ISSN: 2347-467X, Vol. 12, No. (1) 2024, Pg. 408-422

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Studies on Physicochemical Properties of Rice Bean (*Vigna Umbellata*) Starch: An Underutilized Legume

SAPNA DHAWAN MUNJAL[1], JYOTIKA DHANKHAR[1]*, ALKA SHARMA[2] and PRIXIT GULERIA[1]

[1]Dept. of Food Technology, M D University, Rohtak, Haryana, India.
[2]Department of Food Technology, GJUS & T, Hisar, Haryana, India.

Abstract

Starch is a reserve carbohydrate present in plants. It is an important component for maintaining various quality attributes like texture, consistency, thickening in the food industry. The aim of the present study was to isolate starch from a legume named rice bean that is not very much famous and considered as underutilized-legume. The isolated starch was studied for various physicochemical properties. One-way analysis of variance was used in this study. The starch was isolated as a white powder from the dry legume by using standard method and the starch yield was observed to be 25.79%. Legume starch was further assessed for the chemical composition. The moisture, ash, fat, protein, fiber and carbohydrate content were 9.56%, 0.35%, 0.21%, 0.69%, 0.33%, and 89.19% respectively. The apparent and total amylose content was 37.62% and 40.83% with least gelation concentration at 8% of starch gel. Starch was further assessed for functional properties. The leached amylose displayed a rise in content from 6.56% to 9.50% with the corresponding increase in temperature from 65°C to 95°C that may affect gelatinization properties of starch. The water absorption capacity was 3.60 g/g while oil absorption capacity was 3.54 g/g. Further, the emulsion stability and emulsion capacity of native starch was 79.00% and 68.33% respectively that showed good stability of emulsion. Similarly, the swelling power and solubility of rice bean starch increased with the increase of temperature from 55-95°C. However, the opposite trend was observed for an increase in starch concentration from 1-4%. The effect of different concentrations at 6, 8 and 10% on freeze-thaw stability and storage studies showed that the expelled water decreased with the rise in starch concentration up to three consecutive storage days while no change was observed later during frozen gel storage. It showed good freeze thaw

Article History
Received: 14 December 2023
Accepted: 20 February 2024

Keywords
Amylose;
Color Analysis;
Freeze Thaw;
Paste Clarity;
Rice Bean;
Starch;
Swelling Power.

CONTACT Jyotika Dhankhar jyotika.ft@mdurohtak.ac.in Dept. of Food Technology, M D University, Rohtak, Haryana, India.

Doi: https://dx.doi.org/10.12944/CRNFSJ.12.1.33

stability of starch. This underutilized legume attracted more attention from researchers due of its affordability and ease of availability. In dry farming circumstances with limited soil fertility, the legume yield consistently. Thus, this neglected legume that has minimal agronomic development but many qualities is a good substitute for starch. However, thorough study on the unique functional properties and other qualities of starch from rice bean has not been carried out. The results of this underutilized legume starch can provide opportunities to starch researchers and to the food industries that are frequently using conventional sources of starch from cereals, tubers and rhizomes.

Introduction

Pulses and grain legumes are the cheapest sources of fibers, proteins, minerals, and vitamins along with various phytochemicals that provide health benefits.[1] The management of diabetes, cancer, heart disease, healthy aging, irregular bowel movements, obesity, and diabetes are among the chronic disorders for which the legume diet is advised.[2] Most of the legumes are used to enhance technological properties and nutritive value in various modern and traditional foods like snacks, pasta, breakfast food, soups etc.[3-4] Legumes are a rich source of carbohydrates mainly resistant starch, and fiber, devoid of allergic gluten proteins, so can be used in dietetic and gluten-free foods.[5] Legumes have a higher concentration of resistant starch than other conventional starches like cassava, corn, rice, and potatoes, which makes them useful for use in the creation of anti-diabetic diets.[6]

Vigna umbellata is an underutilized and multipurpose legume commonly named rice bean, mambi bean, climbing mountain bean and oriental bean.[7] In certain regions of India and Southeast Asia, people consume this legume as food8. It grows well in humid subtropical regions as well as temperate (warm and chilly) climates, and can withstand the conditions of drought. Therefore, it can be cultivated by the poor farmers at low cost and in a low fertile soil.[9] Rice bean is used as a biological barrier of the living fence, as a cover crop and as green manure.[10] Because of fibrous mucilage, the rice bean seeds cannot be easily processed to dhal. As a result, hulling and cotyledon separation are more difficult with this legume than with other common sources like mung beans, peas, cowpeas, etc.[11] Its seeds are rich source of proteins, starch, and fiber. In general, starch in rice bean mostly varies between 52-57% and amylose content generally ranges from 20-60%.[12] The amylose content in rice bean is higher than other conventional sources (Cereals and tubers) of starch that makes it more important in the food industry because amylose affects various physicochemical parameters of starch.[12]

Starch is a complex carbohydrate polymer and semi-crystalline in nature. It is an abundant carbohydrate reserve stored in a variety of plants.[13] It is used in food industry to improve various qualities like texture, quality, consistency, and thickening to enhance the overall quality and acceptability of food.[14] It is also used to develop biodegradable materials like edible coatings and polymers.[15] The various properties of starch like gelation, swelling power and solubility, water absorption capacity, oil absorption capacity, emulsification properties, etc further depend on chemical composition and major constituents of starch.[16] Because of the desired characteristics, starch is also used in polymer industries, and biomedical and pharmaceutical industries.[17-19] Starch from conventional sources like cereals, rhizomes and tubers has been extensively studied to date[19] but the non-conventional starch sources (legumes) still need to be studied because they are the cheapest source sources of starch.

As little is known about the properties of rice bean starch, it has not been exploited economically and is hence still underutilized. The physicochemical characteristics of rice bean starch are poorly understood[12] because the less research has been conducted by the researchers on this. Consequently, since food industries only rely on traditional sources of starch like grains and tubers, extensive research is required to examine its varied qualitative attributes and expand its applicability in food sector. Further, the cultivation of this legume may play a role for livelihood of poor farmers. Therefore, the present study was conducted to observe the physicochemical characteristics of native rice bean starch.

Material and Methods

Materials

The rice bean legume was purchased from Haryana Agriculture University, Hisar, Haryana. All the chemicals used i.e., toluene, sulphuric acid, hydrochloric acid, potassium iodide, dimethyl sulfoxide (DMSO), n-propanol, etc. for the research were of analytical grade from Himedia, Sigma-Aldrich and Merck.

Isolation of Rice Bean Starch

Isolation of starch from rice beans was carried out by the method of Schoch and Maywald.[20] The seeds were soaked in distilled water for a night with 0.5% toluene (to prevent fermentation during soaking) and washed properly with distilled water. Slurry was prepared by using Waring blender and passed from a nylon cloth (60-mesh size). The slurry was filtered again through a screen (220-mesh size) after washing with distilled water. The process of double sieving provided better starch yield. The slurry was allowed to stand for 1 hour. The upper pale layer was scrapped off and the remaining white layer was re-suspended in distilled water and centrifuged (Laboratory centrifuge-REMI) for 5 minutes at 4000 revolutions per minute. The process was repeated 4 times to obtain clear white layer of starch. It was dried in a convection oven at 40˚ C for 24 hours, ground with pestle mortar, sieved (80-mesh size) and stored in an air-tight container for further analysis.

Chemical Composition of Isolated Starch

The moisture, ash, lipid, protein, and fiber content of isolated rice bean starch were analyzed by using the Official Methods of Analysis-(AOAC).[21]

Amylose Content

Total and apparent amylose percent were analyzed by the method of Hoover and Ratnayake.[22]

Total Amylose Content

Defatted native starch (20 milligram starch) was taken in a 10 millilitre screw-cap reaction vial and dissolved in 8 millilitre of 90% Dimethyl sulfoxide (DMSO). It was mixed with a Spinix vortex shaker (Tarsons, India) for 2 minutes and heated at 85oC in a water bath for 15 minutes with intermittent shaking. It was allowed to cool at room temperature. The content was diluted to 25 millilitre in a volumetric flask. 1 millilitre of this solution was taken in a test tube and mixed with 40 millilitre water and 5 millilitre I2/KI solution (0.0025 m I2 and 0.0065 M KI). The final volume was adjusted to 50 millilitre. This was then left for 15 minutes at room temperature and absorbance was measured at 600 nanometre (nm) by double beam spectrophometer (ELICO, India).

Apparent Amylose Content

The apparent amylose content of starch was estimated by the above same procedure except starch was taken 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

$$Y=0.004\,X-0.011 \quad ...(1)$$

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

This was done to correct the overestimation of both types of amylose because of the complex formation between I_2 (Iodine)and the amylopectin's outer branches.

Amylose Complexed with Lipids

This was observed after obtaining apparent and total amylase content and calculated by a formula as under Hoover and Ratnayake.[22]

$$(\text{Total amylose-apparent amylose})/(\text{Total amylose}) \times 100 \quad ...(2)$$

Effect of Temperatures on Amylose Leaching

The effect of various temperatures on amylose leaching was assessed by the method of Ambigaipalan *et al.*[23] In a test tube 20 mg starch and 10 ml of distilled water was taken. The content was heated to different temperatures (65, 75, 85, and 95 ^{o}C) in a boiling water bath for 30 minutes while being shaken intermittently. The tubes were cooled at room temperature and centrifuged for 10 minutes at 2000xg. Now, 1 milliliter of its supernatant was withdrawn, and the Hoover and Ratnayake[22] method was used to assess the amylose content as before.

Least Gelation Concentration (Lgc)

The Adebowal and Lawal[24] method was used to assess the LGC of rice bean starch. The sample of starch was dissolved in 5 milliliters of distilled water to get concentrations of 2, 4, 6, 8, 10, 12, and 14% (w/v). The tubes were cooled to room temperature after 30 minutes at 90 ^oC in a water bath. All of the suspensions were stored in a refrigerator at 10±2^oC for 2 hours. By inverting the tube, the coagulum's strength was assessed. The gelation end point, or least gelation concentration, was defined as the sample concentration at which no longer formed a stable gel and remained in an inverted test tube.

Emulsion Capacity and Emulsion Stability

Analysis of emulsion capacity and emulsion stability was done by using Kinsella[25] method. 5 millilitres of distilled water was added to half a gram of starch (0.5 g) in an Erlenmeyer flask. It was mixed with a magnetic stirrer (TARSONS, India) at 1000 rpm for 15 minutes. Over a 5-minute period, 5 ml of refined soybean oil was added and the mixture was agitated at 1000 rpm. The suspension was transferred to a centrifuge tube treated in a shaking water bath (NSW-100, India) maintained at 85˚C for 15 minutes. This was cooled for 15 minutes in a water bath at 25^oC and then centrifuged at 3500 rpm till the height of the oil was constant. Results were expressed as a percent of emulsion after separating the upper layer from the emulsion.

Emulsion capacity (%) = (Height of emulsified layer in tube)/(Height of total content in tube) x100 ...(3)

Emulsion stability (%) = (Height of emulsified layer after heating)/(Height of emulsified layer before heating) x100 ...(3)

Water and Oil Absorption Capacity

Analysis of water and oil absorption capacity was done by the method of Beuchat26. 10 ml of distilled water and oil was added to 1.0 gm of starch sample in different test tubes. The content of both the tubes were mixed properly. It was let to stand at 21˚C for 30 minutes. The material was centrifuged at 5000xg for 30 minutes, A 10 milliliter graduated cylinder was used to record the volume of supernatant. The mass of oil/ water absorbed is expressed as g/g starch on a dry weight basis.

Color Analysis

Hunter Colorlab (45/0 LAV) was used to measure the color of the isolated starch in terms of whiteness, and L*, a*, and b* values were anticipated. A sample cup was used, and it was set on the port plate to hold the sample cup. The sample cup was covered with an opaque cover and the color value was measured directly that was displayed on the screen.

Effect of Temperatures on Swelling Power and Solubility of Starch

The solubility and swelling power of starch was determined at concentrations of 1%, 2%, 3%, and 4% by the method of Lauzpnet *et al.*,[27] The starch suspension—0.25, 0.50, 0.75, and 1.0g of starch, respectively, in 25 milliliters of distilled water—was heated for 30 minutes at various temperatures (55, 65, 75, 85, and 95^0C), while being continuously shaken. This was followed by a quick cooling period to room temperature and 15-minute centrifugation at 1900xg. The % solubility and swelling power were then calculated by the standard formula.

% Solubility = (weight of dried sample)/(weight of sample) x100 ...(5)

Swelling power (g/g) = (weight of wet sample)/(sample weight X (100-% Solubility)) x100 ...(6)

Effect of Temperature and Concentration on Freeze-Thaw Stability

The method of Hoover and Ratnayake[28] was used to study freeze-thaw stability of starch. It was conducted at three concentrations for rice bean starch (6%, 8%, and 10%) and stability was checked for five freezing cycles (day 1-5). For sixteen hours, starch gels were kept at 4 ^oC for 16 hours. The gels were further kept in frozen storage for a full day at -16^oC. The gel tubes were centrifuged at 2000xg for 20 minutes after being frozen for 6 hours at 25 ^oC. The amount of water (ml) removed from each tube was calculated.

Effect of Storage and Temperature on Paste Clarity of Starch

The Peera and Hoover[29] approach was used to determine it. The starch suspension (1% starch solution in distilled water) was boiled in a boiling water bath for an hour while being continuously stirred. It was then cooled to room temperature in the two different test tubes. The tubes were stored for 1-7 days at 4°C and 30°C. The paste clarity was assessed by obtaining absorbance values of suspensions displayed on the UV Spectrophotometer (ELICO, SL-177 Scanning mini spec) at 640 nm against distilled water taken as blank. The observation was continued for 7 days to study the storage effect on the clarity of starch paste.

Statistical Analysis

All the results were reported in triplicates for all the properties and their mean values ±SD (standard deviation of mean) were predicted. Statistical software (SPSS 19.0) was used for statistical analysis. One way analysis of variance (ANOVA) at a significant difference level of $p<0.05$ was used to analyze the data.

Results and Discussion

Table 1: Chemical composition of rice bean starch

Parameters (%)	Mean±SD
Starch yield	25.79±2.51
Moisture	9.56±0.12
Ash	0.35±0.02
Lipid	0.21±0.05
Protein	0.69±0.02
Fiber	0.33±0.05
Carbohydrate	89.19±0.08
Apparent amylose	37.62±1.02
Total amylose	40.83±0.02
Amylose complexed with lipids	7.90±0.13

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

Chemical Composition

The isolated starch from legume was analyzed for the chemical composition and the results were displayed in Table 1. The values are presented as mean±SD. The extraction yield of starch was 25.79% (Table 1) It was comparatively higher than beach pea (12.30%) legume starch previously studied[30] but lower compared to starches extracted from black gram (45%), pea (40%), and red bean (46%) and this may be attributed to the compact association of granules of starch with other biomolecules.[31] It is challenging to extract the starch from legume seeds because they include fine fiber and flocculent proteins, which sedimented as a brown layer with the starch during separation.[20] The dilute alkali has been used by some investigators for more efficient isolation of starch.[32]

The moisture, protein, and fiber were found to be 9.56%, 0.69%, and 0.33% respectively (Table 1). The low moisture level of the starch (0–10%) made it safe for storage and subsequent analysis without running the risk of fatty acid microbial degradation.[33] The ash content of rice bean starch was 0.35%. The "Fine fibers" are components that contribute to the greater ash content of starch and also affect the whiteness of starch.[34] The present study shows the lipid content in starch was 0.21%. Similar results were also reported for ash content (0.24-0.36%) and crude fat content (0.15 to 0.54%) of starches isolated from Nigerian legume starch.[35] The total carbohydrates (sum of moisture, ash, lipid and protein) were found to be 89.19% in rice bean starch. The quantity of fiber, fat, protein, and ash is usually considered as the purity index of legume starches. The less quantity of these components contribute to the purity of isolated starch.[36]

Amylose Content

In the present study, apparent amylose content and total amylose content of native rice bean starch were 37.62% and 40.83% respectively (Table 1) which was within the range (21.19% - 60.14%) as previously studied for rice bean starch grown in Himalyan region by Kaur *et al.*,[12] Total amylose content refers to the defatted amylose content, while apparent amylose content is the assessed amylose before defatting. Amylose content significantly affects starch properties, impacting food characteristics. High and low amylose starches display different structures, affecting physicochemical characteristics (pasting, swelling, texture etc) during various applications.[37-38] Colorimetric techniques based on iodine complex production can be used to estimate amylose. However, amylopectin interference may cause an overestimation. Consequently, propanol should be used to defatt starch prior to the final evaluation of amylose.[39] The amylose content in legume starches ranged from 24%-88% as reported previously in various legumes34. Moreover, during the studies of mung bean starch the researchers reported amylose content varying from 37-42%.[40] It is preferable for the starches to have a higher amylose content while making noodles.[41] The lipids that are bound to amylose are called as amylose-lipid complex. The rice bean starch contains 7.90% of amylose-lipid complex. Amylose-lipid complexes are insoluble in water and require higher energy for dissociation, while amylose bonded with lipids remains intact within starch granules.[42-43]

Effect of Temperature on Amylose Leaching

Fig. 1 illustrates the amylose leaching of rice bean starch and the impact of temperature on leaching. As the temperature increased from 65°C to 95°C, there was a significant variation in the leaching of amylose ($p < 0.05$), ranging from 6.56% to 9.50%. Below 65°C, there was no amylose leaching, indicating that the starch molecules in the structure had strong bonds w.[34] ith one another.[28] Legumes have high amylose concentration, reducing swelling and amylose leaching at low temperatures (< 65°C).[44] However, at 90°C, amylose leaching increases due to crystalline amylopectin swelling in water, causing the suspension to leach out.[45] Heat can weaken amylopectin structures, releasing amylose, potentially affecting gelatinization properties of starch.[46] Relationship between swelling power, starch solubility, and amylose leaching may exist.[47-49] Similar results of amylose leaching were also observed in the previous studies for navy beans and northern bean as 3.5%-9.0% and 3.8%-9.50% respectively.[47] Amylose leaching is influenced by the apparent amylose content, bound lipids, and interactions between amylose-amylopectin and amylose-amylose chains, with a higher amylose lipid complex causing less leaching.[50.]

Gelation Properties

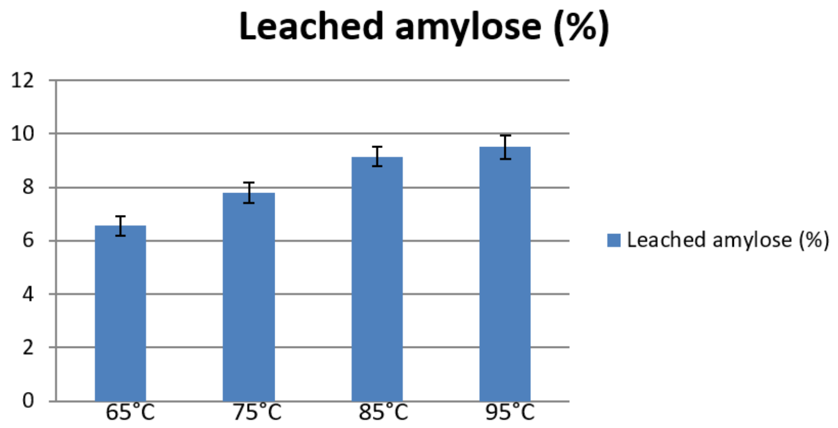

Fig. 1: Effect of temperature on amylose leaching of rice bean starch

The least gelation concentration of native rice bean starch was observed at 8.0% (Table 2). At this concentration, a soft, complete gel was seen to develop. However, as the concentrations increased higher, the gel hardened and fractured. In previous studies the LGC of a legume (Jack bean, Nigeria) was observed at 8% similar to the present study and gel become harder at increased concentrations.[51] Since the LGC and water absorption capacity of starches are connected, the high water absorption capacity of starches contribute to good gel forming abilit.[51] Moreover, it is affected by the physical competition of water for its availability in starch gelatinization and protein gelation.[52] The gelation temperature is the point at which starch takes on the consistency of gel, whereas the least gelation concentration is the lowest concentration of starch required to make a soft gel.[53] Starch absorbs water during gelation, which causes the granules to enlarge and ultimately create a three-dimensional structure through the process of gelatinization. It occurs due to the formation of the structural network in the starch granules by H-bonding as intergranular binding.[54]

Emulsion Capacity and Stability

The emulsion capacity and emulsion stability of native rice bean starch were 68.33% and 79.00% respectively (Table 2). 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 stability of the emulsion is assessed by evaluating the proteins' ability to fortify the emulsion's resistance to alterations and stress. As a result, it is related to the interfacial area's consistency throughout a certain time period.[55] The findings were in line with research conducted by Du *et al*., that the emulsion capacity of black beans, navy beans, and black eye beans was 67.82%, 66.94%, and 67.02% respectively. Additionally, the pinto bean and red kidney bean emulsion stability was 84.15% and 86.54%, respectively. The researchers also observed that water and protein interactions occur in the polar amino acid areas of protein molecules.[56] Most proteins have different polar side chains that contain peptide groups from parent chains. These chains result in hydrophobic areas that affect the molecules' solubility and emulsification potential.[57]

Table 2: Hydration and physical properties of rice bean starch

Parameters	Mean±SD
Water absorption capacity	3.60±0.55 (g/g)
Oil absorption capacity	3.54±0.43(g/g)
Emulsion capacity	68.33±0.07(%)
Emulsion stability	79.00±0.05(%)
Least gelation concentration	8.0±0.00(%)
L*(lightness)	94.23±0.50
a*(red/green)	0.48±0.40
b*(yellow/blue)	5.78±0.70

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

Water and Oil Absorption Capacity

The water and oil absorption capacity of native rice bean starch was found to be 2.60% and 2.54% respectively (Table 2). Water absorption capacity plays a crucial role in the preparation of food as it affects sensory attributes and other functional properties.[58] It is the amount of water retained after compression or external centrifugal force whereas oil absorption capacity is a functional property related to the physical entrapment of oil.[59]The ability of starch to retain oil is a measure of emulsifying property that is important to retain flavour and increase the mouth feel of the food product.[60] Water absorption and retention are crucial for food system texture, with legume starches having lower water absorption capacity due to less amylose content.[61] It is affected by various parameters like conformational characteristics, size, shape, steric properties, fats and carbohydrates associated with proteins, hydrophilic-hydrophobic balance in starch molecules, thermodynamic properties of the system, solubility of the starch molecule and physicochemical environment etc.[62] The present results were comparable with the studies of Sathe & Salunke.[63] They observed the water and oil absorption of great northen bean starch as 2.93g/g and 2.94 g/g respectively. The swelling power and the solubility of starch are inversely correlated with the water absorption capacity of legume starches.[61] Researchers reported that raw legume starches had a water absorption capacity of less than 10 g/g63–65. These findings were consistent with the findings of the current investigation.

Pigmentation

The L*, a*, and b* values of native rice bean starch were 94.23, 0.48 and 5.78 respectively (Table 2). The components like beta-carotene and polyphenolic compounds are present in plants and may influence the degree of whiteness in starch. Additionally, these phytochemicals affect the quality of starch, which may affect the final color of the product36. To satisfy consumer preferences, it is essential that the starch must have a low chroma value (b*) and high lightness value (L*) The red-green color axis is quantified using the a* value. More red tones are present when a* has a positive value, while more green tones are present when a* has a negative value. Similarly, a metric for measuring the color axis that runs from yellow to blue is the b* value. More yellow tones are present when the b* value is positive, while more blue tones are present when the b* value is negative. Rice bean starch can be used in food applications i.e., pasta products.[66] The value of L* (94.23) in the present study showed that the value is very high towards the whiteness so the isolated starch is pure and light in color which further indicates its applications in various foods where the equality in color is predominant. Moreover, protein and ash in starch are negatively related to the lightness of starch.[67]

Effect of temperature on swelling power and solubility

Swelling power and solubility depict the interaction within crystalline and amorphous regions within the starch chains.[68] The swelling power and solubility at different temperatures (55 to 95°C) and the effect of concentration (1 to 4%) are shown in Fig. 2-3 and Table 3-4. The swelling power of rice bean starch significantly increased ($p < 0.05$) as the temperature increased from 55- 95°C . It ranged 2.50 - 20.53g/g, 2.19 - 12.08g/g , 2.00 - 10.79g/g and 1.94 - 9.80g/g at 1%, 2%, 3% and 4% starch concentrations respectively (Table 3). Similarly, solubility of native rice bean starch was in the range of 2.31% - 12.85%, 1.56% - 7.84%, 1.14% - 6.53% and 1.04% - 6.37% at 1%, 2%, 3% and 4% respectively with the elevated temperature from 55- 95°C (Table 4). The internal associative forces that keep the structure of granule intact, diminished as temperature increased.[69]

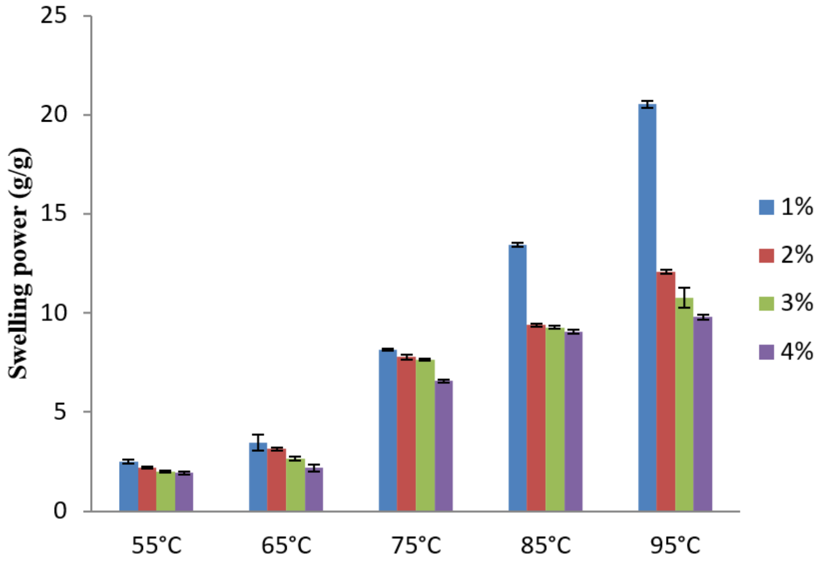

Fig. 2: Effect of temperature on swelling power of rice bean starch (1, 2, 3 and 4 shows the 1%, 2%, 3% and, 4% starch concentration respectively)

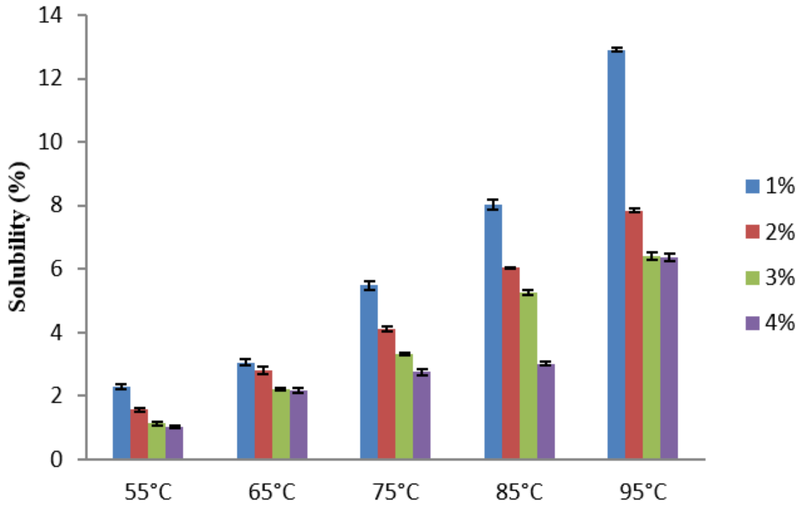

Fig. 3: Effect of temperature on solubility of rice bean starch (1, 2, 3 and 4 shows the 1%, 2%, 3% and, 4% starch concentration respectively)

Swelling power indicates water absorption, while solubility measures dissolution. Heat causes disruption in starch granules, causing hydroxyl groups to link with water molecules, increasing swelling and solubility.[70] The swelling power and solubility of starch are significantly influenced by factors such as amylose-to-amylopectin ratio, chain length distribution, phosphate concentration, amylose leaching, and amylose lipid complexes.[71-72] Furthermore, swelling power and solubility showed the reverse trend and decreased as the concentration of starch increased from 1% - 4%. The swelling power decreased to 1.93g/g as concentration increased to 4% at 55°C. While, at the temperature 95°C the swelling power decreased to 9.65g/g as concentration increased to 4%. A similar trend was observed for solubility. It decreased to 1.00% as concentration increased to 4% at 55°C and at temperature 95°C it decreased to 6.39% as concentration increased to 4%.

Table 3: Effect of temperatures and concentrations on swelling power (g/g) of rice bean starch

Conc. (%)	55°C	65°C	75°C	85°C	95°C
1	2.5±0.09^{c}	3.46±0.40^{c}	8.15±0.06^{c}	13.46±0.10^{c}	20.53±0.20^{d}
2	2.19±0.06^{b}	3.15±0.07^{c}	7.77±0.14^{b}	9.4±0.07^{b}	12.08±0.09^{c}
3	2.00±0.06^{a}	2.67±0.10^{b}	7.64±0.04^{b}	9.28±0.08^{b}	10.79±0.50^{b}
4	1.94±0.07^{a}	2.19±0.17^{a}	6.56±0.08^{a}	9.04±0.10^{a}	9.8±0.13^{a}

The 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 4: Effect of temperatures and concentrations on solubility (%) of rice bean starch

Conc. (%)	55°C	65°C	75°C	85°C	95°C
1	2.31±0.08^{c}	3.05±0.10^{c}	5.48±0.14^{d}	8.03±0.14^{d}	12.91±0.06^{c}
2	1.56±0.07^{b}	2.81±0.11^{b}	4.11±0.09^{c}	6.04±0.03^{c}	7.84±0.06^{b}
3	1.14±0.06^{a}	2.21±0.05^{a}	3.31±0.04^{b}	5.26±0.06^{b}	6.41±0.11^{a}
4	1.04±0.04^{a}	2.17±0.09^{a}	2.76±0.10^{a}	3.01±0.06^{a}	6.37±0.11^{a}

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

Effect of Storage and Concentration on Freeze-Thaw Stability

Freezing is one of the method used to enhance the shelf life of perishable foods and could be used as an indicator of tendency to retrograde starch.[73] In the present study the starch gels at 6%, 8% and 10% concentrations were stored in freezing conditions for 5 days. The gels were taken out each day, centrifuged and water released was measured in ml. The results in Table 5 depicted that the water leached out was significantly more (p<0.05) on the first day at all the concentrations and decreased till 3rd day of storage. The expelled water was 9.80 ml, 1.67 ml, and 0.9 ml on 1st, 2nd and 3rd day storage respectively (6% concentration) while it was 7.84 ml, 1.03 ml and 0.6 ml (8% concentration), and 4.34 ml, 0.98 ml and 0.42 ml (10% concentration) on 1st, 2nd and 3rd days of successive storage respectively. Moreover, there was no water expelled after 3rd day of storage. This can be ascribed to the reason that the water content of gels decreased after the first and second storage intervals due to its re-freezing after the first storage cycle.[74]Temperature and storage changes can cause ice crystals in frozen food products, causing deterioration due to thermal fluctuations and water phase changes.[74-75]

Moreover, retrogradation led to the removal of water from rice bean starch gels during the production of ice crystals when the gels were kept in a freezing conditions. These ice crystals melt to separate water as they thaw at room temperature. No syneresis on

4th and 5th day showed good freeze-thaw stability. Furthermore, the gels changed from a smooth structure to a sponge-like structure (rough textured porous gel) during the freeze-thaw cycles. The separated water was able to reabsorb due to rough and porous gel structure.[76]Therefore, there was no syneresis on the 4th and 5th day of gel storage.

The present study also observed the effect of concentration of starch on freeze-thaw stability (Table 5). The expelled water was significantly ($p<0.05$) more at 6% concentration. The increased concentration of starch in gels or pastes led to lower syneresis and long-term thermally reversible gel stiffness, attributed to amylopectin involved in crystallization.[77] Additionally, in earlier research, the maximum amount of free water in both wheat flour paste and regular corn starch was 6% rather than 8% or 10% concentration.[78] The extent of syneresis in legume starches depends on their higher amylose content as it leads to a higher extent of retrogradation.[79]

Table 5: Effect of concentration and storage on freeze-thaw stability of rice bean starch

Starch conc (%)	Expelled water (ml)				
	Day 1	Day 2	Day 3	Day 4	Day 5
6	9.80±0.11^{c}	1.67±0.10b	0.97±0.04^{c}	0.00	0.00
8	7.84±0.19^{b}	1.03±0.03^{a}	0.61±0.05^{b}	0.00	0.00
10	4.34±0.29^{a}	0.98±0.03^{a}	0.42±0.00^{a}	0.00	0.00

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

Effect of Storage and Temperature on Paste Clarity (Turbidity) of Starch

Paste clarity is an important factor in determining the quality of starch paste as high reflection and less light intensity improve color in the finished product.[80] The paste clarity of starch suspensions was evaluated at two temperatures (30°C and 4°C) (Table 6). The values for the starch paste clarity are given as the percentage absorbance read directly in a spectrophotometer. On the first day, it was found to 0.267 followed by 0.715, 1.002, 1.008, 1.022, 1.027 and 1.031 on successive days at 30°C. Similarly, the absorbance values increased from 0.0849 on the first day followed by 1.200, 1.211, 1.345, 1.418, and 1.534 on successive days at 4°C. It was depicted from the table that absorbance values increased with the storage time and more at refrigeration temperature than the room temperature. The rearrangement of solubilized starch chains and the retrograded starch formation at lower temperatures, there is a rise in turbidity in starch suspensions during cold storage.[81]

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

Temperature (°C)	Storage Period with absorbance values						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
30	0.267	0.715	1.002	1.008	1.022	1.028	1.031
4	-	0.0849	1.200	1.212	1.346	1.419	1.534

The effect of storage (1-7 days) on paste clarity was also observed (Table 6) in the present study. The extent of retrogradation of starch increased with the enhancement of storage period so gel became more and more cloudy as storage exceeded.[82] Starch gels stored at room temperature have less retrogradation tendency and better paste clarity compared to those stored in refrigeration.[83] Polymer-polymer aggregation plays a significant role in a polymer-solvent system, with amylose chains causing crystallization within hours and amylopectin chains causing crystallization over longer storage days, resulting in increased light absorption.[82,84-85]

Conclusion

In the present study, we examined the physicochemical properties of rice bean starch. Various parameters like moisture, ash, fat, protein, fiber, carbohydrate content was evaluated. Further, water and oil absorption capacity, emulsion capacity and stability, least gelation concentration, amylose content, complexed amylose with lipids, and starch pigmentation properties were evaluated. The effect of temperature showed a positive relationship with amylose leaching. Similarly, there was a positive relationship between swelling power and solubility with temperature but a negative relationship with the concentration of starch. Moreover, freeze-thaw stability increased with the storage period and concentration of starch. The starch paste clarity was more at 30°C than at 4°C. Our experimental results would provide necessary information to the food industry making use of non-conventional source of starch rather than conventional starch sources like potato, corn, rice, cassava, chestnut, wheat, etc. Understanding the properties of this legume starch will provide valuable perspectives on the innovative application of legume starch. The rice bean starch can be used to modify the texture of food products such as soups, frozen foods, noodles, cookies, pasta, crackers, sauces and extruded snacks. The future food scientists may study the various properties of starch by blending of 2 or more starches (conventional or non-conventional) that may enhance the application of legume starch without going any kind of modification because modification leads to structural changes and sometimes affect health as some kind of enzymes or chemicals used in such starch modification process. Consuming native starch has no health issues. Therefore, this study may be helpful for future scientists to better understand its properties for various uses in food industries.

Acknowledgments

We acknowledge the Department of Food Technology, Maharshi Dayanand University, Rohtak for providing the infrastructure, chemicals, and continuous support during the research.

Funding Sources

The funding for the research was provided as the University Research Fellowship by Maharshi Dayanand University, Rohtak, Haryana, India.

Conflict of Interest

There is no conflict of interest declared by the authors.

Author’s Contribution

Sapna Dhawan Munjal: Writing – original draft, Methodology, Conceptualization, Visualization, Data curation. Jyotika Dhankhar: Writing – review & editing. Alka Sharma: editing, and, Supervision. Prixit Guleria: Statistical analysis of results

Data Availability Statement

Not applicable

Ethics Approval Statement

Not applicable

References

1. Shevkani K., Singh N., Patil C., Awasthi A., Paul M. Antioxidative and antimicrobial properties of pulse proteins and their applications in gluten free foods and sports nutrition. *Int. J. Food Sci. Technol.* 2022; 57: 5571-5584.
2. Brennan A. B., Lan T., Brennan C. S. Synergistic effects of barley, oat and legume material on physicochemical and glycemic properties of extruded cereal breakfast products. *J. Food Process. Preserv.* 2016; 40(3): 405-413.
3. Sozer N., Holopainen-Mantila U., Poutanen K. Traditional and new food uses of pulses. *Cereal Chem.* 2017; 94: 66-73.
4. Escobedo, A., Mojica I. Pulse based snacks as functional foods: processing challenges and biological potential. *Compr. Rev. Food*

Sci. Food Saf. 2021; 20: 4678-4702.

5. Singh N. Pulses: an overview. *J. Food Sci. Technol*. 2021; 54: 853-857.
6. Keskin S. O., Ali T. M., Ahmed J., Shaikh M., Siddiq M., Uebersax M. A. Physico-chemical and functional properties of legume protein, starch and dietary fiber- a review. *Legum. sci.* 2022; 4 (1): e117.
7. Bisht I. S., Singh M. Asian Vigna. In: Singh M, Upadhayay HD, Bisht IS (eds). Genetic and genomic resources of grain legume improvement. 2013; Elsevier, Amsterdam. 237-267.
8. Sujayanand G. K., Chandra A., Jagadeeswaran R., Dubey S., Sheelamary S. Rice bean: Potential vine legume for achieving nutritional self-sufficiency in India. *Vigyan Varta.* 2021; 2(12): 10-14.
9. Tomooka N., Kaga A., Isemura T., Vaughan D. 2011. In:Chittaranjan K (Ed.) Wild crop relatives; Genomics and breeding resources, Legume crops and forages. Chapter-15.
10. Khanal A. R., Khadka K., Poudel I., Joshi K. D., Hollington P. 2009. Report on farmerslocal knowledge associated with the production, utilization and diversity of ricebean (*Vigna umbellata*) in Nepal. In: The Ricebean Network: Farmers indigenous knowledge of ricebean in Nepal (report N 4), EC. 6th, Project no. 032055, FORSIN.
11. Rajerison R., 2006. Vigna umbelatta (Thunb.) Ohwi & H. Ohashi. In: Brink M, Belay G (Eds.). PROTA 1: Cerals and pulses, Wageningen, Pays Bas.
12. Kaur A., Kaur P., Singh N., Singh V. S., Singh P., Chand R. J. Grain, starch and protein characteristics of rice bean (*Vigna umbellata*) grown in Himalyan regions. *Food Res. Int.* 2013; 54(1): 102-110.
13. Blazek J., Copeland L. Pasting and swelling properties of wheat flour and starch in relation to amylose content. *Carbohydr. polym.* 2008; 71(3): 380-387.
14. Das D., Jha S., Kumar K. J. Isolation and release characteristics of starch from the rhizome of Indian Palo. *Int. J. Biological Macromol*. 2015; 72: 341-346.
15. Yuliana M., Huynh L. H., Ho Q. P., Truong C. T., Ju Y. H. Defatted cashew nut shell starch as renewable polymeric material: Isolation and characterization. *Carbohydr. polym.* 2012; 87(4): 2576-2581.
16. Sreerama Y. N., Sashikala W. B., Pratape V., Singh V. 2012. Nutrients and anti-nutrients in cow-pea and horse gram flours in comparison to chickpea flour: evaluation of their flour funtionality. *Food Chem.* 2012; 131: 462-468.
17. Ma M., Wang Y., Wang M., Jane J. I., Du S. K. Physicochemical properties and in vitro digestibility of legume starches. *Food Hydrocoll*. 2017; 63: 249-255.
18. Reddy C. K., Kimi L., Haripriya S. Variety difference in molecular structure, physicochemical and thermal properties of starches from pigmented rice. *Int. J. Food Eng.* 2016; 12(6): 557-565.
19. Sukhija S., Singh S., Riar C.S. Isolation of starches from different tubers and study of their physicochemical, thermal, rheological and morphological characteristics. *Starch Staerke*. 2016; 68(1-2): 160-168.
20. Schoch T.J., Maywald E. C. Preparation and properties of various legume starches. *Cereal chem*. 1968; 45. 564-573.
21. AOAC. 2006. Official methods of analysis. 18th ed. Association of Official Analytical Chemists, Washington, DC.
22. Hoover R., Ratnayake W. S. Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. *Food Chem.* 2002; 78(4): 489-98.
23. Ambigaipalan P., Hoover R., Donner E., Liu Q. Retrogradation characteristics of pulse starches. *Food Res. Int.* 2013; 54(1): 203-212.
24. Adebowale K. O., Lawal O. S. Microstructure, physicochemicalproperties and retrogradation behaviour of mucuna bean (Mucuna pruriens) starch on heat moisture treatments. *Food Hydrocoll.* 2003; 17(3): 265-272.
25. Kinsella J. E. Functional properties of soy proteins. *J. Am. Chem. Soc.* 1979; 56(3): 242-258.
26. Beuchat L. R. Functional and electrophoretic characterstics of succinylated peanut flour proteins. *J. Agric. Food Chem.* 1977; 25:258.
27. Leach H. W., McCowen L. D., Schoch T. J. Structure of the starch granule. I- Swelling and solubility patterns of various starches. *Cereal Chem.* 1959; 36: 534-544.
28. Hoover R., Ratnayake W. S. Starch characteristics of black bean, chick pea, lentil,

navy bean and pinto bean cultivars grown in Canada. *Food Chem.* 2002; 78(4): 489-98.
29. Peera C., Hoover R. Influence of hydroxypropylation on retrogradation properties of native, defatted and heat-moisture treated potato starches. *Food Chem.* 1999; 64: 361-375.
30. Naivikul O. Appolonia B. L. Carbohydrates of legume flours compared with wheat flour. II. Starch. *Cereal Chem*. 1979; 56:24-28.
31. Hoover R., Sosulski F. W. Composition, structure functionality and chemichal modification of legume starches-a review. *Canadian J Physiol. Pharmacol.* 1991; 69: 79-92.
32. Hoover R., Sosulski F. W. Studies on functional characteristics and digestibility of starches from Phaseolus vulgaris biotypes. *Starch/Staerke.* 1985; 37(6): 181-191.
33. Chinma C. E., Ariahu C. C., Abu J. O. Chemichal composition, functional and pasting properties of cassava starch and soy protein concentrate blends. *J. Food Sci. Technol.* 2013; 50 (6): 1179-1185.
34. Ratnayake W. S., Hoover R., Shahidi F., Prera C. Composition, molecular structure and physicochemical properties of starches from four field pea (*Pisum Sativum L.*) *cultivars. Food Chem.* 2001; 72(2): 189-202.
35. Ashogbon A. O., Akintayo E. T. Morphological and functional properties of starches from cereal and legumes: a comparitive study. *J. Int. J. Biotechnol. Food Sci.* 2013a; 1(4): 72-83.
36. Galvez F. C. F., Resurreccion A. V. A. The effect of decortication and method of extraction on the physical and chemichal properties of starch from mung bean (Vigna radiate (L) wilczec). *J. Food Process Preserv.* 1993; 17: 93-107.
37. Schirmer M., Hochstotter A., Jekle M., Arendt E., Becker T. Physicochemichal and morphologichal characterization of different starches with variable amylose/amylopectin ratio. *Food Hydrocoll.* 2013; 32(1): 52-63.
38. Chanapamokkhot H., Thongngam M. The chemichal and physico-chemichal properties of sorghum starch and flour. *Kasetsart J. Nat. Sci.* 2007; 41: 343-349.
39. Hoover R., Ratnayake W. S. Determination of total amylose content of starch. *Current protocols in food analytical chemistry.* 2001; 2.3.1-2.3.5.
40. Gunaratne A., Gan R., Wu K., Kong X., Collado L., Arachchi L.V., Kumara K., Pathirana S. M., Corke H. Physicochemical Properties of Mung Bean Starches Isolated From Four Varieties Grown in Sri Lanka. Starch/Starke. 2018; 70: 1–6.
41. Lii C.Y., Chang S. M. 1981. Characterization of red bean (Phaseolus radiatus var. Aurea) starch and its noodle quality. *J. Food Sci.* 1981; 46: 78-81.
42. Morrison W. R. Lipids in cereal starches: a review. *J. Cereal Sci.* 1988; 8:1-15.
43. Raphaelides S., Karkalas J. Thermal dissociator of amylose-fatty acid complexes. *Carbohydr. Res.* 1988; 172: 65.
44. Hoover R., Hughes T., Chung H. J., Liu Q. Composition, molecular structure, properties and modification of pulse starches: A review. *Food Res. Int.* 2010; 43: 399-413.
45. Romero H. M., Zhang Y. Physicochemical properties and rheological behaviour of flours and starches from four bean varities for gluten free pasta formulation. *J. Agric. Food Res.* 2019;1: 100001.
46. Van Hung P., Maeda T., Morita N. Study on physicochemical characteristics of waxy and high-amylose wheat starches in comparison with normal wheat starch. 2007; Starch/ Starke. 59: 125-131.
47. Chung H. J., Liu Q., Donner E., Hoover R., Warkentin T. D., Vandenberg B. Composition, molecular structure, properties and in vitro digestibility of starches from newly released Canadian pulse cultivars. *Cereal Chem.* 2008a; 85: 471-479.
48. Chung H., Liu Q., Hoover R., Warkentin T. D., Vandenberg B. In vitro starch digestibility, expected glycemic indexand thermal and pasting properties of flours from pea, lentil and chickpea cultivars. *Food Chem.* 2008b; 111 (2): 316-321.
49. Vandeputte G. E., Derycke V., Geeroms J., Delcour J. A. Rice starches. II. Structural aspects provideinsight into swelling and pasting properties. *J. Cereal Sci.* 2003; 38 (1) 53-59.
50. Chung H. J., Hoover R., Liu Q. The effect of single and dual hydrothermal modification on the molecular structure and physicochemical

properties of normal corn starch. *Int. J. Biol. Macromol*. 2009; 221:203-210.

51. Lawal A. O., Adebowale K. O. An assessement of changes in thermal and physic-chemical parameters of jack bean (Canavalia ensiformis) starch following hydrothermal modifications. *Eur. Food Res. Technol.* 2005; 4 (6): 621-638.
52. Singh J., Singh N. Study on morphological, thermal and rheological properties of starch from some Indian potato cultivars. *Food Chem.* 2001; 75. 67-77.
53. Schmidt R. H. Gelation and coagulation. Protein Functionality in Foods, 1981. ASC symposium series 147; Cherry, J.P.; Ed: American Chemichal Society: Washington DC. 131-147.
54. Lawal A. O., Adebowale K. O. The acylated protein derivatives of Canavalia ensiformis (jack bean): A study of functional characteristics. *Food Sci. Technol.* 2006; 39(8): 918-929.
55. Singh N., Kaur N., Rana J. C., Sharma S. K. Diversity in seed and flour properties in field pea (Pisum sativum) *germplasm. Food Chem.* 2010; 122: 518-525.
56. Du S. K., Jiang H., Yu X., Jane J. L. Physicochemichal and functional properties of whole legume flour. *J. Food Sci. Technol.* 2014; 55: 308-313.
57. James C. O., Norman N. P. Physico-chemichal and functional properties of cowpea powders processed to reduce beany flavor. *J. Food Sci*. 1979; 44: 1235-1240.
58. Dossou V. M., Agbenorhevi J. K., Alemawor F., Oduro I. Physicochemichal and functional properties of full fat and defatted Ackee (Blighia sapida) Aril flours. *Am. J. Food Sci. Technol*. 2014; 2(6). 187-191.
59. Hasmadi M., Noorfarahzilah M., Noraidah H., Zainol M. K., Jahurul M. H. A. Functional properties of composite flour: a review. Food Res. 2020. 4(6), 1820-1831.
60. Olu-Owolabi B. I., Afolabi T. A., Adebowale K. O. Pasting, thermal, hydration and functional properties of annealed and heat-moisture treated starch of sword bean (*Canavalia gladiata*). 2011; 14:157-174.
61. Halbrook W. V., Kurtzman R. H. Water uptake of bean and other starches at high temperatures and pressures. *Cereal Chem.* 1975; 52: 156-159.
62. Chou D. H., Morr C. V. Protein waret interactions and functional properties. *J. Am. Oil Chem. Soc.* 1979; 56: 53-62.
63. Sathe S. K., Salunkhe D. K. Preparation and utilization of protein concentrates and isolates for nutritional and functional improvements of foods. *J. Food Qual.* 1981; 4: 145-233.
64. Deshpande S. S., Sathe S. K., Rangnekar P. D., Salunkhe D. K. Functional properties of modified black gram (*Phaseolus mungo* L.) starch. *J. Food Sci.* 1982; 47: 1528-1533.
65. Sathe S. K., Iyer V., Salunkhe D. K. Investigation of great Northen bean (*Phaseolus vulgaris* L.). Starch solubility, swelling interaction with free fatty acids and alkaline water retention capacity of blends with wheat flours. *J. Food Sci.* 1981; 46: 1914-1917.
66. Reddy C. K., Luan F., Xu B. Morphology, crystallinity, pasting, thermal and quality characteristics of starches from adzuki bean (Vigna angularis L.) and edible kudzu (Pueraria thomsonii Benth). *Int. J. Biol. Macromol*. 2017; 105: 354-362.
67. Liu Y., Xu M., Wu H., Jing L., Gong B., Gou M., Zhao K., Li W. The compositional, physicochemical and functional properties of germinated mung bean flour and its addition on quality of wheat flour noodle. *J Food Sci Technol.* 2018; 55 (12): 5142-45.
68. Singh N., Singh J., Kaur L., Sodhi S., Gill B. S. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chem.* 2003; 81 (2): 219-213.
69. Peroni F. H. G., Rocha T. S., Franco C. M. L. Some structural and physicochemical characteristics of tuber and root starches. *Food Sci. Tech. Int*. 2006; 12 (6): 505-513.
70. Carcea M., Acquistucci R. Isolation and physicochemichal characterization of fonio (Digitaria exilis stapf) starch. Starch/Staerke. 1997; 49 (4): 131-135.
71. Hoover R.. Composition, molecular structure and physico-chemichal properties of tuber and root starches: a review. *Carbohydr. Polym.* 2001; 45(3): 253-267.
72. Zuluaga M., Baena Y., Mora C., Ponce D'Leon L. Physicochemichal chracterization and application of yam (Dioscorea cayenensis-rotundata) starch as a pharmaceutical

excipient. Starch/Starke. 2007; 59: 307-317.

73. Karim A. A., Norziah M. H., Seow C. C. Methods for the study of starch retrogradation. *Food Chem.* 2000. 71: 9-36.
74. Hussain S., Alamri M. S., Mohamed A. A. Rheological, thermal and textural properties of starch blends prepared from wheat and turkish bean starches. *Food Sci. Technol. Res*. 2013; 19 (6): 1141-1147.
75. Pongsawatmanit R., Temsiripong T., Ikeda S., Nishinari K. Influence of tamarind seed xyloglucan on rheological properties and thermal stability of tapioca starch. *J. Food Eng.* 2006; 77: 41-50.
76. Varavinit S., Shobsngob S., Varanyanond W., Chinachoti P., Naivikul O. Freezing and thawing conditions affects the gel stability of different varieties of rice flour. *Starch-Starke.* 2002; 54 (1): 31-36.
77. Orford P. D., Ring S. G., Carroll V., Miles M. J., Morris V. J. The effect of concentratin and botanical source on the gelation and retrogradation of starch. *J. Sci. Food Agric.* 1987; 39: 169.
78. Zheng G. H., Sosulski F. W. Determination of water separation from cooked starch and flour paste after refrigeration and freeze thaw. *J. Food Sci.* 1998; 63: 134-139.
79. Zhang Y., Liu W., Liu C., Luo S. Rertrogradation behaviour of high aylose rice starch prepared by improved extrusion cooking technology. *Food Chem.* 2014; 158: 255-261.
80. Segura M., Chel L., Betancur D. Effect of Octenylsccinylation on functional properties of Lima Bean (Phaseolus lunatus) *starch. J. Food Process Eng*. 2010; 33: 712-727.
81. Goswami K., Yadav R. B., Yadav B. S., Yadav R. L. Physico-chemical , textural and crystallinity properties of oxidized, cross-linked and dual-modified white sorghum starch. *Int. Food Res. J*. 2018; 25: 2104-2111.
82. Denchai N., Suwannaporn P., Lin J., Soontaranon S., Kiatponglarp W., Huand T. C. Retrogradation and digestibility of rice starch gels: the joint effect of degree of gelatinization ans storage*. J. Food Sci*. 2019; 84(6): 1400-1410.
83. Silverio J., Svensson E., Eliasson A. C. Isothermal microcalorimetric studies on starch retrogradation. *J. Therm. Anal.* 1996. 47: 1179-1200.
84. Yu S., Ma Y., Menager L., Sun D. Physicochemichal properties of starch and flour from different rice cultivars. *Food Bioproc. Technol*. 2012; 5(2): 626-637.
85. Hoover R., Hadziyev D. The effect of monoglycerides on amylose complexing during a potato granule process. *Starch Stareke*. 1981; 33:346-255.