https://dergipark.org.tr/en/pub/turkager 2024, 5(1): 1-20 DOI: 10.46592/turkager.1304379

Turkish Journal of Agricultural Engineering Research
(Turk J Agr Eng Res)
e-ISSN: 2717-8420

TURKAGER

Manufacturing of Healthy and Functional Savoury Flavours Using Over-Fermented Tempeh Hydrolisate Flour

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ARTICLE INFO: Research Article
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Received: 28 May 2023 **/ Accepted:** 20 December 2023 **/ Published:** 30 June 2024

Cite this article: Wignyanto, W and Mujianto M (2024). Manufacturing of Healthy and Functional Savoury Flavours Using Over-Fermented Tempeh Hydrolisate Flour. *Turkish Journal of Agricultural Engineering Research, 5(1), 1-20. https://doi.org/10.46592/turkager.1304379*

ABSTRACT

This research aims to: 1) determine the closeness of the properties of overfermented tempeh protein hydrolyzate flour to the properties of 11 cooking spices, 2) determine the potential of overfermented tempeh protein hydrolyzate flour as a raw material for the cooking spices industry. Fourier Transform Infrared (FTIR) Spectroscopy absorbance patterns were analyzed using principal component analysis (PCA) and hierarchical cluster analysis (HCA). Based on the PCA, the study's findings indicated that over-fermented tempeh hydrolisate flour 19/25H with a loading factor value of 0.617, 22/12H with a loading factor value of 0.609, 5/1H with a loading factor value of 0.533, 14/7H with a loading factor value of 0.533, 15/8H with a loading factor value of 0.528, 20/17H with a loading factor value of 0.513, As the primary ingredient for the savoury flavors of grilled chicken (SF01), over-fermented tempeh hydrolisate flour 4/15H with a loading factor value of 0.504 and 9/10H with a loading factor value of 0.505 both offer potential flavor character, Balado (SF02), Barbeque (SF03), Spicy Corn (SF04), Cheese (SF05), Salty Cheese (SF06), Sweet Spicy (SF07), Roasted Beef (SF08) and Tiramisu (SF09). Based on the result of hierarchical cluster analysis of over-fermented tempeh hydrolisate flour 9/10H and 23/23H, they have flavour character and functional properties as the main ingredient for the Savoury Flavours of Grilled Chicken (SF01) with closeness value of 453.406 (9/10H) and 465.536 (23/23H), Balado (SF02) with closeness value of 506.061 (9/10H) and 544.227 (23/23H), Barbeque (SF03) with a closeness value of 593.029 (9/10H) and 652.165 (23/23H), Spicy Corn (SF04) with closeness value of 595.097 (9/10H) and 632.614 (23/23H), Cheese (SF05) with closeness value of 482.596 (9/10H) and 520.814 (23/23H), Salty Cheese (SF06) with closeness value of 469.605 (9/10H) and 475.465 (23/23H), Sweet Spicy (SF07) with closeness value of 515.754 (9/10H) and 563.700 (23/23H), Roasted Beef (SF08) with closeness value of 526.120 (9/10H) and 525.428 (23/23H) and Tiramisu (SF09) with closeness value of 520.196 (9/10H) and 551.815 (23/23H).

Keywords: *Fourie Transform Infrared (FTIR), Hierarchical Cluster Analysis (HCA), Principal Component Analysis (PCA), Savoury flavors*

INTRODUCTION

Tempeh is a fermented food made of soybeans. It is a nutritious, affordable, and sustainable functional source of protein. Tempeh has been a widely accepted fermented product (Ahnan-Winarno *et al.*, 2021). Savoury flavour is flavour which gives taste to dry food (snacks), such as those made of potato (*Solanum tuberosum*), cassava (*Manihot utilissima*), banana (*Musa balbisiana*), sweet potato (*Ipomoea batatas*), breadfruit (*Artocarpus incissus*), melinjo chips (*Gnetum gnemon*), gayam fruit (*Inocarpus edulis*), corn (*Zea mays*), peanut (Arachis hypogeae), bean (*Canavilia gladiata*), pruriens (*Dolichos lablab*), chickpeas (*Pisum sativum*), bogor beans (*Voadzeia subterranea*), and biscuits and fruits which are generally sliced thinly and fried. The savoury flavours distributed in commercial markets are available in various tastes, such as salty savoury, grilled chicken, barbeque chicken, onion chicken, black pepper chicken, roasted chicken, citrus-scented balado, red balado, super red balado, orange balado, roasted balado, spicy balado, barbeque, spicy barbeque, chocolate tiramisu seasoning, grilled squid, green tea seasoning, grilled corn, sweet corn, chicken broth, balado chili sauce, green chili sauce, black pepper beef, roasted beef and many other taste variants. The savoury flavour is defined as far as possible and discussed with examples of delicious foods. According to a study, there is a direct causal relationship between 2 variations of MSG and 9 (nine) brands of seasoning (savoury flavors) created from food additives and supportive materials with eigenvalues equal to 8.854 or 98.374 of variant. The seasonings with grilled chicken flavor (SF7), salted cheese flavor (SF5), roasted beef flavor (SF3), barbeque flavor (SF9), tiramisu flavor (SF6), spicy corn flavor (SF10), and monosodium glutamate (MSG) with eigenvalues score of 7.416 or 67.416% of variant all exhibit this direct causative correlation. Balado flavored seasoning (SF8), cheese flavored seasoning (SF4), sweet, spicy flavored seasoning (SF11), and Chinese flavor enhancing ingredients have direct causality with Chinese flavor enhancing ingredients, with eigenvalues scores of 1.517 or 13.792% of the variant. Monosodium glutamate (SF1) and Chinese flavor enhancing ingredient have similar eigenvalues score, which is 1.108 or 10.071% of variant (Mujianto *et al.*, 2020). It was characterized by the complexity of its taste, odour and trigeminally mediated attributes as by its combination of stimulation by mouth with lack of sweetness (Land, 1994) the moisture content of white bean (55.20%) and red lentil (55.99%) tempeh was similar to that of soy tempeh (55.45%) (Erkan *et al.*, 2020).

The characteristics of protein hydrolyzed from enzymatic hydrolysis process of rejected tempeh. The result of this study indicates that the highest Hydrolysis Level (HL) belongs to Flavorzyme enzyme (10.3% HL), Protamex (8.4% HL) and Calontropin (7.1% HL). The enzymatic reaction rate for Flavorzyme enzyme is V max, as much as 0.01727 mg per ml per minute, while the content of glutamate acid in hydrolyzed over fermented-tempeh reaches 15.95% (Mujianto *et al.*, 2018). Several groups of microorganism from fermented food such as tempeh and tape were reported; the potential LAB (Lactic Acid Bacteria) derived from those food sources is limited (Novarina *et al.*, 2020). Tempeh aroma and texture showed strong correlation between the numbers of bacterial population during the period or stages of tempeh maturation (Nur *et al.*, 2020). For centuries, fermented soy foods have been dietary staples in Asia and, now, in response to consumer demand, they are available

throughout the world. Fermentation produces unique flavors, boosts nutritional values, and increases or adds new functional properties (Cao *et al.*, 2019). The content of polyphenols and the antioxidative properties were determined in soybean tempeh fermented by four strains of Rhizopus oligosporus (Kuligowski *et al.*, 2017). In 2004, the value of savoury snack market in Thailand was expected to be worth 10-12 billion baht and this market consisted of puffed snacks (40%), potato chips (30 %), puffed rice (9%), prawn crackers (8-9%), fish snacks (8%), and others flavors for snack-food application (Wangcharoen *et al.*, 2006). The need for high nutritional value and healthy savoury flavors opens up opportunities for industries made from over fermented tempeh hydrolysate flour.

MATERIALS and METHODS

Materials

This study uses 9 (nine) kinds of savoury flavor, 1. Grilled Chicken (SF01), 2. Balado (SF02), 3. Barbeque (SF03), 4. Spicy Corn (SF04), 5. Cheese (SF05), 6. Salted Cheese (SF06), 7. Sweet Spicy (SF07), 8. Roasted Beef (SF08) and, 9. Tiramisu (SF09). Several savoury flavor stores in Surabaya provided the nine (9) different types of savoury flavors. Other ingredients include over-fermented tempeh flour (TTA01), over-fermented tempeh flour is made by crushing over-fermented tempeh obtained from traditional markets in Sidoarjo. Xingtai Sinobest Biotech Co.LTD is the manufacturer of the enzymes used, which include mannase (M), cellulose neutral enzyme (NC), and neutral protease enzyme (NP) and 2 (two) variants of monosodium glutamate, namely: 1. Monosodium glutamate (MSG) and, 2. Chinese Vetsin (VC).

Methods

Research method with Central Composite Design (CCD) model Quadratic Response Surface Method (QRSM) using Design Expert for Design of Experiment (DOE) software version 7.1. Data from Absorbance observations from Fourier Transform Infrared (FTIR) spectroscopy was processed using OriginLab software version 8.5. For PCA and HCA using SPSS software version 25.

RESULTS AND DISCUSSION

The descriptive statistic analysis results of 27 (twenty-seven) experiment units, 9 (nine) savoury flavours, 2 (two) types of MSG and over-fermented tempeh flour as seen in Table 2, which indicates that 23/23 H experiment unit has minimal absorbance average value of 27.4601 ± 9.06326. Meanwhile, cheese flavor or SF02 has a maximum absorbance average value of 66.3262 ± 27.13280. The total absorbance point observation from those 39 (thirty-nine) variables was 72.852 (seventy-two thousand eight hundred and fifty-two).

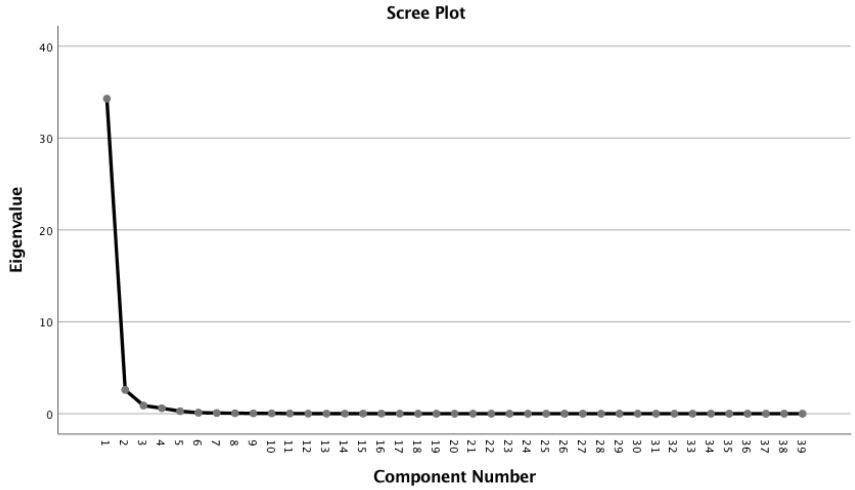

***Figure 1**. The correlation between eigenvalues and the 39 (thirty-nine) Principal Component.*

Figure 1 shows the correlation between eigenvalue and 39 principal components. The correlation between the eigenvalues score and the principal component in the above figure shows that up to the 10th principal component (F10), the eigenvalues score approaches 0 (zero). Cumulatively, the percentage (%) of variants explained wass 99.903%. For nine (9) savory flavors, the KMO test result was 0.949 and the Barlett's test result was 421,242,458 with degrees of freedom of 741, suggesting that the data was normally distributed. The test result of KMO score > 0.50 is 0.949 > 0.500, which means that the variable can be predicted and further analysed. Bartlett's test < 0.05 is 0.000 < 0.050 indicating that inter-variables have a high correlation.

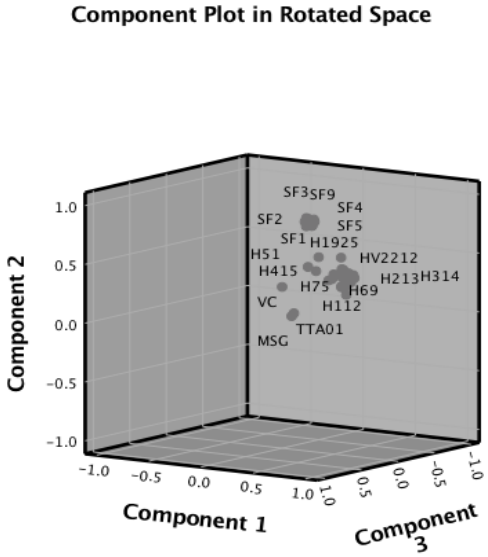

***Figure 2**. Correlation between the first principal component (F1), second principal component (F2) and third principal component (F3) of 27 (twenty-seven) experiment units, 2 (two) MSG Variants, over-fermented tempeh flour and 9 (nine) savoury flavours from FTIR absorbance observation result.*

Based on the Figure 2, from 39 (thirty-nine) variables of principal component constituent, 54.56% is in the 1st (first) principal component, 36.825% is in the 2nd (second) principal component, and 8.615% is in the 3rd (third) principal component.

The suitability of attenuated total reflection (ATR) mid-infrared (MIR) spectroscopy, combined with principal component analysis (PCA) and partial least squares (PLS) regression, was evaluated as a rapid analytical technique for the classification of sparkling wine style and quality (Culbert *et al.*, 2015). Principal Component Analysis (PCA) is frequently used to display and discover patterns in SNP data from humans, animals, plants, and microbes, especially to elucidate population structure. Given the popularity of PCA, one might expect that PCA is understood well and applied effectively (Gauch *et al.*, 2019). FTIR-ATR combined with chemometrics analysis such as hierarchical cluster analysis (HCA), principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) was used for classification and discrimination of gelatin gummy candies related to their gelatin source (Cebi *et al.*, 2019). Principal component analysis and hierarchical cluster analysis can be used to determine the similarity or proximity of material characteristics. Table 1 below shows the multi-enzyme hydrolysis surface response design layout of over-fermented tempeh flour.

Table 1*. Multi-enzyme hydrolysis surface respond design layout of over-fermented tempeh flour.*

No	Std	RUN	Block	A T°C	B L/S (%)	C NP (%)	D NC (%)	E M (%)
1	4	15	Block 1	50	0.4	2	1	1
2	13	4	Block 1	45	0.3	1.5	1.5	1.5
3	3	14	Block 1	40	0.4	2	1	2
4	10	6	Block 1	40	0.4	2	2	1
5	6	9	Block 1	50	0.2	1	2	2
6	8	3	Block 1	40	0.4	1	2	2
7	1	12	Block 1	50	0.4	1	2	1
8	5	1	Block 1	50	0.4	1	1	2
9	14	7	Block 1	45	0.3	1.5	1.5	1.5
10	9	10	Block 1	50	0.2	2	1	2
11	2	13	Block 1	50	0.2	2	2	1
12	15	8	Block 1	45	0.3	1.5	1.5	1.5
13	12	11	Block 1	45	0.3	1.5	1.5	1.5
14	11	2	Block 1	40	0.2	1	1	1
15	7	5	Block 1	40	0.2	2	2	2
16	20	17	Block 2	45	0.3	0.58942	1.5	1.5
17	19	25	Block 2	45	0.482116	1.5	1.5	1.5
18	17	18	Block 2	54.1058	0.3	1.5	1.5	1.5
19	21	27	Block 2	45	0.3	2.41058	1.5	1.5
20	24	24	Block 2	45	0.3	1.5	1.5	0.58942
21	26	16	Block 2	45	0.3	1.5	1.5	1.5
22	16	19	Block 2	35.8942	0.3	1.5	1.5	1.5
23	25	26	Block 2	45	0.3	1.5	1.5	2.41058
24	23	23	Block 2	45	0.3	1.5	2.41058	1.5
25	22	22	Block 2	45	0.3	1.5	0.58942	1.5
26	27	21	Block 2	45	0.3	1.5	1.5	1.5
27	18	20	Block 2	45	0.117884	1.5	1.5	1.5

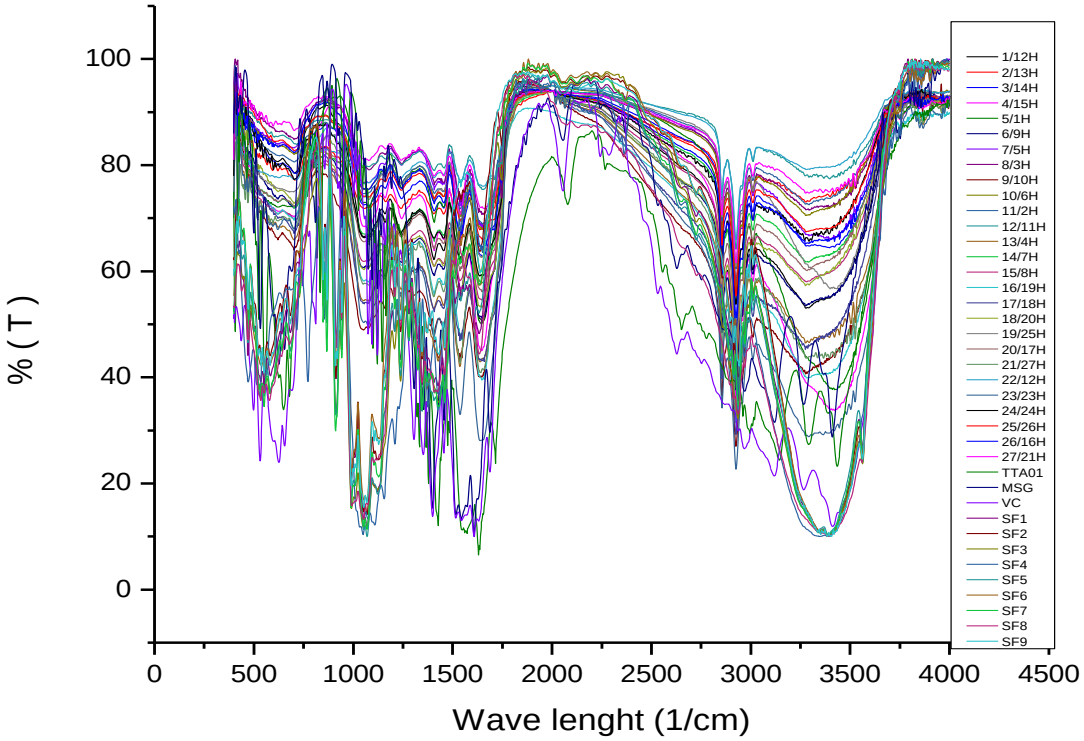

Figure 3. *The absorbance pattern of 27 (twenty-seven) experiment units, over-fermented tempeh flour (TTA01), 2 (two) MSG variants and 9 (nine) savoury flavours in Indonesia.*

Table 2. *The descriptive statistics of 27 (twenty-seven) experiment units, 9 (nine) savoury flavours, 2 (two) MSG variants and over-fermented tempeh flour (TTA01).*

Descriptive Statistics				
No	**Variable**	**Mean**	**Std. Deviation**	**N Analysis**
1	1/12H	76.6104	14.20904	1868
2	2/13H	81.4654	8.20171	1868
3	3/14H	80.6243	9.85148	1868
4	4/15H	73.4221	17.79953	1868
5	5/1H	76.5199	16.25822	1868
6	6/9H	73.9939	13.88617	1868
7	7/5H	83.4050	9.03077	1868
8	8/3H	84.6223	7.87576	1868
9	9/10H	66.9074	18.14567	1868
10	10/6H	83.5856	8.48006	1868
11	11/2H	83.9339	7.89538	1868
12	12/11H	85.9742	5.93450	1868
13	13/4H	69.8087	16.12828	1868
14	14/7H	77.7141	11.14317	1868
15	15/8H	75.1172	12.60613	1868
16	16/19H	69.6567	16.52951	1868
17	17/18H	69.9997	16.82641	1868
18	18/20H	75.0469	12.86786	1868
19	19/25H	80.6327	10.49047	1868
20	20/17H	77.0628	11.73228	1868
21	21/27H	70.6065	16.57611	1868
22	22/12H	84.9985	6.66885	1868
23	23/23H	65.7346	21.61881	1868
24	24/24H	79.5129	10.59181	1868
25	25/26H	82.1704	9.00046	1868
26	26/16H	81.3369	9.91432	1868
27	27/21H	85.3122	7.00052	1868
28	TTA01	58.4175	23.21023	1868
29	MSG	67.3225	23.42716	1868
30	VC	54.8081	27.70221	1868
31	SF1	62.4048	27.22395	1868
32	SF2	66.3262	27.13280	1868
33	SF3	61.4711	28.48600	1868
34	SF4	60.3558	27.67728	1868
35	SF5	61.1741	27.51418	1868
36	SF6	65.7723	26.78064	1868
37	SF7	62.4748	27.94771	1868
38	SF8	58.7726	27.04615	1868
39	SF9	65.6665	27.08503	1868

Table 3. *The result of FTIR absorbance pattern variable ınterpretation of over-fermented tempeh flour, 27 (twenty-seven) experiment units and 2 (two) MSG variants from 59,776 observation points.*

No	Variable	Factor	Eigen Values	Loading Factor	% Variance	Cumulative %
1	2/13H	The similarity of flavour character and material functional properties with over-fermented tempeh flour, 27 (twenty-seven) over-fermented tempeh hydrolisate flour and 2 (two) MSG variants (F1)	18.107	0.855	46.428	46.428
2	24/24H			0.848		
3	18/20H			0.833		
4	12/11H			0.824		
5	10/6H			0.821		
6	27/21H			0.820		
7	11/2H			0.814		
8	25/26H			0.805		
9	17/18H			0.803		
10	6/9H			0.796		
11	8/3H			0.793		
12	20/17H			0.790		
13	15/8H			0.790		
14	1/12H			0.786		
15	26/16H			0.782		
16	13/4H			0.781		
17	14/7H			0.778		
18	9/10H			0.775		
19	3/14H			0.775		
20	21/27H			0.735		
21	16/19H			0.720		
22	7/5H			0.710		
23	22/12H			0.702		
24	23/23H			0.701		
25	4/15H			0.573		
26	19/25H			0.572		
27	TTA01			0.596		
28	MSG			0.616		
29	VC			0.502		
30	5/1H			0.499		

Figure 3 above shows the absorbance pattern of 27 (twenty-seven) experimental units, over-fermented tempeh flour (TTA01), 2 (two) MSG variants and 9 (nine) savory flavors in Indonesia. The 1st principal component (F1), after variable extraction and rotation using varimax method, has 30 (thirty) variables of the 1st principal component (F1) with eigenvalues score more than or equivalent to 0.50 respectively as follows:

Based on Table 3, the equation for the 1st principal component (F1) is the function of flavour character similarities and material functional properties 18/20H, 12/11H, 10/6H, 27/21H, 11/2H, 25/26H, 17/18H, 6/9H, 8/3H, 20/17H, 15/8H, 1/12H, 26/16H, 13/4H, 14/7H, 9/10H, 3/14H, 21/27H, 16/19H, 7/5H, 22/12H, 23/23H, 4/15H, 19/25H, TTA01, MSG, VC and 5/1H with eigenvalues score of 18.107 and total with over-

fermented tempeh flour, 27 (twenty-seven) over-fermented tempeh hydrolisate flour of the 1st (first) variant and 2 (two) MSG variants as in equation (1) below :

F1 = 0.855 (2/13H) + 0.848 (24/24H) + 0.833 (18/20H) + 0.824 (12/11H) + 0.821 (10/6H) + 0.820 (27/21H) + 0.814 (11/2H) + 0.805 (25/26H) + 0.803 (17/18H) + 0.796 (6/9H) + 0.793 (8/3H) + 0.790 (20/17H) + 0.790 (15/8H) + 0.786 (1/12H) + 0.782 (26/16H) +0.781 (13/4H) + 0.778 (14/7H) + 0.775 (9/10H) + 0.775 (3/14H) + 0.735 (21/27H) + 0.720 (16/19H) + 0.710 (7/5H) + 0.702 (22/12H) + 0.701 (23/23H) + 0.573 (4/15H) + 0.572 (19/25H) + 0.596 (TTA01) + 0.616 (MSG) + 0.502 (VC) + 0.499 (5/1H) (1)

Remark:

F1 = The Similarity of Flavour Character and Material Functional Properties of Over-Fermented Tempeh Flour (TTA01), 27 (twenty-seven) Over-Fermented Tempeh Hydrolisate Flour, and 2 (two) MSG Variants.

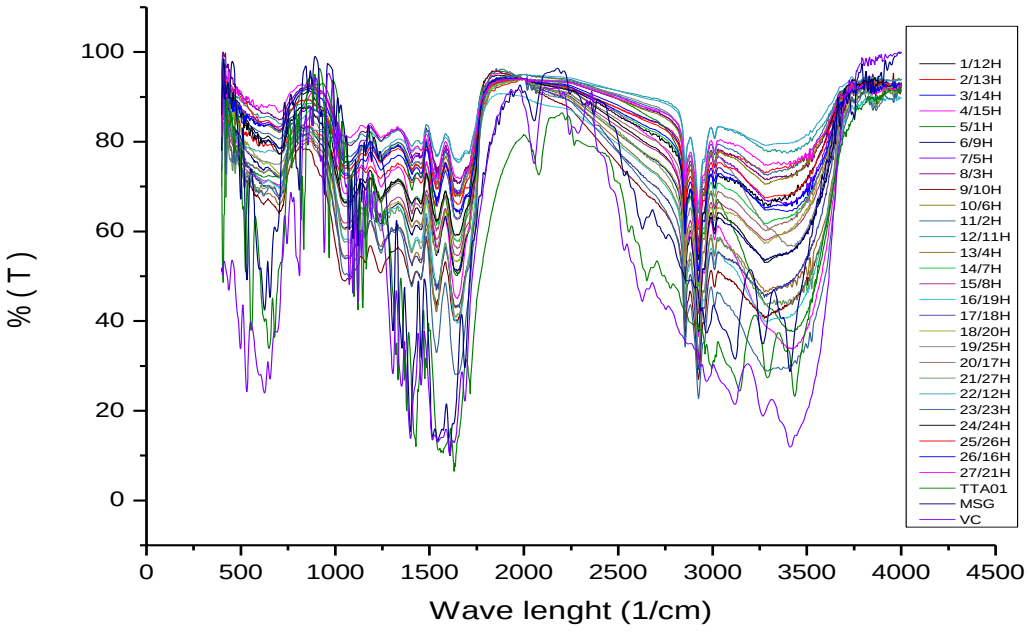

Figure 4*. The absorbance pattern of the 1st principal component constituent variable (F1).*

Figure 4 above shows the absorbance pattern of the 1st principal component constituent variable (F1). Based on equation (1) above, there are 9 (nine) variables with coefficient value (NK) of $0.803 \leq NK \leq 0.855$, namely 2/13H, 24/24H, 18/20H, 12/11H, 10/6H, 27/21H, 11/2H, 25/26H and 17/18H. There are 14 (fourteen) variables with coefficient value (NK) of $0.701 \leq NK \leq 0.796$, namely 6/9H, 8/3H, 20/17H, 15/8H, 1/12H, 26/16H, 13/4H, 14/7H, 9/10H, 3/14H, 21/27H, 16/19H, 7/5H, 22/12H and 23/23H. There are 6 (six) variables with coefficient value (NK) of $0.499 \leq NK \leq 0.616$, namely 4/15H, 19/25H, TTA01, MSG, VC and 5/1H. The closeness of coefficient value (NK) indicates the proximity of flavour characteristic properties and material functional properties.

The analytical method using infrared spectrophotometry requires a little sample preparation, and evaluation of the spectrogram was carried out in the protein and carbohydrate absorbance area (Kos *et al.*, 2016). Spectral information can also be exploited for strain typing purposes, which are particularly important for epidemiological analyses and some technological applications. Accordingly, in recent years, FTIR spectroscopy has been increasingly used for typing and classifying microorganisms below the species level (Wenning and Scherer, 2013).

Table 4. *FTIR absorbance pattern variable ınterpretation of over-fermented tempeh flour and 22 (twenty-two) experiment units and 9 (nine) savoury flavours from 57,908 observation points.*

No	Variable	Factor	Eigen Values	Loading Factor	%Variance	Cumulative %
1	2/13H	The similarity of flavour character and material functional properties of 19 (Nineteen) over-fermented tempeh hydrolisate flour of the 1st variant with 9 (nine) savoury flavours (F2)		0.455		
2	18/20H			0.454		
3	12/11H			0.473		
4	10/6H			0.447		
5	11/2H			0.497		
6	17/18H			0,457		
7	6/9H			0.484		
8	20/17H			0.513		
9	15/8H			0.528		
10	13/4H			0.499		
11	14/7H			0.533		
12	9/10H			0.505		
13	3/14H			0.473		
14	21/27H		13.283	0.491	34.058	80.487
15	16/19H			0.449		
16	7/5H			0.463		
17	22/12H			0.609		
18	4/15H			0.504		
19	SF9			0.903		
20	SF2			0.898		
21	SF3			0.895		
22	SF6			0.880		
23	SF7			0.879		
24	SF4			0.872		
25	SF5			0.860		
26	SF1			0.855		
27	SF8			0.850		
28	19/25H			0.617		
29	5/1H			0.533		

The 2nd principal component (F2), after doing variable extraction and rotation using varimax method, has 29 (twenty-nine) 2nd principal components (F2) with eigenvalues score more than or equivalent to 0.50 respectively as follows: 2/13H, 18/20H, 12/11H, 10/6H, 11/2H, 17/18H, 6/9H, 20/17H, 15/8H, 13/4H, 14/7H, 9/10H, 3/14H, 21/27H, 16/19H, 7/5H, 22/12H, 4/15H, SF9, SF2, SF3, SF6, SF7, SF4, SF5, SF1, SF8, 19/25H and 5/1H with eigenvalues score of 13.283 and total cumulative variant of 34.058%. Based on Table 4 above, the equation for the 2nd principal component (F2) that is the function of flavour character similarities and material functional properties of 20 (twenty) over-fermented tempeh hydrolisate flour of the 2nd variants with 9 (nine) savoury flavours is in equation (2). The present review

describes the implementation of these complementary vibrational spectroscopy techniques and their potentials, advantages and disadvantages for GAG analysis (Mohamed *et al.*, 2017).

F2 = 0.455 (2/13H) + 0.454 (18/20H) + 0.473 (12/11H) + 0.447 (10/6H) + 0.497 (11/2H) + 0.457 (17/18H) + 0.484 (6/9H) + 0.513 (20/17H) + 0.528 (15/8H) + 0.499 (13/4H) + 0.533 (14/7H) + 0.505 (9/10H) + 0.473 (3/14H) + 0.491 (21/27H) + 0.449 (16/19H) + 0.463 (7/5H) + 0.609 (22/12H) + 0.504 (4/15H) + 0.903 (SF9) + 0.898 (SF2) + 0.895 (SF3) + 0.880 (SF6) + 0.879 (SF7) + 0.872 (SF4) + 0.860 (SF5) + 0.855 (SF1) + 0.850 (SF8) + 0.617 (19/25H) + 0.533 (5/1H) (2)

Remark :
F2 = The similarity of flavour character and material functional properties of 20 (twenty) over-fermented tempeh hydrolisate flour of the 1st (first) variant with 9 (nine) savoury flavours.

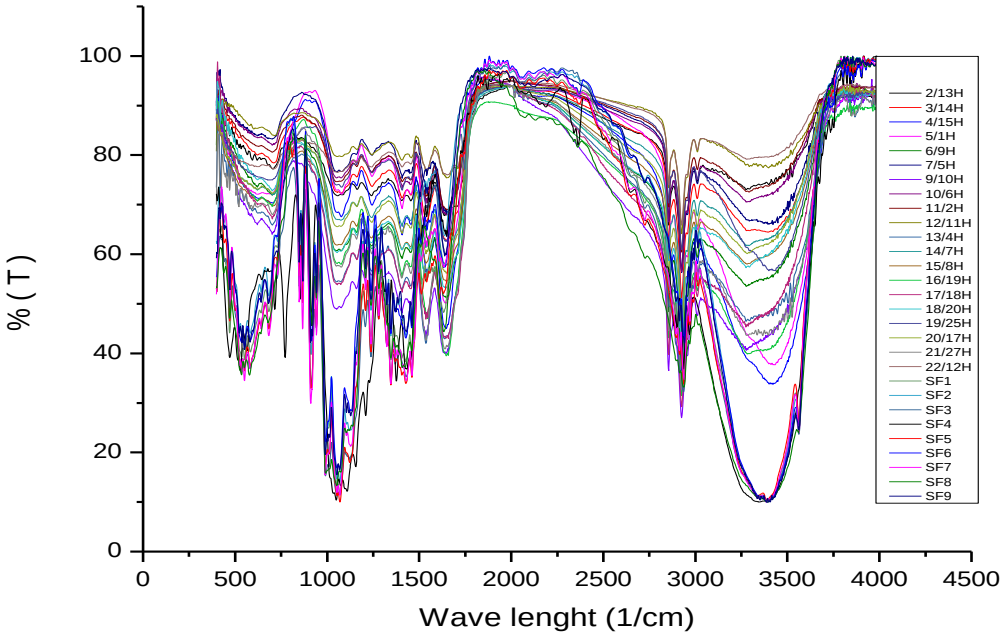

Figure 5*. Absorbance pattern of the 2nd principal component constituent variable (F2).*

Figure 5 above shows the absorbance pattern of the 2nd principal component constituent variable (F2). Based on equation (1) above, there are 9 (nine) variables with coefficient value (NK) of $0.850 \leq NK \leq 0.903$, namely SF8, SF1, SF5, SF04, SF7, SF6, SF3, SF2 and SF9. There are 12 (twelve) variables with coefficient value (NK) of $0.447 \leq NK \leq 0.499$, namely 10/6H, 16/19H, 18/20H, 2/13H, 17/18H, 7/5H, 3/14H, 12/11H, 6/9H, 11/2H, 21/27H, and 13/4H. There are 7 (seven) variables with coefficient value (NK) of $0.504 \leq NK \leq 0.617$, namely 4/15H, 9/10H, 20/17H, 15/8H, 14/7H, 22/12H, and 19/25H. The closeness of coefficient value (NK) indicates the closeness of flavour characteristic properties and the closeness of material functional properties, that is, the flavour characteristic and functional properties of over-fermented tempeh hydrolisate flour with 9 (nine) savoury flavours. The extraction of

FS from S. binderi was further optimized with central composite design (CCD) using RSM (Hii *et al.*, 2014).

Previous studies have reported that the umami taste of monosodium l-glutamate (MSG) and saltysmelling odors (e.g., soy sauce, bacon, sardines) enhance the perception of saltiness. This study aimed to investigate the neural basis of the enhancement of saltiness in human participants using functional near-infrared spectroscopy (Onuma *et al.*, 2018). Using Response Surface Methodology (RSM), we evaluated the culture conditions (nitrogen source, carbon source, pH and agitation rate) that increase the biomass of Acidocella facilis strain USBA-GBX-505 and therefore enhance the production of its lipolytic enzyme, 505 LIP (Bernal et al. 2017). Principal component analysis and partial least regression models were developed using the first derivative DRIFT spectra (400–4000 cm−1) to predict fructose and glucose sugars (Olale *et al.*, 2017). Enzymatic mungbean meal protein hydrolysate with PCA showed that 72.87% of the total variance confirmed the correlation between DH, S0, DPPH, ABTS, sensory characteristics and volatile flavour compounds (Sonklin *et al.*, 2018).

Table 5*. Interpretation Results of over-fermented tempeh flour and 2 (two) MSG variants FTIR absorbance pattern variable from 3,372 (three thousand three hundred and seventy two) observation points.*

No	Variable	Factor	Eigen Values	Loading Factor	% Variance	Cumulative %
1	TTA01	Similarities of flavour character and material functional properties of over-fermented tempeh flour with 2 (two) MSG variants (F3)	4.230	0.722	10.847	91.334
2	MSG			0.720		
3	VC			0.718		

The 3rd principal component (F3), after variable extraction and rotation using the varimax method, has 3 (three) 3rd principal components (F3) with eigenvalues score more than or equivalent to 0.50, respectively, as follows: TTA01, MSG and VC with eigenvalues score of 4.230 and total cumulative variant of 10.847%. Based on Table 5 above, the equation for the 3rd principal component (F3) is the function of flavour character similarities and material functional properties of MSG and VC with over-fermented tempeh hydrolisate flour (TTA01) as in Equation (3) below:

$$F3 = 0.722\ (TTA01) + 0.720\ (MSG) + 0.718\ (VC) \quad (3)$$

Remark :
F3 = The similarity of flavour character and material functional properties of over-fermented tempeh flour with 2 (two) MSG Variants

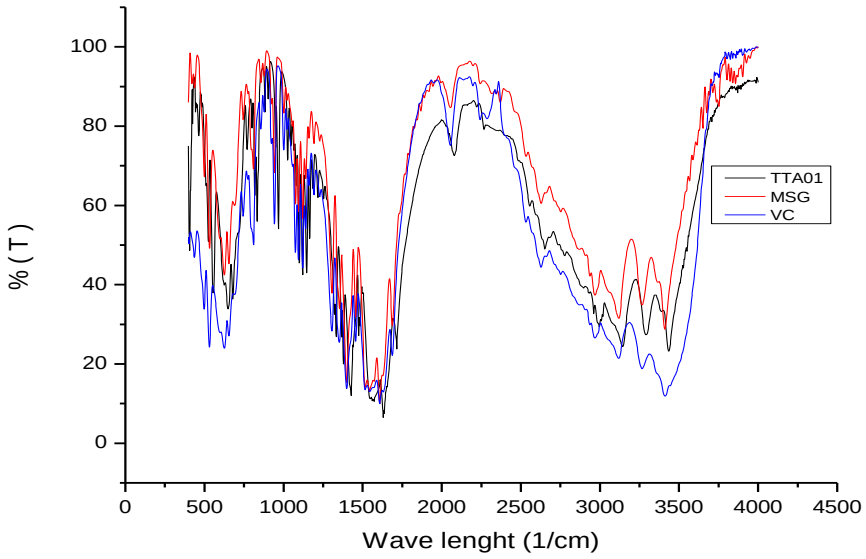

Figure 6*. Absorbance pattern of the 3rd principal component constituent variable (F3)*

Table 6. *Interpretation results of 1 (one) experiment unit FTIR absorbance pattern variable from 1,868 observation points.*

No	Variable	Factor	Eigen Values	Loading Factor	% Variance	Cumulative %
1	5/1H	Similarities of flavour character and material functional properties with over-fermented tempeh hydrolisate flour of the 3rd variant (F4)	2.7031	0.553	7.031	98.364

Figure 6 above shows the absorbance pattern of the 3rd principal component constituent variable (F3). The 4th principal component (F4), after doing variable extraction and rotation using varimax method, has 1 (one) 4th principal component (F4) with eigenvalues score more than or equivalent to 0.50 respectively as follows: 5/1H with eigenvalues score of 2.7031 and total cumulative variant of 7.031%. Based on Table 6 above, the equation for the 4th principal component (F4) is the function of flavour character similarities and material functional properties with over-fermented tempeh hydrolisate flour of the 4th (fourth) variants as in equation (4) below:

$$F4 = 0.553\ (5/1H) \quad (4)$$

Remark :
F4 = The similarities of flavour character and material functional properties with over-fermented tempeh hydrolisate flour of the 3rd (third) variants.

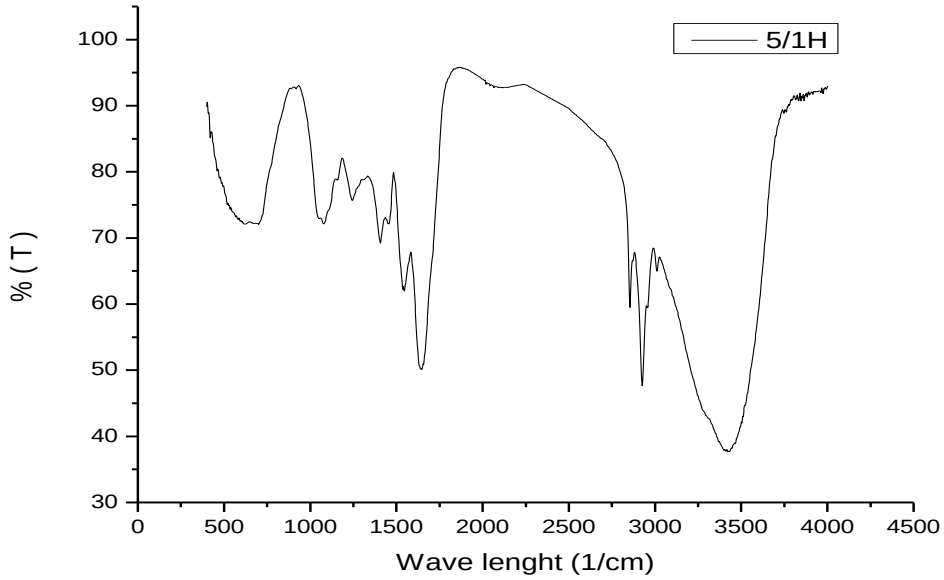

Figure 7*. Absorbance pattern of the 4th principal component constituent variable.*

Figure 7 above shows the absorbance pattern of the 4th principal component constituent variable. Table 7 below shows the component matrix after varimax rotation. Figure 8 below shows the dendogram of 27 (twenty-seven) experimental units, 9 (nine) savory flavors, over-fermented tempeh flour (TTA01) and 2 (two) MSG variants, 1) over-fermented tempeh hydrolisate flour, 2) over-fermented tempeh flour, and 2 (two) MSG variants, 3) 9 (nine) savory flavors.

***Table 7**. Matrix component after varimax rotation.*

Rotated Component Matrix[a]				
	Component			
	1	2	3	4
2/13H	0.855	0.455	0.233	-0.023
24/24H	0.848	0.424	0.271	0.112
18/20H	0.833	0.454	0.27	0.125
12/11H	0.824	0.473	0.203	0.177
10/6H	0.821	0.447	0.272	0.225
27/21H	0.82	0.318	0.293	0.356
11/2H	0.814	0.497	0.256	0.134
25/26H	0.805	0.42	0.267	0.311
17/18H	0.803	0.457	0.305	0.194
6/9H	0.796	0.484	0.29	0.201
8/3H	0.793	0.369	0.278	0.385
20/17H	0.79	0.513	0.303	0.124
15/8H	0.79	0.528	0.292	0.095
1/12H	0.786	0.392	0.308	0.353
26/16H	0.782	0.382	0.308	0.373
13/4H	0.781	0.499	0.313	0.192
14/7H	0.778	0.533	0.296	0.111
9/10H	0.775	0.505	0.292	0.186
3/14H	0.775	0.473	0.284	0.302
21/27H	0.735	0.491	0.345	0.298
16/19H	0.72	0.449	0.346	0.396
7/5H	0.71	0.463	0.289	0.438
22/12H	0.702	0.609	0.196	-0.18
23/23H	0.701	0.439	0.368	0.409
4/15H	0.573	0.504	0.354	0.528
SF9	0.336	0.903	0.149	0.216
SF2	0.352	0.898	0.151	0.203
SF3	0.401	0.895	0.139	0.111
SF6	0.359	0.88	0.202	0.22
SF7	0.403	0.879	0.201	0.123
SF4	0.392	0.872	0.118	0.16
SF5	0.441	0.86	0.218	0.106
SF1	0.439	0.855	0.219	0.145
SF8	0.4	0.85	0.24	0.184
19/25H	0.572	0.617	0.317	0.366
TTA01	0.596	0.176	0.722	0.197
MSG	0.616	0.203	0.72	0.156
VC	0.502	0.415	0.718	0.163
5/1H	0.499	0.533	0.359	0.553
Extraction Method: Principal Component Analysis.				

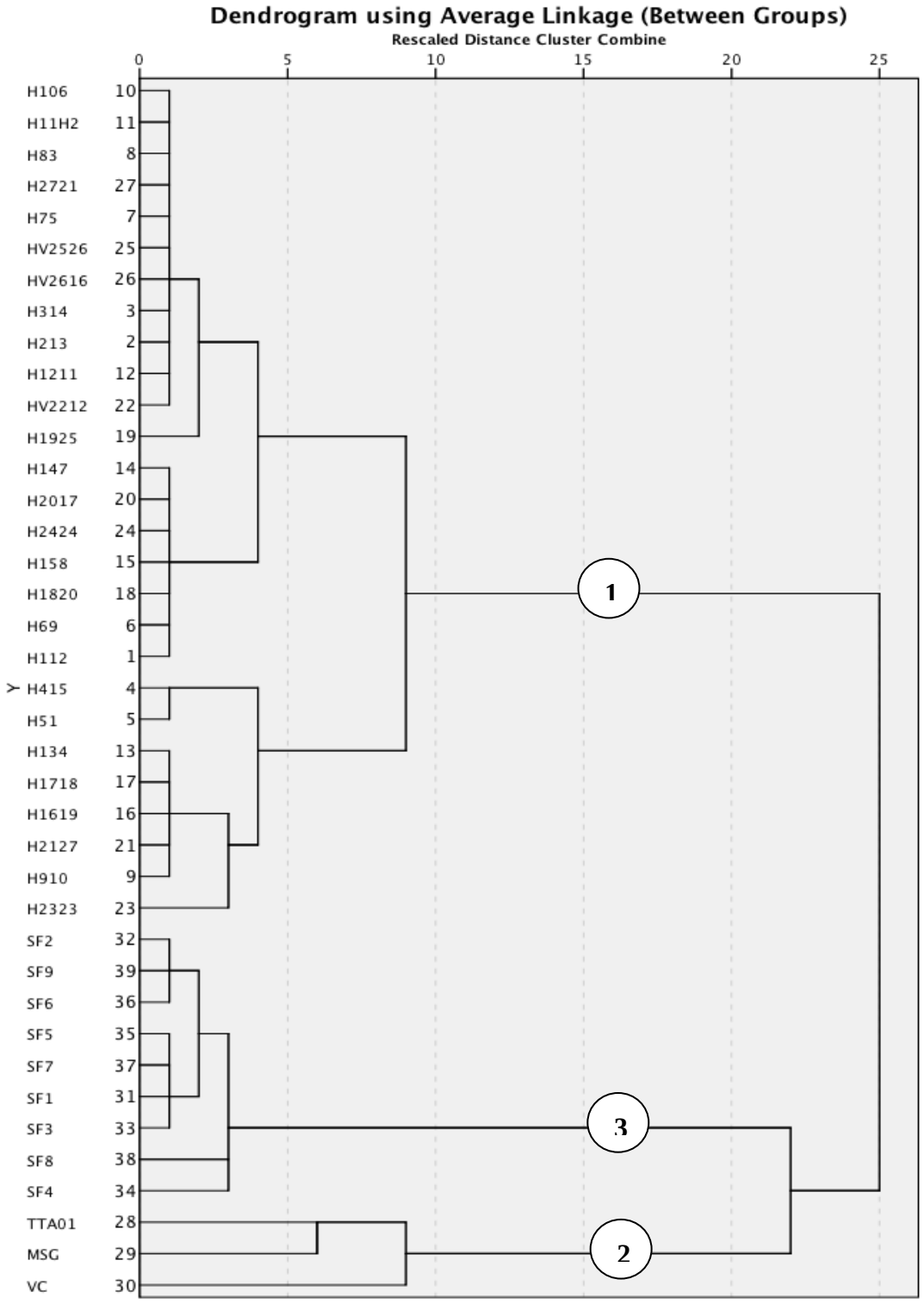

Figure 8*. Dendogram of 27 (twenty-seven) experiment units, 9 (nine) savoury flavors, over-fermented tempeh flour (TTA01) and 2 (two) MSG variants, 1) over-fermented tempeh hydrolisate flour, 2) over-fermented tempeh flour, and 2 (two) MSG variants, 3) 9 (nine) savoury flavours.*

***Table 8**. Closeness of functional properties and flavour character of 27 (twenty-seven) experiment units and 9 (nine) savoury flavours, 2 (two) MSG variants, and over-fermented tempeh flour.*

Case	TTA01	MSG	VC
1/12H	976,872.52	512,297.52	1,472,312.01
2/13H	1,588,322.37	961,598.81	2,222,509.79
3/14H	1,416,467.25	826,040.41	2,003,459.91
4/15H	810,350.01	467,060.21	1,112,303.80
5/1H	1,053,677.75	610,795.41	1,409,830.62
6/9H	847,189.55	461,095.48	1,268,478.76
7/5H	1,696,063.28	1,018,682.09	2,342,049.08
8/3H	1,845,458.95	1,126,285.97	2,551,162.88
9/10H	507,303.17	339,872.04	740,207.11
10/6H	1,727,745.46	1,035,887.41	2,389,502.88
11/2H	1,800,856.72	1,097,041.12	2,466,659.83
12/11H	2,098,851.12	1,336,128.50	2,832,728.26
13/4H	598,267.22	344,988.94	903,143.34
14/7H	1,170,788.66	667,537.57	1,665,964.94
15/8H	962,336.08	539,293.72	1,395,609.83
16/19H	554,829.53	320,844.64	884,027.69
17/18H	592,598.74	330,721.66	923,927.89
18/20H	941,212.90	517,183.79	1,420,882.81
19/25H	1,456,227.85	869,570.32	1,976,431.19
20/17H	1,101,325.15	615,192.64	1,578,289.86
H2127	610,472.35	338,362.49	907,450.61
22/12H	2,049,736.57	1,311,738.47	2,712,758.53
23/23H	412,620.60	299,421.94	583,485.00
24/24H	1,308,228.82	744,997.72	1,899,505.33
25/26H	1,576,321.05	935,151.67	2,224,049.56
26/16H	1,460,196.38	848,496.05	2,071,357.44
27/21H	1,946,456.91	1,204,332.28	2,676,168.16
TTA01	0.00	270,781.21	318,027.15
MSG	270,781.21	0.00	511,428.97
VC	318,027.15	511,428.97	0.00
SF1	1,001,661.57	953,618.13	811,731.55
SF2	1,268,717.63	1,107,104.91	1,134,018.22
SF3	1,244,703.62	1,237,744.35	1,000,691.97
SF4	1,225,055.12	1,271,692.03	992,494.01
SF5	1,011,645.21	994,867.61	803,018.33
SF6	1,143,963.16	991,629.56	1,001,238.34
SF7	1,117,982.78	1,063,922.35	909,226.60
SF8	975,607.37	1,065,292.30	703,497.57
SF9	1,264,075.83	1,135,857.22	1,115,278.29

Table 8 above shows the closeness of functional properties and flavor characteristics of 27 (twenty-seven) experimental units and 9 (nine) savory flavors, 2 (two) MSG variants, and over-fermented tempeh flour.

The clustering of 39 (thirty-nine) variables which consists of 27 (twenty-seven) experiment units, 9 (nine) savoury flavours, 2 (two) MSG variants and over-fermented tempeh flour results in 3 (three) clusters was as follows :

1. The 1st (first) cluster indicated the similarities of flavour character and material functional properties of 27 (twenty-seven) over-fermented tempeh hydrolisate flour as the product of multi enzyme hydrolization as shown on Figure 9, point 1 (one).
2. The 2nd (second) cluster indicated the similarities of flavour character and material functional properties of 2 (two) MSG variants with over-fermented tempeh flour (TTA01) as shown in Figure 9, point 2 (two).
3. The 3rd (third) cluster indicated the similarities of flavour character and material functional properties of 9 (nine) savoury flavors as shown in Figure 9, point 3 (three).

The HCA indicated that those 3 (three) clusters in more details can be seen in Figure 9 of flavor character dendogram and material functional properties, which are the results of cluster analysis hierarchy using agglomerative method, namely the average linkage method. ELISA and high content analysis (HCA) were employed to examine the disruptive effects of MSG on the secretion of enteroendocrine hormone glucagon-like peptide-1 (GLP-1) and GLP-1 receptor (GLP-1R), respectively (Shannon *et al.*, 2017). Detection of L-Cysteine in wheat flour was accomplished successfully using Raman microscopy combined chemometrics of PCA and HCA (Cebi *et al.*, 2017).

CONCLUSION

Based on the results of the research with the experimental design above, the conclusions are:

1. The results of principal component analysis of 28 (twenty-eight) experiment units, 2 (two) MSG variants and 9 (nine) savoury flavours in Indonesia, over-fermented tempeh hydrolisate flour 19/25H with loading factor value of 0.617, 22/12H with loading factor value of 0.609, 5/1H with loading factor value of 0.533, 14/7H with loading factor value of 0.533, 15/8H with loading factor value of 0.528, 20/17H with loading factor value of 0.513, 9/10H with loading factor value of 0.505 and over-fermented tempeh hydrolisate flour 4/15H with loading factor value of 0.504, show that they have potential flavour character and functional properties as the main ingredient for the Savoury Flavors of Grilled Chicken (SF01), Balado (SF02), Barbeque (SF03), Spicy Corn (SF04), Cheese (SF05), Salty Cheese (SF06), Sweet Spicy (SF07), Roasted Beef (SF08) and Tiramisu (SF09).
2. The results of hierarchical cluster analysis of 28 (twenty-eight) experiment units and 9 (nine) savoury flavours in Indonesia, over-fermented tempeh hydrolisate flour 9/10H and 23/23H, show that they have flavour character and functional properties as the main ingredient of Savoury Flavours of Grilled Chicken (SF01) with closeness value of 453.406 (9/10H) and 465.536 (23/23H), Balado (SF02) with closeness value of 506.061 (9/10H) and 544.227 (23/23H), Barbeque (SF03) with closeness value of 593.029 (9/10H) and 652.165 (23/23H), Spicy Corn (SF04) with closeness value of 595.097 (9/10H) and 632.614 (23/23H), Cheese (SF05) with closeness value of 482.596 (9/10H) and 520.814 (23/23H), Salty Cheese (SF06) with

closeness value of 469.605 (9/10H) and 475.465 (23/23H), Sweet Spicy (SF07) with closeness value of 515.754 (9/10H) and 563.700 (23/23H), Roasted Beef (SF08) with closeness value of 526.120 (9/10H) and 525.428 (23/23H) and Tiramisu (SF09) with closeness value of 520.196 (9/10H) and 551.815 (23/23H).

ACKNOWLEDGMENTS

This expression of gratitude is sent to Republic of Indonesia Finance Department Research Fund Management Insitution that has provided research grant funding.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Wignyanto Wignyanto: Investigation, methodology, conceptualization, formal analysis, data curation, validation, writing-original draft, review, and editing, visualization.
Mujianto Mujianto: Investigation, methodology, conceptualization, formal analysis, data curation, validation, writing-original draft, review, and editing, visualization.

ETHICS COMMITTEE DECISION

This article does not require any ethical committee decision.

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