

Physicochemical characterization of jack bean (*Canavalia ensiformis*) tempeh

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Abstract

The objective of our research was to investigate the properties of jack bean tempeh during fermentation with two different packaging types, banana leaves and LDPE (Low-Density Poly Ethylene) Plastic. After 24 hrs mycelia of *Rhizopus* has not been formed, but it fully formed after 36 hrs fermentation for all formulated tempeh with two different packaging. The mycelia started the change to form brownish colour after 36 hrs of fermentation. Solid state fermentation (SSF) for 0, 24, 36, 48 and 60 h showed influence in different parameter e.g. total protein, the colour parameter (L, a, b, C, Hue, and ΔE) and pH during fermentation ($p < 0.05$). Optimum condition of tempeh was chosen after 36 hrs fermentation that showed the influence of different packaging type to the lipid content and antioxidant activity ($p < 0.05$), but no significant different to the water content, ash content, protein content, dietary fiber content and carbohydrate by the different content of tempeh ($p > 0.05$). In the present research work, we demonstrated that different fermentation time and packaging will give influence to several physicochemical characteristics including total soluble protein, colour, and pH.

1. Introduction

Tempeh is an Indonesian staple food produced through a solid-state fungal fermentation of legumes, resulting to a mycelia-knitted compact cake of beans (Gibbs *et al.*, 2004). The key microorganism leading the process of tempeh production is a fungus from the *Rhizopus* genus family, which digests the raw materials; thereby increasing its nutritional value for human consumption (Cuevas-Rodríguez *et al.*, 2006; Azeke *et al.*, 2007).

In Indonesian, although tempeh is mostly made from soybeans, it can also be produced from a wide variety of legumes, one of them is jack bean (*Canavalia ensiformis*). While the use of jack bean for making tempeh is still restricted to few places, especially the Yogyakarta region of Central Java province, it is becoming a potential substitute for soybean across Indonesia, because there is evidence that jack bean tempeh resembles soybean tempeh in many aspects, including color, flavor, texture and overall acceptability (Widaningrum *et al.*, 2012). Additionally, jack bean has potential medicinal value. For example, the methanolic extract of jack bean seeds contains acceptable levels of

free phenolics with promising antioxidant and type II diabetes-related enzyme inhibition properties (Vadivel *et al.*, 2012). Moreover, the seed decoction or powdered seeds from jack bean are used as an antibiotic and antiseptic. Furthermore, urease-derived peptide from jack bean represents a new example of the membrane-active peptide with insecticidal and fungi toxic activities (Martinelli *et al.*, 2014). Therefore, a further step towards encouraging the idea that jack bean is a suitable ingredient for tempeh production would be to determine its physicochemical characteristic and functional value. Indeed, several investigations have shown that fermentation especially solid-state fermentation during tempeh production can decrease the physicochemical characteristic and functional value of tempeh, including reduction of value e.g. phytic acid and substance that can cause flatulence (Eklund-Jonsson *et al.*, 2006; Cuevas-Rodríguez *et al.*, 2006; Azeke *et al.*, 2007; Duli and Starzy, 2016). The objective of this study was to investigate the physicochemical characteristic and functional property of jack bean tempeh, by examining the solid-state fermentation of jack bean tempeh under different packaging material (banana leaves and plastic packaging) and fermentation time (0, 24, 36, 48 and 60 hrs).

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2. Materials and methods

2.1 Material

Jack bean (*C. ensiformis*) seeds (white color seed coat) were collected from Playen District, GunungKidul, Yogyakarta by the cooperation with UST (Universitas Sarjanawiyata Taman Siswa), Yogyakarta. Commercial tempeh culture was *R. oligosporus* from Raprima Cultures (Indonesia). All chemicals were from Merck, Darmstadt, Germany. The packaging of tempeh e.g. banana leaves and plastic were purchased in a local market.

2.2 Tempeh production

The tempeh was made by the addition of starter tempeh (commercial tempeh culture. The tempeh was prepared in the laboratory according to the procedure described by Kustyawati *et al.* (2017) as follows: First, the jack beans (300 g) were washed and soaked in clean tap water for 1 hr at room temperature (28°C), and then boiled for 30 mins; the ratio of water to soybean was 3:1. This was followed by dehulling to remove the jack bean skin from the cotyledon manually, and another boiling for 30 mins. Subsequently, the boiled jack beans were drained, air-dried, inoculated with 0.02 g ragi for every 100 g cooked jack beans, and packed into the perforated banana leaves and plastic bags, then incubated for 0, 24, 36, 48 and 60 hrs at 32°C. All samples were prepared in duplicates according to Kustyawati *et al.*, (2017).

2.3 Colour

The colour was measured with Chroma Meter (Minolta CR 400, Japan). The instrument was calibrated with the white calibration plate before the measurement. The internal color (International Commission on Illumination L*, a*, b*) of samples were measured. To determine differences in color values to visually perceived differences, the calculation of ΔE was made using the method of Hidayat *et al.* (2018).

2.4 Total Soluble Protein

The soluble protein content was measured by following the Modified Lowry's method (Lowry *et al.*, 1951).

2.5 pH

Five grams (5 g) of tempeh were homogenized in 50 mL of water, and the filtrate was obtained using Whatman filter paper. Then the pH was measured using a pH meter that has been calibrated with buffer pH 4 and pH 7

2.6 Proximate analysis

Moisture, ash, protein, and fat contents were

determined according to the reference methods described by AOAC (AOAC, 1998). Moisture content was determined by oven drying at 105°C until constant weight was obtained. Crude fat was determined by the Soxhlet method using ether extraction (AOAC method 4.5.05). Crude protein was analyzed by the Kjeldahl method (AOAC method 960.52). Ash was quantified after burning at 550°C for 20 hrs (AOAC method 942.05). The total dietary fiber content (TDF) was evaluated by an enzymatic-gravimetric method (Leão *et al.*, 2017)

2.7 Antioxidant activity

The radical scavenging activity against DPPH of the methanolic extract was analyzed for measuring total antioxidant, according to Vadivel *et al.* (2012) and Kumar *et al.* (2018). Briefly, methanolic extract sample (0.1 mL) was added to 3.9 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (6×10^{-5} mol/L) in methanol and incubated for 30 mins at room temperature ($30 \pm 1^\circ\text{C}$); then absorbance was measured at 515 nm. DPPH solution was used as the control and methanol was used as the blank.

2.8 Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear model in Minitab 17 Statistical Software (Minitab Inc., State College, PA, USA) and Turkey's multiple comparison tests for the parameters of color (L, a, b, C, Hue), total soluble protein, pH, proximate analysis, dietary fiber and antioxidant activity. $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Effect during fermentation time and packaging type on mycelia formation

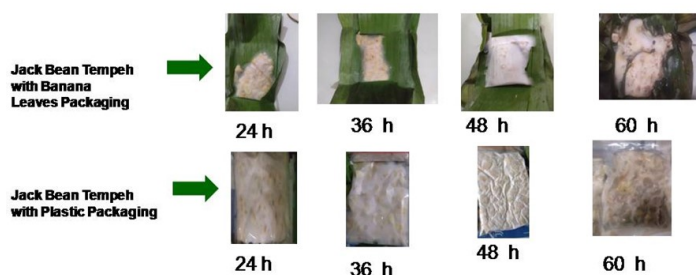


Figure 1. Tempeh after different time of fermentation with two different packaging type

Figure 1 represents the structural appearance of jack bean during fermentation with two different packaging materials and four-time regimes, while Table 1 depicts changes in physiochemical elements resulting from both packaging and incubation time. Accordingly, the growth of mycelia in different packaging showed that tempeh

Table 1. Physicochemical change of tempeh during fermentation time with different packaging type

Parameter	Packaging Type	Fermentation Time (hours)				
		0	24	36	48	60
Total Soluble Protein (g/mL)	Banana Leaves	0.02±0.01 ^{ab}	0.03±0.01 ^{aAB}	0.04±0.01 ^{aAB}	0.03±0.00 ^{aA}	0.03±0.00 ^{ab}
	Plastic	0.02±0.00 ^{ab}	0.03±0.00 ^{aAB}	0.03±0.00 ^{aAB}	0.07±0.00 ^{aA}	0.02±0.00 ^{ab}
Color						
L (Lightness)	Banana Leaves	75.2±0.14 ^{aCD}	71.88±0.01 ^{aD}	79.35±0.01 ^{aAB}	81.45±0.03 ^{aA}	72.26±0.02 ^{aBC}
	Plastic	75.88±0.00 ^{aCD}	70.18±0.00 ^{aD}	81.45±0.00 ^{aAB}	80.35±0.00 ^{aA}	80.26±0.00 ^{aBC}
a	Banana Leaves	1.77±0.00 ^{aC}	1.95±0.01 ^{ab}	4.41±0.01 ^{aA}	4.45±0.00 ^{aA}	4.37±0.00 ^{aA}
	Plastic	1.77±0.00 ^{bc}	3.12±0.00 ^{bb}	4.31±0.00 ^{ba}	4.79±0.00 ^{ba}	4.37±0.00 ^{ba}
b	Banana Leaves	12.53±0.00 ^{aB}	17.65±0.01 ^{aA}	6.04±0.01 ^{aCD}	7.02±0.00 ^{aC}	3.46±0.00 ^{aD}
	Plastic	10.46±0.00 ^{bb}	12.33±0.00 ^{ba}	5.24±0.01 ^{bCD}	5.2±0.01 ^{bc}	3.46±0.01 ^{bd}
C	Banana Leaves	12.65±0.00 ^{aB}	17.76±0.00 ^{aA}	7.48±0.00 ^{aC}	8.31±0.01 ^{aC}	5.57±0.03 ^{aC}
	Plastic	10.61±0.01 ^{bb}	12.72±0.01 ^{ba}	6.78±0.01 ^{bc}	7.07±0.01 ^{bc}	5.57±0.01 ^{bc}
Hue	Banana Leaves	1.43±0.00 ^{aA}	1.46±0.00 ^{aA}	0.94±0.00 ^{ab}	1.01±0.00 ^{ab}	0.67±0.00 ^{aC}
	Plastic	1.4±0.01 ^{ba}	1.32±0.01 ^{ba}	0.88±0.01 ^{bb}	0.83±0.01 ^{bb}	0.67±0.01 ^{bc}
ΔE	Banana Leaves	0±0.00 ^{aD}	6.11±0.00 ^{aC}	15.47±0.00 ^{ab}	15.01±0.00 ^{aA}	18.02±0.00 ^{aA}
	Plastic	0±0.01 ^{bd}	6.11±0.01 ^{bc}	16.09±0.01 ^{bb}	16.22±0.01 ^{ba}	18.01±0.01 ^{ba}
pH	Banana Leaves	6.34±0.00 ^{ab}	6.26±0.00 ^{ab}	6.16±0.00 ^{ab}	6.34±0.00 ^{ab}	7.76±0.00 ^{aA}
	Plastic	6.18±0.00 ^{ab}	6.39±0.00 ^{ab}	6.40±0.00 ^{ab}	6.59±0.00 ^{ab}	7.53±0.00 ^{aA}

Means with small alphabet superscripts within columns, and those with capital alphabet superscripts within rows are significantly different ($p < 0.05$).

packed with plastic had more compact mycelia than tempeh packed with banana leaves. Different packaging type could influence the growth of mycelia (Sukasih and Purwani, 2015). With respect to fermentation time, formation of brown mycelia started to appear after 36 hrs regardless of the type of packaging material used, which is consistent with the reports by other researchers (Sanberg *et al.*, 2006; Angulo-Bejarano *et al.*, 2008; Azeke *et al.*, 2007; Duli and Starzy, 2016).

3.2 Effect during fermentation time and packaging type on physicochemical parameters

3.2.1 Total soluble protein

Total soluble protein content in jack bean tempeh showed no significant ($p > 0.05$) difference between different type of packaging used, although there was significant ($p > 0.05$) difference with respect to fermentation time (Table 1). Similar findings have been reported on nutritional parameters and the in vitro protein and carbohydrates bioavailability in cooked quinoa seeds (Duli and Starzy, 2016). Also, the amino nitrogen concentrations showed a considerable increase between fermentation during tempeh processing, indicating the degradation of peptide bonds by microbial proteases, without large differences between the starters used (Moa *et al.*, 2013).

3.2.2 pH

During fermentation, although pH values changed a little with packaging materials, they increased

significantly ($p > 0.05$) with time to over 7 after 60 hrs (Table 1). Indeed, it's well known that fermentation also causes changes in some organic acids such as acetic acid, oxalic acid, citric acid and succinic acid, which will influence the pH, as observed on soybean fermentation using *R. oligosporus* starter (Vong *et al.*, 2018) or *Rhizopus* starter (Moa *et al.*, 2013).

3.2.3 Color

Based on the data in Figure 1, L value showed no significant difference ($p > 0.05$) in the packaging influence on the lightness of the sample, whereas the values changed significantly ($p > 0.05$) over the course of the fermentation process (Table 1). On the other hand, a, b, C, Hue and ΔE values showed significant ($p > 0.05$) difference with both packaging material and fermentation time (Table 1).

3.2.4 Water, ash, protein, lipid, carbohydrates, antioxidants and dietary fiber

Table 2 shows the moisture, ash, protein, lipid, carbohydrates, antioxidants and dietary fiber contents of jack bean tempeh recovered after 36 hrs fermentation with banana or plastic packaging. It is evident that lipids and antioxidants were affected by the packaging; lipid values were significantly higher ($p > 0.05$) with banana leaves, while antioxidant values were high with plastic packing. We assume that higher lipase activity could be responsible for the increase in the amount of lipid tempeh packed with plastic, while the increase in

Table 2. Proximate analysis and antioxidant activity of tempeh after 36 hrs fermentation

Packaging type	Water (%)	Ash (%)	Protein (%)	Lipid (%)	Carbohydrate (%)	Antioxidant activity (%)	Dietary fiber (%)
Plastic	62.49±0.08 ^a	0.94±0.01 ^a	9.86±0.16 ^a	1.62±0.06 ^b	25.1±0.30 ^a	3.59±0.07 ^b	0.79±0.04 ^a
Banana Leaves	61.63±0.45 ^a	0.97±0.03 ^a	9.64±0.24 ^a	1.75±0.01 ^a	26.02±0.66 ^a	2.53±0.15 ^a	0.75±0.01 ^a

Means with different superscript within the same column/parameter are significantly different ($p < 0.05$).

antioxidant activity could be due to increased activity of selected microbe enzyme, as demonstrated with fermented soybean by-product (Chan *et al.*, 2018). The other parameter, including water, ash, protein, carbohydrate and dietary fiber did show any no significant difference between tempeh packed with plastic or banana leaves.

4. Conclusion

In this study, we demonstrated that different fermentation time and packaging influenced several physiochemical characteristics, including total soluble protein, colour, and pH. The optimum condition for fermentation of jack bean tempeh was chosen after 36 hrs, and the harvested tempeh packed with plastic contains high levels of lipids, whereas tempeh packed with banana leaves contains high levels of antioxidants, indicating that lipid-rich jack bean tempeh and antioxidant-rich tempeh can be produced using banana leaves packaging and plastic packaging, respectively.

Conflict of Interest

The authors declare no conflict of interest in the manuscript.

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