

PHYSICAL CHARACTERISTICS, SENSORY EVALUATION, AND AMINO ACID CONTENT OF POWDERED OVER FERMENTED *MLANDING TEMPEH*

[*Karakteristik Fisik, Evaluasi Sensoris, dan Kadar Asam Amino Bubuk Tempe Mlanding Over Fermented*]

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ABSTRACT

Mlanding tempeh is non-soybean *tempeh* made from ripe *lamtoro* (*Leucaena leucocephala*) seed. In Wonogiri, an area in Indonesia, over fermented *mlanding tempeh* (OFMT) is often used as a seasoning for traditional food since it produces a special taste. OFMT is also dried to extend the shelf life, making it easier to use as a seasoning. This research aims to study the physical characteristics, sensory evaluation, and amino acid content of powdered over fermented *mlanding tempeh* (POFMT). The drying of the OFMT used a cabinet dryer with variations in drying temperatures of 55, 60, and 65°C for eight hours. Then, it was mashed and sifted to obtain POFMT. The obtained powder was then analyzed for its physical characteristics in yield, water absorption capacity, solubility, bulk density, sensory evaluation with the hedonic test, and amino acid content. This study found that different drying temperatures significantly affected the yield and water absorption but did not significantly affect the solubility and bulk density. The yield values varied between 7.06-12.92%; water absorption capacity varied between 0.36-0.55 g/g; solubility varied between 36.45-41.33%; bulk density varied between 0.38-0.42 g/mL. The sensory evaluation results showed that the panelists' preference for POFMT with variations in drying temperature was not significantly different. In addition, the amino acid content of POFMT varied between 0.99-5.43% (db), with the highest levels being glutamic acid and aspartic acid.

Keywords: amino acid, drying, *mlanding tempeh*, powdered over fermented *mlanding tempeh*, seasoning

ABSTRAK

Tempe mlanding merupakan *tempe* yang terbuat dari biji *lamtoro tua*. Di Wonogiri, *tempe mlanding* yang sudah mengalami fermentasi lanjutan (*over fermented*) seringkali dimanfaatkan sebagai bumbu berbagai makanan tradisional. *Tempe mlanding over fermented* ini dikeringkan untuk memperpanjang masa simpan dan mempermudah penggunaannya sebagai bumbu. Penelitian ini bertujuan untuk mempelajari karakteristik fisik, evaluasi sensori, serta kadar asam amino dari bubuk *tempe mlanding over fermented*. Pengeringan *tempe mlanding over fermented* ini menggunakan cabinet dryer dengan variasi suhu pengeringan 55, 60, dan 65°C selama 8 jam. Kemudian dihaluskan dan diayak sehingga diperoleh bubuk *tempe mlanding over fermented*. Bubuk yang diperoleh, dianalisis karakteristik fisik yang berupa rendemen, daya serap air, kelarutan, bulk density, evaluasi sensori dengan uji kesukaan, dan kadar asam amino. Pada penelitian diperoleh hasil bahwa suhu pengeringan yang berbeda memberikan pengaruh yang nyata terhadap rendemen dan daya serap air dan tidak memberikan pengaruh yang nyata terhadap kelarutan dan bulk density. Nilai yield bervariasi antara 7,06-12,92% dan daya serap air bervariasi antara 0,36-0,55 g/g, sedangkan untuk kelarutan bervariasi sebesar 36,45-41,33% dan bulk density bervariasi antara 0,38-0,42 g/mL. Hasil dari evaluasi sensori menunjukkan bahwa kesukaan panelis terhadap bubuk *tempe mlanding over fermented* dengan variasi suhu pengeringan tidak berbeda secara nyata. Kadar asam amino bubuk *tempe mlanding over fermented* bervariasi antara 0,99-5,43% (db), dengan kadar yang paling tinggi adalah asam glutamat dan asam aspartat.

Kata kunci: asam amino, bubuk *tempe mlanding over fermented*, bumbu, pengeringan, *tempe mlanding*

INTRODUCTION

Lamtoro (*Leucaena leucocephala*) is included in the legume family *Fabaceae* (Nwokocha and Williams, 2012) that grows well in tropical countries such as Indonesia (Zayed *et al.*, 2018). The protein content in *lamtoro* seeds is high (24.5-46%) (Verma *et al.*, 2018). In Gunungkidul, Wonogiri, and Pacitan, regions in Indonesia, ripe *lamtoro* seeds are processed into *lamtoro tempeh* (Nursiwi *et al.*, 2018). Traditionally, the manufacture of *lamtoro tempeh* uses *laru* or *usar* as an inoculum. *Laru* or *usar* is an inoculum in tempeh fermentation consisting of a group of mold spores. *Rhizopus oryzae* and *Rhizopus oligosporus* were found in the traditional tempeh inoculum (Dunijaji *et al.*, 2019). Some people call *lamtoro tempeh* as *mlanding tempeh*, in Wonogiri. It is non-soybeans tempeh, made from ripe *lamtoro* seeds through fermentation using *usar*. *Usar* is a tempeh inoculum that contains *Rhizopus* sp. and some microbial contaminants (Triyono *et al.*, 2017). During 36 hours of fermentation of *lamtoro* seeds using commercial inoculum, there is an increase in protein content and dissolved protein levels, from 26.48% (db) to 34.39% (db) and from 3.92% (db) to 7.28% (db), respectively (Nursiwi *et al.*, 2018). In the fermentation of soybean tempeh, the longer the fermentation time, the higher the amino acid content. It is resulted from protein hydrolysis by the activity of *R. oligosporus*. The highest free amino acids are produced during 24 to 72 hours of fermentation (Handoyo and Morita, 2006).

In the Wonogiri area, *mlanding tempeh* has undergone further fermentation or referred to as over fermented *mlanding tempeh*, which is fermented 1-5 days longer than fresh *mlanding tempeh*. The local community adds it as a traditional food seasoning, such as for making *sambal tumpang*. It is the same as some communities in Java that utilize over fermented soybean tempeh as a seasoning for various traditional foods. This continued fermentation increases the volatile component, contributing to the special flavor produced. Increased glutamic acid and stronger flavor intensity show the potential of over fermented tempeh as a seasoning (Wijaya and Gunawan-Puteri, 2015). In 50 to 90 hours of fermentation, the mold growth decreases and is replaced by bacteria so that amino acids are degraded, and ammonia is formed, which has a characteristic pungent odor (Gunawan-Puteri *et al.*, 2015; Hassanein *et al.*, 2015). Pungent odor is also produced from fat hydrolysis. The over fermented tempeh is usually fermented for longer than normal tempeh, about 2-5 days longer (Hassanein *et al.*, 2015).

As a seasoning, over fermented tempeh is usually used in a fresh form. The over fermented tempeh, if not immediately used, will change. It is

caused by fermentation that still continues, especially the flavor produced. In addition, the shelf life of over fermented tempeh is limited, only three days, after which the product becomes unsuitable for consumption with very soft textures, darker colors, and very strong odor (Syahrial and Muchalal, 2001). In this case, one method that can be used to stop the continued fermentation is the drying process. The drying process followed by grinding into powder can extend shelf life and facilitate the use of powdered tempeh as a seasoning more easily (Andriani and Nurhartadi, 2014).

One important factor in drying is the drying temperature. Drying over fermented soybean tempeh at temperatures of 55, 60, and 65°C resulted in higher drying temperature, lower yield and bulk density, and higher water absorption. While the solubility increased when the temperature increased, the solubility was constant at 60 and 65°C. The results also showed that the drying temperature influenced the physical characteristic (Andriani and Nurhartadi, 2014). Therefore, this study intended to study the physical characteristics, sensory evaluation, and amino acid content of powdered over fermented *mlanding tempeh* (POFMT), dried using a cabinet dryer.

MATERIALS AND METHOD

Materials

The ripe *lamtoro* seed (brown color) and the traditional inoculum were obtained from a small industry that produced *mlanding tempeh* in Wonogiri, Indonesia.

POFMT sample preparation

The ripe *lamtoro* seeds (4 kg) were boiled (first boiling) by adding 500 g of ash into ten liters of water for two hours. Then, the hull was separated from the *lamtoro* seed. Afterward, the seed was soaked for 24 hours (changing the water after 15 hours of soaking), with the ratio between the water and seed being 4:3. Next, the *lamtoro* seeds were boiled (second boiling) for two hours, drained and cooled down (Nursiwi *et al.*, 2018). After that, it was inoculated with traditional inoculum (5% w/w), packaged, and incubated for 60 hours at room temperature. The resulting OFMT was then dried using a cabinet dryer at various temperatures of 55, 60, and 65°C for eight hours. Then, it was powdered and sieved with an 80-mesh sieve.

Analysis of POFMT

The POFMT was analyzed for physical characteristics, namely yield using method described by Andriani *et al.* (2013), bulk density using the method described by Kumalasari *et al.* (2015), solubility

using method described by Buwono *et al.* (2018), water absorption capacity using the method described by (Ariyantoro *et al.*, 2016), and amino acid content using UPLC Waters H Class with Photodiode Array Detector (PDA) and column C18 (Waters, 2012). Meanwhile, the sensory evaluation employed a 7-points hedonic scale (1= dislike very much; 7= like very much). Then, 30 untrained panels were asked to rate their preferences for color, aroma, taste, and overall (Marand *et al.*, 2020).

Data analysis

The data (three sample replicates) were statistically analyzed using one-way analysis of variants at a significance level of 5% ($\alpha = 0.05$) to determine the effect of differences in drying temperatures on the POFMT physical characteristic, amino acid content, and sensory evaluation. If there were differences, it was followed by analysis using the Duncan Multiple Range Test (DMRT) at a significance level of 5%.

RESULTS AND DISCUSSION

Physical characteristic

Physical characteristics analyzed in POFMT were yield, water absorption capacity, solubility, and bulk density. The results of physical characteristics are shown in Table 1.

Yield

The yield calculation was based on a comparison between the powder's final weight and the tempeh's initial weight before drying. According to Table 1, the yield of POFMT with the drying temperature of 55°C was significantly different from that of 65°C. The yield of the POFMT with the drying temperature of 55°C was significantly higher than the drying temperature of 65°C. Meanwhile, POFMT with the drying temperature of 55°C was not significantly different from that of 60°C, and POFMT with the temperature of 60°C was not significantly different from that of 65°C. The yield of POFMT with the drying temperatures of 55, 60, and 65°C was 12.92, 10.37, and 7.06%, respectively. These results indicate the same trend with the drying jack bean (*Canavalia ensiformis*) tempeh into tempeh flour. The yield of drying jack bean tempeh using a tem-

perature of 55°C was significantly higher than drying at 65°C (Affandi *et al.*, 2020). In this regard, the yield value of a process decreases if the drying temperature increases. The drying process causes the moisture content of the material to be reduced and leads to a decrease in yield (Purbasari, 2019).

Water absorption capacity

Water absorption capacity is the ability of the powder to absorb water. Particle size, moisture content, and differences in chemical content and material affect water absorption (Ntau *et al.*, 2017). According to Table 1, the water absorption capacity of POFMT with a drying temperature of 55°C was significantly different from that of 65°C. In contrast, the drying temperature of 60°C was not significantly different from that of 55 and 65°C. The water absorption capacity of POFMT dried with the drying temperature of 55, 60, and 65°C were 0.55, 0.45 and 0.36 g/g, respectively. These results showed the same with research conducted by Affandi *et al.* (2020) in making jack bean tempeh flour. In their study, the water absorption capacity of the flour resulted in the drying temperature of 55°C being significantly higher than the drying temperature of 65°C. In this case, water absorption is related to hydrophilic molecules, especially protein and carbohydrates, so it is influenced by the number of these molecules in food ingredients (Abe-Inge *et al.*, 2018). Water absorption capacity is also influenced by the protein quality and the polar amino acid content, which has hydrophilic groups in the food (Affandi *et al.*, 2020).

Solubility

Solubility is an important characteristic of seasoning since it will be added to a food product. Seasoning powder is expected to dissolve completely in food products (Andriani and Nurhartadi, 2014). According to Table 1, the solubility of POFMT with a drying temperature of 55, 60, and 65°C was not significantly different. The solubility of POFMT with a drying temperature of 55, 60, and 65°C was 36.45, 41.13, and 42.33%, respectively. In line with that, the drying of jack bean tempeh with different temperatures of 55, 60, and 65°C also did not significantly affect the solubility of the flour produced (Affandi *et al.*, 2020).

Table 1. The physical characteristic of powdered over fermented *mlanding tempeh*

Physical Characteristics	Drying Temperature (°C)		
	55	60	65
Yield (%)	12.92 ^b ±0.54	10.37 ^{ab} ±3.28	7.06 ^a ±1.81
Water absorption (g/g)	0.55 ^b ±0.09	0.45 ^{ab} ±0.09	0.36 ^a ±0.02
Solubility (%)	36.45 ^a ±3.84	41.13 ^a ±4.82	41.33 ^a ±4.14
Bulk density (g/mL)	0.42 ^a ±0.43	0.43 ^a ±0.05	0.35 ^a ±0.99

Note: Numbers with similar alphabets along the same line show no significant differences ($P > 0.05$)

Bulk density

Bulk density is the mass of a material that fills a certain volume unit. It expresses the conciseness of material in occupying the volume. The greater the bulk density, the more concise the material occupies the same volume with a greater mass (Astawan *et al.*, 2016). According to Table 1, the bulk density of POFMT was not significantly different for the three drying temperatures. The bulk density of POFMT with the drying temperatures of 55, 60, and 65°C was 0.42, 0.43, and 0.35 g/mL, respectively.

Sensory evaluation

Sensory evaluation was conducted to determine the panelists' preferences for the color, aroma, taste, and overall POFMT, dried at different temperatures.

Color

According to Table 2, the panelists' preferences for color had no significant differences in the three temperatures, 5.02 to 5.15. However, the highest score was drying temperature of 60°C. According to the panelists, the POFMT color is a fairly light yellowish-brown. The brown color in POFMT is due to the color change during the continued fermentation of the tempeh. The study of Djunaidi *et al.* (2017) showed that the value of L (lightness) decreased in the 72 hours overripe tempeh, between 48.96-56.9, compared to 24 hours overripe tempeh with an L value of 72. A lower L value indicates a darker color. It is related to microbial growth; the color of immature mold is white, while the mature mold has a darker color. Due to the mold maturation, it lowers enzyme production and causes a darker color in overripe tempeh.

Table 2. Sensory evaluation of powdered over fermented *mlanding tempeh*

Parameter	Drying Temperature (°C)		
	55	60	65
Color	5.02 ^a	5.31 ^a	5.15 ^a
Aroma	4.50 ^a	4.71 ^a	4.52 ^a
Taste	4.33 ^a	4.35 ^a	4.31 ^a
Overall	4.42 ^a	4.60 ^a	4.56 ^a

Note: Scoring scale 1= dislike very much; 2= dislike; 3= dislike slightly; 4= neither like nor dislike; 5= like slightly; 6= like; 7= like very much. Numbers with similar alphabets along the same column show no significant differences ($P>0.05$)

Aroma

According to Table 2, the panelists' preferences for aroma were not significantly different between three drying temperatures, namely between 4.50 and 4.52. However, the highest score was the drying temperature of 60°C. The aroma formed in OFMT originates from compounds formed during fermentation, namely degradation of amino acid and forming

ammonia with a characteristic pungent odor (Gunawan-Puteri *et al.*, 2015; Hassanein *et al.*, 2015). The pungent odor is also produced from fat hydrolysis (Hassanein *et al.*, 2015).

Taste

According to Table 2, the panelists' preferences for the taste parameters of the three variations of the drying temperature were not significantly different, namely 4.31 to 4.35. However, the highest score was the drying temperature of 60°C. Besides, powdered over fermented *mlanding tempeh* had a savory or umami taste since the glutamic acid was 4.44 to 4.91%. This glutamic acid was the highest amino acid content in POFMT (Table 3). Here, glutamic acid is an essential amino acid that contributes to umami flavor and has been used throughout the world to produce savory seasoning even though this flavor has been masked by fat or spice flavor (Gunawan-Puteri *et al.*, 2015).

Table 3. The amino acid content of powdered over fermented *mlanding tempeh*

Amino Acid	Amino Acid Content (% db) in Various Drying Temperatures		
	55°C	60°C	65°C
L-Phenylalanine	2.59	2.20	2.88
L-Isoleucine	1.88	1.55	1.84
L-Valin	2.13	1.82	2.14
L-Arginine	2.92	2.36	2.93
L-Lysine	2.91	2.57	2.78
L-Leusin	3.31	2.75	3.11
L-Threonine	1.75	1.54	1.95
L-Histidin	1.41	0.99	1.42
L-Serine	1.83	1.55	1.75
L-Glutamic Acid	5.50	5.05	5.43
L-Alanin	2.25	2.17	2.40
Glisin	1.94	1.68	2.04
L-Aspartic acid	3.79	3.46	3.73
L-Tyrosine	1.65	1.43	1.74
L-Prolin	1.47	1.34	1.35

Overall

According to Table 2, the panelists' preferences as a whole signified no significant differences in the three drying temperatures with scores of 4.42 to 4.60. However, the highest score was the drying temperature of 60°C.

Amino acid content

The amino acid in all POFMT was identified and quantified (Table 3, the example of the obtained chromatogram is presented in Figure 1). Amino acids are building blocks compound of protein, divided into two groups, essential and non-essential amino acids. Table 3 presents the content of amino acids in the POFMT. The highest amino acid level was glutamic acid, followed by aspartic acid, about 14 and 10% of the 15 amino acids, respectively.

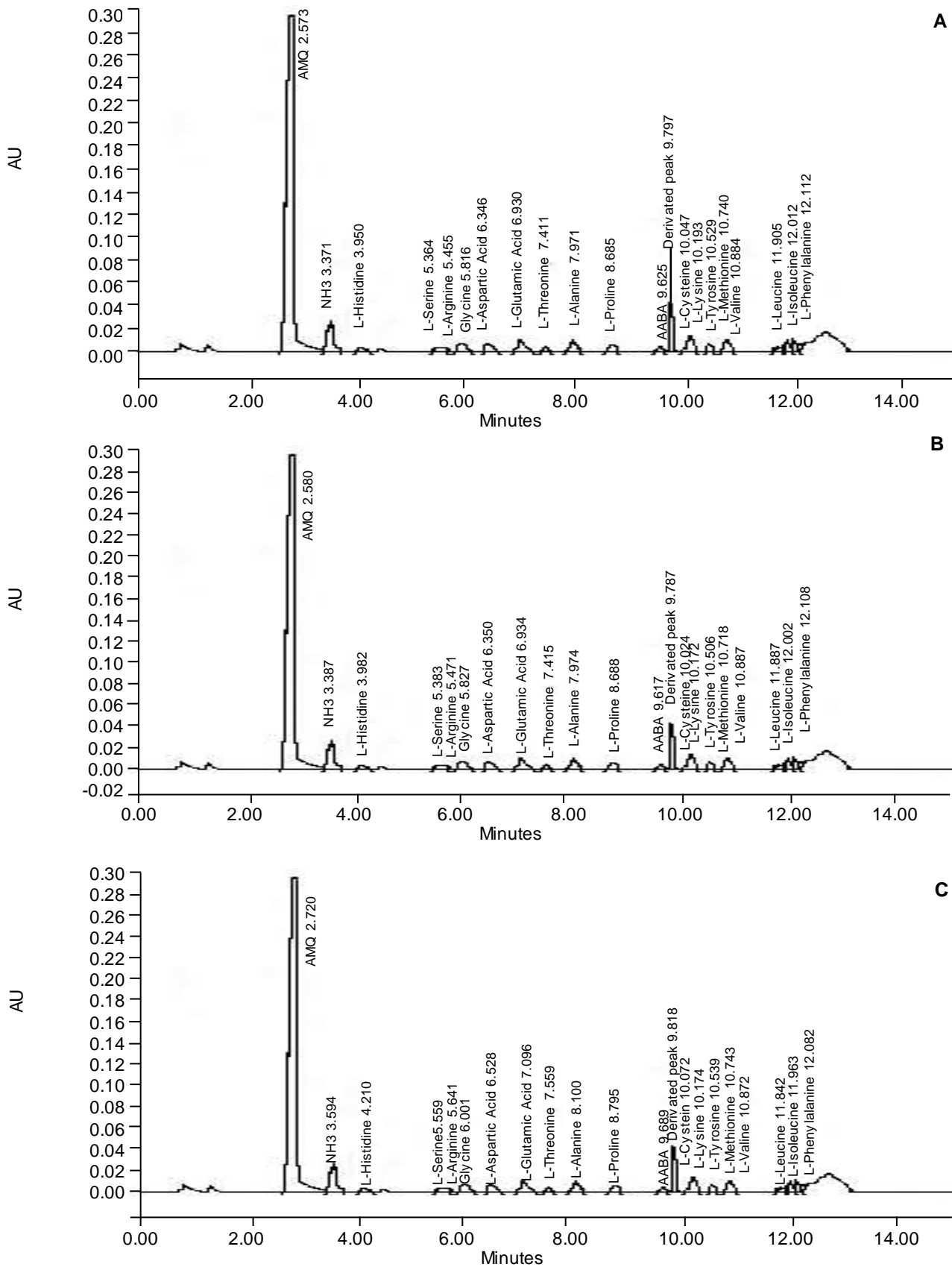


Figure 1. Amino acid profile chromatogram for powdered over fermented *mlanding tempeh*, dried in 55 (A), 60 (B), and 65°C (C).

The glutamic acid content of POFMT, which was dried at 55, 60, and 65°C, was 5.50, 5.05, and 5.43%, respectively. Whereas aspartic acid content of POFMT dried at 55, 60, and 65°C were 3.79, 3.46, and 3.73%, respectively. In this case, glutamic acid is crucial in forming umami (savory) taste and has long been used worldwide as savory seasoning. These results confirm that tempeh is a source of protein that can be used as an alternative to meat (Gunawan-Puteri *et al.*, 2015).

The presence of glutamic acid as the dominant amino acid in powdered over fermented soybean tempeh also indicates its potential as a seasoning (Gunawan-Puteri *et al.*, 2015). Likewise, it is potential in POFMT as a seasoning. The results align with the research conducted by Gunawan-Puteri *et al.* (2015) that in oven-dried overripe tempeh powder, the composition of glutamic acid and aspartic acids was 15.9 and 13.39%.

In fermented products, glutamic acid is the highest level among the amino acids and contributes to the product's taste. Peptides consisting of hydrophilic amino acids (acidic), such as glutamic acid and aspartic acid in N terminals, have an umami taste. Besides, the taste of some fermented soybean food can be predicted by the free amino acid composition. The intensity of the umami taste can also be determined from the concentration of two acidic amino acids: glutamic acid and aspartic acid (Utami *et al.*, 2016).

CONCLUSION

The research concludes that the different drying temperatures had a significant effect on yield and water absorption capacity but had no significant effect on the solubility and bulk density of the POFMT. The yield varied between 7.06-12.92%, and the water absorption capacity varied between 0.36-0.55 g/g. While the solubility of the POFMT varied between 36.45-41.33%, the bulk density varied between 0.38-0.42 g/mL. However, the different drying temperatures did not significantly affect the panelist's preferences. In addition, the amino acid content of POFMT varied between 0.99-5.43% (db), with the two highest content being glutamic acid and aspartic acid. Therefore, POFMT indicates its potential as a seasoning.

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