Physicochemical and sensory characteristics of soy sauce substituted with pigeon pea (*Cajanus cajan* (Linn.)

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Abstract. The objective of this study wasto investigate the physicochemical and sensory properties of the soy sauce substituted with pigeon pea. Soybean was substituted by 20%, 50%, 75%, and 100% of pigeon pea. The observation included viscosity, total solids, protein levels, antioxidant activity, and sensory characteristics. The results showed that the more substitution of pigeon pea, the less the protein content of soy sauce and the more the antioxidant activity as well as total solids. The most favored group was 25% pigeon pea substitution. It is suggested that soy sauce could be prepared using 25% to 75% pigeon pea substitution.

Keywords: soy sauce, pigeon pea

1. Introduction

Pigeon pea *(Cajanuscajan* (Linn.))is a group of nuts with 20.7% of proteinand 1.4% of fat contents and it could be used as black soybean substitution in making soy sauce due to their similar properties in shape, color, and seed size [1]. Pigeon pea also contains antioxidants such as polyphenols, isoflavones, phenols, phenolic acids and derivatives, flavonoids, tocols, phospholipids, amino acids, peptides, phytic acid, ascorbic acid, pigments, and sterols [1]. However, pigeon pea containsa low level of antinutrition compounds such as antitrypsin, hemagglutinin, and phytic acid. Phytic acid is not desirable because it can bind minerals and proteins and decrease the bioavailability of iron in the body. The content of phytic acid in pigeon pea flour is 0.77% and can be eliminated. Washing, soaking, boiling, steaming, and fermentation process can decrease phytic acid by 91-97% [2].

Cikuray and Merapi black soybean are two best black soybean varieties and suitable for producing soy sauce due to their high protein content at around 37% [3]. Soybean has globular protein which is soluble in water or salt solution at a pH value below or above the isoelectric point [4]. Amino acids found in black soybean are leucine and lysine, essential amino acids needed by soy-breaking enzymes to produce soy sauce with tasty and distinctive tastes.

In Indonesia, soy sauce is *kecap manis*, literary means sweetened soy sauce. Soy sauce is produced by fermentation process which activates the growth of the required microorganisms. Fermentation process breaks down the long molecular chains into simpler ones. Fermented foods have higher nutritional values than their original ingredients because the microorganisms convert the complex proteins, fats, and polysaccharides into simpler and higher-digesting agents. Fermentation process alsotraditionally improvesflavor and the food quality as the products are easier to digest and lower in anti-nutritive substances [5]. This research aims to investigate the physicochemical and sensory properties of the soy sauce substituted with pigeon pea.

2. Materials and Methods

2.1. Materials and Tools
Materials used in this study includedblack soybeans, pigeon peas obtained from Gita Pertiwi Foundation in Surakarta, inoculum *tempe*Raprima, brown sugar, salt, galangal, bay leaf, lemongrass leaves, orange leaves, deaf, K_2SO_4, HgO, concentrated H_2SO_4, $Na_2S_2O_3$-NaOH , 4% boric acid, methyl red and methylene blue indicator, 0.1 N HCl, methanol, DPPH (2,2-diphenyl1-1-picrylhydrazyl), and aquades. The tools used were stove, pot, *tampah*, filter cloth, bottle, plastic bucket, strainer, wood stirrer, analytical scales, cormorant, pounder, *cobek*, viskotester, destructive, destilator, refractometer, burette, statif, erlenmeyer, bekker glass, pumpkin powder, porcelain cup, spectrophotometer, waterbath, and hotplate.

2.2. *Materials Preparation.*
Black soybeans and pigeon peas were sorted and cleaned through running water. Black soybeans were soaked in 3 liters of water for 24 hours while pigeon peas were soaked for 48 hours in water which was changed every 6 hours. The black soybeans and pigeon peas were boiled for 60 minutes to soften the texture, drained, and fully peeled. Then, they were steamed for 30 minutes, drained, and chilled at 30^0C. Last, the materials were prepared into 4 groups which contained 1 kg of materials for each group. The groups respectively contained 25%, 50%, 75%, and 100% of pigeon peas.

2.2.1. Koji Fermentation. The yeast required in koji fermentation was 1% of the soybean net weight of each group, sprinkled over the materials on a *tampah* (clean tray). The materials were incubated at room temperature for 3-4 days in a rather dark place. After the hyphae were formed and the structure of the materials became compact, the koji then was dried [2].

2.2.2. Moromi Fermentation. The dried koji was soaked into a 20% salt solution for up to 2 months. One kg of materials requires 2 liters of 20% salt solution. During this incubation process, the mixture needed to be sunbathed and stirredevery morning and closed and stored every afternoon [2].

2.2.3. Cooking Moromi Filtrate with Seasoning Spices. After 2 months, the soy sauce filtratewas filtered and cooked with Javanese sugar and mashed-roasted spices until it boiled, caramelized, and thickened, forming the desired texture. During the cooking process, the filtrate should be continuously stirred so that the heat evenly spread and all the ingredients well-blended. Next, the boiled soy sauce was filtered using a filter cloth to remove the solids [6]. The pigeon pea soy sauce formulasare modified was presented in Table 1.

Table 1. The pigeon pea soy sauce formula.

Materials	Pigeon pea 25%	Pigeon pea 50%	Pigeon pea 75%	Pigeon pea 100%
Pigeon pea (g)	250	500	750	1000
Black soybean (g)	750	500	250	-
Lemongrass (g)	10	10	10	10
Citrus leaf (g)	10	10	10	10
Bay leaf (g)	10	10	10	10
Salt (g)	200	200	200	200
Brown sugar (g)	2000	2000	2000	2000
Star anise (g)	10	10	10	10
Water (ml)	3000	3000	3000	3000

2.3. Physicochemical and Sensory Analysis

2.3.1. Viscosity. A 100 ml of the samples from each group was measured using rotor viscotester three [7].

2.3.2. Total Solids. The process included putting the porcelain cup into the kiln for 1 hour with a temperature of 100^0C, weighing it with a desiccator (a gram), putting 5 ml of soy sauce on the porcelain cup, weighing it (b gram), inserting the porcelain cup into an oven at 100^0C for 24 hours, cooling the cup in desiccator for 10 minutes, and weighing the cup (c gram). The total dissolved solid (TDS) is calculated by the formula [8]:

$$TDS = \frac{c - a}{b} \times 100\,\%$$

Information : a : weighing the cup
b : weighing the cup + sample before dried
c : weighing the cup + sample after dried
(Apriyantono *et al.,* 1989).

2.3.3. Sugar Level and Salinity. The process included diluting 1 g of sample in 10 ml of aquades, cleaning the refractometer prism and the lid with alcohol, dripping the solution on the refractometer prism, and directing light through the refractometer prism until the focal point between bright regions and dark areas was clear. The values appearing on the brix refractometer are calculated as thebrix value in soy sauce [9].

2.3.4. Protein Level. The process included putting 1 g of sample (1 g of aquades for blanko) into the destruction flask, adding 7 grams of K_2SO_4, 0.35 gram HgO, and 15 ml of concentrated H_2SO_4 to the flask, destructing the solution for 20 minutes at 420^0C or 60 minutes at 370^0C, cooling the solution, transferring the solution into a distillation flask while rinsing it with 50 ml of aquades, adding 400 grams of $Na_2S_2O_3$ and 25 grams of NaOH, and diluting the solution in the distillation flask until it reached a volume of 1000 ml. Next, 25 ml of 4% boric acid was prepared in a distillate erlenmeyer. The distillation was carried out until 100 ml distillate was obtained. Then, the distillate was added by 2 drops of methyl red and methylene blue mixture and titrated with 0.1 N HCl until the distillate became purple[6]. The formulas to calculate % N and% proteins are:

$$\% \text{ Nitrogen} = \frac{\text{HCl volume (sample-blanco) x N HCl}}{\text{Sample weighing (gram) x 100}} \text{ x } 14{,}008 \text{ x } 100\%$$

% protein =% N x conversion factor (6.25)

*2.3.5. Antioxidant Activity.*The process included measuring 0.5 ml of the sample (methanol as blanko) and extracting it using 5 ml methanol for 2 hours, taking 0.1 ml of the extraction and mixing itwith 3.9 ml of DPPH solution (2,2-diphenyl1-1-picrylhydrazyl) for 30 minutes of chemical reaction, and measuring the absorbance at t = 0 for control and t = 30 for sample using a 517 nm wavelength [10]. The antioxidant activity was measured as% discoloration calculated using formula:

% discoloration = [1- (At30 / At0)] x 100

Information

At 30 = sample, At 0 = control

*2.3.6. Sensory Characteristics.*The 4 groups of pigeon pea soy sauce and commercial soy sauce as control were evaluated for their color, odor, taste, and overall appearance using hedonic rating methodand 30 panelists. The attributes was expressed by a score of 1 to 5 written in a questionnaire sheet, with 1 as least preferredand 5 as the most preferred. The highest average of the resultsdetermined the most preferred group [11].

2.4. *Statistical Analysis*
Data were subjected to ANOVA (and Duncan test for any significant results with p <0.05) to determine significant differences between groups using SPSS 13 for Windows statistical application program. The Mann-Whitney test was used to determine the real difference between variables. Sensory characteristics data were analyzed using Kruskal-Wallis and Mann Whitney test [12].

3. Results and Discussion
In this study, the characteristics of the pigeon pea soy sauce were compared to the characteristics of the commercial soy sauceas control. Each group of the pigeon pea soy sauce yielded about 2,125 ml. The data of viscosity, sugar level, and salt level are presented in Table 2.

Table 2. Viscosity, sugar, and salt level of the commercial soy sauce and pigeon pea soy sauce.

Soy Sauce	Viscosity (dPaS)	Sugar level (%)	Salt level (%)
Commercial	8.5	74	7
Pigeon pea 25%	8.5	74	6
Pigeon pea 50%	8.5	74	6
Pigeon pea 75%	8.5	74	6
Pigeon pea 100%	8.5	74	6

The result showed that there were no differencesof viscosity and sugar level between pigeon pea soy sauce and commercial soy sauce. Commercial soy sauce had a higher salt level compare to pigeon pea soy sauce. The salt level might be influenced by the different formulas and moromi fermentation process. The immersion of koji in salt solution increased the soy sauce saltiness. This process also meant to prevent thegrowth of harmful microbeswhile still allowing the growth of bacteria and taste-shaping yeast. The salt solution serves to extract the dissolved nitrogen compounds resulting from the hydrolysis of the mold enzymes in soybeans. Without salt, an undesirable anaerobic fermentation process occurs [4,13]. During the salting process, every morning the koji needed to be sunbathed and stirred, while every afternoon it needed to be closed and stored again. During this incubation process, the koji must be stirred so that the salt solution can homogeneously touch the surface of the substrate and provide air to stimulate the growth of yeast and flavor-forming bacteria [13,14].

Viscosity is one of the physical attributes that determines the quality of soy sauce. Viscosity equalization is needed so that the quality of the pigeon pea soy sauce and commercial soy sauce is similar with standard viscosity of 8.5 dPaS. Therefore, the cooking process of all groups stopped when they reached the same viscosity as the commercial soy sauce. The total content of solids, protein level, and antioxidant activity of the soy sauce groups are presented in Table 3.

Table 3. Total dissolved solids, protein, and antioxidants activityof the commercial soy sauce and pigeon pea soy sauce^{AB}.

Soy Sauce	Total solids (%)	Protein level (%)	Antioxidant activity (%)
Commercial	15.67±0.26^{d}	3.51±0.02^{b}	90.31±0.84^{a}
Pigeon pea 25%	13.21±0.25^{a}	3.70±0.04^{e}	92.59±0.29^{b}
Pigeon pea 50%	13.72±0.12^{b}	3.63±0.02^{d}	93.15±0.20^{c}
Pigeon pea 75%	14.38±0.32^{c}	3.54±0.02^{c}	93.55±0.21^{cd}
Pigeon pea 100%	15.41±0.36^{d}	3.46±0.02^{a}	93.91±0.14$_{d}$

^{A}The result is the mean value ± standard deviation.
^{B}Different letters show significant difference values ($p<0.05$).

The total solids of commercial soy sauce had the same value as 100% pigeon pea soy sauce. The protein levels in all groups of soy sauce were significantly different due to the different amount of pigeon pea substitution. The more amount of pigeon pea used for substitution in soy sauce, the fewer the protein level of soy sauce because the protein level of pigeon pea is lower than black soybean. However, the higher concentration of pigeon pea added, the more the antioxidant activity of soy sauce. The carbohydrate level of pigeon pea is higher than black soybean, resulting in increasing the total solids of soy sauce. In food material, some solids are in soluble form and some are in non-solvent form. Soluble solids usually consist of organic substances, inorganic salts and, dissolved gases [2].

Protein level in pigeon pea soy sauce and commercial soy sauce are between 3-4%. According to *Standar Nasional Indonesia* (SNI) 3543-01-2013, the minimum protein levelof soy sauce is 2%. The pigeon pea substitution decreased the protein level of soy sauce [15]. Soy sauce has more carbohydrate than protein level due to the use of brown sugar as one of the seasoning spices. Moromi extract has complete nutrients with amino acids. Almost all sorts of amino acids are present in soy sauce, including hydrophobic amino acids (valine, leucine, and isoleucine), hydrophilic amino acids (glutamic, glycine, and aspartate) and aromatic amino acids (phenylalanine) [16].

Sensory analysis on pigeon pea and commercial soy sauce (Table 4) showed that 25% pigeon pea soy sauce had the highest score on the overall parameter. It showed that pigeon pea can substitute black soybean for producing soy sauce.

Table 4. Sensory Characteristics of the commercial soy sauce and pigeon pea soy sauce.

Soy Sauce	Color	Aroma	Taste	Mouth feel	Overall
Commercial	3,60^{b}	3,20^{b}	3,73^{b}	3,47^{ab}	3,67^{ab}
Pigeon pea 25%	4,27^{bc}	3,83^{b}	3,60^{ab}	3,77^{b}	4,20^{b}
Pigeon pea 50%	3,87^{bc}	3,63^{b}	3,27^{ab}	3,13^{a}	3,37^{a}
Pigeon pea 75%	3,00^{a}	3,43^{ab}	3,60^{ab}	3,17^{a}	3,40^{a}
Pigeon pea 100%	2,63^{a}	2,97^{a}	3,10^{a}	2,90^{a}	3,33^{a}

[A] The result is the mean value ± standard deviation.
[B] Different letters show significant difference values (p<0.05).
[C] The score interpretation are 1 = very unacceptable, 2 = unacceptable, 3 = quite acceptable, 4 = acceptable, and 5 = very acceptable.

The mouthfeel of pigeon pea 25% had the best score among other concentrations. It is related to the total solids presented in soy sauce. Pigeon pea 100% had a high total solid content which gave a specific taste in mouth feel attribute. Besides, pigeon pea 100% had a stronger flavor of pigeon pea which made it less favored by the panelists.

4. Conclusion

Based on the sensory analysis and protein level results, the best formula of pigeon pea soy sauce waspigeon pea 25%. The more amount of pigeon pea used for substitution in soy sauce, the fewer the protein level of soy sauce and the more the total solids and antioxidant activity of soy sauce. Higher amount of pigeon pea decreased the panelists' acceptance of soy sauce.

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