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THE DETERMINATION OF THE PHYSICOCHEMICAL, FUNCTIONAL & STRUCTURAL PROPERTIES OF YELLOW PEA STARCH

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ABSTRACT

The purpose of this study was to estimate the physicochemical, functional and structural characteristics of starch powder extracted from yellow pea (Pisumsativum L.)by wet-milling process. The yellow pea starch contained about 9.40% moisture, 0.03% fat, 0.85% ash, 62.65% amylose and 0.914 g/ ml bulk density. The water solubility and swelling power began to increase from 7.6% and 5.5 g/g at70°C and reached 28 % and 18.1 g/g at 95°C, respectively. Scanning image of starch was displayed mostly oval, irregular and some spherical shaped granules at 500x and 1000x magnification with 20 µm mean diameters. Yellow pea starch exhibited diffraction peak at 4.8°, 14.96º, 16.90º, 17.2º, 23.02º, 34.04º and 47.38º 2θ which is possess the C-type crystalline structure. The IR spectra of yellow pea starch exhibited Methylene peaks at 2928.32 cm^{-1} and amide peaks at 3212.04 cm^{-1}in functional group region. This paper focuses on physicochemical, functional & structural properties of pea starch, leading towards the future application of starch as a functional component in the food sector.

KEYWORDS: Yellow Pea Starch, Functional Properties, Morphology & C-type Starch

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Original Article

1. INTRODUCTION

Starch is the principal carbohydrate obtained from plant sources e. g. cereal, legume, root and tuber and green fruit are good source of starches (AshogbonandAkintayo 2014). It is one of the most important polysaccharide in the human and animal diet. Starches are composed with a mixture of six-member-ring glucose units (amylose and amylopectin polymers) and its molecular weight $l0^4$ to $l0^7$daltons (French 1997). Amylose possesses an α-1, 4 linkage backbone structure and can be sparsely branched via α-1, 6 linkages and amylopectin possesses more than 100-fold larger than amylose and contains clustered α-1, 6 linkages. Amylopectin clustering in granules of starch results in 9-nm alternating branched amorphous and more-linear crystalline, double-helical lamellae (Damager et al. 2010; Perez and Bertoft 2010). Most of starches from corn, wheat and potato, contain about 25% amylose and 75% amylopectin (Haasse 1993; Be- Miller and Whistler 1996), but some legume starches from pea species are characterized by a high-amylose content 24% -65% (Hoover andSosulski 1991). Two types A and B polymorph or crystallite structure have been recognized in the starch particles, which can be distinguished by the packing density of the double helices. A polymorphs structure have been denser then B polymorphs. C-polymorph has been intermediate of both A and B polymorph (Cairns et al. 1997; Bogracheva etal. 1998). Starches from different plant parts have different structure such cereal (A- polymorph), tuber (B- polymorph) and legumes (C- polymorph) and this can be identified by position of diffraction peak. Starches and their derivatives are very versatile raw material with a wide applications in dairy and food sector for film formation and modify the physical

properties of food products such texture, gel formation, viscosity, binding, adhesion and moisture holding (Thomas and Atwell 1997; Liu 2005). Starch is a most abundant, renewable, biodegradable and inexpensive polymer after cellulose (Visakh and Yu2016). Yellow pea (*Pisumsativum L.)* is one of the major legumes in the world used for food and feedstuff. Pea starch contains an intermediate level of amylose (30-40%) possesses both heat and acid stability Comer and Fry (1978).

The objective of this study was to focus on the new sources of starch isolated from yellow pea which have good functional and structural characteristics, beside this yellow peas are cheap and easily available source.

2. MATERIALS AND METHODS

2.1 Materials

Split yellow peas (Pisumsativum L.) were purchased from local market, Varanasi (Uttar Pradesh). All chemicals were analytical grade obtained from Centre of Food Sciences and Technology, IAS, BHU, Varanasi (Uttar Pradesh). X-Ray Diffraction (XRD) and Scanning Electron Microscope (SEM) study were performed in Instrumentation Lab, IIT, BHU. Fourier Transform Infrared Spectrometer (FTIR) was performed in Department of Chemistry, Faculty of Science, BHU.

2.2. Starch Extraction

Starch was isolated as by-product from liquid extract of yellow pea by wet milling process of seeds. The mixture was filtered through cheese cloth. The starch content was sediment from the filtrate. The starch was washed several times with 0.1 N NaoH solutions to remove protein from starch and then it dried in an air oven at 50ºC for 16 hrs and grinded with pestle mortar.

2.3. Physicochemical Analysis

2.3.1 Moisture, Fat and Ash Content

Moisture and ash content of pea starch powder were determined bystandard method described by AOAC (2000).

2.3.2 Bulk Density

Bulk density of starch measured by taking 10ml capacity graduated cylinder was weighed and filled at 10 ml mark with starch with gently tapped.

2.3.3 Amylose Content

Amylose content was determined by the iodine binding assay as described by Shen et al. (2016). Ten mg of yellow pea starch was first suspended in 95% ethanol and dissolved in 1 mL of 1 N sodium hydroxide (NaOH). The prepared solution was diluted with 10 times distilled water and neutralized with 0.1 N hydrochloric acids (Hcl). Finally, the solution was diluted to a final 0.25 mg/ ml as starch stock solution. Starch stock solution (0.1 ml) added with distilled water (1.8 ml) and 0.1 ml of KI–I2 solution (2% Potassium iodide and 0.2% Iodine) and kept it for 30 min at room temperature. The absorbance was recorded at 625 nm. Amylose content in starch was calculated by the following formula with reference to amylose standard curve.

2.4 Functional Properties

2.4.1 Solubility and Swelling Power

Swelling power and solubility of starch sample was determined by mixing of 500 mg sample with 50 ml distilled water and heated in a shaking water bath at 50º, 60º, 70º, 80º and 90 °C for 30 min. then it was cooled and centrifuged at 1500 rpm for 15 min. Supernatants and sediments were separated and analyzed for solubility and swelling power. For determination of starch solubility10 ml of supernatant was placed in moisture tin and dried at 105 °C for 20 h. The dried residue was used to calculate solubility according to Leach (1959). Swelling power (SP) was determined according to Tester and Morrison (1990).

2.5 X-Ray Diffraction (XRD)

The crystalline and amorphous nature of yellow pea starch was determined by using a RigakuMiniFlex 300/600 X-ray diffractometer (XRD). The sample of pea starch was kept in test holder and afterward test was performed under X-ray beam at 40 kV, 15mA. The operating conditions for sample were scanned over the range 3-50° 2θ, at a speed of 5° 2θ /min, scan axis θ/2θ, incident slit 1.250º, length limiting slit 10.0mm, receiving slit(1) 13.0mm, receiving slit (2) 13.0mm and with a step size of 0.0200° 2θ.

2.6 Scanning Electron Microscope (SEM)

Morphology of pea starch granule was determined by using ZEISS Supra-40 Scanning electron microscope (SEM) at an accelerating potential of 20 kV. Samples were mounted on circular aluminum stubbed and coated with the help of silver glue and examined under 500x and 1000x magnification.

2.7 Fourier Transform Infrared Spectrometer (FTIR)

FTIR analysis of yellow pea starch was carried out using PerkinElmer, Spectrum version 10.4.3. FTIR spectrum was used to analyse the short-range ordered structure in the external region of yellow pea starch granule. In this procedure IR beam was penetrate the starch granules about few micrometres that penetration depth is normally smaller than the mean size of starch granules.

3. RESULTS AND DISCUSSIONS

3.1 Physicochemical Composition

The data of yellow pea starch regarding to physicochemical composition are presented in Table 1. Yellow pea contained 9.40% moisture, 0.03% fat, 0.85% ash, 62.65% amylose and 0.914 g/ml bulk density. The moisture content in yellow pea starch was lower than jack beans (10.16%) reported by Betancur and Chel (1997). This variation may be due to the different physical condition (drying) of a process. The ash content of yellow pea starch was higher than 0.50 % ash had reported in rice starch (Swinkels 1985), but was similar to that of wrinkled pea 0.80-0.84% ash content (Zhou et al. 2004; Ratnayake et al. 2001). The fat content of yellow pea starch was lower than the beach pea (0.16%), green pea (0.19%) and grass pea (0.12%) had reported by Chavan et al. (1999). The amylose content of yellow pea was higher than the starches of field pea, high amylose corn and jack beans which contain 34.2%, 51.8% and 37.5% amylose had reported by Gujska et al. (1994); Han and Tyler (2003);Betancur and Chel (1997), respectively. Amylose content of yellow pea comparable to that of the wrinkled pea starch (60.5-88%) had reported by Ratnayake et al. (2001).

Table 1: Physicochemical Properties of Yellow Pea Starch

Parameters	Yellow pea starch
Moisture (%)	9.40 ± 0.36
Fat (%)	0.03 ± 0.10
Ash (%)	0.85 ± 0.01
Amylose (%)	62.65 ± 0.46
Bulk density (g/ml)	0.914 ± 0.01

Notes: Values are in mean± SD

3.2 Starch Solubility and Swelling Power

The solubility and swelling power of yellow pea starch at different temperature are shown in Table 2. The water solubility and swelling power of starch began to increase from 7.6% and 5.5 g/g at 70°C and reached 28 % and 18.1 g/g at 95°C, respectively. The starch solubility and swelling power increased with increasing the temperature and it increased after 70 ºC. A similar study had reported by Wang et al. (2018) for pea starch. According to Ekissi et al. (2015) who reported that coulaedulis starch were ruptured, dispersed and started for leaching out the soluble content after increasing temperature to 95°C that lead to decreased swelling power. The amylose, amylopectin, granule size, morphology, protein and fat content of starch affect the swelling power and water solubility. (Caiet al. 2014; Fan et al. 2016). According to Fuke and Matsuoka (1984) kiwi fruit had low solubility due to the low content to amylose.

Table 2: Functional Properties of Yellow Pea Starch

Parameter	50ºC	60ºC	70ºC	80ºC	90ºC
Solubility (%)	0.7 ±0.10	1.5±0.30	7.6±0.15	20.3±0.70	28±0.75
Swelling power(g/g)	1.9±0.36	2.1±0.20	5.5±0.10	10.20±0.65	18.1±0.40

Notes: Values are in mean± SD

3.3 Scanning Electron Microscope (SEM)

The scanning images of yellow pea starch granules at 500x and 1000x magnification are shown in Figure1. Yellow pea starch showed mostly oval, irregular and some spherical shaped granules have also been observed in samples with 20µm. The morphology of starch granules showed smooth surface of particles that indicate the amorphous nature of the starch powder. Similar morphology of pea starch had showed by the Ratnayake et al. (2001) and Barron et al. (2000). Variation in the raw starch granules could be due to the use of different condition in physical operations such as drying, grinding and sieving. According to Andrade-Mahecha et al. (2012) starch morphology was highly changed by the pH modification and extraction using chemical reagents like hydrochloric acid and sodium hydroxide then physical operation.

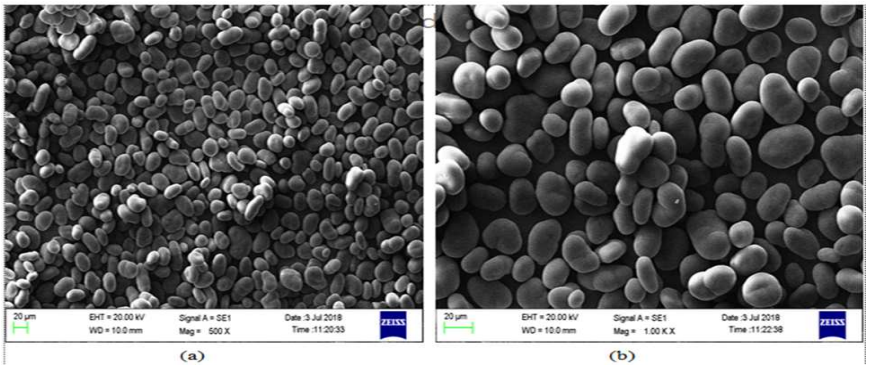

Figure 1: Scanning Image of Yellow Pea Starch Granules

3.4 X-Ray Diffraction (XRD)

X-ray diffraction pattern of Yellow pea starch is shown in Figure 2. Yellow Pea starch showed characteristic peaks at 4.8°, 14.96º, 16.90º, 17.2º, 23.02º, 34.04º and 47.38º 2θ, which are possesses a typical characteristic peak of A-type and B-type polymorphs. The sharp peaks (referred to as a Bragg peak) obtained from crystalline nature of powder while broad peak obtained from an amorphous starch powder. X-ray diffractograph of yellow pea starch shows peaks are not sharp, which is amorphous behaviour of starch sample. Similar, starch nature had shown by Wang and Yu (2008) who reported amorphous nature for pea starch. According to Colonna et al. (1982) wrinkled pea starch had exhibit a 'B' type X-ray pattern with peaks that are both broad and weak. The X-ray diffraction pattern was B-type even though it contained low amylose content (Fuke and Matsuoka 1984). Buleon et al. (1998) reported that both A and B polymorphs present in pea starch that the B polymorphs are arranged centrally with the A polymorph located peripherally within the granules. In the study of Bogracheva et al. (1998); Sarko and Wu (1978); Cairns et al. (1997) had reported starches exhibit a weak peak at 5.6° 2θ (characteristic of B polymorphs), strong peaks at 17.9° 2θ (characteristic of A polymorphs) and 17.0° 2θ (characteristic of both A and B polymorphs). Cassava starch was reported to possesses 'A', 'C' or a mixed pattern with three major peaks at 2 θ = 15.3, 17.1 and 23.5° (Kawabata et al. 1984; Rickard et al.1991).

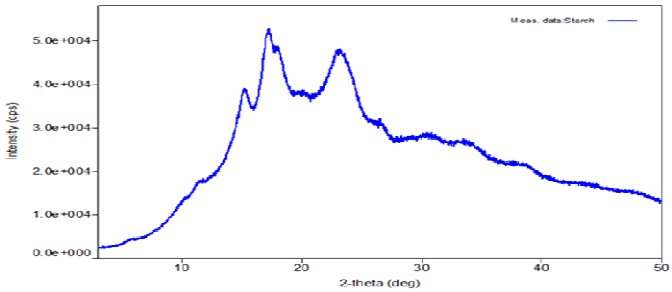

Figure 2: X-ray Diffraction Pattern of Yellow Pea Starch

3.5 Fourier Transform Infrared Spectrometer (FTIR)

The short-range order of starch reflects double helical order and can be measured by Fourier Transform Infrared Spectrometer. ATR-FTIR spectra of yellow pea starch granule in the region 4000–400 cm^{-1}which showed the typical peaks for the starch backbone are given in Figure3. An IR spectrum usually extends from radiation around 4000 cm^{-1} to 600 cm^{-1} and divided into two main regions- functional group region and fingerprint region. The functional group region extend from 4000-1500 cm^{-1}, is very useful to easily interpret because it identified functional group of molecules. Further, the functional group region split into three regions which include the X–H stretching region (4000–2500 cm^{-1}), the triple-bond region (2500–2000 cm^{-1}), and the double-bond region (2000–1500 cm^{-1}). However, other region from 1500–600 $cm^{-1}$is called fingerprint region, is relatively very hard to interpret due to bending of bond resulting complex of absorption pattern. The IR spectra region from 4000 to 2500 cm^{-1} is used to locate the following bonds: the alcohol O-H, the terminal alkyne C-H, and the amine or amide N-H. The presence of alkyne C-H bonds gives strong peaks around 3300 cm^{-1}butsimilar band was not present in the FTIR spectrum of yellow pea starch. A characteristic broad and widepeak was found at 3212.04 cm^{-1}, which was attributed of the amide group. The strong peak at 2928.32 cm^{-1} and 1423.4 cm^{-1} were a characteristic of methylene group and alkene group. According Sacithraa et al. (2013) C–H stretching bands from aliphatic compounds occur in the range 3000–2850 cm^{-1}. The presence of alkyne triple bonds and nitrile triple bonds are found 2300 to 2100

cm^{-1}. C=C stretching is much weaker and occurs at 1654.96 cm^{-1}but this band is often absent for dipole moment reasons. The peaks of IR spectra at 860.36 cm^{-1} and 933.4 $cm^{-1}$are showed presence of alkenesgroup, 1055 cm^{-1} and 1089 $cm^{-1}$are showed COH bending and CH_2-related modes, 764.53 cm^{-1} is showed presence of pyrrole, 519.18 cm^{-1} and 578.96 cm^{-1} are showed the presence of bromide group in pea starch.

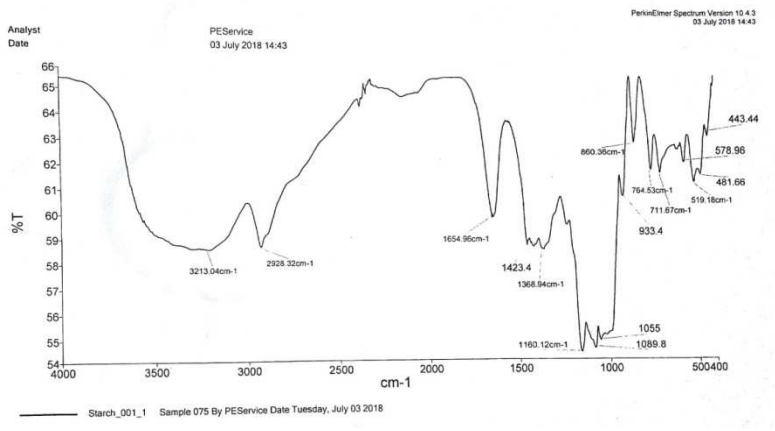

Figure 3: FT-IR Spectra of Yellow Pea Starch

4. CONCLUSIONS

Dry legume yellow pea is one of the important sources of starch. Scanning image of starch was displayed mostly oval, irregular and some spherical shaped granules and diffraction peak was showed that it exhibited C-type crystallinity. Fourier Transform Infrared Spectrometer was showed methylene and amide group in functional region of pea starch spectrum. The yellow pea starch contains 62.65% amylose that is indicating crystalline and amorphous characteristics of starch. The morphological and functional properties of pea starch showed good properties (water solubility, swelling power, crystallite structure, granular size and smooth shape), which are important for its application in the food sector.

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