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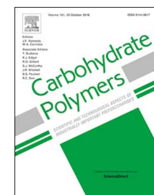

Structure of New Zealand sweetpotato starch

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ABSTRACT

New Zealand sweetpotatoes (kumara) (*Ipomoea batatas*) represent unique genetic resources for sweetpotato diversity, though they are much under-studied. In this study, 7 New Zealand sweetpotato varieties with commercial significance were collected for the characterization of the molecular and granular structure of the starches. In particular, the internal molecular structure of the amylopectins was detailed by chromatographic and enzymatic techniques. Maize and potato starches with normal amylose contents, which are among the most important commercial starch sources, were employed for comparison. The results revealed a degree of diversity in amylose composition, unit and internal chain composition, granule size distribution, and degree of crystallinity among the 7 sweetpotato starches. All the sweetpotato starches showed C_A-type polymorph. The sweetpotato amylopectins have intermediate amounts of both short and long internal unit chains among amylopectins of different botanical sources. The differences in the structure of sweetpotato starches suggest differences in physicochemical properties.

1. Introduction

Sweetpotato (*Ipomoea batatas*) is the sixth most important food crop (after rice, wheat, potato, maize, and cassava) (Zhu & Wang, 2014). The global annual production quantity is over 105 million tonnes (FAOSTAT, 2017). Major producers are China, Nigeria, Tanzania, Ethiopia, Mozambique, and Indonesia (FAOSTAT, 2017).

Sweetpotato has been increasingly recognised as a “healthy” food product in recent years (Wang, Nie, & Zhu, 2016). A range of bioactive components have been identified in the root, including dietary fiber, proteins, carotenoids, anthocyanins, phenolic acids, and certain types of minerals (Wang et al., 2016). These nutrients contribute to various health effects of sweetpotato, including antioxidant, antiinflammatory, hepatoprotective, antitumor, antidiabetic, anti-obese, antimicrobial, and antiaging effects. Therefore, sweetpotato has great potential to be further developed to formulate value-added and nutritionally enhanced foods for human health. There are sweetpotato based food products available in the market. These include noodles, vermicelli, fried chips, bread and steamed bread, cake, and alcoholic beverages (Zhu, Yang, Cai, Bertoft, & Corke, 2011; Zhu & Wang, 2014). Starch is the major component of sweetpotato roots. It is expected that the starch quality greatly determines the quality of sweetpotato based products. Furthermore, the starch itself has potential for different food and industrial applications (Zhu & Wang, 2014). The starch has been used to produce thermoplastic films, as substrate for producing a range of industrial products (e.g., bioethanol and reducing sugars), and for pharmacological uses (e.g., tablet formation) (Zhu & Wang, 2014). Therefore, understanding the structure and properties of the starch contributes to the development of sweetpotato as a sustainable crop. Especially, assessing genetic resources for sweetpoato starch quality remains to be better explored to provide a basis for the uses of the starch (Zhu & Wang, 2014).

Starch is consisted of amylose and amylopectin which are assembled in the form of granules. The former is linear with a few branches, while the latter is much branched with a larger molecular weight (Pérez & Bertoft, 2010). The branches of the amylopectin are arranged in a clustered manner. The part with a high concentration of the branches is termed “building block” by Bertoft (2017). These building blocks contribute to the formation of backbone of amylopectin, which is the internal part of the amylopectin and forms the amorphous lamellae of the granules (Bertoft, 2017). The external part of amylopectin is between the non-reducing ends and the branches. This part of the amylopectin interacts with each other to form the crystalline region of the starch granules. Understanding the structure of starch contributes to understanding the molecular basis of functional properties. In particular, there has been increasing interest to study the internal molecular structure of amylopectin (Vamadevan & Bertoft, 2015). This is because the internal structural feature of amylopectin has been correlated with physicochemical properties (e.g., gelatinization) and biosynthesis of starch (Bertoft, 2017; Vamadevan & Bertoft, 2015). So far, only one study reported the internal unit chain composition of amylopectins of 11 Chinese sweetpotato genotypes (Zhu, Corke, & Bertoft, 2011a). The

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cluster and building block structure of amylopectin (1 genotype) has also been studied (Zhu, Corke, Åman, & Bertoft, 2011b, 2011c). More genetic resources are to be studied to reveal the structural diversity of this starch.

In New Zealand, sweetpotato is commonly known as kumara (a Maori word). Maori people grew sweetpotatoes with Polynesian origin before the European contact (Yen, 1963). It was a major food crop before the rise of potatoes and other crops (Lewthwaite, 2006). Nowadays, there has been increasing interest in utilizing New Zealand sweetpotato for food production. New cultivars with targeted agricultural and nutritional properties are being developed. Apart from being a food item, sweetpotato has strong social and cultural significance associated with New Zealand people (Lewthwaite, 2006; Yen, 1963). However, lack of information on starch properties of the sweetpotatoes seriously hinders their development for sustainable production.

This study aims to explore the molecular and granular structure of starches from 7 sweetpotato varieties with commercial significance in New Zealand (Supplementary Table S1, Supplementary Fig. S1). There has been a large inconsistency in the methodology in structural characterization of starch among different studies in our research community. This inconsistency makes the results of different studies difficult for direct comparison (Zhu, 2015). Therefore, in this study under the same experimental conditions, the structure of these starches was compared with that of maize and potato starches which are among the most well studied and industrial starches. The results of this study contribute to our knowledge of the diversity in sweetpotato starch, providing a fundamental basis for the development of sweetpotato as a sustainable cash crop. The readers are encouraged to refer to the supplementary material which also contains a significant amount of related information.

2. Materials and methods

2.1. Sweetpotatoes, starches, and enzymes

Seven sweetpotato samples from Kaipara Kumara were grown in Ruawai, Northland, New Zealand. They were harvested in the year of 2016 (root images shown in Supplementary Fig. S1). The sweetpotato varieties used in this study have great diversity in the appearances and general quality attributes. The names and some general quality attributes including recommended uses are summarised in Supplementary Table S1. Three varieties including Orange kumara (Beauregard), Red kumara (Owairaka), and Gold kumara (Toka Toka) are New Zealand local varieties with great commercial significance. Owairaka is a major variety in the current market. It is a traditional New Zealand cultivar derived from a mutant well established in the 19th century. Toka Toka was selected and introduced to local New Zealand industry in 1972. The Maori name is after a peak around Dargaville of Northland in New Zealand. Beauregard was originally from the USA and was introduced into New Zealand for cultivation in 1993 (Lewthwaite, 2006). Orange Sunset (OS) and Purple Dawn (PD) are two new cultivars bred by Plant & Food Research of New Zealand since 2014 and are being developed for commercial production. Kokei and Koganesengan (Kogan) are two varieties originated from Japan and were released there in 1945 and 1966, respectively (Tsutsui, Shiga, & Mikami, 2016). They were brought to New Zealand probably in the 1980s and are now cultivated in New Zealand. A modern potato variety with red skin was purchased from Countdown Supermarket (Auckland, New Zealand). The roots and tubers were immediately processed for starch isolation upon arrival in the laboratory as described in the next Section 2.2.

GELOSE 50, waxy maize, and normal maize (Melogel) starches used in this study were from Ingredion (Auckland, New Zealand). GELOSE 50 and waxy maize were used as standards for amylose content measurement as described in the Section 2.5.1 below. The normal maize starch (Melogel) was used for comparative purpose. A commercial modern potato variety with red skin for starch isolation was purchased from Countdown Supermarket (Auckland, New Zealand).

Barley β-amylase (EC 3.2.1.2, specific activity 600 U/mg), *Klebsiella planticola* pullulanase (EC 3.2.1.41, specific activity 30 U/mg), and *Pseudomonas* sp. isoamylase (EC 3.2.1.68, specific activity 280 U/mg) were from Megazyme International (Wicklow, Ireland). The enzyme activities are according to the suppliers.

2.2. Starch isolation

The isolation of starch from sweetpotato roots followed a previous description (Zhu, Yang et al., 2011). The isolation of starch from potato tubers followed a previous description (Singh, McCarthy, & Singh, 2006). Basically, the roots or tubers were washed, peeled, and cut into small pieces before macerating with water in a blender. The resulting slurry was washed and sieved a few times to remove impurities. The brown/yellow layer at the top of the starch cake after centrifugation was removed. The purified starch was air-dried in an oven (40 °C).

2.3. Amylopectin fractionation

Amylopectin was isolated from starch by using the 1-butanol and isoamyl alcohol precipitation based method as detailed previously (Klucinec & Thompson, 1998). The fractionation process was repeated once to ensure that the amylopectin is highly pure.

2.4. Production of β-limit dextrins (β-LDs) from amylopectin

Amylopectin was hydrolysed by β-amylase to obtain β-LDs. The method in general followed a previous report (Zhu et al., 2011a). Only β-amylolysis was conducted 3 times to reach the β-limit. The maltose was removed by using a tangential flow filtration with an Omega 10 K membrane in Minimate™ TFF Capsule System (Pall Life Sciences, Ann Arbor, MI, USA). The β-LDs were freeze-dried and stored in a sealed plastic tube.

2.5. Analytical methods

2.5.1. Composition

The amylose contents of starch were determined by 3 different methods. Apparent amylose content of starch was measured by iodine binding-spectrometry based method as described previously with modifications in that GELOSE 50 (amylose content, 50%) and waxy maize (amylose content, 0%) starches were used as standards (Hoover & Ratnayake, 2005). The determination of true amylose contents of starch was based on concanavalin A precipitation based method using a Megazyme assay kit (Wicklow, Ireland). Amylose content of starch was also analyzed by running debranched starch on gel permeation chromatography (GPC) of Sepharose CL 6B as described in the Section 2.5.2.

Phosphorus content of starch was analyzed by inductively coupled plasma mass spectrometry (ICP-MS) as described previously (D. Li & Zhu, 2017).

2.5.2. Chromatographic analysis

Starch, amylopectin, and β-LDs were debranched by using a combination of isoamylase and pullulanase at pH 5.5, following a previous study (Zhu et al., 2011a).

The analysis of molecular size distribution of debranched starch and amylopectin was conducted on a GPC of Sepharose CL 6B as described previously (Zhu et al., 2011a).

The analysis of unit chain composition of debranched amylopectin and β-LDs was conducted on a HPAEC-PAD system (high-performance anion-exchange chromatography-pulsed amperometric detection) (Dionex ICS 5000^{+}) (Sunnyvale, CA, USA). The procedures and settings of HPAEC followed a previous study (Zhu et al., 2011a). Calculation of diverse chain length values followed the methods as described

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