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Physicochemical characteristics of jack bean (*Canavalia ensiformis* (L.) DC.) milk, a non-dairy milk alternative developed using various pretreatment methods

*Virly, Sutedja, A.M., Trisnawati, C.Y., Kaharso, V.C., Ivana, F. and Asali, K.

Department of Food Technology, Faculty of Agricultural Technology, Widya Mandala Surabaya Catholic University, 60265, Indonesia

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Abstract

The endeavor to turn jack bean (*Canavalia ensiformis* (L.) DC.) into jack bean milk faced a challenge regarding its hard skin, which makes pretreatment of the jack bean before processing essential to soften and ease the dehulling of beans, as well as aid the grinding process. However, different pretreatment methods influence the characteristics of treated jack beans and jack bean milk as the final product. This study, thus, aimed to investigate the effect of different jack bean pretreatment methods such as soaking in $NaHCO_3$ solution (P_1), soaking in hot water (95°C) (P_2), and a combination of boiling and soaking in water (P_3), on the physicochemical properties of jack bean milk. Jack bean milk was prepared by subjecting jack beans to pretreatment, dehulling, wet grinding, filtering, and heating before proceeding with analyses based on proximate composition, phase behavior, color and viscosity. The results demonstrated that P_2 produced the highest protein (7.01±0.11%), fat (0.10±0.00%), and ash contents (0.11±0.00%) of jack bean milk. P_1 yielded higher protein and ash contents of jack bean milk than that of P_3, while no significant differences in fat contents were discovered in both P_1 and P_3. Besides, a slight difference in carbohydrate contents was found among all treatments, ranging from 1.17 to 1.20%. The phase behavior and color analyses showed P_1 and P_2 to significantly generate higher colloidal stability and whiteness index value of jack bean milk than P_3. In addition, jack bean milk from P_2 possessed the highest viscosity value of 48.1±1.4 cP, followed by P_1 (28.6±0.6 cP) and P_3 (12.6±0.5 cP). Accordingly, the recent study proposes the pretreatment of soaking in hot water (95°C) to be applied in jack bean milk processing and provides new insights into how pretreatment methods affect the characteristics of plant-based milk products.

1. Introduction

Milk is the liquid secreted by the mammary glands of mammals to fulfill the nutrition of newborn mammals. The wide array of nutrients in milk makes people adopt milk consumption to meet the body's daily nutritional needs, with cow's milk dominating the world's consumption. However, cow's milk cannot be consumed by lactose-intolerant people due to the body's limitation in producing lactase to digest lactose in cow's milk, which results in digestive system disorders after consumption (Szilagyi and Ishayek, 2018). Furthermore, the adoption of a vegan diet that is limited only to food comprising plants by some community groups has also increased the demand for plant-based food and beverage products. The mentioned phenomena thus urge the innovation of plant-based lactose-free milk products as an alternative to cow's milk (Jeske *et al*., 2017; Munekata *et al*., 2020).

Plant-based milk can be referred to as homogenized plant matrices, such as cereals, nuts, legumes, and seeds, which can be obtained by extracting the soluble parts from a wet grinding process (Bocker and Silva, 2022). One example of a plant-based milk product that has been widely sold in the market is soybean milk. The fact that the availability of soybeans in Indonesia is still dependent on imports from foreign countries makes research on local food ingredients that can be processed into plant-based milk deemed necessary to be conducted (Harsono *et al*., 2021).

Jack bean (*Canavalia ensiformis* (L.) DC.) is one type of legume easily found in Indonesia but still

*Corresponding author.
Email: *virly@ukwms.ac.id*

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underutilized by the local community. The nutritional profile of the jack bean comprises relatively high amounts of carbohydrate (58.4%) and protein (25.2%) while low in fat content (5.21%) (Okomoda *et al*., 2016), which has led to findings of jack bean potency as fermented food (tempeh-like product) (Andriati *et al*., 2018) and a soybean substitute in animal feed production (Solomon *et al*., 2018). A recent study by Sutedja *et al*. (2020) also discovered jack bean to contain a bioactive compound called kaempferol glycoside that could inhibit α-glucosidase activity that links to anti-diabetic properties. These characteristics further address the potential of the jack bean to be further developed into plant-based milk with functional properties.

The basic processing steps in making plant-based milk consist of pretreatment, dehulling, wet grinding, filtering, and pasteurization/sterilization. Here, the pretreatment step is essential for several purposes, including softening and easing the dehulling of raw materials and enhancing the extraction of proximate and other solid components (Aydar *et al*., 2020; Li, Cao, Tong *et al*., 2021). Previous studies reported several pretreatment methods to be applied in plant-based milk processing, such as soaking using various mediums (alkaline or acidic solution) (Li *et al*., 2019), heat treatment (soaking in hot water, boiling) (Pan *et al*., 2019), and a combination of boiling and soaking in water (Sutedja *et al*., 2022). However, different pretreatment methods produced different characteristics of the end products. For example, higher soaking temperatures increased solid loss from soybean and lowered the protein content of soymilk. Besides, soaking soybean in a basic pH medium induced higher protein extraction than neutral and acid pH and affected the whiteness of soymilk (Pan and Tangratanavalee, 2003; Li *et al*., 2019). To the best of our knowledge, no studies have focused on varying pretreatment methods in jack bean milk processing and its effect on the quality of the end product.

Hence, this study aimed to evaluate different pretreatment methods for jack beans on the physicochemical properties of the produced jack bean milk. Three pretreatment methods of soaking in $NaHCO_3$ solution, soaking in hot water (95°C), and a combination of boiling and soaking in water were chosen by considering the ease of adoption by the local community. The present study results are thus expected to provide new insights into jack bean milk processing to produce an alternative lactose-free plant-based milk product.

2. Materials and methods

2.1 Materials

Jack beans with white color and oval shape were purchased from a local supplier in Temanggung, Indonesia. Drinking water and sodium bicarbonate were purchased from local markets in Surabaya, Indonesia. The chemicals used were of analytical grade. All experiments were conducted at the laboratory of the Agricultural Technology Department, Widya Mandala Surabaya Catholic University, Indonesia.

2.2 Pretreatment of jack beans

Jack beans that were free from dirt and contaminants were divided into three parts and treated with three different pretreatment methods based on procedures of Sutedja *et al*. (2021) with some modifications, such as soaking in 0.4% sodium bicarbonate ($NaHCO_3$) solution (P_1), soaking in hot water (95°C) (P_2), and a combination of boiling and soaking in water (P_3). P_1 was performed by soaking the jack beans (50 g) in 0.4% $NaHCO_3$ solution (250 mL) for 24 hrs at room temperature (25°C). P_2 was done by soaking the jack beans (50 g) in hot water with an initial temperature of 95°C (250 mL) for 24 hrs at room temperature (25°C). The combination of pretreatments (P_3) was performed by first boiling the jack beans (50 g) in water (250 mL) for 30 mins, followed by draining and soaking the boiled jack beans in water (250 mL) for 24 hrs at room temperature (25°C). The soaking solution of every pretreatment method was replaced with the same amount and condition as the fresh one every 12 hrs. Jack beans from every pretreatment method were subsequently dehulled, soaked in water (250 mL) for 1 hr at room temperature (25°C), drained, and used for making jack bean milk.

2.3 Preparation of jack bean milk

Jack bean milk was prepared by mixing the previously treated, dehulled jack beans with water (500 mL). The mixture was ground to a slurry using a blender (Philips HR 2115, Indonesia) with a speed of 2 for 1 min. The resulting slurry was manually strained using a filter cloth and the obtained liquid was heated for 3 mins at 85°C. The jack bean milk was cooled down at room temperature prior to analysis.

2.4 Proximate analysis

Proximate analysis carried out on jack bean milk included moisture, ash, protein, fat, and carbohydrate contents with the procedures outlined in Association of the Official Analytical Collaboration (AOAC) International (2016). The moisture and ash contents were determined thermogravimetrically using oven drying and dry ashing methods, respectively. The crude protein

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content was estimated using the macro-Kjeldahl method. The crude fat content was determined using the Soxhlet extraction method. Carbohydrate content was determined by difference.

2.5 Phase behavior

The phase behavior analysis was done according to the procedure by An *et al*. (2019) with some modifications. The jack bean milk was poured into a 100 mL graduated cylinder and let to stand for 12 hrs. Visual observation of the phase separation phenomenon and measurement of the volume of the separated layers were done every 1 hr.

2.6 Color

The color of the jack bean milk was measured using a color reader (Konica Minolta CR-10, Japan) with Commission Internationale de I'Eclairage (CIE) system. The results were expressed as lightness (L*), redness/ greenness (a*), and yellowness/blueness (b*). In addition, further characterization of jack bean milk's whiteness index (WI) that indicated the degree of jack bean milk's whiteness was performed according to Jeske *et al*. (2017) and defined as:

$$\mathrm{WI} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

2.7 Viscosity

The viscosity of the jack bean milk was analyzed using a viscometer (Brookfield DV-II+ Pro, Massachusetts, USA). The measurements were performed at 25°C and the results were expressed in centipoise (cP).

2.8 Statistical analysis

The experimental design used was a randomized block design (RBD) with one factor of pretreatment of jack beans; soaking in $NaHCO_3$ solution (P_1), soaking in hot water (95°C) (P_2), and a combination of boiling and soaking in water (P_3). All the analyses were done in triplicate, and the results were expressed as mean values ± standard deviation (SD). Any differences between treatments were analyzed using analysis of variance (ANOVA). The level of significance was determined at p<0.05 using Tukey's Test.

3. Results and discussion

3.1 Effect of pretreatment methods on proximate composition

The proximate composition of all produced jack bean milk obtained by three different pretreatment methods (P_1, P_2 and P_3) in Table 1 shows jack bean milk from P_2 had the lowest moisture content, which indicated higher solids in the jack bean milk suspension and further proved by containing the highest protein (7.01±0.11%, wb), fat (0.1±0.00%, wb), and ash (0.11±0.00%, wb) contents compared to P_1 and P_3. On the other hand, jack bean milk from P_1 possessed higher protein and ash contents than P_3. However, both P_1 and P_3 generated jack bean milk with the same fat content (0.07±0.00%, wb). Besides, insignificant differences in total carbohydrates were found in all treatments, ranging from 1.17 to 1.20% (wb).

The overall results depicted in Table 1 could conclude that different pretreatment methods interacted differently with the jack bean matrices, thus affecting the extractability of solid components of jack beans into the milk filtrate. In terms of the protein content, interactions derived from pretreatments affected the protein properties of jack beans, presumably resulting in a certain amount of protein still being contained in the jack bean dreg as the by-product of the filtration process. Therefore, the protein content of jack bean dreg from all treatments was analyzed to extend the aforementioned discoveries and results. As expected, jack bean dreg from P_2 had the lowest protein content (20.72±0.60%, db). In contrast, jack bean dreg obtained from P_1 and P_3 possessed higher protein contents with values of 26.72±1.00 and 25,24±0.87% (db), respectively.

Table 1. Proximate composition of jack bean milk made with different pretreatment methods.

Component (% w/w, wb)	Pretreatment methods		
	P_1	P_2	P_3
Moisture	92.66±0.05^b	91.61±0.07^a	93.20±0.20^c
Protein	5.98±0.14^b	7.01±0.11^c	5.45±0.04^a
Fat	0.07±0.00^a	0.10±0.00^b	0.07±0.00^a
Ash	0.10±0.00^b	0.11±0.00^c	0.08±0.00^a
Carbohydrate (by difference)	1.19±0.17^a	1.17±0.05^a	1.20±0.19^a

Values are presented as mean±SD of triplicates. Values with different superscripts within the same row are statistically significantly different (p < 0.05). P_1: soaking in 0.4% of sodium bicarbonate solution, P_2: soaking in hot water (95°C), P_3: a combination of boiling and soaking in water.

Several factors, such as temperature, influence protein properties. The boiling process in P_3 with a constant high temperature for a period of time can denature the protein within the jack beans and reduce its solubility. Sashikala *et al*. (2015) also found a similar finding in cooked green gram legumes. The heat treatment given to the green gram legumes eventually denatured the protein and led to the formation of aggregations of the unfolded chains, making the protein fraction less soluble. Another proposed possibility is that the starch in the jack beans can interact with the protein, form a starch-protein complex, and further aggregate during starch gelatinization along with continuous heating. This complex consequently traps the protein within the carbohydrate-protein gel matrix of jack beans and lowers its extractability and solubility (Shim and Mulvaney, 2001). Johansson *et al*. (2022) also addressed the starch-protein gel complex formation in faba beans after being subjected to heat. Hence, after being ground and filtered, a certain amount of the protein may still be retained within the jack bean dreg instead of dissolved in the filtrate, thus making the produced jack bean milk's protein content the lowest among other treatments. Although soaking in hot water with an initial temperature of 95°C (P_2) was also performed on the jack beans, it is worth mentioning that the hot water temperature gradually decreased over time. Therefore, the heat exposure was not harsh enough to cause a higher degree of protein or starch-protein gelation, making the final protein content of jack bean milk with P_2 the highest in all treatments.

Soaking jack beans in $NaHCO_3$ solution (pH 8.2) performed in P_1 also retained a higher amount of protein within the jack bean dreg. The results suggested that $NaHCO_3$ altered the jack bean's protein conformation and structure, reducing protein solubility in water. According to Lu *et al*. (2021), $NaHCO_3$ could induce protein denaturation by turning the random coil into a β-sheet structure and exposing the buried hydrophobic residues to the hydrophilic environment. A similar result was also reported by Li, Zhang, Lu *et al*. (2021) that the addition of $NaHCO_3$ exposed the internal hydrophobic amino residues to the molecular surface and increased the surface hydrophobicity of protein, as well as by Muhammad *et al*. (2018) mentioned crude protein content reduction in liquid whey as a result of neutralization with $NaHCO_3$ that caused precipitation and denaturation of protein. The conditions resulted in less maximum protein extraction into the water during jack bean grinding and lower protein content in the final jack bean milk.

Fat content was found to be significantly higher in jack bean milk from P_2 rather than P_1 and P_3 ($p<0.05$), which could be related to a higher degree of protein denaturation that occurred in jack beans after being boiled and soaked in $NaHCO_3$ solution. Protein unfolding due to denaturation exposes amino acids that can interact hydrophobically with fats to form insoluble gels, and this occurrence increases at higher temperatures and more extended time. This finding is in line with a previous study by Niyibituronsa *et al*. (2019) about soymilk prepared with different soybean preparation methods, where treating soybeans with cooking and blanching with $NaHCO_3$ solution generated lower fat contents of soymilk compared to soaking in water.

Ash content describes the amount of total minerals within foods. Significant differences in ash content results (Table 1) indicate that different pretreatment methods also affected the number of minerals contained in the jack bean milk. Moreover, the values tend to be the same as the protein content, where exposing jack beans to boiling and soaking (P_3) was found to generate jack bean milk with the lowest ash content, followed by soaking in 0.4% of $NaHCO_3$ solution (P_1) and soaking in hot water (95°C) (P_2). Therefore, mineral content reduction in jack bean milk is also suggested to have a relationship with the altering protein profile of jack bean milk after being subjected to pretreatment procedures. Heating becomes one factor that can change the conformation of chemical composition in the food matrix, including proteins, fats, carbohydrates and minerals. The boiling process (P_3) denatures the proteins and turns them insoluble. In this case, the solubility of minerals that are tightly bound to the proteins decreases by forming insoluble complexes with proteins after cooking; thus, a lesser amount of minerals can be extracted from the beans into the milk filtrate (Oliveira *et al*., 2018; Niyibituronsa *et al*., 2019). A similar insoluble protein-mineral complex formation due to protein denaturation might also occur after soaking jack beans in a higher pH medium (P_1). Besides preventing minerals from being less accessible, pretreatment methods performed by immersing beans in a liquid medium can raise the possibility of considerable changes in jack bean matrices that eventually lead to mineral leaching. During the immersion process, water will get into the beans' matrix and dissolve several water-soluble molecules, thus increasing the chance for the molecules to be carried out of the beans (Yulianti *et al*., 2022). Soaking beans in salt solutions, such as $NaHCO_3$, weakens the pectin layer that builds up plant cell walls by exchanging calcium ions contained within the pectin with sodium ions (Leng *et al*., 2019). Indeed, an increase in energy due to a higher temperature for an extended time favors further disintegration and separation of the cell membrane, as well as an increase in mass transfer rate, such as minerals (Huma *et al*., 2008; Pereira *et al*., 2020). In addition, a previous study by Rousseau *et al*. (2020) also discovered that certain minerals, such as Mg, are more sensitive to leaching during cooking at high temperatures than soaking alone. As a result, a higher rate of mineral leaching occurs during the boiling and soaking process (P_3) rather than soaking in $NaHCO_3$ (P_1) or hot water (P_2).

Total carbohydrate estimation of jack bean milk by difference is obtained by deducting the percentage of

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moisture, protein, fat, and ash from 100. The same notation on all carbohydrate percentages (P_1, P_2, and P_3) indicated that different pretreatment methods had insignificant effects on the jack bean's carbohydrate extractability along the processing steps and the final carbohydrate contents of all jack bean milk.

Apart from various compositions of jack bean milk due to different pretreatment methods, it is worth mentioning that all the produced jack bean milk had considerably high protein contents that were ranging from 5.45 to 7.01% compared to cow's milk (3.96 to 4.02%; Manzocchi *et al*., 2020) as well as several plant-based milk, including soy, oat, rice, and almond milk with the values of 3.4, 0.70, 0.32 and 2.11%, respectively (Jeske *et al*., 2017; Niyibituronsa *et al*., 2019). Meanwhile, all jack bean milk was low in fat content ranging from 0.07 to 0.10%, and the values were much lower than other types of plant-based milk, such as those derived from almond (8.25%), soy (1.72%), oat (0.28%), quinoa (2.40%), and rice (0.87%) (Mäkinen *et al*., 2015; Kundu *et al*., 2018). In addition, low ash contents of jack bean milk ranging from 0.08 to 0.11%, were found to be quite similar to rice milk (0.13%) (Mäkinen *et al*., 2015).

3.2 Effect of pretreatment methods on phase behavior

In general, plant-based milk is a colloidal fluid composed of size-reduced particles of plant materials extracted and dispersed within the water system. The colloidal system is susceptible to experiencing sedimentation within a short storage time due to the large particle size consisting mainly of fat globules, protein aggregates and starch granules. Therefore, to evaluate the colloidal stability of the produced jack bean milk, the phase separation test was done by placing each produced jack bean milk in a 100 mL graduated cylinder. The observation was carried out until the sedimentation height remained stable. Figures 1A and 1B depict the phase behavior observation carried out before and after standing for 12 hrs at room temperature. All the jack bean milk samples were in a homogeneous mixture prior to the test, and as expected, the separation occurred in each sample with different characteristics. The results revealed that jack bean milk particles from P_3 tended to completely separate in a short time (3 hrs) and form a noticeable layer at around 11-12 mL of the meniscus. Meanwhile, the separation in jack bean milk from P_1 and P_2 was not clearly visible in standard room lighting. For that reason, the observation was extended using an intense flashlight to help visualize the separation that might occur in the sample. The results depicted in Figure 1C highlighted the occurrence of phase separation in jack bean milk P_1 and P_2 after 6 h, albeit the interface border looked blurry with both layers having almost similar color, indicating that these two pretreatments produced a more stable colloidal system of jack bean milk compared to P_3.

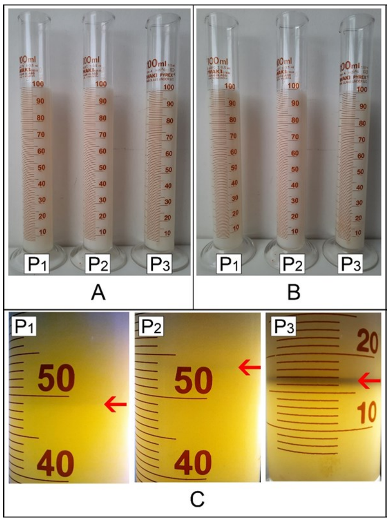

Figure 1. Jack bean milk before standing (A) and after standing for 12 hrs (B) at room temperature, and the visualization of phase separation (C). P_1: soaking in 0.4% of sodium bicarbonate solution, P_2: soaking in hot water (95°C), P_3: a combination of boiling and soaking in water.

Different phase behavior links to different pretreatments given to the jack beans. Boiling as a pre-extraction heating step in P_3 induced changes in several components, including starch-protein gel and protein denaturation within the jack bean, to form coarse and insoluble matrices before being ground into a slurry (Shim and Mulvaney, 2001). Therefore, the formed matrices that passed the filtration process did not contribute to creating a stable colloidal system within jack bean milk but tended to precipitate during storage. A similar phenomenon occurred in the pre-extraction heating of soybean applied in the soymilk process generating larger particle sizes of insoluble protein aggregates and leading to the lower physical stability of the produced soymilk (Rosenthal *et al*., 2003). Moreover, the particle size and hydrophobicity of the aggregates increase with elevated heating temperature (Shimoyamada *et al*., 2014). The findings mentioned also support the evidence of P_1 and P_2 to produce higher stability of jack bean milk by allowing the components in jack beans, starch, protein, and fat globules, to experience less significant changes and remain as is after being extracted from the wet grinding process. All the extracted components then interacted with each other when subjected to pasteurization to form a complex that could facilitate the structure and stability of the jack bean milk colloidal system (Liu and Chang, 2007; Yang *et al*., 2020).

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3.3 Effect of pretreatment methods on color

Color is the first attribute of food products being recognized by consumers. Therefore, the jack bean milk is expected to have a white color resembling cow's milk. The whiteness index (WI) results in Table 2 and the documentation of jack bean milk in Figure 2B indicate that all produced jack bean milk was white. However, boiling jack beans prior to milk extraction (P_3) produced jack bean milk with the lowest WI (49.97±0.57) and significantly different ($p<0.05$) from those of P_1 (60.40±0.87) and P_2 (60.60±0.52). In addition, it was noticed that the jack bean milk processed with different pretreatment methods possessed different lightness values (L^*) with the same pattern as its WI.

Table 2. Color parameters of jack bean milk with different pretreatment methods.

Color parameter	Jack bean milk		
	P_1	P_2	P_3
L^*	60.40±0.87^b	60.60±0.52^b	49.97±0.57^a
a^*	-3.97±0.06^a	-3.27±0.06^b	-3.23±0.06^b
b^*	-1.70±0.17^b	-0.03±0.06^c	-3.80±0.10^a
WI	60.17±0.87^b	60.46±0.52^b	49.72±0.55^a

Values are presented as mean±SD of triplicates. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$). P_1: soaking in 0.4% of sodium bicarbonate solution, P_2: soaking in hot water (95°C), P_3: a combination of boiling and soaking in water.

To further understand this phenomenon, comparing dehulled treated jack beans with the untreated bean as control was carried out to observe any effect the pretreatment methods had on the color of jack beans. The documentation in Figure 2A and the quantification of color attributes in Table 3 elucidate that the yellow color was still observable on the bean soaked in 0.4% of $NaHCO_3$ solution (P_1) and hot water (P_2). Although the yellow intensity was visibly higher for the bean from P_1, the same notation for all values ranging from 17.33 to 18.57 shows the difference in b^* value between both treated jack beans was still insignificant. Meanwhile, the a^* values had a slight variation between treatments. Therefore, it was suggested that the color differences between the treated beans were contributed by different pH and initial temperatures of the soaking medium. The carotenoid pigments with red-orange-yellow shades in jack beans, such as lycopene, α-carotene, and β-carotene (Okla *et al*., 2021), are known to have higher stability at a higher pH, making the beans soaked in 0.4% of $NaHCO_3$ solution (pH 8.2) had more intense, brighter yellow color than those soaked in hot water (pH 7.0). Furthermore, exposing jack beans to a higher temperature can degrade the carotenoids and make them colorless, accompanied by changes in color (D'evoli *et al*., 2013; Song *et al*., 2017).

Higher significant discoloration from heat exposure occurred on the jack bean that had been through 30-min boiling and soaking (P_3), where the light-yellow color of the original beans turned off-white with a slight brown shade. Moreover, the L^* and b^* values were the lowest among the treated beans. The tendency of the boiled jack beans to darken along with the loss of yellow color is suggested to be caused by prolonged heat treatment during boiling that induced the Maillard reaction and the degradation of carotenoids. The effect of heat treatment on the lightness decline of jack beans was similar to that observed during the heat treatment given to kidney beans (Li *et al*., 2022) and soybeans (Žilić *et al*., 2014).

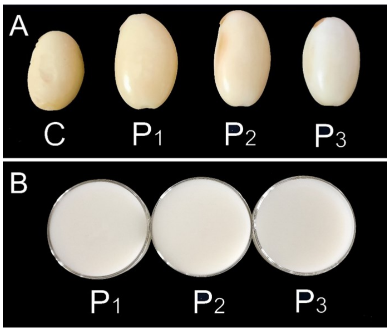

Figure 2. Dehulled jack beans (A) and jack bean milk (B) with different pretreatment methods. C: control, P_1: soaking in 0.4% of sodium bicarbonate solution, P_2: soaking in hot water (95°C), P_3: a combination of boiling and soaking in water.

Table 3. Color parameters of dehulled jack bean with different pretreatment methods.

Color parameter	Dehulled jack bean			
	Control	P_1	P_2	P_3
L^*	76.93±0.47^b	74.30±0.36^a	81.77±0.64^c	74.03±0.61^a
a^*	3.43±0.06^c	1.07±0.06^a	1.13±0.06^a	1.77±0.06^b
b^*	25.67±0.12^b	18.57±0.23^a	17.47±0.21^a	17.33±1.16^a

Values are presented as mean±SD of triplicates. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$). P_1: soaking in 0.4% of sodium bicarbonate solution, P_2: soaking in hot water (95°C), P_3: a combination of boiling and soaking in water.

3.4 Effect of pretreatment methods on viscosity

Viscosity is a characteristic of fluid that depicts the resistance to deformation at given shear stress and can reflect several sensory attributes of the products, such as creaminess and thickness (Deblais *et al*., 2021). The viscosity of all jack bean milk was 28.6±0.6, 48.1±1.4, and 12.6±0.5 cP for P_1, P_2, and P_3, respectively. Additionally, all values were significantly different ($p < 0.05$). Indeed, the viscosity trend corresponds to the moisture content of the jack bean milk. Jack bean milk from P_2 with the lowest moisture content (91.61±0.07%)

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was found to have the highest viscosity compared to other pretreatments. This is due to the higher content of solid components extracted from jack beans during the grinding process, including protein, fat, and carbohydrate, compared to P_1 and P_3. All extracted components interacted and formed hydrophobic gel-like matrices when subjected to pasteurization, which contributed to building the consistency and increasing the viscosity of jack bean milk.

On the contrary, P_1 and P_3 that subjected the jack beans to soaking in a higher pH and pre-extraction boiling process, respectively, increased the components' insolubility due to protein denaturation, starch-protein gelation, and protein-fat interaction. Consequently, components responsible for providing the body structure and increasing the viscosity of the fluid system were extracted in a lesser amount during the grinding process, as discussed earlier. These results agree with the previous study by Ringgenberg *et al*. (2012), which reported that higher protein concentration in soymilk would increase the total solids content and viscosity. The significance of total solid in affecting the viscosity of fluid product was also proven by Penna *et al*. (2006) that highlighted higher total solid of milk and gelling of solid constituents to increase the apparent viscosity of the produced stirred yogurt.

4. Conclusion

Various pretreatment methods on jack bean generated different physicochemical characteristics of jack bean milk. Jack bean milk pretreated with soaking in hot water (95°C) (P_3) possessed the highest protein (7.01±0.11%), fat (0.10±0.00%), and ash contents (0.11±0.00%) of jack bean milk than those of soaking in $NaHCO_3$ and combination of boiling and soaking, which in turn generated the highest viscosity value (48.1±1.4 cP) among other treatments. Meanwhile, the protein and ash contents of jack bean milk from P_1 were still higher than P_3, but both treatments resulted in an insignificant difference in fat content. On the contrary, none of the treatments showed significant differences in the carbohydrate contents (ranging from 1.17 to 1.20%), which indicated that different pretreatment methods did not remarkably affect the carbohydrate extractability during the process. In addition, the boiling process performed in P_3 produced jack bean milk with the tendency to separate in a short time and also the lowest WI compared to P_1 and P_2. Therefore, taking in consideration the final properties of the jack bean milk, the pretreatment of soaking in hot water (95°C) can be considered optimal to produce the best nutritional contents and physical attributes of jack bean milk.

Conflict of interest

The authors declare no conflict of interest.

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