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FULL PAPER

Development of instant vegetarian cream soup from tempeh powder to increase antioxidant capacities

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Abstract

Tempeh is an Indonesian traditional soyfood made by *Rhizopus* spp. fermentation. Tempeh has been well known as a vegetarian-friendly food and has been processed into various derived food products. This study aimed to develop an instant vegetarian cream soup from tempeh powder. The addition of tempeh powder was expected to increase the product's antioxidant activity without compromising its physicochemical properties as an instant product. The five different formulations of cream soups containing 0–30% tempeh powder were analysed for their physicochemical properties (dissolving time, density, viscosity, colour, pH), antioxidant capacity (total phenolic content, total flavonoid content, DPPH radical scavenging activity, reducing power, and ferrous ion chelating ability), and hedonic test. Soup cream made with more tempeh power had a higher dissolving time, but lower viscosity, lightness value, and pH value. However, their antioxidant activities also increased with the increase of tempeh powder added. From the hedonic analysis, the formulation with 15% tempeh powder showed the highest hedonic rate and therefore, was chosen as the best formulation. In conclusion, the addition of tempeh powder did not only give a better consumer acceptance but also higher antioxidant activities.

1. Introduction

Tempeh is a traditional fermented soyfood from Indonesia that takes an integral part in the food culture among Indonesians (Handajani *et al*., 2020). It is commercially sold in a cake form or slab (Malav *et al*., 2015). Tempeh is an example of a solid-state fermentation product using *Rhizopus* spp. as a fermentation starter. The fermentation process of tempeh is known to increase the flavour, taste, and nutrition of soybean (Gunawan-Puteri *et al*., 2015). Soybeans are the most common basic ingredients used to produce tempeh, apart from other legumes such as chickpeas (Abu-Salem and Abaou-Arab, 2011), common beans (*Phaseolus vulgaris*) (Starzynska-Janiszewska *et al.,* 2014), *mlanding* or *Leucaena leucocephala* (Ishartani *et al.,* 2019), mungbeans, kidney beans, and jojoba seeds (Abu-Salem *et al.,* 2014). Tempeh has been associated with many health benefits that make it considered a potential functional food (Ishartani *et al.,* 2019).

Tempeh is believed to provide higher nutritional values compared to soybeans since it is more easily digested than soybeans due to the fermentation taking place (Stephanie *et al.,* 2017). During tempeh fermentation, the antioxidant activity increases due to fungal activities transforming isoflavone glucosides into aglycones. Indeed, such compounds contribute the most to the antioxidant activities of tempeh. Studies have shown that tempeh had a higher antioxidant activity (Ahmad *et al.,* 2015; Barus *et al.,* 2019) and demonstrated a higher chelating ability compared to soybeans (Ahmad *et al.,* 2015). Isoflavones in tempeh have been shown to lower cellular ROS and upregulate the expression of antioxidant enzymes (Surya *et al.*, 2021). Other studies also showed that tempeh extract protected HepG2 cells from TBHP-induced oxidative stress *in vitro* (Surya and Romulo, 2020).

Besides all the health benefits of tempeh, studies regarding the use of tempeh as an ingredient in processed food products are still limited. Some studies have shown the possibility of using tempeh in the production of chips, tempeh milk powder, yoghurt, tempeh cider drinks, tempeh ice cream and sports drink (Dewayani *et al.,* 2020). All these studies have come to the common conclusion that tempeh could be used as a basic ingredient in the development of new food products. However, using tempeh as a based product without

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adding a flavour component would render the product unpleasant to consume. Therefore, food products derived from tempeh often require other ingredients that would increase their sensory characteristics (Dewayani *et al.,* 2020). Vegetarian cream soup is an example of a trending food product in Indonesia with increasing market demand. In this study, we analysed the use of tempeh as a substitute for maltodextrin in the production of vegetarian cream soup in order to increase the antioxidant activity of the cream soup. We aimed at diversity in the use of tempeh as a commercial functional food.

2. Materials and methods

2.1 Sample preparation

2.1.1 Tempeh powder preparation

In making tempeh, soybeans are used as the main ingredient. At the beginning of processing, non-GMO soybeans (SB&B, USA) were soaked for 4 hrs. After soaking, the soybeans are washed and then peeled. After peeling, the soybean skin is then separated from the soybean seeds. Soybean seeds that have been cleaned from the skin then being washed. After washing, the soybean seeds were then cooked for 30 mins using a 1% lactic acid solution. After cooking, the soybeans were drained and allowed to stand for 30 mins until the temperature of the soybean seeds reached 30°C. The *Rhizopus* spp., the starter, was then inoculated into soybean seeds. The starter is stirred until it is mixed properly. Then after stirring, the inoculated soybean seeds will be put into PP plastic and shaped according to the desired shape. The plastic that has been filled with inoculated soybean seeds will then be perforated with a tiny hole. After being perforated, soybean seeds will be fermented using an incubator at 35°C with a fermentation time of 48 hrs. After the tempeh has been fermented, the tempeh is cut and then dried using an oven at 50°C for 24 hrs then it will be blended into a powder.

2.1.2 Cream soup preparation

In the preparation of cream soup, tempeh powder will be used as one of the main ingredients of the product. Tempeh powder will be mixed with other ingredients such as maltodextrin, creamer powder, sugar, salt, corn starch, yeast extract, and spices. All ingredients were then mixed thoroughly. The ratio of tempeh powder with maltodextrin was 0: 100 for Formulation A, 10: 90 for Formulation B, 15: 85 for Formulation C, 20: 80 for Formulation D and 30: 70 for Formulation E.

2.2 Antioxidant analysis

2.2.1 Total phenolic

In total phenolic analysis, 1 mL of the sample will be mixed with 5 mL of Folin-Ciocalteu reagent. After mixing, the solution was then allowed to stand for 5 mins. After being allowed to stand for 5 mins, the solution was then added with 4 mL of 7.5% Na_2CO_3 and left in a dark room for 2 hrs at room temperature. After being left at room temperature for 2 hrs, the absorbance of the solution was measured using a UV-Vis spectrophotometer where the wavelength used was 765 nm. Gallic acid will be used as a standard in total phenolic analysis (Murad *et al.*, 2013). The concentrations of gallic acid solution used in the experiment were 0 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm.

2.2.2 Total flavonoid

Total flavonoid analysis was carried out according to Azizah *et al.* (2014) by mixing 0.5 mL of the sample into 1.5 mL of 95% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COOK, and 2.8 mL of distilled water in a sealed test tube that had been wrapped using aluminium foil. The mixed solution was then vortexed until homogeneous and incubated for 30 mins at room temperature. The incubated solution was then measured for its absorbance at a wavelength of 415 nm on a UV-Vis spectrophotometer. In the total flavonoid analysis, Quercetin was used as the standard.

2.2.3 Free radical scavenging assay (DPPH)

DPPH analysis will be carried out according to Barus *et al.* (2019) with modification, where 1 mL of the sample or filtrate was put into a test tube and then 0.2 mM of 2 mL of DPPH and 7 mL of ethanol were added. The solution was then vortexed until homogeneous and left in a dark room for 30 mins. Ethanol will be used as a negative control. Then after being allowed to stand for 30 mins, the absorbance of the solution will be measured using a UV-Vis spectrophotometer with a wavelength of 517 nm.

$$\text{Inhibition activity} = (\text{Abs.}_{\text{control}}-\text{Abs.}_{\text{sample}})/(\text{Abs.}_{\text{control}}) \times 100\%$$

2.2.4 Reducing power

Reducing power analysis will be carried out according to Canabady-Rochelle *et al.* (2015) with modification, where 0.7 mL of sample extract was added to 0.7 mL of 0.2M phosphate buffer pH 6.6. After that, 0.35 mL of 1% $K_3[Fe(CN)_6]$ was added to the mixture and then incubated at 50°C for 20 mins. Then after incubation, 1.35 mL of distilled water, 0.33 mL of 10%

TCA, and 0.27 mL of 0.1% $FeCl_3$ were added to the incubated solution. After that, the solution was then allowed to stand for 10 mins at room temperature. Then after being allowed to stand for 10 mins, the absorbance of the solution will be measured using a UV-Vis spectrophotometer with a wavelength of 700 nm. Ascorbic acid will be used as standard.

2.2.5 Ferrous ion chelating

Ferrous ion chelating analysis will be carried out according to Chai *et al*. (2014) with modification, where 7.5 L of 2 mM $FeCl_2$ was added to 277.5 L of sample solution. Then after adding $FeCl_2$ solution, the sample was then incubated for 3 mins at room temperature. After the incubation period, 15 L of 5 mM ferrozine solution was added. After 10 mins after adding the solution, the absorbance of the solution was then measured using a UV-Vis spectrophotometer with a wavelength of 560 nm. In the analysis of ferrous ion chelating Ethylenediaminetetraacetic acid (EDTA) was used as a control. A decrease in absorbance indicates an increase in chelating capacity.

2.3 Physical analysis

In physical analysis, water content was measured according to AOAC (2005), colour analysis was analysed using an NH310 colourimeter, density using pycnometer, pH on samples was measured using PH 700 EUTECH, dissolving time was measured using a stopwatch, and viscosity was measured with Brookfield DVE-RV viscometer using spindle number 2.

2.4 Sensory analysis

Hedonic analysis was carried out on the attributes of taste, colour, aroma, texture, the flavour also overall using a 7-point scale where 1 - very dislike and 7 - very like the product. The panellists will be asked for their opinion about their level of preference for the product in the form of a hedonic scale. This will be displayed in the form of numbers so that it can be analysed further (Tarwendah, 2017). After the data was obtained, the data was then processed using statistical analysis, namely One -Way ANOVA and the Post-Hoc Duncan Test.

3. Results and discussion

In this study, five different product formulations were applied by varying the concentration of maltodextrin and tempeh powder. Tempeh powder was used as a substitute for maltodextrin, ranging from 0% to 30% of tempeh powder. Physical analysis was conducted on different parameters such as colour analysis, viscosity, pH, dissolving time, water content, and density of the instant cream soup product (Tables 1 and 2). Colour is one of the factors that affect the sensory of a product. The colour analysis used in this research is CIE or *Comission Internationale de I'Eclairage*. The addition of tempeh powder changed the colour into more yellowish red and reduced the lightness of the cream soup (Figure 1). The change in colour was mainly due to the Maillard browning reaction taking place during the heat-involving tempeh powder production (Witono *et al*., 2015).

Table 1. Colour of cream soup with different formulations of tempeh powder

Sample	L	a	b
Formulation A	$88.14±2.15^d$	$1.40±0.60^a$	$19.56±1.98^a$
Formulation B	$76.48±0.41^c$	$1.98±0.25^a$	$20.17±1.25^a$
Formulation C	$70.51±0.48^b$	$4.45±0.03^b$	$22.10±0.03^b$
Formulation D	$69.24±0.31^b$	$4.59±0.20^b$	$22.22±0.08^b$
Formulation E	$63.76±0.32^a$	$4.74±0.62^b$	$25.13±0.34^c$

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

The pH value of cream soup varied depending on the ingredients and process included in the product making. Studies have shown that different fungal cultures gave a different range of pH values in tempeh (Omosebi and Otunola, 2013). In this study, it was observed that the higher the concentration of tempeh powder used to substitute maltodextrin in the cream soup, the lower the pH in the final product. Indeed, the pH value of tempeh could range from 4.6 to 6.6 following a 46-hour fermentation (Ahnan-Winarno *et al*., 2021). The acidity of tempeh is mainly due to the boiling process of soybeans prior to fermentation, during which soybeans are boiled in lactic acid solution to support fungal growth.

The water content seemed to be higher in tempeh cream soup compared to the cream soup without tempeh.

Table 2. Water content and pH of cream soup with different formulations of tempeh powder

Sample	Water content (%)	pH	Dissolving time (s)	Density (g/mL)	Viscosity (Cp)
Formulation A	$5.26±0.043^a$	$6.55±0.02^d$	$5.11±0.93^a$	$1.07±0.01^a$	$174.4±4.08^c$
Formulation B	$5.35±0.12^{ab}$	$6.49±0.14^c$	$9.21±1.11^b$	$1.06±0.001^a$	$163.6±16.21^c$
Formulation C	$5.26±0.04^a$	$6.37±0.01^b$	$12.49±0.58^c$	$1.06±0.003^a$	$140.4±21.64^b$
Formulation D	$5.44±0.01^b$	$6.38±0.03^b$	$14.27±1.062^d$	$1.06±0.004^a$	$104.9±5.49^a$
Formulation E	$5.26±0.14^a$	$6.15±0.03^a$	$16.85±1.38^e$	$1.06±0.007^a$	$108.2±6.45^a$

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

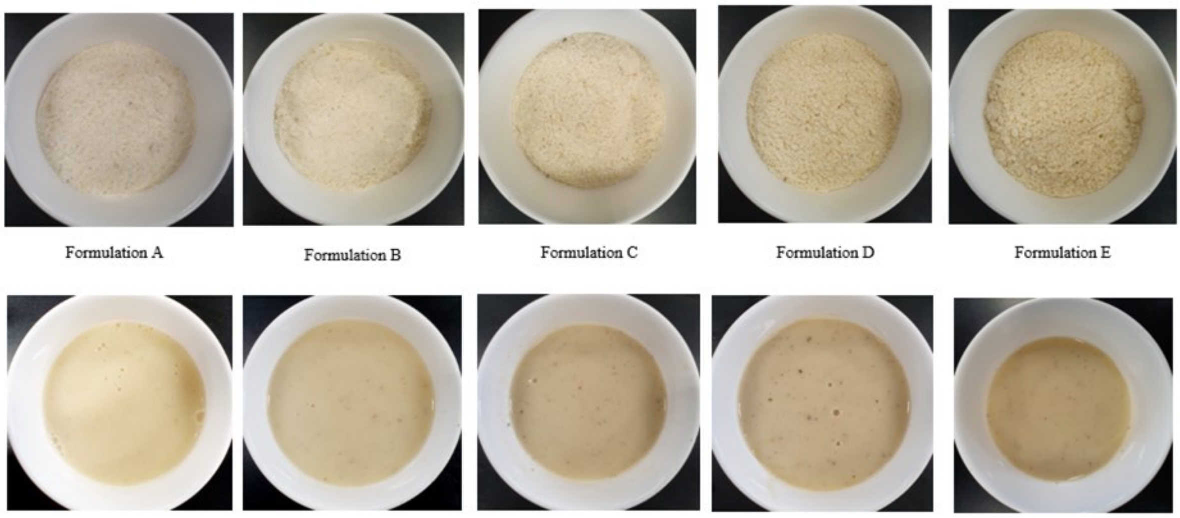

Figure 1. The appearance of powder (upper panel) and hydrated (lower panel) forms of different tempeh-based cream soup formulations

Such a difference could be explained by the fact that maltodextrin has a lower moisture content compared to tempeh powder, ranging from 2.82% to 6.47% (Yakeiti *et al*., 2010). However, since the moisture content of tempeh cream soup was less than 10%, it was still considered a dried product (Sunyoto *et al*., 2018).

The increase in dissolving time was demonstrated to be positively correlated with the higher amount of tempeh powder used to substitute maltodextrin in tempeh cream soup. This may be due to the protein inside the tempeh powder tending to be less soluble when it is denatured. In addition, the gelatinization of starch in tempeh also plays a role in increasing the dissolving time. Thus, the longer steaming time, drying time, and the higher temperature used to dry the tempeh will lower the solubility of tempeh powder (Reyes-Bastidas *et al*., 2010; Wijayanti *et al*., 2020).

There was no significant difference between all formulae regarding the density. The density of cream soup was higher than water due to the presence of solutes in the cream soup. The viscosity of cream soup was found to be higher in the formulae with less tempeh powder. In this formulae, viscosity was mainly determined by gelatinized starch and maltodextrin which became highly soluble in water and formed a viscous matrix. In the formulation, corn starch is known to contain a higher proportion of amylopectin/amylose was used (Ramesh *et al*., 2018).

Total phenolic compounds in the cream soup increased significantly with the addition of tempeh powder in the formulation (Table 3). The phenolic compounds in tempeh were isoflavones that are known to exert antioxidant properties. Isoflavones have been reported to prevent chronic illnesses, such as cancer, hypercholesterolemia, and osteoporosis (Kuligowski *et al*., 2017). Isoflavones exist mainly in two forms: glucosides (isoflavones attached chemically to sugar molecules) and aglycones (free isoflavones) with higher bioavailability. Isoflavones in soybeans are mostly present in the form of glucosides. During tempeh fermentation, *Rhizopus* spp. produces a beta-glucosidase enzyme that transforms isoflavone glucosides into aglycones with higher antioxidant activities (Ahmad *et al*., 2015). Such a phenomenon could also explain the higher antioxidant activities observed in the formulae with higher tempeh powder (Table 4). Previous studies have also reported that tempeh exerted higher antioxidant activities (Ahmad *et al*., 2015; Barus *et al*., 2019) and a higher chelating ability (Ahmad *et al*., 2015) compared to soybeans.

Table 3. Phenolic compounds of cream soup with different formulations of tempeh powder

Sample	Total Phenolic (µg GAE/mL sample)	Total Flavonoid (µg QE/mL sample)
Formulation A	64.78±2.26^{a}	18.48±2.70^{a}
Formulation B	78.81±3.72^{b}	37.40±4.71^{b}
Formulation C	94.74±9.54^{c}	57.86±2.11^{c}
Formulation D	115.96±5.08^{d}	79.55±12.49^{d}
Formulation E	149.26±9.38^{e}	97.02±3.34^{e}

Values are presented as mean±SD. Values with different superscript within the same column are significantly different (p<0.05)

The hedonic analysis revealed that formulae C had the highest overall rate among other formulae (Table 5). In formulae C, 15% of the maltodextrin was substituted by tempeh powder. In terms of flavour, it was noted that tempeh powder also contributed to a slightly pungent smell and bitter taste. Therefore, a higher substitution rate of tempeh extract did not lead to a higher consumer acceptance. Besides, the higher the substitution rate of tempeh powder in the formulation, the lower the viscosity of the cream soup. Our panellists expressed that formulae A and B were too thick while formulae D and

Table 4. Antioxidant activities of cream soup with different formulations of tempeh powder

Sample	DPPH radical scavenging activity (%)	Reducing power (µg AAE/ mL sample)	Ferrous ion chelating (%)
Formulation A	17.44±5.11[a]	76.97±3.33[a]	0.38±0.38[a]
Formulation B	22.35±1.83[b]	118.62±3.64[b]	6.11±0.61[b]
Formulation C	24.79±1.27[b]	124.31±2.52[c]	8.40±0.86[c]
Formulation D	31.41±0.52[c]	131.46±2.07[d]	10.38±0.93[d]
Formulation E	33.13±0.80[c]	147.90±2.83[e]	20.78±0.75[e]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different (p<0.05).

Table 5. Sensory evaluation of cream soup with different formulations of tempeh powder

Sample	Aroma	Colour	Flavour	Texture	Overall
Formulation A	5.26±1.21[ab]	5.58±1.23[a]	5.16±1.29[b]	5.39±1.09[ab]	5.45±1.09[bc]
Formulation B	4.77±1.23[a]	5.26±1.44[a]	4.58±1.39[ab]	5.03±1.40[a]	4.94±1.21[ab]
Formulation C	5.55±1.06[b]	5.48±1.03[a]	5.97±1.02[c]	5.84±1.10[b]	5.61±1.09[c]
Formulation D	5.23±1.36[ab]	5.19±1.60[a]	5.00±1.21[b]	5.19±1.38[ab]	5.00±1.10[bc]
Formulation E	5.26±1.37[ab]	5.55±1.43[a]	4.03±1.66[a]	5.23±1.33[ab]	4.35±1.47[a]

Values are presented as mean±SD. Values with different superscript within the same column are significantly different (p<0.05).

E were too thin. Formulae C was considered to have the right thickness and viscosity.

Taken together, the results showed that tempeh could be used in the production of vegetarian cream soup. The addition of tempeh powder in the cream soup formulae could be considered a strategy to create a tempeh-based functional food. The use of tempeh could bring health benefits, including an increase in the antioxidant activities of the product. In addition, the presence of tempeh powder in the cream soup formulae could also improve the sensory value of the product.

Conflict of interest

The authors declare no conflict of interest.

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