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*Research Article*

# Physicochemical properties of native Jack bean (*Canavalia ensiformis*) starch: An underutilised legume

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**Abstract**
Legumes are a cost-effective source of proteins and abundant starch, a biodegradable substance, providing human nutrition and serving various food sectors globally. Some of the neglected (underutilised) legumes can also be used as the cheapest source of starch. Therefore, the present study was conducted to determine the physicochemical characteristics of jack bean (*Canavalia ensiformis*) starch - a legume not widely known so underutilised. The starch was isolated from the bean by standard method to study its various properties. One-way analysis of variance was employed to verify a significant difference at the 5% significance level. The jack bean yielded 30.98% of starch. The starch's moisture, ash, fat, protein, fiber, and carbohydrate content were 9.67%, 0.19%, 0.27%, 0.56%, 0.27%, and 89.28% respectively. The physicochemical properties were also determined. The apparent and total amylose contents were 43.82% and 47.78%, respectively, with 7.66% of amylose leaching at 95° C. The water and oil absorption capacities were 2.31 and 2.56 g/g, respectively, while emulsion capacity and stability were 62.30 and 71.38 %, respectively. The solubility and swelling power of jack bean starch increased with temperature from 55 to 95°C. The effect of starch concentrations (6, 8, and 10%) on freeze-thaw stability revealed that water expelled decreased as starch content increased. Nevertheless, a comprehensive investigation has not been conducted into the distinct functional characteristics and other attributes of jack bean starch. This study could provide new opportunities for conventional starch industries that rely on starch from sources like cereals, tubers, and rhizomes.

**Keywords**: Amylose, Freeze thaw, Jack bean legume, Starch, Swelling, Turbidity, Underutilized

## INTRODUCTION

*Canavalia ensiformis*, a member of the Leguminosae family and underutilised legume, is called the "jack bean." There are 12,000 species and about 480 genera worldwide (Akinyemi, *et al*., 2020). Jack beans have an average productivity of 7 tons/hectare, with a potential yield of 12 tons/hectare. Moreover, the average yearly production (Indonesia) of 5 tons of jack beans comes from 1,590 hectares of land (Ariyantoro *et al*., 2021). It is widely accessible throughout Latin America, Africa, Asia, the West Indies, Indonesia, and India (Marimuthu and Gurumoorthi, 2013). In India it is grown in the states of Sikkim, Assam, Nagaland, Arunachal Pradesh, Tripura, Manipur, Mizoram, and Meghalya in the northeast (Patel *et al*., 2016). The Indian tribal sects, Erula, Malayali, Kurumba, and other Dravidian people, boil the mature seeds before consuming them. Although it is frequently used as animal feed, it is rarely employed for human nutrition (Mitre, 1991). It is tolerant of acidic dry land and may thrive in a range of soil types (Bamiro *et al*., 1994). Its roots go deep into the ground to get water, and it is incredibly drought resistant. These characteristics facilitate its cultivation. Low-altitude locations with high temperatures and relative humidity have a high yield (Patel *et al*., 2016). In West-

ern countries, the crop is used as a cover crop and the seeds of the jack bean are pulverised, roasted, and used to make a beverage like coffee (Bressani *et al*., 1987). Its cotyledon separation and hulling are a little more challenging than other legumes, such as cowpea, mung bean, pea, etc. because of the fibrous mucilage (Rajerison, 2006) of seeds. Due to its favourable physicochemical characteristics, its starch can positively compete with other well-known sources of starch such as corn and cassava (Akinyemi *et al*., 2020).

Pulses and grain legumes are easily available and one of the cheapest sources of nutrition. The nutrients present in legumes provide various health benefits (Shevkani *et al*., 2022), like cancer, heart disease, diabetes, healthy aging, and obesity (Brennan *et al*., 2016). Most legumes are used in various dishes like soups, pasta, breakfast foods and snacks to improve their nutritional value and technological qualities (Sozer *et al*., 2017 ; Escobedo and Mojica, 2021). Because legumes lack allergic gluten proteins and are a rich source of resistant starch and fiber, they can be utilised in dietetic and gluten-free recipes (Singh, 2017). Beans are a superior ingredient for anti-diabetic diets due to their high percentage of resistant starch compared to other starches. (Keskin *et al*., 2022).

Starch is a complex semi-crystalline carbohydrate polymer found in various plants as a plentiful source of carbohydrates (Blazek and Copeland, 2008). It is utilised in the food and non-food industries to enhance texture, quality, consistency, and thickening, and to create biodegradable materials like polymers and edible coatings (Das *et al*., 2015; Yuliana *et al*., 2012). Starch's chemical makeup and primary components of starch influence physicochemical properties, including gelation, swelling power, solubility, water and oil absorption capacity, and emulsification capabilities (Sreerama *et al*., 2012). Starch finds application in the culinary, polymer, biomedical, and pharmaceutical industries due to its desired properties (Mengting *et al*., 2016; Reddy *et al*., 2017). Even though the traditional sources of starch like grains, rhizomes, and tubers have been extensively studied to date, non-conventional sources of starch still need to be looked into because they are the least expensive sources of starch (Sukhija *et al*., 2015).

The least popular crop for starch production is the jack bean. Due to a lack of knowledge about its accessibility, jack bean starch has not been utilised and is therefore overlooked. The physicochemical characteristics of jack bean starch are poorly understood. Therefore, further research is required to explore its varied quality attributes to improve its applications in industries. The present study aimed to examine the physicochemical properties of native jack bean (*Canavalia ensiformis*) starch.

## MATERIALS AND METHODS

### Material

Jack bean legume (*Canavalia ensiformis*) was purchased from Ruhi Enterprises, New Delhi, India. The reagents like sulphuric acid, hydrochloric acid, potassium iodide, dimethyl sulfoxide, *n*-propanol, etc., were of analytical grade from Himedia, Sigma-Aldrich and Merck.

### Isolation of Jack bean starch

Jack bean starch was extracted using Lawal and Adebowale-, (2005) method. The beans were soaked in distilled water with 1M NaOH (sodium hydroxide) to make its pH 8.0 for 12 hours at 4˚C. The seed coats were removed manually and blended with the Warring blender. Slurry was suspended in distilled water and pH was adjusted to 8.0 by adding 0.5M NaOH. The suspension was stirred manually for 30 minutes and screened with a 75 µm sieve. It was centrifuged at 5000 rpm (revolution per minute) for 20 minutes and washed 5 times with distilled water. The resultant starch was dried at 30˚C for 48 hours and stored in air-tight containers for further use.

### Chemical composition of starch

Isolated jack bean starch was analysed for its chemical composition using the methods of Association of Official Analytical Chemist (AOAC 2006). The ash content was measured in a muffle furnace at a temperature of 550$^{o}$C. The moisture content was assessed using hot air oven till achievement of constant weight at 105$^{o}$C by gravimetric method. Lipid content was analysed with the help of a solvent extraction technique. Protein was assessed by using standard Kjeldhal apparatus in which 6.25xN was taken as the conversion factor and fiber was estimated by automatic fiber estimation analyser.

### Amylose content

Total and apparent amylose were analysed using the method of Hoover and Ratnayake (2002).

### Total amylose content

The jack bean starch was defatted with hot *n*-propanol-water at 3:1v/v for 7 hours. In 10ml screw-cap reaction vial 20mg of starch (db) was dissolved in 8ml of 90% Dimethyl sulfoxide (DMSO). The content was mixed with a Vortex shaker for 20 minutes and heated at 85$^{o}$C in a water bath for 15 minutes with intermittent shaking. The vial was then allowed to cool at room temperature. The content was diluted to 25ml in a volumetric flask.1ml of this solution was taken in a test tube and mixed with 40ml of distilled water and 5ml of $I_2$/KI solution (0.0025 m $I_2$ and 0.0065 M KI). The final volume

was adjusted to 50ml. This was then left for 15 minutes at room temperature and absorbance was measured at 600 nm (Hoover and Ratnayake, 2002).

**Apparent amylose content**

The above procedure was also used to estimate the apparent amylase content of jack bean starch except in the first step. In this estimation, the starch was analysed without defatting. Further, the amylose content was estimated by drawing a standard curve with standard potato amylose (conc. 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) (range 0-100% amylose). A line of calibration was plotted. The amount of apparent and total amylose was calculated using a regression equation.

Y = 0.004 X - 0.011 .....Eq.1

(Y = absorbance at 600 nm; X = % amylose)

This was done to correct the over-estimation of both types of amylose because of the complex formation between Iodine and the amylopectin's outer branches.

**Amylose complexed with lipids**

After estimating apparent and total amylose, the amylose complexed with lipids was estimated by the formula (Hoover and Ratnayake, 2002):

$$\frac{\text{Total amylose} - \text{apparent amylose}}{\text{Total amylose}} \times 100 \quad \text{.....Eq. 2}$$

**Effect of temperatures on amylose leaching**

The effect of various temperatures on amylose leaching was assessed using the method of Ambigaipalan *et al*. ( 2013). For this, 20 mg db starch was taken in a test tube and 10 ml of water was added. The content was heated with intermittent shaking for 30 minutes in a water bath under various temperatures (65,75,85 and 95$^{o}$C) . The tubes were cooled at room temperature and centrifuged for 10 minutes at 2000xg. Further, 1ml of its supernatant was taken and amylose content was estimated as above by the method of Hoover and Ratnayake (2004). The amylose leaching was expressed as the percentage of amylose leached out per 100 gm of dry starch.

**Least gelation concentration (LGC)**

LGC of jack bean starch was analysed using the method of Adebowal and Lawal (2003). The starch sample was dissolved in 5 ml of distilled water to prepare 2, 4, 6, 8, 10 , 12, 14% (w/v) concentration. They were heated in the water bath for 30 min at 90$^{o}$C and cooled down to room temperature. All the suspensions were kept in refrigerator (10±2$^{o}$C) for 2 hours. The strength of the coagulum was evaluated by inverting the tube. The lowest sample concentration, which formed a stable gel (remained in the inverted test tube), was considered the gelation end-point, i.e., the least gelation concentration.

**Emulsion capacity and stability**

Emulsion capacity and the stability of starch were analysed using Kinsella's method (1979). In short, 0.5 g of sample was taken and 5ml of distilled water was added to an Erlenmeyer flask. It was stirred at 1000 rpm for 15 minutes with a magnetic stirrer. 5ml of soybean refined oil was added over a period of 5 minutes and stirred at 1000 rpm. This was transferred to a centrifuge tube treated in a water bath maintained at 85°C for 15 minutes with occasional stirring. This was cooled for 15 minutes in a water bath at 25$^{o}$C and then centrifuged at 3500 rpm till the height of the oil was constant. Results were expressed as a percent of the emulsion after separating the upper layer from the emulsion.

Emulsion capacity (%) =

$$\frac{\text{Height of emulsified layer in tube}}{\text{Height of total content in tube}} x100 \quad \text{...Eq. 3}$$

Emulsion stability (%) =

$$\frac{\text{Height of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} x100 \quad \text{..... .Eq. 4}$$

**Water and oil absorption capacities**

It was estimated using Beuchat's method (1977). In two separate test tubes 10 ml of distilled water and oil was added to 1.0 gm of starch sample. It was mixed thoroughly for 30 seconds and allowed to stand for 30 minutes at 21°C. It was centrifuged at 5000xg for 30 minutes. The volume of supernatant was recorded in 10ml graduated cylinder. The oil/ water absorbed mass was expressed as g/g starch on dry weight basis.

**Color evaluation**

The colour of isolated starch, i.e., how much the starch whiteness is, was measured by the Hunter Colorlab model 45/0 LAV, and the values of L*, a*, and b* were predicted. The samples were taken in a sample cup that was then placed on port plate. The sample cup was covered with an opaque cover and the colour value was measured directly.

**Effect of temperatures and concentrations on swelling power and solubility of starch**

The swelling and solubility of starch were determined-at concentrations of 1%, 2%, 3% and 4% by the method of Leach *et al.,*- (1959). The starch suspensions (0.25, 0.5, 0.75 and 1.0g starch, respectively, in 25ml of distilled water) were heated at different temperatures of 55, 65, 75, 85 and 95$^{0}$C for 30 minutes with continuous shaking followed by rapid cooling to room temperature and centrifugation at 1000 x g for 20 minutes. The solubility and swelling power were then calculated by the standard formula.

% Solubility = weight of dried sample/ weight of sample x 100 .....Eq. 5

Swelling power (g/g) =

$$\frac{weight\ of\ wet\ sample}{sample\ weight\ X\ (100-\%\ Solubility)}\ x100 \quad \text{.....Eq. 6}$$

**Effect of temperature and concentration on freeze-thaw stability**

The study was conducted at three concentrations for jack bean starch, i.e., 6%, 8% and 10% and stability was checked for five freezing cycles, i.e. day 1- day 5. The method of Hoover and Ratnayake,- (2002) was used. Starch gels with 6%, 8% and 10% concentrations were stored at 4°C for 16 hours to increase nucleation. After this, gels were frozen at -16 °C for 24 hours. The gel tubes were thawed at 25 °C for 6 hours and centrifuged for 20 min at 2000xg. The water excluded was determined.

**Effect of storage and temperature on paste clarity (turbidity) of starch**

It was determined by the method of Peera and Hoover (1999). For this 1% starch suspension was heated in a boiling water bath for 1 hour with constant stirring and cooled to room temperature. The suspensions were stored for 0-6 days at 4°C and 30°C. The absorbance was measured with a UV Spectrophotometer (ELICO, SL-177 Scanning mini spec) at 640 nm against water taken as blank and paste clarity was assessed.

**Statistical analysis**

Results were assessed in triplicates for all the samples and their mean values ±SD were predicted. The results were analysed by one-way analysis of variance (ANOVA) method at a significant difference level ($p<0.05$) by Tukey's HSD (Honestly Significant Difference) test. Statistical software (SPSS 19.0) was used for statistical analysis.

## RESULTS AND DISCUSSION

**Chemical composition**

The present study shows that the isolated starch yield of jack bean (*Canavalia ensiformis*) was 30.98% (Table 1), which was more compared to the jack bean (Canavalia ensiformis, Nigeria) starch examined by Akinyemi *et al*. (2020), while for the starches such as black gram (45%), pea (40%), and red bean (46%) it was comparatively lower. This could be explained by the compact interaction that starch granules have with specific other biomolecules (Hoover and Sosulski, 1991; Yusuf *et al*., 2007). The chemical composition of starch isolated from dry jack bean seeds is given in Table 1. The moisture, protein, and fiber content were found to be 9.67%, 0.56%, and 0.27%, respectively. Starch with a low moisture content (0–10%) is considered safe to store and analyse further without risking fatty acid microbial degradation (Chinma, 2013). In the present study, the amount of ash in the jack bean starch was 0.19%, like in the starch of native jack bean (*Canavalia ensiformis*) of Auchi area, Nigeria (Akinyemi *et al*., 2020). Occasionally, the presence of substances referred to as "fine fibers" is the source of the greater ash level in starch (Ratnayake *et al*., 2001). The present study showed that 0.30% of starch was fat, with a total carbohydrate of 89.28%. Similar findings for the crude fat content in jack bean of Abeokuta, Nigeria, starch (0.36%) (Yusuf *et al*., 2007) and from starch isolated from Nigerian legume starch (0.15 to 0.54%) were also previously reported (Ashogbon and Akintayo, 2013).

**Amylose content**

The evaluation of amylose content is of significance as it has been observed that high and low amylose starches display different structures and, consequently, affect various physicochemical characteristics during various applications (Schirmer *et al*., 2013). Total amylose content refers to the amount of defatted amylose, while apparent amylose content refers to the amount of amylose that was analysed without defatting. The removal of fat from the starch is imperative due to the potential of fat to impede accurate amylose measurement (Hoover and Ratnayake, 2001). The apparent and total amylose contents (Table 1) of native jack bean starch in the present study were 43.82% and 47.78%, respectively. Legume starches ranged in amylose concentration from 24% to 88% (Ratnayake *et al*., 2001). The amylose concentration of jack bean (*Canavalia ensiformis* of Nigeria and Mexico, respectively) starch ranged from 20.20 to 37.50% (Akinyemi *et al*., 2020; Betancur-Ancona *et al*., 2002). The starches that are used to make noodles should have a higher amylose content (Lii and Chang, 1981). The term "amylose-lipid complex" refers to the lipids that are attached to amylose. The present study for native jack bean starch also observed the amylose-lipid complex of 8.29% (Table 1). Water does not dissolve the amylose-lipid complexes, and their dissociation requires more energy. Furthermore, lipid-bound amylose is resistant to being leached out and stays inside the starch granules. (Morrison, 1988; Raphaelides and Karkalas, 1988).

**Effect of temperature on amylose leaching**

The effect of temperature on amylose leaching is illustrated in Table 2. The percentage of amylose leaching increased with temperature from 65°C to 95°C, ranging from 5.38% to 7.66%. Below 65°C, there was no amylose leaching, indicating that the starch molecules in the structure have strong bonding forces (Hoover and Ratnayake, 2002). Furthermore, because of the high amylose content of the legumes, starches exhibit limited swelling so less amylose leaching at temperatures

**Table 1.** Composition of jack bean starch

| Parameters | Mean±SD | Parameters | Mean±SD |
|---|---|---|---|
| Starch yield | 30.98±1.86(%) | Water absorption capacity | 2.31±0.06 (g/g) |
| Moisture | 9.67±0.57 | Oil absorption capacity | 2.56±0.44(g/g) |
| Ash | 0.19±0.02(%) | Emulsion capacity | 62.30±0.63(%) |
| Lipid | 0.30±0.02(%) | Emulsion stability | 71.38±0.67(%) |
| Protein | 0.56±0.05(%) | Least gelation concentration | 8.0±0.00(%) |
| Fiber | 0.27±0.06(%) | L*(lightness) | 97.14±0.01 |
| Carbohydrate | 89.28±0.62 (%) | a*(red/green) | -0.45±0.02 |
| Apparent amylose | 43.82±0.23(%) | b*(yellow/blue) | 2.38±0.01 |
| Total amylose | 47.78±0.05(%) | Amylose-lipid complex | 8.29±0.32 |

The values are depicted as the mean ±SD of three independent determinations

**Table 2.** Effect of temperature on amylose leaching of jack bean starch

| Temperature (°C) | Leached amylose (%) |
|---|---|
| 65 | 5.38±0.47 |
| 75 | 6.88±0.75 |
| 85 | 7.43±0.89 |
| 95 | 7.66±0.96 |

Values are depicted as the mean ±SD of three independent determinations

below 65°C. This results from the densely packed legume starch within the amorphous regions, which causes intense hydrogen bonding interactions between nearby amylose strands. But as the temperature increased to 90°C, amylose leaching also increased (Hoover *et al*., 2010). Amylose is forced to leach out of the hot water-starch suspension due to the crystalline amylopectin swelling when starch is heated in water (Romero and Zhang, 2019). The weak amylopectin structures may be destroyed by heat, allowing amylose to be released more readily. This could impact starch's gelatinisation characteristics (Van Hung *et al*., 2007). The more amylose lipid complex there is, the less amylose leaching (Chung *et al*., 2009).

**Gelation properties**

When starch can produce a soft gel at a minimum concentration, this is known as the least gelation concentration and is highly valued by the food industry. The temperature at which starch achieved a gel like consistency is termed as gelation temperature (Ubwa *et al*., 2012). The gelation index was typically predicted using LGC (least gelation concentration). The better the gelling property, the lower the LGC. The lowest gelation concentration was 8.0% for native jack bean starch (Table 1). At this concentration, a soft, complete gel was seen to develop. However, as concentrations were increased higher, the gel hardened and fractured. Similar results of LGC for native jack bean (*Canavalia ensiformis*, Nigeria) starch was observed by Lawal and Adebowale (2005). Starch absorbs water during gelation, which causes the granules to enlarge and ultimately create a three-dimensional structure through the process of gelatinisation. They formed a structural network in the starch granules because of intergranular interaction via H-bonding (Lawal and Adebowale, 2006).

**Emulsion capacity and emulsion stability**

The ability of proteins to produce a stable emulsion is referred to as emulsion capacity, and it is associated with the ability of proteins to absorb water and oil in the interfacial area of an emulsion. The emulsion stability test shows the ability of proteins to offer strength for resistance to stress and emulsion alterations (Singh *et al*., 2010). Nonetheless, emulsion stability assesses the capacity of an emulsion to maintain its original composition over time (Marimuthu and Gurumoorthi, 2013). In the present study native jack bean starch had an emulsion capacity and stability of 62.30% and 71.38%, respectively (Table 1). The findings align with the research conducted by Du *et al*. (2014). They found that the emulsion capacity of black beans, navy beans, and black eye beans obtained from the local market of China was 67.82%, 66.94%, and 67.02%, respectively. Further, the emulsion capacity of jack bean starch in a previous study was 55.14% (Marimuthu and Gurumoorthi, 2013).

**Water and oil absorption capacity**

Water and oil absorption capacity influences sensory qualities and other functional features. The water absorption capacity (WAC) is important in food preparation (Dossou *et al*., 2014) and is defined as the amount of water retained following compression (Hasmadi *et al*., 2020). Factors like the solubility of the starch, its size, shape, steric properties, fats and carbohydrates linked to proteins, etc., influence WAC (Chou and Morr, 1979). Oil absorption capacity (OAC) is a property related to the physical trapping of oil (Hasmadi *et al*., 2020). A key emulsifying characteristic for preserving flavor and improving the mouthfeel of food products is the capacity of starch to hold oil (Olu-Owolabi *et al*., 2011). Native jack bean starch was shown to have an absorption capacity of 2.31% for water and 2.56% for oil (Table 1). The present findings align with the research conducted by Sathe and Salunke (1981) that

the great northern bean (*Phaseolus vulgaris*) starch absorbed 2.93 g/g of water and 2.94 g/g of oil, respectively. Legume starches are less able to absorb water than cereal starches because legumes contain more amylose.The ability of legume starch to absorb water is negatively related to starch solubility and directly related to swelling power (Halbrook and Kurtzman, 1975). Some researchers (Sathe *et al*., 1981; Deshpande *et al*., 1982) reported the water absorption capacity of raw legume (Great Northern beans (*P. vulgaris* L., Idaho, US) and black gram *( P. mungo,* California) starches to be less than 10g/g and their findings were consistent with the present study, respectively.

**Color evaluation**

The degree of whiteness in starch may vary due to the presence of some minor components like polyphenolic chemicals and beta-carotene. These phytochemicals also impact starch quality as well, which could impact the color of finished products. As a result, it may lower the acceptance of starch products. (Galvez and Resurreccion, 1993). The starch needs to have a high lightness value (L*) and a low chroma value (b*) to meet customer preferences (Reddy *et al*., 2017). Furthermore, the lightness of starch is inversely related to its protein and ash content (Liu *et al*., 2018). Table 1 shows that the L*, a*, and b* values of native jack bean starch were 97.14, -0.45, and 2.38, respectively. A slight greenish tint is indicated by the negative value of a*, and a mild yellow tint is shown by the positive value of b* in starch. As the value of L* toward whiteness was very high in the present study, the isolated starch was pure and light in colour, indicating its potential applications in various cuisines where colour equality predominates.

**Effect of temperature and concentration on swelling power and solubility**

Swelling power and solubility are affected by the amylose-to-amylopectin ratio, the chain length distribution of amylopectin, phosphate content, amylose leaching, and amylose lipid complexes (Hoover, 2001). Tables 3 and 4 present the findings of jack bean starch swelling power and solubility at various temperatures ranging from 55°C to 95°C and the effect of concentration varied from 1 to 4%. The swelling power of jack bean starch varied from 2.30 - 17.66 g/g, 1.94 - 11.31 g/g, 1.83 - 10.09 g/g, and 1.79 - 8.82g/g at 1%, 2%, 3%, and 4% starch concentrations, respectively. This increase was observed when the temperature increased from 55°C to 95°C (Table 3). Similar to this, at 1%, 2%, 3%, and 4%, respectively, the solubility of native jack bean starch ranged from 2.56% - 17.28%, 1.99% - 8.84%, 1.52% - 7.99%, and 1.50% - 7.05% at temperatures from 55°C to 95°C (Table 3). The swelling power and solubility of jack bean starch at 1% concentration were 17.34 g/g and 24.56 % respectively ( Marimuthu and Gurumoorthi, 2013). The intrinsic associative forces that keep the granule structurally intact (Peroni *et al*., 2006) diminished as the temperature enhanced. The crystalline area of starch granules is disrupted when they are heated in enough water. By forming hydrogen bonds with the water molecules, the exposed hydroxyl groups of amylopectin and amylose further increased the swelling and solubility of the starch granules.

In the present study, swelling power and solubility demonstrated a negative trend and declined as starch concentration rose from 1% to 4%. At 55°C, the swelling power dropped from 2.30 to 1.79 g/g as the concentration increased from 1% to 4%. Further, at 95°C, it dropped to 8.82 from 17.66 g/g as the concentration increased from 1% to 4%. For solubility, a comparable pattern was noted. It dropped from 2.56% to 1.50% as the concentration climbed from 1% to 4% at 55°C, and when the temperature rose to 95°C, it dropped from 17.28% to 7.05% as the concentration increased from 1% to 4%.

**Effect of storage and concentration on freeze-thaw stability**

The tendency to retrograde starch may be indicated by freeze-thaw stability (Karim *et al*., 2000). Frozen food products can thaw and refreeze, potentially causing ice crystals that can damage food structure, and their freeze-thaw stability is influenced by temperature and storage duration. (Hussain *et al*., 2013). To evaluate the freeze-thaw stability of jack bean starch gels, a syneresis study was carried out. Starch gels at concentrations of 6%, 8%, and 10% were made for the current investigation and kept frozen for five days. The gels were removed and centrifuged daily, and the amount of water released was measured. The data of Table 5 shows that, water released was considerably more after first day of storage at all concentrations and mini-

**Table 3.** Effect of temperatures and concentrations on swelling power (g/g) of jack bean starch

| Starch Conc. (%) | 55°C | 65°C Swelling | 75°C Power | 85°C | 95°C |
|---|---|---|---|---|---|
| 1 | 2.30±0.03$^{b}$ | 3.17±0.12$^{d}$ | 5.85±0.05$^{d}$ | 13.91±0.09d | 17.66±0.07$^{d}$ |
| 2 | 1.94±0.05$^{a}$ | 3.00±0.04$^{c}$ | 4.89±0.03$^{c}$ | 8.63±0.06$^{c}$ | 11.31±0.04$^{c}$ |
| 3 | 1.83±0.08$^{a}$ | 2.24±0.06$^{b}$ | 4.14±0.07$^{b}$ | 7.00±0.09$^{b}$ | 10.09±0.04$^{b}$ |
| 4 | 1.79±0.06$^{a}$ | 2.00±0.10$^{a}$ | 3.21±0.05$^{a}$ | 6.14±0.05$^{a}$ | 8.82±0.04$^{a}$ |

Values are depicted as the mean ±SD of three independent determinations; Values in the same column having different superscripts are different significantly ($p<0.05$)

**Table 4.** Effect of temperatures and concentrations on solubility (%) of jack bean

| Starch Conc. (%) | 55°C | 65°C Solubility | 75°C | 85°C | 95°C |
|---|---|---|---|---|---|
| 1 | 2.56±0.07$^{c}$ | 3.69±0.03$^{d}$ | 7.01±0.11$^{d}$ | 11.83±0.04$^{d}$ | 17.28±0.03$^{d}$ |
| 2 | 1.99±0.09$^{b}$ | 3.00±0.12$^{c}$ | 5.72±0.07$^{c}$ | 6.58±0.13$^{c}$ | 8.84±0.09$^{c}$ |
| 3 | 1.52±0.06$^{a}$ | 2.76±0.05$^{b}$ | 4.29±0.07$^{b}$ | 5.21±0.06$^{b}$ | 7.99±0.09$^{b}$ |
| 4 | 1.50±0.03$^{a}$ | 2.24±0.06$^{a}$ | 2.72±0.07$^{a}$ | 3.01±0.06$^{a}$ | 7.05±0.20$^{a}$ |

Values are depicted as the mean ±SD of three independent determinations; Values in same column having different superscripts are different significantly ($p<0.05$)

**Table 5.** Effect of concentration and storage on freeze-thaw stability of jack bean

| Starch conc (%) | Storage days Expelled water (ml) | | | | |
|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| 6 | 11.11±0.10$^{c}$ | 2.61±0.06$^{c}$ | 1.29±0.11$^{b}$ | 0.00 | 0.00 |
| 8 | 9.97±0.12$^{b}$ | 1.83±0.08$^{b}$ | 0.92±0.07$^{a}$ | 0.00 | 0.00 |
| 10 | 7.29±0.08$^{a}$ | 1.20±0.09$^{a}$ | 0.85±0.10$^{a}$ | 0.00 | 0.00 |

Values are depicted as the mean ±SD of three independent determinations; Values in same column having different superscripts are different significantly ($p<0.05$)

mum after the third day. On the first, second, and third days of storage at 6% starch concentration, the amount of water expelled was 11.11 ml, 2.61 ml, and 1.29 ml, respectively. Further, at 8% and 10% starch concentration, it was 9.97 ml, 1.83 ml, and 0.92 ml, and 7.29 ml, 1.20 ml, and 0.85 ml on the first, second, and third days of successive storage.

Furthermore, no water was released for all concentrations on the fourth day of storage. This can be explained by the fact that, following the first day's freeze-thaw cycle, the same gel was refrozen and used for the syneresis for the next day. The gel had less water than the first and second days of storage intervals (Hussain *et al*., 2013). Jack bean starch gels undergo retrogradation, removing water during ice crystal production. After freezing, ice crystals divide water, resulting in good freeze-thaw stability. The gels change from smooth to porous, allowing water to readsorb (Varavinit *et al*., 2002), resulting in no syneresis after the fourth-day storage. At 6% concentration, more water was expelled than at 8% and 10% concentration. The reason for this was the osmotic pressure. Because starch retrogradation is closely correlated with the concentration of starch in the gels or pastes, the higher concentration led to a lower degree of syneresis (Orford *et al*., 1987).

**Effect of storage and temperature on paste clarity (turbidity) of starch**

Paste clarity is a crucial factor in evaluating the quality of the starch paste. This is because of the gel's molecular chains, which exhibit significant reflection, further reduce the amount of light that could pass through them (Segura *et al*., 2010). Table 6 displays the paste clarity of gelatinised starch suspensions made from jack bean starch. The absorbance values were observed by spectrophotometer to predict the starch paste clarity. The paste clarity was evaluated after storing the starch suspensions for one to seven days at two different temperatures (30°C and 4°C). It was found to be 0.052 on the first day and 0.878, 1.057, 1.063, 1.079, 1.090, and 1.145 on the days that followed at 30°C. Comparably, on the first day at 4°C, the absorbance values increased from 0.9562 to 1.525, 1.549, 1.570, 1.609, and 1.664. The results show that turbidity increased with storage time and more during refrigeration storage. The rearrangement of molecules in the solubilised starch chains was identified as the cause of the increased turbidity seen in all of the starch suspensions after refrigerated storage (Goswami *et al*., 2018). Moreover, synthesising more retrograded starch at lower temperatures, which reduces clarity, may cause high absorbance values at refrigeration temperatures. Similarly, paste clarity is also influenced by storage time. Gel became cloudy after a long storage period because the amount of starch retrogradation increased with the lengthening of the storage period (Denchai *et al*., 2019). This light absorption behavior revealed that starch gels held at ambient temperature have superior paste clarity and less chances to retrograde than those stored under refrigeration. Increased retrogradation with increased amylose leaching increases light absorption greatly (Denchai *et al*., 2019).

**Table 6.** Effect of storage and temperature on paste clarity (turbidity) of starch

| Temperature (°C) | Storage period (Days) Optical densities as absorbance units | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
| 30 | 0.052 | 0.878 | 1.057 | 1.063 | 1.079 | 1.090 | 1.145 |
| 4 | - | 0.9562 | 1.525 | 1.549 | 1.570 | 1.609 | 1.664 |

## Conclusion

The assessment of physicochemical characteristics, including the amount of moisture, ash, fat, protein, fiber, amylose content, complexed amylose with lipids, least gelation concentration, emulsion stability and capacity, and starch whiteness of jack bean (*Canavalia ensiformis*) starch, indicated a positive relationship between temperature and amylose leaching because a high temperature might have weakened the linkages that allow amylose to reach out. Likewise, swelling power and solubility showed a positive relation with temperature but a negative relation with starch concentration. Furthermore, freeze-thaw stability increased as starch concentration and storage time increased due to the change of gel structure from a smooth to a sponge-like structure during the freeze-thaw cycles. At 30°C, the starch paste had more clarity than at 4°C because the low temperature promotes early retrogradation. The present results will be helpful to the food sector when it comes to using unconventional starch sources instead of traditional ones like potatoes, corn, rice, cassava, chestnuts, wheat, etc.

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### Conflict of Interest

The authors declare that they have no conflict of interest.

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