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Overripe tempe as source of protein in development of ready to eat porridge

[1*]Puteri, M. D. P. T. G., [1]Fortunata, S. A., [1,2]Prabawati, E. K., [1]Kristianti, F. and [3]Wijaya, C. H.

[1]*Department of Food Technology, Faculty of Life Sciences and Technology, Swiss German University, Tangerang 15143, Indonesia*
[2]*School of Agriculture and Food Sciences, University of Queensland, St. Lucia, 4072, Australia*
[3]*Study Program Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Indonesia*

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Abstract

Recent study and local wisdom had shown the potencies of overripe tempe as source of umami flavor due to its high glutamic acid content. Despite the popularity of tempe as low cost high quality plant-based protein source, overripe tempe potencies as source of protein have not yet been explored. The protein profile development showed that overripe tempe is a promising source of protein and might even have better protein utilization than its original tempe and soybean. Therefore in this research the utilization of overripe tempe as part of food for school age children, more specifically as ingredient for ready to eat porridge was investigated. Target of the product was to fulfill at least one third of RDI of protein age 1-3 years old per serving with higher acceptance based on taste. Ratio of stock to rice used and also heating time required in the production of porridge, as well as formula adjustment were determined using sensory evaluation employing trained panelist in series of focus group discussion to describe the sensory character and acceptance of the porridge. Selected porridges were evaluated against commercial products, for their hedonic sensory acceptance, employing pre-screened naive panelist. The resulted porridge was described to have texture similar to porridge with no rice-like form remained and had dominant umami taste. One packaging containing 200 g of instant porridge was able to contribute to 48% RDI of protein of 1-3 years children. Additionally, the selected product had better overall acceptance in comparison to commercial product.

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Introduction

Stunting due to lack of protein consumption and poverty is an important issue in developing countries including Indonesia. And search for new source of low cost high quality protein ingredient isrequired to support infant and toddler growth, a period where dietary protein is highly required to assist maintenance and formation of tissue. Overripe tempe, fermented soybean with longer solid state fermentation period than tempe, is a common flavoring ingredients in Indonesian traditional cuisine, traditionally used as medication to increase appetite, and also commonly eat as children snack (Yudianto, 1997). Tempe has been known as a superior plant-based protein source. Prior study had shown that longer mould activity in tempe processing improve the protein quality and reduce anti-nutrients content (Abu-Salem *et al.*, 2014). Therefore prolonged fermentation in overripe tempe may support its development as protein source ingredient that may support children growth in developing country, especially those in lower economic group.

Recent studies have also showed the higher glutamic acid composition in overripe tempe in comparison to its previous form of tempe and soybean (Gunawan-Puteri *et al.*, 2015) and the waters soluble extracts of overripe tempe also shown higher umami taste activity value coming from its hydrophilic components (Utami *et al.*, 2016). However, despite of its historical and scientific support for overripe tempe utilization as flavoring agent (Yudianto, 1997; Setiadharma *et al.*, 2015; Gunawan-Puteri *et al.*, 2017), there were still scarce discussion in its potencies as source of protein. Review by Astuti *et al.* (2000) and Babu *et al.* (2009) highlighted the

*Corresponding author.
Email: maria.gunawanputeri@sgu.ac.id

biochemical changes during tempe fermentation that increase the nutritional value and the potencies of tempe as low-cost nutrient source. Amongst other, the review mentioned the better protein quality, lower phytic acid content, and the conversion of complex into simple carbohydrate are those supporting factors that may play roles in better digestion and nutrient absorption of tempe in comparison to its original soybean. Tamang (2015) and Roubos-van den Hil *et al.* (2010) also showed that soybean tempe contained arabinose that has beneficial effect in controlling the enterotoxigenic Escherichia coli inducing diarrhea and therefore might play role as protective ingredient in children food. Longer fermentation in overripe tempe also had shown to reduce the total coliform number even more (Hassanein *et al.*, 2015)

Indonesia is among the watched countries for malnutrition and subsequent stunting in the country reach 27.5% of the children population (National Institute of Health Research and Development, 2016) and the condition was even more severe in urban area as the number reach up to 42% of the children population (National Institute of Health Research and Development, 2013). In urban area, per capita monthly expenditure is dominated by non-food (55.43%) leaving on average only 460,639 Indonesian Rupiah (or about 30 USD) per month for food (Suhartini and Hakiki, 2016). Protein consumption are essential factors in promoting tissue maintenance and formation in children growth (Manary et. al., 2016), However, in compliance to the limited budget available for food, protein consumption of Indonesian children was recorded to be as low as only 11.5% of Recommended Dietary Allowances. Breakfast problem was also found to be one contributing factors in the insufficiency provision of children nutrition in Indonesia as 44.6% of primary school-aged children ate breakfast with low quality nutrients or even does not have breakfast at all (Khomsan *et al.*, 2005; National Institute of Health Research and Development, 2010; Aries *et al.*, 2012;). Review by Nicklas *et al.* (1998) emphasized the importance of breakfast in children nutrition, as missing breakfast or consuming an inadequate breakfast contributed to dietary shortages that were rarely compensated for at other meals. The study also showed that ready-to-eat breakfast cereal may have significant effect in nutritional intervention due to its ability to contain fortification of essential nutrition. And therefore, the formulation of high-protein food for children breakfast might become option in the national nutritional improvement of children.

Fortunata (2017) showed that on lower economy society, porridge as type of food and three times a day as feeding frequency had highest score as feeding pattern in children age 0-6 years old. The survey also showed that nutrition, usage of natural ingredients, and taste were the major attributes affecting the selection of children food. While utilization of overripe tempe might meet the expectation of "usage of natural ingredients", it is important to also meet the expected nutrition fulfillment and acceptable taste in the development of the children food product. In this research development of protein profile was evaluated during overripe tempe production. The overripe tempe was formulated into liquid stock and the stock was used to cook the raw rice into porridge inside retortable aluminium packaging to produce ready to eat porridge. The overripe tempe stock act as source of protein in the porridge and formulation was done to fulfill at least on third of RDI of protein age 1-6 and high score of taste acceptance.

Materials and methods

Materials

Soybean seeds as raw materials and Raprima® tempe starter used in producing overripe tempe were obtained in the local market Pasar Modern BSD City, Tangerang Selatan, Indonesia. Pancreatin from porcine pancreas was acquired from Sigma-Aldrich (Singapore) while other chemicals were analytical grade and acquired from Merck (Germany) unless stated otherwise. Rice, oil, and other seasonings for the formulation were bought from local supermarket Giant Alam Sutera, Tangerang, Indonesia.

Overripe tempe production

Overripe tempe was produced using method described in previous study (Hassanein *et al.*, 2015; Gunawan-Puteri *et al.*, 2015; Djunaidi *et al.*, 2017). The soybean was washed, sorted, soaked overnight (12 h), and then de-hulled. The de-hulled soybean was boiled for 30 min, drained, dried, and cooled down. Solid-state fermentation was started by the inoculation of Raprima® tempe starter with ratio 2 gs inoculum for each kilogram of soybean and take place at room temperature for about 24 h to obtain tempe pera (or half ripen tempe), 48 h to obtain tempe and 72 h further fermentation was done to obtain overripe tempe that has brown color (L* 52.96, a* 6.84, and b* 18.52), and 50.32 – 61.77 mg BSA eq/g dry base protein content and 357.39 – 418.78 mg tyrosine eq/g dry base soluble amino acid according to standard determined in previous study (Djunaidi *et al.*, 2017). Evaluation of the development of protein profile during tempe fermentation were observed from soybean, soaked bean, tempe *pera*, tempe, and up to overripe tempe.

Overripe tempe eolor evaluation

Colorimeter is applied to analyze the color of tempe as sample. Colorimeter was calibrated with white standard calibration kit that is connected to the application and the measurement was obtained. The output of the measurement was in L* which represents lightness, a* and b* represent color opponent direction; a* for green to red and b* for yellow to blue.

Protein profile evaluation

Sample extraction was done for protein, soluble amino acids and protein digestibility analysis with modification. The extraction of samples was started by freezing the samples in -20ºC and was dried using freeze dried for about 24 hours in order to facilitate the crushing and grinding process of samples. Water was used as solvent with ratio one to five (20 g samples in 100 ml distilled water). The extraction processed was done under room temperature (± 25ºC) with constant agitation for about 6 hours. The collected solutions were further to be centrifuged at 8000 rpm for 15 minutes to obtain supernatant. Sample extract was then stored in freezer under freezer temperature until further analysis.

Protein profile was shown as the value of total nitrogen content, oligopeptide content, soluble amino acid (SAA), and of the protein enzymatic hydrolysis rate. Total nitrogen was evaluated using Kjehdahl method (AOAC, 2001), oligopeptide content was measured in the principle of its reaction with Biuret and Folin-ciocalteu in comparison to Bovine Serum Albumin (BSA) standard curve. While SAA was evaluated in the principle of its reaction with Na_2CO_3 and Folin-ciocalteu in comparison to Tyrosine standard curve as described in Djunaidi *et al.* (2017). The protein enzymatic hydrolysis rate was calculated as the increment of SAA after pancreatin enzymatic digestion divided by the average oligopeptide content per gram samples. The result was analyzed statistically by ANOVA and continued with Tukey's Post-Hoc Analysis.

BSA standard curve was constructed using several concentration of BSA solution. A 0.3 ml of each concentration of BSA solution was placed in each test tube and was mixed homogeneously with 1.5 ml biuret reagent, incubated at room temperature for 10 minutes, and further incubated at room temperature for 30 minutes following the addition of 75 µl Folin-ciocalteu reagent. Absorbance at 650 nm of each concentration was plotted in Y-axis against the BSA concentration plotted in X-axis (Djunaidi *et al.*, 2017).

Tyrosine standard curve was constructed using several concentration of Tyrosine solution. A 0.25 ml was added with 0.25 ml water and 0.25 ml phosphate buffer pH 8. The mixture was incubated at room temperature for 5 minutes and then added with 0.75 ml of 10% TCA. The mixture (0.3 ml) was mixed homogenously with 1 ml of 0.5 M Na2CO3 and 0.2 ml Folin-ciocalteu reagent. Absorbance at 578 nm of each concentration was plotted in Y-axis against the Tyrosine concentration plotted in X-axis (Djunaidi *et al.*, 2017).

Formulation of overripe tempe porridge

Overripe tempe powder was produced using method described in previous study (Hassanein *et al.*, 2015; Gunawan-Puteri *et al.*, 2015). Overripe tempe was sliced thinly (2-3 mm) then spread into the tray and dried in the oven at 60°C for 6 h until the moisture content reach below 10%. The dried overripe tempe was then grounded using home-scale disk-mill (Ramesia, FCT - Z100) for 2 min (28,000 rpm, 100 g per batch). The resulted overripe tempe was then formulated with oil and seasonings into overripe tempe stock using previous research as reference of basic formula (Setiadharma *et al.*, 2015) with adjustment to fulfill 1/3 RDI of protein and create balanced-taste formula, which consist 29.36% overripe tempe powder 6.48% garlic, 2% pepper, 22.16% salt, 20% oil, and 20% caramel syrup. The resulted stock paste was further diluted into water (1:25) into stock solution that is used to cook the rice in the next step. Ready-to-eat overripe tempe porridge was produced by cooking grounded uncooked rice and overripe tempe stock that were placed inside retortable aluminium pouch using pressure cooker. There were 6 variations of stock to rice ratio (1:3, 1:4, 1:5, 1:6, 1:7, 1:8, w/v) and 3 variations of cooking time (10 min, 12 min, and 15 min), in total 18 variations were conducted. Formula was selected based on most preferred texture according to trained sensory panels and further taste adjustment for the taste balance of the overripe tempe porridge was conducted using focus group discussion session of the trained sensory panels to select the best balance. Best formula was compared to selected commercial product.

Sensory evaluation

Sensory evaluations by trained panelist were done in the taste adjustment of overripe tempe stock and ready-to-eat overripe tempe porridge. Prior to the analysis, the panelist went through 50 h training of sensory attributes and protocol, taste,

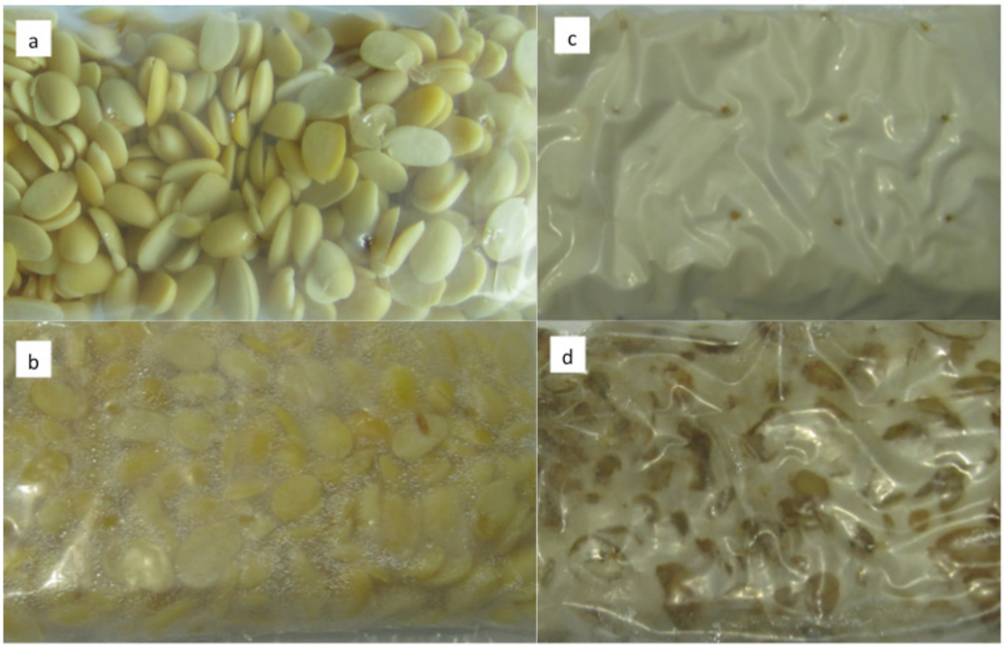

Figure 1. (a) Soaked and inoculated soy bean; (b) Tempe pero; (c) Tempe; (d) Overripe tempe

aroma, and flavor recognition and ranking, descriptor familiarization, vocabulary training, scale reference and evaluation, attribute generation, and descriptive scaling. During focus group discussion, the panelists were requested to generate attributes related to overripe tempe stock and porridge, to describe the samples according to the attributes, to define criteria for sample selection and also to select best samples based on the samples' description and criteria defined.

Hedonic sensory evaluation by naive panelist was done to compare the acceptance between four selected ready-to-eat overripe tempe porridge and one selected commercial product. There were five different sensory attributes that should be evaluated, which were appearance (color), aroma, taste, texture, and overall acceptance. The results were analyzed statistically by Friedman's Test and continued with Wilcoxon's Test. Forty naive panelist were selected based on criteria (1) mothers with children age 3-6 years old; (2) live in the urban area; (3) monthly income of less than 1,500,000 millions Indonesian Rupiah (or about 100 USD). Commercial product was selected from ready-to-eat or ready-to-serve porridge product for age 0-6 years old that received highest score on consumption frequency.

Results and discussion

Tempe solid state fermentation was initiated by the inoculation of commercial tempe starter. During fermentation process the mould grow and form white mycelium that covered and penetrated the bean to create compact cake commonly known as tempe. Based on the fermentation stages, two other variant of tempe is also known, which are tempe *pero* and overripe tempe (Figure 1). Tempe *pero* is term used for half fermented tempe that commonly formed at 24-36 h fermentation. The microbial growth is indicated with the condensation showed in the surface of the packaging and while the mycelium has not covered the whole bean, the mycelium growth has started to strengthen the bond between the beans, but was yet able to retain its shape when sliced. The overripe tempe is term used for tempe that undergone 96-120 h of further fermentation, where the bacterial fermentation override the mould as shown by shrinkage of the mould mycelium after it become tempe. This tempe is characterized by pungent odor, darker color, and softer texture. Handoyo and Morita (2006) stated that trend line color from tempe (48 h fermentation time) to overripe tempe (72 h fermentation time) is decreasing from light to dark. This phenomenon happens due to growth of mold where immature mold is shown in white color while mature mold is shown in darker color because mature mold produce lower enzymes than immature mold. The standard visual appearance of tempe is regulated in Indonesian National Standard for Soybean Tempe (SNI 3144:2009), which can be used as guidance if tempe artisan wants to produce tempe, while overripe tempe was made using standard of visual appearance using quantification of lightness and chromaticity in the color as stated in previous publication (Djunaidi *et al.*, 2017) as shown in Table 1.

Tempe considered as complex fermentation which includes several microorganisms including lactic

Table 1. Texture, colour, and protein profile development during overripe tempe production

	Raw Soybean	Soaked & Inoculated Bean	Tempe Pero	Tempe	Overripe Tempe
			Texture & Color		
Texture	n/a	n/a	n/a	14.79 - 21.85	10.38 - 24.34
Color (index value)*:					
L	n/a	n/a	n/a	55.53 - 87.46	31.74 - 61.92
a				2.12 - 9.55	8.18 - 12.05
b				7.23 - 26.75	17.81 - 33.11
			Protein Profile		
Total Nitrogen (%)	29.74 ± 1.20[a]	35.70 ± 1.57[ab]	41.03 ± 4.44[abc]	45.81 ± 0.99bc	49.56 ± 1.41[c]
Oligopeptide content (mg eq. BSA/g dry base)	16.17 ± 0.07[a]	4.32 ± 0.71[b]	4.90 ± 0.11[b]	2.80 ± 1.14[b]	3.72 ± 1.63[b]
SAA (mg eq. Tyrosine/g dry base)	7.35 ± 0.01[a]	64.03 ± 1.96[a]	129.11 ± 27.77[a]	302.46 ± 39.84[b]	465.14 ± 0.01[c]
Protein enzymatic hydrolysis rate (%)	2.47	5.45	6.85	13.36	22.35

Different letters in the same group of chart indicate a significant difference at $p \leq 0.05$; *L indicates lightness; a indicates chromaticity coordinates of green (-) to red (+); b indicates chromaticity coordinates of blue (-) to yellow (+)

acid bacteria, and several moulds such as *Rhizopus oligosporus, R. oryzae, R. stolonifer* (Dwinaningsih, 2010). Since there are a lot of microorganisms took place during the processing, tempeh called polymicrobial fermentation (Seumahu *et al.*, 2013). During fermentation, these microorganisms increase the protein qualities through various ways. The mycelium growth increases the amino acid content in tempe in comparison to its original soybean (Murata *et al.*, 1967). The microbial fermentation also hydrolyze protein into soluble amino acid and anti-nutritional factors such as phytic acid, supporting better protein digestion and absorption for human-nutrition (Astuti *et al.*, 2000).

In this study, it was shown that along with further fermentation, changes in the overripe tempe showed more desirable profile of protein that indicate better protein qualities (Table 1). The total protein content significantly increased during solid state fermentation, especially following the mould growth as shown in the measurement of total nitrogen content. Not only increasing in amount, the protein enzymatic hydrolysis rate, that indicate better protein digestibility by mammalian protease, increased along with fermentation. Higher leaps of protein hydrolysis rate were observed especially between tempe *pero*, tempe, and overripe tempe (6.85%; 13.36%; 22.35%; respectively). Microbial digestion on the protein during fermentation was also shown with the significant decrease of oligopeptide and significant increase of soluble amino acid. The data indicate that the microbial activity during solid-state fermentation hydrolyzed oligopeptide into soluble amino acid. Observation in this study also shown that though there was no significant difference between oligopeptide content of tempe *pero*, tempe and overripe tempe, there was a significant increase of soluble amino acid content during this three stages (129.11; 302.46; 465.14 mg Tyrosine eq./100 g dry basis; respectively). This fact support the increase of total nitrogen and suggest that the amount of protein increased during further fermentation from tempe to overripe tempe might be in the form of soluble amino acid, which are the preferable form of protein as nutrition (Liebau *et al.*, 2015). In overall, the data indicated that overripe tempe has better protein profile compared to tempe and therefore also play role as a promising source of protein.

Overripe tempe was oven-dried and formulated into overripe tempe stock according to methods and formula described in Setiadharma *et al.* (2015). Proximate analysis of the porridge ingredients (data not shown) indicated overripe tempe powder as major contributors of protein source (55.51% total nitrogen), though rice will also add up to the protein content of the resulting product (8.04% total nitrogen). In accordance to the major attributes affecting acceptance in children food (Fortunata, 2017), for the first stage of formulation adjustment in the overripe stock were made by employing minimum limit of overripe tempe usage that will fulfil 1/3 RDI of protein in children, which were 26 g for 1-3 years old and 35 g for 3-6 years old (Health Minister of the Republic of Indonesia, 2013), and equal to the utilization of 139.22 and 87.42 g of overripe tempe powder per portion for, 1 - 3 years, and 4-6 years children, respectively.

The selection of the formula was done based on taste balance and acceptance of trained panelist over focus group discussion. The selected formula of overripe tempe stock contain 27 g of overripe tempe powder, 4 g of vegetable oil, and 69 g of other seasonings for each 100 g formula of stock paste that will be diluted in the ratio of stock paste to water 1:25 (w/v) to produce overripe tempe stock solution. The resulting stock solution was described to have very intense umami flavor with balanced proportion of sweet and saltiness. The result was in conjunction with the proposed usage of overripe tempe that will also bring out the umami taste in the food product due to its higher glutamic acid and aspartic acid known to contribute the umami flavor amino acid (Lioe *et al.*, 2007; Gunawan-Puteri *et al.*, 2015; Witono *et al.*, 2015).

In the second stage of formulation the ratio of grounded uncooked-rice to overripe stock and cooking time was determined based texture of the resulted porridge. Uncooked-rice was grounded coarsely and placed in retortable aluminium pouch, sealed, and was cooked using pressure cooker to produce ready to eat porridge. Trained panelists in focus group discussion were employed to describe the expected texture in porridge, not too thick and not too runny, and selecting the variations that meet the criteria. From all 18 variations produced, five samples met the criteria, and sample produced from uncooked-rice and overripe stock with ratio 1:5 (w/v) and 12 min cooking time resulted in the best texture of porridge and determined to be a model ratio and cooking time in the next stage of formulation (Table 2).

Table 2. Selected samples in the determination of ratio of grounded uncooked-rice : overripe stock and cooking time that met the criteria of porridge texture

Ratio of uncooked-rice : overripe stock (w/v)	Cooking time (min)	Description
1:6	10	No water left from the container, the water was absorbed completely to the rice. The texture was rather hard and more like cooked-rice rather than porridge.
1:7	10	The rice became swollen, there was still water left. This variation had a porridge-like texture but still similar like soft cooked rice.
1:5	12	There was a little water left, the texture was completely turn into porridge. Soft enough, there was no rice-like form from its appearance.
1:6	12	There was a little water left, the texture was completely turn into porridge. There was no rice-like form from its appearance, but has very soft and runny texture.
1:8	15	The rice was cooked, however there was still much water left. The texture was very mushy like soft cooked-rice.

Due to the inclusion of rice in the porridge, further formulation was required, especially to enhance the taste and aroma intensity. The adjustment includes adjustment of overripe tempe powder and salt proportion in the formula and usage of garlic oil or palm oil as vegetable oil used in stock formulation. Samples were selected based on best balance of sweetness, saltiness, and umami taste using trained panelist under focus group discussion. Four selected samples were then compared to selected commercial product for hedonic sensory evaluation employing naive respondents. Higher acceptance based on taste was determined as the selection point of the porridge formula. According to the hedonic evaluation on, all four porridges have significantly better taste and aroma acceptance compared to commercial product. These may contribute to significantly better overall acceptance of all four porridges compared to the commercial product, despite their lower acceptance of appearance and texture (Table 3). The result was in conjunction to the previous survey indicating that taste was an important attribute that supports the selection of the food product (Fortunata, 2017). Lower saltiness in porridge A and B seemed to contribute in better taste acceptance. Some comments indicated that lower saltiness was thought to be healthier for children food, and it may affect the taste acceptance of the product. In the ratio 1.6 g/1000 ml water, garlic oil seemed to increase the aroma acceptance compared to palm oil, however no significant difference in aroma acceptance was observed in the ratio of 1.1 g/1000 ml water. Taste was considered as important factor affecting the sensory acceptance and therefore the samples were further selected based on these two attributes. Porridge A and B acceptance for taste was significantly higher than other porridge. Though the acceptance for taste and also aroma between porridge

Table 3. Hedonic Sensory Evaluation of Selected Porridge formula

	Commercial Porridge	Porridge A	Porridge B	Porridge C	Porridge D
Formula of Selected Porridge Samples					
Ingredients (g/1000 ml water):					
- Rice	not available	200.0	200.0	200.0	200.0
- Overripe tempe powder		9.0	9.0	12.0	12.0
- Salt		7.8	7.8	10.4	10.4
- Palm oil		1.2	0.0	1.6	1.6
- Garlic oil		0.0	1.2	0.0	0.0
- Other seasoning		10.8	10.8	14.4	14.4
Hedonic Sensory Evaluation of Selected Samples*					
Attribute evaluated:					
- Appearance	5.03 ± 1.46[a]	3.85 ± 1.92[d]	4.30 ± 1.82b	4.10 ± 1.57[bc]	4.28 ± 1.52[c]
- Aroma	4.35 ± 1.66[c]	5.30 ± 1.29[a]	5.48 ± 1.04[a]	4.98 ± 1.14[b]	5.50 ± 0.75[a]
- Taste	4.73 ± 1.60[c]	5.25 ± 1.46[a]	5.20 ± 1.49[a]	4.85 ± 1.73[bc]	4.95 ± 1.71[b]
- Texture	4.98 ± 1.31[a]	3.88 ± 1.34[d]	4.10 ± 1.41[c]	4.58 ± 1.26[b]	4.48 ± 1.41[b]
- Overall	4.10 ± 1.37[b]	4.43 ± 1.58[a]	4.43 ± 1.57[a]	4.38 ± 1.08[a]	4.35 ± 1.27[a]

* Scored on a 9-point hedonic scale: 9 = like extremely, 1 = dislike extremely. Different letters in the same group of chart indicate a significant difference at $p \leq 0.05$

A and B was not significantly different, porridge B was selected because it has better texture and appearance compared to porridge A. Porridge B contain 6.23% total nitrogen (complete proximate data not shown), and therefore 200 g portion of the porridge was able to contribute to 48% and 36% RDI of protein of 1-3 years old and 4-6 years old toddler.

Conclusion

The protein profile of overripe tempe showed better overall protein quality as indicated with higher nitrogen content, higher rate of protein enzymatic hydrolysis, and higher soluble amino acid content compared to tempe, that has been know as protein source. Formulation of ready-to-eat overripe tempe porridge as food for 0-6 years old children resulted in four selected formula, had better taste and overall acceptance in comparison to selected commercial product. Formula with best sensory acceptance was also successfully able to fulfill more than 1/3 RDI of protein for children age 0-6 years old, which is sufficient for three times a day feeding pattern. For further development of children food, other nutritional requirements and limitations might also be important aspect to be evaluated.

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