



# Physicochemical properties and in vitro digestibility of legume starches



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## ABSTRACT

The physicochemical properties and in vitro digestibility of starches from different cultivars (genus) of legumes, namely, black eye bean, baby lima bean, lentil, chick pea, small red bean, and mung bean, were analyzed. All legume starches had oval and spherical granules, with an average diameter of 18.6–27.8  $\mu\text{m}$ . Baby lima bean starch, with high amylose content, high molecular weight ( $M_w$ ) of amylopectin, and low short amylopectin branch chains (DP 6–12), showed high gelatinization temperature, gelatinization enthalpy ( $\Delta H$ ), final viscosity, setback viscosity, and pasting temperature. By contrast, chick pea starch, with low amylose content and  $M_w$  of amylopectin as well as high short amylopectin branch chains, exhibited low gelatinization temperature,  $\Delta H$ , peak viscosity, breakdown viscosity, and pasting temperature. Baby lima bean starch was highly resistant to enzyme digestion. The contents of slowly digestible and resistant starches of cooked small red bean were significantly higher than those of other cooked starches.

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## 1. Introduction

Legumes are dicotyledonous seeds of plants belonging to the Leguminosae family, which comprises 16,000–19,000 species in approximately 750 genera (Hoover, Hughes, Chung, & Liu, 2010). Legumes are an important part of the human diet in many countries worldwide and are second to cereals as a source of human and animal food (Chung & Liu, 2012). More than 20 legume species are widely cultivated in China. Faba bean, pea, mung bean, small red bean, and black eye pea are the main leguminous plants currently grown in China (Du, Jiang, Yu, & Jane, 2014a). Legumes are an excellent source of carbohydrates, possess high levels of proteins, and contain various vitamins and minerals (Chung & Liu, 2012). Legumes are also important in several beneficial physiological responses, such as preventing and controlling metabolic diseases, including diabetes mellitus, colon cancer, and coronary heart diseases (Wilson, 2013).

Starch is the main carbohydrate reserve in higher plants and is deposited in partially crystalline granules; the morphology and molecular structure of starch vary among and within plant species

(Blazek & Copeland, 2008). Starch is the principal component of many food matrices and contributes to the functional properties and nutritional characteristics of processed food products (Pérez-Pacheco et al., 2014). Starch is also the most abundant carbohydrate (22%–45%) in the legume seed (Hoover & Ratnayake, 2002). Compared with cereals, legume starches exhibit poor digestibility, resulting in slow promotion of postprandial glucose, insulin responses, and glycemic index (Jenkins, Wolever, Taylor, Barker, & Fielder, 1980). Current opinion on healthy-eating habits may induce the use of legume starches as alternative to cereals. According to Englyst, Kingman, and Cummings (1992), starch can be classified based on enzyme digestion rate into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). The digestibility of starch widely varies in foods and is influenced by plant type, processing conditions, physicochemical characteristics, and plant microstructure and composition (Ring, Gee, Whittam, Orford, & Johnson, 1988).

Previous studies on legume starches primarily focused on molecular structure as well as physicochemical and functional properties (Hoover & Ratnayake, 2002; Sharma, Singh, Virdi, & Rana, 2015). Studies were also conducted on the physicochemical and in vitro digestibility properties of single cultivars or genus of legume starches (Chung & Liu, 2012; Chung, Liu, Pauls, Fan, & Yada, 2008; Du, Jiang, Ai, & Jane, 2014b; Kaur, Sandhu, & Lim, 2010; Liu,

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Wang, Copeland, & Wang, 2015). The present work mainly aims to determine the physicochemical and in vitro digestibility properties of starches extracted from six cultivars (four genus) of black eye bean (*Vigna sinensis* S.), chick pea (*Cicer arietinum* L.), mung bean (*Vigna radiate* L.), lentil (*Lens culinaris* M.), small red bean (*Vigna umbellata* L.), and baby lima bean (*Phaseolus vulgaris* L.). The in vitro digestibility of RDS, SDS, and RS is compared between raw and cooked legumes of different cultivars. This study provides a basis for optimizing applications in food and nonfood industries and increasing nutritional intake from different cultivars of legumes to enable consumers to select suitable varieties for health benefits. Furthermore, the results can be used for further investigations on physical and chemical modifications to improve the functional properties of the studied starches.

## 2. Materials and methods

### 2.1. Materials

Six legume seeds, namely, black eye bean, chick pea, mung bean, lentil, small red bean, and baby lima bean, were purchased from a local supermarket. Legume starches were isolated following the method described by Li, Jiang, Campbell, Blanco, and Jane (2008). The purity of the isolated starches which was tested using total starch assay kit was more than 95%. All the chemicals used were of reagent grade.

### 2.2. Starch granule morphology and size distribution

Starch samples were placed on a double-sided carbon adhesive tape and vertically coated with palladium–gold. Scanning electron micrographs were obtained using a scanning electron microscope (SEM; JSM-6360LV, Japan Electron Optics Laboratory Co., Ltd.). The size distribution of starches was determined with a laser light-scattering particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., England) following the method described by Du et al. (2014b).

### 2.3. Amylose content

The amylose content of legume starches that was defatted with 85% methanol solution was determined using a potentiometric autotitrator (702 SM Titrimo, Brinkmann Instrument, Westbury, NY) by following the method of Du et al. (2014b). Amylose content was calculated by dividing the iodine affinity of starch by 20%.

### 2.4. Thermal properties

Starch (3 mg, dry basis) was weighed into an aluminum pan and added to distilled water (9  $\mu$ L), sealed in an aluminum pan, and equilibrated at 25 °C for 2 h. The thermal properties of starch were analyzed using a differential scanning calorimeter (DSC-7, Perkin–Elmer, Norwalk, CT) with thermal analysis database and recording software by using the methods described by Du et al. (2014b).

### 2.5. Pasting properties

The pasting properties of starch were determined using a Rapid Visco Analyzer (Newport Scientific, Sydney, Australia) following the method described by Du et al. (2014b). Viscosity parameters of starches were recorded using starch suspensions (8%, w/w; 28 g total weight).

### 2.6. Molecular weight and gyration radius of amylopectin

Starch samples were suspended in 90% dimethyl sulfoxide to obtain a suspension (1.0%, w/v). The suspension was heated in a boiling water-bath for 1 h with stirring and then stirred for 16 h in room temperature. The starch dispersion (1.0%, w/v) was mixed with four volumes of ethanol to precipitate starch and then separated by centrifugation at 7000 r/min for 20 min. The starch pellet was redissolved in boiling water to obtain a suspension (0.4 mg/mL) and then stirred for 1 h in a boiling water-bath. The starch sample solutions were filtered using a nylon membrane filter (5.0  $\mu$ m). The molecular weight and gyration radius of amylopectin were measured using a high-performance size exclusion chromatograph, which consists of Shodex SB-804 and SB-803 analytical columns with a Shodex OH pak SB-G guard column (Showa Denko K.K., Tokyo, Japan) equipped with multi-angle laser scattering and refractive index detectors, by using the method described by Yoo and Jane (2002).

### 2.7. Branch chain length distribution of amylopectin

Amylopectin was separated from amylose through gel permeation chromatography and precipitated using excess ethanol. The collected amylopectin was dispersed using 90% dimethyl sulfoxide, debranched using isoamylase, and labeled with 8-aminopyrene-1,3,6-trisulfonic acid. The branch chain length distributions of amylopectin of large and small molecular weights were determined using fluorophore-assisted capillary electrophoresis (FACE) (P/ACE MDQ, Beckman Coulter, Fullerton, CA) following the method by Jiang, Campbell, Blanco, and Jane (2010).

### 2.8. In vitro digestibility of cooked starch

The in vitro digestibility of cooked starch was determined according to the method of Englyst et al. (1992) modified by Du et al. (2014b). Starch (0.50 g, db) in 10 mL of sodium acetate buffer (0.1 M, pH 5.2) was cooked in a boiling water-bath for 30 min with stirring. The cooked starch was cooled down to 37 °C, mixed with an enzyme solution (2.5 mL) consisting of pancreatin extract and amyloglucosidase, and incubated in a water-bath at 37 °C.

## 3. Results and discussion

### 3.1. Morphological properties of starches

The SEM images of isolated starches are shown in Fig. 1. Legume starches presented similar shapes of round, elliptical, and oval. The surfaces of all starch granules appeared smooth and showed no evidence of fissures or ruptures (Fig. 1). The characteristic features of legume starches are similar to those reported previously (Du et al., 2014b; Hoover & Ratnayake, 2002; Liu et al., 2015; Sharma et al., 2015). The different morphologies of starches might be attributed to biological origin, amyloplast biochemistry, and plant physiology (Singh, Singh, Kaur, Sodhi, & Gill, 2003).

The size distributions of starch granules are presented in Table 1. The average granule diameters of legume starches ranged from 18.6  $\mu$ m to 27.8  $\mu$ m (Table 1), which are within the range of mean diameters reported for other legume starches (Hoover et al., 2010). The mean granule diameters of starches evaluated in the present study were arranged in the following order: baby lima bean starch > small red bean starch > lentil starch > black eye bean starch > chick pea starch > mung bean starch (Table 1). Lindeboom, Chang, and Tyler (2004) attributed the differences in the size of starch granules to biological sources. Starch biosynthesis induces natural variability in amylose and amylopectin molecules, which

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