amino acids according to AOAC 994.12. The sample was cooled and filtered through 0.45 µm Whatman filter paper to remove any remaining particles. The filtrate was injected into the HPLC and fractionated using gradient composition system (Acetonitrile 60 %, 1.0 mL · min^{-1} , 37 ºC, column 3.9 mm × 150 mm). The HPLC analysis separate various individual amino acids (Excitation = 250 nm, emission = 395 nm) (references). Amino acids react with ninhydrin reagent that detects amino acids. The amount of an amino acid standard is analyzed at the same time to quantify the amount of amino acid that may be present in the sample.

3. Result and discussion

The shelf life of overripe tempeh is limited 3 d, after which the product becomes unsuitable for consumption as it is already classified as spoiled tempeh with too tender texture, darker color, and stronger stink odor with the higher productions of volatiles such as ethenyl butanoic; 2-methyl-3-(methylethenyl)ciclohexyl ethanoic and 3,7-dimethyl-5-octenyl ethanoic[15]. The growing importance of overripe tempeh as food condiment and ingredient has lead to investigation to prolong the shelf life and increase the convenience of its use.

Two drying methods were used in the experiments, oven drying in 60 °C and freeze drying -80 °C, to produce overripe tempeh powders ad fresh tempeh powders, respectively. Comparison of the taste, aroma, and color of the powders showed that freeze drying method was able to maintain the aroma (Table 2) and brightness while heat treatment in the oven drying process increased color intensity of the seasoning powders (Figure 2).

Table 2. Sensory characteristics of dried tempeh powders

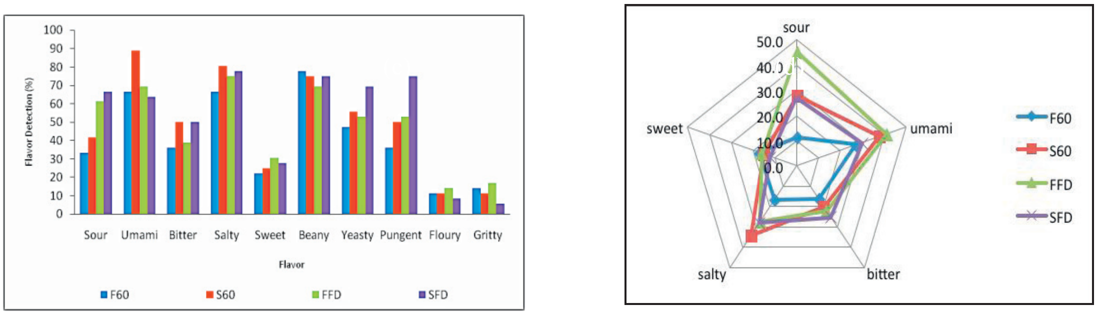
Sample	Intensity of taste	Intensity of aroma	Intensity of color
Oven dried fresh tempeh powder (F60)	Weak	Weak	Light
Oven dried overripe tempeh powder (S60)	Strong	Medium	Darkest
Freeze dried fresh tempeh powder (FFD)	Weak	Medium	Lightest
Freeze dried overripe tempeh powder (SFD)	Strong	Strong	Light

In this study, seasoning powders were developed from overripe tempeh and fresh tempeh (as control). In the analysis of overripe tempeh potencies as condiment, sensory evaluation was applied in the basic tastes and soybean related flavour perceived by human subjects, resulting in higher frequencies in the perception of sour, umami, bitter and salty tastes, and also pungent flavour of the overripe tempeh powder in comparison to fresh tempeh (control) (Figure 3).

The development of flavour in tempeh is due to the interactions of components of soybean with the solid food fermentation of *R. oligosporus* and the unit operations that are attached[16]. The mould fermentation itself will result in mushroom odour containing volatiles like 1-octen-3-ol and 3-octanone. During fermentation, soybean as the raw materials interacts with the mould and produce certain volatiles and amino acid that affects the flavour of the product, tempeh. Volatile compounds such as 3-hexanone, (E)-2-hexenal, 1-hexanol, and 3-octanone develop during early maturity stage, as well as hexanal, (E)-2-heptenal, (E)-2-octenal, ethanol, 1-hexanol, and 1-octen-3-ol has been detected under fermentation and a pleasant mushroom odour has been associated with tempeh[16].

Sensory and chemical changes during fermentation of over-ripe tempeh have been reported[17]. The study observed changes in sensory profiles of the fermented products including the tenderness of the texture and increased color intensity. Also, the aromatic substances increased and then decreased. During late fermentation stage of tempeh (50 h to 90 h after mould inoculation), mould growth declined and is replaced by bacteria fermentation resulted in the amino acid degradation to form ammonia and its unique pungent odour. The dominant aromatic compound of overripe tempeh was ammonia and its rancid and pungent odour[18]. Even though acid produced during soaking of the soybean may neutralize the ammonia produced by bacterial proteolytic enzymes, in later stage, however, the amount of ammonia increases in an unbalanced rate of the neutralization resulting in the unique odour[18]. During fermentation, chemical reactions continuously take place, thus altering the composition of volatile compounds at each fermentation stage. The rancid odour after 5 d and 8 d fermentation seems to be the effect of lipid hydrolysis by lipase enzymes resulting in short fatty acids production that undergo chemical esterification to produce the acids. As shown in Table 3, longer fermentation time produced more intense odour due to more production of simple compounds with higher volatility like ammonia[15]. Other than ammonia, the lactic acid bacteria also produce enzymes to degrade fat substances in soybean into volatile esters and short chain fatty acids[19].

Figure 2. Visual appearances of: (a) Oven dried fresh tempeh powder (F60), (b) Oven dried overripe tempeh powder (S60), (c) Freeze dried fresh tempeh powder (FFD), and (d) Freeze dried overripe tempeh powder (SFD)



(a) (b)

Fig. 3. (a) Free choice profiling and (b) Basic tastes of oven dried fresh tempeh powder (F60), Oven dried overripe tempeh powder (S60), Freeze dried fresh tempeh powder (FFD), and Freeze dried overripe tempeh powder (SFD)

Table 3. Sensory characteristics of *tempeh* after 2, 5, and 8 days fermentation[15]

Type of *tempeh*	Texture	Color	Odour	Volatile compounds
Fresh *tempeh*-2 days fermentation	Chewy	White	Fresh *tempeh* odour	Nonalal; α-pinene; 2,4-dicadienal; 5-phenyldecane; 5-phenylundecane; 4-phenylundecane; 5-phenyldodecane; 4-phenyldodecane; 3-phenyldodecane; 2-phenyldodecane; 5-phenyltridecane; and carrophyllene.
Overripe *tempeh*-5 days fermentation	Slightly tender	Light brown	Slightly stink odour	Nonalal; carrophyllene; 4-phenylundecane; 5-phenyldodecane; 4-phenyldodecane; 3-phenyldodecane, and 2-phenyldodecane.
Spoiled *tempeh*-8 days fermentation	Tender	Brown	Stink odour	ethenyl butanoic; 2-methyl-3-(methylethenyl)ciclohexyl ethanoic and 3,7-dimethyl-5-octenyl ethanoic.

According to the taste characteristics described by Tseng et al.[20], amino acids were grouped as monosodium glutamate-like (MSG-like) (aspartic acid + glutamic acid), sweet (alanine + glycine + serine + threonine), bitter (arginine + histydine + ileleusine + leusine + methionine + phenylalanine + triptophane + trypsine + valine) and tasteless (cystidine + lysine + proline). Alterations in the free amino acid composition are well connected with the flavor alterations during fermentation. These low molecular weight compounds are responsible for the flavor of soybean and soybean products [20-25]. The production of fermented seasonings from soybean and marine products are based on these dynamic processes[24-27].

Soybean fermentation has been shown to increase the MSG-like related amino acid glutamic acid (Glu)[27-28]. Kawai et al[24] reported that glutamic acid affects the chemical senses not only in the oral cavity but also in the gastrointestinal tract, and it modulates the ingestion, digestion and metabolism of proteins. Glutamic acid was found to be important for umami (savory) taste and has been used for savory seasonings around the world, although its taste is masked by flavors from fat or herbs. The results of this study confirm that fermented soybean (tempeh) is protein–rich, and can be alternative to meat. Glutamic acid was found to be the important substance for umami taste and has been used for savory seasonings around the world, although its taste is masked by flavors from fat or herbs. Oven drying at 60 °C increased glutamic acid composition in overripe tempeh powder (S60 15.9 %) and fresh tempeh powder (F60 14.5 %) compared to the freeze–drying method and its original tempeh cake (Table 4). The existence of glutamic acid as dominating amino acid in overripe tempeh powder indicates its potencies of the oven–dried tempeh powder as fermented soybean seasoning.

Processing of fresh tempeh and overripe tempeh into powder may have altered the composition of some AA such as glutamic acid, which is associated with umami taste, and also arginine and proline, which are associated with the bitter taste[26]. Despite changes in the amino acid amount due to the longer solid state fermentation, the fermentation period did not alter most of the composition of amino acid, except alanine, which is often associated with sweet taste (Table 4).

Table 4. Amino acid composition dried tempeh

Amino Acid	Tempeh	Semangit	F60	S60	FFD	SFD	Sensory attribute[26]
Aspartic Acid	13.09	12.64	13.29	13.39	13.16	12.56	Umami
Glutamatic Acid	**12.8**	**12.57**	**14.53**	**15.9**	**13.9**	**13.89**	**Umami**
Serine	5.26	5.31	5.84	5.61	5.73	5.44	Sweet
Glysine	4.77	4.9	4.8	4.8	4.78	4.93	Sweet
Histidine	3.3	3.48	3.54	3.19	3.56	3.55	Bitter
Arginine	6.65	6.88	8.14	7.22	7.56	7.03	Bitter
Threonin	4.24	4.37	4.66	4.52	4.79	4.68	Sweet
Alanine	4.97	5.72	4.62	5.23	4.76	5.96	Sweet
Proline	7.74	7.52	5.64	5.66	5.96	6.1	Sweet, Bitter
Valine	6.08	6.19	5.91	5.98	6.16	6.07	Bitter
Metheonine	0.65	0.8	0.67	0.99	0.73	0.77	Bitter
Isouleucine	5.89	6.07	5.81	5.76	5.99	5.71	Bitter
Leucine	9.42	9.6	9.18	9.11	9.38	8.96	Bitter
Phenylalanin	6.67	6.98	6.59	6.15	6.83	6.59	Bitter
Lysine	8.45	6.98	6.78	6.49	6.71	7.74	Sweet, Bitter
Total 15 Amino Acid	100	100	100	100	100	100	

Notes: Oven dried fresh tempeh powder (F60), Oven dried overripe tempeh powder (S60), Freeze dried fresh tempeh powder (FFD), and Freeze dried overripe tempeh powder (SFD)

4. Conclusion

Tempeh fermentation produces flavor compounds and nutritional and functional compounds. Extension of tempeh fermentation period increased volatile compounds that contribute to the unique taste of overripe tempeh.

Increasing glutamic acid composition and also the intensity of perceived taste and aroma of overripe tempeh powders showed their potencies as fermented soybean seasoning.

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