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Synthesis of Fructooligosaccharides in Banana 'Prata' and Its Relation to Invertase Activity and Sucrose Accumulation

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Levels of sucrose and total fructooligosaccharides (FOS) were quantified in different phases of banana 'Prata' ripening during storage at ambient (~19 °C) and low (~10 °C) temperature. Total FOS levels were detected in the first days after harvest, whereas 1-kestose remained undetectable until the sucrose levels reached approximately 200 mg/g (dry weight) in both groups. Sucrose levels increased slowly but constantly at low temperature, but they elevated rapidly when the temperature was raised to 19 °C. Total FOS and sucrose levels were higher in bananas stored at low temperature than in the control group. In both samples, total FOS levels were higher than those of 1-kestose. The carbohydrate profiles obtained by HPLC and TLC suggest the presence of neokestose, 6-kestose, and bifurcose. The enzymes putatively involved in banana fructosyltransferase activity were also evaluated. Results obtained indicate that the banana enzyme responsible for the synthesis of FOS by transfructosylation is an invertase rather than a sucrose-sucrosyl transferase-like enzyme.

KEYWORDS: Banana 'Prata'; fructoligosaccharides; low-temperature storage; invertases; transfructosylation activity; sucrose

INTRODUCTION

Fresh fruits and certain vegetables are major sources of unprocessed sugars in the human diet (*1*). Banana is currently indicated as a good source of carbohydrate and an important source of fiber, vitamins, and minerals. To extend the shelf life, particularly for maritime transport, bananas have been submitted to low-temperature storage (*2*). However, low-temperature conditions (nonfreezing) may cause chilling or unknown changes in the metabolism of bananas during ripening. It is already known that plant cells subjected to chilling temperatures accumulate soluble sugars—mainly sucrose, followed by glucose and fructose—in response to low temperatures (*3*, *4*). Also, low temperatures can trigger fructan synthesis in some monocotyledons, depending on the amount of accumulated sucrose (*5*).

A strong correlation was proposed between a specific sucrose level [~200 mg/g of dry matter (DM)] and the accumulation of 1-kestose, the first member of the fructooligosaccharide (FOS) series, in bananas (*6*). The synthesis and accumulation of 1-kestose and nystose levels in bananas were dependent on both the cultivar in question and the ripening stage of the fruit. In eight cultivars previously analyzed, 1-kestose accumulated during ripening, but nystose (the second member of the FOS series, resulting from further polymerization of fructose) was detected solely in cultivar 'Prata'. Total FOS levels, however, were not measured (*6*).

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The precise role of fructans in the physiological adjustment to low temperatures remains unclear. Fructans may play a role in osmoregulation because they are osmotically inert and can be rapidly converted into osmotically active monosaccharides (*7*). Therefore, the accumulation of fructans may be an important part of physiological acclimation and survival at low temperatures (*8*).

Whereas other cultivars suffer severe chilling injuries at temperatures below 14 °C (*9*), the cultivar 'Prata' tolerates storage at temperatures lower than 12 °C. The factors that confer temperature tolerance to this cultivar are unknown.

The ability to accumulate fructans is related to increasing fructosyltransferase activities, mainly by sucrose-sucrosyltransferase (SST), during low-temperature exposure, as observed in wheat (*10*). However, the synthesis of fructans could also result from the transfructosylation activity of invertases (*11*). There is no information about which of these enzymes are involved in fructan synthesis in bananas.

In this work, the sucrose and total FOS levels were quantified in different phases of banana 'Prata' ripening, in fruits stored at both ambient and low temperatures. Enzymes potentially involved in their synthesis were also analyzed.

MATERIALS AND METHODS

Material. Mature green bananas (*Musa acuminata* × *Musa balbisiana*, AAB, cv. 'Prata') were obtained at a local market within 1 day of harvest. The fruits were separated into two groups and stored in separate chambers at 19 °C (the control group) and 10 °C (the cold-stored group). After 15 days, the cold-acclimated fruits were elevated to 19 °C to complete their

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ripening. Samples were taken on a daily basis for ethylene and respiration measurements, as described previously (*6*) (data not shown). Representative samples of control and cold-stored fruits were peeled, sliced, frozen in liquid N_2, and stored at −80 °C for future analysis.

Carbohydrate Determination. Soluble sugars were extracted three times with 80% ethanol, and the supernatants were combined and adjusted to 25 mL. Aliquots of 1 mL were taken from each sample and evaporated under vacuum (Savant model 110A). The residues were reconstituted with water, filtered through 0.22 μm membrane filters, and analyzed by high-pressure liquid chromatography with pulse amperometric detection (HPAEC-PAD; Dionex, Sunnyvale, CA), using a Carbopac PA1 column (Dionex) and an isocratic run of 18 mM NaOH over 25 min.

The total FOS were extracted three times from 0.5 g of banana samples with deionized water (10 mL) at 80 °C for 15 min. The supernatants were combined and adjusted to 50 mL. Analyses of FOS by HPAEC-PAD and enzymatic methods were conducted after filtration through 0.22 μm membrane filters. For thin layer chromatography (TLC) analyses, a 1 mL aliquot of FOS extract was evaporated under vacuum and reconstituted with water to a final volume of 250 μL.

Analysis of FOS by HPAEC-PAD was performed on a HPAEC-PAD, using a Carbopac PA1 column. The flow rate was 1 mL/min with a 50 min gradient composed of three solvents: 1 M NaOH (solution A), H_2O (solution B), and 1 M sodium acetate (solution C). The elution program began at 10% A and 90% B (0−12 min), followed by 30% A and 70% B (12−15 min); 30% A, 50% B and 20% C (15−25 min); 30% A, 20% B and 50% C (25−40 min); and then re-equilibration at 10% A and 90% B (40−50 min).

The enzymatic analysis of total FOS was performed using the Fructan Assay Kit (Megazyme Ltd., Wicklow, Ireland).

Analysis of FOS by TLC was done with the same FOS extracts employed for the enzymatic analyses. Aliquots (10 μL) of FOS extract and 3 μL of an extract from *Helianthus tuberosus* (40 μg/μL; made with the same method and used as standard) were spotted on TLC foil (silica gel on aluminum, 20 × 20; Merck), which was developed three times in *n*-butanol/isopropanol/water (3:12:4) (v/v). The bands were stained with a Fru-specific urea−phosphoric acid spray (*12*).

Protein Extraction and Invertase Activity. Banana samples (20 g) were homogenized using a 1:2.5 tissue-to-buffer extraction ratio with mechanical shaking. The extraction buffer (buffer A) contained 50 mM MES buffer (pH 5.5), 1 mM DTT, 1 mM EDTA, 1 mM benzamidine, 50 mM cysteine, and 1% PVP (40000 MW). The homogenate was centrifuged (10000*g*, 15 min), and the supernatant was collected. Ammonium sulfate solution was added to a level of 40% saturation, and the resulting mixture was centrifuged (10000*g*, 15 min). The pellet was discarded, and additional ammonium sulfate was added to give a saturation of 75%. The resulting precipitate (40−75% fraction) was dissolved and dialyzed against buffer A without PVP (buffer B). After 24 h, the dialysate was centrifuged again (10000*g*, 15 min) to clarify the protein extract. All of these operations were conducted at approximately 4 °C.

For further purification, the protein extract was passed through a DEAE-cellulose column, equilibrated with buffer B. The enzymes were eluted with 0.5 NaCl in buffer B. The protein fraction was concentrated by precipitation with 75% saturated ammonium sulfate solution. The precipitate formed after 3 h of gentle stirring and was collected by centrifugation at 10000*g* for 5 min. The resulting residue was reconstituted with 2 mL of buffer B (pH 5.5) and dialyzed overnight. The resulting partially purified protein extract was designated "treated extract." Protein concentration was estimated according to the method of Bradford (*13*) using BSA as the standard.

Fructosyltransferase activity was measured in response to substrate concentration, reaction time, and the presence of an inhibitor. Substrate concentration effects were evaluated as follows: 200 μL of crude and partially purified protein extracts was added to 200 μL of buffer (50 mM MES, pH 5.5). Sucrose was added to the mixtures up to final concentrations of 20 mM, 100 mM, 600 mM, 1.2 M, and 2 M. The reaction mixtures were incubated at 28 °C for 6 h and then heated to 90 °C for 5 min in a heating block to stop the reaction. The amount of fructosyltransferase activity present in partially purified invertase samples was determined by HPAEC-PAD, as described above, to measure the amount of trisaccharides formed. The resulting reaction mixtures were diluted to a final concentration of 50 mM sucrose (except for the 20 mM sucrose sample) and filtered through a 0.45 μm membrane. To construct a time course for the production of trisaccharides, the fructosyltransferase assays were performed using 600 mM sucrose. To test for an inhibitor effect, the protein extract was added to MES buffer (pH 5.5) and sucrose (600 mM) along with pyridoxal HCl (10 mM). The trisaccharide products were also measured, using turanose as an internal standard.

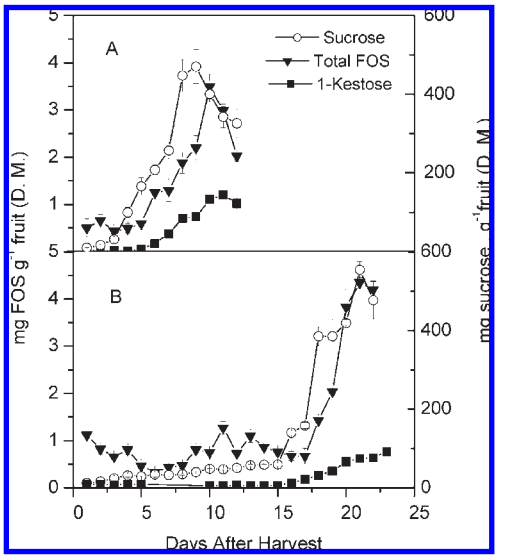

Figure 1. Sucrose, 1-kestose, and total FOS profiles of banana 'Prata' (**A**) control and (**B**) cold-stored during ripening. Data presented are means of triplicate assays ± standard errors.

To confirm that the trisaccharides formed are FOS, the mixture was treated with fructanase (Megazyme) (454 U of exoinulinase/mL and 4.54 U of endoinulinase/mL) for 2 h at 40 °C in acetate buffer (pH 5.5). The products were analyzed by HPAEC-PAD, according to the protocol described above.

The hydrolysis activity of invertase was evaluated with 20 mM sucrose. The enzyme reaction was the same as that established for transferase activity. The sugars were analyzed by HPAEC-PAD using a Carbopac PA1 column, 18 mM NaOH as the mobile phase, and a constant flow rate of 1 mL/min.

RESULTS AND DISCUSSION

Total FOS and Sucrose Levels. In the low-temperature case, all analyzed events were delayed by 15 days (**Figure 1**). Sucrose levels increased slightly and constantly while bananas were stored at low temperature, followed by a burst after 15 days when the temperature was raised to the same temperature as the control samples (19 °C). FOS were detected in the first days after harvest, whereas 1-kestose remained undetectable until the sucrose levels reached approximately 200 mg/g of DM, in both groups. After that, both 1-kestose and total FOS accumulated continually until the end of the experiment, when the signals of senescence in bananas were evident (**Figure 1**).

Total FOS levels were higher in bananas stored at low temperature than in the control group, and the levels of sucrose were also higher (**Figure 1**). The FOS/sucrose ratios were similar in both groups (control, 3692 μg/FOS/470 mg/g of sucrose = 7.8; cold stored, 4345 μg/g FOS/554 mg/g sucrose = 7.8), indicating a correlation between the syntheses of these carbohydrates; this is to be expected, once sucrose becomes the substrate for FOS synthesis. In both samples, total FOS levels were higher than those of 1-kestose, suggesting the presence of higher oligofructans, because 1-kestose is just the first member of the FOS series accumulated in banana (*14*, *15*). FOS levels in banana are controversial among researchers: Campbell et al. found the highest levels (~10 mg), in contrast with lower levels found by

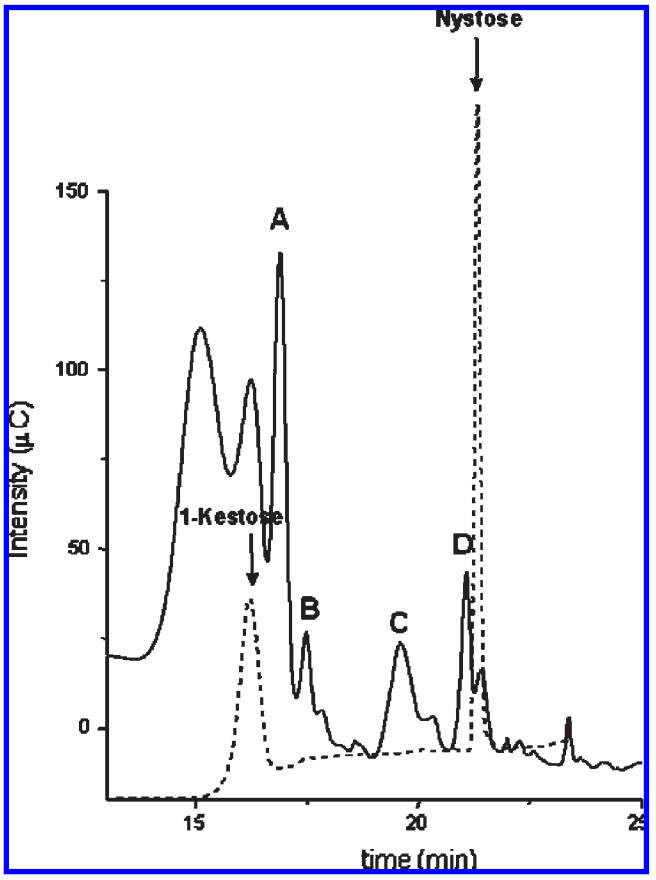

Figure 2. HPAEC-PAD chromatogram of soluble sugars in pulp extracts for banana 'Prata' 10 days after harvest. The chromatogram of 1-kestose and nystose standards (dashed line) are superimposed to the sample one to indicate the presence of the peaks at the same retention time. A−D correspond to peaks of possible fructans type between 1-kestose and nystose.

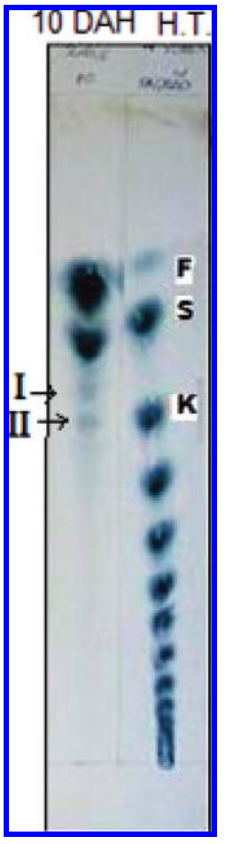

Figure 3. Thin layer chromatography in silica gel or sugar extract of banana 'Prata' control 10 days after harvest (DAH). The standard corresponds to *Helianthus tuberosus* extract. The system propanol/ethyl acetate/water was developed three times and stained with a fructose-specific staining (urea−phosphoric acid according to ref *12*). F, fructose; S, sucrose; K, 1-kestose.

other researchers (0.2−2.0 mg of DM) (*6*, *15*). However, differences in cultivars, degree of ripening, and the presence of fructans outside the inulin neo-series (e.g., neokestose, 6-kestose, and bifurcose) could explain the discrepancies reported.

Generally, the identification of FOS other than 1-kestose in banana fruit is difficult because of the lack of fructan-type standards. This is one reason we analyzed the banana carbohydrates by HPLC and TLC, as well as by an enzymatic method. For these analyses, we chose the sample in which we observed the maximum level of total FOS: banana 'Prata' control 10 DAH (**Figure 1**).

Analysis by HPAEC-PAD (**Figure 2**) showed two peaks—coincident with 1-kestose and nystose standards that already had their identity confirmed by GC-MS in banana (*6*)—along with four additional peaks (**Figure 2**, A−D) between 1-kestose and nystose. These had retention indices similar to 6-kestose, neokestose, and bifurcose, already identified by Vijn et al. (*16*) and Kawakami et al. (*17*) in onion and wheat, respectively. When the same extract was submitted to enzymatic hydrolysis with fructanase (exo- and endoinulinase; Megazyme) before chromatographic analysis, all peaks disappeared (data not shown). This confirms that all peaks corresponded to compounds containing a FOS-type linkage.

Analysis by TLC, in which a sugar extract from *H. tuberosus* was employed as a standard, revealed two fructose-containing carbohydrates that generated spots (I and II) over sucrose (**Figure 3**). Spot II was equivalent in terms of RF to the *H. tuberosus* (H.T.) standard and was subsequently identified as 1-kestose. Spot I, between sucrose and 1-kestose, showed mobility identical to that of 6-kestose as described in a similar analysis performed by Sims et al. (*18*). This suggests the presence of this sugar in the FOS extracted from banana pulp.

FOS Biosynthesis in Banana Fruit. The different types of fructans found in bananas would seemingly require the same enzymatic pathway present in species that utilize fructans as primary stored carbohydrates. The presence of several fructans with distinct glycosidic linkages depends on the consecutive action of various fructosyltransferases enzymes (*19*). Inulin that is composed of linear β-(2,1)-fructosyl linkages is generated by the action of two enzymes: sucrose:sucrose 1-fructosyltransferase (1-SST, EC 2.4.1.99), which catalyzes 1-kestotriose formation; and fructan:fructan 1-fructosyltransferase (1-FFT, EC 2.4.1.100), which catalyzes transfructosylation between fructans (*20*). Members of the inulin neoseries are produced by 1-SST and fructan:fructan 6-G-fructosyltransferases (6G-FT or 6G-FFT) that transfer a fructosyl unit from a fructan to the six-position of the glucosyl unit of sucrose, resulting in the formation of 6G-kestotriose (*21*, *22*). Bifurcose and extensions of sucrose through β-(2,6)-linked fructosyl units, with and without branches of β-(2,1)-linked fructosyl units, appear to be synthesized by 1-SST and sucrose:fructan 6-fructosyltransferase (6-SFT) (*19*, *23*).

Because several forms of FOS were found in banana 'Prata' pulp, we hypothesized that some of these frutosyltransferases are present in banana fruit—namely, 1-SST and 1-FFT. After several biochemical and molecular biology experiments failed to find these classical fructosyltransferases in banana (data not shown),

we sought to determine the impact of invertase transglycosyl activity upon fructan synthesis.

Despite the lack of evidence pointing to the presence of known fructan-producing enzymes, earlier studies had already suggested that transferase activity by banana invertases could be involved in the synthesis of fructans, but this assertion was not experimentally confirmed (*24*).

Fructosyltransferase Activity in Banana Is Performed by an Invertase. Crude and partially purified banana 'Prata' protein extracts from 10 DAH samples were assayed for fructosyltransferase activity at different sucrose concentrations. **Figure 4** shows that transfructosylation activity was detected (measured through the direction of FOS synthesis) at high sucrose concentrations. This observation is more typical of an invertase than a fructosyltransferase: those fructosyltransferases that are classically associated with FOS synthesis exhibit transfructosylation activity even at small sucrose concentrations.

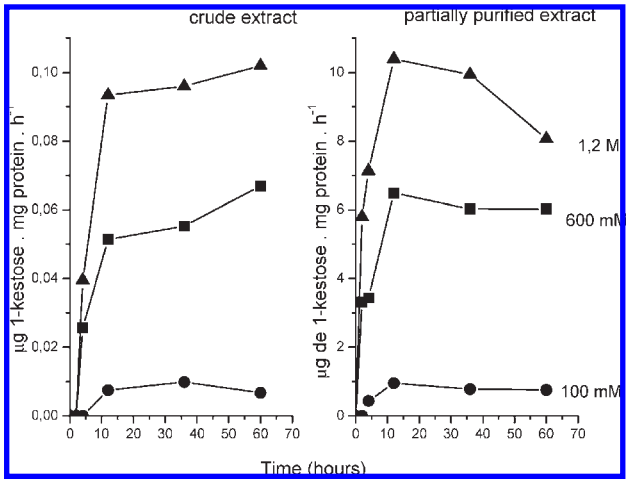

Figure 4. Transglycosylation activity of invertase in crude and partially purified extracts from banana 'Prata' (10 DAH) incubated at different sucrose concentrations (100 mM, 600 mM, and 1.2 M). 1-Kestose was quantified as sugar product by HPAEC-PAD after 0, 2, 4, 12, 36, and 60 h.

A general property of fructosyltransferases is the specific transfructosylation between fructans, whereas invertases are able to conduct both synthesis and hydrolysis, depending on substrate concentration (*25*, *26*). The HPAEC-PAD analysis of carbohydrates produced by banana protein extract (10 DAH) at different sucrose concentrations showed FOS synthesis only from 100 mM sucrose (**Figure 5**). (A discrete peak does appear at 17 min with a 20 mM sucrose concentration, but it does not appear to correspond to a FOS-type product.) The enzyme activity was largely dependent on sucrose concentration (**Figure 4**) and time of incubation (**Figure 5**).

Due to the lack of pure FOS standards, it is difficult to calculate the quantity of FOS-like substances indicated in the chromatograms. Even the use an internal standard, such as turanose (used in these analyses), is limited by the fact that the pulsed amperometry detector responds differently to different sugars. However, it is possible to use the area of peak corresponding to turanose to normalize the other areas with the aim to estimate the increase in FOS as a product of the protein extracts incubated with different sucrose concentrations. This estimation indicates that the four main peaks (indicated as I, II, III, and IV in **Figure 5A**) increased, respectively, 85-, 8-, 6-, and 28-fold when compared to the areas of the chromatograms of the samples incubated with 100 and 600 mM sucrose. Samples incubated with 1.2 M sucrose presented increments of 125-, 12-, 10-, and 49-fold for each peak. Almost the same values were found for the samples incubated with 2.0 M sucrose. This clearly indicates that synthetic activity is selective for some FOS and, possibly, there is a sucrose saturating limit.

This agrees with previous reports that invertase transfructosylation activity is generally observed at or above a sucrose concentration of 100 mM and that this activity increases with higher sucrose concentrations. By contrast, relatively low sucrose levels increase hydrolytic activity, which remains constant from 100 mM sucrose onward (*11*). Moreover, fructosyltransferases do not share the kinetic characteristics of invertases, as they synthesize FOS at low sucrose concentrations (*26*−*28*). The ability of an invertase to synthesize fructans appears to be associated with a sucrose binding box motif present in the enzyme that is often highly conserved in fructosyltransferases (*29*). A recent study on

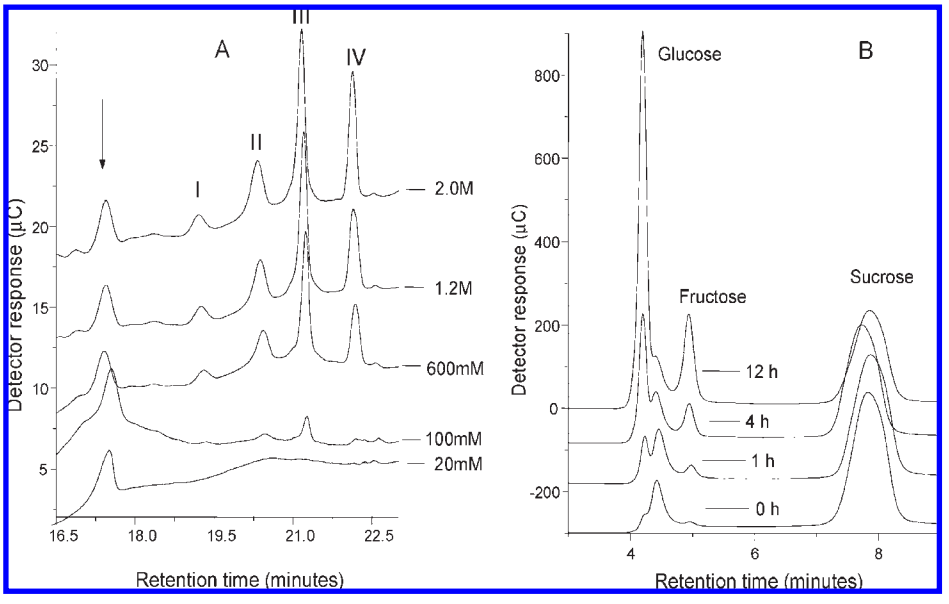

Figure 5. (**A**) Analysis of sugar products generated by protein extract of banana 'Prata' control (10 DAH) incubation during 6 h with different sugar concentrations. The arrow indicates a peak of different FOS-type formation. (**B**) Hydrolytic activities of invertase present in protein extract of banana 'Prata' control (10 DAH) after incubation with 20 mM sucrose during different times. I−IV indicate the main FOS-like substances synthesized.

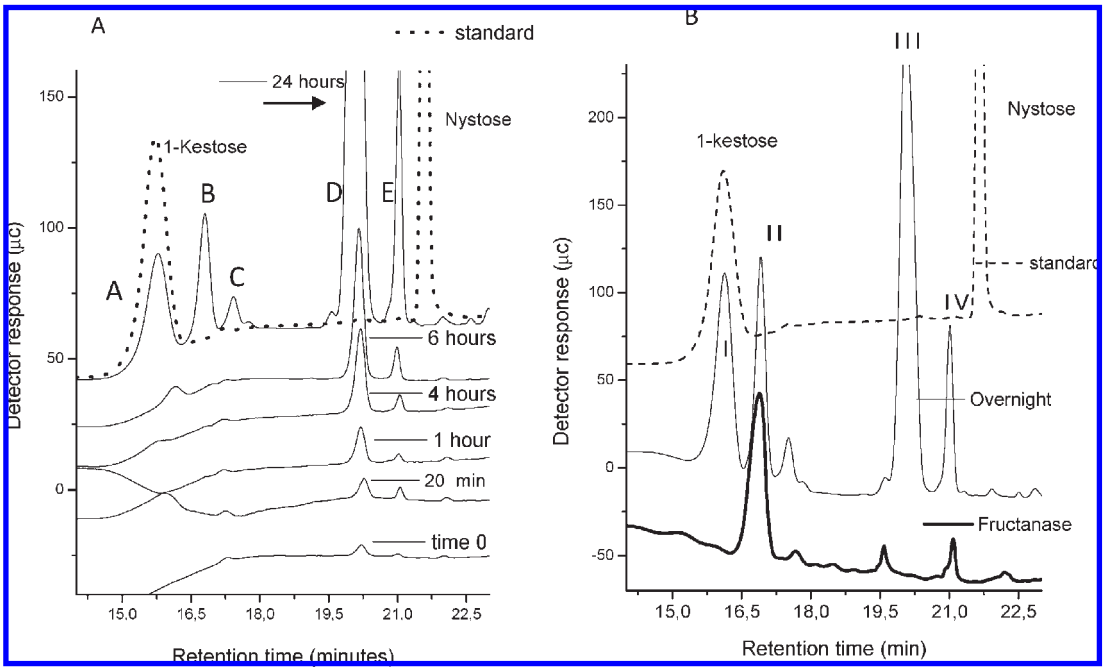

Figure 6. (**A**) Analysis of sugar products after incubation for different times with the protein extract from banana 'Prata' (control sample, 10 DAH) at 600 mM sucrose. The arrow indicates the major peak after 24 h of incubation time. A−E indicate the main FOS-like substances synthesized. (**B**) Analysis after hydrolysis of the sugar products with fructanase (24 h incubation time). I, III, and IV correspond to FOS products; II corresponds to unidentified sugar.

onion vacuolar invertase showed that a relatively small change in amino acid positions within the sucrose binding box significantly increased the transglycosylation capacity of the enzyme (*11*).

To investigate the influence of reaction time on FOS synthesis, the partially purified extracts were incubated in the presence of 600 mM sucrose substrate, and the reactions were stopped at different time intervals (**Figure 6A**). Chromatographic peaks were observed at the same retention times as those of sugar products (**Figure 6A**) shown in **Figure 5**, as well as the peaks A−D that appeared in **Figure 2**. To confirm the identity of those peaks as FOS, the extracts were incubated with fructanase (exo- and endoinulinase, specific to FOS hydrolysis) (**Figure 6B**), and only one peak failed to disappear (peak II, at 17 min), suggesting a different sugar product as discussed earlier (**Figure 5**). Using the same approach (based on peak areas) between 1 and 24 h of incubation, FOS increased about 126-, 125-, 10-, 30-, and 32-fold for peaks A, B, C, D, and E, respectively. As the sucrose concentration was the same for all samples, the results indicate that FOS are differentially synthesized with time.

It should be noted that the FOS peaks detected after overnight incubation were much higher than those obtained after a FOS reaction time of up to 6 h (**Figure 6A**). However, the transfructosylation action of invertase appears to reach maximum activity only after 12 h of incubation (**Figure 4**). Furthermore, a significant peak with a 20 min retention time emerges after a prolonged period of invertase transfructosylation. This could explain the higher FOS levels in banana proposed by previous papers (*30*). It is possible that this peak corresponds to peak I noted on TLC analysis (**Figure 3**), due to the low sensitivity of TLC as compared with the HPAEC-PAD method. On the basis of TLC, this peak appears to correspond to 6-kestose. Ritsema et al. (*11*) observed that the incubation of invertase with 100 mM sucrose over a short period of time (20 min) resulted in 1.2% of its total activity being directed toward transfructosylation. Overnight incubation completely hydrolyzed the sucrose and degraded the 1-kestose initially formed. Furthermore, in the same experiment, overnight incubation of invertase with 1 M sucrose resulted in 6.4% transglycosylation

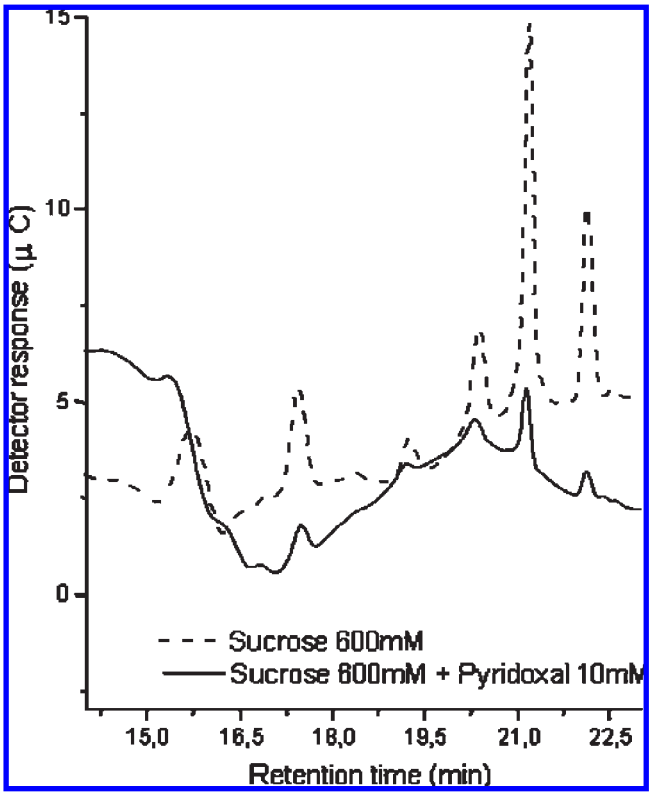

Figure 7. Analysis of the pyridoxal (10 mM) inhibition effect on transglycosylation activity of the protein extract from banana 'Prata' (control samples, 10 DAH).

activity. Our results with banana protein extracts are in accordance with the observation that both time and sucrose concentration are important parameters for invertase transfructosylation activity.

The central issue in developing the ability to synthesize fructans is the evolution of transfructosylating enzymes from the hydro-

lyzing invertases (*25*, *31*, *32*). However, fructosyltransferase and invertase enzymes conserve specific features that can be used to distinguish between their respective activities. For example, the hydrolytic and transferase activities of purified yeast invertases do not respond equally to changes in pH and temperature, giving a false impression that there are two enzymes acting (*33*). Also, it has been proved that both invertase activities may be inhibited in parallel by pyridoxal (10 mM) (*34*, *35*). In fact, inhibition by pyridoxal is a useful way to differentiate between invertase and SST activities (*34*). To gain further evidence of fructosyltransferase activity by invertase in banana samples, we have measured the effect of pyridoxal on protein extracts from banana 'Prata' (control 10 DAH) (**Figure 7**). The results show a significant decrease in FOS synthesis, indicating that fructosyltransferase activity in banana can be attributed to invertases, rather than other "classical" types of fructosyltransferases.

In conclusion, our results suggest that invertases have the capacity to synthesize FOS in banana 'Prata'. Moreover, the analysis of FOS levels and sugar products indicates the presence of structurally distinct trisaccharides; this observation could explain the higher FOS levels found in banana in previous works. The higher levels of FOS in banana samples stored at low temperature could indicate that these carbohydrates play a role in cold acclimation and that this might also hold true for other tissues that have been observed to accumulate FOS. The higher sucrose levels found in bananas stored at low temperature could be the result of changes to the activity of biosynthetic and degradative enzymes, for example, sucrose-synthase, sucrose-phosphate-synthase, and invertases. The next steps of this work should include the purification and structural analysis of the FOS-like sugars detected in the banana pulp, as well as the cloning and heterologous expression of banana invertases, to test their ability to synthesize FOS in vitro.

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LITERATURE CITED

(1) Torija, E.; Díez, C.; Matallana, C.; Camara, M.; Camacho, E.; Mazarío, P. Influence of freezing process and free sugars content of papaya and banana fruits. *J. Sci. Food Agric.* **1998**, *76* (3), 315–319.

(2) Chauhan, O. P.; Bajei, P. S.; Dargupta, D. K.; Bawa, A. S. Instrumental texture changes in banana during ripening under active and passive modified atmosphere. *Int. J. Food Prop.* **2006**, *9* (4), 237–253.

(3) Guy, C. L.; Huber, J. L. A.; Huber, S. C. Sucrose phosphate synthase and sucrose accumulation at low temperature. *Plant Physiol.* **1992**, *100*, 502–508.

(4) Gounaris, Y. A qualitative model for the mechanism of sugar accumulation in cold-stressed plant tissues. *Theor. Biosci.* **2001**, *120* (2), 149–165.

(5) Pollock, C. J.; Lloyd, E. J. The effect of low temperature upon starch, sucrose and fructan synthesis in leaves. *Ann. Bot.* **1987**, *60* (2), 231–235.

(6) Agopian, R. G. D.; Soares, C. A.; Purgatto, E.; Cordenunsi, B. R.; Lajolo, F. M. Identification of fructooligosaccharides in different banana cultivars. *J. Agric. Food Chem.* **2008**, *56*, 3305–3310.

(7) Wiemken, A.; Sprenger, N.; Boller, T. Fructan − an extension of sucrose by sucrose. In Pontes H. G., Salerno G. L., Echeverria E. J., Eds.; *Am. Soc. Plant Physiol.* **1995**, 179−189.

(8) Pollock, C. J. Fructans and the metabolism of sucrose in vascular plants. *New Phytol.* **1986**, *104* (1), 1–24.

(9) Lichtemberg, L. A.; Malburg, J. L.; Hinz, R. H. Susceptibilidade varietal de frutos de bananeira ao frio. *Rev. Bras. Frutic.* **2001**, *23* (3), 568–572.

(10) Jeong, B. R.; Housley, J. Fructan metabolism in wheat in alternating warm and cold temperatures. *Plant Physiol.* **1990**, *93*, 902–906.

(11) Ritsema, T.; Hernández, L.; Verhaar, A.; Altenbach, D.; Boller, T.; Wiemken, A; Smeekens, S. D Developing fructan-synthesizing capability in a plant invertase via mutations in the sucrose-binding box. *Plant J.* **2006**, *48* (2), 228–237.

(12) Wise, C. S.; Dimler, R. J.; Davis, H. A.; Rist, C. E. Determination of easily hydrolysable fructose units in dextran preparations. *Anal. Chem.* **1955**, *27*, 33–36.

(13) Bradford, M. M. Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein−dye binding. *Anal. Biochem.* **1976**, *72* (1−2), 248–254.

(14) Homme, C. L.; Peschet, J. L.; Puigserver, A.; Biagini, A. Evaluation of fructans in various fresh and stewed fruits by high-performance anion-exchange chromatography with pulsed amperiometric detection. *J. Chromatogr., A* **2001**, *920* (1−2), 291–297.

(15) Hogarth, A. J. C. L.; Hunter, D. E.; Jacobs, W. A.; Garleb, K. A.; Wolf, B. W. Ion chromatography determination of three fructooligosaccharide oligomers in prepared and preserved foods. *J. Agric. Food Chem.* **2000**, *48*, 5326–5330.

(16) Vijn, I.; Van Dijken, A.; Luscher, M.; Bos, A.; Smeets, E.; Weisbeek, P; Wiemken, A.; Smeekens, S. Cloning of sucrose: sucrose 1-fructosyltransferase from onion and synthesis of structurally defined fructan molecules from sucrose. *Plant Physiol.* **1998**, *117*, 1507–1513.

(17) Kawakami, A.; Yoshida, M. Fructan: fructan 1-fructosyltransferase, a key enzyme for biosynthesis of graminan oligomers in hardened wheat. *Planta* **2005**, *223* (1), 90–104.

(18) Sims, I. M. Structural diversity of fructans from members of the order Asparagales in New Zealand. *Phytochemistry* **2003**, *63* (3), 351–359.

(19) Vijn, I.; Smeekens, S. Fructan: more than a reserve carbohydrate? *Plant Physiol.* **1999**, *120*, 351–359.

(20) Edelman, J.; Jefford, T. G. The mechanism of fructosan metabolism in higher plants as exemplified in *Helianthus tuberosus*. *New Phytol.* **1968**, *67* (3), 517–531.

(21) Shiomi, N. Purification and characterization of 6^G-fructosyltransferase from the roots of asparagus (*Asparagus officinalis* L.). *Carbohydr. Res.* **1982**, *99* (2), 157–1592.

(22) Vijn, I.; Van Dijken, A.; Sprenger, N.; Van Dun, K.; Weisbeek, P.; Wiemken, A.; Smeekens, S. Fructan of the inulin neoseries is synthesized in transgenic chicory plants (*Cichorium intybus* L.) harbouring onion (*Allium cepa* L.) fructan: fructan 6G-fructosyltransferase. *Plant J.* **1997**, *11* (3), 387–398.

(23) Duchateau, N.; Bortlik, K.; Simmen, U.; Wiemken, A.; Bancal, P. Sucrose: fructan 6-fructosyltransferase, a key enzyme for diverting carbon from sucrose to fructan in barley leaves. *Plant Physiol.* **1995**, *107*, 1249–1255.

(24) Henderson, R. W.; Morton, R. K.; Rawlinson, W. A. Oligosaccharide Synthesis in banana and its relationship to the transferase activity of invertase. *Biochem. J.* **1959**, *72* (2), 340–344.

(25) Ende, W.; Michielis, A.; De Roover, J.; Van Laere, A. Fructan biosynthetic and breakdown enzymes in dicots evolved from different invertases. Expression of fructan genes throughout chicory development. *Sci. World J.* **2002**, *2*, 1281–1295.

(26) Ende, W.; Van Wonterghem, D.; Dewil, D.; Verhaert, P.; De Loof, A.; Van Laere, A. Purification and characterization of 1-SST, the key enzyme initiating fructan biosynthesis in young chicory roots (*Chicory intybus* L). *Physiol. Plant.* **1996**, *98* (3), 455–466.

(27) Luscher, M.; Hoscstrasser, U.; Vogel, G.; Aeschbacher, R.; Galati, V.; Nelson, C. J.; Boller, T.; Wiemken, A. Cloning and functional analysis of sucrose: sucrose 1-fructosyltransferase from tall fescue. *Plant Physiol.* **2000**, *124*, 1217–1228.

(28) Cairns, A. J.; Winters, A.; Pollock, C. J. Fructan biosynthesis in excised leaves of *Lolium temulentum* L. III. A comparison of the *in vitro* properties of fructosyl transferase activities with the characteristics of *in vivo* fructan accumulation. *New Phytol.* **1989**, *112* (3), 343–352.

(29) Ritsema, T.; Verhaar, A.; Vijn, I.; Smeekens, S. Fructosyltransferase mutants specify a function for the β-fructosidase motif of the sucrose-binding box in specifying the fructan type synthesized. *Plant Mol. Biol.* **2004**, *54* (6), 853–863.

(30) Campbell, J. M.; Bauer, L. L.; Fashey, G. C.Jr.; Hogarth, A. J. C. L.; Wolf, Br. W.; Hunter, D. E. Selected fructooligosaccharides (1-kestose, nystose and 1^F-β-fructofuranosylnystose) composition of foods and feeds. *J. Agric. Food Chem.* **1997**, *45*, 3076–3082.

(31) Hendry, G. A. F. Evolutionary origins and natural functions of fructans − a climatological, biogeographic and mechanistic appraisal. *New Phytol.* **1993**, *123* (1), 3–14.

(32) Wei, J. Z.; Chatterton, N. J. Fructan biosynthesis and fructosyltransferase evolution: expression of the 6-SFT (sucrose: fructan 6-fructosyltransferase) gene in crested wheat-grass (*Agropyron cristatum*). *J. Plant Physiol.* **2001**, *158* (9), 1203–1213.

(33) Cairns, A. J.; Ashton, J. E. The interpretation of in vitro measurements of fructosyl transferase activity: an analysis of patterns of fructosyl transferase by fungal invertase. *New Phytol.* **1991**, *118* (1), 23–34.

(34) Wagner, W.; Wiemken, A. Properties and subcellular localization of fructan hydrolyze in the leaves of barley (*Hordeum vulgare* L. cv Gerbel). *J. Plant Physiol.* **1986**, *123*, 429–439.

(35) Simmen, U.; Obenland, D.; Boller, T.; Wiemken, A. Fructan synthesis in excised barley leaves. Identification of two sucrose-sucrose fructosyltransferase induced by light and their separation from constitutive invertases. *Plant Physiol.* **1993**, *101*, 459–468.

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