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SELECTED PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF EXTRACTED SOYBEAN STARCH

Shristy CHETTRI[1] RIZWANA[2] Ashish M. MOHITE[1]

Abstract: *Legumes are one of the most potent sources of starches. However, soybean has a comparatively low content of starch as compared to other legumes. This study was conducted to analyze selected physicochemical and functional properties of the extracted soybean starch by two different methods. The proximate analysis of the extracted soybean starch was reported as average values as 10% for moisture content, 0.22% for fat, 1.68% for ash 1.79% for crude fibre, and 0.49% for protein for T1sample. Similarly, for T2 sample the values found were 12, 0.19, 2.11, 2.05 and 0.74% for moisture content, fat, ash, crude fibre, and protein, respectively. The physical properties of the extracted soybean starch samples found variation for bulk density, tap density, angle of repose and coefficient of friction for Plywood surface, and Mild steel surface and SS 304 surface properties for T1 and T2 samples. The swelling power of T1sample was found 2.86 at 60˚C, 4.32 at 70˚C 5.36 at 80˚C while the values for water absorption capacity and least gelation concentration were reported as 1.2ml/g and 6%, respectively. The results reveal that samples prepared from T1 samples had better physic-chemical and functional properties compared to T2 sample.*

Key words*: starch, soyabean, physical, functional.*

1. Introduction

Soybean has been utilized as one of the major sources of plant protein all over the world owing to its high protein profile [34], i.e., 35-40% [17]. The use of soybean seeds as an important plant protein source in Asia has been evident since past generations [2]. Soybean is also nowadays used as an appropriate substitute for meat protein in the diet by the vegetarian and vegan population. Soybean is highly loaded with various nutrients along with peptides, iso-flavonoids and other functional components [16]. Soybean is an appropriate blend of a source for oil production, animal feed and a potentially nutrient-loaded legume for human consumption. It stands after palm oil in the global contribution to vegetable oil [17]. Soy products viz, miso, tofu, tempeh etc. hold background as traditional eatables in numerous countries of Africa and Asia while the recent products that

[1] Amity Institute of Food Technology, Noida, Amity University, U.P, India;
[2] Department of Food Technology, Bhaskarocharya College of Applied Sciences, New Delhi, India;
Correspondence: Ashish M. Mohite; email: ashishmohite1251@gmail.com.

are derived from soybeans that have gained importance are meat and dairy substitutes, sprouts, soy bread, flour etc. [9].

Starch ranks behind cellulose as the 2nd bulky biomass on the planet [18]. The molecules of starch are composed of units of anhydrous glucose making up the polymers [15]. It is one of the primary energy storages in plants and has a wide spectrum of uses in both the non-food and food industries [18]. One of the most crucial carbohydrates for humans is starch which has been used in the processing of; pasta, noodles, cereals, tortilla, porridge, pancake and bread in the food industry [10].

The starch content in soybean seeds have been reported to be at around 20% of the total seed weight [10], and 10-15% of total dry matter almost 20 days before harvesting [30], however, the starch content in soybean seeds can be to be as low as 0.19-0.91% or less than 1% which might be on the basis of higher degree of phosphorolysis [10].

The increase in the study of the structure and functionality of starch could be backed by its importance in human feed and innovations that can be beneficial for the food industry. The concern of the starch industry is the estimation of the interaction with other basic constituents of food based on their structures [6]. The literature has numerous studies on conventional food starches from numerous legumes, cereals, and other plant sources. There are only a few handfuls of literature on the structure and characterization of soybean starch, therefore, this study will be useful to scientist and researchers for future research on analysis of physicochemical and functional properties of soybean starch which will be useful for Industrial application.

2. Materials and Methods

2.1. Soybean Starch Extraction

The soybean (cv PDKV-AMS 1001) was used for the extraction of starch and was procured from Akola, Maharashtra. The procured samples were stored at 4˚C until the commencement of the starch extraction process. The first method followed for the extraction of starch was taken from Stevenson et al. [30] with the implementation of minor changes. The soybean seeds were soaked overnight in distilled water with a concentration of 0.5% sodium metabisulphite. The soaked seeds were ground into a puree form with the help of the lab mixer grinder (M/s Inalsa, India, Model: MAXIE plus). The puree was then passed through a mesh screen of 60 BSS, mesh sieve. The filtrate was taken separately and centrifuged at 10,000 RPM for 35-40 minutes. The pellet after the removal of supernatant was mixed in a 10% toluene solution in a concentration of 0.1 M NaCl. The supernatant fraction was discarded, and the pellet was further washed with water and ethanol. The final pellet obtained was then dried in a tray drier for 40˚C for 12-14 hrs.

For extraction of starch by second method from soyabean, seeds were grinded in Laboratory scale mill (hammer type) (M/s Sanco India Pvt Ltd) for 15 min then the grounded sample was passed through BSS 100 mesh sieve [20]. The obtained sample was extracted using petroleum ether for 90°C for 150 minutes. The obtained defatted soybean meal residue was again further extracted in 95% ethanol for 120 minutes to remove any

traces of organic solvents which were left behind in the samples. Received samples were bought to room temperature naturally. The sample obtain after extraction by first and second method was denoted as T1 and T2 in further study.

2.2. Proximate Analysis

2.2.1. Moisture Content

The moisture (1) was calculated as per the procedure given by Association of Official Analytical Chemists [3]. 3 g of the sample was taken in a pre-weighed Petri plate and kept in a hot air oven at 105˚C for 3 hrs. The weight of the sample was measured after taking the sample out of the oven and cooling the Petri plate with sample in the desiccator.

$$\text{Moisture} = \frac{\text{Wpd} - \text{Wad}}{\text{Wpd}} \cdot 100 \qquad (1)$$

where:

Moisture is the moisture content [%];
Wpd – the weight of the sample prior drying [g];
Wad – the weight of the sample post drying [g].

2.2.2. Fat Content

For the determination of fat content (2), the method given by Carpenter, 2010, was adopted [5]. 3 g of the extracted soybean starch was poured in an extraction thimble the weight of which was measured and placed in the Soxh let unit which was fitted into a beaker containing 250 ml of petroleum ether. After a minimum of 6 hrs of extraction, the thimble was taken out of the beaker, dried at 70˚C followed by cooling in a desiccator and reweighing.

$$\text{Fat} = \frac{\text{Wti} - \text{Wtf}}{\text{Wti}} \cdot 100 \qquad (2)$$

where:

Fat is the fat content [%];
Wti – the initial weight of the sample [g];
Wtf – the final weight of the sample [g].

2.2.3. Ash Content

The ash content (3) was calculated based on the protocol mentioned by Ismail [8]. 3 g of the soybean starch whose ash content was to be found was taken in a pre-weighed silica crucible and kept over a Bunsen burner to a point where no fumes were visible. The soybean starch sample was placed in the muffle furnace at 550˚C for 24 hrs. The sample was the cooled in a desiccatorand weighed.

$$\text{Ash} = \frac{\text{Wt}}{\text{Wti}} \cdot 100 \qquad (3)$$

where:

Ash is the ash content [%];
Wt – the weight of the ash [mg];
Wti – the initial weight of the sample [mg].

2.2.4. Crude Fibre Content

The method used for the estimation of fibre (4) was cited by Mohite and Sharma [22]. 3 g of the soybean starch sample devoid of fat was placed in a digestion flask and to it 200 ml of hot H_2S0_4 was poured immediately attaching the flask to a condenser. The flask was then heated for 30 minutes with constant rotation. The solution was passed through a filter cloth and the residue obtained was cleaned with hot water and placed back in the digestion flask to which 200 ml of NaOH

was added to boil at around for 30 minutes. The residue obtained after filtration of the boiled solution from the flask through a muslin cloth was cleaned and was placed in a crucible followed by alcohol washing and drying for 120 minutes at 130°C. The weight of the crucible was recorded after cooling and before placing it in the muffle furnace for 30 minutes at a temperature of 600°C. The crucible was taken out of the furnace and weight was taken only after cooling it in a desiccator, the weight loss, gave the percentage of crude fibre in the sample.

$$\text{Crude fibre} = \frac{\text{Waod - Wta}}{\text{Wto}} \cdot 100 \quad (4)$$

where:

Crude fibre is the fibre proportion [%];
Waod – the weight of crucible with sample after over drying [g];
Wta – the weight of crucible and sample with ash [g];
Wt – the weight of the original sample [g].

2.2.5. Protein Content

For protein estimation (5), the method used by Jung et al. [11] was used. 1 gm of the extracted soybean starch was weighed and placed in a digestion tube to which 6 ml of conc. H_2SO_4, 0.3 g of potassium sulphate and 0.2 g of cupric selenite were added for digestion. 40% of NaOH was used to create an alkaline environment for distillation and boric acid at a concentration of 4% was used for the collection of distilled ammonia. The final step was the titration of the distilled solution using 0.1 N HCL. The end point during titration was detected by Tashiro's indicator which is a combination of 0.250 gms methylene blue with 0.375 gms methyl red in ethanol at a concentration of 95%).

$$\text{Nitrogen} = \frac{\text{V1 - V2} \cdot \text{HCL} \cdot \text{Atomic N}}{\text{Wt} \cdot 10} \quad (4)$$

$$\text{Protein} = \text{Nitrogen} \cdot \text{F} \quad (5)$$

where:

Nitrogen is Kjeldahl Nitrogen [%];
V1 – the volume of standardized acid used in the titration of test [ml];
V2 – the volume of standardized acid used in titration of blank reagent [ml];
HCL – the HCL molairty [M];
Atomic N – the Atomic weight of N [mg];
Wt – the weight of test portion [gm];
10 – the factor conversion of mg/gm to percent;
F – the Nitrogen to Protein conversion factor.

2.3. Physical Properties

2.3.1. Bulk Density

Bulk density (6) also known as apparent density is the ratio between the sample mass and the sample volume including the spaces between the pores [13].

$$\text{Pb} = \frac{\text{m}}{\text{v}} \quad (6)$$

where: *m* represents the mass [kg], and *v* is the volume [m^3].

A 10ml measuring cylinder was filled with the starch sample from a given selected height [4]. The bulk density of the soybean starch was given by the ratio of the mass of the soybean starch to its volume. For the determination of bulk

density were taken 3 replications, in average.

2.3.2. Tap Density

The tap density was calculated in accordance with the method of Mohite et al. [23]. The starch sample was filled in a 10ml measuring cylinder taking care that the sample was evenly layered followed by gentle tapping to a point where no decrease in volume was seen. The ratio between the sample weight and the weight of the measuring cylinder gave the tap density. 3 replications of the same process were taken for the average calculation of the tap density.

2.3.3. Angle of Repose

The method to determine the angle of repose of the soybean starch was adopted from Sacilik et al. [27], where a box 300 mm x 300 mm x 300 mm in dimensions, with an adjustable front area slide, was designed. The soybean starch sample was placed in the box followed by speedy removal of the adjustable front slide for naturing sloping of the soybean starch.

2.3.4. Coefficient of Friction

For the determination of static coefficient of friction; a rectangular box with no lid on sides, loading pans and frictionless pulley was utilized where a specific amount of soybean starch was added to the box and the given load was placed on the pansupported by the pulley. The reading of the load was recorded when even a mild slide of the rectangular box was witnessed. The same process was performed in at least three different surfaces (SS-304 sheet, Plywood, Mild steel Sheet) and an average reading of a minimum of 3 replications were taken [22]. The ratio between the weights loaded, i.e., frictional force and the mass of the material, i.e., normal force gave the resulting coefficient of static friction.

2.3.5. Color Analysis

The portable colorimeter NS 810 (M/s Shenzhen 3nh Technology Pvt Ltd, China) was used for the color analysis of the extracted soybean starch samples. The colorimeter was calibrated using black and white plates, the starch samples were placed on sample plate for the reading [12]. Color dispersion in terms of L*, *a** and b* values were collected for each sample separately. Hue angle and Chroma values calculated using L*, *a** and b* values were also recorded. Average of three readings were taken for the extracted soybean starch.

2.4. Characterization Properties

2.4.1. Swelling Power

Swelling power (*SP*) was calculated as per the method used by Adebowale et al. [1]. 0.5 gms of soybean starch sample was weighed and poured in a centrifuge tube and the weight was measured and noted as W1. To it 20 ml of distilled water was added and heated at temperatures viz; 60, 70 and 80˚C each for 30 minutess in a water bath with continuous mixing in between followed by cooling and centrifugation for 15 minutes at 3,000 g. W2 was taken as the weight of the centrifuge tube with residue and retained water. The swelling power was calculated as (7):

$$SP = W2 - W1 \quad (7)$$

where:
Sp is the swelling power [g/g]:
W1 – the weight of soybean starch sample poured in a centrifuge tube [g];
W2 – the weight of the centrifuge tube with residue and retained water [g].

2.4.2. Least Gelation Concentration

For the determination of Least Gelation Concentration (LGC) the method used by Sangokunle et al. [28] cited from Ratnayake and Jackson [26] with slight modification was utilized. The extracted soybean starch in (w/v) ratios as; 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% were taken separately with 1ml water in covered glass tubes and heated for 60mins at 100°C followed by speedy cooling by placing under running water and storage at 4°C for a period of 120 minutes. The complete formation of gel was noted among the given ratios which gave the least gelation concentration for the soybean starch.

2.4.3. Water Absorption Capacity

To calculate (8) [32] the Water Absorption Capacity (WAC) almost 1 gm of soybean starch sample was added to water in the ratio 1:10 and properly vortexed followed by centrifugation for 15 minutes at 3,000 RPM, the supernatant was decanted while the water absorbed was determined as, retained moisture in gm/gm of the soybean starch sample [28]. The water absorbed by the given amount of extracted soybean starch sample was calculated and shown as the water absorption capacity of the sample.

$$WAC = \frac{Wtf - Wti}{Wti} \quad (8)$$

where:
WAC is the Water Absorption Capacity [g/g];
Wtf – the final weight of sample after water absorption [g];
Wti – the initial weight of sample [g].

3. Results and Discussion

3.1. Proximate Analysis

3.1.1. Moisture Content

The determination of moisture as per the protocol mentioned in AOAC [3] for the extracted soybean starch samples found 10% value for T1 and 12% for T2 sample on a dry basis. The estimation of moisture is an important aspect in determining some of the moisture-dependent properties of the starch extracted from soybean. The literature shows the moisture content of the soybean seeds to be at around 8-16% on a dry basis [14, 21].

3.1.2. Fat Content

The calculation for the percentage of fat content in the extracted soybean starch samples were found to be 0.22% for T1 and 0.19% for T2 sample. These differences can be due to different method used for extraction of soybean starch. The amount of fat in the sample also influences other physic-chemical and functional properties of the extracted starch sample (Table 1). The proportion of fat in soybean seeds was found to be ranging between 17.6-18.9% based on the findings given by Stevenson et al. [30].

3.1.3. Ash Content

The quantity of ash is a crucial parameter in predicting the quality of a

given sample and is indicative of the leftover inorganic residue after oxidation or burning of the organic proportions of the given sample [30]. The content of ash from extracted soybean starch samples was found 2.11% for T1 and 1.68% for T2 sample, respectively. As a comparative study from literature, the ash content of soybean flour was given as 4.95% by Warle et al. [31].

3.1.4. Crude Fibre Content

The content of crude fibre in a sample reveals the amount of residue obtained after solvent extraction and digestion of a given sample with an acid and an alkali in dilute concentrations [12]. The percentage of crude fibre in the extracted soybean starch sample was 2.01% for T1 and 1.79% for T2 sample. Ogodo et al. [25] reported the fibre content in unfermented and fermented soybean flour as 4.35% and 0.86%, respectively.

Table 1

Physico-chemical and functional properties of extracted soybean starch samples

Properties		T1	T2
Moisture [%]		10.00	12.00
Fat [%]		0.22	0.19
Ash [%]		1.68	2.11
Crude Fibre [%]		1.79	2.01
Protein [%]		0.49	0.74
Bulk density [kg/m^3]		413.00	402.00
Tap density [kg/m^3]		526.00	509.00
Coefficient of friction (Plywood surface)		0.54	0.50
(Mild steel surface)		0.43	0.38
Coefficient of friction (SS304 surface)		0.26	0.24
Angle of Repose [˚]		33.68	24.67
Swelling power	60˚	2.86	2.18
	70˚	4.38	4.32
	80˚	5.36	5.11
Water absorption capacity [ml/g]		1.20	1.30
Least gelation concentration [%]		6.00	5.50

3.1.5. Protein Content

The near to precise estimation of protein is an important step as the shows the accuracy of the starch extraction and purity of the starch sample. The protein percentage of extracted soybean starch was found to be at around 0.74% for T1 and 0.49% for T2 sample. The amount of water-soluble protein was reported to be around 26.5-36.0% in various soybean germplasms [33].

3.2. Physical Properties

3.2.1. Bulk Density

The bulk density of the extracted soybean starches was calculated as 413 kg/m^3 for T1 and 402 kg/m^3 for T2 sample. These differences are due to volumetric difference of starches which were

extracted by two different method. The bulk density of soybean seeds ranged between 733.6-832.0 kg/m^3 at different moisture ranges [19].

3.2.2. Tap Density

The calculation of the tap density of the starch sample extracted from soybean seeds showed a value of 526 kg/m^{3}for T1 and 509 kg/m^3 for T2 sample, respectively. The tap density of soybean flour was shown between the ranges of 380-760 kg/m^3 for different soybean flour samples [24].

3.2.3 Angle of Repose

Angle of repose values were found to be 33.83 and 24.67 for extracted starch samples as T1 and T2, respectively. Very good flow properties were represented by T1 sample and fair by T2 samples as per [27].

3.2.4. Coefficient of Friction

The coefficient of friction on three different surfaces gave different results for T1 and T2 samples. The calculation for coefficient of friction showed the highest value in plywood surface i.e., 0.54, while the second successive value was found in Mild Steel surface, 0.43 among the three surfaces considered in this study. The coefficient of friction gave the least value as 0.26 in SS 304 (stainless steel) surface for T1 samples. Similarly, for T2 samples coefficient of friction values for plywood surface, Mild Steel surface and SS304 surface was found 0.50, 0.38 and 0.24, respectively.

3.2.5. Color Analysis

The color analysis of the extracted soybean starch sample gave the color property values as shown in the Table 2. It represented a variation in color values as the different methods for extraction included organic solvents, and their traces color had a marginal influence on the obtained powder. Hue angle (99.46 and 95.03) and Chroma values (6.12 and 5.45) found for T1 and T2 sample indicated the influence of methods used for extraction on color values.

Color characteristics of the extracted soybean starch samples Table 2

Extracted soybean starch	L*	a*	b*	c*	h˚
T1	22.61	-1.01	6.04	6.12	99.46
T2	15.55	-0.48	5.43	5.45	95.03

3.3. Characterization Properties

3.3.1. Swelling Power

The swelling power for the extracted soybean starch was calculated at three different temperatures (Figure 1). The values for all the temperatures were different, however, with the increase in temperature the readings for swelling power also elevated. The relationship between temperature and swelling power was directly proportional. The calculation of swelling power gave the values as 1.86, 4.32, and 5.36 in gm/gm at temperatures 60, 70, and 80˚C, respectively. A similar trend was found in the study conducted

for rice starches where the values for swelling increased with an increase in the temperature [7]. The swelling power value increased from; 5.1 to 11.2 in Mushkbudij variety rice starch (IMN), 4.4 to 11.8 in the SR1 variety starch (LSN), and 7.8 to 15.2 in Aghoni bora variety rice starch (WAN) when the temperature was increased from 55 to 95˚C in the research performed by Iftikhar and Dutta [7].

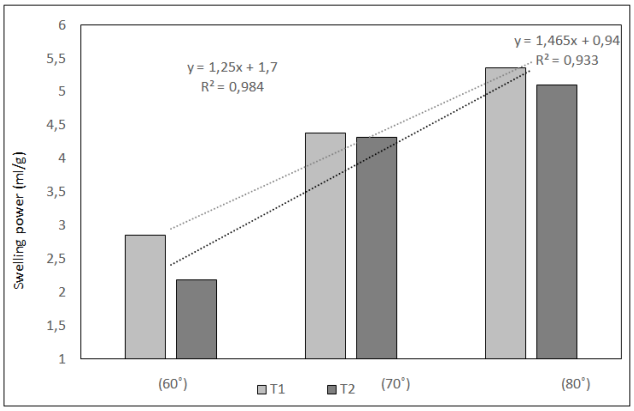

Fig. 1. *Swelling power of extracted soybean starch samples at 60, 70 and 80˚C temperature*

3.3.2. Least Gelation Concentration

The second characterization property undertaken for the extracted soybean starch was the least gelation concentration (LGC) which is a minimum concentration of starch needed for gel formation post-heating at a temperature of 100°C [28]. The lower values of gelling concentration show that the potential of any given starch sample in forming gel is potentially higher. In the case of food formulations, the smaller values for LGC tend to be beneficial as a lesser amount of starch is needed for gelling [29]. The LGC shows an inversely proportional relationship with the gelling potential of any given sample. The LGC of the extracted soybean starch T1 sample was found to be at 6% (w/v) and for T2 sample it was found 5.5 % (w/v).

3.3.3. Water Absorption Capacity

The water absorption capacity (WAC) for the extracted soybean starch sample after addition of water by 10 times sample volume followed by centrifugation and measurement of absorbed water by the soybean starch sample was found as 1.2 ml/g and for T2 sample it was 1.1 ml/g. This means that the amount of water retained by 1 gm of extracted soybean starch sample was 1.2 gm. The higher the water penetration the more is the water absorption capacity for any given sample. The other factors, viz; the size of the starch particles, the biochemistry of the starch molecules also play a role in determining the water absorption capacity. The breaking down of starch particles to finer pieces while grinding after extraction increase the area for water absorption, thereby increasing the water absorption capacity. The penetration of the water to starch could

also be facilitated by the increased gaps between the amylopectin and amylose structures because of heating or increased temperature.

4. Conclusion

The study was conducted for the proximate analysis, physical and functional properties of starch extracted by two different methods from soybean seeds. The proximate analysis for the extracted soybean starch was found to be higher values in T1 samples compared to T2 sample this was due to the variation in method undertaken for extraction. The bulk density, tap density, angle of repose and coefficient of friction (Plywood surface, Mild steel surface and SS 304 surface) were reportedas 413 and 402 kg/m^3 for T1 & T2 samples, 526 and 509 kg/m^3 for T1 & T2 samples, 33.68 and 24.67˚ for T1 & T2 samples, (0.54, 0.43, and 0.26) and (0.50, 0.38, and 0.24) for T1 & T2 samples, respectively. The swelling power was found to be (2.86 and 2.18) at 60˚C, (4.86 and 4.32) at 70˚C and (5.36 and 5.11) at 80˚C. The results for water absorption capacity and least gelation concentration found slight variation in results. Therefore, from this research it can be concluded that soybean starch extracted by T1 method has better physic-chemical and functional properties compared to T2 sample and can good source of starch for Industry purpose.

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