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Profile of aroma compounds and acceptability of modified tempeh

Kustyawati, M.E., Nawansih, O. and *Nurdjanah, S.

Department of Agro-Product Technology, Faculty of Agriculture, University of Lampung , Jalan Soemantri Brojonegoro No 1 Lampung, Indonesia 35145

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Abstract

Modified tempeh was prepared by the addition of *Saccharomyces cerevisiae* during the fermentation of soybeans for tempeh production. The sensory characteristics of modified tempeh were tested for their acceptability using hedonic test and descriptive test with Free Choice Profiling/FCP. The volatile compounds of tempeh were analyzed using GC-MS. The result showed that tempeh modified by the addition of 2% *S.cerevisiae* was acceptable by the panelists and had the scored of like for its color, flavor, aroma, and overall acceptability. These sensory characteristics were not different from those of non-modified tempeh. The raw modified tempeh had pleasant yeast/tapai aroma. This aroma was persisted after frying. On the other hand, the regular tempeh had beany aroma. The aroma compounds of modified tempeh were dominated by alcohol, ester, and aromatic group such as styrene, caryophyllene, phenol, and maltol; whereas, the aroma compounds of the regular tempeh were characterized by furan and sulfur. Nevertheless, both regular and modified tempeh contained 1-octen-3-ol, butanoic acid, and caryophyllene. Deeper and more comprehensive research for understanding the mechanisms of volatile compounds metabolism affected by the addition of *S.cerevisiae* is needed.

Introduction

Modified tempeh could be prepared from a tempeh of which its microbial characteristics has been modified through the addition of certain microorganism during fermentation, or the disruption of its original characteristic by using several treatments, in order to improve its characteristics. Tempeh is a kind of miracle food because it contains long chain non saturated fatty acid, vitamin B-12, D and E, phytoestrogen and other functional compounds (Klus *et al*., 1993 ; Keuth and Bisping, 1994; Wuryani, 1995; Nakajima *et al*., 2005; Watanabe, 2007; Boniglia *et al*., 2009). However, tempeh has unpleasant beany aroma which impart its acceptability (Nout dan Kiers, 2005).

Tempeh is one type of fermented soybean products. The fermentation of soybeans for tempeh production solely depends on the activity of *R. oligosporus*, However, some researchers reported that yeast can grow in the presence of bacteria and fungi during tempeh fermentation (Mulyowidarso *et al*., 1990 ; Feng *et al*., 2007; Kustyawati, 2009). Yet, the role of yeast on the tempeh fermentation is still unclear. In this research, tempeh was modified by the addition of *Saccharomyces cerevisiae* during soybeans fermentation. The addition of *S. cerevisiae* on tempeh production was expected to improve the aroma and to mask the unpleasant beany aroma which is dominant in regular tempeh. Yeast has been used for bread making, various fermented food and drink for a long time, partly because of its ability to produce enzymes and its contribution to the aroma of the fermented product (Fleet, 1990; Welthagen dan Vilijoen, 1999; Torrens *et al*., 2008). Other researchers also explained that the fermentation of food and beverages undergone by *S. cerevisiae* was characterized by the production of isoamylalcohol, and 2,3-butan. These compounds are responsible for the pleasant aroma (Romano *et al*., 2003 and Cordente *et al*., 2007). Therefore, the incorporation of yeast into tempeh fermentation could be expected to alter flavor by eliminating the unpleasant beany flavor of tempeh. This research was aimed to evaluate the change of sensory properties and aroma compounds generated by the modified tempeh.

Materials and Methods

The materials used in this experiment were American variety soybeans purchased from a local market in Bandar Lampung; starter used for tempeh preparation (Ragi) bought from Agency for Assessment and Application of Technology

*Corresponding author.
Email: siti.*nurdjanah@fp.unila.ac.id*
Tel: +6281272453460

(Badan Pengkajian dan Penerapan Teknologi/BPPT), Bandung Indonesia; pure culture of Saccharomyces cerevisiae bought from The Department of Agriculture Technology, University of Gadjah Mada, Indonesia; and various chemicals for aroma identification using GC-MS obtained from the Laboratorium of Flavor Analysis, Sukamandi, Indonesia.

Modified tempeh preparation

Modified tempeh was made by the addition of *S. cerevisiae*. Tempeh was prepared in laboratory according to the procedure described by Mulyowidarso *et al.* (1990) as follows: First, soybean (300g) was washed, and then soaked in clean tap water for overnight at room temperature (28°C). Then the process was followed by dehulling. The clean dehulled soybean was boiled for 30 minutes with the ratio of water and soybean was 3:1. The boiled soybean was drained, air-dried and inoculated with 0.02 g ragi for every 100 g cooked soybean. *S. cerevisiae* was added to the beans according to designated treatments. After that, inoculated soybean was packed into the perforated plastic bags (5x10 cm) and incubated for 48 h at 32°C. Three types of tempeh sampels prepared for this experiment were, (1) tempeh inoculated with ragi only, as a control, (2) tempeh inoculated with ragi and added *S. cerevisiae* at the concentrations of 1%, 2%, and 5% (w/w), and (3) tempeh inoculated solely with *S. cerevisiae*. All samples were prepared in duplo.

Sensory evaluation

Sensory evaluation of tempeh was conducted using Hedonic test followed the procedures explained in Meilgaard *et al*. (2000). Evaluation was performed by 50 semi-trained panelists who regularly consume tempeh. They were asked to evaluate both raw and fried tempeh. The parameters of raw tempeh included color, compactness, and aroma, whereas those for fried tempeh were texture, aroma and overall acceptability. The fried tempeh was prepared by frying the sliced tempeh in a deep frying pan containing palm oil for 5 minutes at 155 °C -170°C. The data were analyzed statistically using ANOVA to find the effect of the treatment, and then continued tested using the Honest Significant Differences (HSD) to find the most preferred tempeh. The most preferred tempeh then was further tested using Free Choice Profiling (FCP) to evaluate how the 50 panelists perceived and described the sensory properties of raw and fried modified tempeh as well as raw and fried regular tempeh, according to the procedure used by Guardia *et al.* (2011) with some modifications.

Aroma analysis

The most acceptable tempeh was selected and analyzed for its volatile compounds and compared to those of the regular tempeh. The samples were extracted using a head space solid phase micro extraction (SPME) method. Fifty grams of sample was put into an Erlenmeyer flask, sealed with aluminum foil with protective seal and fitted with a space solid phase micro extraction gastight syringe in such a way that the syringe would be directed to just above the food sample. The flask was placed in a water bath at 50°C with the syringe still be fitted. The volatiles released from the sample during 30 minutes at 50°C were absorbed onto SPME. The volatile collected from samples were analyzed using a HP 5890A gas chromatograph connected to a HP 5970 mass selective detector (Hewlett Packard). GC-MS was operated at 70 eV in the EI mode over the range 35-450 amu, column used was BP-5x column (30 mx0.25 mm) with 0.25um film thickness to resolve the volatiles (Supelco, Sigma-Aldrich Co.). Helium was used as carrier at flow rate of 1 ml/minute. The collected volatiles were thermally desorbed at 250°C for 2 minute after desorption, the oven was heated rapidly to 60°C and maintained at this temperature for 5 min before the temperature was increased at 5°C min-1 to 220°C (10 min). The constituents of samples were tentatively identified by matching their mass spectra with those recorded in the computer library (NIST98 and Wiley library).

Results and Discussion

Sensory analysis

The sensory qualities of tempeh were based on the whole appearance, color, and texture. The appearances of tempeh are the results of the growth of mycelia covering the whole tempeh. The mycelium growth produces the white color of tempeh. In addition. during the growth, the hyphae of the mycelia are kneading together to bind the beans to construct the compactness of the tempeh. The modified tempeh made by the addition of 1% and 2% of *S. cerevisiae* had the appearance of white color of mycelia which covered the whole tempeh and compact texture similar to those of regular tempeh. This indicated that *S. cerevisiae* was able to grow together with *R. oligosporus* during the fermentation of the tempeh making. However, *R. oligosporus* mycelia were only seen on a little part of tempeh when *S.cerevisiae* was added as much as 5%. This phenomenon indicated that high concentration (5% addition) of *S. cerevisiae* could retard the growth of *R. oligosporus*.

Table 1. Recapitulation of HSD test for color, compactness, aroma, texture, and over all acceptability of all treatments of raw tempeh and fried tempeh

	Raw tempeh			Fried tempeh			
Treatments	Color	Compactness	Aroma	Texture	Taste	Aroma	Overall acceptability
T_0	4.10^{ab}	4.02^{a}	3.66^{ab}	3.70^{ab}	3.78^{ab}	3.72^{b}	3.82^{b}
T_1	3.92^{b}	3.94^{b}	3.48^{b}	3.82^{a}	4.04^{a}	4.02^{a}	4.06^{a}
T_2	3.70^{b}	3.80^{b}	3.12^{bc}	3.56^{ab}	3.60^{bc}	3.58^{bc}	3.82^{b}
T_3	3.02^{c}	3.36^{c}	2.80^{c}	3.52^{b}	3.48^{c}	3.44^{c}	3.38^{c}
T_4	1.70^{d}	1.94^{d}	1.82^{d}	2.78^{c}	2.54^{d}	2.86^{d}	2.42^{d}

Note: Values followed by different superscript letters in the same column are significantly difference ($\rho < 0.05$). T0 = regular tempeh/control, T_1 = tempeh modified by the addition of 1% of *S. cerevisiae* T_2 = Tempeh modified by the addition of 2% *S. cerevisiae,* T_3 = tempeh modified by the addition of 5% *S. cerevisiae*, T_4 = tempeh fermented solely by 5% of *S. cerevisiae.*
Hedonic score: 1 = dislike very much, 2 = dislike, 3 = neutral (either dislike nor like), 4 = like, 5 = like very much

Hedonic test

Hedonic test was performed to evaluate the acceptability of modified tempeh and regular tempeh to the panelists. It was found that the color, the compactness and the aroma of the raw tempeh modified with 1 and 2% of S. cerevisiae were not significantly different, but different from those of regular tempeh. The aroma was scored around 3-3.6 (between neutral and like). In contrast to the fried tempeh, the results showed that the addition of 1% of *S. cerevisiae* to the tempeh resulted in the highest hedonic score of the the taste, aroma and overall acceptablility (Tabel 1). This was because the frying process of tempeh could generate various volatiles compounds which some of them might originate from the versatile growth of the mycela as well as *Saccharomycess,* and resulted in pleasant aroma preferred by the panelists. In addition, *S. cerevisiae* has a function of the conversion of carbohydrates into alcohols and other aroma components such as esters, organic acids and carbonyl compounds which have a great impact on the flavor of fermented food products. It was also found that inoculation of pure *S. cerevisiae* or in combination with other bacteria into rice, maize dough or into indigenous fermented foods such as "pito", "ogi" increased the organoleptic scores significantly (Jespersen, 2003; Adeniran *et al.*, 2012).

Descriptive sensory analysis: Free-Choice Profiling

The majority of the panelists described the raw modified tempeh as having yellow-white, white, and gray-white colors; dense, compact and integrated textures; typical tempeh, tapai/alcohol, pleasant, and yeasty aromas ; bitter, astringent, savory, sour, umami, and sweet taste (Table 2). On the other hand, the fried modified tempeh had a typically brown color, hard crispy, oily, alcohol and pleasant odor , and umami taste. It showed that raw modified tempeh had a typical tapai/alcohol aroma, of which might masked the beany aroma, and was persisted after frying. Tapai or tape is produced by fermenting cooked cassava using various moulds, yeast mainly *Saccharomyces* and some bacteria. Tapai, one of popular indigenous foods among Southeast Asia primarily Indonesia and Malaysia, has sweet or sour taste and is consumed as snack (Law *et al*., 2011).

The beany aroma was found in most fermented foods of soybean based. The beany aroma was generated by a mixture volatile compounds such as methyl-1-butanol, hexanal, 2,4-decadienal, dimethyl disulfide (Boatright and Lei, 1999; Blagden and Gilliland, 2005; Jelen *et al*., 2013). The bitter taste was also not described by the panelists in fried tempeh. This was possibly due to degradation or conversion of compounds responsible for bitter taste. The interaction between amino acids and dicarbonyl compounds during heating process of tempeh produced other compounds such as alkyl pyrazines. Bitter taste of tempeh could be caused by the present of peptide and amino acids in raw soybeans or the growth of bacteria in tempeh. The enzymatic hydrolysis of proteins frequently leads to the production of bitter taste caused by the hydrophobic peptides, as a result of the degradation product of proteolytic reaction (Leejeerajumnean

Table 2. Recapitulation of the description of color, texture, taste and aromas of raw and fried modified tempeh by the addition of 2% *S. cerevisiae*

Attributes	Descriptions	Frequency (%)		Average Intensity (I)	
		Raw	Fried	Raw	Fried
Color	yellow-white	90		6,4	
	white	30		5,7	
	gray-white	26		4,1	
	Yellow-brown		70		7,3
	Brown		24		6,1
	Golden yellow		12		6
Textures	dense	56	40	5,3	7,2
	compact	82	70	6,4	6,8
	integrated	30	30	6	4,7
	crispy	0	40	0	5,2
Aroma	fungi unpleasant	38	none	5,3	none
	beany	56	12	5,9	3
	specific tempeh	80	84	7,5	7.3
	tapai (alcohol)	6	8	4,3	5
	Raw soybean	48	none	6,2	none
	pleasant	12	50	6,8	6.8
	Yeast	10	10	5,8	5.5
Taste	bitter	32	none	3,6	none
	astringent	50	16	4,5	3.5
	savory	32	76	5,8	6.9
	sour	34	20	3,6	3.5
	Umami	14	56	5,3	7
	sweet	12	14	3,5	5.1
	bland	8	none	4,5	none
	salty	6	66	3	5.5

Note: Frequency = Relative frequency describing how many percent of panelists describing each particular description (number of panelists describing the same describtion / total panelists x 100%). I = Average of relative intensity of each description ranges from 0 to 10. The data given were the average of 2 replicates.

et al., 2001; Boue *et al*., 2005). Jelen *et al*. (2013) found that frying process of tempeh induced the increase of the main key odorants such as 2-acetyl-1-pyrroline, 2-ethyl-3,5-dimethylpyrazine, dimethyl trisulfide, methional, 2-methylpropanal, and (E,E)-2,4-decadienal. Qin and Ding (2007) found that arginine, glutamic acid, phenylalanine, leucine and lysine were the most free amino acids (FAA) induced the bitterness in Chinese fermented-soy Duchiba. Whereas, Barus *et al.* (2008) found that the bitter taste was found in tempeh containing abundance bacterial population.

The analysis of aroma compounds of regular tempeh and modified tempeh

The aroma profile of modified tempeh and regular tempeh were identified with GC-MS method. Some typical chromatograms are shown in Figure 1 and Figure 2. The compounds identified in both regular and modified tempeh were presented in Table 3. The results showed that the regular tempe generated 23 volatile compounds consisted of alcohol (7 compounds), ketone (2 compounds), furans (2 compounds),fatty acid (4 compounds), ester (1 compound), hydrocarbon (1 compound), sesquiterpenes (2 compounds), benzenoid (3 compounds), and sulfur containing compound (1 compound). Whereas 26 volatile compounds were identified in modified tempeh. They were alcohol (8 compounds), ketone (5 compounds), fatty acid (3

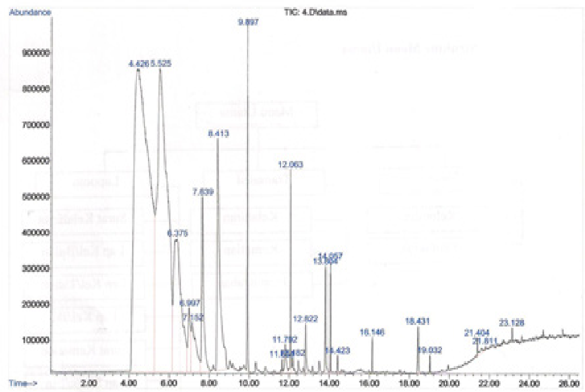

Figure 1. Profile of volatiles aromas SPME from regular tempeh.
Retention times and area in the chromatograms are mentioned in Table 3.

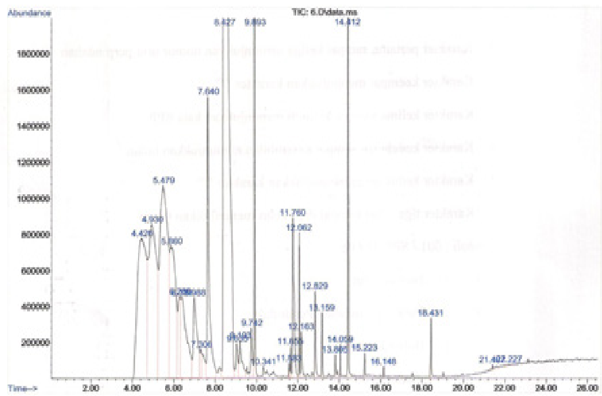

Figure 2. Profile of volatiles aromas SPME from modified tempeh by addition of 1% *S. cerevisiae*.
Retention times and area in the chromatograms are mentioned in Table 3.

Table 3. Volatile compounds of modified tempeh by the addition of 2% *S. cerevisiae* and regular tempeh

Modified tempeh			Regular tempeh		
Volatile compounds	Retentio n time	Area	Volatile compounds	Retention time	Area
Alcohol			**Alcohol**		
Etyl alcohol	5.5073	561109	Ethyl alcohol	5.5189	2938147
2-butanol	6.2529	237576	2-methyl-1-propanol	6.9847	1562462
2-pentanol	6.3107	118062	3-methyl-1-butanol	8.4103	9164302
1-propanol	6.3969	149215	2-methyl-3-buten-2ol	9.6205	70784
1-butanol	10.3379	459893	1-octanol	10.336	215578
1-octen-3-ol	11.5812	496971	1-octen-3-ol	11.5792	45210
2-metyl-1-propanol	6.9914	3998097	1-propanol	13.9187	11874
3-methyl-1-butanol	8.4188	1.080008			
Ketones			**Ketones**		
Butanone	5,4193	215295	3-hydroxy-3-methyl-2-butanone	7.1635	582048
2-octanone	8.2399	60946	3-hydroxy-2-butanone	9.7563	30555
3-hydroxy-3-methyl-2-butanone	10.505	86950	**Furans**		
3-hydroxy-2-butanone	9.7394	2785107	Dihydro-5-methyl-2(3H)-furanone	22.6851	11576
			Furan	9.0314	245625
Fatty acid			**Fatty acid**		
Acetic acid	11.7565	7894178	Acetic acid	11.7906	610766
Propanoic acid	12.828	2857683	Propanoic acid, 2-methyl-	13.1595	103775
Butanoic acid	13.9177	132104	Butanoic acid, 2-methyl-	14.4217	215257
			Butanoic acid	16.8845	15434
Ester			**Ester**		
Butanoic acid, 2-methyl-, ethyl ester	11.8079	1252389	Butanoic acid, 2-methyl-, ethyl ester	18.6874	12343
Propanoic acid, 2-methyl-ester	13.1568	2153178	**Hydrocarbon**		
Butanoic acid, 3-methyl-ester	14.4077	10107363	Undecane	9.1819	41399
Terpenoid			**Terpenoid**		
Caryophyllene	13.802	770245	Caryophyllene	13.8012	1851732
α-caryophyllene	14.743	7057	α-caryophyllene	147446	9130
Styrene	9.3397	256249			
Benzenoid			**Benzenoid**		
Phenol, 2-methoxy-	17.5388	143878	Phenol, 2-methoxy-	17.5404	48660
Phenylethyl alcohol	18.4296	2562389	Phenylethyl alcohol	18.4282	1034690
Maltol	19.6097	11634	Phenol	19.9436	80673
Phenol	19.9409	29321	**Sulfur containing compound**		
			Dimethyl disulfide	12.5851	1550

Note: The values given were the average of 2 replicates.

compounds), ester (3 compounds), Sesquiterpenes (3 compounds), and benzenoid (4 compounds). The volatile aroma compounds of the modified tempeh were dominated by alcohol, ester, and aromatic groups, while the regular tempeh contained of furan, undecane and dimethyl disulfide. Nevertheless, 1-octen-3-ol, a mushroom smelling compound originated from the beans was presence in both regular and modified tempeh.This finding was in agreement with Feng *et al.* (2007), found that the volatile compounds produced during soybean tempeh fermentation included ethanol, acetone, 2-butanone and 3-methyl-1-butanol as well as 3-octanone and 1-octen-3-ol. Even though, Jelen *et al.* (2013) found that key odorants in tempeh after five days of fermentation included pyrazine, sulfides, aldehydes and 8-carbon-alcohols and ketones. Compound of 1-octen-3-ol was originated from the conversion of linolenic acid in soybeans into C8 and C10 by the presence of hydroperoxide cleavage enzymes which cleavage of 13- and 9-hydroperoxide into C8 and C10 compounds (Feng *et al.*, 2007; Jelen *et al.*, 2013). In addition, α-caryophyllene and caryophyllene were identified in regular tempeh as well as modified tempeh in our experiment. It is likely that tempeh of soybean based fermented by *R. oligosporus* had the characteristic of having 1-octen-3-ol, butanoic acid, and α-caryophyllene. Caryophyllene is aromatic hydrocarbon which orignated from the soybeans and this compound is reported to be responsible for flavor in herbal spices(Shylaja and Peter, 2004). Feng *et al.* (2007) also detected a volatile sesquiterpenes, β-caryophyllene during *R. oligosporus* growth on soybean. Furans and hydrocarbon, identified in regular tempeh were not detected in modified tempeh. It is supposed that those compounds were either broken down, conversed to other compounds, or masked by the other volatile compounds because they were present in very low odor intensity. Damian *et al.* (2004) reported that dimethyl disulfide , one of the key odorant of tempeh fermentation of soybean based, was found at a very low odor intensity in fermented soybean curd. Sulfides are formed from the degradation of methionine to become methional which is a starting point for methanethiol formations, and subsequently yields dimethyl-disulfide, trimethyl, and tetramethyl-disulfide. Sulfides compounds did not decrease during frying of tempeh because it was attributed to its formation from S-methylmetionine during heating (Jelen *et al.*, 2013). In addition, Dajantaa *et al.* (2013) found that dimethylsulfides were noted to increase during the fermentation of thua-nao, a soy based bacterial fermentation. However, in this study , sulfides were not detected.

The componens of esters found in modified tempeh were butanoic acid, 2-methyl-, ethyl ester, propanoic acid, 2-methyl-ester, and butanoic acid, 3-methyl-ester , whereas butanoic acid, 2-methyl-, ethyl ester was the only ester compound in regular tempeh . This was true as the modified tempeh containing *S. cerevisiae*, of which is central in the production of aroma compounds during fermentation. In the wine making, esters are the most important yeast-derived aroma compounds produced during fermentation (Romano *et al.*, 2003; Cordente *et al.*, 2007). Esters are formed intracellularly in an enzyme-catalyzed reaction between a higher alcohol and an activated acyl-CoA molecules. Ester compounds imparted the aroma of fruity or flowery (Torren *et al.*, 2008; Pereira *et al.*, 2014). Modified tempeh also contained styrene compounds, an aromatic hydrocarbon, which was not identified in regular tempeh. Styerene or vynil benzene contributes to sweet smell (Dennis *et al.*, 2005).

It is likely that the variety of the volatile compounds identified in both types of the tempeh was responsible for aroma differences generated among those tempeh. The sensory analysis indicated that the aroma of modified tempeh was yeasty and the regular tempeh was beany. This demonstrates a complementary role of S.cerevisiae associated with tempeh quality through the synthesis of yeast-specific volatile compounds.

Conclusion

Raw modified tempeh had aroma of pleasant “yeasty tempeh” which was typical tapai or alcohol aroma. This unique aroma still persisted after the modified tempeh was fried, although it was at low intensity. Fried tempeh modified by the addition 1% of *S. cerevisiae* had the highest hedonic score for aroma and overall acceptability. This study shows that the use of mixed cultures of *R.oligosporus* and *S.cerevisiae* in fermentation processes could be a strategy to minimize the beany flavor of tempeh.

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