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# Effect of alkali treatment on structure and function of pea starch granules

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ABSTRACT

The effect of alkaline treatment on the structural and functional properties of pea starch granules was studied using a range of characterization methods including amylose content, scanning electron microscopy (SEM), X-ray diffraction (XRD), $^{13}C$ nuclear magnetic resonance (NMR), swelling power, differential scanning calorimetry (DSC), the Rapid Visco Analyser (RVA) and *in vitro* digestibility. The amylose content decreased by about 20–25% after 15 days of alkaline treatment and there were small decreases in relative crystallinity and double helix content. Deformations were observed on the surface of alkali-treated granules, and there was evidence of adhesion between some of the granules. There was a 25–30% reduction in peak and final RVA pasting viscosities, but only a small reduction in swelling power. The endothermic transition of alkali-treated starch was broadened with a shift of the endothermic peak to higher temperature. However, the endothermic enthalpy remained largely unaffected. Alkali-treatment greatly increased the rate of *in vitro* enzymatic breakdown of the pea starch. More prolonged alkaline treatment for 30 days did not cause further significant changes to the structural and functional properties of the starch granules. The effects of alkali on structure and function of pea starch are explained on the basis of limited gelatinization of the granules.



## 1. Introduction

Starch is the main storage reserve carbohydrate of higher plants and a biopolymer of considerable significance for humans. It is the major source of energy in the human diet and has many industrial applications. Plant starches are synthesized inside plastids by the coordinated action of multiple biosynthetic enzymes and deposited as insoluble, semi-crystalline granules that vary greatly in size, shape and internal crystalline and molecular organisation, depending on the botanical source (Tetlow, 2011). Native starch granules contain two glucose polymers: amylose and amylopectin. Amylose is an essentially linear macromolecule with mostly α(1–4)-linked D-glucopyranosyl units and less than 0.5% of the glucose residues in α(1–6) linkages. Amylopectin is highly branched with α(1–4) linked chains and up to about 5% of the glucose residues in α(1–6) branch points (Buléon, Colonna, Planchot, & Ball, 1998). Normal native starches contain 20–30% amylose, although pea starches have higher amylose content, mostly ranging between 30% and 40% (Hoover, Hughes, Chung, & Liu, 2010; Wang, Sharp, & Copeland, 2011).

Native starch granules have a hierarchical structural periodicity, with alternating amorphous and semi-crystalline growth rings of 120–400 nm thickness that surround an amorphous core (Tester, Karkalas, & Qi, 2004; Wang, Blazek, Gilbert, & Copeland, 2012; Wang, Yu, & Yu, 2008). The semi-crystalline growth rings are characterised by alternating crystalline and amorphous lamellae with a repetition period of 9–10 nm (Blazek & Gilbert, 2011). The nature of the amorphous regions in the core and growth rings is still not well understood, although it has been proposed that this is where a large portion of the amylose occurs (Jenkins & Donald, 1995; Wang et al., 2012). Amylopectin chains that are unbranched (A chains) and singly branched (B1 chains), and that have more than 10 glucose units, may form double helices which are arranged into either A- or B- crystalline structures (polymorphs) that can be differentiated by characteristic powder X-ray diffraction (XRD) or solid-state $^{13}C$ nuclear magnetic resonance (NMR) spectra (Gidley, 1987; Gidley & Bociek, 1985). The A-type polymorphs occur mainly in cereal starches, and the B-type in root, tuber and high-amylose starches. Starches from pulses, rhizomes and some fruits yield XRD patterns that contain both A- and B-type polymorphs, and these are termed C-type starches. The B-type polymorph accounts for between 4% and 49% of the C-type starch of pea seeds (Hoover et al., 2010; Wang et al., 2011) and is located predominantly in the centre of the granule surrounded by the peripheral A-type polymorphs (Bogracheva, Morris, Ring, & Hedley, 1998; Wang et al., 2008).

Despite the wide range of botanical sources, native starch is not always suitable for its various industrial applications because of functional limitations (Hermansson & Svegmark, 1996). Hence, native starch is often modified with various chemical reagents or through acid, alkaline, enzymatic or hydrothermal treatments for

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use in industry (BeMiller & Huber, 2010). Alkaline treatment by agents such as sodium hydroxide is widely used in the production of many food products, including tortillas, waxy rice dumplings, instant noodles and yellow alkaline noodles, to enhance quality characteristics of colour, flavour and texture (Nadiha, Fazilah, Bhat, & Karim, 2010). It is also used in the manufacture of starch-based adhesives. Alkaline washing is regarded as an effective step in the isolation of starch granules with high purity and well-defined physical properties (Cardoso, Putaux, Samion, & Silveira, 2007; Cardoso, Samios, & Silveira, 2006; Thys et al., 2008). However, the effects of alkali on starch structure and properties have received relatively less attention compared to other methods of starch modification. Alkaline steeping was shown to alter the structural properties of sago, corn and potato starch granules, resulting in changes in functional properties such as swelling power, gelatinization and pasting profiles (Karim et al., 2008; Nadiha et al., 2010). To the best of our knowledge, there is no information regarding the effect of alkaline treatment on the *in vitro* digestibility of starch granules. Moreover, the mechanism underlying the action of alkali on starch structure and functionality has yet to be elucidated. In the present paper, relatively minor structural changes induced in pea starch granules by alkali treatment are shown to result in large consequential changes in functional properties. The results are interpreted on the basis that the alkali treatment disrupts the molecular order of granules in a way that resembles limited gelatinisation.

## 2. Materials and methods

### 2.1. Materials

Pea seeds (*Pisum sativum* L, variety Kaspa, grown in 2009) were obtained from the Plant Breeding Institute of The University of Sydney. The pea variety (an Australian commercial variety) and the method for starch isolation are described in Wang et al. (2011).

### 2.2. Preparation of alkali-treated starch

The alkali-treated starch was prepared according to the method of Nadiha et al. (2010) with some modifications. Duplicate samples of pea starch (10 g dry weight) were each suspended in 100 ml of 0.1 M NaOH solution containing 0.1% sodium azide. After 15 and 30 days at 35 °C with intermittent shaking by hand to resuspend the starch granules, the slurry was filtered through Whatman No. 1 filter paper with suction. The residue was washed with distilled water until the filtrate was at neutral pH and then twice with 95% ethanol. The resulting alkali-treated starch was dried overnight at room temperature under a gentle air stream. The yield of alkali-treated starch was calculated from the dry weight of starch after alkali treatment divided by the dry weight of starch before treatment.

### 2.3. Apparent amylose content

Apparent amylose content was determined by the iodine binding colorimetric method (Williams, Kuzina, & Hlynka, 1970).

### 2.4. X-ray diffraction measurements

XRD patterns from the powders were obtained using a Bruker D8 Focus X-ray diffractometer (Bruker AXS, Germany) with a Cu–$K_\alpha$ source ($\lambda = 0.154$ nm) operating at 40 kV and 40 mA. The specimens were stored in a constant humidity atmosphere of 75% in a desiccator over a saturated solution of NaCl for one week before spectra were collected. The intensity was measured from 4° to 35° as a function of $2\theta$ and at a scanning speed of 0.5°/min and a step size of 0.04°. Relative crystallinity of the starch samples was estimated from the XRD patterns as previously described (Wang, Yu, Yu, Chen, & Pang, 2007).

### 2.5. NMR measurements

High-resolution solid-state $^{13}C$ Cross polarisation/Magic Angle Spinning (CP/MAS) NMR experiments were conducted using a Varian Infinity Plus 300 WB spectrometer at a $^{13}C$ frequency of 75.46 MHz. NMR spectra were observed under high power decoupling (DD) conditions. Samples were spun at the magic angle (54.51 or 54.71). Magic angle spinning rates of 2.5 kHz and DD field of 61 kHz were used. The 901 pulse width was 4 ms with a recycle time of 5 s. A contact time of 1 ms was used for all the samples; spectral width was 20 kHz; and 27 ms acquisition time. Starch samples (200–240 mg) were packed into a 7 mm sample rotor with tight push-fitting caps. Spectra were referenced to external $Me_4Si$ via the low field resonance of adamantane (38.6 ppm). About 10,000 scans were accumulated for each spectrum to obtain a satisfactory signal–noise ratio. A polynomial baseline was corrected manually where necessary after Fourier transformation and phasing. The percentage of double helix content of the starch samples was evaluated according to the method of Bogracheva, Wang, and Hedley (2001). The percentage of amorphous regions in a starch sample is given by the proportion of C4 peak area relative to the total area of the spectrum divided by the same ratio for gelatinised pea starch as an amorphous starch reference. Double helix content (%) is 100 minus the amorphous part (%).

### 2.6. Field emission scanning electron microscopy

Native and alkali-treated starches were fixed onto the surface of double-sided, carbon-coated adhesive tape attached to an aluminium stub. The mounted starch granules were coated with palladium/gold prior to imaging in a field emission scanning electron microscope (SEM) (Carl Zeiss ULTRA plus, Germany). The accelerating voltage was 1.01 kV.

### 2.7. Swelling power

Swelling power (SP) of native and alkali-treated starches was determined using a 40-mg test according to the method of Konik-Rose et al. (2001). Starch swelling power was calculated as the ratio of the weight of the swollen starch to the initial dry weight of starch.

### 2.8. Differential scanning calorimetry

DSC measurements were made using a Modulated Differential Scanning Calorimeter MDSC 2920 instrument (TA Instruments Inc., Delaware, USA) equipped with a thermal analysis data station and data recording software. Approximately 3 mg of starch was weighed accurately into an aluminium sample pan. Distilled water was added with a microsyringe to obtain a starch:water ratio of 1:2 (w/w) in the DSC pans. Care was taken to ensure that the starch samples were completely immersed in the water by gentle shaking before the pans were sealed, reweighed and left overnight at room temperature before DSC analysis. An empty pan was used as a reference. The pans were heated from 30 to 100 °C at a scanning rate of 10 °C /min. The instrument was calibrated using indium as a standard. The DSC trace was used to make measurements of the start ($T_s$), peak ($T_p$) and conclusion ($T_c$) temperatures and enthalpy change ($\Delta H$) of the endotherm. The start and conclusion temperatures are defined as the point at which the DSC trace first starts and finally ceases to deviate from a flat baseline, respectively. The peak