

## Identification of Fructooligosaccharides in Different Banana Cultivars

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Banana has been currently indicated as a good source of fructooligosaccharides (FOS), which are considered to be functional components of foods. However, significant differences in their amounts in bananas have been observed in the literature. This work aims to identify and quantify FOS during ripening in different banana cultivars belonging to the most common genomic groups cultivated in Brazil. Considering that these differences can be due to cultivar, stage of ripening, and the methodologies used for FOS analyses, sugar contents were analyzed by high performance anion exchange chromatography-pulsed amperometric detection (HPAEC-PAD) and gas chromatography–mass spectrometry (GC-MS). An initial screening of eight cultivars (Ouro, Nanicao, Prata, Maça, Mysore, Pacovan, Terra, and Figo) in a full-ripe stage showed that 1-kestose, the first member of the FOS series (amounts between 297 and 1600  $\mu\text{g/g}$  of DM), was accumulated in all of them. Nystose, the second member, was detected only in Prata cultivar. Five of the cultivars were analyzed during ripening, and a strong correlation could be established with a specific sucrose level ( $\sim 200$  mg/g of DM), which seems to trigger the synthesis of 1-kestose (the low amounts of FOS, below the functional recommended dose, indicates that banana cannot be considered a good source of FOS).

**KEYWORDS:** Banana; cultivars; FOS; sucrose; nystose; 1-kestose; gas chromatography; HPAE-PAD

### INTRODUCTION

Fructans is a general term used for carbohydrates constituted by fructosylfructose units and a glucosidic unit. Fructooligosaccharides (FOS) have been defined as a combination of three sugars, 1-kestose [ $\beta$ -D-Fru-(2 $\rightarrow$ 1) $_2$ - $\alpha$ -D-glucopyranoside, GF $_2$ ], nystose [ $\beta$ -D-Fru(2 $\rightarrow$ 1) $_3$ - $\alpha$ -D-glucopyranoside, GF $_3$ ], and fructofuranosylnystose [ $\beta$ -D-Fru-(2 $\rightarrow$ 1) $_4$ - $\alpha$ -D-glucopyranoside, GF $_4$ ], in which the fructosyl units (F) are bound at the  $\beta$  (2 $\rightarrow$ 1) position of sucrose (1). Fructans with longer chains are named inulin.

Inulin and FOS are considered to be functional ingredients of food due to their effects on gastrointestinal functions. They are named prebiotics because of their resistance to digestive enzyme hydrolysis, being hydrolyzed only by the bacterial activity in the colon. The resultant process of fermentation stimulates the intestinal motility, changing the intestinal flora composition and causing a selective bifidobacteria biomass increase, which can reduce the risk of some diseases, such as colon cancer (2–7). Other benefits credited to FOS include glycemia and insulinemia reduction in rats (8) and decrease of

triglyceridemia associated with lower lipogenic enzymes activity in diabetic patients (9, 10).

Because of several scientific assertions of benefits of these carbohydrates with a “bioactive” or “functional” fiber action in intestinal health, the food industry has increased the inclusion of FOS in their products. However, the consumption of natural sources of FOS has advantages for its lower price in comparison to industrialized products. Moreover, natural sources offer values highly aggregated with the associated intake of other nutrients such as fibers, vitamins, and minerals. Some of the natural sources of FOS indicated are asparagus, garlic, leak, onion, Jerusalem artichoke, chicory (11), and fruits.

Although banana is currently indicated as a good source of FOS (12–14), significant differences in the amounts were observed in reported studies: 10.9 mg/g of dry mass (12), 4.3 and 6.0 mg at different maturity stages of banana (14), and 1.3 mg/g FOS in banana puree (13). These differences can be due to the cultivar, stage of ripening, processing (including heating and low pH), and methodologies applied in the FOS analyses.

The AOAC method for FOS analysis comprises an enzymatic hydrolysis of the fructose chain followed by fructose, glucose, and sucrose molecule analysis, which does not indicate the concentration of each FOS in the food matrix. Suitable alternatives to this AOAC method are high-performance liquid

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chromatography (HPLC) and gas chromatography (GC), which are also limited in terms of sensitivity, selectivity, and applicability (15).

High-performance anion-exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD) offers a powerful alternative to traditional HPLC methods. Most of the HPAEC-PAD applications have been performed using a CarboPac PA1 and PA100 column applications. However, according to the AOAC Dietary Fiber (16), this analytical method does not measure short-chain FOS because of their ethanol/water mixture solubility. The alternative methodology, a direct extraction followed by HPAEC-PAD, suffers interference of the maltooligosaccharides derived from starch hydrolysis, which elute at the same retention times as FOS short chains. Consequently, investigation of the kind of oligosaccharides in foods containing FOS, such as bananas, should be carefully conducted.

In fact, the presence of FOS in banana is not completely consistent because there are no previous reports about enzymatic activities linked to the synthesis of FOS in banana. The enzyme generally considered to be involved in plant fructan synthesis is the sucrose-sucrose fructosyl transferase (EC 2.4.1.99), which catalyzes the transference of a fructose molecule from one sucrose molecule to another, leading to kestose formation (glucosyl-1,2-fructosyl-1,2-fructose). Chain elongation is mediated by either 1<sup>F</sup> or 6<sup>F</sup> fructan-fructan fructosyl (EC 2.4.1.100) transferase, leading to inulin and levans, respectively (17). It is already known that sucrose is the main substrate for FOS biosynthesis and that banana fruit accumulates a significant amount of sucrose (6–16% FW) during ripening, with a high dependence on the banana cultivar (18, 19). Nevertheless, there is no previous report about the fructosyl transferase activity needed for this synthesis in banana fruit. Although some authors had assigned this role to the invertases, the presence of some fructosyltransferases, such as sucrose:sucrosyl transferase, cannot be ruled out.

The natural variability of the carbohydrate metabolism in different banana cultivars can contribute to the different levels of FOS observed in previous works, as well as the fruit stage of ripening. This work aims to show the levels of sucrose and FOS measured in the most consumed banana cultivars in Brazil, as well as their correlation during ripening through their simultaneous identification and quantification.

## MATERIALS AND METHODS

**Material.** Banana fruit (*Musa acuminata* L.) cultivars Ouro colatina (AA), Nanicão (AAA), Prata comum (AAB), Mysore (AAB), Maçã (AAB), Terra (AAB), Pacovan (AAB), and Figo (ABB) were harvested at a plantation located in Itapetininga (São Paulo State, Brazil). These cultivars were chosen on the basis of their amounts of starch accumulated during fruit development and sucrose synthesized during fruit ripening (~14–23% and 6–16%, respectively) (19). Bananas at approximately 110 days after anthesis (daa) were stored at 20 °C and 90% moisture under control. They were sampled on the basis of their respiration and ethylene production, along pre- and post-climacteric, including the first stage of senescence. The samples composed of 10 banana fingers, at least, were peeled, sliced, immediately frozen in liquid N<sub>2</sub>, and stored at -80 °C. For carbohydrate analysis, samples were thoroughly homogenized by powdering in liquid nitrogen.

**Ethylene and CO<sub>2</sub> Emission Measurements.** For the ethylene and respiration analysis, bananas were enclosed in 1.5 L jars (three fingers per jar; six jars per each cultivar). After 1 h, samples of 10 mL for ethylene analysis and 1 mL for CO<sub>2</sub> analysis were taken from the jar headspace by using a gastight syringe and injected in a gas chromatograph (HP-6890, Agilent Technologies). A flame ionization detector was employed for ethylene analysis and a thermal conductivity detector

for CO<sub>2</sub> analysis. For both gases, the column used was HP-Plot Q (30 Mts., i.d. 0.53 mm, Agilent Technologies); injector and detector temperatures were 250 °C, and the runs were isothermic at 30 °C. Fluxes of helium carrier gas were 1 mL/min for ethylene and 4 mL min<sup>-1</sup> for CO<sub>2</sub>. The injections were made in pulsed splitless mode for ethylene and in split mode for CO<sub>2</sub> analysis (50:1). Ethylene and CO<sub>2</sub> standards, both in synthetic air (Air-Liquid LTD), were used for calibration curves.

**Carbohydrate Determinations.** Soluble sugars were extracted three times with 80% ethanol at 80 °C. The supernatants were combined, and the ethanol was evaporated under vacuum. The residues were reconstituted with water, filtered through 0.22 μm membrane filters, and analyzed by HPAEC-PAD. The chromatographic analysis was performed on a Dionex DX 500 instrument equipped with a PAD system (ED 40). The analytical column employed was a CarboPac PA1 (250 × 4 mm, 5 μm particle size). The mobile phase was 18 mM NaOH, and the flow rate was kept constant at 1.0 mL/min.

Analysis of FOS by HPAEC-PAD was done in the same ethanolic extracts of sucrose analysis with the same column. The flow was of 1 mL/min at a gradient elution using two solvents: solution A (150 mM NaOH) and solution B (500 mM sodium acetate in 150 mM NaOH), under a total of 65 min run time. The elution program was initially 95% A and 5% B, followed by 90% A, 10% B in 8 min; 81% A, 19% B in 12 min; 30% A, 70% B in 19.7 min; and a cleaning step at 39.8 min (0% A, 100% B) maintained for 10 min. The column was then re-equilibrated for 15 min with 95% A and 5% B. To distinguish between FOS and starch hydrolysis products, the samples were analyzed with and without enzymatic treatment with invertase during 6 h at 40 °C (1326 units/L; 1 unit of invertase activity is defined as the amount of enzyme that produces 1 mol of fructose per minute at 60 °C in a sucrose solution 0.5 mol/L in 0.1 mol/L acetate buffer at pH 4.5). After treatment, ethanol was added to obtain an 80% solution to precipitate proteins. The mixture was centrifuged at 17000g during 15 min at 25 °C. The supernatant was evaporated under vacuum, and the pellet was resuspended with 1 mL of deionized water, filtered in a 0.45 μm micro filter, and injected on a liquid chromatograph.

The FOS yield was calculated as follows (16):

$$\left\{ \left[ \left( \text{g of } 1 - \text{kestose} + \text{maltose} \right) - \text{g of maltose} \right] + \left[ \left( \text{g of nystose} + \text{maltotriose} \right) - \text{g of maltotriose} \right] \right\} \times 100 / \left( \text{g of } 1 - \text{kestose} + \text{g of nystose} \right)$$

For the analysis of FOS by GC-MS, an aliquot of 2 mL of the soluble sugars extract in ethanol 80% was evaporated under vacuum. To the dry extract was added 100 μL of *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) to sample derivatization, which was shaken in a heating block at 800 rpm at 37 °C during 1 h, followed by centrifugation at 14000g during 1 min. The supernatant was then transferred to a glass vial and analyzed by GC-MS. The chromatographic conditions were as follows: injection of 1 μL of sample in splitless mode in column HP5MS (50 m, 0.25 μm of film thickness, and 0.32 mm i.d.). The carrier gas was helium at flux of 1.4 mL/min. The temperature program was as follows: 70 °C 2 min; raised from 70 to 315 °C at 15 °C/min and from 315 to 325 °C at 10 °C/min. The mass selective detector (model HP 5973) was operated in SIM (selected ion monitored) mode scanning ions at *m/z* 217 and 361 and in scan mode (from *m/z* 50 to 550).

**Moisture Analysis.** The moisture content of the sample was calculated on the basis of weight loss after the sample had been heated in an oven at 105 °C.

**Statistical Analysis.** The data were subjected to analysis of variance (ANOVA) and Turkey's comparison using Statistica 6.0 software. Means evaluated were considered to be significantly different at *p* < 0.05 and very significantly different at *p* < 0.01.

## RESULTS AND DISCUSSION

**HPAEC-PAD and GC-MS Methodologies for FOS Identification and Quantification.** During the process of optimizing the HPAEC-PAD method to quantify FOS, it was observed that some maltooligosaccharides, such as maltose and maltotriose, eluted at the same time as the 1-kestose and nystose, respec-

tively. Changes in the acetate gradient of the mobile phase did not succeed in achieving a better separation between the malto- and fructooligosaccharides. The samples were treated with invertase to confirm the presence of 1-kestose and nystose (16), and the disappearance of their corresponding peaks in the chromatograms was indicative of their identities (data not shown).

The validation of these results was made through GC-MS-SIM analysis. **Figure 1A,B** shows the partial GC-MS chromatograms of the 1-kestose standard (1  $\mu\text{g}/\mu\text{L}$ ) and the Mysore banana extract, respectively. The retention time of 1-kestose was 20.2 min, and the respective mass spectrum of both standard and sample (**Figure 1C,D**, respectively) shared a similarity of 0.98, mainly due to the ratio between the characteristic ions at mass/charge relations ( $m/z$ ) 217 and 361 (20). The same methodology used to quantify 1-kestose by HPAEC-PAD was tested for nystose analysis. However, due to the relative low concentration of this sugar in banana, the nystose levels were estimated directly by GC-MS.

A peak with high definition in Prata banana sample (**Figure 2**) was detected at the same retention time as the nystose standard (28.2 min). The partial MS spectrum of the nystose standard and the equivalent peak in Prata banana (**Figure 3**) indicate a structural similarity in both (match >98% according to NIST MS Search 1.6 software). However, a small rise in the baseline at 28.2 min was also observed in the other banana cultivars, but the structural identities were not equivalent.

The nystose GC-MS chromatograms of the different banana cultivars (**Figure 2**) were submitted to peak purity analysis through software AMDIS 32, which revealed the presence of two components that coeluted at the same retention time (28.2 min) for all samples, except for Prata banana.

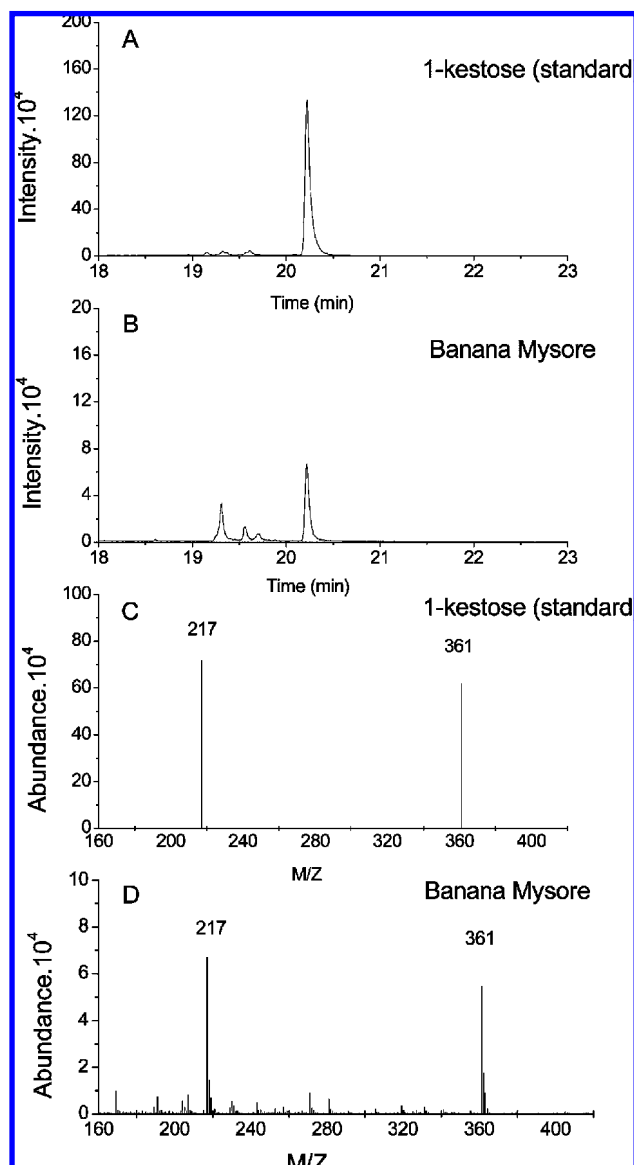
In the nystose spectrum were observed two typical abundant ions ( $m/z$  217 and 361), as in the 1-kestose spectrum (**Figure 1C,D**), but the ratio between them was different. However, these peak ratios could be used as a way to characterize some fructooligosaccharides. The ratio 217:361 value is 1.2 for 1-kestose and 1.79 for nystose.

Statistical analysis (**Figure 3**) of the peak abundance ratio at  $m/z$  217 and 361 revealed significant similarity ( $p > 0.05$ ) only for the nystose standard and the corresponding peak observed in sugar extract from Prata banana. This high detection sensitivity of the GC-MS analysis and the structural information provide reliable data, apart from the high reproducibility of the HPAEC-PAD method. Thus, it is important to emphasize that FOS was only detected in banana fruit by using the GC-MS method. Because of the presence of maltooligosaccharides, this method (CG-MS) is an alternative to HPAEC-PAD for the analysis of FOS on starch rich material.

**FOS Detection and Quantification in Different Banana Cultivars.** For initial screening, eight banana cultivars in the full-ripe stage, from different genomic groups (AA, AAA, AAB, and ABB), were analyzed (**Table 1**). 1-Kestose was detected in all cultivars with amounts varying between 297 and 1600  $\mu\text{g}/\text{g}$  of DM of banana pulp. Nystose was detected only in the Prata cultivar, which was found to produce more 1-kestose.

To detect the ripening stage at which the synthesis of 1-kestose occurred, five cultivars sampled of pre- and post-climacteric fruits, on the basis of their respiratory and ethylene profiles, were analyzed during all ripening processes. Results showed different maximum amounts of 1-kestose in full-ripe bananas (**Table 1**) and in the profile during ripening (**Figure 4**).

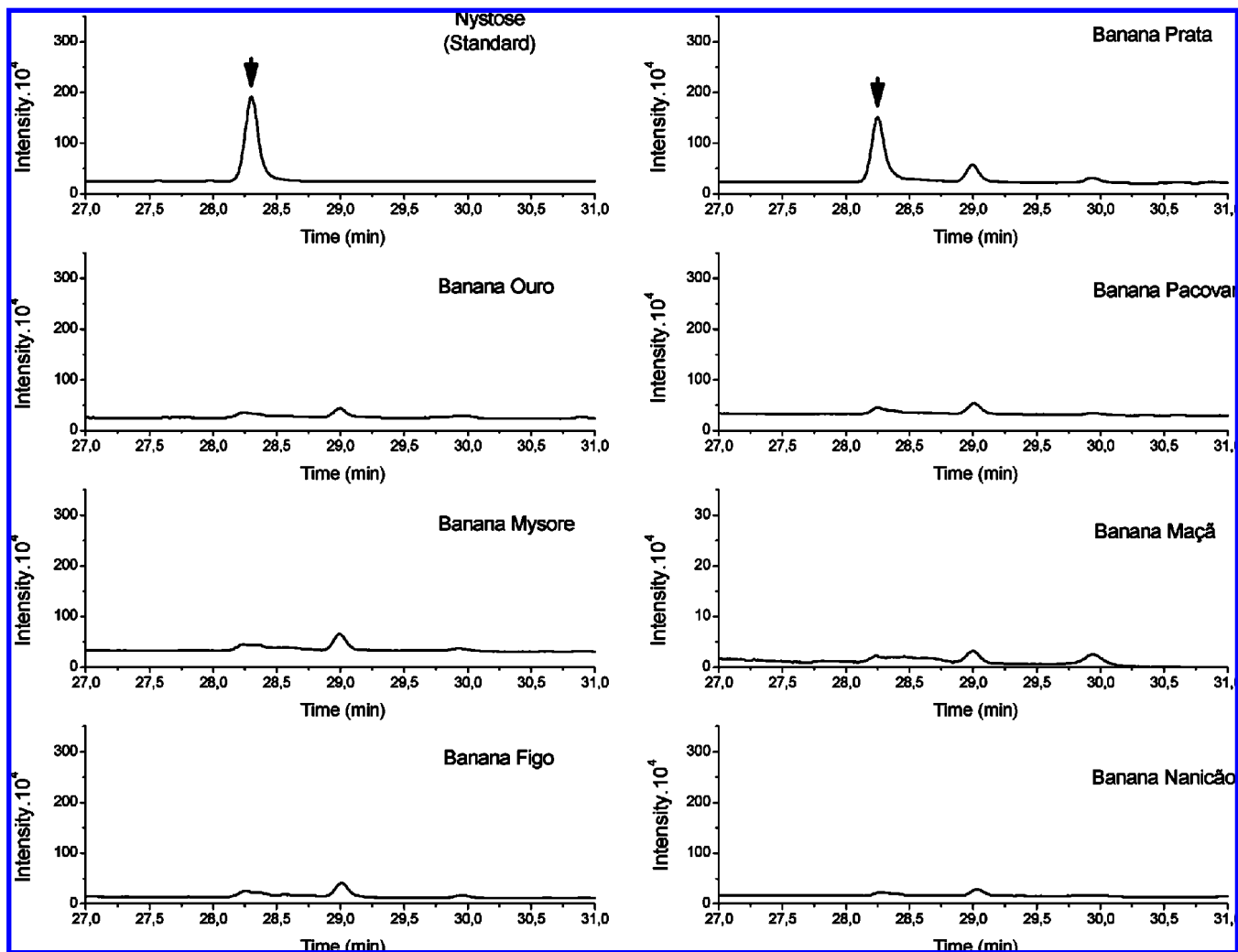
A weak correlation ( $R = 0.14$ ) was found between total maximum sucrose and total 1-kestose levels, as can be seen in



**Figure 1.** GC-MS chromatograms of 1-kestose standard and sugar extract from banana Mysore (**A** and **B**, respectively). The peak at 20.02 min represents the 1-kestose peak. Panels **C** and **D** are partial mass spectrum of 1-kestose indicating the characteristic ions at  $m/z$  217 and 361.

Mysore, having the second higher amount of the trisaccharide and the lowest sucrose level, and in Prata, having the second highest level of sucrose and the highest level of 1-kestose (1630  $\mu\text{g}/\text{g}$  of DM), among cultivars. Nystose, the subsequent member of FOS series, was also detected (**Table 1**). The higher amount of 1-kestose (1100  $\mu\text{g}/\text{g}$  of DM) was similar to that obtained by Hogarth et al. (13). They did not find nystose because their detection limit (200  $\mu\text{g}/\text{g}$  of DM) was higher than the detected nystose quantified in this study, once our nystose analysis presented high detection sensitivity by GC-MS-SIM. Hogarth et al. (13) also found  $1^F$ - $\beta$ -fructofuranosylnystose ( $\text{GF}_4$ ) (0.02 g/100 mL), which was not detected in this study despite the high sensitivity of GC-MS. The fructooligosaccharide composition seems to be dependent on both the cultivar and the ripening stage of the fruit (conditions not indicated in the work), impairing the comparison.

Sucrose accumulation (**Figure 4**) occurred mostly during banana ripening, with different timings among cultivars, but timely correlated to the climacteric. The sucrose levels reached similar values in Mysore, Terra, and Figo (207, 210, and 227



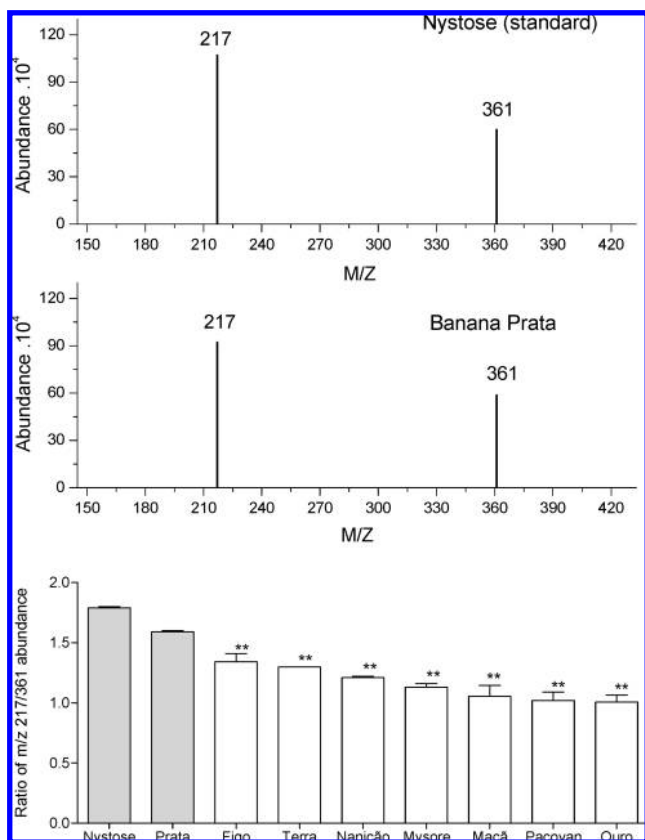
**Figure 2.** Partial chromatograms of sugar extract from different banana samples. The arrow indicates nystose in the sample and in standard solution ( $0.5 \mu\text{g}/\mu\text{L}$ ).

mg/g of dry weight, respectively), whereas they accumulated almost 2-fold more in Pacovan and Prata (430 and 470 mg/g, respectively). The levels of 1-kestose (**Figure 4**), the first member of the FOS series, rise concomitantly with sucrose, peaking at the end of the ripening, and differ among cultivars. As expected, the maximum amounts of sucrose were several times higher ( $\sim 100$  times) than those of 1-kestose.

The results suggest that the start of 1-kestose accumulation is highly dependent on the specific amount of the sucrose accumulated. In fact, the synthesis and accumulation of 1-kestose started only after sucrose had achieved approximately 200 mg/g of DW (**Figure 4**). This amount of sucrose, on the first day of 1-kestose appearance, was not statistically different among cultivars (ANOVA  $P = 0.623$  and  $F = 0.722$ ) (**Figure 3**). However, it seems that there is not a correlation between the final amounts of sucrose and 1-kestose reached during the ripening: Prata, compared to other cultivars, had the highest values for sucrose and 1-kestose. Mysore, Figo, and Terra accumulated different 1-kestose amounts (1.3, 0.9, and 0.3 mg/g of DM), although having comparable sucrose amounts (207, 227, and 210 mg/g of DM). Despite this, the profiles of sucrose and 1-kestose (**Figure 4**) suggest that even a slight increase in sucrose amounts for all cultivars caused a significant 1-kestose increase. This can be better visualized through the plot of 1-kestose amounts against the sucrose of Terra cultivar, in a semilog scale (**Figure 4**). The same test was applied for the

other three cultivars, resulting in similar patterns (data not shown). When other cultivars belonging to different genomic groups were analyzed in terms of sucrose and 1-kestose maximum amounts, the non-cultivar-dependence was reinforced.

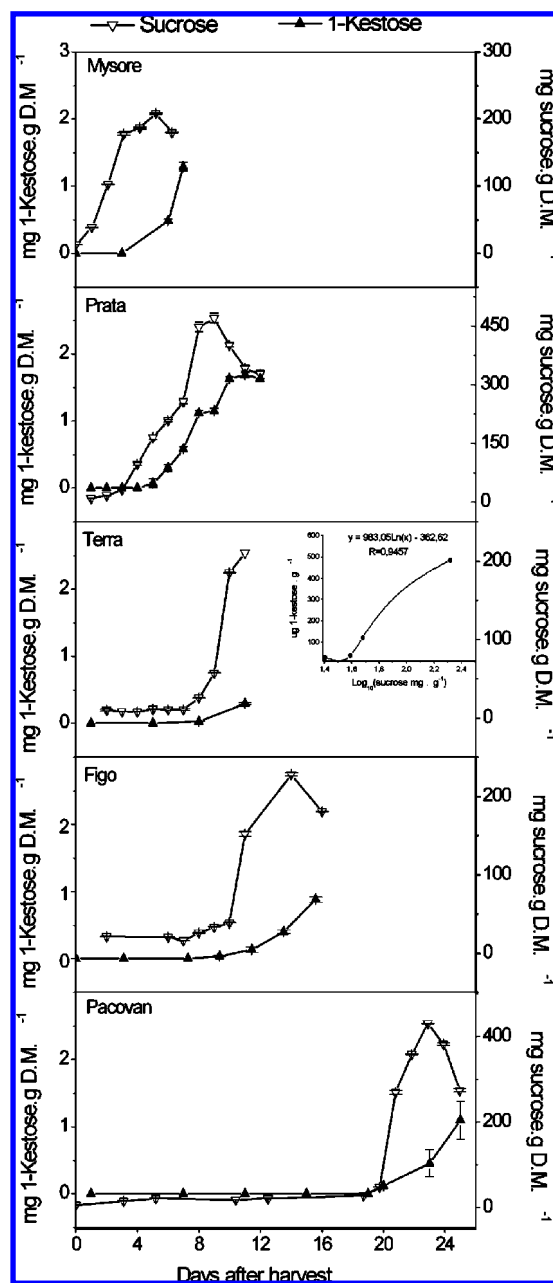
The importance of the cultivars and the ripening stage in influencing the FOS levels in banana was mentioned by Homme et al. (14) and confirmed in the present study. However, the influence of the cultivars in FOS amount are independent of the genomic group because the highest and lowest levels of 1-kestose were found in Prata and Terra cultivars, respectively (**Table 1**), both belonging to the AAB group. The results presented in **Table 1** and **Figure 4** show that even though the synthesis and accumulation of sucrose and 1-kestose seem to be dependent on the cultivar, there is no correlation between them and the genomic group. For example, Nanicaõ and Pacovan, belonging to the AAA and AAB groups, respectively, accumulated similar 1-kestose levels (1016 and 1097  $\mu\text{g}/\text{g}$  of DM). Campbell et al. (12) mentioned that the FOS level reached 0.2–2.0 mg/g of DM in more than 50% of different fruits analyzed, which is similar to the data found here (0.3–1.6 mg/g of DM, among different cultivars). However, as no cultivar or postharvest treatment was conveniently specified by the authors concerning the highest levels ( $\sim 10$  mg) found in banana fruit, it proved to be difficult to explain the discrepancies reported.



**Figure 3.** El mass spectra of nystose in standard solution and sugar extract from Prata banana. Ions at  $m/z$  217 and 361 are the more abundant; the ratio between ions 217 and 361 for nystose standard and Prata banana were statistically (\*\*,  $P < 0.01$ ) different from the other cultivars.

The results found herein support the Farrar et al. (21) hypothesis that sucrose acts both as a signal triggering the 1-kestose synthesis and as a substrate to the fructan's synthesis. In fact, the authors proposed a link between elevated sucrose contents and the induction of changes in the patterns of gene expression during the fructan accumulation in leaves of C3 grasses and cereals. The progressive up-regulation of the mRNA species had been suggested previously, one of these up-regulated mRNA species being a fructosyl transferase cloned from barley, which was demonstrated by Spenger et al. (22).

It was confirmed in the present work that the fructooligosaccharides 1-kestose and nystose are not only present in banana fruit but also depend on the cultivar rather than on the genomic group. Due to the low quantities present, below the functional recommended dose, banana cannot be considered a good source of FOS. The synthesis of 1-kestose seems to be triggered by a specific sucrose amount ( $\sim 200$  mg/g of DM), during banana



**Figure 4.** Sucrose and 1-kestose profiles of five banana cultivars. The cultivars were sampled at pre- and post-climacteric stages of the fruits, covering the entire ripening process. Data presented are means of triplicate assays  $\pm$  standard errors. The semilog plot shows the correlation between 1-kestose and sucrose levels during Terra banana ripening.

ripening, suggesting that sucrose works as a signal for the synthesis of 1-kestose in bananas.

**Table 1.** Sugar Levels of Banana Cultivars from Different Genomic Groups Determined at the Full-Ripe Stage<sup>a</sup>

cultivar	genomic group	moisture (%)	sucrose (mg/g of DM)	1-kestose <sup>b</sup> ( $\mu$ g/g of DM)	nystose ( $\mu$ g/g of DM)
Ouro	AA	69.2 $\pm$ 0.3	190.7 $\pm$ 5.7	918 $\pm$ 18 g	ND
Nanicão	AAA	75.9 $\pm$ 0.2	354.7 $\pm$ 3.1	1016 $\pm$ 40 f	ND
Prata	AAB	71.1 $\pm$ 0.2	326.5 $\pm$ 18.9	1630 $\pm$ 29 e	105 $\pm$ 8
Maçã	AAB	73.1 $\pm$ 0.1	224.5 $\pm$ 8.4	699 $\pm$ 15 d	ND
Mysore	AAB	76.6 $\pm$ 0.2	179.2 $\pm$ 3.5	1292 $\pm$ 50 c	ND
Pacovan	AAB	75.1 $\pm$ 0.1	274.3 $\pm$ 15.7	1097 $\pm$ 28 b	ND
Terra	AAB	61.8 $\pm$ 0.2	210.1 $\pm$ 2.2	297 $\pm$ 13 a	ND
Figo	ABB	68.7 $\pm$ 0.1	180.1 $\pm$ 2.0	881 $\pm$ 38 g	ND

<sup>a</sup> Each value is the mean of at least three determinations that are indicated with the standard deviation, respectively. DM, dry matter; ND, not detected. <sup>b</sup> Different letters indicate statistically significant differences ( $p > 0.05$ ).

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## LITERATURE CITED

- (1) Lewis, D. H. Nomenclature and diagrammatic representation of oligomeric fructans: a paper for discussion. *New Phytologist* **1993**, *124*, 583–594.
- (2) Hidaka, H.; Eida, T.; Takiwaza, T.; Tokunga, T.; Tashiro, Y. Effects of fructooligosaccharides on intestinal flora and human health. *Bifidobac. Microflora* **1986**, *5*, 37–50.
- (3) Wang, X.; Gibson, G. R. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *J. Appl. Bacteriol.* **1993**, *75*, 373–380.
- (4) Gibson, G. R.; Wang, X. Enrichment of bifidobacteria from human gut content by oligofructose using continuous culture. *FEMS Microbiol.* **1994**, *118*, 121–128.
- (5) Gibson, G. R.; Beatty, E. R.; Wang, X.; Cummings, J. H. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* **1995**, *108*, 975–982.
- (6) Roberfroid, M. B.; Bornet, F.; Bouley, C. H.; Cummings, J. H. Colonic microflora: nutrition and health. *Nutr. Rev.* **1995**, *53*, 127–130.
- (7) Cumming, J. H.; MacFarlane, G. T.; Englyst, H. N. Prebiotic digestion and fermentation. *Am. J. Clin. Nutr. (Suppl.)* **2001**, *73*, 415–420.
- (8) Oku, T.; Tokunga, T.; Hosoya, H. Non-digestibility of a new sweetener “neosugars” in the rat. *J. Nutr.* **1984**, *114*, 1574–1581.
- (9) Yamashita, K.; Kawaik, K.; Itakura, K. Effect of fructooligosaccharides on blood glucose and serum lipids in diabetic subjects. *Nutr. Res. (N.Y.)* **1984**, *4*, 961–966.
- (10) Arbeeny, C. A.; Meysers, D. S.; Bergquist, K. E.; Gregg, R. E. Inhibitions of fatty acid synthesis decrease very low-density lipoprotein secretion in the hamster. *J. Lipid Res.* **1992**, *33*, 843–851.
- (11) Van Loo, H. J.; Coussement, P.; Leenheer, L. D. E.; Hoebregs, H.; Smits, G. On the presence of inulin and oligofructose as a natural ingredients in the western diet. *Crit. Rev. Food Sci. Nutr.* **1995**, *35* (6), 525–552.
- (12) Campbell, J. M.; Bauer, L. L.; Fahey, G. C. J. R.; Hogarth, A. J. C. L.; Wolf, B. W.; Hunter, D. E. Selected fructooligosaccharide (1-kestose, nystose, and 1<sup>F</sup>- $\beta$ -fructofuranosyl-nystose) composition of foods and feeds. *J. Agric. Food Chem.* **1997**, *45*, 3076–3082.
- (13) Hogarth, A. J. C. L.; Hunter, D. E.; Jacobs, W. A.; Garleb, K. A.; Wolf, B. W. Ion chromatographic determination of three fructooligosaccharide oligomers in prepared and preserved foods. *J. Agric. Food Chem.* **2000**, *48*, 5326–5330.
- (14) Homme, C. L.; Peschet, J. L.; Peigserver, A.; Biagini, A. Evaluation of fructans in various fresh and stewed fruits by high performance anion exchange chromatography with pulsed amperometric detection. *J. Chromatogr., A* **2001**, *920*, 291–297.
- (15) Verzele, M.; Simoens, G.; Van Damme, F. A critical review of some liquid chromatography systems for the separation of sugars. *Chromatographia* **1987**, *23*, 292–300.
- (16) Ouarne, F.; Geuibert, A.; Brown, D.; Bornet, F. A sensitive and reproducible analytical method to measure fructooligosaccharide in food products. In *Complex Carbohydrate in Foods*; Cho, S. S., Prosky, L., Dreher, M., Eds.; Marcel Dekker: New York, 1994; pp 191–201.
- (17) Gupta, A. K.; Kaur, N. Fructan metabolism in Jerusalem artichoke and chicory. In *Carbohydrate Reserves in Plants—Synthesis and Regulation*; Elsevier Science: Amsterdam, The Netherlands, 2000; pp 223–248.
- (18) Cordenunsi, B. R.; Lajolo, F. M. Starch transformation during banana ripening: sucrose synthase and sucrose phosphate synthase behavior. *J. Agric. Food Chem.* **1995**, *43*, 347–351.
- (19) Mota, R. V. D. Da.; Lajolo, F. M.; Cordenunsi, B. R. Composição em carboidratos de alguns cultivares de banana (*Musa ssp.*) durante o amadurecimento. *Cienc. Tecnol. Aliment.* **1997**, *17*, 94–97.
- (20) Medeiros, P. M.; Simoneit, B. R. T. Analysis of sugars in environmental samples by gas chromatography–mass spectrometry. *J. Chromatogr., A* **2007**, *1141*, 271–278.
- (21) Farrar, J.; Pollock, C.; Gallagher, J. Sucrose and the integration of metabolism in vascular plants. *Plant Sci.* **2000**, *154*, 1–11.
- (22) Sprenger, N.; Bortlik, K.; Brandt, A.; Boller, T.; Wlrmken, A. Purification, cloning and functional expression of sucrose fructan 6-transferase, a key enzyme of fructan synthesis in barley. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11652–11656.

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