



Freeze-thaw procedure as an alternative method to reduce the cooking time of Jack bean (*Canavalia ensiformis* (L.) DC) while retaining its nutritional quality

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ABSTRACT

This study explored the effect of freeze-thaw procedures on the Jack bean's cell wall, cooking times, and nutritional quality.

Confocal laser microscopy was used to examine bean's microstructure. Optimal cooking times, hemagglutination activity, and mineral content were evaluated on beans subjected to freeze-thaw procedures and those cooked with sodium bicarbonate (AC) and demineralized water (DC). The freeze-thaw procedure that yield the largest reduction in cooking times, AC, and DC samples were used for *in vitro* protein and starch digestion.

Freeze-thaw procedure at -20°C for 24 h resulted in the biggest enlargement of intercellular spaces and the largest reduction in cooking time although it was less effective compared to alkaline salt cooking. No hemagglutination activity was observed in any cooked beans and mineral loss was correlated with duration of cooking times. Protein digestibility increased in freeze-thaw treated beans compared to control beans cooked in demineralized water, with no effect on starch digestibility. The findings suggest that enlargement of intercellular space due to freeze-thawing treatment can reduce cooking times and that the shorter duration of cooking may increase protein digestibility. The absence of hemagglutination activity and the observed mineral loss emphasize the importance of efficient cooking methods in preserving nutritional properties.

1. Introduction

One of the global challenges established by FAO (FAO, 2017) is to find a more sustainable source of protein, and increasing the consumption of legumes plays a crucial role in addressing this issue (Willett et al., 2019). Legumes are renowned for being rich in protein, high in fiber and minerals, and they require less land and water, and producing fewer greenhouse gas emissions compared to animal husbandry and other crops (Singh, Jain, Ujinal, & Langyan, 2022). Among legumes, there are underutilized legumes that are not consumed on a global scale. One of them is Jack bean, which protein content ranges from 23 to 35 g/100 g while having low lipid levels and high fiber content (Sridhar & Seena, 2006). Jack bean contains antinutrient lectin concanavalin A, which has hemagglutination activity but can be inactivated by thermal processes (Carlini & Udedibie, 1997). Despite its nutritional potential, Jack bean utilization is limited by long cooking time (Akpapunam &

Sefa-dede, 1997).

Longer cooking time in Jack bean is associated with more cell connections and more covalently bond pectin within the cell walls (Purwandari, Fogliano, de Ruijter, & Capuano, 2023). These factors may result in a more pronounced difficulty in separating the cells, which is a crucial microstructural feature linked to bean softening. Specifically, softening during cooking is driven by the solubilization of pectin in the middle lamella, which in turn, leads to cell separation upon mechanical disintegration (Chigwedere et al., 2018).

Various approaches have been explored to reduce cooking times for beans such as soaking and dehulling (Kinyanjui et al., 2015). Nevertheless, the role of bean seed coat in softening during cooking was minor (Chigwedere et al., 2018). Methods such as infrared heating, gamma irradiation, pressure cooking, and germination were reported to decrease cooking durations (Bellido, Arntfield, Cenkowski, & Scanlon, 2006; Çelik, Yalçın, Başman, & Köksel, 2004; Haileslassie, Henry, &

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Tyler, 2019; Lima, Miano, Augusto, & Arthur, 2019; Sangsukiam & Duangmal, 2022; Siqueira, Pereira, Flávia, & Zaczk, 2013). Alkaline solutions were used to reduce cooking times either as a soaking or cooking medium (Chigwedere et al., 2018; Mubaiwa, Fogliano, Chidewe, & Linnemann, 2019; Schoeninger, Coelho, Christ, & Sampaio, 2014). This technique offers a simple way to shorten cooking times, suitable for resource-constrained regions. However, the use of alkaline solutions may darken the beans, impacting sensory qualities and overall acceptability (de Leon, Elias, & Bressani, 1992; Schoeninger et al., 2014).

Freezing is a commonly used process for preserving the quality and extending the shelf life of fruits and vegetables. Freeze-thaw process is usually deleterious for food quality due to the cellular damage and textural loss after thawing, especially in plant tissue with high water content (Jha, Xanthakis, Chevallier, Jury, & Le-Bail, 2019). Freezing involves the crystallization of water in the cellular structure, forming ice crystals. Fast freezing forms small intracellular ice crystals, while slower rates causes intracellular water to leave the cell and form larger extracellular ice crystals (Kaale, Eikevik, Bardal, Kjorsvik, & Nordtvedt, 2013; Schudel, Prawiranto, & Defraeye, 2021), creating more cellular damage. Slow freezing rates at -20°C resulted in significant damage in the cellular structure of mango cell walls, whereas fast freezing rates at -80°C yielded cells similar to those found in fresh tissues (Charoenrein & Owcharoen, 2016). In the case of Jack beans, our hypothesis is that a freeze-thaw treatment may damage the beans structure or facilitate enlargement of intercellular space, thus potentially leading to a reduction in cooking time. Moreover, since the effect on bean microstructure depends on the formation of ice crystals, it is essential to assess the kinetics of water absorption to achieve optimal water uptake prior to the freezing stage.

Cooking durations may impact the leaching of mineral as well as the effectiveness of thermal inactivation of lectin with hemagglutination activity. Concanavalin A is a lectin in Jack bean that require 3 h of boiling for inactivation (Udedibie & Carlini, 1998). Thus, it is crucial to investigate the hemagglutination activity especially when the cooking duration of Jack bean becomes shorter. In addition, pectin solubilization is associated with cell wall permeability, which influences the accessibility of amylase and proteases and subsequently affects starch and

protein digestion (Pallares Pallares et al., 2018). Our previous investigation showed that cooking times affect starch digestion but not protein digestion in Jack bean (Purwandari, Westerbos, Lee, Fogliano, & Capuano, 2023). Nevertheless, the investigations examined the duration of cooking times relative to alterations in textural hardness. The impact on protein and starch digestion in Jack bean cooked at varying cooking times while maintaining comparable hardness remains unclear.

To the best of our knowledge, no literature has reported the use of freeze-thaw treatments to reduce cooking time in beans. This study aimed to investigate the influence of freeze-thaw treatments on the Jack bean cell wall, cooking times, and nutritional quality such as mineral content, hemagglutination activity, and *in vitro* protein and starch digestibility.

2. Materials and methods

2.1. Materials

Jack bean was grown and harvested in Malang (Indonesia). The bean was stored in the warehouse at ambient temperature before being shipped to the laboratory in the Netherlands. All other chemicals were of analytical grade unless stated otherwise. The flow diagram of the experiments can be found in Fig. 1.

2.2. Determination of water absorption kinetic of Jack bean

Fully soaked stage is important for the effectiveness of freeze-thaw procedure on the reduction of the cooking time. In a preliminary study, we observed a statistically significant difference ($p < 0.05$) in the cooking time of beans soaked until 50% of full hydration was achieved compared to fully hydrated beans, both of which were subsequently frozen for 24 h, on another batch of the Malang collection (data not shown). At the best of our knowledge, there is no information related to water absorption kinetics during soaking of Jack bean. In this study, we therefore evaluated the kinetics of water absorption during soaking of Jack beans according to a previous report on common beans (Kinyanjui et al., 2015), to determine the time to achieve fully hydrated beans. In brief, the whole beans (10 g) were soaked in 100 mL demineralized

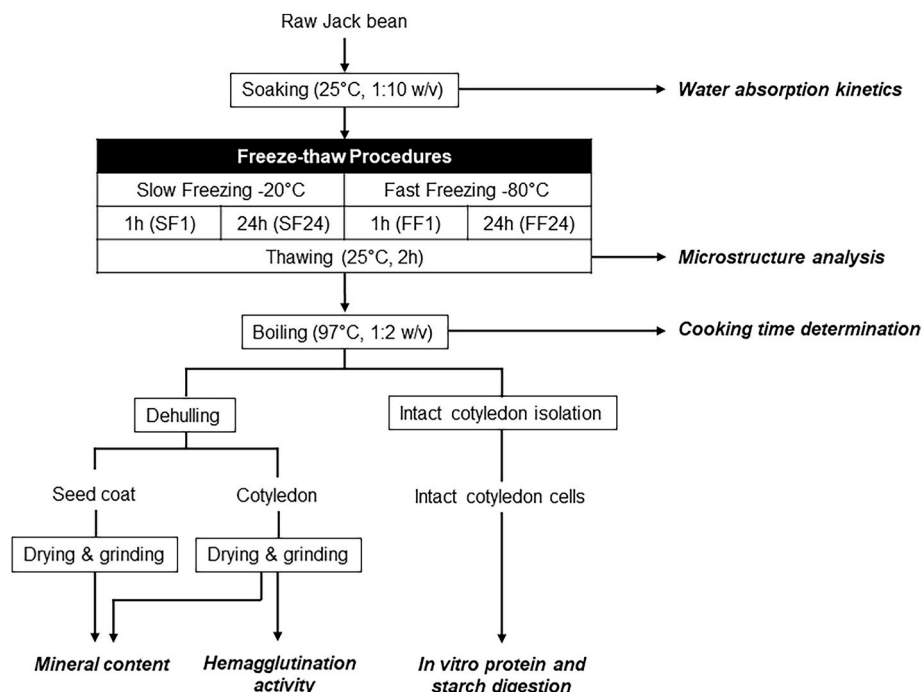


Fig. 1. Schematic illustration of the experimental design.

water. Soaking was performed in a water bath at 25 °C with a total soaking time of 40 h. The weight of the soaked beans was monitored every 30 min during the 0–8, 14–28, and 36–40 h and the beans were returned to the soaking water. Excess surface water was removed from the beans with paper towels. The soaking tests were carried out in duplicate. The results were expressed as g water/g dry matter, representing moisture content for Peleg model below.

The dry matter content of the whole beans was determined by weighing a known amount of beans powders and stored in an incubator oven at 105 °C overnight. The dried powders were weighed until constant weight.

Data on the water absorption were modelled by using a Peleg model (Eq. (1)) (Peleg, 1988) from duplicate data.

$$M(t) = M_0 + \frac{t}{(k_1 + k_2 t)} \quad (1)$$

where M_t (g/100 g db) is the moisture content at time t , M_0 (g/100 g db) is initial moisture content, t (h) is soaking time, k_1 (h/(g/100 g)) is the Peleg rate constant, and k_2 (1/(g/100 g)) is the Peleg capacity constant. The parameters were estimated by minimizing the residual sum of squares (RSS) using Solver. The goodness of fit between the experimental data and the model was examined using the mean relative percentage deviation modulus (E) as described in Eq. (2).

$$E = \frac{1}{N} \sum_{i=1}^n \left| \frac{M_{exp} - M_{pred}}{M_{exp}} \right| \times 100\% \quad (2)$$

where M_{exp} is the experimental data, M_{pred} is the predicted value, N is the number of experimental data. It is widely adopted that E value below 10% indicates a good fit for practical purposes (Peng, Chen, Wu, & Jiang, 2007). The moisture equilibrium (M_E), when $t \rightarrow \infty$, was estimated using the following equation (Eq. (3)) (Peleg, 1988).

$$M_E = M_0 + \frac{1}{k_2} \quad (3)$$

2.3. Freezing treatment

Jack beans (500 g) were soaked in demineralized water (10 mL/g) for 24 h to ensure optimum water absorptions were obtained. This soaking time was based on the experimental results determined by the method described in Section 2.2. The objective of the freezing treatment is to convert the water inside the soaked bean into ice crystal in an attempt to induce microstructural alterations. The soaked beans were randomly divided into four groups and subjected to different freezing treatments: slow freezing at −20 °C for 1 h (SF1) and 24 h (SF24), and fast freezing at −80 °C for 1 h (FF1) and 24 h (FF24). The freezing durations were selected based on our preliminary study showing a significant difference. The frozen bean samples were thawed in an incubator at 25 °C for 2 h and immediately used for further analysis. The thawing condition and duration are sufficient to ensure that the inner temperature of the beans reaches equilibrium despite the prior freezing conditions.

2.4. Microstructural analysis

Microstructural analysis was performed on thawed beans from various freezing conditions (SF1, SF24, FF1, FF24) to evaluate the impact of freezing on the microstructure of the Jack bean. A control (NF) was prepared from beans that had been soaked in demineralized water (10 mL/g) for 24 h. Cotyledon from each treatment and control was thinly sliced (0.5–0.7 cm) to generate two slices per bean cotyledon. The sliced samples were stained with 1 mL/L calcofluor white to examine the cell wall and left overnight to ensure complete dye diffusion and homogeneity (Rovalino-Córdova, Fogliano, & Capuano, 2019). Cell microstructure was observed with a confocal laser microscope Zeiss 510 (Carl Zeiss microscopy, Oberkochen, Germany), with an argon laser set

at 405 nm. Images were taken at objective 20× Plan-Neofluar (0.30) and processed with Zen blue software v.2.3 (Carl Zeiss, Oberkochen, Germany). At least 10 images were randomly captured from different areas for each sample treatment. We measured the shortest perpendicular distance between two adjacent cells to assess enlargement of intercellular spaces. A total of 100 measurements were carried out for each sample group using Image J 1.52 (Fiji) software (NIH, Maryland, USA).

2.5. Cooking time determination

Cooking times were determined on the thawed beans using finger pressing method according to our previous study (Purwandari, Westerbos, et al., 2023). In brief, the thawed Jack beans were cooked in boiling demineralized water (2 mL/g) at 97 °C. Cooking times were also determined on the unfrozen beans i.e. soaked in demineralized water and cooked in alkaline solution (NaHCO₃, 5 g/L) (AC) and soaked and cooked in demineralized water (DC), representing positive and negative control, respectively. During the cooking process, ten beans were removed every 30 min and classified as cooked when they are easily disintegrated upon pressing between thumb and forefinger. The results were expressed as a percentage of the cooked beans (%) and modelled using a logistic model as described in the following equation (Eq. (4)):

$$y = \frac{a}{1 + e^{(b-kt)}} \quad (4)$$

where y (%) is the percentage of cooked beans, t (min) is the cooking time, and a , b , and k are the kinetic parameters. The parameters were estimated by minimizing the residual sum of squares (RSS) using Solver. The model was used to determine the cooking time, defined as the time at which 9 out of 10 beans were cooked (i.e. $y = 90\%$, CT_{90}). The cooking time was determined in duplicate.

2.6. Preparation of Jack bean flours and intact cells

Jack bean was frozen using the condition as described in Section 2.3. The thawed beans were cooked in demineralized water at 97 °C as previously mentioned. The beans were cooked for different times for SF1, SF24, FF1, FF24, AC and DC corresponding to the optimal cooking times previously determined in Section 2.5. The cotyledons and seed coat were separated and freeze-dried. To generate flours, the dry samples were milled using a ball mill MM 400 (Retsch, Haan, Germany) at 30 Hz for 60 s followed by sieving through a 425 µm filter. The flours that passed through the filter were collected and stored at room temperature until further use for hemagglutination and mineral analysis. In addition, flours were prepared from the cotyledon of raw Jack beans (dry beans) (as a control sample for hemagglutination analysis) and cotyledon and seed coat of raw beans (soaked beans) (as control sample for mineral analysis).

Intact cells were isolated from cotyledon samples of beans that were subjected to the freeze-thaw condition that resulted in the largest reduction in cooking times, as well as from AC, and DC. Briefly, the cooked cotyledons were mashed with mortar and pestle and wet-sieved. Particles between 71 and 125 µm filters were collected and flushed with demineralized water three times to remove free starch granules. This range was used to collect only individual cells without the presence of cluster cells. Intact cells were collected, added with sodium azide (0.2 g/L in the final mixture), and stored at 5 °C until further use.

2.7. Hemagglutination activity

Hemagglutination activity was determined on the protein extract of raw and cooked Jack bean flours (SF1, SF24, FF1, FF24, AC, DC). Phosphate saline buffer (PBS) (0.2 mol/L, pH 7.4) was used to suspend bean flours (0.2 g/mL). The mixtures were stirred (450 rpm) for 2 h at 4 °C followed by centrifugation of 4500 g for 15 min. The supernatant

was dialyzed against demineralized water at 4 °C for 20 h using dialysis tubes (MWCO 12–14 kDa). The extracted protein was stored at –20 °C until further use. Protein concentration was determined using the Bicinchoninic Acid (BCA) Protein Assay kit (Thermo Fisher Scientific, Waltham, USA). Hemagglutination activity was performed according to a previous study (Udedibie & Carlini, 1998). In brief, erythrocyte suspension was prepared by diluting 5 times erythrocyte stock (MBS539320) (MyBioSource Inc., San Diego, USA) with PBS. Protein extracts were prepared in serial dilutions (2-fold). In duplicates, 25 µL of diluted protein extracts were placed in a 96-well round-bottom microplate, followed by the addition of 25 µL erythrocyte suspension and incubation for 2 h at room temperature. One hemagglutination unit (HU) is the minimal concentration of protein expressed in µg/mL that could agglutinate 10^6 erythrocyte cells.

2.8. Mineral content

Mineral content was analyzed on the cotyledon and seed coat flours from cooked beans (SF1, SF24, FF1, FF24, AC, DC) and raw soaked beans. The ash determination method according to AOAC 923.03 (AOAC, 2002) was used to determine the mineral content by using a muffle furnace at 550 °C overnight. Dry matter content was analyzed by drying a known amount of bean flour followed by oven incubation at 105 °C overnight. Mineral data were expressed as g/100 g dry weight.

2.9. Protein and starch digestibility

Static *in vitro* intestinal digestion was performed on intact cells of SF24, AC, and DC based on INFOGEST protocol as previously described (Brodkorb et al., 2019). For the oral phase, bean samples (5 g) were mixed with simulated salivary fluid (SSF) (with no salivary α -amylase), CaCl₂ and Milli-Q water to produce a final volume of 10 mL. Subsequently, the bolus was mixed with simulated gastric fluid (SGF) and the pH was adjusted to 3 with HCl. CaCl₂, Milli-Q water, and finally porcine pepsin enzymes were added to the mixture (enzyme concentration in the final mixture was 2000 U/mL) before incubation for 2 h at 37 °C. To stop the enzymatic reaction in the gastric phase, the pH was adjusted to 7 with NaOH. Gastric chyme was combined with simulated intestinal fluid (SIF), bile salt solution, CaCl₂, porcine trypsin, and pancreatic enzyme (100 U/mL trypsin activity and 200 U/mL amylase activity in the final mixture). The tubes were incubated for 2 h at 37 °C. Blank digestion was prepared by replacing the sample with Milli-Q water. All samples were run in triplicate. During the digestion phase, aliquots were collected at different times (time 0 and 120 min in the gastric phase and time 15, 30, 60, and 120 min in the intestinal phase). Absolute ethanol and Pefabloc were used to stop the amylase and protease activity, respectively, and the samples were stored at –20 °C until further analysis. The concentration of free amino groups was determined by o-phthalaldehyde (OPA) on the pefabloc-added samples as described in a previous study (Mulet-Cabero, Rigby, Brodkorb, & Mackie, 2017). The degree of protein hydrolysis (DH%) was calculated based on the level of free amino groups in digested samples, undigested samples, and completely hydrolyzed samples in 6 mol/L HCl at 110 °C for 24 h (Zahir, Fogliano, & Capuano, 2020). To the ethanol-added supernatants, amyloglucosidase treatment was applied to convert the amylase hydrolysis products into glucose (Rovalino-Córdova et al., 2019) followed by D-glucose assay using GOPOD protocol (Megazyme Inc., Bray, Ireland). Starch hydrolysis was expressed as grams of hydrolyzed starch/100 g of dry starch.

2.10. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics v.26 (IBM, New York, USA). One-way analysis of variance (ANOVA) was used to analyze the effect of different freezing temperatures and freezing times on the cooking time as well as the mineral data. Correlation (2-tailed) between cooking time and mineral content in cotyledon was

assessed using Pearson correlation. Mood's median test was used to analyze the effect of different freeze-thaw conditions on the distance between adjacent cells. A significance level was determined at the $p < 0.05$.

3. Results

3.1. Water absorption kinetics

Fig. 2 shows the water absorption profile during the soaking of whole Jack beans and reflects the increase of moisture content during soaking. The Jack bean initial moisture content was 14.4 (g/100 g dry weight). The seed absorbed a larger quantity of water during the initial stage of soaking, and the rate of water absorption decreased as the soaking time increased. The Peleg model was used to estimate the parameter kinetics, the k_1 and k_2 values were 0.57×10^{-2} h/(g/100 g) and 3.71×10^{-2} h/(g/100 g), respectively. The E value and M_E were 3.7% and 190.2 g/100 g, respectively. An E value less than 10% implied a good fit of the Peleg model to model water absorption (Peng et al., 2007).

3.2. Freeze-thaw procedures change beans microstructure

Fig. 3 displays representative microstructure images of Jack beans and the measured distance between adjacent cells after freeze-thaw procedures compared to soaked beans as a control. Jack bean cells had an ovoid shape and no occurrence of cell wall damage was apparent in any of the images after freeze-thaw procedures. Changes in the microstructure after freeze-thaw were observed as the distance between adjacent cells indicating the presence of intercellular spaces. SF1, SF24 and SF1 showed a significant increase in distance between adjacent cells compared to NF ($p < 0.05$). SF24 showed the largest distance between adjacent cells compared to the other treatments. Meanwhile, FF procedures were likely to maintain the bean microstructure where small changes in the enlargement of intercellular spaces could only be detected after 24 h.

3.3. Freeze-thaw procedures reduce cooking time of the beans

Cooking times of Jack beans after different freeze-thaw procedures and soaked beans cooked with two different mediums are summarized in Table 1. The cooking times of Jack beans after the freeze-thaw procedures were not significantly different compared to DC ($p > 0.05$). Based on our observations, SF24 showed the biggest reduction effect on bean cooking time (by 23%). The result indicates that the freeze-thaw procedure could not significantly affect the cooking times of Jack beans ($p = 0.07$). The alkaline cooking procedure (AC) remained the most effective in reducing cooking time, a golden standard to speed up the optimal cooking times in beans.

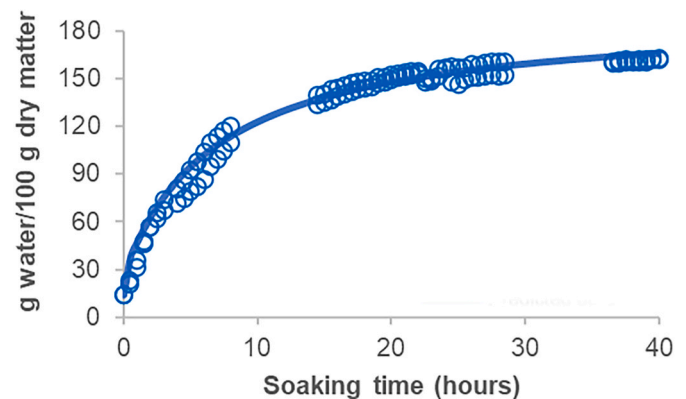


Fig. 2. Water absorption profile of Jack beans during soaking. Circles indicate experimental data. Line indicates predicted data.

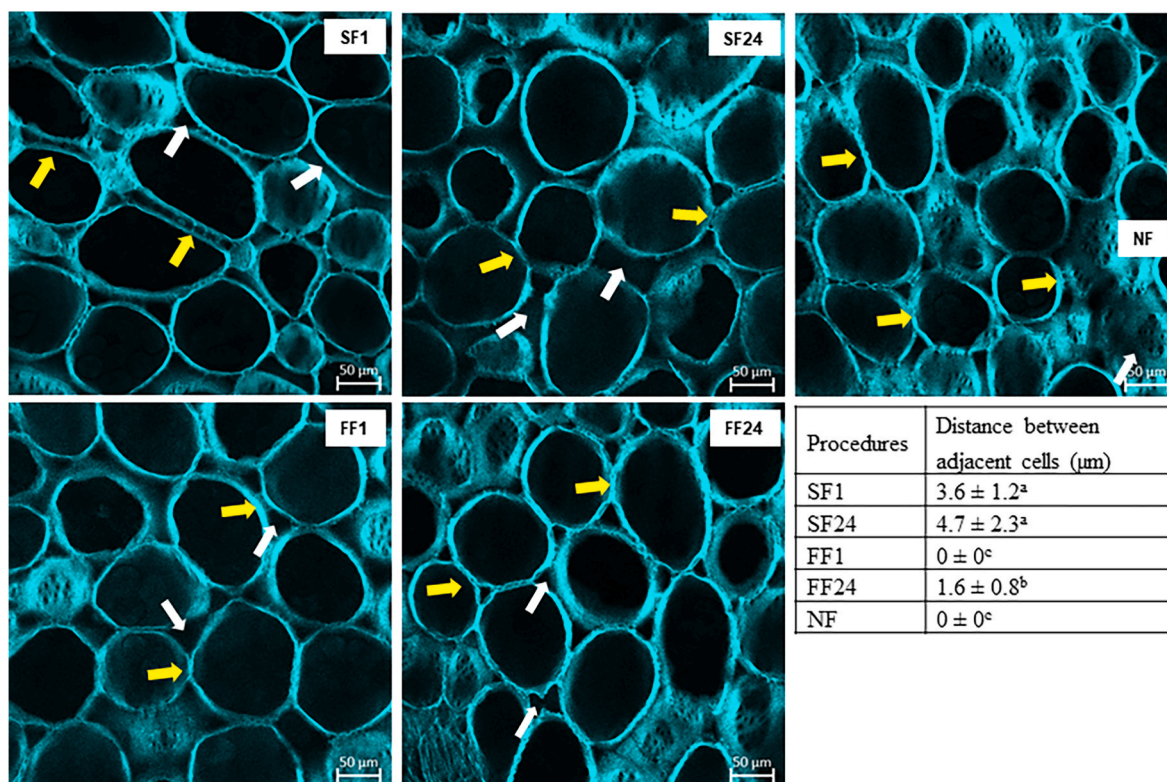


Fig. 3. Representative micrographs and the distance between adjacent cells of Jack bean after freeze-thaw procedures (SF1, SF24, FF1, FF24) compared to soaked bean (NF). White and yellow arrows indicate intercellular spaces and cell contacts, respectively. SF1: slow freezing at -20°C for 1 h, SF24: slow freezing at -20°C for 24 h, FF1: fast freezing at -80°C for 1 h, FF24: fast freezing at -80°C 24 h. In the table, distance between adjacent cells is presented as median and 95% confidence Interval ($N = 100$). Different letters indicate a significant difference in distance between adjacent cells among treatments ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1

Optimal cooking times of Jack bean after frozen and cooked in demineralized water (SF1, SF24, FF1, FF24), soaked and cooked in demineralized water (DC), and soaked in demineralized water and cooked in alkaline solution (AC) extrapolated from cooking profiles.

Procedures	Cooking times (min)
SF1	212.6 ± 6.5^a
SF24	166.1 ± 16.5^{ab}
FF1	214.2 ± 3.9^a
FF24	192.8 ± 10.2^a
DC	215.9 ± 0.0^a
AC	124.2 ± 11.2^b

SF1: slow freezing at -20°C for 1 h, SF24: slow freezing at -20°C for 24 h, FF1: fast freezing at -80°C for 1 h, FF24: fast freezing at -80°C 24 h. Different letters indicate significant difference at $p < 0.05$.

3.4. Hemagglutination activity and mineral content of frozen-thawed and cooked Jack beans

The levels of hemagglutination activity of the cotyledon and mineral content of cotyledon and seed coats of cooked Jack beans with and without freeze-thaw treatments are reported in Table 2. Hemagglutination activity was only present in raw cotyledon (0.0128 HU) and no hemagglutination activity was detected in all cooked beans. The mineral contents did not show a significant difference for both cotyledon and seed coat for all Jack beans cooked in demineralized water with/without freezing treatments (SF1, SF24, FF1, FF24). Higher mineral content in cotyledon and seed coat was found in alkaline cooked beans (AC) as compared to DC. In comparison to raw bean, all cooked Jack beans that

Table 2

Hemagglutination activity and mineral content of Jack beans after freeze-thawing, and cooking in demineralized water (SF1, SF24, FF1, FF24), soaking and cooking in demineralized water (DC), and soaking in demineralized water and freezing in alkaline solution (AC) as compared to raw bean (Mean and standard deviations, $N = 2$).

Procedures	Hemagglutination activity (HU)	Mineral content (% dry weight)	
		Cotyledon	Seed coat
SF1	N.H.	1.8 ± 0.1^c	3.6 ± 0.1^b
SF24	N.H.	2.2 ± 0.2^{bc}	3.4 ± 0.4^b
FF1	N.H.	2.1 ± 0.3^{bc}	3.6 ± 0.0^b
FF24	N.H.	1.9 ± 0.0^c	3.6 ± 0.0^b
DC	N.H.	1.8 ± 0.0^c	3.6 ± 0.1^b
AC	N.H.	2.6 ± 0.2^{ab}	5.4 ± 0.3^a
Raw bean	0.0128	3.1 ± 0.0^a	3.6 ± 0.5^b

SF1: slow freezing at -20°C for 1 h, SF24: slow freezing at -20°C for 24 h, FF1: fast freezing at -80°C for 1 h, FF24: fast freezing at -80°C 24 h. HU: hemagglutination unit. N.H.: no hemagglutination was detected. Different letters indicate significant difference at $p < 0.05$.

were subjected to the freeze-thawing procedures and DC showed lower mineral contents for cotyledon but similar mineral contents for seed coat. No significant variation in the mineral content of all treatments compared to the raw bean, except for AC, was observed.

3.5. In vitro protein and starch digestibility of frozen-thawed and cooked Jack beans

Fig. 4 displayed the protein digestibility of intact cotyledon cells of Jack beans after different treatments (SF24, AC, and DC). Protein digestibility was higher in AC compared to SF24 and DC at early digestion

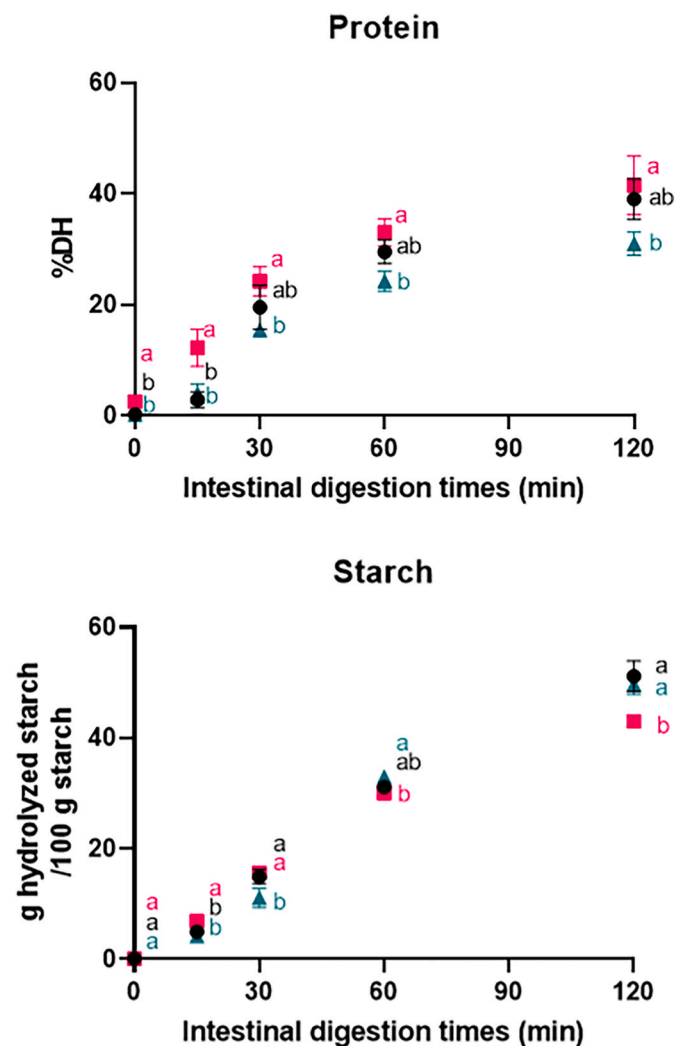


Fig. 4. *In vitro* protein and starch digestion of Jack bean after frozen and cooked SF24 (dot), soaked and cooked in alkaline solution AC (square), and soaked and cooked in demineralized water DC (triangle). Error bar indicates standard deviation of the mean (N = 3). Different letters indicate significant differences (p < 0.05).

times but it was only higher in AC at the end of the intestinal digestion phase when no effect of freezing was observed. We observed that despite different cooking times, SF24 and DC showed similar starch digestibility. In addition, AC showed a lower starch digestibility compared to SF24 and DC.

4. Discussion

Freeze-thawing has been known to alter the microstructure of plant cells (Charoenrein & Owcharoen, 2016; Jha et al., 2019; Schudel et al., 2021), offering a potential solution to the long cooking time in beans. This is the first study that investigated the influence of freeze-thaw procedures on the cell wall of legumes in relation to their cooking durations, as well as the implications of the procedures on the nutritional quality of the cooked beans, using Jack beans as model food.

Jack beans exhibited a rapid initial water absorption with no appearance of a lag phase at the beginning of the soaking process (Fig. 2). The initial rapid water absorption could be attributed to the filling up of capillaries located on the surface of the seed coat and the hilum (Kumar, Prasad, Chandra, & Debnath, 2018). As soaking continued, the rate of water absorption slowed down, which was due to the increased extraction of soluble materials as well as the filling up of

the free capillary and other intercellular spaces (Abu-Ghannam & McKenna, 1997). For Jack bean, the water absorption rate began to decrease at time 15 h of soaking. To optimize water absorption while preventing fermentation (Abu-Ghannam & McKenna, 1997) and avoiding extensive solid loss, we chose a soaking time of 24 h at the equilibrium phase to prepare an optimum hydrated beans before freeze-thaw treatments. This hydration duration corresponds to 136.9 g water/100 g dry matter.

We observed that the freeze-thaw procedure increased enlargement of intercellular spaces. We did not find any changes related to the cell shape nor damage in the cell wall on frozen-thawed Jack beans. Our finding is in line with a previous report on soybeans where no cell rupture was visible in cotyledon cells after freezing at -5°C for 4 days (Lili, Yeming, & Zaigui, 2013). Enlargement of intercellular space can be a result of the formation of extracellular ice crystals that may separate the cells and solubilize pectin induced by freezing (Charoenrein & Owcharoen, 2016). The formation of extracellular ice crystals could cause the expansion of intercellular spaces, weakening of intercellular adhesion (Delgado & Rubiolo, 2005).

Freezing conditions, such as the rates and durations, affected the severity of microstructure changes. In apple tissue, larger intercellular spaces were found after freezing at -20°C while no difference in intercellular space was found after freezing at -80°C compared to fresh samples (Chassagne-Berces et al., 2009). This may explain the fact that the microstructure of the beans after freezing at -80°C looks similar to that of the control beans. During prolonged freezing times, recrystallization tends to occur naturally when ice crystals change shape, increase in size, and decrease in number, due to the distribution of water from small to large ice crystals (Kaale et al., 2013). In the present study, increasing trends of distance between adjacent cells were found with the longer freezing procedures, suggesting that the freezing duration may induce larger ice recrystallization in frozen-thawed beans.

Slow freezing for 24 h (SF24) on soaked beans yielded the highest reduction on the optimal cooking times compared to other freeze-thaw procedures even though the difference was not significant at $p = 0.05$ level. Slow freezing in mango increased the level of water-soluble pectin fractions (Charoenrein & Owcharoen, 2016). Moreover, electron micrographs showed that slow freezing process in carrots caused a loosening of cellulose microfibrils (Roy, Taylor, & Kramer, 2001). During the hydrothermal process, pectin in the middle lamella will solubilize causing the cotyledon cells to separate upon mechanical disintegration (Chigwedere et al., 2018). It is possible that freezing may induce modifications in cell wall pectin in Jack beans allowing faster pectin solubilization and reducing cooking times. In the present study, beans cooked in alkaline solution (AC) have the shortest cooking time, indicating a fast pectin solubilization rate. It can be assumed that combination of freeze-thaw and alkaline cooking may further enhance the rate of pectin solubilization during cooking. Cooking with sodium bicarbonate was reported to promote the rearrangement of side chains of rhamnogalacturonan-I and β -elimination reactions that degrade the backbone of the chelated pectin chains, and lead to degradation into short-chain molecules (Liu et al., 2022).

Whereas a reduction of cooking time has practical advantage, it is important that it does not impact nutritional (i.e. mineral loss and nutrients digestibility) or toxicological aspects (i.e. the inactivation of hemagglutination activity). Therefore, we assessed whether the strategies to reduce cooking time would compromise Jack bean nutritional qualities. We found that raw bean showed hemagglutination activity and no hemagglutination activity was observed in all treated samples, regardless of the different pretreatments and cooking methods employed (Table 2). This finding suggests that reducing cooking time to the degree achieved in this study can effectively eliminate the hemagglutination activity, without compromising the safety of Jack bean consumption.

Table 2 shows that a significant mineral loss occurred in the cotyledon part. Moreover, mineral loss was significantly correlated ($p < 0.05$) with cooking times (Pearson correlation coefficients were

–0.935). We found no changes in the mineral content in the seed coat part after freezing and cooking treatments as compared to raw beans. This result was in agreement with a previous study showing that the changes of mineral content occurred in the cotyledon and not the seed coat (Chigwedere et al., 2019). During cooking, minerals will diffuse from the cotyledon cell through the cell walls causing mineral leaching. The minerals can form chelates with compounds such as phytic acid and dietary fiber such as pectin (Rousseau, Kyomugasho, Celus, Hendrickx, & Grauwet, 2020). The seed coat contains more galacturonic acid and free carboxyl groups than the cotyledon, whereas the cotyledon contains more phytate than the seed coat (Yi et al., 2016). Seed coat pectin is less sensitive to β -elimination, due to its lower degree of methoxylation compared to cotyledons, and that might explain the retained mineral content in the seed coat, i.e. more pectin is left in the seed coat that can chelate the minerals diffusing out of the cotyledon cells. In addition, cooking in alkaline solutions (AC) resulted in an increase in the mineral content in the seed coat compared to the raw beans. The use of bicarbonate solution may provide additional source of minerals. The mineral binding capacity of phytic acid and pectin can be changed with the pH. It is possible that the pH of AC induces more dissociation of phytic acid and pectin, causing mineral binding despite the leaching and solubilization of these molecules. Moreover, the effect of mineral binding is more pronounced in the seed coat, having more pectin with less susceptible of β -elimination, resulting in an increase of mineral content in AC in the present study.

We evaluated the protein and starch digestibility of three cooked Jack beans (SF24, AC, and DC) as depicted in Fig. 3. There was a trend of reduction of optimal cooking times yielded a higher protein digestibility despite the fact that at optimal cooking times, we could expect a similar degree of pectin solubilization and cell wall permeability to proteases (Pallares Pallares et al., 2018). However, how different pre-treatment conditions affect pectin solubilization is still unclear and we cannot exclude that different levels of cell wall permeability are produced in AC and SF24. More protein aggregation possibly occurred when the beans are cooked longer, causing a decrease in protein digestibility (Miraji, Linnemann, Fogliano, Laswai, & Capuano, 2021; Torres, Rutherford, Muñoz, Peters, & Montoya, 2016; Zahir, Fogliano, & Capuano, 2021). Hence, the observed decrease of protein digestibility with optimal cooking times may not depend on differences in cell wall permeability to proteases but different levels of protein aggregation. It is likely that similar cell wall permeability may cause a similar diffusion rate of amylase thus can be observed as a similar starch digestibility (SF24 and DC). In the case of AC, alkaline cooking conditions can induce pH changes in intracellular regions. The higher pH within the isolated cells may not be effectively neutralized after adjustment of pH in the *in vitro* digestion simulation. This higher pH “as sensed by amylase” might potentially affect its activity during the *in vitro* digestion.

In this study, we demonstrated that freeze-thaw procedures can reduce Jack bean cooking times. To the best of our knowledge, this is the first investigation on the use of freeze-thaw methods to shorten bean cooking durations. Freezing induces enlargement of intercellular spaces without any signs of cell rupture. These changes in microstructure appear to be the primary factors responsible for the reduction in Jack bean cooking times. It is important to note that the rates and duration of freezing play crucial roles in altering the microstructure of Jack beans. However, it is not clear whether the microstructural changes can be directly linked to the changes in the pectin fractions. Further exploration may be performed as well on the effect of freeze-thaw treatments in the protein or starch structure in the cytoplasmic matrix. In summary, freeze-thawing can be used as a simple, practical alternative method to reduce Jack bean cooking time, which in turn may prevent loss of minerals without impacts on protein and starch digestibility but its application at industrial or domestic scale may depend on resource availability as well as sustainability considerations.

CRediT authorship contribution statement

Fiametta Ayu Purwandari: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Raehana Saria Gahari:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Vincenzo Fogliano:** Writing – review & editing, Visualization, Supervision, Project administration, Conceptualization. **Edoardo Capuano:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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