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Characterization of polymer chain fractions of kiwifruit starch

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ABSTRACT

In this report, the amylose composition and molecular structure of starches from the core and outer pericarp of 3 golden kiwifruit varieties were characterised, using enzymatic and chromatographic techniques. Starches from the core tissues of kiwifruit tend to have higher amylose contents (by ~3–5%) and longer unit chains of both amylopectins and their φ, β-limit dextrins (LDs) than those of the outer pericarp starches. The contents of short B-chains of the φ, β-LDs of amylopectins from the outer pericarp were higher (by ~3%) than those of φ, β-LDs of the core amylopectins. Overall, the composition and structure of starches from the outer pericarp and core tissues of a golden kiwifruit were different. This study provides a structural basis to further investigate the starch degradation in kiwifruit, which may be of importance for the storage and eating quality of the fruit.

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1. Introduction

Kiwifruit (*Actinidia* spp.) or Chinese gooseberry is native to China, and now is widely cultivated over the world. The most popular commercial species are green (*A. deliciosa*) and golden (*A. chinensis*) kiwifruits (Testolin & Ferguson, 2009). Their popularity are mainly contributed by the pleasant taste and important nutrients such as vitamin C and polyphenols. The commercial value of the golden kiwifruit tends to be higher than the green ones (Testolin & Ferguson, 2009). Kiwifruit is economically important for the New Zealand horticulture, and the export exceeds $1 billion per year. Hort16A, which played a significant role in the kiwifruit commerce, was infected by *Pseudomonas syringae* pv. *actinidiae* (PSA). New varieties such as Gold3 and Gold9 which are resistant to PSA have been cultivated in New Zealand to replace Hort16A. However, the shelf life of some of the new varieties during post-harvest storage may be short.

Starch is a major component in developing kiwifruit. The starch accumulates as the kiwifruit grows and the content reaches a peak (~40% of the dry weight) around one month before the commercial harvesting (Richardson, McAneney, & Dawson, 1997). The starch is then gradually hydrolysed to simple sugars as the fruit becomes ripe during storage (Beever & Hopkirk, 1990). The starch degradation may play a role in the kiwifruit shelf life during the storage. Understanding the molecular structure of kiwifruit starch would provide a basis for studying the starch degradation in ripening fruits. Furthermore, as the outer pericarp (flesh) of kiwifruit becomes eating-ripe, the core tissue inside the fruit may still be hard. The eating quality of kiwifruit can be largely affected by heterogeneous distribution of hardness in the fruit. The considerable amount of starch in the core tissue and the starch degradation in the outer pericarp contribute to such a phenomenon (MacRae, Lallu, Searle, & Bowen, 1989). The different degradation behaviours of the starches from the two types of tissue may suggest some differences in the structure. To the best of our knowledge, the molecular structure of kiwifruit starch remains largely unknown. Only one study presented some structural information on green kiwifruit starch, and the kiwifruit starch studied was not specific to the tissue type (Stevenson, Johnson, Jane, & Inglett, 2006).

Starch molecules are categorized into amylose and amylopectin. Amylose is linear with the glucosyl units being connected by α-(1–4)-linkages. A few branches of α-(1–6)-linkages may exist in amylose. Amylopectin has highly branched structure, in which the glucosyl units are branched by α-(1–6)-bonds (Bertoft, 2004a). The molecules are assembled in granular forms with amorphous and semi-crystalline rings (Bertoft, 2004a). The semi-crystalline rings are composed of alternating amorphous and crystalline lamellae. The external unit chains of amylopectin tend to form the double helices, contributing to the formation of the crystalline lamellae. The internal part of amylopectin tends to form the amorphous lamellae in clustered manner (Bertoft, 2013). The amylopectin molecular structure are critically responsible for the physicochemical properties of starch (Vamadevan & Bertoft,

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2015). In particular, there is increasing research interest in understanding the importance of amylopectin internal structure in various physicochemical properties and biosynthesis of starch (Bertoft, 2013; Vamadevan & Bertoft, 2015). The internal part of amylopectin can be obtained by using phosphorylase and/or β-amylase to remove the external parts (Bertoft, 2004a, 2004b). For example, all the internal chains appear as B-chains and all the A-chains appear as maltose stubs when the amylopectin is in the form of φ, β-limit dextrins (φ, β-LDs) (Bertoft, 2004a, 2004b). The unit chain length distribution of the φ, β-LDs can be obtained by high-performance anion exchange chromatography after debranching with isoamylase and/or pullulanase (Bertoft, 2004a). To the best of our knowledge, there is no report on the amylopectin structure of golden kiwifruit and internal molecular structure of kiwifruit amylopectin. The lack of structural knowledge of this starch hinders the understanding of starch degradation in kiwifruit, which may be important for the fruit storage and final eating quality. The physicochemical properties of starches from 3 golden kiwifruit varieties (Hort16A, Gold3, and Gold9) have been studied recently (D. Li & Zhu, 2017). Kiwifruit starch has a B-type polymorph. The particle size of the granules is ~8–9 μm (volume-weighted mean value) (D. Li & Zhu, 2017). Variations in the properties such as rheology and gelatinization among different starches were noted. Interestingly, the starch contents and properties from the outer pericarp and core tissues of a golden kiwifruit are different. The amylose contents of outer pericarp and core starches were 15.5–17.8% and 20.7–23.3%, respectively, according to the concanavalin A precipitation-based method (D. Li & Zhu, 2017). This suggests different molecular structure and biosynthetic properties of the starches in these tissues. It would be interesting to study if the molecular structure of golden kiwifruit starch is tissue-specific. The results would contribute to the knowledge of the synthesis-structure-function relationships of kiwifruit starch, while providing useful information for developing kiwifruit-based food products. For example, a research topic in our laboratory is to develop starch-rich flour from whole unripen kiwifruits for novel food formulations.

The chemical compositions and molecular structures of starches from various botanical source have been studied by enzymatic and chromatographic techniques (Bertoft, 2004b; Bertoft, Piyachomkwan, Chatakanonda, & Sriroth, 2008; Kong, Bertoft, Bao, & Corke, 2008; G. Li & Zhu, 2017a; Zhu, Corke, & Bertoft, 2011). Our study aims to apply the methodology to investigate the composition and structure of starches from 3 golden kiwifruit varieties (*A. chinensis*) which were used in a previous study (D. Li & Zhu, 2017). The study highlights the differences in internal molecular structures of the amylopectin components between core and outer pericarp starches. A large number of nomenclatures related to the internal molecular structure of amylopectin are employed in this report, which are summarized in Supplementary Table 1. Potato and maize starches, as two of the most known starches, were employed as references for comparison.

2. Materials and methods

2.1. Starches and enzymes

Three golden kiwifruit varieties (Gold3, Gold9, and Hort16A) were collected from an orchard at Pukekohe, Auckland, New Zealand. The kiwifruits were picked on the 161st, 166th, and 176th days after anthesis for Gold3, Gold9, and Hort16A, respectively. The background information on the starches from 3 golden kiwifruit varieties have been described in a previous paper (D. Li & Zhu, 2017). The starches from both outer pericarp and core tissues of the fruit (Supplementary Fig. 1) were extracted according to the report of Fuke and Matsuoka (1984) with some modifications (D. Li & Zhu, 2017). Maize and potato starches were studied as reference samples, the background information of which were detailed in a previous study (D. Li & Zhu, 2017).

β-Amylase from barley (EC 3.2.1.2, specific activity ~600 U/mg), *Pseudomonas* sp. isoamylase (EC 3.2.1.68, specific activity ~280 U/mg), and *Klebsiella planticola* pullulanase (EC 3.2.1.41, specific activity ~30 U/mg) are from Megazyme International (Wicklow, Ireland). Rabbit muscle phosphorylase *a* (EC 2.4.1.1, specific activity ~1.2 U/mg) was from Sigma-Aldrich (Deisenhofen, Germany). The given enzyme activities are according to the suppliers.

2.2. Isolation of amylopectin

Amylopectin was isolated from the starch based on a previous method (Zhu et al., 2011) with some modifications. Starch (2.5 g) was dispersed in 35 mL 90% dimethyl sulfoxide (DMSO) in a 500 mL Erlenmeyer flask sealed with an aluminium foil. The flask was heated in a boiling water bath with constant stirring for 3 h to obtain a clear starch solution before cooling to ~28 °C. 6% 1-butanol and 6% isoamyl alcohol (24 mL) well dispersed in water (352 mL) was poured to the starch solution. The whole mixture was stirred for 15 min before placing in a water bath at 95 °C for 1 h. The entire system was slowly cooled for around 20 h to ~28 °C before centrifugation (10,000×*g*) at 4 °C for 15 min. The supernatant was collected and condensed to ~100 mL at 60 °C using a rotary evaporator (R114 Rotavapor equipped with a B480 water bath) (Flawil, Switzerland). The solution was then mixed with ethanol (~900 mL) to precipitate the amylopectin. The precipitate was collected by centrifugation at 3000×*g* for 15 min. The precipitate was washed with 100 mL ethanol twice and with 100 mL acetone once before drying in an air-forced oven at 60 °C.

The starch from outer pericarps had a higher amylose content than that from the core tissues (D. Li & Zhu, 2017). Amylopectin of the former was purified once more by repeating the isolation process. This purification process little changed the amylose content of the resulting amylopectins as measured by concanavalin A precipitation-based method (D. Li & Zhu, 2017). Therefore, all the amylopectins purified once were considered pure.

2.3. Production of φ, β-limit dextrins (φ, β-LDs)

φ, β-LDs of amylopectin were produced based on previous reports with some modifications (Bertoft, 2004a; G. Li & Zhu, 2017a). The amylopectin (25 mg) was dissolved in 3 mL 90% DMSO in a Pyrex test tube (30 × 195 mm). The tube was sealed with aluminium foil before heating in a boiling water bath for 15 min with constant stirring to completely dissolve the amylopectin. The solution was diluted by adding 40 mL hot water, sodium phosphate buffer (3.6 mL, pH 6.8, 1.1 M) and Na-EDTA (1.7 mL, 2.8 mM). After cooling to ~35 °C, rabbit muscle phosphorylase *a* (1 mg, ~1.2 U/mg) was added before placing the solution in a water bath at 35 °C overnight with constant stirring. The glucose 1-phosphate was removed by using a tangential flow filtration (an Omega 10 K membrane in Minimate™ TFF Capsule System) (Pall Life Sciences, Ann Arbor, MI, USA) until no more carbohydrate was detected from the discarded solution, which was analysed by the phenol-sulfuric acid based method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Li, Wang, & Zhu, 2016). The sample was boiled for 10 min to inactivate the enzyme. The phosphorolysis procedure above was repeated one more time to ensure the production of φ-LDs. A sodium acetate buffer (0.01 M, pH 6.0) (0.325 vol) and β-amylase (10 μL, ~2 U/mg starch) were subsequently added before placing at a 50 °C water bath overnight with constant stirring. The solution was then transferred to a Vivaspin 20 centrifugal concentrator (MWCO 10,000 Da, Sartorius Stedim

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