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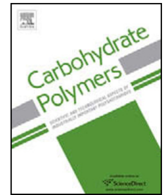

The cold storage of green bananas affects the starch degradation during ripening at higher temperature

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ABSTRACT

The aim of this work was to investigate the starch degradation of bananas stored at low temperature (13 °C, cold-stored group) and bananas stored at 19 °C (control group) during ripening. The starch granules were isolated during different stages of banana ripening, and their structure was investigated using different techniques. The activities of α-amylase and β-amylase associated to the starch granules were determined, and their presence was confirmed using immunolocalization assays. The increased molecular mobility likely facilitated the intake and action of α-amylase on the granule surface, where it was the prevalent enzyme in bananas stored at low temperature. The 10 days of storage at low temperature also influenced the sizes and shapes of the granules, with a predominance of rounded granules and pits on the surface along with superior amylose content, the higher amounts of amylopectin A-chains and the subtle increase in the A-type allomorph content.

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1. Introduction

Banana is a typical climacteric fruit, and important physico-chemical changes take place during ripening. Thus, because this fruit has a short green-life, *i.e.*, the elapsed time between harvest and the beginning of ethylene production, the manipulation of environmental conditions, mainly the atmosphere and the temperature, is used to extend the storage time.

Storage at low temperatures is a step in the cold chain, from the harvest to market, to extend the green-life of fruit. In general terms, this condition can substantially reduce the rate of many metabolic pathways that lead to fruit senescence, deterioration and decay of quality (Chauhan, Rajei, Dargupta, & Bawa, 2006). Low temperatures temporarily impair ripening by maintaining the lowest possible ethylene concentrations (Seymour, Taylor, & Tucker, 1993; Wills, McGlasson, Graham, & Joyce, 1998), but most tropical fruits undergo physiological disorders and deterioration of quality when exposed to low temperatures. The surface pitting at temperatures lower than 10 °C is a typical symptom of chilling injury (Imahori, Takemura, & Bai, 2008; Martínez-Romero, Serrano, & Valero, 2003; Zamorano et al., 1994), and in the case of bananas, although the pulp will not be affected for several days, the skin may turn dark, which negatively affects the quality.

In bananas, the symptoms of chilling injury appear to be cultivar dependent and related to the genomic group. In Brazil, the Nanicão cv., a member of the AAA group, is commercially relevant but less resistant to low temperatures than Prata, a cultivar of the AAB group (Agopian et al., 2011). According to Lichtemberg, Malburg, and Hinz (2001), the B genome appears to confer cold resistance.

Green Nanicão bananas may accumulate high levels of starch (25%), which is mostly degraded during ripening, resulting in high amounts of soluble sugars in the fully ripe fruit (22%) (Peroni-Okita et al., 2010). However, the low cold resistance could be a disadvantage for the long-term storage of Nanicão bananas because the fruits exposed to low temperatures for several days may accumulate a lower amount of sugars during ripening, although a marginal

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increase in the sucrose levels was observed during storage, which is reportedly a cyroprotective effect (Agopian et al., 2011).

This lower amount of soluble sugars in cold-stored Nanicão bananas was followed by significant changes in the activities and expression of the enzymes linked to carbon partitioning and starch levels, such as β-amylase, starch-phosphorylases, sucrose-phosphate-synthase and sucrose-synthase (Agopian et al., 2011). Although these changes could be partially responsible for the marginal cryoprotective effect, suggesting they may contribute to cold acclimation, the net result was a decrease in fruit quality, as the end amount of soluble sugars in the ripe fruit was lower. Therefore, a better understanding on the effects of cold on the starch-to-sucrose metabolism of commercially relevant, cold sensitive bananas is important in terms of food quality. In this regard, the structural features of the starch granules, which are the substrates for soluble sugar synthesis, may have been overlooked in the previous study, as the differences in the pattern of degradation of the granules and other parameters, such as the levels of amylose and amylopectin or even the crystallinity, may provide clues about the changes occurring in banana pulp tissue during cold acclimation.

Therefore, the aims of this study were to investigate the degradation of starch in Nanicão bananas stored at low temperature at the starch granule level. The structural characteristics of the starch granules from this cultivar were investigated to determine the crystallinity and the ratio of A- to B-type starch allomorphs, the structural water content of starch granule, the chain length distribution of amylopectin, and the amylose content. Additionally, microscopy was used to study the internal and external structural features of the granules in relation to the phosphate and amylose distribution and the occurrence of the granule-attached forms of the main enzymes involved in starch degradation.

2. Materials and methods

2.1. Material

Mature green bananas, *Musa acuminata*, AAA, cv. 'Nanicão', were obtained at CEAGESP (Companhia de Entrepostos e Armazéns Gerais do Estado de São Paulo, Brazil) immediately post-harvest. The fruits were washed with sodium hypochlorite solution (0.1%, w/v) and were separated into two groups that were stored in different chambers as follows: fruits were stored at 19 °C (control group) until fully ripe and fruits were stored at 13 °C (cold-stored group) for 15 days. Then, the cold-stored group was stored at 19 °C until fully ripe. The degree of ripening was monitored through both the CO_2 and ethylene levels and the results obtained were presented in the previous work (Agopian et al. (2011). The samples were collected, peeled, sliced, frozen in liquid N_2 and stored at −80 °C for future analyses.

2.2. Isolation of starch granules

Based on the starch degradation profile, starch granules were isolated from the pulp tissue according to Soares et al. (2011) during different stages of ripening: starches were isolated from the control fruits with 1, 10, 15, 17, 21 and 22 days after harvest, and starches were isolated from the cold-stored fruits (at 13 °C) with 10, 17, 21 and 22 days after harvest.

2.3. Laser differential interference contrast microscopy

Starch grains suspended in water were placed on a slide and covered by a cover slip. The starches were visualized using a Confocal laser scanning microscope (CLSM, Zeiss, Jena, Turingia, Germany, LSM 510) and the images were analyzed in a Laser Scanning Microscopes (LSM) Image Browser Program.

2.4. Optical microscopy

A mixture of dried starch and 50% glycerol was fixed on a glass slide, and the analyses were conducted using a Polarizing Optics microscope (Zeiss-Axioplan 2 microscope, Carl Zeiss, Göttingen, Germany) equipped with a 3CCD camera (Color Vision Camera Module, Donpisha).

2.5. Scanning electron microscopy (SEM)

The samples were fixed in stubs by double face tape and coated with a 10-nm-thick platinum layer in a Bal-tec MED-020 Coating System (Kettleshulme, UK). The samples were analyzed in an FEI Quanta 600 FEG Scanning Electron Microscope (FEI Company, Oregon, USA). SEM observations were performed in the secondary electron mode operating at 2 kV, 5 kV and 10 kV.

2.6. Amylose content

To measure the amylose content of starch, the Megazyme enzymatic method (Kit K-AMYL 04/06, Megazyme International Ireland Ltd, Wicklow, Ireland) was used, according to Peroni-Okita et al. (2010).

2.7. APTS Staining

The APTS staining was performed as described by Blennow et al. (2003), and Glaring, Koch, and Blennow (2006), with the following modifications. The starch granules (5 mg) were incubated in 10 μL of freshly made APTS solution (20 mM 8-amino-1,3,6-pyrenetrisulfonic acid, Molecular Probes, Carlsbad, USA, dissolved in 15% acetic acid) and 10 μL of 1 M sodium cyanoborohydride. The mixture was incubated at 30 °C for 18 h. The granules were washed five times with 1 mL of distilled water and suspended in 20 μL of 50% glycerol. For microscopy, 2 μL of the granule mixture was fixed on a glass slide. A confocal laser scanning microscope (CLSM, Zeiss, Jena, Turingia, Germany, LSM 510) was used for detection of the fluorescence signal from the stained starch grains. For APTS, a 488 nm laser line was used for excitation, and light was detected between 500 and 535 nm. For each starch granule, a stack of horizontal optical sections was obtained according to Blennow et al. (2003). The images were analyzed in a laser scanning microscopes (LSM) image browser program.

2.8. Amylopectin branch chain-length distribution

The starch was debranched using isoamylase of *Pseudomonas* sp. (Megazyme International Ireland Ltd., Ireland), according to the procedure described by Peroni-Okita et al. (2010). The branch chain-length distribution of amylopectin was analyzed and calculated by using a high-performance anion exchange chromatograph equipped with a pulsed amperometric detector (HPAEC-PAD, Dionex, Sunnyvale, CA, USA), as described by Peroni-Okita et al. (2010).

2.9. Wide-angle X-ray diffraction (WAXD)

The WAXD diagrams were recorded using a rotating-anode X-ray diffractometer (Rigaku corporation, Danvers, MA, USA) with Ni-filtered CuKα radiation (λ = 1.542 Å) operating at 50 kV and 100 mA at the Brazilian Synchrotron Light Laboratory (Campinas, Brazil), according to Cardoso and Westfahl (2010). The diffraction patterns were recorded during 10 min exposures on a mar345

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