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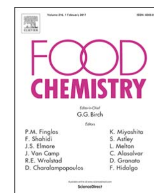

Changes in molecular structure of chickpea starch during processing treatments: A thin layer chromatography study

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ABSTRACT

To detect the changes in molecular structure of chickpea starch during processing treatments, a thin layer chromatographic method for characterizing the molecular structure of chickpea starch was developed. With this method, the components in chickpea starch could be divided into amylopectin, small linear molecules and large linear molecules, and their contents could be determined. It was found that the degrees of polymerization of the large linear molecules and small linear molecules in chickpea enzyme-resistant starch were about 40 and below 15, respectively. Furthermore, the small linear molecules were more susceptible to α-amylase hydrolysis than the large linear molecules. The results suggested that the large linear molecules and small linear molecules in chickpea enzyme-resistant starch might mainly originate from the amylose and amylopectin of native chickpea starch, respectively, based on the retrogradation properties of amylose and amylopectin and the enzymatic degradation behavior of the large linear molecules and small linear molecules.

1. Introduction

Chickpea (*Cicer arietinuml* L.), mainly cultivated in India, Pakistan, Iran, Australia and Turkey, is the third major pulse crop in the world on the basis of total production after beans and peas (FAOSTAT, 2013). It is a good source of carbohydrates, fibers and proteins in human diet and may provide health benefits for the consumer (Chino et al., 2017; Gupta et al., 2017; Wallace, Murray, & Zelman, 2016; Zhang, Chen et al., 2017; Zhang, Su et al., 2017). Based on seed morphology and geographical distribution, there are two types of chickpeas: Desi (small and dark, mostly cultivated in Asia and Africa) and Kabuli (large and light color, mostly cultivated in Europe, North America) (Chavan, Kadam, & Salunkhe, 1986; Jukanti, Gaur, Gowda, & Chibbar, 2012).

Starch consists of amylose and amylopectin, and the ratio of amylose/amylopectin in starch varies depending on starch sources (Tester, Karkalas, & Qi, 2004). The molecular structure (such as amylose content and molecular size distribution) has significant influences on the physical and functional properties of starch such as gel-texture, crystallinity and digestibility property (Bertoft et al., 2016; Li, Prakash, Nicholson, Fitzgerald, & Gilbert, 2016; Li & Zhu, 2017; Xu et al., 2017). At present, starch molecular structure is usually determined by gel permeation chromatography (Hoyos-Leyva, Bello-Pérez, Alvarez-Ramirez, & Agama-Acevedo, 2017; Zhang, Chen et al., 2017; Zhang, Su et al., 2017). Other methods are also used for starch analysis, such as field-flow fractionation (Chiaramonte, Rhazi, Aussenac, & White, 2012; Juna & Huber, 2012), high performance liquid chromatography (Kärkkäinen, Lappalainen, Joensuu, & Lajunen, 2011), cellulose column (Patil & Kale, 1973) and paper chromatography (Talib, Karve, Bhide, & Kale, 1987).

Thin layer chromatography (TLC) is an easy, simple, rapid and relatively inexpensive method for mixture analysis. It can be used as a preliminary method in the analysis of saccharides, particularly in the analysis of monosaccharides (Ferey et al., 2016; Lucia, Manfredini, Bernardi, & Vertuani, 2015), disaccharides (Fukutomi et al., 2013; Ranta et al., 2013) and oligosaccharides (Fukutomi et al., 2013; Ranta et al., 2013), and less in the analysis of polysaccharides (Bischel, Austin, Kemeny, Hubble, & Lear, 1966). As far as we know, this method has not been applied to analyze starch molecular structure. In our previous study, we investigated the changes in crystal structure of chickpea starch during processing treatments (Sun et al., 2015). In order to detect the changes in molecular structure of chickpea starch during processing treatments, TLC is specifically developed in the present study and can serve as a useful method for preliminary characterization of starch molecular structures.

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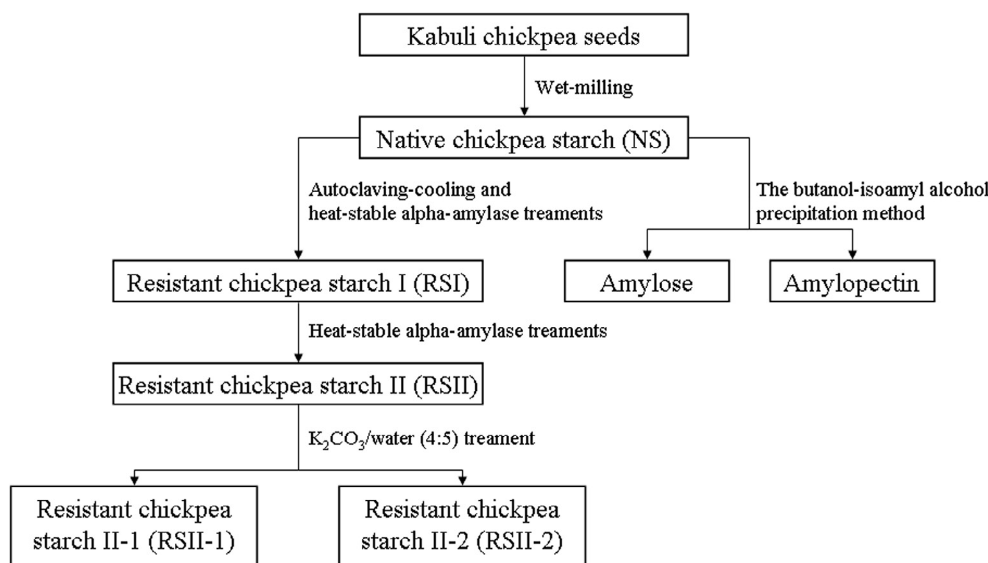

Fig. 1. General scheme for preparation of chickpea starch samples.

2. Materials and methods

2.1. Materials and reagents

Kabuli chickpea seeds were provided by Xinjiang Agricultural University (Urumqi, China). Amyloglucosidase from *Aspergillus niger* (100 U/mg) was purchased from Shanghai Kayon Biological Technology Co., Ltd (Shanghai, China), heat-stable amylase from *Bacillus licheniformis* (20000 U/mL) was purchased from Jiangsu Ruiyang Biotech Co., Ltd (Wuxi, China). Silica gel G plate (20 × 10 cm) was purchased from Qingdao Jiyida Silica Reagent Factory (Qingdao, China). All other reagents were of analytical grade.

2.2. Preparation of chickpea starch samples

The procedures for preparation of chickpea starch samples are outlined in Fig. 1. The preparation of native chickpea starch (NS), resistant chickpea starch I (RSI), resistant chickpea starch II (RSII) was performed according to the reported method (Sun et al., 2014). Briefly, NS was prepared from Kabuli chickpea seeds by a wet-milling process. RSI was prepared from NS by autoclaving treatment and amylase digestion, and RSII was obtained from RSI by extensive hydrolysis with amylase. These starch samples were all lyophilized.

The samples of resistant chickpea starch II-1 (RSII-1) and II-2 (RSII-2) were prepared from RSII. Suspending of RSII in high concentration of K_2CO_3 solution (ratio of K_2CO_3/water, 4:5 (w/v)), RSII was divided into two components: the floated matter and the settled matter. These two components were washed by successive centrifugation in water until neutrality and lyophilized to afford RSII-1 and RSII-2, respectively.

The chickpea amylose and amylopectin were prepared according to the butanol-isoamyl alcohol precipitation method (Klucinec & Thompson, 1998) with modifications. NS was defatted, deproteinized and dissolved in 0.5 M warm NaOH solution. The pH of the solution was adjusted to be neutral with HCl solution, 0.45 vol of mixture of 1-butanol and isoamyl alcohol (3:1, v/v) was then added to the solution. The mixture was incubated in a boiling water bath for 20 min, decreased to room temperature and kept at 4 °C for 24 h. The mixture was centrifuged to afford amylose (precipitates) and amylopectin (supernatants), respectively. The precipitates were resuspended in warm water-saturated butanol, kept at 4 °C for 24 h and centrifuged. This process was repeated six times. Finally, the resulting precipitates were washed with ethanol and dried in an oven of 70 °C as chickpea amylose. The latex-like liquid after standing and demixing of supernatant was taken, redispersed by adding 0.1 vol of mixture of 1-butanol and isoamyl alcohol (1:1, v/v), kept at 4 °C for 48 h and centrifuged. This process was repeated five times. At the end, the soluble polysaccharides in the mixture were precipitated by two volumes of cold ethanol, washed with ethanol and dried in an oven of 70 °C as chickpea amylopectin.

2.3. Preparation of starch solution

The starch solution was prepared by dissolving chickpea starch sample (50 mg dry weight, but 25 mg for chickpea amylose) in dimethylsulfoxide (DMSO, 5 mL) containing 50 mM $NaNO_3$ at 90 °C for 24 h with constant stirring. The polysaccharide solution was made by dispersing polysaccharide, isolated from the starch solution by alcohol precipitation, in water.

2.4. Determination of degree of polymerization (DP)

The DP of chickpea starch sample was calculated from the following Eq. (1) using the corresponding polysaccharide solution as the test solution (Eerlingen, Deceuninck, & Delcour, 1993):

$$\mathrm{DP} = \frac{2 \times \text{Total sugar content (as } \mu\text{g maltose)}}{\text{Reducing sugar content (as } \mu\text{g maltose)}} \quad (1)$$

2.5. Determination of branch number

Starch is a polymer of glucoses joined by α-1,4- and α-1,6-glycosidic bonds. It has only one reducing end and many non-reducing ends, in which the total number of reducing end and α-1,6-glycosidic bonds is equal to the total number of non-reducing ends. The periodate oxidation of the reducing end or α-1,4-glycosidic bond would not liberate formic acid, while periodate oxidation of the non-reducing end or α-1,6-glycosidic bond would lead to the liberation of one molecule of formic acid. Thus, the total number of α-1,6-glycosidic bonds and non-reducing ends (equaled to the number of reducing end plus twice the number of α-1,6-glycosidic bonds) is equal to the total number of formic acid molecule liberated after periodate oxidation. And the branch number of chickpea starch sample could be determined by the following Eq. (2) using the corresponding polysaccharide solution as the test solution:

$$\text{Branch number} = \frac{\text{Formic acid (mM)} - \text{Reducing sugar (mM)}}{2 \times \text{Reducing sugar (mM)}} \quad (2)$$

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