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Structure of black pepper (*Piper nigrum*) starch

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A R T I C L E I N F O

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A B S T R A C T

Black pepper (*Piper nigrum*) is one of the most used spices. Starch is the major component of the dried peppercorns. However, little is known about this starch. In this study, starches of black and white peppers were isolated and structurally studied. The total starch content of the white peppers amounted up to 52% of the total dry weight. Starches from the two peppers had similar granule and molecular structures and amylose composition. The amylose contents of pepper starches were ~18% according to concanavalin A precipitation-based method. The unit and internal chain profiles of pepper amylopectins were studied by chromatographic and enzymatic means. Pepper amylopectin was structurally similar to that of some A-type starches such as rice starch. Pepper starch had A-type polymorph. The granules were irregularly polygonal and the volume-weighted mean diameter was 3–5 μm. The results of this study suggest that pepper starch can be a novel starch resource.

1. Introduction

Black pepper (*Piper nigrum*) is one of the most popular spices. It is a flowering vine of the family Piperaceae and is widely cultivated in tropical regions for the fruits (drupes). The world production of black pepper reached 462,955 tonnes in 2014. The major producers are Viet Nam, Indonesia, Brazil, India, China, Sri Lanka, and Malaysia (FAOSTAT, 2017). The commercial products mostly appear in the form of berries or flour with different colours. Black pepper is made from the unripe, green drupes. The drupes are heated in hot water to develop the black color due to enzymatic browning. The heated drupes are dried to develop the dark, wrinkled layer. The white pepper consists of the seed but with the skin removed. The green pepper is produced from the unripe, green drupes with special treatments such as sulphur dioxide or freeze-drying to retain the original color (Wikipedia, 2017). It should be stressed that the pink peppers (*Schinus* spp.) are the fruits of *Schinus molle* (Peruvian pepper) from the family Anacardiaceae (Wikipedia, 2017).

Black pepper has a range of therapeutic potential against various disorders (Butt et al., 2013; Srinivasan, 2007). The health claims include antioxidant, anti-inflammatory, antimicrobial, gastro-protective, anti-cancer, antidepressant activities and so on (Butt et al., 2013; Srinivasan, 2007). These health effects are due to the chemical constituents present in the pepper. The most well-known component is piperine. Others include vitamins (A & K), essential oils, polyphenols and so on. These components have been well studied (Butt et al., 2013; Srinivasan, 2007). In fact, starch is the major component of black pepper and may amount up to 50% of the dry weight (Bhat & Tharanathan, 1983). However, there has been only very few studies on the starch from black peppers. Starch may contribute to the cooking and nutritional quality of black peppers. For example, black pepper cooked in a soup dish may contribute to the texture of the foods. Starch may interact with other pepper components such as piperine and polyphenols as well as other food components, altering their nutritional properties (Zhu, 2015). Furthermore, the digestion of starch is part of the nutritional quality of black peppers and related food products. However, there is a lack of systematic knowledge on black pepper starch. The molecular structure of this starch is essentially unknown.

Starch is consisted of two major α-glucan molecules, namely amylose and amylopectin (Pérez & Bertoft, 2010). Amylose is smaller with few branches, whereas amylopectin is larger with many branches. The branches in amylopectin are arranged in a clustered fashion. The tightly branched unit in the amylopectin is termed building block which has an internal chain length of 1–3 glucosyl residues (Pérez & Bertoft, 2010). The internal part of amylopectin refers to the region between the branches and the sole reducing end. The external part of amylopectin is the region between the branches and the non-reducing ends (Pérez & Bertoft, 2010). Interestingly, recent studies showed that the internal

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molecular structure of amylopectin can be critical for the physicochemical properties of starch (Vamadevan & Bertoft, 2015). However, there has been no report on the internal structure of black pepper amylopectin so far. The amylose and amylopectin molecules are naturally assembled in the form of granules with the size ranging from 1 to 100 μm, depending on the botanical sources and plant genetics (Pérez & Bertoft, 2010). The external part of amylopectin molecules contributes to the formation of crystallites and the internal part forms the amorphous lamellae in the granules. A previous study showed that black pepper starch (1 variety) has A-type polymorph with a granule size of ~2.5 μm (Bhat & Tharanathan, 1983). The granule size and polymorph type of this starch are, therefore, similar to those of quinoa and amaranth starches (Li & Zhu, 2017b; Zhu, 2017). So far, there is a dearth of information on the structure of black pepper starch.

In this study, the granular and molecular structures of 2 commercial black pepper samples were studied by diverse analytical techniques. The pepper samples included 1 black and 1 white pepper of *P. nigrum*. A pink pepper of *S. molle* was also collected and analysed for total starch content. The study fills in the blank area of the structural knowledge of black pepper starch, while providing a basis to sustainably develop this spice for food applications. A large number of nomenclatures related to amylopectin molecular structure were employed in this report. Readers are encouraged to read some previous publications, especially those related to the internal molecular structure of amylopectin (Bertoft, 2004; Pérez & Bertoft, 2010), to have background information for the current report. The abbreviations of nomenclatures used were presented in Supplementary Table 1. Readers should also pay attention to the information of Supplementary material which helps to increase the readability of the report.

2. Materials and methods

2.1. Materials

Three varieties of peppercorns, namely black (brand: Pams), white (brand: Cock brand), and pink (brand: farro fresh) peppers, were purchased from New World, Tai Ping Trading, and Farro supermarkets in Auckland, New Zealand, respectively. Their country origins were Vietnam, Thailand, and Peru, respectively.

GELOSE 50, a maize starch with an apparent amylose content of 50%, was a gift from Ingredion ANZ Pty Ltd. (Auckland, New Zealand). A normal maize starch (Melogel from Ingredion), which is one of the mostly studied starches, was employed in this study for comparison. Waxy maize starch were purchased from Sigma–Aldrich Chemical, Co. (Auckland, New Zealand). The source of enzymes used in this study was described previously by Li and Zhu (2017a).

2.2. Methods

2.2.1. Total starch content

The peppers were ground into flour and sealed in plastic bags before total starch content measurement. The total starch content of pepper flours was quantified using a total starch assay kit (Megazyme, Wicklow, Ireland).

2.2.2. Starch isolation

Starch isolation followed a previous method of Annor, Marcone, Bertoft, and Seetharaman (2014a) with some modifications. Peppercorns (600 g) were soaked in a sodium metabisulfite solution (3.6 L, 0.5%) for 24 h before centrifugation. The soaked samples were suspended in iced water before grinding in a blender. The resulting slurry was passed through a cheesecloth under vacuum. This procedure was repeated 2 more times to remove the impurities. The collected slurry was passed through a series of mesh (140, 50, and 30 μm) before centrifugation to recover the starchy cake. The supernatant was discarded and the protein layer on the top was scraped off. Sodium dodecyl sulfate (SDS) solution (0.5%) was added to remove the protein. This procedure was repeated and starch was extensively washed with water before drying at 45 °C.

2.2.3. Amylopectin isolation

The amylopectin fractionation followed a previous method using butanol and isoamyl alcohol as described by Klucinec and Thompson (1998), which was slightly modified by Zhu, Corke, and Bertoft (2011).

2.2.4. Production of β-limit dextrins (LDs) of amylopectin

The production of β-LDs of amylopectin followed a previous method as described by Bertoft (2004). The amylopectin of pepper starch was extensively hydrolysed by β-amylase to produce the β-LDs.

2.2.5. Amylose content determination

2.2.5.1. Iodine-binding spectrometry-based method. The measurement of apparent amylose content (AAM) of pepper starch followed a previous method (Hoover & Ratnayake, 2005). AAM of GELOSE 50 and waxy maize starches were treated as 50 and 0%, respectively, for establishing the standard curve. It should be noted that the amylose contents measured by this method is apparent as the amylopectin and intermediate material of GELOSE 50 may also interact with the iodine.

2.2.5.2. Concanavalin A precipitation-based method. The measurement of true amylose content of starch was based on concanavalin A precipitation method, using an amylose/amylopectin assay kit (Megazyme, Wicklow, Ireland).

2.2.6. Gel-permeation chromatography (GPC)

Starch and amylopectin were debranched by using isoamylase and pullulanase as described previously by Zhu et al. (2011). The debranched starch and amylopectin were analysed on GPC of Sepharose CL 6B as described previously by Li and Zhu (2017a).

2.2.7. High-performance anion-exchange chromatography (HPAEC)

The unit chain profiles of amylopectin and β-LDs were analysed by a HPAEC system with pulsed amperometric detection (PAD) as described by Li and Zhu (2017a).

2.2.8. Granule size distribution

The particle size distribution of starch granules was measured by a Master 2000 particle size analyser (Malvern Instruments, Worcestershire, UK). The procedures followed a previous report as described by Li and Zhu (2017b). D[4,3] (volume-weighted mean diameter), D[3,2] (surface-weighted mean diameter), D[n, 0.5] (number median diameter), and d(0.9) (90% of the particles are smaller than this diameter) were recorded (Allen, 2003).

2.2.9. Scanning electron microscopy (SEM)

The morphology of starch granules were recorded by a Hitachi S-3200 N scanning electron microscope (Tokyo, Japan) at an accelerating voltage of 15 kV.

2.2.10. Wide-angle X-ray diffractometry

The nature and relative degree of crystallinity of black pepper starch were analysed by using a PANalytical Empyrean X-ray Diffractometer (Almelo, The Netherlands). The calculation of the degree of crystallinity followed the method 1 of a previous report