

Effect of tempe protein isolates addition on beef meatballs characteristics

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Abstract

Meatball is a processed meat emulsion product made from starches, meat, water and seasonings. Problems that often occurred in meatball production are the lack of compactness of the product texture, as well as low water holding capacity due to poor emulsification process during the forming of the meatball dough. Soy protein isolate (SPI) is usually used to improve the texture quality and act as a binder on meat emulsion products. Tempe protein isolates (TPI) can be used as an alternative for SPI because of their higher protein quality compared to commercial SPI due to the fermentation process. TPI used in this study was made from germinated soybean tempe (GTI) and non-germinated soybean tempe (NGTI). The purpose of this study was to examine the effect of SPI, GTI and NGTI application and concentration on the quality of the meatballs produced. The protein isolate concentration used were 1, 2 and 3% based on the weight of the meat. Results showed that the WHC value of meatballs increased with the increasing concentration of protein isolates used. The highest WHC value (91.60%) was obtained in GTI treatment with 3% concentration, not significantly different from 3% NGTI (91.06%) and significantly higher than 3% SPI (90.93%). In the overall texture parameters, GTI produces meatballs with higher scores than SPI, but it was not significantly different from NGTI. The highest protein content (13.39%), was achieved by GTI (3%), not significantly different from GTI (1%) (12.69%) but significantly higher than SPI (3%) (10.70%). GTI (1%) meatballs had the highest sensory acceptance scores on the attributes of colour, taste, hardness, juiciness, chewiness and overall preferences. It showed that GTI was useful to reduce the concentration of protein isolate used.

1. Introduction

Meatball is one of the meats processed food made from a mixture of ground beef, flour or starch as filler and various mixture of seasoning to increase the flavour. The fillers that are commonly used as meatball fillers are sago starch (*Metroxylon sago*) and palm starch (*Arenga pinnata*). The types of starch that are used affects the outcome of the meatball texture. The high price of meat makes meatball producers use more starch compared to meat, which alters the texture and taste. Alternatively, soy protein isolate (SPI) is added as an emulsifier and has the ability to increase the quality of texture in emulsified meat such as meatballs, sausage and corned beef (Zheng *et al.*, 2022). Isolate or protein concentrate

that is commonly used by food industries usually originates from plant-based protein, such as soybean. SPI in food industries can also be used as an emulsifier, filler, and stabilizer, as well as to improve texture and gel power (Santhi *et al.*, 2017).

Tempe is known to have the potential as an alternative protein isolate due to the protein quality it possesses (Puteri *et al.*, 2018). Tempe protein isolate (TPI) had higher protein content, better water absorption and oil absorption capacity, emulsion capacity and stability and gel power compared with SPI (Astawan, Wresdiyati, Subarna *et al.*, 2020). In addition, the antinutritive compounds such as trypsin inhibitors

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declined during the soybean fermentation process. This process increased the tempe protein bioavailability compared with soy protein (Astawan *et al.*, 2022). TPI is classified as germinated soybean (GTI) and non-germinated soybean (NGTI). The germination process could alter the carbohydrate, fat, protein, minerals, isoflavone and amino acid compounds in soybean (Astawan, Rahmawati, Cahyani *et al.*, 2020), which produces better functional properties.

The application of TPI in chicken sausage products also resulted in sausage with improved physicochemical and functional properties (Prayudani *et al.*, 2020). Based on the abovementioned properties, this research focused on the effect of the addition of TPI, specifically GTI on the texture quality and sensory profile improvement in beef meatballs. Therefore, the optimum concentration of TPI added to beef meatball products needs to be determined and compared with commercial SPI. The functional characteristics resulting from the use of TPI in meatballs can be considered for the meat processing industry to modify the physical properties of the product and provide information about the optimum concentration that can be added.

2. Materials and methods

2.1 Production of beef meatball

The study was conducted in the Food Science and Technology Laboratory, at IPB University. The ingredients used for this study were lean beef and connective tissues, ice, starch, protein isolate, salt, seasoning, flour, palm oil and sodium tripolyphosphate (STPP). The ingredients were obtained from the local market. The compositions of ingredients used for this study were shown in Table 1. Commercial SPI for this

Table 1. Beef meatball formulation.

Main ingredient	Weight (g)		
Beef	250		
Additives	F1*	F2*	F3*
Protein isolate	2.5	5	7.5
Sago flour	50	50	50
Ice	62.5	62.5	62.5
MSG	3	3	3
Salt	6	6	6
Sugar	1	1	1
Pepper	2	2	2
Garlic powder	2	2	2
Fried onion	2	2	2
STPP	1.1	1.1	1.1
Total	382.1	384.6	387.1

*Each formula was made using each SPI, GTI, and NGTI; resulting of a total of 9 meatball formulas. F1: Formula 1 with 1% protein isolate, F2: Formula 2 with 2% protein isolate and F3: Formula 3 with 3% protein isolate.

study was obtained from Nutrifood Indonesia, Co., while GTI and NGTI were obtained from the previous study (Astawan, Wresdiyati, Yoshari *et al.*, 2020).

The beef was grounded using a meat grinder (Formac MGD-12A), then the grounded beef was mixed with ice and salt using a food processor (Panasonic MK-5076M), then proceeded with the addition of protein isolate (SPI/GTI/NGTI), palm oil, STPP, sugar, starch and seasoning for 5 mins. The meatball dough was moulded manually into a spherical shape and boiled in two steps. In the first step, the dough was boiled at 60–70°C for 10 min, then in the second step, the meatball was boiled until cooked at 80–100°C.

2.2 Analysis of objective characteristics

The beef meatballs were analysed for water holding capacity (Sukarno *et al.*, 2014) and texture profile analysis (Huda *et al.*, 2010), which included the hardness, springiness, cohesiveness and chewiness.

2.3 Sensory test

Based on the analysis of variance from samples that have been tested previously, several representative samples were selected to be tested subjectively with sensory tests. The beef meatball samples with different types of protein isolates and protein isolates concentrations were served randomly to 75 panellists (Singh-Ackbarali and Maharaj, 2014). The beef meatballs were boiled for 2-3 mins in boiling water and served with no broth to be assessed by the panellists (Hermanianto and Andayani, 2002).

In the hedonic ranking test, the panellists were instructed to rank the samples that they highly preferred, with rank 1 as highly like to rank 5 as highly dislike. In the hedonic rating test, the panellists were instructed to give a rating from 1 to 7, with a scale of 1 indicating strongly dislike, scale of 2 dislike, scale of 3 somewhat dislike, scale of 4 neutral, scale of 5 somewhat like, scale of 6 like and scale of 7 strongly like. The attributes assessed were colour, taste, aroma, hardness, chewiness, juiciness and overall preference.

2.4 Proximate analysis

The selected samples were then analysed for their moisture, ash, protein, fat content and carbohydrate by difference (AOAC, 2012).

2.5 Data analysis

The data processing and analysis were done using SPSS 20.0 software for Windows (SPSS Inc., Richmond, CA, USA). Each test was performed in two repetitions. The resulting data were analysed using analysis of

variance (ANOVA) and proceeded with DMRT with a confidence level of 95%. Meanwhile, the hedonic ranking test result was analysed using the Friedman test and then followed with the LSD post hoc test.

3. Results and discussion

3.1 Objective analysis

3.1.1 Water holding capacity

The water holding capacity has an important role in determining the final texture of a product, especially in meat-processed products. Based on the results of the analysis (Figure 1), the highest WHC was found in GTI (3%) treatment with a value of 91.6%, while the lowest WHC was found in SPI (1%) treatment (87.57%). Based on the ANOVA result, only the concentration of protein isolate factor resulted in significantly different WHC values. The protein isolate type factor did not significantly affect WHC at each concentration of protein isolate added. The increased WHC was directly proportional to the protein isolate concentration due to the ability of protein in holding water, both physically and physiochemically. This occurs due to the presence of polar amino acids which have roles in protein interaction with water due to the formation of hydrogen bonds with the water molecules (Cheftel *et al.*, 2017).

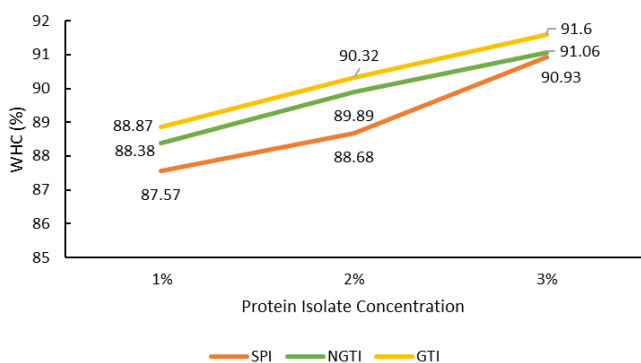


Figure 1. Effect of concentration and protein isolates on the water holding capacity of meatball. Protein isolate concentration significantly improved WHC value ($p < 0.05$), while protein isolate types do not significantly affect WHC value. SPI: Soy Protein Isolate, GTI: Germinated Soybean Tempe Protein Isolate, NGTI: Non-germinated Soybean Tempe Protein Isolate.

The process of tempe fermentation using *Rhizopus* spp. mould results in protease enzyme which can breakdown the protein molecules into short chain peptides and free amino acids (Kadar *et al.*, 2018), thus the WHC from GTI and NGTI values were higher compared to SPI, although statistically not significantly different. According to Zhang *et al.* (2021), the main factors that affect WHC are intrinsic and protein concentration, ionic power, pH, temperature and process conditions such as denaturation and unfolding. The

heating process during cooking increases the WHC due to the opening of protein bonds, so the side chains of the protein are opened and able to bind water. Besides, the presence of other food components like hydrophilic polysaccharides, fat, and salt also affects the water binding properties.

3.1.2 Texture profile

In addition to taste and aroma, texture is also one of the parameters that determine the consumers liking of solid food products. Several parameters that can be measured and related to texture are hardness, cohesiveness, springiness and chewiness. The values of each parameter were shown in Table 2. Based on the result of ANOVA, the types and concentration of protein isolate factors significantly contributed to hardness, cohesiveness, and chewiness, but there was no interaction found in both factors. In the meatball springiness score, the types and concentrations of protein isolate treatment in meatballs added with GTI and NGTI were not significantly different, but they became significantly different and lower in SPI-added meatballs.

The higher protein content can increase the hardness, cohesiveness and chewiness values in meatballs. This is in accordance with the study by Wee *et al.* (2018) who reported that protein content in solid food products was positively-correlated with hardness and chewiness. This was caused by the binding ability of protein, especially between myosin protein from the meat and globulin 11s protein from soybean at temperatures below 100°C (Sha and Xiong, 2020). The increased protein content results in a denser protein matrix in the product and a more rigid structure, which is more able in retaining the form from the applied force (Li *et al.*, 2021). The addition of GTI in chicken sausage can also increase the hardness value of the product (Prayudani *et al.*, 2020). The higher protein content in GTI can solidify the meat protein matrix structure. The texture of the product also varies based on the added non-meat protein ingredient.

3.2 Sensory test

Based on the texture analysis result, GTI and NGTI meatballs had no significantly different results. The result was also similar in the treatment of 1% and 2% protein isolate. Therefore, four meatball samples [SPI (1%), SPI (3%), GTI (1%) and GTI (3%)] which represented the result were chosen for the sensory test. The selection of GTI meatball was based on the purpose of determining the effects of soybean germination and fermentation process on the resulting meatball characteristics. The result of the meatball sensory test can be seen in Table 3 and Figure 2.

In terms of colour parameter, meatball that was too

Table 2. Effect of protein isolate types and concentrations on meatball texture profiles.

Texture parameter values on the protein isolate types	Protein Isolate Concentration			Mean of protein isolate types effect	
	1%	2%	3%		
Hardness (gf)	SPI	3617.5	3912.3	4739.3	4089.6 ^a
	GTI	4034.1	4257.5	5068.8	4453.5 ^b
	NGTI	3943.6	4187.5	5019.6	4383.5 ^b
	Mean of protein isolate concentration effect	3865.0 ^a	4119.1 ^b	4942.6 ^c	(-)
Cohesivness	SPI	0.72	0.72	0.76	0.74 ^a
	GTI	0.75	0.75	0.79	0.76 ^b
	NGTI	0.74	0.75	0.79	0.76 ^b
	Mean of protein isolate concentration effect	0.74 ^a	0.74 ^b	0.78 ^c	(-)
Springiness (mm)	SPI	0.99	0.99	0.98	0.988 ^a
	GTI	0.98	0.99	0.99	0.989 ^a
	NGTI	0.99	0.99	0.98	0.989 ^a
	Mean of protein isolate concentration effect	0.986 ^a	0.992 ^a	0.988 ^a	(-)
Chewiness (gf mm)	SPI	2563	2804.6	3560.3	2975.9 ^a
	GTI	2962	3186.9	3980.9	3376.6 ^b
	NGTI	2907.6	3130.1	3885	3307.6 ^b
	Mean of protein isolate concentration effect	2810 ^a	3040.5 ^b	3808.8 ^c	(-)

Values with the different superscripts within the same row and column are statistically significantly different ($p < 0.05$). SPI: Soy Protein Isolate, GTI: Germinated Soybean Tempe Protein Isolate, NGTI: Non-germinated Soybean Tempe Protein Isolate, -: No significant interaction between protein isolate types and concentration.

dark in colour (SPI with 3% treatment) was least liked by the consumers. The high protein content results in the Maillard reaction which involves a nucleophile group from the meat amino acid or isolate and a reactive carbonyl group from the starch that was added into the mixture. The boiling process results in the formation of darker colour in meatballs (Xu *et al.*, 2020; Shaheen *et al.*, 2021). The GTI meatball was preferred because it had brighter colour compared to SPI due to the decreased fat content during the fermentation process (Ahnann-Winarno *et al.*, 2021).

In terms of taste parameters, both treatments of GTI concentration had no significantly different taste, but both were significantly different compared with SPI-treated meatballs. The soybean (beany) taste in meatballs was detected more in SPI compared with GTI meatballs. The aroma had no significantly different result, except for the 3% SPI treatment which tended to be lower due to the soybean aroma.

Based on the hardness parameter result, the higher the protein content in the meatball, the hardness value of the meatball increased as well. However, this was not always parallel with the increased liking of consumers. Consumers tend to prefer meatball with tender texture compared to the harder ones, but they also prefer meatball that is not easily ruptured (Beniwal *et al.*, 2021). The addition of more protein isolate results in meatballs with denser starch matrix structures that become difficult to rupture (Beniwal *et al.*, 2021).

Furthermore, it will also result in increased meatball chewiness. However, meatball with chewy texture was less preferred by consumers due to the tough texture when bitten.

Although the increase in protein isolate concentration could increase the water holding capacity which affected the meatball juiciness, this was not always the case with the increase in consumer preferences. Overall, meatball with 1% GTI treatment had the highest rating score and was significantly different from other treatments. Sequentially, the results were as followed: 3% GTI, 1% SPI and 3% SPI meatballs.

Based on the result of the hedonic ranking test in Figure 2, it was shown that 1% GTI meatballs had the lowest score. This implies that 1% GTI meatball was preferred by the consumers. The LSD post hoc test showed that the preferences ranking in 1% GTI meatballs were significantly different with 1% SPI, 3% GTI and 3% SPI meatballs, but there were no significant differences found in 1% SPI and 3% GTI meatballs.

3.3 Proximate analysis

Based on the results of the proximate analysis (Table 4), from four meatball samples that had been tested organoleptically, only 3% GTI meatball fulfilled the requirements of the Indonesian National Standard 3818:2014 about beef meatball, with requirements of moisture content to be not more than 70%, ash content

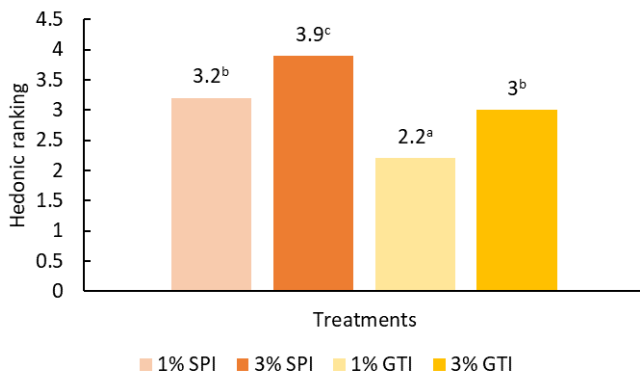


Figure 2. Hedonic ranking tests of meatballs with selected treatments. Values with different superscript are statistically significantly different as determined by Fisher's LSD ($P < 0.05$). SPI: Soy Protein Isolate, GTI: Germinated Soybean Tempe Protein Isolate

Table 4. Chemical composition of meatballs with selected treatments.

Parameters (%wb)	Treatments			
	1% SPI	1% GTI	3% SPI	3% GTI
Moisture	71.4±0.0 ^b	70.8±0.0 ^b	70.6±0.1 ^b	68.6±0.0 ^a
Ash	1.5±0.0 ^a	1.5±0.1 ^a	1.5±0.0 ^a	1.5±0.0 ^a
Protein	9.8±0.1 ^a	12.7±0.0 ^c	10.7±0.0 ^b	13.4±0.2 ^d
Fat	1.7±0.0 ^a	1.8±0.0 ^a	2.1±0.0 ^b	2.1±0.2 ^b
Carbohydrate	15.7±0.4 ^c	13.3±0.1 ^a	15.2±0.7 ^c	14.3±0.1 ^b

Values with different superscripts within the same row are statistically significantly different ($p < 0.05$). SPI: Soy Protein Isolate, GTI: Germinated Soybean Tempe Protein Isolate

not more than 3%, minimum protein content of 11% and fat content not more than 10% (BSN, 2014). Meatballs with other treatments were more suitable to be classified as combination beef meatballs.

The analysis of variance result showed that the type of protein isolate had significant effects on the moisture, protein, fat, and carbohydrate content of the meatball, but had no significant effect on the ash content. The differences found in protein content on the meatballs tested were caused by the different protein content of the protein isolate. GTI had higher protein content compared to SPI, due to the soybean fermentation process into tempe which initiates the elimination of soluble components such as minerals and sugars from the soybean (Wresdiyati *et al.*, 2021), thus resulting in the increased protein content.

In GTI-treated meatballs, the germination of soybean resulted in the enzymatic breakdown of protein into simpler compounds, so more amino acids were released during the germination (Abdurrasyid *et al.*, 2020). The fat content was higher in 3% SPI and 3% GTI meatballs due to the more protein isolate added to the mixture. Although GTI had lower fat content compared with SPI (Astawan, Wresdiyati, Yoshari *et al.*, 2020), since the

percentage of the added isolate was threefold higher, the fat content in 3% GTI meatballs became higher compared with 1% SPI and 1% GTI meatballs. The ash content of the meatball samples did not show significant differences. This was caused by the mineral content of the added protein isolates being relatively in similar amounts.

4. Conclusion

GTI and NGTI meatballs had the highest overall WHC and texture parameters compared with SPI meatballs. Meatball with 1% GTI is the recommended formula because it has better sensory acceptance compared to other treatments, as well as suitable protein content based on the requirements of Indonesian National Standard 3818:2014 about beef meatballs. Therefore, the 1% GTI addition results in meatballs with chemical composition, texture, and sensory score that were not significantly different from the usage of 3% SPI, which will result in the reduction of production cost. The result also indicates that the soybean germination and tempe fermentation processes significantly affected the quality of the produced protein isolate.

Conflict of interest

The authors declare no conflict of interest.

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