Sys Rev Pharm 2021;12(1):1437-1451
A multifaceted review journal in the field of pharmacy

The Potential of Refined Tempeh Hydrolyzated Flour as a Raw Material for Functional and Natural Cooking Spices in Indonesia

*Mujianto[1], Wignyanto[2], Indarwati[3]

[1]Agriculture Industry Technology, Wijaya Kusuma University of Surabaya, Indonesia
[2]Agro Industrial Department, Universitas Brawijaya, Indonesia
[3]Faculty of Agriculture, Wijaya Kusuma University of Surabaya, Indonesia

ABSTRACT

This study is aimed at finding out the potential of refined tempeh hydrolyzated flour from multi-enzyme hydrolysis (neutral protease, neutral cellulase and mannase) as a raw material for functional and natural cooking spices in Indonesia. The method of the study was the response surface method with Central Composite Design of Quadratic model using Design Expert for Design of Experiment (DOE) software of version 7.1. The observation is conducted to 28 (twenty-eight) experiment units consisting of 10 (ten) cooking spice brands using Fourier Transform Infrared (FTIR) Spectroscopy. The observation results then were processed using principal component analysis and hierarchical cluster analysis. The result of study indicates that from the analysis of principal component of 28 (twenty-eight) experiment units of 10 (ten) cooking spice brands in Indonesia. The refined tempeh hydrolyzate flour coded 4/15H has a loading factor of 0.646, code 5/1H a loading factor of 0.579, and the flour coded 19/25H has a loading factor of 0.618. Those three experiment units have the potential of flavor character and functional property as the main natural flavor enhancer which has similarities to monosodium glutamate (MSG) with a loading factor of 0.602, Chinese MSG (VC) with a loading factor of 0.581, and curry cooking spice (BM03) with a loading factor of 0.681. The result of the hierarchical cluster analysis of28 (twenty-eight) experiment units of 10 (ten) cooking spice brands in Indonesia, the refined tempeh hydrolyzate flour coded 23/23H has the flavor characters and functional properties ofthe main ingredient for natural flavor enhancers close to monosodium glutamate (MSG) with a closeness value of 519.542 and Chinese MSG (VC) with a closeness value of 531.641. The refined tempe hydrolyzate flour with code 3/14H has flavor characters and functional properties close to curry cooking spice (BM03) with a closeness value of 236.961.

Keywords: Fourier Transform Infrared (FTIR, Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), Indonesian Natural Cooking Spices and Refined Tempe Hydrolyzate Flour

Correspondence:
Mujianto
Agriculture Industry Technology, Wijaya Kusuma University of Surabaya, Indonesia
Email: mujianto@uwks.ac.id

INTRODUCTION

Tempeh is an Indonesian original food with high nutritional value and is healthy. Total diet fibre innatto and tempeh isdecreasingslightly during fermentation (Owen R.Fennema, Y.H. Hui, Marcus Karel, Pieter Walstra, 1999). Study is to be acknowledged of the characteristics of protein hydrolyzed from enzymatic hydrolysis process of rejected tempe(Mujianto, Witono, Wignyanto, Kumalaningsih, & , 2018).The refined tempeh hydrolyzated flour is the flour made from refined tempeh multienzyme hydrolysis that is nutritionous and healthy.Using overripe tempeh extract as protein source could fulfill infant protein requirement with acceptable sensory properties(Dewi Puspitasari Tirtaningtyas Gunawan Puteri, Christli, Kartika Prabawati, & Muzi Marpaung, 2018). Wheat fiber gel has many functional properties that make it ideal as a fat replacer: viscosity, creamy mouthfeel, body, and substance(Owen R.Fennema, Y.H. Hui, Marcus Karel, Pieter Walstra, 1999). Tempeh is an indigenous fermented foods of Southeast Asia(David Owens, 2014). Antibacterial activities against Staphylococcus aureus and Bacillus subtilis were found in an ethanol fraction of tempeh, anIndonesian fermented soybean produced using Rhizopus oligosporus(Kusumah et al., 2020). Protein hydrolysates were produced from shrimp waste mainly comprising head and shell of Penaeus monodon by enzymatic hydrolysis for 90 min using four microbial proteases(Dey & Dora, 2014). Interractive similarity maps may identify more accurately the segmentations than the hierarchical clustering based approaches, and thus are a viable and due to their interactive nature attractive alternative to hierarchical clustering.As the former is much more efficient in time and memory, our results suggest another less resource demanding alternative for annotating large spectral images(Zhong et al., 2013). Enzymatic mungbean meal protein hydrolysate is a novel natural flavour/antioxidant source(Sonklin, Laohakunjit, Kerdchoechuen, & Ratanakhanokchai, 2018). Herbs and spices are traditional role in flavouring and colouring foods (Peter, 2004). the safety of protein, protein hydrolysates, fractions thereof and free amino acids on relevant food legislation is reviewed and evaluated. A new concept for the safety assessment of protein hydrolysates and fractions thereof is developed(Gertjan J. Schaafsma, 2009)

MATERIALS AND METHOD

The research materials were 10 (ten) kinds of cooking spice, namely monosodium glutamate (MSG), balado cooking spice (BM02), curry cooking spice (BM03), Indofood magic cooking spice (BM04), mushroom broth cooking spice (BM05), Chinese MSG (VC)cooking spice, mama cooking spice (BM07), beef broth cooking spice (BM08), chicken broth cookingspice (BM09) and Totole mushroom cooking spice (BM10) purchased from a commercial market in Surabaya. Other materials were refined tempeh flour (TTA01), neutral protease enzyme (NP), neutral cellulase enzyme (NC) and mannase enzyme (M).Important polymers in the two former tissues are cellulose, pectic substances with associated polysaccharides, xyloglucans, and some glycoproteins, while in the lignified tissues cellulose, lignin, glucuronoarabinoxylan, and smaller amounts of glycoprotein and pectic substances are important macromolecules(Owen R.Fennema, Y.H. Hui, Marcus Karel, Pieter Walstra, 1999). The method of the study was

the response surface method with Central Composite Design of Quadratic model using Design Expert for Design of Experiment (DOE) software ofversion 7.1. The effects of four enzymatic hydrolysis parameterswere optimized using response surface methodology(Sangani, Patel, Bhatt, Davara, & Antala, 2014).Using Response Surface Methodology (RSM), we evaluated the culture conditions (nitrogen source, carbon source, pH and agitation rate) that increased the biomass of Acidocella facilis strain USBA-GBX-505 and therefore enhanced the production of its lipolytic enzyme, 505 LIP (Bernal et al., 2017). The results of Fourier Transform Infrared (FTIR) spectroscopy absorbance are further processed using OriginLab software version 8.5. SPSS software of version 25 was used to analyze the principal component analysis and Hierarchical Cluster Analysis.Statistical optimisation techniques were applied in the present study to extract fermentable sugar from indigenous brown seaweed, Sargassum binderi(Hii, Lip, Loh, & Wong, 2014). Microwave-assisted extraction was applied for extracting rice bran protein usingresponse surface methodology (Phongthai, Lim, & Rawdkuen, 2016).

RESULTS AND DISCUSSION

Principal component analysis isa valid data analysis tool. Interpretation is usually made by principal component analysis and canonical variate analysis (Satyajit D.Sarker, Zahid Latif, 2006). Detection of L-Cysteine in wheat flour was accomplished successfully using Raman microscopy combined chemometrics of PCA (Principal Component Analysis) and Hierarchical Cluster Analysis(Cebi, Dogan, Develioglu, Yayla, & Sagdic, 2017). The main advantage of using PCA is the discovery of data patterns by reducing the number of dimensions without losing a lot of information (5).

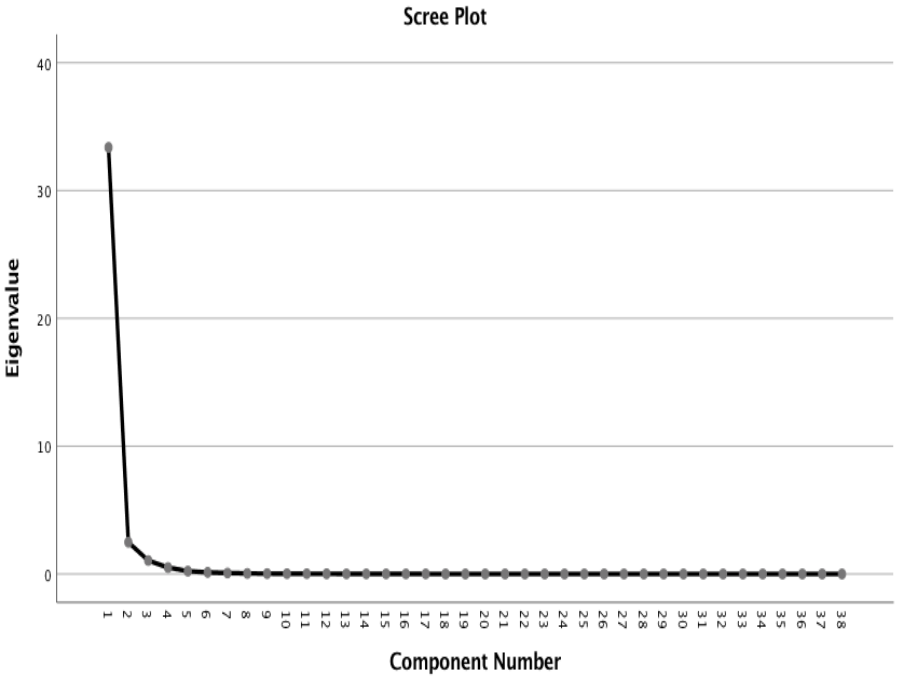

Figure 1. The correlation between Eigenvalues and the Principal Component

The correlation between the score of Eigenvalues and the principal component in figure 1 above indicates that the firstprincipal component (F1) has the total eigenvalue score of 55.571%. The eigenvalues possibly may be complex numbers(Helge Toutenburg, n.d.). The second principal component (F2) has the total eigenvalue score of 11.166 with variant percentage (%) of 29.385%. The third principal component (F3) has a total eigenvalue score of 4.268 with variant percentage (%) of 11.230%.Then, the fourthprincipal component (F4), has the eigenvalue score of0.569 and the variant percentage (%) that can be cummulatively explained is 97.685% which means that out of 100% of total variance, 97.685% can be explained up to the 4th principal component. Atomic emission spectra are inherently well suited for multivariate analysis(Gauglitz, 2003).

Table 1. The Results of KMO Teast and Barlett's Test of FTIR Absorbance of 10 (ten) Cooking Spices and 28 Experiment Units

KMO and Bartlett's Test		
Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		0.945
Bartlett's Test of Sphericity	Approx. Chi-Square	404967.318
	df	703
	Sig.	0.000

The result of the KMO value test is > 0.50. Thus, 0.945 > 0.500 means that the variable can be further predicted and analyzed. Barlett's Test is < 0.05. Thus, 0.000 < 0.050 indicates that there is a high correlation between variables.

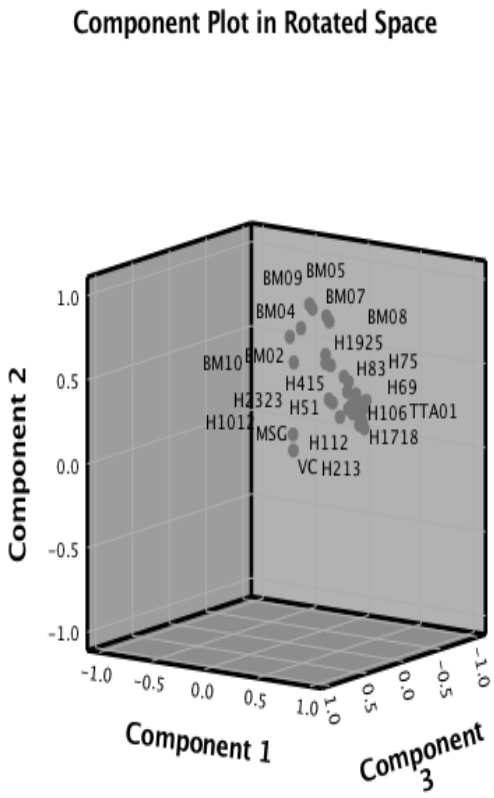

Figure 2. The Correlation among the firstPrincipal Component (F1), the second Principal Component (F2) and the third Principal Component of 28 Experiment Units and 10 (ten) Cooking Spices in Indonesia

The result of the descriptive analysis of table 2 shows that from 28 (twentyeight) experiment units and 10 (ten) cooking spices in Indonesia with observation as many as 74,850 (seventyfour thousand eight hundred and fifty) points of absorbance indicates that the lowest absorbance percentage is the experiment unit coded as 23/23H, namely the treatment under order standard number (23), running number (23), block number (2) with process temperature of 45^{0}C, densed liquid ratio (0.3), protease neutral enzyme (1.5%), cellulase neutral enzyme (2,42058%) and mannanase enzyme (1.5%) and absorbance percentage as much as 27.460 ± 9,063.The average highest absorbance percentage is from the experiment unit coded 22/22H, namely the treatment under order standard number (22), running number (22), block number (2) with process temperature of 45^{0}C, densed liquid ratio (0.3), protease neutral enzyme (1.5%), cellulase neutral enzyme (0.58942%) and mannanase enzyme (1.5%) and absorbance process as much as 37,053 ± 2,554.

It can be seen in table 3 that the firstprincipal component (F1) is the function of flavour charactersand functional properties similarity of the refined tempeh hydrolyzated flour of the first variant, namely the experiment units coded 17/18H, 11/2H, 10/6H, 8/3H, 7/5H, 1/12H, 6/9H, 9/10H, 12/11H, 24/24H, 25/26H, 14/7H, 15/8H, 18/20H, 20/17H, 22/22H, 2/13H, 26/16H, 27/21H, 4/15H, 5/1H, 19/25H, 21/27H, 13/4H, 23/23H, 3/14H, 16/19H and TTA01 with curry cooking spice (BM03), mama cooking spice (BM09), Totole mushroom cooking spice (BM10), refined tempeh flour (TTA01), monosodium glutamate (MSG) and Chinese MSG (VC).The results of the analysis show that the ten brands of cooking spice are made from similar food additives and supportive materials with the eigenvalues of 9.477 or 94.771% of variant(Mujianto, Wignyanto, Hidayat N., & Aulianni'Am, 2020).

Based on table 3, it can be seen that the refined tempeh hydrolyzated flour is the result of multi-enzyme hydrolysis with standard order number (4), running number (15), block number (1), process temperature 50^0 C, densed liquid ratio (0.4), proteases neutral enzyme (2%), cellulase neutral enzyme (1%) and mannanase enzyme (1%) or 4/15H with a loading factor value of 0.646. The refined tempeh hydrolyzate flour under order standard number (5), running number (1), block number (1), process temperature 50^0 C, densed liquid ratio (0,4), protease neutral enzyme (1%), cellulase neutral enzyme (1%) and mannanase enzyme (2%) or 5/1H with a loading factor value of 0,579 and the refined tempeh hydrolyzated flour under order standard number (19), running number (25), block number (2), process temperature 45^0 C, densed liquid ratio (0.482116), protease neutral enzyme (1,5%), cellulase neutral enzyme (1,5%) and mannanase enzyme (1,5%) or 19/25H with a loading factor value of 0,618 have flavour characters and functional properties those are similar tomonosodium glutamate (MSG) with a loading factor of 0,602, Chinese MSG (VC) with a loading factor of 0,581, and curry cooking spice (BM03) witha loading factor of 0,681. The spectral data from the seven methodologies were integrated into one data model usingthe consensus principal component analysis, in order to obtain the relations between the

molecular signatures traced by different techniques.(Ba, Zimmermann, & Kohler, 2015).The results of the peak analysis calculation using the Gaussian method that uses the following equation:

Y = Yo + (A/(w*sqrt(PI/2)))*exp(-2*((x-xc)/w)^2)

For the first principal component (F1) in table 4, it is shown that the minimumYo value is 23,374 for the experiment unit with the code 13/4H and the maximumYo is the experiment unit with the code 16/19H of 38,558. The minimumA value is -42.923,540 for the experimental unit with the code BM10 (Totole mushroom cooking spice) and themaximumA value is 11.264,792 for the experiment unit with the code 13/4H. W score is minimally 7,726 for the experiment unit with the code BM10 (Totole mushroom cooking spice) and maximumW score is 11.264,792 for the experiment unit with code 23/23H. The minimumXc value for the experiment unit with code 23/23H is -400,778 and the maximumXc value for the experiment unit with code BM10 (Totole mushroom cooking spice) is 5.142,556. Blanching and steaming of mushrooms (Shittake), green beans, and green bell peppers resulted in a high conversion of RNA into GMP; potatoes have an intermediate conversion and cabbage doesn't have any conversion of RNA into GMP(R.Scott, 1997). Other food containing natural umami flavor are Parmesan cheese, Shiitake mushrooms, and naturally fermented soy sauce (Davies, 2003).

For the second principal component (F2) in table 6 shows that the minimumYo value is 25,792 for the experiment unit with code 21/27H and the maximumYo for the experiment unit with code 16/19H is 38,558. The minimumA score is - 42.923,540for the experiment unit with code BM10 (Totole mushroom cooking spice) and the maximumA score is 10.501,021 for the experiment unit with code 5/1H. The minimumW value is 7,726for the experiment unit with code with code BM10 (Totolemushroomcooking spice) and the maximumW value is 25.121,033for the experiment unit with code 23/23H. The minimumXc value is - 400,778for the experiment unit with code 23/23H and the maximumXc value is 5.142,556for the experiment unit with code BM10 (Totole mushrom cooking spice).

For the thirdprincipal component (F3) in table 8, it is shown that the minimumYo value is 27,670 for the experiment unit with code 23/23H and the maximumYo value for the experiment unit with code BM10 (Totole mushroom cooking spice) is 32,565. The minimumA value is -42.923,540for the experiment unit with code BM10 (Totole mushroom cooking spice) and the maximumA value is 7.090,872for the experiment unit with code VC (Chinese MSG). The minimumW value is 7,726for the experiment unit with code BM10 (Totole mushroom cooking spice) and the maximumW value is 25.121,033for the experiment unit with code 23/23H. The minimumXc value is - 400,778for the experiment unit with code 23/23H and the minimumXc value is 5.142,556for the experiment unit with code BM10 (Totole mushroom cooking spice). FTIR as in NIR, chemometric techniques are required to discriminate samples or to obtain quantitative information about the taste components (B.M. Nicolai, A.Berna, K.Beullens, 2008).

Table 2. Descriptive Statistics of 10 Cooking Spices and 28 Experiment Units

No	Descriptive Statistics				
	Variable	Mean	Std. Deviation	Analysis N	Missing N
1	TTA01	33.924	8.408	1868	13
2	MSG	26.770	10.686	1868	13
3	VC	33.181	5.411	1868	13
4	17/18H	28.348	6.929	1868	13
5	11/2H	34.523	3.216	1868	13
6	10/6H	34.441	3.642	1868	13
7	8/3H	35.154	3.321	1868	13
8	7/5H	33.617	3.770	1868	13
9	1/12H	30.317	5.644	1868	13
10	6/9H	29.913	5.799	1868	13
11	9/10H	27.962	7.538	1868	13
12	12/11H	35.763	2.763	1868	13
13	24/24H	31.966	4.257	1868	13
14	25/26H	32.819	3.874	1868	13
15	14/7H	33.121	4.684	1868	13
16	15/8H	31.371	5.165	1868	13
17	18/20H	30.797	5.319	1868	13
18	20/17H	32.196	4.818	1868	13
19	22/22H	37.053	2.554	1868	13
20	2/13H	33.868	3.329	1868	13

21	26/16H	33.825	4.172	1868	13
22	27/21H	35.248	2.923	1868	13
23	4/15H	28.321	7.118	1868	13
24	5/1H	30.149	6.789	1868	13
25	19/25H	33.498	4.346	1868	13
26	21/27H	30.094	7.049	1868	13
27	13/4H	28.478	6.521	1868	13
28	23/23H	27.460	9.063	1868	13
29	3/14H	32.573	4.184	1868	13
30	16/19H	36.066	8.874	1868	13
31	BM02	33.665	4.211	1881	0
32	BM03	35.449	3.042	1881	0
33	BM04	31.864	7.127	1881	0
34	BM05	32.317	8.223	1881	0
35	BM07	34.050	3.389	1881	0
36	BM08	32.110	5.009	1881	0
37	BM09	31.565	7.812	1881	0
38	BM10	31.012	6.732	1881	0

Table 3. The Interpretation Result of FTIR Absorbance Pattern Variable of 5 (five) Cooking Spices and 28 (twentyeight) Experiment Units of the FirstPrincipal Component (F1) Which Were Composed with Observation Points As Much As 61,683 Points.

NO	VARIABLE	FACTOR	EIGEN VALUES	LOADING FACTOR	% VARIANCE	CUMULATIVE %
1	TTA01	The Similarity of Flavor Characters and Functional Properties of the first Refined Tempeh Hydrolyzated Flour Variant with BM03, BM07, BM10, TTA01, MSG and Chinese MSG (F1)	**21.117**	0.879	**55.571**	**55.571**
2	MSG			0.602		
3	VC			0.581		
4	17/18H			0.883		
5	11/2H			0.854		
6	10/6H			0.871		
7	8/3H			0.844		
8	7/5H			0.771		
9	1/12H			0.836		
10	6/9H			0.875		
11	9/10H			0.850		
12	12/11H			0.830		
13	24/24H			0.894		
14	25/26H			0.858		
15	14/7H			0.854		
16	15/8H			0.872		
17	18/20H			0.903		
18	20/17H			0.859		
19	22/22H			0.872		
20	2/13H			0.914		
21	26/16H			0.837		
22	27/21H			0.789		
23	4/15H			0.646		
24	5/1H			0.579		
25	19/25H			0.618		
26	21/27H			0.740		
27	13/4H			0.831		
28	23/23H			0.716		
29	3/14H			0.823		
30	16/19H			0.794		
31	BM03			0.681		
32	BM07			0.462		
33	BM10			0.445		

The results of the paired "t" samples test toward the values of Yo, Xc, W and A from the calculation of peak analysis by means of Gaussian's method for the firstprincipal component (F1) in table 4 show that variable 4/15H with 5/1H ("t" count = -0,963 ns) , 4/15H with 19/25H ("t" count = -0,943 ns), 4/15H with VC ("t" count = 1,345 ns), 4/15H with BM03 ("t" count = -0,932 ns), 5/1H with 19/25H ("t" count = 1,007 ns), 5/1H with MSG ("t" count = 1,074 ns), 5/1H with VC("t" count = 1,059 ns), 19/25H with MSG ("t" count = 1,093 ns), 19/25H with VC ("t" count = -0,763 ns), 19/25H with BM03 ("t" count = -1,231 ns), MSG with VC ("t" count = -1,077 ns), MSG with BM03 ("t" count = -1,101 ns), VC with BM03 ("t" count = -2,130 ns) all indicate no significant difference at the level of 5%.

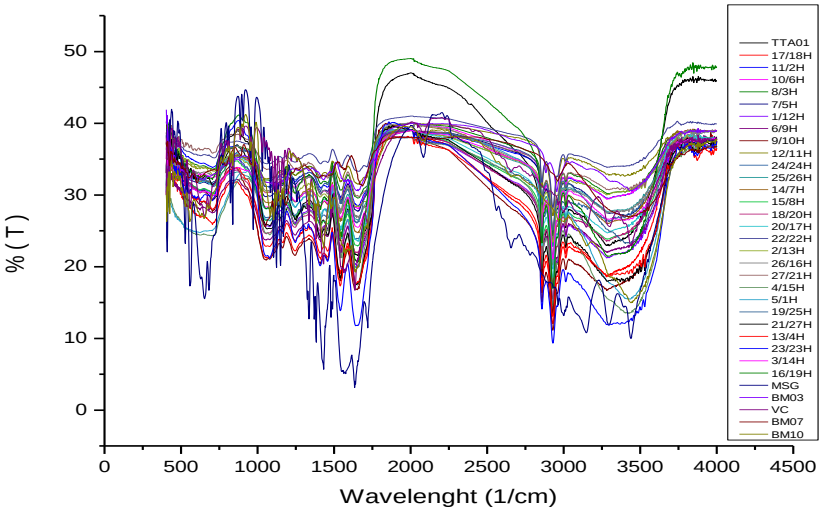

Figure 3.The absorbance pattern (spectogram) of 5 (five) Cooking Spices and 28 (twenty eight) Experiment Units of the FirstPrincipal Component (F1)Composed

Table 4. Calculation Results of Peak Analysis from Figure 3

THE WAVE LENGHT OF FOURIER TRANSFORM INFRARED (FTIR) OF 900 cm^{-1} - 3.150 cm^{-1} GAUSSIAN'S EQUATION y=y0 + (A/(w*sqrt(PI/2)))*exp(-2*((x-xc)/w)^2) (F1)									
NO	**VARIABEL**	**Yo**	**STDEV**	**Xc**	**STDEV**	**W**	**STDEV**	**A**	**STDEV**
1	TTA01	35.542	0.256	2,953.201	8.163	209.141	17.717	-3,846.614	296.565
2	MSG	30.345	0.303	1,555.413	6.067	281.718	13.103	-8,612.176	393.251
3	VC	30.403	0.195	2,234.287	8.859	503.867	21.586	7,090.872	338.673
4	17/18H	29.536	0.209	2,961.214	9.531	242.875	21.523	-3,488.922	275.677
5	11/2H	31.848	0.106	2,202.768	6.463	621.223	17.524	5,988.858	204.370
6	10/6H	31.183	0.117	2,211.037	5.721	649.264	15.955	7,618.169	231.191
7	8/3H	32.525	0.108	2,205.763	6.256	623.944	17.002	6,338.574	209.362
8	7/5H	30.951	0.118	2,220.053	6.091	650.859	16.978	7,198.952	232.103
9	1/12H	31.505	0.162	2,962.230	7.694	223.893	17.099	-2,915.023	199.684
10	6/9H	31.037	0.179	2,957.846	8.957	216.953	19.684	-2,610.355	213.952
11	9/10H	29.282	0.217	2,968.766	10.471	279.538	24.151	-4,374.105	333.147
12	12/11H	33.021	0.097	2,254.746	5.554	725.467	16.547	6,829.468	202.865
13	24/24H	32.273	0.135	2,950.373	9.227	198.071	19.756	-1,647.933	150.540
14	25/26H	29.278	0.116	2,208.567	5.133	666.923	14.609	8,460.533	231.947
15	14/7H	29.313	0.143	2,198.792	5.918	617.212	15.990	8,824.268	275.760
16	15/8H	32.325	0.153	2,956.874	8.001	221.093	17.641	-2,570.465	185.135
17	18/20H	25.821	0.148	2,170.577	5.176	608.577	13.907	10,340.859	283.338
18	20/17H	28.355	0.149	2,170.368	6.291	595.286	16.668	8,451.011	280.995
19	22/22H	34.789	0.083	2,203.254	5.683	653.743	15.959	5,437.147	164.510
20	2/13H	35.175	0.122	1,372.921	11.822	489.002	27.580	-3,614.652	214.457
21	26/16H	30.737	0.131	2,181.372	6.357	588.059	16.704	7,316.465	245.480
22	27/21H	35.819	0.084	2,945.921	6.002	183.865	12.656	-1,398.848	88.741
23	4/15H	31.316	0.176	2,997.353	10.306	257.614	23.613	-3,422.363	273.015
24	5/1H	27.530	0.187	2,234.361	6.714	657.623	18.773	10,501.021	372.138
25	19/25H	31.650	0.125	2,232.016	7.232	633.062	19.734	6,381.086	242.556
26	21/27H	25.792	0.217	2,172.433	7.512	555.574	19.145	9,930.810	395.052
27	13/4H	23.374	0.190	2,168.536	5.964	586.119	15.659	11,264.792	355.010
28	23/23H	27.670	0.247	-400.778	0.000	25,121.033	0.000	8.000	0.000

29	3/14H	29.221	0.130	2,217.744	5.720	647.837	15.901	8,463.947	256.222
30	16/19H	38.558	0.255	2,980.134	10.098	244.497	23.110	-4,264.078	353.520
31	BM03	32.732	0.087	2,280.700	4.785	735.587	14.201	7,291.375	183.916
32	BM07	36.486	0.081	1,153.753	12.872	541.156	29.319	-4,031.426	208.704
33	BM10	32.565	0.161	5,142.556	0.000	7.726	0.000	-42,923.540	0.000

Table 5. The Interpretation Result of FTIR Absorbance Pattern Variable of 8 (eight) Cooking Spices and 8 (eight) Exeriment Units of the SecondPrincipal Component (F2) Compose with Observation Points as Much as 29,992 Points.

NO	VARIABLE	FACTOR	EIGEN VALUES	LOADING FACTOR	% VARIANCE	CUMULATIVE %
1	7/5H	The Similarity of Flavor Characters and Functional Properties of the Second Refined Hydrolyzated Flour Variant with BM02, BM03, BM04, BM05, BM07, BM08, BM09 andBM10 (F2)	**11.166**	0.555	**29.385**	**84.956**
2	4/15H			0.650		
3	5/1H			0.701		
4	19/25H			0.663		
5	21/27H			0.451		
6	23/23H			0.471		
7	3/14H			0.482		
8	16/19H			0.504		
9	BM02			0.797		
10	BM03			0.566		
11	BM04			0.845		
12	BM05			0.945		
13	BM07			0.856		
14	BM08			0.884		
15	BM09			0.926		
16	BM10			0.677		

Based on table 5, the similarity of flavor characters and functional properties of the refined tempeh hydrolyzate flour 7/5H with a loading factor of 0.555 and 16/19H with a loading factor of 0.504 have a character closeness to the currycooking spice (BM03) with a loading factor of 0.566. The refined tempeh hydrolyzated flour 4/15H with a loadingfactor of 0.650 and 19/15H with a loading factor of 0.663 have flavor characters and functional properties closeness with Totole mushroom cooking spices with aloading of 0.677. Figure 4 shows that the said closeness of flavor characters and functional properties is at the wave lenght between 1750 cm^{-1} and 2750cm^{-1}. The practical applicability of FT-IR microspectroscopy technique for early and rapid identification of these species based on the spectral data showed striking difference with their major biomolecules such as lipids, proteins, and nucleic acids produced by them(Subburaj, Barathkumar, Prem, Thangaraj, & Sivasubramanian, 2013).

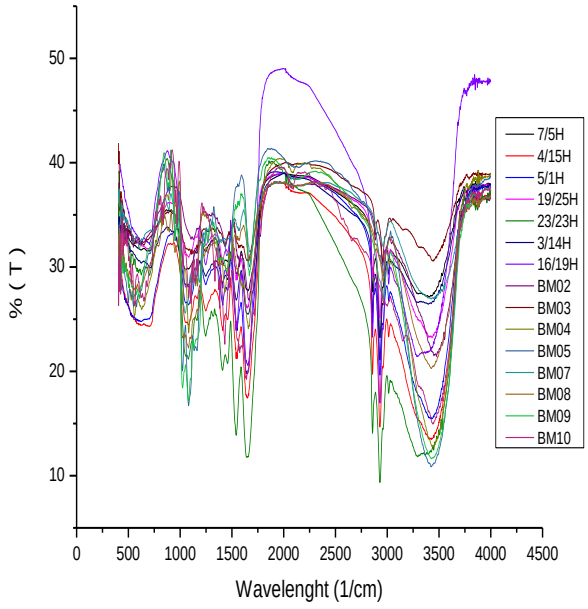

Figure 4. The absorbance pattern (spectogram) of 8 (eight) Cooking Spices and 8 (eight) Experiment Units of the 2nd Principal Component (F2) Composed

Table 6. Calculation Results of Peak Analysis from Figure 4

THE WAVE LENGHT OF FAURIER TRANSFORM INFRARED (FTIR) OF 900 cm^{-1} - 3.150 cm^{-1} GAUSSIAN'S EQUATION y=y0 + (A/(w*sqrt(PI/2)))*exp(-2*((x-xc)/w)^2) (F2)									
NO	**VARIABEL**	**Yo**	**STDEV**	**Xc**	**STDEV**	**W**	**STDEV**	**A**	**STDEV**
1	7/5H	30.951	0.118	2,220.053	6.091	650.859	16.978	7,198.952	232.103
2	4/15H	31.316	0.176	2,997.353	10.306	257.614	23.613	-3,422.363	273.015
3	5/1H	27.530	0.187	2,234.361	6.714	657.623	18.773	10,501.021	372.138
4	19/25H	31.650	0.125	2,232.016	7.232	633.062	19.734	6,381.086	242.556
5	21/27H	25.792	0.217	2,172.433	7.512	555.574	19.145	9,930.810	395.052
6	23/23H	27.670	0.247	-400.778	0.000	25,121.033	0.000	8.000	0.000
7	3/14H	29.221	0.130	2,217.744	5.720	647.837	15.901	8,463.947	256.222
8	16/19H	38.558	0.255	2,980.134	10.098	244.497	23.110	-4,264.078	353.520
9	BM02	35.814	0.070	1,637.327	3.985	172.551	8.304	-1,576.387	70.675
10	BM03	32.732	0.087	2,280.700	4.785	735.587	14.201	7,291.375	183.916
11	BM04	30.597	0.161	2,229.900	7.128	644.600	19.688	8,413.083	316.022
12	BM05	36.850	0.112	1,101.440	2.458	150.816	5.090	-3,439.199	106.732
13	BM07	36.486	0.081	1,153.753	12.872	541.156	29.319	-4,031.426	208.704
14	BM08	35.750	0.102	1,139.783	10.975	507.351	24.900	-5,467.568	253.536
15	BM09	35.556	0.120	1,097.332	2.725	149.318	5.641	-3,286.147	114.052
16	BM10	32.565	0.161	5,142.556	0.000	7.726	0.000	-42,923.540	0.000

Table 7. The Interpretation Result of FTIR Absorbance Pattern Variable of 3 (three) Cooking Spices and 1 (one) Exeriment Unit of the ThirdPrincipal Component (F3) Compose with Observation Points As Much As 7,511 Points.

NO	VARIABLE	FACTOR	EIGEN VALUES	LOADING FACTOR	% VARIANCE	CUMULATIVE %
1		The Similarity of Flavor Characters and Functional Properties of the 3rd (third)Refined Hydrolyzate Flour Variant with BM10, MSG and Chinese MSG(F3)	**4.268**		**11.23**	**96.187**
2	MSG			0.766		
3	VC			0.741		
4	23/23H			0.449		
5	BM10			0.537		
6						

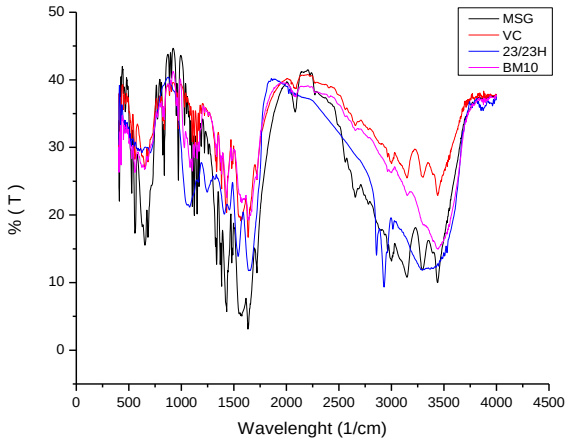

Figure 5. The absorbance pattern (spectogram) of 3 (three) Cooking Spices and 1 (one) Experiment Unit of the ThirdPrincipal Component (F3)Compose

Table 8. Calculation Results of Peak Analysis from Figure 5

	Cluster Membership				
	Case	**5 Clusters**	**4 Clusters**	**3 Clusters**	**2 Clusters**
1	TTA01	1	1	1	1
2	MSG	2	2	2	1
3	VC	2	2	2	1
4	17/18H	1	1	1	1
5	11/2H	1	1	1	1
6	10/6H	1	1	1	1
7	8/3H	1	1	1	1
8	7/5H	1	1	1	1
9	1/12H	1	1	1	1
10	6/9H	1	1	1	1
11	9/10H	1	1	1	1
12	12/11H	3	1	1	1
13	24/24H	1	1	1	1
14	25/26H	1	1	1	1
15	14/7H	1	1	1	1
16	15/8H	1	1	1	1
17	18/20H	1	1	1	1
18	20/17H	1	1	1	1
19	22/22H	1	1	1	1
20	2/13H	1	1	1	1
21	26/16H	1	1	1	1
22	27/21H	1	1	1	1
23	4/15H	4	3	3	2
24	5/1H	4	3	3	2
25	19/25H	4	3	3	2
26	21/27H	1	1	1	1
27	13/4H	1	1	1	1
28	23/23H	1	1	1	1
29	3/14H	1	1	1	1
30	16/19H	1	1	1	1
31	BM02	4	3	3	2
32	BM03	3	1	1	1
33	BM04	4	3	3	2
34	BM05	5	4	3	2
35	BM07	5	4	3	2
36	BM08	5	4	3	2
37	BM09	5	4	3	2
38	BM10	4	3	3	2

Table 9. Cluster Membership of 28 (twentyeight) Experiment Units and 10 (ten) Types of Cooking Spice in Indonesia

THE WAVE LENGHT OF FAURIER TRANSFORM INFRARED (FTIR) OF 900 cm^{-1} - 3.150 cm^{-1} GAUSSIAN'S EQUATION
y=y0 + (A/(w*sqrt(PI/2)))*exp(-2*((x-xc)/w)^2)
(F3)

NO	VARIABEL	Yo	STDEV	Xc	STDEV	W	STDEV	A	STDEV
1	MSG	30.345	0.303	1,555.413	6.067	281.718	13.103	-8,612.176	393.251
2	VC	30.403	0.195	2,234.287	8.859	503.867	21.586	7,090.872	338.673
3	23/23H	27.670	0.247	-400.778	0.000	25,121.033	0.000	8.000	0.000
4	BM10	32.565	0.161	5,142.556	0.000	7.726	0.000	-42,923.540	0.000

Table 9 indicates that there is a cluster of 28 (twentyeight) experiment units and 10 (ten) types of cooking spice in Indonesia can be clustered into five. Trilinolein is present in virgin olive oil at trace level, thus high content of trilinolein indicated that virgin olive oil has been adulterated with other kinds of vegetable oil. Classical multivariate procedures were applied which are principal components regression and cluster analysis (Fei, Lujia, & Xian, 2017). Such clustering result is that the second cluster is divided into 2 (two) groups, namely the first group that is consistedof experiment units each with the code TTA01, MSG, VC, 17/18H, 11/2H, 10/6H, 8/3H, 7/5H, 1/12H, 6/9H, 9/10H, 12/11H, 24/24H, 25/26H, 14/7H, 15/8H, 18/20H, 20/17H, 22/22H, 2/13H, 26/16H, 27/21H, 21/27H, 13/4H, 23/23H, 3/14H, 16/19H, and BM03. The second group is consistedof experiment units each with the code 4/15H, 5/1H, 19/25H, BM02, BM04, BM005, BM07, BM08, BM09, and BM10. The third cluster is divided into 3 (three) groups as seen in figure 6 (six), namely the first group that is consistedofexperiment units each with the code TTA01, 17/18H, 11/2H, 10/6H, 8/3H, 7/5H, 1/12H, 6/9H, 9/10H, 12/11H, 24/24H, 25/26H, 14/7H, 15/8H, 18/20H, 20/17H, 22/22H, 2/13H, 26/16H, 27/21H, 21/27H, 13/4H, 23/23H, 3/14H, 16/19H, and BM03 (the first variant of refined tempeh hydrolyzated flour from multi-enzyme hydrolysis). The second group is consistedof experiment units each with the code MSG and VC (the similarity of MSG and Chinese MSG). The third group is consistedof experiment units each with the code 4/15H, 5/1H, 19/25H, BM02, BM04, BM05, BM07, BM08, BM09, and BM 10 (the similarity of refined tempe hydrolyzated flours 4/15H, 5/1H and 19/25H with 7 (seven) Cooking Spices.

The fourth cluster is divided into 4 (four) groups, namely the first group that is consistedof experiment units each with the code TTA01, 17/18H, 11/2H, 10/6H, 8/3H, 7/5H, 1/12H, 6/9H, 9/10H, 12/11H, 24/24H, 25/26H, 14/7H, 15/8H, 18/20H, 20/17H, 22/22H, 2/13H, 26/16H, 27/21H, 21/27H, 13/4H, 23/23H, 3/14H, and 16/19H.In particular, we utilize this scheme to quantitatively compare different hierarchical clustering approaches to interactive similarity maps(Zhong et al., 2013)

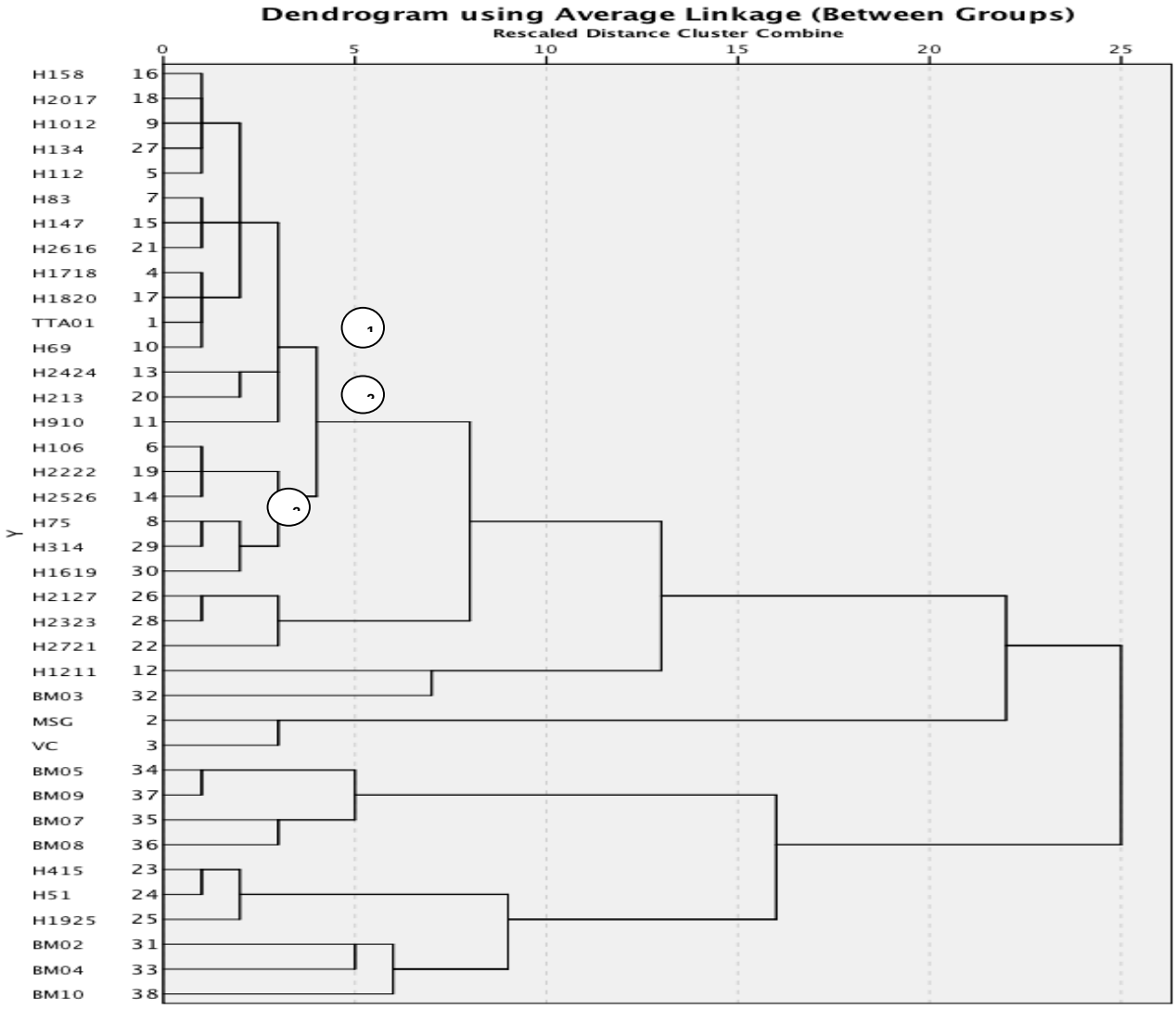

Figure 6. A dendrogram of 28 (twentyeight) Experiment Units and 10 (ten) Cooking Spices in Indonesia, 1) the first variant of refined tempeh hydrolyzated flour from multi-enzyme hydrolysis, 2) the similarity of MSG and Chinese MSG, 3) the the similarity of refined tempeh hydrolyzated flours 4/15H, 5/1H and 19/25H with 7 (seven) Cooking Spices.

The second group is consistedof experiment units each with the code MSG and VC. The third group is consistedof experiment units each with the code 4/15H, 5/1H, 19/25H, BM02, BM04, and BM 10. The fourth group isconsistedof experiment units each with the code BM05, BM07, BM08, and BM09. HCA computes the Euclidian distance between the spectra using the Ward's algorithm. This classification allows the combination of spectra with a minimum Euclidian distance in the same cluster (Mohamed, Untereiner, & Sockalingum, 2017).

The fifth cluster is divided into 5 (five) groups, namely the first group that is consistedof experiment units each with the code TTA01, 17/18H, 11/2H, 10/6H, 8/3H, 7/5H, 1/12H, 6/9H, 9/10H, 12/11H, 24/24H, 25/26H, 14/7H, 15/8H, 18/20H, 20/17H, 22/22H, 2/13H, 26/16H, 27/21H, 21/27H, 13/4H, 23/23H, 3/14H, and 16/19H. The second group isconsistedof experiment units each with the code MSG and VC. The third group is consistedof experiment units each with the code 12/11H and BM03. The fourth group isconsistedof experiment units each with the code 4/15H, 5/1H, 19/25H, BM02, BM04,andBM10. The fifth group isconsistedof experiment units each with the code BM05, BM07, BM08, and BM09.

Based on the dendogram in figure 6 above, it indicates that the multi-enzyme hydrolysis of refined tempe flour produces several variants of refined tempeh hydrolyzated flour, which in general can bedivided into 3 (three) clusters, namely: the first cluster that is a refined tempeh hydrolyzated flour which has flavor character and material functional property that those isclose with refined tempeh flour (TTA01), the second cluster is the cluster in which the flavor character and the functional property of those that are close with the character of monosodium glutamate (MSG), and the third cluster that is the cluster in which the flavor character and the material functional property of those that are close with the character of balado cooking spice (BM02), Indofood magic cooking spice (BM04), mushroom broth cooking spice (BM05), mama cooking spice (BM07), beef broth cooking spice (BM08), chicken broth cooking spice (BM09), and Totole mushroom cooking spice (BM10).

Table 10 below is a matrix table of the closeness of flavor character and functional property of 27 (twentyseven) refined tempeh hydrolyzated flour and 10 (ten) types of cookingspice circulated in Indonesia. The refined tempeh hydrolyzated flour under order standard number (17), running number (18), block number (2), process temperature 54,1058^{0}C, densed liquid ratio (0,3), protease neutral enzyme (1,5%), cellulase neutral enzyme (1,5%) and mannanase enzyme (1,5%) has the flavor character and functional properties that is close to therefined tempeh flour (TTA01). The refined tempeh hydrolyzated flour under order standard number (23), running number (23), block number (2), process temperature 45^{0}C, densed liquid ratio (0,3), protease neutral enzyme (1,5%), cellulase neutral enzyme (2, 41058%) and mannanase enzyme (1,5%) has the flavor character and functional properties that is close tomonosodium glutamate (MSG) chinese MSG (VC).

The refined tempeh hydrolyzated flour under order standard number (19), running number (25), block number (2), process temperature 45^{0}C, densed liquid ratio (0,482116), protease neutral enzyme (1,5%), cellulase neutral enzyme (1,5%) and mannanase enzyme (1,5%) has the flavor character and functional properties that is close tobalado cooking spice (BM02), mama cooking spice (BM07), and Totole mushroom cooking spice (BM10).

The refined tempeh hydrolyzated flour under order standard number (3), running number (14), block number (1), process temperature 40^{0}C, densed liquid ratio (0,4), protease neutral enzyme (2%), cellulase neutral enzyme (1%) and mannanase enzyme (2%) has the flavor character and functional properties that is close tocurry cooking spice (BM03).The physico-chemical features of tempehs produced from different sources of legume (soybean (control), chickpea, lentils (red and green), and beans (white, black, and broad)) using Rhizopus oligosporus and to specify the best tempeh by sensory evaluation(Erkan, Gürler, Bilgin, Germec, & Turhan, 2020).

The refined tempeh hydrolyzated flour under order standard number (5), running number (1), block number (1), process temperature 50^{0}C, densed liquid ratio (0,4), protease neutral enzyme (1%), cellulase neutral enzyme (1%) and mannanase enzyme (2%) has the flavor character and functional properties that is close toindofood magic cooking spice (BM04), mushroom broth cooking spice (BM05), beef broth cooking spice (BM08) and chicken broth cooking spice (BM09).

Tropical diseases can be prevented by consuming fermented food that have antimicrobial activity. One of them is gembustempeh that has short shelf life(Noviana, Dieny, Rustanti, Anjani, & Afifah, 2018). Tempeh, a kind of traditional fermented food from Indonesia, is rich in soluble fibers and microbial consortia(Stephanie, Kartawidjajaputra, Silo, Yogiara, & Suwanto, 2019). tempeh is highly perishable, with a shelf life of 24-48 hours(Kustyawati, Pratama, Saputra, & Wijaya, 2020).It is concluded that tempeh has the potential to be developed as phytoestrogens formenopausal women with certain dose settings(Maran et al., 2019)

Table 10. The Closeness Matrix of Flavor Characters and Functional Properties of Refined Tempeh Hydrolyzated Flour with 10 (ten) Types ofCooking Spice in Indonesia

No	Case	Proximity Matrix										
		TTA01	MSG	VC	BM02	BM03	BM04	BM05	BM07	BM08	BM09	BM10
1	TTA01	0.000	788.480	727.589	1,172.570	343.523	935.864	1,321.001	930.665	920.327	1,222.671	808.708
2	MSG	788.480	0.000	103.117	1,311.465	1,197.085	1,301.884	2,040.162	1,820.267	1,856.453	1,910.963	719.178
3	VC	727.589	103.117	0.000	1,036.548	901.596	1,031.631	1,798.602	1,543.437	1,553.658	1,704.275	556.233
4	17/18H	37.680	672.948	652.793	1,180.006	476.446	963.549	1,360.270	977.015	1,011.085	1,238.856	764.553
5	11/2H	127.909	665.251	612.896	999.757	522.416	891.689	1,273.151	861.784	982.745	1,143.490	656.023
6	10/6H	36.910	825.827	698.643	1,115.818	298.586	896.546	1,283.264	855.855	868.319	1,191.119	786.519
7	8/3H	71.534	726.380	628.037	924.463	374.721	773.031	1,170.165	801.680	844.884	1,068.739	621.051
8	7/5H	124.208	841.665	672.744	687.180	272.711	506.569	851.689	577.579	564.031	781.772	476.325
9	1/12H	134.760	590.019	567.155	920.228	604.360	827.711	1,232.910	914.978	997.620	1,095.577	560.652
10	6/9H	23.041	766.631	688.519	1,123.887	379.491	889.652	1,262.008	883.087	885.640	1,154.278	754.122
11	9/10H	169.284	645.639	678.330	1,129.946	703.446	967.428	1,311.193	982.774	1,074.765	1,162.766	710.799
12	12/11H	188.411	1,253.064	1,002.437	1,265.638	232.608	1,013.380	1,334.587	863.744	819.385	1,281.927	1,057.940
13	24/24H	116.796	668.280	653.598	1,286.620	582.478	1,141.523	1,530.694	1,049.045	1,171.511	1,381.058	854.500
14	25/26H	45.684	954.503	792.373	1,136.849	242.104	871.544	1,230.760	820.565	785.246	1,156.075	838.607
15	14/7H	45.256	667.617	587.413	959.761	379.813	799.500	1,213.790	844.593	884.334	1,109.579	625.648
16	15/8H	64.929	639.850	623.171	1,084.809	517.464	913.146	1,304.042	938.846	999.570	1,175.671	690.869
17	18/20H	59.402	709.082	690.098	1,295.471	513.968	1,090.596	1,469.403	1,018.549	1,093.427	1,337.417	869.032
18	20/17H	95.247	596.568	581.551	1,029.367	562.064	890.050	1,298.774	957.968	1,026.658	1,169.628	642.720
19	22/22H	55.870	934.270	807.338	1,170.519	295.493	935.668	1,295.054	874.262	872.549	1,216.096	877.198
20	2/13H	99.319	752.248	706.613	1,367.257	527.260	1,221.572	1,624.057	1,097.568	1,209.782	1,489.831	970.235
21	26/16H	85.465	651.121	595.778	889.317	499.973	774.135	1,179.237	882.507	916.243	1,063.254	564.649
22	27/21H	366.610	583.666	573.915	915.174	892.059	981.343	1,407.507	1,081.161	1,253.978	1,248.325	595.195
23	4/15H	334.830	875.952	672.130	365.729	397.277	247.145	629.609	542.548	480.310	592.130	258.323
24	5/1H	471.354	1,069.555	787.041	331.639	366.847	185.446	547.213	500.520	389.141	545.425	297.805
25	19/25H	438.938	871.337	658.332	295.876	474.585	234.247	603.969	497.350	497.777	561.644	225.805
26	21/27H	367.152	535.968	532.470	719.084	852.204	741.850	1,149.141	928.312	1,061.646	1,001.404	402.139
27	13/4H	144.341	578.332	548.773	922.104	596.212	826.489	1,227.888	889.497	986.204	1,088.723	558.504
28	23/23H	402.137	519.542	531.641	698.671	881.761	699.765	1,090.091	908.264	1,031.616	940.748	362.876
29	3/14H	63.296	853.775	682.229	878.398	236.961	684.785	1,070.817	710.786	700.169	997.930	628.256
30	16/19H	84.099	685.723	567.142	752.201	339.135	570.508	978.374	710.781	697.947	893.059	458.284
31	BM02	1,172.570	1,311.465	1,036.548	0.000	893.824	138.346	459.691	590.932	568.301	459.952	186.404
32	BM03	343.523	1,197.085	901.596	893.824	0.000	642.244	993.233	578.718	530.119	986.017	770.567
33	BM04	935.864	1,301.884	1,031.631	138.346	642.244	0.000	230.235	362.448	257.544	248.320	189.918
34	BM05	1,321.001	2,040.162	1,798.602	459.691	993.233	230.235	0.000	209.251	123.606	23.420	674.758

Continued Table 10

35	BM07	930.665	1,820.267	1,543.437	590.932	578.718	362.448	209.251	0.000	82.727	170.112	707.786
36	BM08	920.327	1,856.453	1,553.658	568.301	530.119	257.544	123.606	82.727	0.000	129.229	698.838
37	BM09	1,222.671	1,910.963	1,704.275	459.952	986.017	248.320	23.420	170.112	129.229	0.000	623.592
38	BM10	808.708	719.178	556.233	186.404	770.567	189.918	674.758	707.786	698.838	623.592	0.000

Based on table 10, the results of the closeness test for functional properties and flavor characters of 28 (twenty eight) experiment units and 10 (ten) cooking spices circulated in Indonesia show that refined tempeh hydrolyzated flour (TTA01) has the functional properties and flavor characters that are close to the experiment unit with the code 6/9H and closeness value of 23.041, experiment unit with the code 10/6H and closeness value of 36.910, and the experiment unit with the code 17/18H and closeness value of 37.680.

Monosodium glutamate (MSG) cooking spice has the functional properties and flavor characters that is close tothe experiment unit with the code VC (Chinese MSG) with the closeness value of 103.117, experiment unit with the code 23/23H with the closeness value of 519,542, and the experiment unit with the code 21/27H with the closeness value of 535.968.

Chinese MSG (VC) has the functional properties and flavor characters that is close tothe experiment unit with the code 23/23H with the closeness value of 531.641, experiment unit with the code 21/27H with the closeness value of 532.470, the experiment unit with the code BM10 (Totole mushroom cooking spice) with the closeness value of 556.233, and the experiment unit with the code 16/19H with the closeness of 567.142.

Balado cooking spice (BM02) has the functional properties and flavor characters that is close tothe experiment unit with the code BM04 (Indofood magic cooking spice) with the closeness value of 138.346, experiment unit with the code BM10 (Totole mushroom cooking spice) with the closeness value of 186.404, experiment unit with the code 19/25H with the closeness value of 295.876, experiment unit with the code 5/1H with the closeness value of 331.639, and the experiment unit with the code 4/15H with the closeness value of 365.729.

Curry cooking spice (BM03) has the functional propertiesand flavor characters that is close tothe experiment unit with the code 3/14H with the closeness value of 236.961, experiment unit with the code 7/5H with the closeness value of 272.711 and the experiment unit with the code 10/6H with the closeness value of 298.586.

Indofood magic cooking spice (BM04) has the functional properties and flavor characters that is close tothe experiment unit with the code BM02 (balado cooking spice) with the closeness value of 138.346, experiment unit with the code kode 5/1H with the closeness value of 185.446, experiment unit with the code BM 10 (Totole mushroom cooking spice) with the closeness value of 189.918, experiment unit with the code 19/25H with the closeness value of 234.247, experiment unit with the code BM05 (mushroom broth cooking spice) with the closeness value of 230.235, and the experiment unit with the code 4/15H with the closeness value of 247.14.

Mushroom broth cooking spice (BM05) has the functional properties and flavor characters that is close tothe experiment unit with the code BM09 (chicken broth cooking spice) with the closeness value of 23.420, experiment unit with the code BM08 (beef broth cooking spice) with the closeness value of 123.606, experiment unit with the code 5/1H with the closeness value of 547.213, and the experiment unit with the code 4/15H with the closeness value of 629.609.

Mama cooking spice (BM07) has the functional properties and flavor characters that is close tothe experiment unit with the code 19/25H with the closeness value of 497.350, experiment unit with the code 5/1H with the closeness value of 500.520, experiment unit with the code 4/15H with the closeness value of 542.548, experiment unit with the code BM08 (beef broth cooking spice) with the closeness value of 82.727, and the experiment unit with the code BM05 (mushroom broth cooking spice) with the closeness value of 209.251.

Beef broth cooking spice (BM08) has the functional properties and flavor characters that is close to the experiment unit with the code 5/1H with the closeness value of 389.141, experiment unit with the code 4/15H with the closeness value of 480.310, experiment unit with the code 19/25H with the closeness value of 497.777, experiment unit with the code BM07 (mama cooking spice) with the closeness value of 82.727, and the experiment unit with the code BM05 (mushroom broth cooking spice) with the closeness value of 123.606.

Chicken broth cooking spice (BM09) has the functional properties and flavor characters with the experiment unit with the code 5/1H with the closeness value of 545.425, experiment unit with the code 19/25H with the closeness value of 561.644, experiment unit with the code 4/15H with the closeness value of 592.130, experiment unit with the code BM05 (mushroom broth cooking spice) with the closeness value of 23.420, and the experiment unit with the code BM07 (mama cooking spice) with the closeness value of 170.112.

Totole mushroom cooking spice (BM10) has the functional properties and flavor characters that is close to the experiment unit with the code 19/25H with the closeness value of 225.805, experiment unit with the code 4/15H with the closeness value of 258.323, experiment unit with the code 5/1H with the closeness value of 297.805, experiment unit with the code BM02 (balado cooking spice) with the closeness value of 186.404, and the experiment unit with the code BM04 (Indofood Magic cooking spice) with the closeness value of 189.918. Spectral data was used since cluster analysis strongly indicated an increase in charm values between the duplicate (N.Millller, 1998).

CONCLUSION

1. The results of principal component analysis on 28 (twenty eight) experiment units and 10 (ten) cooking spices in Indonesia that arerefined tempeh hydrolyzated flours 4/15H with a loading factor value of 0.646, 5/1H with a loading factor value of 0.579 and refined tempeh hydrolyzated flour 19/25H with loading factor value of 0.618 show thatall three of them have the potential in theirflavor characters and functional properties to bethe main ingredients for natural flavor enhancers which have similarities with monosodium glutamate (MSG) with a loading factor of 0.602, Chinese MSG (VC) with a loading factor of 0.581, and curry cooking spice (BM03) with a loading factor of 0.681.
2. The results of hierarchical cluster analysis of 28 (twenty eight) experiment units and 10 (ten) cooking spices in Indonesia, it shows that the refined tempeh hydrolyzated flour 23/23H has the flavor characters and functional properties as the main ingredient for natural flavor enhancers that are close to monosodium. glutamate (MSG) with the closeness value of 519,542 and ChineseMSG (VC) with the closeness value of 531,641. Refined tempeh hydrolyzated flour 3/14H has the flavor characters and functional properties that are close to curry

cooking spice (BM03) with the closeness value of 236,961.

DEDICATION PAGE

An expression of gratitude is addressed to the Indonesia Endowment Fund for Education (LPDP) who has funded this research through dissertation grant scheme in 2018.

BIBLIOGRAPHY

1. B.M. Nicolai, A.Berna, K.Beullens, S. V. and J. L. (2008). *Fruit and Vegetabble Flavour, Recent Advances and Future Prospects* (B. B. and S. G. Wyllie, Ed.). Woodhead Abington Hall, Abington, Cambridge CB21 6AH, England: CRC Press, Woodhead Publishing Limited.
2. Ba, M., Zimmermann, B., & Kohler, A. (2015). *A Multiscale Vibrational Spectroscopic Approach for Identification and Biochemical Characterization of Pollen*. 1–20. https://doi.org/10.1371/journal.pone.0137899
3. Bernal, L. F., Lopez, G., Ruiz, M., Vera-Bravo, R., Reyes, A., & Baena, S. (2017). Response Surface Methodology (RSM) for analysing culture conditions of Acidocella facilis strain USBA-GBX-505 and Partial Purification and Biochemical Characterization of Lipase 505 LIP. *Universitas Scientiarum*, *22*(1), 45. https://doi.org/10.11144/javeriana.sc22-1.rsmr
4. Cebi, N., Dogan, C. E., Develioglu, A., Yayla, M. E. A., & Sagdic, O. (2017). Detection of L-Cysteine in wheat flour by Raman microspectroscopy combined chemometrics of HCA and PCA. *Food Chemistry*, *228*, 116–124. https://doi.org/10.1016/j.foodchem.2017.01.132
5. David Owens, J. (2014). Indigenous fermented foods of southeast Asia. *Indigenous Fermented Foods of Southeast Asia*, (February 2018), 1–416. https://doi.org/10.1201/b17835
6. Davies, J. R. (2003). *Herbs, Natural Healing & Nutrition*.
7. Dewi Puspitasari Tirtaningtyas Gunawan Puteri, M., Christli, L., Kartika Prabawati, E., & Muzi Marpaung, A. (2018). *Development of Rice Porridge With Overripe Tempeh Extract for Infants*. (January). https://doi.org/10.2991/fanres-18.2018.39
8. Dey, S. S., & Dora, K. C. (2014). Optimization of the production of shrimp waste protein hydrolysate using microbial proteases adopting response surface methodology. In *Journal of Food Science and Technology* (Vol. 51). https://doi.org/10.1007/s13197-011-0455-4
9. Erkan, S. B., Gürler, H. N., Bilgin, D. G., Germec, M., & Turhan, I. (2020). Production and characterization of tempehs from different sources of legume by Rhizopus oligosporus. *Lwt*, *119*(October), 108880. https://doi.org/10.1016/j.lwt.2019.108880
10. Fei, G., Lujia, H., & Xian, L. (2017). *Vibration spectroscopic technique for species identification based on lipid characteristics*. *10*(3). https://doi.org/10.3965/j.ijabe.20171003.3144
11. Gauglitz, E. G. (2003). *Handbook of Spectroscopy Near-Infrared Spectroscopy Handbook of Analytical Techniques In-situ Spectroscopy in Heterogeneous Catalysis*. Weinheim, The Federal Republic of Germany: Wiley-VCH Verlag GmBH & Co KGaA.
12. Gertjan J. Schaafsma. (2009). *Review safety protein hydrolysates in human nutrition.pdf*. Scherpenzeel, Netherlands: European Journal of Clinical Nutrition (2009) 63, 1161-1168.
13. Helge Toutenburg, S. (n.d.). *Statistical Analysis of Designed Experiments* (Third). New York Dordrecht Heidelberg London: Springer Science.
14. Hii, S. L., Lip, K. F., Loh, Y. T., & Wong, C. L. (2014). Statistical optimization of fermentable sugar extraction from the Malaysian brown alga Sargassum binderi. *Journal of Applied Phycology*, *27*(5), 2089–2098. https://doi.org/10.1007/s10811-014-0480-6
15. Kustyawati, M. E., Pratama, F., Saputra, D., & Wijaya, A. (2020). Shelf life of tempeh processed with sub-supercritical carbon dioxides. *Potravinarstvo Slovak Journal of Food Sciences*, *14*(August), 351–357. https://doi.org/10.5219/1247
16. Kusumah, D., Wakui, M., Murakami, M., Xie, X., Yukihito, K., & Maeda, I. (2020). Linoleic acid, α-linolenic acid, and monolinolenins as antibacterial substances in the heat-processed soybean fermented with Rhizopus oligosporus. *Bioscience, Biotechnology and Biochemistry*, *84*(6), 1285–1290. https://doi.org/10.1080/09168451.2020.1731299
17. Maran, G. G., Septaningrum, N. R., Wulandari, R., Utomo, R. Y., Hermawan, A., & Meiyanto, E. (2019). The proliferative effect of tempeh in female ovariectomized rats. *AIP Conference Proceedings*, *2099*(April). https://doi.org/10.1063/1.5098419
18. Mohamed, H. T., Untereiner, V., & Sockalingum, G. D. (2017). *Implementation of infrared and Raman modalities for glycosaminoglycan characterization in complex systems*. 309–323. https://doi.org/10.1007/s10719-016-9743-6
19. Mujianto, , Witono, Y., Wignyanto, , Kumalaningsih, S., & , A. (2018). Hydrolysis Characteristics of Over Fermented Tempe (Fermented Soybean Cake) Product Hydrolyzed by Enzymatic Hydrolysis as Natural Flavor Source (Flavor Enhancer). *The Indian Journal of Nutrition and Dietetics*, *55*(1), 29. https://doi.org/10.21048/ijnd.2018.55.1.18062
20. Mujianto, Wignyanto, Hidayat N., & Aulianni'Am. (2020). *The Similarity of Fourier Transform Infrared (FTIR) Spice in Indonesia Spectroscopy Absorbance in Several Products of Cooking*. *11*(8), 640–650.
21. N.Milller, R. T. M. and. (1998). *Determination of the Cause of Off-Flavors in Milk by Headspace GC/MS and Multivariate Data Analysis* (F. S. and A. M. S. E.T.Contis, C.T.HO, C.J.Mussinan, T.H.Parliment, Ed.). P.O. Box 521, 1000 AM Amsterdam, The Netherlands.: Elsevier Science B.V.
22. Noviana, A., Dieny, F. F., Rustanti, N., Anjani, G., & Afifah, D. N. (2018). Antimicrobial activity of tempeh gembus hydrolyzate. *IOP Conference Series: Earth and Environmental Science*, *116*(1). https://doi.org/10.1088/1755-1315/116/1/012044
23. Owen R.Fennema, Y.H. Hui, Marcus Karel, Pieter Walstra, J. R. W. (1999). *FOOD SCIENCE AND TECHNOLOGY*.
24. Peter, K. V. (2004). *Handbook of Herb and Spices* (2nd ed.). Cambridge England: Woodhead Publishing Limited.
25. Phongthai, S., Lim, S. T., & Rawdkuen, S. (2016). Optimization of microwave-assisted extraction of rice bran protein and its hydrolysates properties. *Journal of Cereal Science*, *70*. https://doi.org/10.1016/j.jcs.2016.06.001
26. R.Scott, C. F. and T. (1997). *Food Flavours Biology and Chemistry*. The Royal Society of Chemistry.

27. Sangani, V. P., Patel, N. C., Bhatt, V. M., Davara, P. R., & Antala, D. K. (2014). Optimization of enzymatic hydrolysis of pigeon pea for cooking quality of dhal. *International Journal of Agricultural and Biological Engineering*, *7*(5), 123–133. https://doi.org/10.3965/j.ijabe.20140705.014
28. Satyajit D.Sarker, Zahid Latif, A. I. G. (2006). *Natural Products Isolation* (Second). Totowa, New Jersey: Humana Press.
29. Sonklin, C., Laohakunjit, N., Kerdchoechuen, O., & Ratanakhanokchai, K. (2018). Volatile flavour compounds, sensory characteristics and antioxidant activities of mungbean meal protein hydrolysed by bromelain. *Journal of Food Science and Technology*, *55*(1), 265–277. https://doi.org/10.1007/s13197-017-2935-7
30. Stephanie, Kartawidjajaputra, F., Silo, W., Yogiara, Y., & Suwanto, A. (2019). Tempeh consumption enhanced beneficial bacteria in the human gut. *Food Research*, *3*(1), 57–63. https://doi.org/10.26656/fr.2017.3(1).230
31. Subburaj, J., Barathkumar, T. R., Prem, V., Thangaraj, M., & Sivasubramanian, J. (2013). *SPECTROSCOPIC ANALYSIS OF FIVE PHYLOGENETICALLY DISTANT FUNGI (DIVISION : ASCOMYCETE) FROM VELLAR ESTUARY , SOUTHEAST COAST OF INDIA – A PILOT STUDY*. *2*(5), 2355–2359.
32. Zhong, Q., Yang, C., Großerüschkamp, F., Kallenbach-thieltges, A., Gerwert, K., & Mosig, A. (2013). *Similarity maps and hierarchical clustering for annotating FT-IR spectral images*.