Food Research 7 (5) : 42 - 52 (October 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Characterization of physicochemical properties and enzymatic digestibility of lotus (*Nelumbo nucifera*) tempeh through different methods

*Ridhowati, S., Lestari, S.D., Rendi, M. and Rachmawati, S.H.

Department of Fisheries Product Technology, Faculty of Agriculture, Sriwijaya University

Article history:
Received: 4 December 2021
Received in revised form: 5 January 2021
Accepted: 8 January 2022
Available Online: 13 September 2023

Keywords:
Boiled,
Digestibility,
Fermented food,
Lotus tempeh,
Steamed

DOI:
https://doi.org/10.26656/fr.2017.7(5).903

Abstract

This study aimed to determine the impact of different methods (boiling and/or steaming) in lotus (*Nelumbo nucifera*) tempeh making on its physical and chemical characteristics. The modified methods through 5 mins (A1), 10 mins (A2), and 15 mins (A3) of steaming, and twice boiling method (A4) were applied as the treatment. The results showed that the A4 method had significantly affected the nutritional value (76.60% water, 2.48% ash, 5.60% fat, 38.38% protein, and 53.55% carbohydrates), and hardness (9.93 N/s) of the sample, not in pH and colour. The twice boiling method in the A4 sample could improve the digestibility of not only protein (64.89%) but also starch (31.09%). However, the protein digestibility of lotus tempeh was higher than that of starch. It was concluded that the A4 treatment was more effective in producing lotus tempeh. Lotus tempeh could be good functional food with physicochemical and digestibility.

1. Introduction

Lotus (*Nelumbo nucifera*) has been widely cultivated in East Asia, China, but the practice is still limited in Indonesia. All parts of the lotus plant can be utilized. Its stamens can be used as herbal tea while the seeds can be used as cake ingredients. The flowers, seeds, leaves, and roots of lotus flowers are edible whether raw or cooked. Lotus has been continuously studied and even cultivated due to its functional properties and commercial value. Lotus seeds contained approximately 16.2% protein, 4.05% ash, 2.05% fat, 8.13% sugar, 55.77% starch, and 14% moisture (Pan *et al*., 1993; Zhang *et al*., 2015). Besides the cuisine, lotus has also been used in traditional medicine due to its phytochemical components. Some studies revealed that those compounds have displayed hypoglycemic, anticancer, and hepatoprotective activity (Zhang *et al*., 2015).

The development of lotus seeds as a fermentation product is still limited. Fermented products developed from lotus seeds are natto and tempeh. The suitability of lotus seeds as soybean replacement material in natto production has been studied (Lestari *et al*., 2017). During the natto fermentation, some changes in the nutritional value took place, and tempeh does. Tempeh is a soy-based fermented food that originated in Indonesia. Its richness in protein makes it an ideal meat replacement in the vegetarian diet. Furthermore, *in vitro* and *in vivo* studies have confirmed its health benefits. The potential of other types of beans as tempeh raw material has been explored (Cueves-Rodriguez *et al*., 2006; Sujak *et al*., 2006; Priatni *et al*., 2013; Wickramasinghe, 2017).

Tempeh made from non-soybeans is still recognized as not so optimal as soybean tempeh, despite some promising results found by studies in the field. Ridhowati *et al.* (2020) showed that the aglycones (genistein) of lotus tempeh were higher than that found by Gil-Izquierdo *et al.* (2012) in soybean tempeh. Natto product wherein the increment was noticeable in protein (27.18 to 34.09%) as well as in fat (2.09 to 3.39%) while the carbohydrate content decreased from 66.33% to 57.49% (Lestari *et al*., 2017), all values were in dry basis. Lotus seeds contain higher carbohydrates and sugar than soybean. Nevertheless, the lotus starch has a B-type starch with a gradual crystalline structure that was easily weakened or broken over time after pressure treatment (Lin *et al*., 2009).

The other treatment, the fermentation process, has resulted in the changing of nutrients, physical appearance, and digestibility. The process of making tempeh was commonly starting with dehulling, cooking, inoculation, and fermentation (Adam and Moss, 2008). Food processing procedures will greatly affect the nutritional content and bioactive compounds of tempeh (Irina and Mohamed, 2012; Sharma *et al*., 2012; Divekar *et al*., 2017). Adam and Moss (2008) and Ferreira *et al.*

*Corresponding author.
Email: *sherlyridhowati@unsri.ac.id*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

(2011) declared that the two bioactive compounds that act as breast-cancer compounds are strongly influenced by processing techniques, such as boiling, steaming (Azeke *et al.*, 2007), and fermentation process. The previous study by Sarti *et al.* (2020) concluded that lotus tempeh has increased the nutritional substances, otherwise, its final pH was below 6.00 which could affect the taste of tempeh. The nutritional and bioactive potential was influenced by many factors including collaboration and restrain of end products after processing, loss of solid and water-soluble antioxidants, and physicochemical of the product (legumes) (López-Cortez *et al.,* 2016; Mir *et al*., 2016).

Based on the description above, an optimal improvement in the effort of making lotus tempeh as a new food product. Lotus tempeh is arguably a good functional food not only physical appearance but also nutritionally the method of lotus tempeh should be modified in time boiling and/or steaming. In addition, the available evidence of the use of lotus seeds in tempeh production remains scarce, especially how much boiling and steaming have affected its quality. Therefore, this study investigates the use of lotus seeds in an attempt to diversify tempeh products and analyse them in terms of substantial nutrient and physical profiles.

2. Materials and methods

2.1 Lotus tempeh preparation

Tempeh was prepared based on Sarti *et al.* (2020) with modifications following the common practices in local tempeh producers. Lotus seeds were purchased from a commercial market, in Indonesia. Firstly, lotus seeds (250 g) were soaked using the soy vinegar (pH 4-5), wherein there are four preparation methods carried out: soaked for 3 hrs and steamed for 5 mins (A1); soaked for 3 hrs and steamed for 10 mins (A2); soaked for 3 hrs and steamed for 15 mins (A3); and boiled for 30 mins prior sooaking, soaked for 24 hrs and then boiled for15 mins (A4). The loose soybean hulls of all treatments were washed to remove the acid water residue. The dehulled beans and seeds were washed and weighed according to the sample proportion (w/w); 250 g. Then, all samples were allowed for evaporative cooling at 25°C. After that, the seeds were inoculated using fungal spores (*Rhizopus* sp.) at a 0.75% ratio (w/w), and sporulated beans were subsequently placed in perforated plastic bags (13 cm × 20 cm). The thickness was approximately 1.5 cm for proper fungal development. Incubation of the samples was done at 28±2°C for 36 hrs.

2.2 Proximate analysis

Proximate analysis was conducted according to the Association of Official Analytical Chemists methods (AOAC, 2005). Moisture content analysis was conducted by drying the sample in an oven at 102-105°C until it reached a constant weight. Ash content was determined by incinerating 5 g of the sample at 600°C for 6 hrs. Crude fat content was analyzed by the Soxhlet extraction method using n-hexane as solvent. Nitrogen was determined using the Micro-Kjeldahl method and the quantity of protein was calculated by multiplying the percentage of nitrogen content by the conversion factor of 6.25. Then, the content of carbohydrates was analyzed using 2% w/v phenol in sulfuric acid.

2.3 Amino acid

The amino acid composition was analyzed by using High-Performance Liquid Chromatography, wherein the procedures were determined by AOAC (2006) methods, a standard solution used Alpha Amino Butyrate Acid (AABA). Wherein, the HPLC system (AccQtag column (3.9 × 150 mm; used for separation purposes)) maintained at 37^{o}C. The mobile phase consisting of acetonitrile 60% – AccqTag Eluent A was flushed through the column at flow rate of 1 mL/min using a linear gradient system. The flouresence absorption detector at a wavelength of 260 nm was employed to monitor amino acids.

2.4 pH and colour

pH samples were analyzed using the pH WN-PH003 model. The colour of tempeh sample surfaces was determined by using a Spectrophotometer CM-3500d, Konica Minolta Sensing, Inc., Osaka, Japan, and Hunter colour values, L* (lightness), C* (chroma), and H* (hue). The instrument was calibrated to standard black and white plates before analysis. The Hunter values were monitored by a computerized system using spectra magic software (Konica Minolta Sensing, Inc., Japan) and the measurements were performed in triplicate.

2.5 Hardness

An Instron Universal Testing Machine (Model 4400, Instron Co., USA) type TA18 (12.7MM DIA) probe was used for hardness analysis. The samples were prepared in a uniform shape (1.0*2.0*0.3 cm) and the hardness was measured by a cylindrical probe (12.7 mm diameter), the probe above the sample dropped until cut the sample for 2 mins. The biting force data (Newton/s)) obtained were stored.

2.6 Protein digestibility

Protein digestibility *in vitro* was carried out according to Genovese and Lajolo (1998) with slight modification. The samples (0.25 g) were placed in a 50

https://doi.org/10.26656/fr.2017.7(5).903

mL Erlenmeyer flask and 0.1 N HCl containing 0.015 g Pepsin (P6887, 49.5 U/mg solid) was added to 15 mL. The mixtures were shaken (50 rpm, at 37°C) for 3 hrs in a water bath shaker (Memmert WNB 7). The sample solution was adjusted to pH 7 by adding 0.1 N NaOH and then added with 7.5 mL phosphate buffer 0.2 M (pH 8) containing sodium azide 0.005 M and 0.4 g pancreatic enzyme (P3292, 64 U/mg solid). Subsequently, the sample solution was shaken (50 rpm, at 37°C) for 24 hrs and filtered using Whatman paper number 41 (the weight of filter paper was previously recorded). The weight of residue on filter paper was determined and the protein content was further analyzed using the Micro-Kjeldahl method. The protein digestibility was calculated using the following formula:

Protein digestibility (%w/w) = (crude protein-residue protein)/(crude protein) × 100

2.7 Starch digestibility

This analysis was determined by Leong *et al.* (2007) with slight modifications. The sample was prepared by dissolving 1 g each of lotus tempeh in 10 mL of aquadest. In the first step, 2 mL of sample was added with 3 mL of Aquadest and 5 mL of phosphate buffer in the test tube, homogenized using a vortex for 2 mins. The test tube was then heated in a water bath at 37°C for 15 mins. In the second step, 5 mL of α-amylase enzyme was added and heated at 37°C for 30 mins. In the third step, 3 mL of the solution was taken from the test tube and 3 mL of DNS reagent was added to the test tube. Then, the tube was covered with aluminum foil and heated in a water bath at 100°C for 10 mins until it became a brownish-red. Finally, in the fourth step, 5 mL of potassium sodium tartrate solution (2% w/v) was added. After cooling, a spectrophotometer (Shimadzu UV/Visible-1800) was used to measure the absorbance at 600 nm. The maltose content of the reaction mixture was calculated using a pure maltose standard curve.

2.8 Statistical analysis

This research used the randomized block design with three replications. The SPSS (Statistical Package for the Social Sciences) version 14 program was used to analyze the data. To measure significant differences between treatments, Duncan's multiple range test was applied. All analyses were repeated three times. All the results were expressed as mean values ± standard deviation (SD).

3. Results and discussion

3.1 Proximate analysis

Food contains water, minerals, protein, fats, and carbohydrates. The moisture content of tempeh ranged from 54.59±3.27 to 76.60±0.07%. Lotus tempeh (A4) was significantly higher than the other's treatments for water content. The results also showed that lotus tempeh was high in protein content, 38.38% on a dry basis (Table 1). There was no significant difference ($P < 0.05$) for the nutritional substances in A1, A2, and A3 treatments, except for carbohydrate contents, wherein all of the samples were just different in steaming time. In all treatments, the protein of lotus tempeh was higher than pea bean tempeh (Rizwan *et al.,* 2016), and lupin (Australian Sweet Lupin) tempeh (Wickramasinghe, 2017), which protein increased only after 48 hrs of fermentation. The results further indicated that the levels of fat content were no significant differences, although the fat content was higher in the A2 treatment, 8.27±0.35%, as seen in Table 1.

In general, the moisture levels were increased during the fermentation of the lotus seed. Priatni *et al.* (2013) declared the condition of moisture contents was correlated to the making process of tempeh. Boiling and soaking could increase the hydration index and water uptake (Sahni *et al*., 2020). The moisture content of all tempeh produced in this study did not exceed the upper limit of the lupin bean tempeh (Priatni *et al*., 2013). The high moisture levels of tempeh are related to its high-water holding capacity and the metabolic reaction of microorganisms throughout the fermentation (Sujak *et al*., 2006). Fermentation, similar to other food processing techniques, contributes to the increase or/and decrease in the nutritional value of food material. The process of making tempeh involves three supporting factors, which are raw materials (grains), microorganisms, and the state of the micro-growing environment. During the fermentation process, the substantial quality could be improved due to changes in chemical composition as

Table 1. Proximate composition of *Tempeh* (% w/w, dry weight).

Treatment	Water (wet basis)	Ash	Fat	Protein	Carbohydrate
A1	54.59±3.27^{b}	4.07±0.34^{b}	6.03±0.78^{b}	24.31±1.03^{b}	65.58±2.55^{c}
A2	61.81±4.75^{b}	5.29±0.51^{b}	8.27±0.35^{b}	27.10±5.10^{b}	59.33±2.96^{b}
A3	61.57±6.00^{b}	4.87±0.47^{b}	7.13±0.72^{b}	30.11±1.75^{bc}	57.90±3.73^{b}
A4	76.60±0.07^{c}	2.48±0.03^{c}	5.60±0.48^{c}	38.38±0. 43^{c}	53.55±0.68^{b}
Lotus seed	10.17±0.53^{a}	4.50±0.21^{a}	6.57±1.24^{a}	16.98±3.68^{a}	71.96±3.17^{a}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($P<0.05$).

https://doi.org/10.26656/fr.2017.7(5).903

well as the possibility of reducing anti-nutrients such as phytic acid and polyphenols compounds (Murtini *et al*., 2011).

The lotus tempeh which has been fermented for 36 h has a significantly higher content of protein and fat compared to the raw materials, as reported in previous studies (Pan *et al*., 1998; Reyes-Moreno *et al*., 2004; Angulo-Bejarano *et al*., 2008; Abu-Salem and Abou-Arab, 2011). Abu-Salem and Abou-Arab (2011) studied that the protein of chickpea flour tempeh was higher than in raw seeds, except for carbohydrate content. This result was in line with Hong *et al.* (2004). Wickramasinghe (2017) also noticed the increase in lupin tempeh protein after 48 hrs fermentation. Hong *et al.* (2004) argued that the increase in protein and fat content is related to the biochemical reactions which involve oligosaccharides, free sugars, and other polysaccharides during fermentation. Various studies have confirmed that the preparation process or experimental procedure, the particle size of seeds, condition of fermentation (pH, time) could be the reason for the final nutritional quality of tempeh (Fudiyansyah *et al*., 1995; Wickramasinghe, 2017). After the mineral and other substrates were leached out during boiling and soaking in the first step, the lotus seed could easily be denatured by heat and microorganism activity. In fact, boiling could cause the loss of many nutrients such as the ash and fat content, more than the steaming method of cooking (Reid *et al*., 2016). The level of crude protein increased although the amount of protein in A3 and A4 treatments has no significant differences. In contrast, Bembem and Sadana (2013) that the nutrients of lotus tempeh have been unstable and may be damaged due to heat processing, such as boiling and steaming (Azeke *et al*., 2007; Omotosho *et al*., 2016). Differences in the nutritional quality of various tempeh were reported by Priatni *et al.* (2013) and Agosin (1989).

The differences have been caused by processing techniques, such as soaking in soy vinegar, boiling, and steaming, before the tempeh fermentation started. Whereas the multistep processing could retain itself more than boiling or steaming, A4 treatment has increased the proximate substances, especially protein contents (38.38% per 100 g tempeh). Lola (2009) has pointed out that the moisture level of boiled products was higher than that of steamed ones. In fact, all products contain more water after being boiled, as affirmed by Bembem and Sadana (2013). The present study has shown that making tempeh using the A4 method, two times boiling has increased the water content. The first boiling is the same as the soaking process in terms of usability, for opening the dormant period of lotus seed. All of the compounds of lotus seed absorbed water during boiling and soaking. The rise of moisture during both processes has made the end of pH neutral, as shown in Table 2. Notably, the protein level contained in lotus tempeh has met the Food and Agriculture Organization (FAO) (2013) requirement.

3.2 Hardness and colour

The hardness, pH, and colour characteristics of tempeh have been shown in Table 2. A4 treatment was significantly different from A1, A2, and A3 treatments in terms of hardness, not in pH and colour. The texture of A4 tempeh was quite soft and easy to cut than that of other treatments which were more compact (Table 2). This indicated that lotus tempeh still shared the same texture profile as soybean tempeh. The hardness value of tempeh in this study ranged from 9.93±1.33 N/s to 52.60±2.16 N/s. This value is comparable to a 48 hrs fermented soybean in Handoyo and Naofumi's study, in which hardness was 7.14 N/s. The length of fermentation time did not necessarily increase the hardness value, although the mycelium was still overgrown and becoming strong after 48 hrs. At 72 hrs of fermentation, tempeh is considered over-fermented. The formation of the typical texture, taste, and flavour of over-fermented tempeh depends on the fermentation process, beans used, boiling process, and microbial consortium (Utami *et al*., 2016). A variety of beans as raw material affects the physical properties of tempeh. The softer texture of over-fermented tempeh is associated with a weaker mycelium network as the number of mature fungi increases and mycelia regeneration decreases (Handoyo and Naofumi, 2006).

The boiling and soaking have made the integrity of the membrane lost before fermentation; the loss of turgor pressure also made the lotus seed easily digested by

Table 2. Hardness, pH, and color of tempeh.

Treatment	pH	Hardness (N/s)	Color		
			L*	C*	H*
A1	7.00±0.14[a]	49.40±7.38[a]	78.17±3.03[a]	17.77±3.87[a]	66.80±2.98[a]
A2	7.05±0.21[a]	52.60±2.16[a]	78.27±2.14[a]	15.47±3.72[a]	62.13±1.17[a]
A3	6.7±0.70[a]	49.47±1.33[a]	83.30±2.21[a]	13.55±1.87[a]	64.25±1.86[a]
A4	6.3±0.84[a]	9.93±1.33 [b]	73.73±2.21[a]	13.20±1.87[a]	66.27±1.86[a]

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (P<0.05).

https://doi.org/10.26656/fr.2017.7(5).903

fungi. The degradation of the seed compound resulted in the nutrient for the *Rhizopus* sp. growing, which comes from protein and carbohydrates. The activity and dissolution of some compounds (soluble, pectin, etc.) have made the hardness decrease during fermentation. Data from Li *et al.* (2017) showed that the steaming process could reduce hardness to 46.02, more than the boiling process could do (52.59) when the two methods of cooking were conducted at the same length of time (60 mins).

As the fermentation proceeded, the colour of tempeh gradually became whiter. This colour continuously changed during the fermentation process which is considered a unique phenomenon. According to Muzdalifah *et al.* (2017), the colour changes were associated with the increased number of *Rhizopus* sp. which entered the death phase, and increased amount of fat in particular unsaturated fatty acids (linoleic and linolenic acid) which were prone to oxidation as well as the formation of red-coloured vitamin B12. In this case, all lotus tempeh has no significantly different values ($P < 0.05$) in colour properties. As presented in Table 2, the data found in this study were in line with those reported by Reyes-Moreno *et al*. (2004); Kaur *et al*. (2005). The redness "C" value was slightly decreased, while the yellowness "H" value was increased. Tempeh made from soybean had a lighter colour and higher L* and H* values. The colour of all samples was the same as the soybean-based tempeh, which was greenish-white. In the previous study, the lightness value of soybean tempeh was 72.00 to 83.02 (Handoyo and Naofumi, 2006). The lightness value for both soybeans and mycelia of the tempeh decreased as the fermentation period increased (Angulo-Bejarano *et al*., 2008; Muzdalifah *et al*., 2017). However, the lightness of lotus tempeh was still acceptable. As the fungus grew and mycelia formed, the L* and H* values increased, indicating a distinct effect on L*, C*, and H* values. The soaking and blanching processes have caused the slight redness "C" to decrease and yellowness "H" to increase in the tempeh product (Reyes-Moreno *et al*., 2004; Kaur *et al*., 2005). Nevertheless, significant differences ($P < 0.05$) in the "L, C, and H" values were not observed between the treatments in this study, so were the pH values. The process of soaking and cooking has increased "L" value significantly, causing a lighter colour. Rather, the colour of tempeh changed slightly darker as a result of the fermentation. It was because of the mycelia fungi and the heat processing.

The pH in this lotus tempeh ranged from 6.30 to 7.05 during the 36 hrs period of the fermentation process. Tahir *et al.* (2018) proved that tempeh has a pH from 4.00 to 6.00 in normal conditions, the initial pH will increase depending on the fermentation time, such as pH 6.00 at 28°C, 26 hrs; pH 8.00 at 28°C, 48 hrs, respectively. It is generally known that the pH range of most fungi growth was from 4.00 to 7.00, and the acidity condition highly varied from 4.00 to 6.00. Those conditions were important for *Rhizopus* sp. and controlling the growth of the pathogen or other organisms that caused the food spoilage. The increase in pH has also made the mycelium grow thicker than the initial pH of the fungus growth. Although the acidic condition doesn't mainly affect the *Rhizopus* microbes, the growth of microbes is more stable in a pH equal to or greater than 3.5. It becomes slower when the lotus seeds absorbed much water from the acidic soaking water. Beuchat (2001) revealed that the pH of tempeh has increased up to 7.60, from the beginning to the end. The most palatable tempeh was when the *Rhizopus* enzymes digested partially the lotus seed, and the tempeh has a pH of 6.50. In this case, there were no significant differences in pH value observed in all treatments. This condition didn't have an acidic effect on the taste of lotus tempeh.

3.3 Amino acid analysis

Table 3 shows the total amino acid in lotus tempeh, where the value of total amino acids in A4 treatment was significantly different at 95% than others. The glutamate acid was the highest of all amino acids, ranging from 3.06% to 6.20%. Meanwhile, histidine and tyrosine were the lowest amino acids in all treatments. It can be concluded that both of them have limited amino acids. Syida *et al.* (2018) have explained that the decrease in total amino acid levels might occur because of the heat processing during tempeh making.

The heat treatment has also affected the pattern of amino acids. A similar pattern was found in A1, A2, and A3 treatments, such as glu > asp > ala > val > ser > phe; a different pattern in the A4 treatment was glu> leu > ser > arg > val > ile. Similar to Paredes-López and Harry (1988) reports, study by Angulo-Bejarano *et al.* (2008) indicated that the fermented (tempeh) flour made through solid-state fermentation process resulted in the increase of the essential amino acids (Met, Cys, Phe, Tyr, Thr, except Trp), more than unfermented flour (Paredes-López and Harry, 1988). A biochemical mechanism such as transamination and deamination might lead to the fluctuation of the amino acid contents (Paredes-López and Harry, 1988). A similar pattern was found in all samples, that is Glu > Leu > Lys > Cys > Ala > Tyr > Thr > Val > Arg > Ser > Asp > Pro > Gly > Phe > His > Met > Iso. They also have reported that legumes are deficient in Sulphur containing amino acids (methionine). Similarly, in this study, Sulphur-containing amino acid was found low in amount. Thermal

https://doi.org/10.26656/fr.2017.7(5).903

Table 3. Amino acid of tempeh (% w/w, dry weight)

Amino acid	A1	A2	A3	A4
Aspartate acid	1.72±0.01[a]	1.86±0.01[b]	1.95±0.01[c]	3.42±0.01[d]
Leucine	1.37±0.01[a]	1.65±0.01[b]	1.82±0.01[c]	3.21±0.01[d]
Tyrosine	0.64±0.01	0.58±0.01	0.70±0.01	1.15±0.01
Proline	0.66±0.01	0.79±0.01	0.86±0.01	1.41±0.01
Serine	1.06±0.01	0.92±0.01	1.54±0.01	2.52±0.01
Glutamic acid	3.06±0.01[a]	3.56±0.01[b]	3.54±0.01[c]	6.20±0.01[d]
Phenylalanine	1.01±0.01	0.97±0.01	1.35±0.01	2.22±0.01
Isoleucine	0.88±0.01[a]	1.07±0.01[b]	1.17±0.01[c]	2.35±0.01[d]
Threonine	0.92±0.01	1.01±0.01	1.14±0.01	1.88±0.01
Histidine	0.64±0.01[a]	0.63±0.01[a]	0.75±0.01[b]	1.58±0.01[c]
Arginine	0.88±0.01	0.79±0.01	1.69±0.01	2.78±0.01
Glycine	1.06±0.01	1.18±0.01	1.35±0.01	2.22±0.01
Lysine	0.95±0.01	1.28±0.01	1.20±0.01	1.97±0.01
Alanine	1.45±0.01	1.86±0.01	1.38±0.01	2.26±0.01
Valine	1.19±0.01	1.44±0.01	1.46±0.01	2.39±0.01

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($P<0.05$).

processing, such as boiling and steaming, have contributed to the breakdown of the cell wall structure of lotus seed. Not only the high temperature but also pressure could make the partial hydrolysis of polysaccharides (Paredes-lopez and Harry, 1988; Kadiri, 2017). Consequently, the thermal processing in lotus tempeh has induced the process of depolymerization and polymerization of a strong complex compound with macromolecules in the food matrix, as proposed by Massaretto *et al.* (2011).

Tempeh of *Phaseolus vulgaris* L. (common beans) yielded approximately 28% of protein content but lacked sulfur amino acids. As Astuti *et al.* (2000) have argued, the protein content of soybean tempeh and soybeans are practically the same, and so are lotus tempeh and its seeds due to the protease activity produced by *Rhizopus* fungi during the fermentation. According to Mesquita *et al.* (2007), the protein value of the white bean tempeh does not increase significantly after fermentation, and the value here was 23.34 g/100 g. In light of the literature, it is concluded that the microorganism (*Rhizopus* sp.) received nitrogen from amino acids contained in lotus seeds. Since many free amino acids were released during the fermentation, the total amino acid content decreased. Therefore, lotus tempeh as a fermented product is more digestible than cooked lotus.

3.4 Protein digestibility

The tempeh made from lotus seed using different methods displayed protein digestibility *in vitro* from 50.41% to 64.89% while its starch digestibility ranged from 17.62% to 31.09% (Table 4). The protein *in vitro* ability to digest lotus tempeh protein was lower than soybean tempeh (Reyes-Moreno *et al*., 2004). Murtini *et al.* (2011) showed that the protein digestibility of sorghum tempeh in their study was 62.05±3.87% for 36 h fermentation. Lotus tempeh in this study contained higher protein digestibility (64.89±1.45%) than the study of Murtini *et al*. (2011). The decrease in protein digestibility was caused by many soluble proteins in water during the tempeh preparation; the process of soaking, whole seed blanching, dehulling, and dehulled blanching might have decreased the protein solubility. The results were in contrast to the previous findings by Abd El-Hady and Habiba (2003) and Reyes-Moreno *et al.* (2000) which showed that the digestibility of the protein *in vitro* reached 82.7% and 77.6 – 83.5%, respectively. Thus, they concluded that the values of *in vitro* protein digestibility were slightly or insignificantly affected by different processing. In this study, as shown by Table 4, the processing step has significantly affected the protein or starch digestibility, that is, A4 (boiled 30 mins, soaked 24 hrs, and boiled 15 mins) contained higher protein and starch digestibility (64.89% and 31.09%) than A1 (50.41% and 17.62%), A2 (57.60% and 24.29%), and A3 (58.89% and 25.37%).

The increase in protein digestibility could be related to changes in the structure of lotus seeds, soybeans, due to the contribution of microbes during fermentation and the decrease in anti-nutrient content. The preparation process, which involves soaking and heating, would change the structure of the seeds to be softer. According to Cueves-Rodriquez *et al.* (2006), the initial heating process leads to protein denaturation, therefore, the protein is readily broken down enzymatically by microorganisms during the fermentation. The

https://doi.org/10.26656/fr.2017.7(5).903

degradation of complex protein increases soluble proteins and make them easily accessed and digested by the protease enzyme. This is in line with González-Castañeda (1992) that argued the increase of *in vitro* digestibility was more likely caused by the elimination of undesirable factors, e.g., tannins, phytic acid during soaking, and dehulling. Although the protein of lotus tempeh was more difficult to digest than soybean tempeh, it could be nominated as a healthy food product since the starch of lotus seeds can be easily digested without causing blood sugar to rise (Lin *et al*., 2009). This distinct attribute is not found in soybean and other seeds.

3.5 Starch digestibility

In terms of starch digestibility, the sample in A4 treatments contained 31.09±0.05% of starch *in vitro* analysis. Statistical analysis showed a significant difference ($P<0.05$) was observed in starch and protein *in vitro* ability between A4 and other treatments. Marconi *et al*. (2000) found starch digestibility of chickpea and common bean increased after traditional cooking. The rise in starch digestibility was caused by the reduction of phytate content and hydrolysis of starch during the incubation process. Also, this process has led to a change in the structure of starch and other compounds, supporting previous findings by Urooj and Puttaraj (1994); Sahni *et al.* (2020). The processing steps, such as dehulling, soaking, boiling, steaming, and fermentation, have resulted in the loss of many inhibitors or anti-nutritional substances. In particular, Urooj and Puttaraj (1994), Marconi *et al.* (2000), Kaur *et al.* (2015) and Rizwan *et al.* (2016) have explained that the levels of the loss have been affected by the processing technique, which includes cooking (boiling and steaming) > fermentation > soaking > dehulling. Elkhalifa *et al.* (2004) specified that the factors that influence the low digestibility of sorghum protein include the hydrophobicity of kafirin, disulfide and non-disulfide bonds, changes in protein structure, and the presence of anti-nutritional compounds such as tannins and phytic acid. The digestibility of rice and corn was 66.73% and 81%. Their protein was 9-14%, which is high enough, but low power. The fermented flour of chickpea legume has 72.2 – 83.2% for protein digestibility, wherein it also has the lower gelatinization of its starch. Zheng *et al.* (2019) have found that the starch structure tended to be weak due to the reduction of amylose leaching and the coating effect of guar gum so the mycelia covering the tempeh probably might make the starch digestion reduced. Many factors could increase digestibility, two of which are crystallinity and ordered structure. Wang *et al.* (2016) have affirmed that starch digestibility has little effect on cooking conditions, for example, 15% starch digestibility in 20 min. Liang *et al.* (2012) concluded that the digestibility of products that are difficult to cook (starchy foods) can be improved by cooking-pressure treatment. On the other hand, carbohydrate digestion can decrease if starch fraction modification occurs. The researchers have reported that the isoflavones in tempeh lost more than 12% after soaking and heat processing, either retention or distribution of such compounds. Even though, the lotus tempeh making using A4 methods contained the genistein compound higher than soybean tempeh, the scavenging activity of DPPH radical in lotus tempeh was the same as in soybean tempeh (Ridhowati *et al*., 2020).

It was reported that the total loss of α-galactoside was as much as 98% in the fermentation process, 5.5% in the immersion process, and 33.2% in the peeling process (Aliyu *et al*., 2017). This condition may be caused by the activity of microbial and/or fungal flora-producing hydrolytic enzymes. In this research, the A4 treatment, which is equal to the traditional method, was more effective than A1, A2, and A3 treatments, in line with Vital *et al.* (2016), Aliyu *et al*. (2017) and Chen *et al.* (2020).

Table 4. Protein and starch digestibility *in vitro* of tempeh

Treatments	Starch digestibility *in vitro* (% w/w)	Protein digestibility *in vitro* (% w/w)
A1	17.62±0.02^{a}	50.41±0.83^{a}
A2	24.29 ±0.49^{b}	57.60±0.05^{b}
A3	25.37±0.34^{b}	58.89±1.25^{b}
A4	31.09±0.05^{c}	64.89±1.45^{c}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($P<0.05$).

4. Conclusion

The differences in the processing step of lotus tempeh making had significantly affected the contents of the nutrient substance, especially in water and protein content, starch, and protein digestibility. However, there were no significant differences observed among the treatments in the physical appearance (colour, pH), except for the hardness of lotus tempeh. These results concluded that the quality of the lotus tempeh in the A4 treatment was almost the good functional food in the physicochemical and enzymatic digestibility quality. In addition, lotus tempeh made by using multistep processing (twice boiling) was more effective than the modified method through steaming. Further research is needed to know more about the morphological structure of lotus tempeh to shed light on the potential of lotus tempeh to be a fermented functional food.

https://doi.org/10.26656/fr.2017.7(5).903

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was funded by the Institute for Research and Community Service of Sriwijaya University, Indonesia, in 2021.

References

Abd El-Hady, E.A. and Habiba, R.A. (2003). Effect of soaking and extrusion conditions on anti nutrients and protein digestibility of legume seeds. *Lebensm.-Wiss.U.-Technology-Food Science and Technology*, 36(3), 285-293. https://doi.org/10.1016/S0023-6438(02)00217-7

Abu-Salem, F.M. and Abou-Arab, E.A. (2011). Physico-chemical properties of tempeh produced from chickpea seeds. *Journal of American Science*, 7(7), 1 -12.

Adam, M.R. and Moss, O.M. (2008). Handbook of Food Microbiology, Vol 3, 3rd ed., p. 99 – 115. United Kingdom: The Royal Society of Chemistry Press.

Agosin, E., Diaz, D., Aravena, R. and Yañez, E. (1989). Chemical and nutritional characterization of Lupine tempeh. *Journal of Food Science*, 54(1), 102-104. https://doi.org/10.1111/j.1365-2621.1989.tb08577.x

Aliyu, M., Atiku, M.K., Abdullahi, N., Zaharaddeen, A. and Imam, A.A. (2017). Comparative evaluation of nutritional qualities of *Nymphaea* lotus and *Nymphaea pubescens* seeds. *International Journal of Biochemistry Research and Review*, 19(3), 36536. https://doi.org/10.9734/IJBCRR/2017/36536

Angulo-Bejarano, P.I., Verdugo-Montoya, N.M., Cuevas -Rodriguez, E.O., Milan-Carrillo, J., Mora-Escobedo, R., Lopez-Valenzuela, J.A., GarzonTiznado, J.A. and Reyes-Moreno, C. (2008). Tempeh flour from chickpea (*Cicer arietinum* L.) nutritional and physicochemical properties. *Food Chemistry*, 106(1), 106-112. https://doi.org/10.1016/j.foodchem.2007.05.049

AOAC. (2005). Official method of Analysis. Vol. 18, p. 935.14 - 992.24. Washington DC, USA: Association of Officiating Analytical Chemists Press.

Astuti, M., Meliala, A., Dalais, F.S. and Wahlqvist, M.L. (2000). Tempeh, a nutritious and healthy food from Indonesia. *Asia Pacific Journal Clinical and Nutrition*, 9(4),322–325. https://doi.org/10.1046/j.1440-6047.2000.00176.x

Azeke, M.A., Barbara, F., Hans, B-P. and Thomas, B. (2007). Comparative effect of boiling and solid substrate fermentation using the tempeh fungus (*Rhizopus oligosporus)* on the flatulence potential of African yambean (*Sphenostylis stenocarpa* L.) seeds. *Food Chemistry*, 103(4), 1420-1425. https://doi.org/10.1016/j.foodchem.2006.10.058.

Bembem, K. and Sadana, B. (2013). Effect of cooking methods on the nutritional composition and antioxidant activity of potato tubers. *International Journal of Food and Nutritional Sciences*, 2(4), 26–30.

Beuchat, L.R. (2001). Traditional Fermented Foods. In Doyle, M.P. and Buchanan, R.L. (Ed.) Food Microbiology: Fundamental and Frontiers. 2nd ed., p. 701-719. Washington DC, USA: American Society for Microbiology Press

Chen, S.Y. and Tengku Rozaina, T.M. (2020). Effect of cooking methods on the nutritional composition and antioxidant properties of lotus (*Nelumbo nucifera*) rhizome. *Food Research*, 4(4), 1207-1216. https://doi.org/10.26656/fr.2017.4(4).359

Cueves-Rodriguez, E.O., Verdugo-Montoya, N.M., Angulo-Bejarano, P.I., Milan-Carrillo, J., Mora-Escobedo, R., Bello-Pérez, L.A., Garzon-Tiznado, J.A. and Reyes-Moreno, C. (2006). Nutritional properties of tempeh flour from quality protein maize (*Zea mays* L.). *Lebensmittel-Wissenschaft and Technologie*, 39(10), 1072-1079. https://doi.org/10.1016/j.lwt.2005.07.003.

Divekar, M.T., Karunakan, C., Lahlali, R., Kumar, S., Chelladurai, V., Liu, X., Borondics, F., Shanmugasundaram, S. and Jayas, D.S. (2017). Effects of microwaves treatments on the cooking and macronutrients qualities of pulses. *International Journal of Food Properties*, 20(2), 409-422. https://doi.org/10.1080/10942912.2016.1163578

Elkhalifa, A.O., Schiffler, B. and Bernhard, R. (2004). Effect of fermentation on the starch digestibility, resistant starch and some physicochemical properties of sorghum flour. *Foods*, 48(2), 91-94. *https://doi.org/10.1002/food.200300322*

Food and Agriculture Organization (FAO). (2013). Dietary Protein Quality Evaluation in Human Nutrition. Report of an FAO Expert Consultation, FAO Food and Nutrition Paper, p. 66-92. Retrieved on February 12, 2021 from FAO website: https://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf

Ferreira, M.P., Neves de Oliveira, M.C.M., Mandarino, J.M.G., Bonifácio da Silva, J., Ida, E.T. and Carrão-Panizzi, M. C. (2011). Changes in the isoflavone profile and in the chemical composition of tempeh during processing and refrigeration. *Pesquisa Agropecuaria Brasileira*, 46(11), 1555-1561. http://doi.org/10.1590/S0100-204X2011001100018.

Fudiyansyah, N., Petterson, D.S., Bell, R.R. and Fairbrother, A.H. (1995). A nutritional, chemical and sensory evaluation of lupin (*L. angustifolius*) tempe. *International Journal of Food Science and Technology*, 30(3), 297-305. https://doi.org/10.1111/j.1365-2621.1995.tb01378.x

Genovese, M.I. and Lajolo, F.M. (1998). Influence of naturally acid soluble protein from beans (*Phaseolus vulgaris* l.) on *in vitro* digestibility determination. *Food Chemistry*, 62(3), 315-323. https://doi.org/10.1016/S0308-8146(97)00224-0

Gil-Izquierdo, A., Penalvo, J.L., Gil, J.I., Medina, S., Horcajada, M.N., Lafay, S., Silberberg, M., Llorach, R., Zafrilla, P., Garcia-Mora, P. and Ferreres, F. (2012). Soy isoflavones and cardiovascular disease epidemiological, clinical and -omics perspectives. *Current Pharmaceutical Biotechnology*, 13(5), 624-631. https://doi.org/10.2174/138920112799857585

González-Castañeda, J. (1992). Aprovechamiento de garbanzo de bajacalidad commercial mediantefermentanciónenestadosólido. Mexico: Universidad Nacional Autonoma De Mexico, BSc. Thesis. [In Spanish].

Handoyo, T. and Naofumi, M. (2006). Structural and functional properties of fermented soybean (tempeh) by using *Rhizopus oligosporus*. *International Journal of Food Properties*, 9(2), 347-355. https://doi.org/10.1080/10942910500224746.

Hong, K-J., Lee, C-H. and Kim, S. (2004). *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *Journal of Medicinal Food*, 7(4), 430-435. https://doi.org/10.1089/jmf.2004.7.430.

Irina, I. and Mohamed, G. (2012). Biological activities and effects of processing on flavonoids as phenolic antioxidant. In Petre, M. (Ed.) Advances in Applied Biotechnology. InTech Open E-Book. https://doi.org/10.5772/30690.

Kadiri, O. (2017). A review on the status of the phenolic compounds and antioxidant capacity of the flour: Effects of cereal processing. *International Journal of Food Properties*, 20(Sup1), S798-S809. https://doi.org/10.1080/10942912.2017.1315130

Kaur, M., Sandhu, K.S. and Singh, N. (2005). Comparative study of the functional, thermal and pasting properties of flours from different field pea (*Pisum sativum* L.) and pigeon pea (*Cajanus cajan* L.) cultivars. *Food Chemistry*, 104(1), 259 – 267. https://doi.org/10.1016/j.foodchem.2006.11.037

Kaur, M., Sandhu, K.S., Ahlawat, R. and Sharama, S. (2015). *In vitro* starch digestibility, pasting and textural properties of mung bean: effect of different processing methods. *Journal of Food Science and Technology*, 52(3), 1642-1648. https://doi.org/10.1007/s13197-013-1136-2

Leong, Y.H., Karim, A.A. and Norziah, M.H. (2007). Effect of *pullulanase* debranching of sago (*Metroxylon sagu*) starch at subgelatinization temperature on the yield of resistant starch. *Starch/Starke*, 59(1), 32. https://doi.org/10.1002/star.200600554

Lestari, S.D., Fatimah, N. and Nopianti, R. (2017). Chemical changes associated with lotus and water lily natto production. *IOP Conference: Materials Science and Engineering*, 193, 012040. https://doi.org/10.1088/1757-899X/193/1/012040

Liang, X-L., Fan, Z-H., Wang, S-Y. and Wu, W. (2012). Effect of pressure cooking *in vitro* carbohydrates digestion properties of five starchy food materials. Retrieved December 10, 2020 from http://en.cnki.com.cn/Article_en/CJFDTotal-SPKJ201204092.htm

Lin, Y., Zeng, S. and Zheng, B. (2009). Study on the *in vitro* digestibility of edible lotus-seed starch film. *China Agricultural Science Bulletin*, 25(22), 100–103.

Li, C.H., Bland, J.M. and Bechtel, P.J. (2017). Effect of precooking and polyphosphate treatment on the quality of microwave cooked catfish fillets. *Food Science and Nutrition*, 5(3), 812-819.

Lola, A. (2009). The effect of boiling on the nutrients and anti-nutrients in two nonconventional vegetables. *Pakistan Journal of Nutrition*, 8(9), 1430 –1433. https://doi.org/10.3923/pjn.2009.1430.1433

López-Cortez, M.D.S., Rosales-Martínez, P., Arellano-Cárdenas, S. and Cornejo-Mazón, M. (2016). Antioxidants properties and effect of processing methods on bioactive compounds of legumes. In Goyal, A.K. (Ed.) Grain Legumes, p. 103–120. InTech Open E-Book. https://doi.org/10.5772/63757

Marconi, E., Ruggeri, S., Cappelloni, M., Leonardi, D. and Carnovale, E. (2000). Physicochemical, nutritional, microstructural characteristics of chickpeas (*Cicer arietinum* L.) and common beans (*Phaseolus vulgaris* L.) following microwave cooking. *Journal of Agricultural and Food Chemistry*, 48(12), 5986-5994. https://doi.org/10.1021/jf0008083

Massaretto, M.A.M., Mussi De, M.N.V., Carmona, A.K. and Lanfer, M.U.M. (2011). Phenolic compounds in raw and cooked rice (*Oryza sativa* L.) and their inhibitory effect on the activity of angiotensin I-converting enzyme. *Journal of Cereal Sciences*, 54 (2), 236–240. https://doi.org/10.1016/

j.jcs.2011.06.006

Mesquita, F.R, Corrêa, A.D., Abreu, C.M.P., Lima, R.A.Z. and Abreu, A.F.B. (2007). Linhagens de feijão (*Phaseolus vulgaris* L.): Composiçãoquímica e digestibilidadeprotéica. *Ciencia e Agrotecnologia*, 31(4), 1114–1121. https://doi.org/10.1590/S1413-70542007000400026 [In Spanish].

Mir, S.A., Wani, S.M., Wani, T.A., Ahmad, M., Gani, A. and Masoodi, F.A. (2016). Comparative evaluation of the proximate composition and antioxidant properties of processed products of quince (*Cydonia oblonga* Miller). *International Food Research Journal*, 23(2), 816–821.

Murtini, E.S., Radite, A.G. and Sutrisno, A. (2011). Characteristic of chemical content and digestibility of brown sorghum (*Sorghum bicolor*) tempeh. *JurnalTeknologi dan Industri Pangan*, XXII(2), 150-155.

Muzdalifah, D., Athaillah, Z.A., Nugrahani, W. and Devi, A.F. (2017). Colour and pH changes of tempeh during extended fermentation. *AIP Conference Proceedings*, 1803, 020036. https://doi.org/10.1063/1.4973163

Omotosho, O.E., Garuba, R., Ayoade, F., Adebayo, A.H., Adedipe, O.E. and Chinedu, S.N. (2016). Effects of deep-fat frying using canola oil, soya oil and vegetable oil on the proximate, vitamins and mineral contents of unripe plantain (*Musa Paradisiaca*). *Journal of Applied Sciences*, 16(3), 103–107. https://doi.org/10.3923/jas.2016.103.107

Pan, L., Liang, H. and Guo, G. (1993). Nutritional value and processing technology of lotus seed. *Science and Technology of Food Industry*, 1, 29–32.

Paredes-Lopez, O. and Harry, G.I. (1988). Changes in selected chemical and antinutrional components during tempeh preparation using fresh and hardened common beans. *Journal of Food Sciences,* 54(4), 969-970. https://doi.org/10.1111/j.1365-2621.1989.tb07923.

Priatni, S., Devi, A.F., Kardono, L.B.S. and Jayasena, V. (2013). Quality and sensory evaluation of tempeh prepared from various particle sizes of lupin beans. *Jurnal Teknologi dan Industri Pangan*, 24(2), 209–214. https://doi.org/10.6066/jtip.2013.24.2.209

Reid, T., Munyanyi, M. and Mduluza, T. (2016). Effect of cooking and preservation on nutritional and phytochemical composition of the mushroom *Amanita zambiana*. *Food Science and Nutrition*, 5 (3), 538–544. https://doi.org/10.1002/fsn3.428.

Reyes-Moreno, C., Cuevas-Rodríguez, E.O., Milán-Carrillo, J., Cárdenas-Valenzuela, O. and Barrón-Hoyos, J. (2004). Solid state fermentation process for production of chickpea (*Cicer arietinum* L.) tempeh flour: Physicochemical and nutritional characteristics of the product. *Journal of the Science of Food and Agriculture*, 84(3), 271–278. https://doi.org/10.1002/jsfa.1637

Ridhowati, R., Lestari, S.D., Wulandari, W. and Rinto, R. (2020). Lotus (*Nelumbo nucifera*) Tempeh Indonesia as Antioxidant and Breast Anticancer Food- A Preliminary Study. *Asian Journal of Plant Sciences*, 19(4), 406-411. https://doi.org/10.3923/ajps.2020.406.411

Rizwan, A.S., Miniyar, U.G. and Sakhale, B.K. (2016). Studies on preparation of tempeh-like product from green peas (*Pisum sativum*). *South Asian Journal Food Technology and Environment*, 2(2), 366-371. https://doi.org/10.46370/sajfte.2016.v02i02.03

Sahni, P., Sharma, S. and Singh, B. (2020). Impact of different processing treatments on techno and biofunctional characteristics of dhaincha (*Sesbania aculeate*). *Food Science and Technology International*, 27(3), 251-263. https://doi.org/10.1177/1082013220949812

Sarti, M.Y., Ridhowati, R., Lestari, S.D., Rinto, R. and Wulandari, W. (2020). Sensory Study of Tempeh from Lotus Seeds (*Nelumbo nucifera*) and Soybean (*Glycine max*). *Journal FishtecH,* 8(2), 34-41. https://doi.org/10.36706/fishtech.v8i2.9665.

Sharma, P., Jha, A.B., Dubey, R.S. and Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, 2012, 217037. https://doi.org/10.1155/2012/217037.

Sujak, A., Kotlarz, A. and Strobel, W. (2006). Compositional and nutritional evaluation of several lupin seeds. *Food Chemistry*, 98(4), 711-719. https://doi.org/10.1016/j.foodchem.2005.06.036

Syida, W.S.W.K., Noriham, A., Normah, I. and Yusuf, M.M. (2018). Changes in chemical composition and amino acid content of soy protein isolate (SPI) from tempeh. *International Food Research Journal*, 25(4), 1528-1533.

Tahir, A., Maria, A., Hira, M. and Shahid, R. (2018). Evaluation of physicochemical and nutritional contents in soybean fermented food tempeh by *Rhizopus oligosporus*. *Journal of Advances in Biology and Biotechnology*, 17(1), 1-9. https://doi.org/10.9734/JABB/2018/26770

Urooj, A. and Puttaraj, S. (1994). Effect of processing on starch digestibility in some legumes—an *in vitro* study. *Foods*, 38(1), 38–46. https://doi.org/10.1002/food.19940380108

Utami, R., Wijaya, C.H. and Lioe, H.N. (2016). Taste of

water-soluble extracts obtained from over-fermented tempe. *International Journal of Food Properties*, 19 (9), 2063-2073. https://doi.org/10.1080/10942912.2015.1104509

Vital, R.J., Priscila, Z.B., Quédma, A.C., Rosângela, N.C., Júlia, C.M. and Aline, O.C. (2018). Production, quality, and acceptance of tempeh and white bean tempeh burger. *Foods*, 7(9), 136. https://doi.org/10.3390/foods7090136

Wang, S., Sun, Y., Wang, J., Wang, S. and Copeland, L. (2016). Molecular disassembly of rice and lotus starches during thermal processing and its effect on starch digestibility. *Food and Function*, 7(2), 1188–1195. https://doi.org/10.1039/C6FO00067C

Wickramasinghe, N.P. (2017). Nutritional composition of (Australian Sweet Lupin) natto and tempeh: Effect of source and fermentation time. Australia: Curtin University, BSc. Thesis.

Zhang, Y., Lu, X., Zeng, S., Huang, X., Guo, Z., Zheng, Y., Tian, Y. and Zheng, B. (2015). Nutritional composition, physiological functions and processing of lotus (*Nelumbo nucifera* Gaertn.) seeds: a review. *Phytochemical Review*, 14, 321-334. https://doi.org/10.1007/s11101-015-9401-9

Zheng, M., You, Q., Lin, Y., Lan, F., Luo, M., Zeng, H. and Zhang, Y. (2019). Effect of guar gum on the physicochemical properties and *in vitro* digestibility of lotus seed starch. *Food Chemistry*, 272, 286–291. https://doi.org/10.1016/j.foodchem.2018.08.029

https://doi.org/10.26656/fr.2017.7(5).903