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FULL PAPER

Quality characteristics of chicken sausages using a combination of jack bean (*Canavalia ensiformis* L.) and soy protein isolate as a binder

[1,]*Canti, M., [2]Murdiati, A., [2]Naruki, S. and [2]Supriyanto

[1]*Department of Food Technology, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jalan Jenderal Sudirman 51, Jakarta 12930, Indonesia*
[2]*Department of Food and Agricultural Products Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jalan Flora 1 Bulaksumur, Sleman, Yogyakarta 55281, Indonesia*

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Abstract

Jack beans are one of the legumes with a high protein content to make protein isolates. This research aimed to evaluate the physical, sensory and proximate qualities of chicken sausages with jack bean protein isolate (JBPI) and the combination of JBPI and soy protein isolate (SPI) as a binder to improve the quality of the chicken sausages. This research included the preparation of JBPI and chicken sausages. The treatments were formulated as follows: control (without JBPI and SPI); T1 (SPI: JBPI = 100: 0); T2 (SPI: JBPI = 80:20); T3 (SPI: JBPI = 60:40); T4 (SPI: JBPI = 40: 60); T5 (SPI: JBPI = 20: 80); T6 (SPI: JBPI = 0: 100). The analysis of the physical, sensory, and proximate properties of sausages have been performed. The results showed that the JBPI protein content was high at 93.98% db, and contained higher essential amino acids than the FAO/WHO standards, i.e., leucine, lysine, phenylalanine + tyrosine, threonine. The combination of JBPI and SPI improved emulsion stability, lightness, yellowness, texture properties, protein content, and reduced cooking loss and redness of chicken sausages compared to control (p<0.05). The results of the sensory evaluation showed that the overall preference, slice properties, and texture attributes of chicken sausage with the addition of a combination of SPI and JBPI were 40:60 (T4) significantly different from the control received by the panellists (p<0.05). The formulation with the addition of a combination of SPI and JBPI of 40:60 was the optimal treatment because it improves the overall physical, sensory, and chemical characteristics of the resulting chicken sausage. JBPI had the potential as an alternative to substitution for SPI.

1. Introduction

Sausage is a processed meat product that is popular with the public. The level of sausage consumption in Indonesia is increasing every year. That is shown in 2019, whereby the sausage consumption level was 0.672 pieces/capita/week, while in 2018, it was 0.578 pieces/ capita/week (BPS, 2019). In making sausages, the important factors to consider are the raw materials used and the emulsification process. The ingredients used in the manufacturing process of sausages consist of the primary and additional components. The main ingredient used is meat, while the additives are binder and filler. Meat as the primary ingredient for sausages is chosen with high protein content, chicken. The protein content of chicken (22.8-24.2%) was higher than meat from other sources such as beef (20-21.9%); pork (18.1-20.8%), sheep (20%); turkeys (19.9-23.6%), and ducks (19.4%) (Ahmad *et al*., 2018). The high protein content of meat will determine this protein's ability to emulsify fat in the sausage emulsion system (Santhi *et al*., 2017). Also, chicken meat is easy to obtain because of its high production in Indonesia (3,495,090.91 tons/year), and the price is affordable (BPS, 2019).

One of the binding agents used in manufacturing sausages is a protein isolate. A protein isolate is a product with a protein content of not less than 90% against dry matter. This product is almost free of carbohydrates, fibre, and lipid, so its functional properties are much better than protein concentrate and protein flour. The high protein content makes protein isolate an excellent binder to stabilize emulsions, increase the water-holding capacity of meat products, reduce shrinkage during cooking, improve the flavour and characteristics of the product slices, increase the

*Corresponding author.
Email: *meda.canti@atmajaya.ac.id*

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nutritional value of a product and reduce formulation costs (Garba and Kaur, 2014). Soy protein isolate (SPI) is widely used in the meat product processing industry. However, soy protein isolates in Indonesia are still imported. Therefore, an alternative source of soy protein isolate is needed. Other food sources that have been developed in manufacturing protein isolates are Indian black gram (Wani, Sogi and Gill, 2015), kidney beans (Wani *et al*., 2015), black soybeans (Evangelho *et al*., 2016), green beans (Brishti *et al*., 2017), chickpea coproduct (Espinosa-Ramirez and Serna-Saldivar, 2019); lentils (Qayyum *et al*., 2012), cowpea (Khalid *et al*., 2012), *Lathyrus clymenum* and *Lathyrus annuus* (Pastor-Cavada *et al*., 2010).

Jack bean is a type of legume that has high nutritional content, especially protein (24.32±0.11%) and carbohydrates (41.26±0.01%) (Marimuthu and Gurumoorthi, 2013). In addition, jack beans contain essential amino acids i.e., leucine (7.03%); lysine (6.55%); phenylalanine (5.24%); valine (4.21%); threonine (3.44%); isoleucine (3.25%); tyrosine (3.12%); histidine (2.64%); cysteine (1.10%) and methionine (1.05%) (Solomon *et al*., 2017). The high productivity of jack bean reaches 12 tons/ha (Kasno, 2016). However, its utilization is still not optimal because it has an off-flavour and high cyanide acid. Therefore, jack beans can be used for the production of protein isolates. The use of jack bean protein isolate (JBPI) is still limited as an additive to improve cake characteristics (Subagio *et al*., 2003). It was necessary to know the concentration of the addition of JBPI as a binder in sausages. This research aimed to evaluate the physical, sensory, and proximate quality of chicken sausages with JBPI and the combination of JBPI and SPI as a binder to improve the chicken sausages quality.

2. Materials and methods

2.1 Materials

Jack bean (*Canavalia ensiformis* L.) was obtained from the TULSID Farmers Group (Tulakan, Sidowayah), Wonogiri, Central Java, Indonesia. The materials used in chicken sausages were chicken meat obtained from Kranggan Market, Yogyakarta, Indonesia, corn oil (CCO Corn Oil), SPI (MarkSoy 90), and sausage casings (Devro). The chemical materials used in this research, i.e., aquadest, technical n-hexane (Bratachem), NaOH (Merck), HCl 37% (Merck), H_2SO_4 97% (Merck), K_2SO_4 (Merck), HgO (Merck), H_3BO_3 (Merck), $Na_2S_2O_3$ (Merck), BCG-MR (Merck), Ortho Phthalaldehyde (OPA) reagent (Sigma-Aldrich), methanol (Merck), 2-mercaptoethanol (Sigma-Aldrich), Brij-30 30% (Merck), potassium borate buffer (Merck), petroleum ether (Merck), filter paper no. 1 (Whatman).

2.2 Preparation of JBPI

JBPI was prepared based on methods by Oo *et al*. (2017). Jack bean seeds were washed and soaked with the seeds' ratio: water = 1:3 for 12 hrs, its shell is then stripped soaked 3 × 12 hrs. Next, it was drained and dried using a cabinet dryer at a temperature of 50^{o}C for 24 hrs. The dried jack bean seeds were then milled and sifted through the 60 mesh. The jack bean flour was defatted using hexane in the ratio 1:3 w/v and stirred for 30 mins, three times. Then, it was dried to remove the remaining solvent. Defatted jack bean flour was extracted with a solution of 0.1 N NaOH and/or 0.1 N HCl at pH 10, the ratio of 1:12 (w/v), for 1 hr. Further, the solution was centrifuged at 3500 rpm for 30 mins. The supernatants obtained were filtered using filter paper. After that, the precipitation was carried out at pH 4 with HCl and separated by centrifuge at 3500 rpm for 30 mins. The protein isolate obtained was washed using 70% ethanol twice to remove sugar, then dried using a freeze dryer and stored at -18^{o}C until used.

2.3 Sausages preparation

The formulation of the chicken sausage was performed by modification, according to Menegas *et al*. (2013). The modified formulation of chicken sausage can be seen in Table 1. The treatments of chicken sausage were as follows: control (without JBPI and SPI); T1 (SPI: JBPI = 100: 0); T2 (SPI: JBPI = 80: 20); T3 (SPI: JBPI = 60: 40); T4 (SPI: JBPI = 40: 60); T5 (SPI: JBPI =

Table 1. Modified Chicken Sausages Formula with JBPI and SPI

Ingredients (%)	Treatments[1]						
	Control	T1	T2	T3	T4	T5	T6
Chicken meat	70.5	70.5	70.5	70.5	70.5	70.5	70.5
Corn oil	17.62	17.62	17.62	17.62	17.62	17.62	17.62
Water	11.88	11.88	11.88	11.88	11.88	11.88	11.88
Total	100	100	100	100	100	100	100
Soy Protein Isolate (SPI)	-	1.72	1.38	1.03	0.69	0.34	-
Jack Bean Protein Isolate (JBPI)	-	-	0.34	0.69	1.03	1.38	1.72

[1]Treatments: Control (and/or SPI and JBPI); Sausage formulation with SPI: JBPI; T1 (SPI: JBPI = 100:0); T2 (SPI: JBPI = 80:20); T3 (SPI: JBPI = 60:40); T4 (SPI: JBPI = 40:60); T5 (SPI: JBPI = 20:80); T6 (SPI: JBPI = 0:100).

20: 80); T6 (SPI: JBPI = 0: 100). The chicken meat was cut to size 5 × 5 × 1 cm. Then sausages were grounded and mixed with binders of SPI and JBPI, and ice water and seasonings. The seasonings used (per 100 g of meat mixture) were 0.44 g of sugar, 2.64 g of salt, 0.005 g of garlic, and 0.249 g of pepper. The next stage was the emulsification process with the addition of corn oil. The sausage emulsion was put into the casing. Then it was twisted with a size of 10 cm. After that, it was steamed for 20 mins, and the sausages were obtained.

2.4 JBPI amino acids profile

The amino acid content of JBPI was analyzed by precolumn derivation method with Ortho Phthalaldehyde (OPA) HPLC (AOAC, 2005). A total of 3 mg of sample was crushed, then the sample was hydrolyzed by acid using 1 mL HCl 6 N, then the sample was heated in an oven at 110°C for 24 hrs. The sample was cooled at room temperature, then the sample was transferred to an evaporator flask and rinsed several times with 2 mL of 0.01 N HCl. The sample was dried using a freeze dryer for 15 to 30 mins. The dry sample was added with 5 mL of 0.01 N HCl, and then the sample was filtered using Millipore filter paper. The sample was added using a buffer solution of potassium borate pH 10.4 with a ratio of 1: 1. A total of 10 µL of the sample was put into an empty vial, then added with 25 µL of OPA reagent, and left for 1 min. The mixture was then filtered using Whatman filter paper. A total of 5 µL of the filter results were injected into the HPLC and then waited for 25 mins until the separation of amino acids. The calculation of the concentration of amino acids in the sample was carried out by making a standard chromatogram using ready-to-use amino acids with the same treatment as the sample. The amino acid content can be calculated by equation (1):

$$\%\ \text{amino acids} = \frac{\text{Sample area x C x DF x MW x 100\%}}{\text{Standard area x sample weight}} \quad (1)$$

Where C = Standard amino acid concentrations (0.5 µmol/mL); DF = dilution factor (5 mL); MW = The molecular weight of each amino acid (g/mol)

2.5 Analysis of the physical properties of chicken sausages

2.5.1 Emulsion stability

The emulsion's stability was performed according to the method by Horita *et al*. (2014). The sample weighed at 25 g is centrifuged at 2600 rpm/5°C for 5 min. The batters were cooked at 40°C for 15 min, followed by 70°C for 20 mins. The exudate was dried at 105°C for 16 hrs and weighed. The emulsion's stability was calculated as a percentage of liquid released after cooking.

2.5.2 Cooking loss

The weight of the sausage was measured before and after cooking to calculate the cooking loss according to Equation (2) (Jeong and Han, 2019):

$$\text{Cooking loss (\%)} = \frac{\text{Before cooking weight (g) - After cooking weight (g)}}{\text{Before cooking weight (g)}} \text{ x } 100\% \quad (2)$$

2.5.3 Colour

The colour analysis was performed using a chromameter. The chromameter works by determining the colour based on the blue, red, and green colour components of the light absorbed by the sample. L*'s value represents the lightness parameter (achromatic colour, 0-100 for black-white). The value of a represents a red-green chromatic colour (a* + = 0-100 for red, a*- = 0 - (- 80) for green). The value of b represents the blue-yellow chromatic colour (b*+ = 0-70 for yellow, b*- = 0 - (- 70) for blue).

2.5.4 Texture

The texture profile on sausages was analysed using Lloyd's Universal Testing Machine (UTM). Chicken sausage samples were placed on the sample holder in the form of a metal plate, right in the middle. Once the instrument switch was turned on, the program was executed. The experimental data were plotted in a graph. The amount of hardness can be found by reading the maximum F value (N) shown in the graph.

2.6 Analysis of the sensory properties of chicken sausages

Sensory analysis in chicken sausages was performed using a preference test. The semi-trained panellists consisted of 30 student's male/female aged 20-30 years from the Faculty of Agricultural Technology, Universitas Gadjah Mada. Sausages were immersed in boiling water and kept at this temperature for 3 mins and then served at 35–40°C. The preference values were measured on hedonic scale (1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like very much) (Meilgaard *et al*., 2016).

2.7 Proximate analysis of JBPI and chicken sausages

2.7.1 Moisture content

Moisture content analysis was performed using the Thermogravimetric method based on AOAC (2000). One gram of the sample was put into a weighing bottle with a known weight, then dried at 105°C for 3 hrs until constant weight. After that, the sample was cooled in a desiccator and weighed.

2.7.2 Ash content

Ash content analysis was carried out using the Gravimetry method following the AOAC (2000). A total of 2 g of the sample was put into a porcelain container. Next, the sample was placed in a furnace at 600°C for 2 hrs. After that, it was cooled in a desiccator and weighed until a constant weight was obtained.

2.7.3 Protein content

Protein content analysis was carried out *using* the Micro Kjeldahl method following the AOAC (2002). The sample was weighed as much as 0.1 g and a catalyst (K2SO4: HgO = 20:1) of 0.75 g was added. Then 3 mL of concentrated H_2SO_4 was added. The sample was digested (410°C) until it was a transparent green, then cooled and distilled water was added until the volume reached 80 mL. Next, the sample was distilled with 20 mL of NaOH-$Na_2S_2O_3$. The distillate was collected in an Erlenmeyer containing 5 mL of 4% boric acid and 2-4 drops BCG-MR. Then the distillate was titrated with 0.02 N HCl. The nitrogen value was converted into protein content by multiplying by a factor of 6.25.

2.7.4 Lipid content

Lipid analysis was performed using the Soxhlet extraction tool based on AOAC (2005). A total of 5 g of the sample was weighed on filter paper, then covered with cotton. After, the lead was placed on the Soxhlet device connected to the Soxhlet flask, whose weight was known. The Soxhlet flask was placed in a distillation apparatus over a heater (80°C), then the petroleum ether solvent was poured until the sample was immersed. Next, reflux was carried out for 6 hrs. The Soxhlet flask was filled with oil and placed in the oven to evaporate the petroleum ether. Soxhlet tubes were weighed to constant weight. The difference between the weight of the initial and final Soxhlet was the weight of the lipid.

2.7.5 Carbohydrate content

Analysis of carbohydrates was carried out by calculating by difference based on AOAC (2005). This method used the principle of a 100% reduction in moisture, ash, protein, and lipid content so that the remaining product was the carbohydrate content in food.

2.8 Statistical analysis

The research was conducted three times of treatment replication; each treatment was carried out two times the analysis *replicates.* The data obtained were then performed statistical tests with analysis of diversity (ANOVA) and the smallest significant difference test of Duncan's Multiple Range Test (DMRT) with a significant level of $p<0.05$. Data were analysed using SPSS Version 24.

3. Results and discussion

3.1 Proximate of Jack bean protein isolate

Protein extraction can be done by various methods such as acids, bases, enzymes, and physical. JBPI in this study was extracted using the alkaline treatment. Alkali treatment is a protein recovery process that dissolves the insoluble protein in an alkaline solution and then precipitates the protein under acidic conditions (protein isoelectric point). The resulting protein recovery was high, at 80% (Liu *et al*., 2013).

Based on the results of the proximate analysis, JBPI had moisture content (9.50±0.15% wb), ash (1.85±0.10% db), protein (93.98±0.43% db), lipid (0.34±0.03% db), and carbohydrates (3.84±0.50% db) (Table 2). The defatted process on the jack bean flour was carried out in the JBPI preparation to low the JBPI lipid content. The purpose of defatted was to ensure that the material's lipid content does not interfere during the protein isolation and protein denaturation. Also, proteins strongly bound to lipid will be difficult to separate (Oo *et al*., 2017). The protein content of this JBPI was higher than the protein content of *Lathyrus clymenum* (82.40±0.80% wb) and *Lathyrus annuus* protein isolates (81.07±0.11% wb) (Pastor-Cavada *et al*., 2010), Indian black gram protein isolate (83.30±0.76-86.30±1.10% wb) (Wani, Sogi and Gill, 2015), red bean protein isolate (77.20±0.40-83.96±0.07% wb) (Wani *et al*., 2015), black soy protein isolate (81.60% wb) (Evangelho *et al*., 2016), chickpea coproduct protein isolates (85.40±0.50% wb) (Espinosa-Ramirez and Serna-Saldivar, 2019), green bean protein isolate (81.53±0.02% wb) (Brishti *et al*., 2017). Betancur -Ancona *et al*. (2008) stated that the JBPI produced had a lower protein content of 73.8%. Chel-Guerrero *et al*. (2002) reported that JBPI had a lower protein content of 73.75±0.30%. This study showed that the isolation process of jack bean protein at the highest protein solubility pH of 10 and the isoelectric pH of 4 was effective because it can produce JBPI with more than 90% protein content. JBPI protein content was following the International Food Standards Codex Alimentarius CXS 175-1989 for SPI products with protein content > 90% db (Codex Standard, 2019). Protein extraction generally increases with increasing solubilization pH. Non-protein components that interfere with protein extraction were dissolved at pH 10, resulted in better protein recovery in isoelectric precipitation (Chen and Houston, 1970). The resulting protein content of JBPI was high, so it can be potentially used as a binder added to sausage emulsion to increase the binding capacity of the protein to protein and fat.

Table 2. Proximate compositions of JBPI

Component	JBPI
Moisture (% wb)	9.50±0.15
Ash (% db)	1.85±0.10
Protein (% db)	93.98±0.43
Lipid (% db)	0.34±0.03
Carbohydrate (% db)	3.84±0.50

Values are expressed as mean±standard deviation

3.2 JBPI amino acids profile

The amino acid profile of JBPI can be seen in Table 3. JBPI had a higher content of amino acids leucine, threonine, histidine, aspartic acid, serine, alanine, and tyrosine compared to soy protein isolates. The content of JBPI's essential amino acids, namely phenylalanine + tyrosine, was relatively high. JBPI had a lower content of amino acids than SPI, i.e., isoleucine, lysine, methionine, phenylalanine, and valine. However, the levels of these essential amino acids (unmeasured tryptophan) still meet the standards of the FAO/WHO intake recommendation for adults, except for methionine. Besides, the levels of essential amino acids in JBPI (unmeasured tryptophan) also met the standards of the FAO/WHO intake recommendations for children except for lysine, methionine, and phenylalanine. The non-essential amino acids in JBPI contain amino acids with a negative R group, namely aspartate acid (9.98±1.73%) and high glutamate (11.27±0.78%). That showed JBPI had acidic characteristics. JBPI had a higher uncharged polar amino acid (13.91%) than SPI (11.26%). However, JBPI had lower hydrophobic, basic, and acid (26.97%; 13.28%; 21.25%) groups of amino acids than SPI (32.86%; 15.12%; 25.98%). The amino acid composition of protein isolates has been reported to affect the structure, hydrophobicity, and functionality of peptides (Kimatu *et al*., 2017).

Table 3. Amino acids profile of JBPI and SPI

Amino Acids	JBPI Total (%)	SPI[a] Total (%)	FAO/WHO[b]	
			Child	Adult
Essential				
Isoleucine	3.22±0.52	4.09±0.00	2.8	1.3
Leucine	7.51±0.65	7.08±0.04	6.6	1.9
Lysine	5.69±0.52	6.11±0.00	5.8	1.6
Methionine	1.06±0.97	1.32±0.00	2.5	1.7
Phenylalanine	4.50±0.32	4.89±0.04	6.3	1.9
Threonine	4.34±0.45	3.03±0.00	3.4	0.9
Tryptophan	n.d.	n.d.	1.1	0.5
Valine	3.57±1.00	4.10±0.02	3.5	1.3
Histidine	2.46±0.08	2.06±0.00	1.9	1.6
Non essential				
Arginine	5.13±1.38	6.95±0.04		
Aspartic acid	9.98±1.73	9.68±0.04		
Glutamic acid	11.27±0.78	15.98±0.04		
Serine	5.99±0.13	4.29±0.01		
Glycine	3.36±0.54	3.43±0.01		
Alanine	3.75±0.87	3.56±0.02		
Tyrosine	3.58±0.56	3.36±0.05		
Cysteine	n.d.	0.58±0.02		
Proline	n.d.	4.39±0.01		
Glutamine	n.d.	n.d.		
Asparagine	n.d.	n.d.		
Hydrophobic[c]	26.97	32.86		
Uncharged polar[d]	13.91	11.26		
Basic[e]	13.28	15.12		
Acidic[f]	21.25	25.98		

Values are expressed as mean±standard deviation. n.d. = not determined.
[a]Vernaza and Chang (2020), [b]Wang, Xu, Li *et al*., (2015), [c]Ala, Val, Ile, Leu, Phe, Trp, Pro, Gly, and Met, [d]Cys, Thr, Tyr, and Ser, [e]Arg, His, and Lys, [f]Asp and Glu.

The JBPI essential amino acid profile, namely leucine, lysine, phenylalanine + tyrosine, and threonine, was higher than the FAO/WHO reference standard (Table 4). JBPI's amino acids, namely isoleucine, methionine + cysteine, and valine, were lower than the FAO/WHO reference standards. When testing amino acids by precolumn derivation HPLC technique with OPA, the tryptophan amino acid could not be detected. Hence, the tryptophan amino acid score could not be calculated or compared with the FAO/WHO reference standard. The JBPI amino acids, which contain sulfur, namely methionine, and cysteine, were the lowest in number than the FAO/WHO reference standard. According to Wang *et al*. (2010), chickpea protein isolates also had limited amino acids, namely methionine and cysteine. In general, amino acids containing sulfur were limiting amino acids in legume proteins (Pandurangan *et al*., 2015). Therefore, it was necessary to mix two sources of protein limiting amino acids that were different but complementary, such as mixing JBPI with food ingredients with a higher methionine content than jack bean. The JBPI results can be considered as a

Table 4. JBPI amino acid score

Amino acids	FAO/WHO *pattern** (mg/g protein)	JBPI (mg/g protein)	Amino Acid Score
Isoleucine	40	32.2	0.81
Leucine	70	75.1	1.07
Lysine	55	56.9	1.03
Methionine + Cysteine	35	10.6	0.3
Phenylalanine + Tyrosine	60	80.8	1.35
Threonine	40	43.4	1.09
Tryptophan	10	n.d.	-
Valine	50	35.7	0.71

n.d. = not determined. *Harper (1981)

good source of essential amino acids for children and adults. In food processing, it can be formulated based on amino acid composition and proportions.

3.3 Physical properties of chicken sausage

Emulsion stability of chicken sausage for all treatments with the addition of JBPI, SPI, and the combination of JBPI and SPI was significantly different from the control (p<0.05) (Figure 1). Emulsion stability of T1 treatment (88.74±2.82%) was not significantly different from T2 treatment (87.48±1.22%) (p>0.05), while T2 treatment was not significantly different from T3 treatment (86.50±0.80%), T4 (85.71±0.49%) (p>0.05). However, significantly different from the treatment of T5 (85.07±0.58%) and T6 (84.15±2.40%) (p<0.05). Control chicken sausage had emulsion stability of 69.44±2.41%, the lowest compared to all treatments. That can occur because the fat content in the sausages decreases, which was caused by a decrease in the ability to stabilize the meat protein matrix/tissue (Alvarez and Barbut, 2013). Chicken sausage treated with JBPI and a combination of JBPI and SPI had good emulsion stability, namely >80%. According to Grizotto *et al*. (2012), stable sausage emulsion was 85.23%. That showed JBPI had good water and fat binding ability to increase the emulsion stability in chicken sausage. Emulsion stability was influenced by the interface protein-membrane and the emulsion globule (Jiang and Xiong, 2015). The higher the emulsion stability, the more protein that wraps the fat lumps, and the fat lump size was uniform (Shanti *et al*., 2017). Chicken sausages with JBPI, SPI, and a combination of SPI and JBPI had a uniform lump size and were evenly distributed compared to the control of chicken sausages. JBPI had a high capacity (52.78±1.67%) and emulsion stability (51.67±2.54%), so it can be used as a binder in chicken sausages (Murdiati *et al*., 2014). JBPI's good emulsion properties had the potential for food products such as meat analogues, especially in products that require heating. Therefore, the protein was adsorbed at the fat/water interface, thereby reducing the interface tension. That can prevent the incorporation of fat lumps and fat leakage from the product (Xu *et al*., 2013). JBPI can also help form and stabilize thin films such as trapped and stable and/or immovable surface fat droplets in the continuous phase of the meat emulsion matrix's complex/surface (Kumar *et al*., 2016).

The cooking loss of chicken sausage with JBPI and SPI can be seen in Figure 1. Control chicken sausage had the highest cooking loss (26.54±0.73%) and was significantly different compared to other treatments (p<0.05). Cooking loss of chicken sausage with T2 treatment was significantly different from T3, T4, T5, and T6 (p<0.05). With JBPI and SPI's addition, the chicken sausage had a lower cooking loss than without protein isolate. That was following the cooking loss results for frankfurter sausages containing SPI, which was lower than without SPI (Kim *et al*., 2009). Cooking loss was influenced by the loss of water during cooking, this condition was influenced by the protein that can bind water, the more water was retained by the protein, the less water comes out so that the cooking loss is reduced (Toldra, 2017). The cooking loss value was influenced by the stability of the chicken sausage emulsion. The higher the emulsion stability, so that the lower the cooking loss of chicken sausage. The lowest cooking loss in control chicken sausage was due to 69.44±2.41% low emulsion stability. Also, the cooking loss was influenced by the water holding capacity (WHC) and oil holding capacity (OHC) of the protein isolates used in making sausages. The cooking loss value of chicken sausage with T2 treatment was lower than other treatments because SPI had WHC and OHC (3.05±0.06 mL water/g; 2.59±0.18 mL oil/g), which were higher than JBPI (1.88 mL±0.97 water/g); 2.33±0.57 mL oil/g) (Murdiati *et al*., 2014). JBPI had a reasonably high WHC

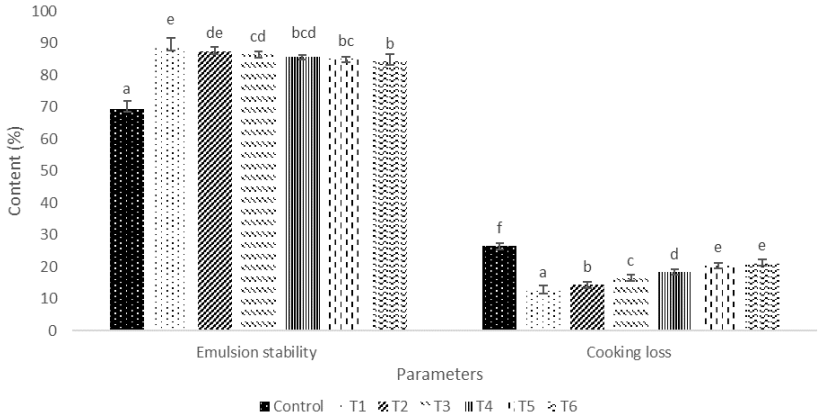

Figure 1. Emulsion stability and cooking loss of chicken sausages with different ratios of JBPI and SPI. Differences in letters indicate significant differences (p<0.05). Treatments: Control (and/or SPI and JBPI); Sausage formulation with SPI: JBPI; T1 (SPI: JBPI = 100:0); T2 (SPI: JBPI = 80:20); T3 (SPI: JBPI = 60:40); T4 (SPI: JBPI = 40:60); T5 (SPI: JBPI = 20:80); T6 (SPI: JBPI = 0:100).

value, so it can be used as a binder in sausages to increase protein binding capacity. The high OHC value in JBPI has the potential for structural interactions in food, especially in taste retention, palatability improvement, and extending the shelf life of meat products through reducing water and oil losses (Chel-Guerrero *et al*., 2002). Also, the high OHC value in JBPI was significant in food formulation, so that it can be used as a binder in sausages, which can increase the protein's capacity to protein and fat (Akinyede and Amoo, 2009; Shad *et al*., 2013). The interaction of oil and water with protein was critical in the food system because they affect the taste and texture of a food product (Foh *et al*., 2012).

The lightness (L* -value), redness (a* -value), and yellowness (b* -value) of chicken sausages with JBPI, SPI, a combination of JBPI and SPI can be seen in Figure 2. The L* value ranges from 0-100 with interpretation dark-bright colours. The highest L* value (lightness) was shown in T6 treatment chicken sausages (74.72±0.35) and was significantly different from all treatments (p<0.05). Chicken sausage with T2 treatment had L * -the value which was not significantly different from the T3, T4, and T5 treatments (p>0.05), but significantly different from the T2, T6, and control treatments (p<0.05). Based on the colour analysis results, chicken sausage with JBPI had a higher L*-value than a chicken sausage with SPI. The higher the JBPI addition ratio, the higher the brightness value of the chicken sausage. JBPI had a higher L*-value (84.51±0.84) compared to SPI (80.65±0.01). The addition of JBPI could increase the lightness value of chicken sausages. L*-value of the control chicken sausage (70.85±0.65) was lower than the sausage with SPI (71.91±0.71). SPI's addition to pork sausages could increase chicken sausages' lightness level compared to meat sausages without SPI and JBPI (Akesowan, 2008; Le *et al*., 2017). The +a (positive) value of 0 - 100 represents a red colour, and –a (negative) value of 0 - (-80) represents a green colour. The a* -values of control chicken sausage was significantly different from treatment T1, T2, and T6 (p<0.05), but not significantly different from treatments T3, T4, and T5 (p>0.05). JBPI had a lower a* -value (-1.48±0.27) compared to SPI (0.85±0.01). The + b (positive) notation of 0 - 70 represents yellow, and the –b (negative) value of 0 - (-70) represents blue. Control chicken sausage had a significantly different b* value with all treatments (p<0.05). The b* value of chicken sausage with T1 treatment was not significantly different from T2, T3 (p>0.05), but significantly different from T4, T5, and T6 (p<0.05). Chicken sausage with JBPI had the lowest b* value (12.73±0.26) because JBPI had a lower b* value (12.83±1.30) than SPI (15.33±0.01). JBPI had a high lightness colour, but low redness and yellowness.

The texture of chicken sausage with different ratios of JBPI and SPI was shown in Figure 3. Control chicken sausage had the lowest texture (0.59 N) and was significantly different from all treatments, except for the T6 treatment (p<0.05). The texture of T1-treated chicken sausage was not significantly different from the other treatments (T2, T3, T4, T5, and T6) (p>0.05). The higher the cooking loss of chicken sausage, the lower the hardness level of the chicken sausage. The lower the hardness of chicken sausage due to the sausage's less stable emulsion so that the sausage's texture was less compact and the presence of air cavities. The sausage formulation's emulsification ability influenced the decrease in hardness, springiness, and cohesiveness (Daros *et al*., 2005). The emulsion's ability to bind water and fat affected the texture of meat products (Yotsuyanagi *et al*., 2016). Also, the higher amount of water and fat in the chicken sausage formulation causes a good texture in the resulting sausage (Zhao *et al*., 2018). JBPI and SPI had high WHC and OHC, leading to increased hardness of chicken sausages produced. The gelation properties of JBPI influenced the texture formation of sausage products. The best gel formation

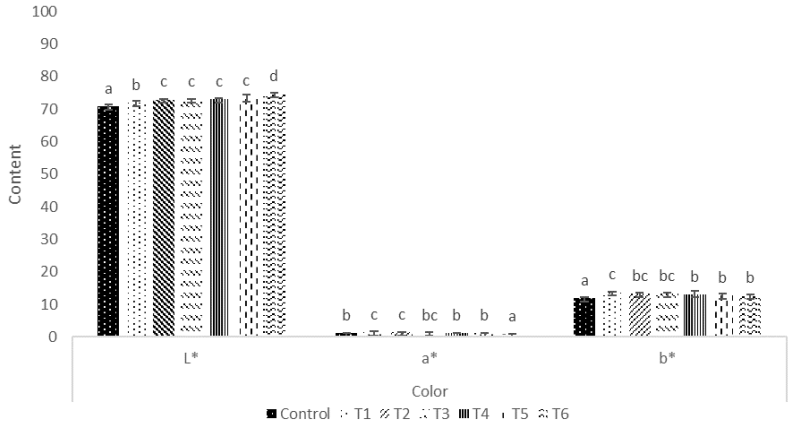

Figure 2. Colour of chicken sausages with different ratios of JBPI and SPI. Differences in letters indicate significant differences (p<0.05). Treatments: Control (and/or SPI and JBPI); Sausage formulation with SPI: JBPI; T1 (SPI: JBPI = 100:0); T2 (SPI: JBPI = 80:20); T3 (SPI: JBPI = 60:40); T4 (SPI: JBPI = 40:60); T5 (SPI: JBPI = 20:80); T6 (SPI: JBPI = 0:100).

concentration was at JBPI 18%, and JBPI gel strength was 0.30±0.02 N (Murdiati *et al*., 2014). The matrix in the gel structure was held water and fat and gave texture to the sausage product. The addition of SPI could increase the hardness of frankfurters (Kim e*t al*., 2009). The increase in hardness was also caused by the fat globules' small size and the high protein content in the sausage gel tissue (Youssef and Barbut, 2010). Other factors that affect texture are nature's protein, water content, type, and percentage of fat, addictive ingredients (Liu, Stevenson and Lainer, 2013; Hashemi and Jafarpour, 2016).

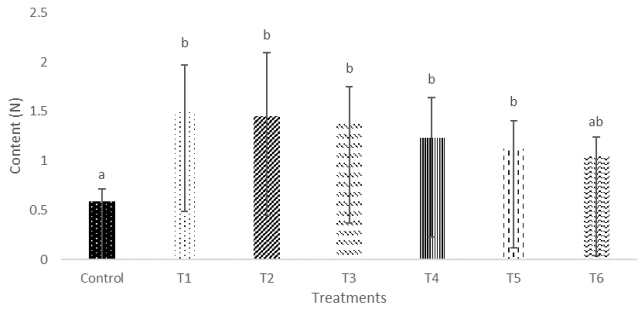

Figure 3. The texture of chicken sausages with different ratios of JBPI and SPI. Differences in letters indicate significant differences (p<0.05). Treatments: Control (and/or SPI and JBPI); Sausage formulation with SPI: JBPI; T1 (SPI: JBPI = 100:0); T2 (SPI: JBPI = 80:20); T3 (SPI: JBPI = 60:40); T4 (SPI: JBPI = 40:60); T5 (SPI: JBPI = 20:80); T6 (SPI: JBPI = 0:100).

3.4 Sensory properties of chicken sausages

The sensory test was carried out, including the preference test of 30 semi-trained panellists. The results of the chicken sausage preference test can be seen in Figure 4. The sensory evaluation results on the colour and flavour attributes were not significantly different in all treatments and controls (p>0.05). The sliced characteristics and texture attributes of chicken sausage with JBPI and the combination of JBPI and SPI were significantly different from the control (p<0.05). The appearance attributes were not significantly different from the control (p>0.05). Based on the results of sensory testing on colour, it showed that all chicken sausage treatments were not significantly different (p>0.05) (Figure 4.). The higher the JBPI ratio added, the higher the chicken sausage's lightness produced, although it did not differ significantly in the panellists' preference. According to Kamani *et al*. (2019), SPI's addition did not affect the chicken sausage produced.

The sensory analysis results on the appearance attribute showed chicken sausage treatment T2, T3, T4 was not significantly different from T1 and control (p>0.05). However, it was significantly different from the T5 and T6 treatment chicken sausages (p<0.05) (Figure 4). The combination of JBPI and SPI produced chicken sausages that were compact compared to control. SPI could increase the firmness of chicken sausages and light pork sausages (Akesowan, 2008; Kamani *et al*., 2019). The type and ratio of binders used in the manufacture of sausages affected the emulsion's stability. The addition of protein isolate could increase water (WHC) and oil holding capacity (OHC) during protein gelation. In the processing of meat products containing protein isolates, WHC and oil absorption during emulsification were significantly increased (Dong *et al*., 2020).

Based on the results of the panellists' preference test on the properties of the chicken sausage slices, all treatments were significantly different from the control (p<0.05) (Figure 4). The properties of T2, T3, T4 treatment of chicken sausage slices were not significantly different from T1 (p>0.05). Besides, the properties of T4 chicken sausage slices were not significantly other than T5 and T6 (p>0.05). The combination of JBPI and SPI and only JBPI resulted in smoother sliced chicken sausage than the control. SPI's addition to meat dough could interfere with gel tissue formation in meat emulsion, resulting in a soft texture (Youssef and Barbut, 2011). The stability of the sausage emulsion also affected the properties of the chicken sausage slices. The more stable emulsion of the chicken sausage, so that the finer the sliced properties.

The panellists' preference for flavour (aroma and taste) of all chicken sausage treatments was not significantly different from the control (p>0.05) (Figure 4). Panellists could not distinguish the flavour, i.e., aroma and taste of chicken sausage with various added binder types and ratios. The basic ingredients of beans (JBPI and SPI) can cause off-flavours to products produced with different intensities, reducing the product's sensory value (Kamani *et al*., 2019). Kim *et al*. (2009) reported that frankfurter-type sausages did not have beany flavour with the addition of 2% SPI and a combination of wheat fibre and SPI, each by 1%. The flavour intensity decreased with the higher the addition of JBPI, but it was not significantly different (p>0.05). JBPI possibly still had an off-flavour component. Panellists could still receive the aroma and taste of chicken sausage products with the addition of JBPI.

The results of panellists' preference for the texture of chicken sausage in all treatments were significantly different from the control (p<0.05) (Figure 4). The texture of chicken sausage between treatments was not significantly different (p>0.05). Wang, Liang, Jiang *et al*. (2015) reported that SPI could increase meat protein's hardness due to increased hydrophobic interactions. SPI

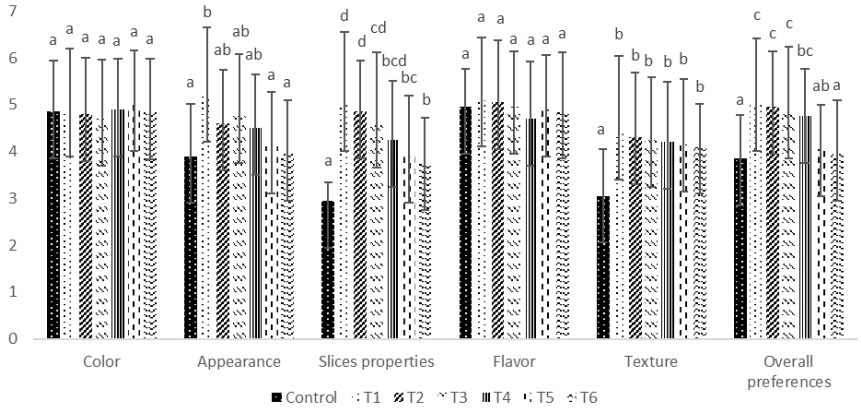

Figure 4. Sensory evaluation of chicken sausages with different ratios of JBPI and SPI. Differences in letters indicate significant differences (p<0.05). Treatments: Control (and/or SPI and JBPI); Sausage formulation with SPI: JBPI; T1 (SPI: JBPI = 100:0); T2 (SPI: JBPI = 80:20); T3 (SPI: JBPI = 60:40); T4 (SPI: JBPI = 40:60); T5 (SPI: JBPI = 20:80); T6 (SPI: JBPI = 0:100).

could also improve analogue sausage texture (Wi *et al*., 2020). The hardness intensity of chicken sausage decreased with the higher the addition of JBPI. Chicken sausage hardness was influenced by the emulsion stability and sausage cooking loss. The addition of JBPI and SPI could increase emulsion stability and reduce cooking loss so that the hardness of chicken sausage produced was higher than the control (without the addition of JBPI and SPI).

Based on the results of the sensory analysis in overall attributes, i.e., colour, appearance, slice properties, flavour, and texture, it can be seen that the chicken sausage treatment T1, T2, T3, and T4 was significantly different from the control (p<0.05) (Figure 4). On the other hand, chicken sausages with T5 and T6 treatments were not significantly different from the control *(p*>0.05). Panellists like T1, T2, T3, and T4 treatment of chicken sausage on all attributes. The T5 and T6 treatment chicken sausages were still acceptable by the panellists in the attributes of colour, slice properties, flavour (aroma and taste), and texture. Otherwise, panellists tended to dislike the chicken sausage appearance in the T5 and T6 treatments, even though the appearance was more compact than the control. In this study, it was interesting that the addition of the combination of JBPI and SPI to chicken sausage was preferred by the panellists so that JBPI could be used as an alternative to SPI substitution.

3.5 Proximate properties of chicken sausages

The results of the proximate analysis of chicken sausage can be seen in Figure 5. The moisture content of chicken sausage in T1 and T3 treatments was significantly different from the control (p<0.05). Meanwhile, chicken sausage with T2, T4, T5, and T6 treatments had no significant moisture content difference with the control (p>0.05). All chicken sausage and control treatments have moisture content following the Indonesian National Standard 3820: 2015 (SNI), with a maximum moisture content of 67% wb (SNI, 2015). Akesowan (2008) reported that chicken sausage's water content with SPI was higher than the control. With the addition of JBPI, the chicken sausage had a lower moisture content than the chicken sausage with SPI. Zhao *et al*. (2018) reported that the water holding capacity (WHC) affected the moisture content of the sausages produced. The higher the WHC of the ingredients formulated into the sausage, the higher the sausage's moisture content. JBPI has a lower WHC (1.88±0.97 mL water/g) compared to SPI (3.05±0.06 mL water/g), thus affected the moisture content of chicken sausages (Murdiati *et al*., 2014).

Ash content of chicken sausage with T1 treatment was not significantly different from T2, T3, T4, and control (p>0.05) (Figure 5). In contrast, chicken sausage with T6 treatment was significantly different from all treatments and controls (p<0.05), while T5-treated sausages were not significantly different from T4 and control (p>0.05). Ash content of chicken sausage in all treatments and controls according to SNI, with a maximum of 3% wb (SNI, 2015). With SPI, the chicken sausage had a higher ash content than a chicken sausage with JBPI. The ash content of the isolates used affected the chicken sausage produced. The ash content of SPI (3.64±0.05% db) was higher than that of JBPI (1.85±0.10% db) (Brishti *et al*., 2017).

The proximate analysis results showed that the protein levels of chicken sausage in all treatments were significantly different from the control (p<0.05) (Figure 5). The protein content of chicken sausage with T1 treatment was significantly other from T4, T5, and T6 (p<0.05). However, it was not significantly different from chicken sausage with T2 and T3 treatments (p>0.05). All chicken sausage and control treatments have protein levels following SNI with a minimum protein of 13% wb (SNI, 2015). The addition of JBPI and SPI resulted in higher protein content of chicken

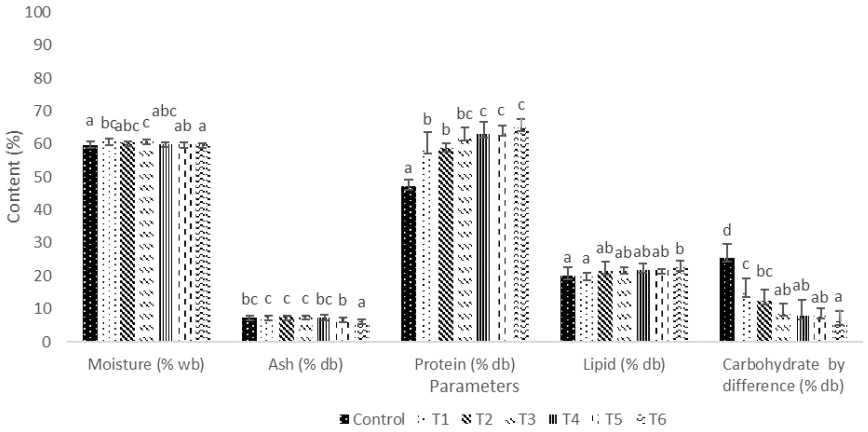

Figure 5. Proximate properties of Chicken Sausages with different ratios of JBPI and SPI. Differences in letters indicate significant differences (p<0.05). Treatments: Control (and/or SPI and JBPI); Sausage formulation with SPI: JBPI; T1 (SPI: JBPI = 100:0); T2 (SPI: JBPI = 80:20); T3 (SPI: JBPI = 60:40); T4 (SPI: JBPI = 40:60); T5 (SPI: JBPI = 20:80); T6 (SPI: JBPI = 0:100).

sausage than the control. Chicken sausage, with the addition of JBPI, had a higher protein content than sausages given SPI. T6 treatment chicken sausage had the highest protein content of 64.87±2.78% db. The protein content of JBPI (93.98±0.43% db) was higher than that of SPI (90.10±0.86% db) (Brishti *et al*., 2017). That shows that JBPI has the potential as an alternative to substitute SPI.

Based on the results of the proximate analysis, the lipid content of chicken sausage in all treatments except T6 was not significantly different from the control (p>0.05) (Figure 5). All treatment and control's lipid content followed SNI, with a maximum of 20% wb (SNI, 2015). With the addition of JBPI, the chicken sausage had a higher lipid content than a sausage with SPI. That showed the isolates' lipid content affected the lipid content of the chicken sausage produced. The lipid content of JBPI (0.34±0.03% db) was higher than that of SPI (0.12±0.01% db) (Brishti *et al*., 2017).

The carbohydrate content of chicken sausage in all treatments was significantly different from the control (p<0.05) (Figure 5). Chicken sausage, with the addition of JBPI, had a higher carbohydrate content than sausages with SPI. JBPI had lower carbohydrate content (3.84±0.50% db) compared to SPI (6.04±0.88% db) (Brishti *et al*., 2017).

4. Conclusion

JBPI had a high protein content of 93.98% db. The profile of essential amino acids, i.e., leucine, lysine, phenylalanine + tyrosine, and threonine, was higher than the FAO/WHO reference standard. JBPI had the potential to improve the characteristics of sausages to be added in several parts of SPI. The combination of JBPI and SPI could improve chicken sausages' physical and chemical characteristics such as emulsion stability, colour lightness, texture properties, protein content, and reduce cooking loss. Chicken sausage formulation with the addition of a combination of SPI and JBPI of 40:60 was the optimal treatment to improve the overall physical, sensory, and chemical characteristics of the resulting chicken sausage. JBPI has the potential as an alternative to substitution for SPI.

Conflict of interest

The authors declare no conflict of interest.

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