Influence of different banana cultivars on volatile compounds during ripening in cold storage

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ABSTRACT

The aroma responsible for the flavor of fruits is highly susceptible to low temperatures in storage. The present study investigated the volatile composition of the Nanicão and Prata banana cultivars by testing pulp and whole fruit under cold storage conditions. The volatile fractions were characterized using headspace solid phase micro-extraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS). The cold storage induced changes in the volatile profile relative to the profile of the control group. The result of principal component analysis revealed that cold storage more strongly affects the Nanicão than the Prata cultivar. Esters such as 2-pentanol acetate, 3-methyl-1-butanol acetate, 2-methylpropyl butanoate, 3-methylbutyl butanoate, 2-methylpropyl 3-methylbutanoate and butyl butanoate were drastically reduced in the cold group of the Nanicão cultivar. Our results suggest that the metabolism responsible for the production of volatile compounds is related to the ability to tolerate low temperatures.

1. Introduction

Several studies have been conducted on the post-harvest quality control of bananas, addressing storage conditions and treatments intended to extend shelf-life, such as short-term anaerobic conditions (Wendakoon, Ueda, Imahori, & Ishimaru, 2006), cold shock treatment (Zhang et al., 2010) and cold storage (Lichtemberg, Malburg, & Hinz, 2001). The maintenance of a cold chain from harvest to market is undoubtedly the most popular technique used to slow down the senescence of plants because low temperatures reduce the rate of enzymatic processes, such as the respiration rate and evolution of ethylene (Wills, Mcglasson, Graham, & Joyce, 1998), extending the plants' shelf life. However, in the case of bananas, the reduction in temperature must be performed carefully because of the recognized chilling injury that these fruits suffer in temperatures below 14 °C. This injury is characterized by changes in the physiology of fruit ripening, with the appearance of dark spots on the skin and brown spots in the pulp (Jiang, Joyce, Jiang, & Lu, 2004; Nguyen, Ketsa, & Van Doorn, 2003; Yang, Ashrafuzzaman, Nakamura, & Hayashi, 2000).

The symptoms of chilling injury appear to vary among banana cultivars and are related to the genomic group. Lichtemberg et al. (2001)

observed that the genome of B group (B—*Musa balbisiana*) cultivars confers greater resistance to low temperatures compared with the AAA group (A—*Musa acuminata*). However, the Prata cultivar (AAB group) seems to be more tolerant to cold storage (around 12 °C) than the Nanicão cultivar (AAA) (similar to Grand Naine) (Lichtemberg et al., 2001), which makes it feasible to transport over long distances without impairing the quality of the fruit. In fact, previous work involving Prata and Nanicão cultivars stored at low temperatures showed that the ripening related events like starch degradation, sucrose accumulation, ethylene and CO_2 levels were affected more in Nanicão than Prata (Agopian et al., 2011). Also higher levels of fructooligosaccharides (FOS) were found in cv. Prata than Nanicão submitted to the storage at low temperature (Agopian, Purgatto, Cordenunsi, & Lajolo, 2009). With these precedents we decided to focus our work in the compounds related to the aroma of the ripe banana that could be affected too by this kind of storage, since it is an important part of the fruit quality.

Volatile compounds of several banana cultivars have been widely studied by many authors in the world: FLHORBAN 920 and Grand Naine (Bugaud, Alter, Daribo, & Brillouet, 2009); Gran Enano, a subgroup of the Cavendish originating from Central and South America (Vermeir et al., 2009); various cultivars grown on Madeira Island (Nogueira, Fernandes, & Nascimento, 2003); banana fruits (*Musa sapientum* L. var. Cavendish) from Honduras and their aqueous essences (Jordan, Tandon, Shaw, & Goodner, 2001); free and glycosidically bound volatile compounds of the Valery and Pequeña Enana

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cultivars (Pérez et al., 1997); banana fruits (*Musa cavendishii* L.) of the Gran Enana and Enana cultivars from the Canary Islands and the Enana cultivar from Colombia (Cano et al., 1997); Philippine bananas (Del Monte, Cavendish cultivar), Taiwanese bananas (Sen-nin cultivar) and Delicious bananas (hybrid between Philippine and Taiwanese) (Shiota, 1993); and fruits, commercial nectars and industrial aromas (Salmon, Martin, Remaud, & Fourel, 1996). Generally esters such as butyl acetate, isoamyl acetate, ethyl acetate, butyl butanoate and isoamyl isobutanoate are responsible for the characteristic aroma of fresh banana and constitute the major class of compounds present in banana's volatile profile (Salmon et al., 1996).

In recent years some new techniques have been developed for the analysis of volatile components, which are attracting the attention of analysts and researchers, once they provide the minimum sample handling, and isolation and enrichment in a single operation. Among them, there is a technique called solid phase microextraction (SPME), developed by Arthur and Pawliszyn (1990). The main advantage of this technique is good analytical performance combined with simplicity and low cost, and it is ideal for mass spectrometry applications (Vas & Vékey, 2004).

In statistical multivariate analysis, principal component analysis (PCA) is a statistical tool widely used to generate volatile profiles, which highlight the differences among samples (Brereton, 2007). Great emphasis has been given to the analysis of multivariate data, in which one can measure many variables simultaneously, when analyzing a sample and it is currently pointed out as the best alternative for the interpretation of data and the acquisition of maximum information (Manly, 2008; Brereton, 2007).

Most works present results of volatiles from the banana pulp, but here, we show that the volatiles can overcome the barrier of the skin with the study of volatiles from whole fruit and are also affected by storage conditions. So, the goal of this study was to determine how cold storage affects the volatile composition of two banana cultivars: 'Nanicão' and 'Prata' from whole fruit and pulp.

2. Materials and methods

2.1. Samples

Mature green bananas were obtained from CEAGESP (Companhia de Entrepostos e Armazéns Gerais de São Paulo). The cv. Nanicão (*M. acuminata*, AAA) were harvested at a plantation located in Vale do Ribeira (São Paulo State, Brazil) and cv. Prata (*M. acuminata* X *M. balbisiana*, AAB), were harvested at a plantation located in Janaúba (Minas Gerais State, Brazil). The fruits were harvested in the morning, transported in box trucks to CEAGESP and then carried to the laboratory for the experiments. The fruits were obtained within 1 day of harvest. A total of 1000 units of fruits of each cultivar were necessary for the experiments.

2.2. Experimental design

Fruits of each cultivar were separated into two groups and stored in distinct chambers at 19 °C (control group) or 13 °C (cold-stored group). Another group of cv. Prata was stored at 10 °C. After 15 days, the cold-acclimated fruits were transferred to 19 °C to complete ripening. Ethylene production and CO_2 emission (respiration) were measured daily throughout the experiment. Sampling was performed according to changes observed in these parameters (about every 2 days during pre-climacteric phase and daily during the climacteric and post-climacteric phases). At least five fruits were sampled from each group (control and cold-stored), peeled, sliced, frozen in liquid N_2 and stored at −80 °C for posterior analysis. Volatile analyses were performed every 3 days until reaching the ethylene peak for the control and cold groups. Volatile analyses were then performed daily.

Note: we performed two sets of experiments for the cold-storage groups; the first with Nanicão and Prata cultivars stored at 13 °C and the second with Prata cultivar stored at 10 °C. This second experiment was performed because we observed that ripening was not completely terminated in Prata fruits stored at 13 °C and because we could not store the Nanicão fruits at 10 °C due to chilling injury. Therefore, in this report, we discuss only the volatile results obtained for Prata at 10 °C and Nanicão at 13 °C.

2.3. Ethylene production and respiration rate

Bananas were enclosed in 3 L jars (three fingers per jar; five jars per treatment) for 1 h for ethylene and respiration analysis. The analyses were performed following procedure used by Agopian et al. (2011).

2.4. Volatile analysis

The volatile fraction of banana was isolated by headspace solid phase micro-extraction (HS-SPME). The fiber used in this study was 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) obtained from Supelco (Sigma-Aldrich, Bellefonte, PA, USA). The fiber was preconditioned at 250 °C for 30 min. The SPME fiber was manually inserted into the headspace of the sample recipient. The volatiles from whole fruit and banana pulp were extracted under the following optimized conditions.

2.4.1. Volatiles from whole fruit
Whole bananas (1 kg) were enclosed in 3 L jars (approximately five fingers per jar). The equilibrium and extraction (fiber exposure) times were 140 and 120 min, respectively. The analyses were carried out at room temperature (~25 °C) in duplicate.

2.4.2. Volatiles from banana pulp
Fresh banana slices were homogenized (Turrax) with distilled water to make banana juice at 33.3% (w/w) and sodium chloride (Merck) (20% w/w) (Liu & Yang, 2002 with modifications). An aliquot of 16 g juice was transferred into a 30 mL vial sealed with a Teflon septum and a plastic cap. The equilibrium and extraction times were 15 and 60 min, respectively, under agitation with a magnetic stir bar. The analyses were carried out at room temperature (~25 °C) in duplicate.

2.5. Chromatographic and mass-spectrometric conditions

The SPME fiber was injected directly into a Hewlett–Packard 6890 (Agilent Technologies Inc., Santa Clara, USA) gas chromatograph–mass spectrometer (GC–MS) and held for 15 min for desorption of volatile compounds and to guarantee the good quality of the SPME extraction procedures. The injection port was lined with a 0.75 mm i.d. splitless glass liner and maintained at 200 °C. Compounds were separated using the capillary column Supelcowax 10 (30 m×0.25 mm×0.25 μm) from Supelco Inc. (Bellefonte, PA, USA) with helium as the carrier gas at a flow rate of 1.0 mL·min^{-1}. The oven temperature was programmed to ramp from 50 to 150 °C at 2 °C/min (Liu & Yang, 2002) and the total GC run time was 55 min. The MS transfer line was maintained at 290 °C, the ionization energy was 70 eV, and the mass range was 50–550 m/z. The total volatile production was estimated by the sum of all peak areas identified in the chromatogram. The retention indexes were calculated according to the equation of Van den Dool and Kratz (Mjos, Meier, & Boitsov, 2006) for an alkane solution (C9–C22) injected under the same GC–MS conditions. The volatile compounds were identified by comparing the results obtained with reference mass spectra from the NIST library (NIST98, version 2.0, Gaithersburg, USA) using the criterion of at least 75% similarity for the mass spectra.

2.6. Statistical analysis

Because of the experiment's complexity, the major esters from the control and cold groups were submitted to linear regression analysis carried out using only the ripe fruit data. Each cultivar data was processed separately with storage conditions as predictor variables (x) and major volatile abundances as response variables (y). The software used was SAS 9.2 program (SAS Institute, Cary, NC, USA).

Principal component analysis (PCA) was used to present and to visualize the high-dimensional data with respect to the influence of cold storage on volatile production. Multivariate statistical analyses were carried out using the software program XLSTAT-MX.

3. Results and discussion

3.1. Respiration and ethylene during ripening

Fig. 1 shows the ethylene and CO_2 production of both cultivars under different storage conditions. It was observed that the low temperature inhibited ethylene and CO_2 production, except for the Prata cold group stored at 13 °C, i.e., the temperature of 13 °C only partially inhibited ethylene and CO_2 production but did not completely stop climacteric ripening. However, when the fruits were stored at 10 °C, ethylene and CO_2 production was successfully inhibited.

The ethylene peak for the control group (19 °C) occurred on the 11th and 4th DAH for the Nanicão and Prata cultivars, respectively (Fig. 1). The Prata cultivar has a postharvest life shorter than the cv. Nanicão. Ethylene levels are higher in the Prata, but CO_2 levels are higher in the cultivar Nanicão. In response to the low temperatures, the basal levels remained low throughout the storage period for the cold group. The increase in the ethylene peak was similar for both cultivars and occurred on the 18th DAH, 3 days after the temperature was raised to 19 °C. So, the cold storage conditions (13 °C for cv. Nanicão and 10 °C for cv. Prata) were enough to delay the ripening, reducing the levels of ethylene and respiration.

3.2. Volatiles in banana fruit

The production of volatiles is in fact dependent on the production of ethylene, the hormone associated with fruit ripening (Kerbauy, 2008). Just 3 days after the ethylene peak (see Fig. 1), there was a burst in the production of the volatiles. Fig. 2 shows the volatile production DAH. From the 1st to the 13th DAH for the Nanicão cultivar (whole fruit and pulp at 19 °C), the chromatograms presented low concentrations of volatile compounds, but on the 14th DAH, the major volatiles appeared and increased in concentration until the 18th DAH. For the Prata control samples (19 °C), the major volatiles appeared and increased from the 9th and 10th day for the whole fruit and pulp, respectively. In the cold group samples, when the temperature was increased to 19 °C after 15 days of storage, the burst occurred on the 21st DAH in all samples.

The volatile components and their retention indexes are listed in Table A.1 (supplementary data). Because of the complexity of the samples, the number of identified volatile compounds and the objective of this work, the presented values for the peak areas obtained by GC–MS do not represent the true quantities of the volatiles in the samples and are only a parameter used to compare the effect of the cold storage conditions on the volatile profiles of the banana samples. For a better presentation of the results, the relative area of the compounds is presented as follows: (i) for green fruits, the areas detected in the cv. Nanicão and cv. Prata samples of the control group were observed on the 10th and 3rd DAH, respectively, and on the 15th DAH for the cold group (in both cultivars); (ii) for ripe fruits, samples in the control group were observed on the 16th (cv. Nanicão) and 11th (cv. Prata) DAH and on the 23rd DAH for samples in the cold group (both cultivars).

Ninety-three compounds were identified in the volatile fraction of two distinct banana cultivars using two methodologies: pulp and whole fruit. For cv. Prata, 45 compounds were identified in the pulp throughout the entire ripening process and 39 in the whole fruit. For cv. Nanicão, 55 and 42 compounds were detected in the pulp and the whole fruit, respectively. Cultivar, maturity, postharvest storage and different biosynthetic pathways are involved in the production of these compounds. With respect to chemical characterization, the frequencies of all functional chemical groups detected in this study in each methodology are listed to provide the relative frequency distribution (Fig. 3). In the green fruits, only alcohol and terpenes were detected in the whole fruits and aldehydes and alcohols in the pulp. In the ripe fruits, the esters were the major group, followed by alcohols, ketones and terpenes.

Terpene compounds were the predominant functional group in green bananas of both cultivars when the whole fruit was used to measure the volatiles (~10 terpenes) (Fig. 3). The main physicochemical properties of terpenes are low water solubility, high volatility and
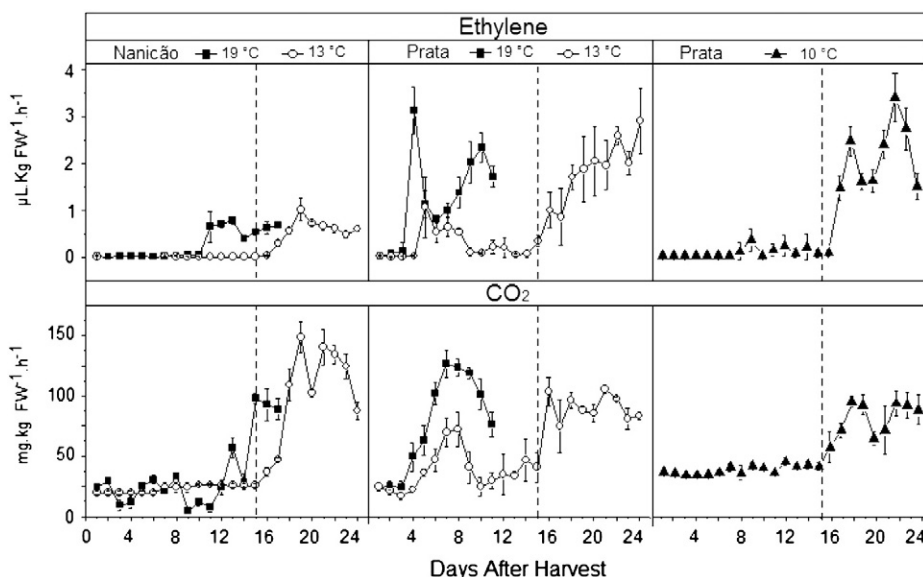

Fig. 1. Ethylene and respiration (CO_2 evolution) of cv. Nanicão and cv. Prata bananas during cold storage and ripening. Standard deviations of the analysis are indicated by vertical bars (n = 4). The lines (− − −) indicate the time of cold storage.
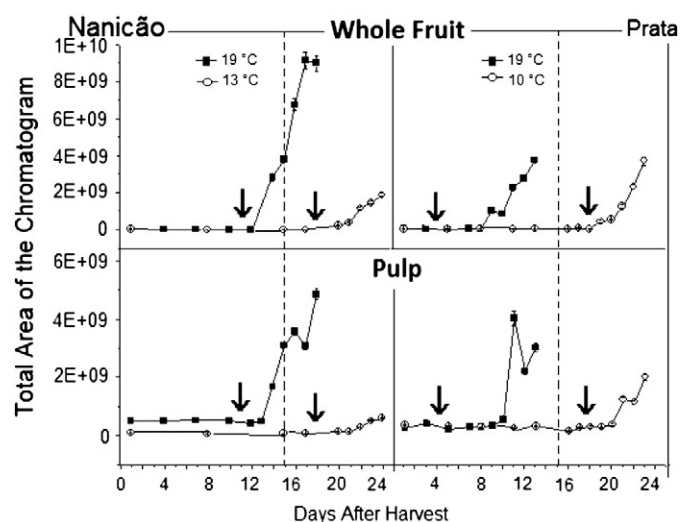
Fig. 2. Volatile production of whole fruit and pulp of cv. Nanicão and cv. Prata bananas during ripening. The lines (− − −) indicate the time of cold storage. The arrows indicate the ethylene peak.

cytotoxicity (Schrader, 2007). Terpenes, particularly monoterpenes, are the major compounds that contribute to the flavor of citrus and other fruits. They are biosynthesized by the isoprenoid pathway, with melavonic acid as the precursor compound (Lindsay, 1996). In cv. Nanicão, terpenes, such as α-pinene, limonene and a small amount of eugenol, were detected (supplementary data). These compounds are frequently found in essential oils and flowers (Hazzit, Baaliouamer, Faleiro, & Miguel, 2006; Kaul, Gujral, & Singh, 1999; Tayoub et al., 2006) and contribute herbal and floral notes (Jordan et al., 2001; Nogueira et al., 2003). Eugenol was not detected in cv. Prata, despite having been detected in a study carried out in various cultivars grown in Madeira Island (Nogueira et al., 2003).

The only alcohol detected in green Nanicão, when measuring the whole fruits, was 2-ethyl-1-hexanol. After ripening, the compounds 3-methyl-1-butanol (isoamyl alcohol) and 2-heptanol were detected in all samples (pulp and whole fruit of both cultivars). These compounds contribute pungent and fruity notes (Jordan et al., 2001).

Compounds such as 1-pentanol and 1-hexanol appeared only in pulp samples. However, 3-hexenol, 1-heptanol, (Z)-3-Octen-1-ol, (Z)-5-Octen-1-ol and nonanol were detected only in the pulp of cv. Nanicão. During ripening, the fruits' metabolism develops the ability to convert some of the fat acids into esters, ketones and alcohols (such as 3-methyl-1-butanol), which are important volatile compounds in many fruits with distinct odor (Jiang & Song, 2010; Lindsay, 1996; Tressl & Drawertl, 1973).

Aldehydes were not detected in whole fruits. In the Nanicão cultivar, they were detected only in the pulp of the green fruits (hexanal, 2-hexenal, (E)-2-heptanal, and others) and were the major functional group in the green fruits. In the Prata cultivar, these compounds appeared in the pulp of green and ripe fruits. Aldehydes are formed by the autoxidation of unsaturated fatty acids. Nonanal and decanal are formed by the autoxidation of oleic acid; hexanal, E-2-octenal and E-2-nonenal by the autoxidation of linoleic acid; and E-2-hexenal, E-2-heptenal and (E,Z)-2,6-nonadienal by the autoxidation of linolenic acid (Belitz, Grosch, & Schieberle, 2009). Each aldehyde has a distinct odor note that could affect the banana's aroma. For example, nonanal has a tallowy and soapy, fruity odor; decanal has an orange peel odor; hexanal has a tallowy and green, leafy odor; E-2-octenal has a fatty and nutty odor; E-2-nonenal has a tallow and cucumber odor; E-2-hexenal has an apple odor; E-2-heptenal has a fatty and bitter almond odor; and (E,Z)-2,6-nonadienal has a cucumber odor (Belitz et al., 2009). However, aldehydes were not detected in whole fruits because their concentration or volatility was too low to overcome the barrier of the banana peel. It has been shown that in apple fruit there is a relationship between low aroma volatile production, low free fatty acids and low ATP content (Song & Bangerth, 2003).

The ketone 2-pentanone was the most abundant ketone in all of the samples, detected only in ripe fruits. In addition, 2-heptanone and 2-undecanone were detected in cv. Nanicão and 2-nonanone in cv. Prata. The presence of these compounds is due to lipid oxidation (Belitz et al., 2009). Methyl ketones contribute fruity and banana-like (2-pentanone), fragrant and herbaceous (2-heptanone), and flowery and fatty (2-nonanone) notes (Belitz et al., 2009).

Esters were the most abundant compounds detected in ripe bananas of both cultivars, accounting for more than 50% of the compounds identified. Fruit esters are formed by the reaction between alcohols and acyl CoAs derived from the fatty acid and amino acid
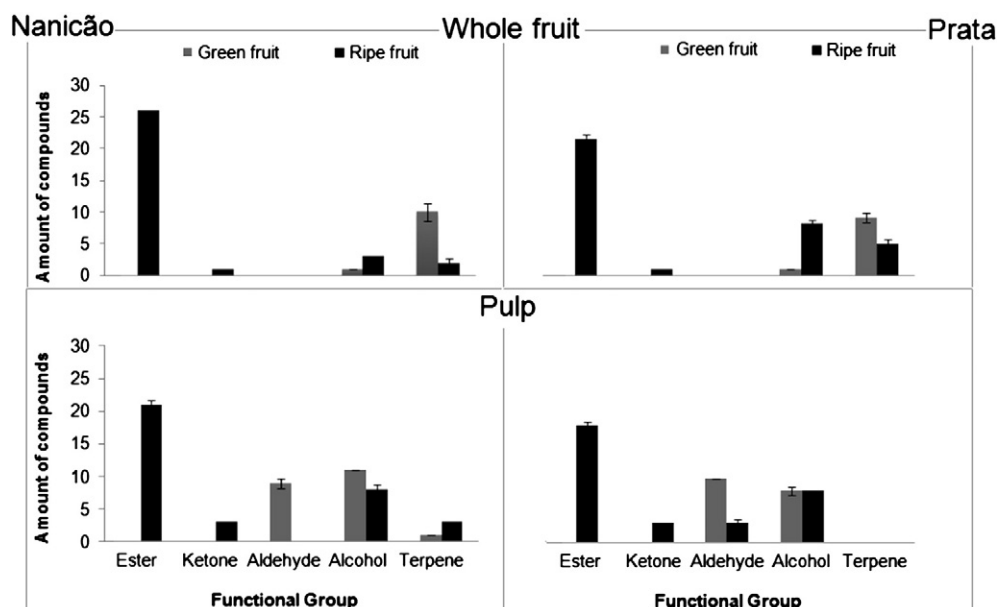

Fig. 3. Amount of volatile compounds by functional chemical group in the Nanicão and Prata samples.

metabolism. This reaction is catalyzed by the enzyme acyl alcohol transferase (AAT) (Pérez et al., 1997).

The development of a pleasant aroma during fruit ripening occurs due to the metabolism of branched amino acids such as leucine with deamination and decarboxylation. Then, an aldehyde is formed, which can be transformed into its corresponding alcohol and, with the addition of acetic acid, produces isoamyl acetate (3-methyl-1-butanol-acetate), which is considered a character impact flavor compound because it has the characteristic aroma of banana (Berger, 1991; Jiang & Song, 2010; McGorrin, 2002; Rodriguez-Amaya, 2003). Terpenes, alcohols, aldehydes and ketones directly contribute to ripe fruit flavor, but esters are the impact compounds. In the volatile composition and the odor-active components of commercial banana essence and fresh banana fruit paste, each ester showed distinct descriptors: 2-pentanol acetate had herbal, sweet and floral notes; 3-methyl-1-butanol acetate (isoamyl acetate) had over-ripe banana and sweet odors; butyl butanoate had spicy and grassy odors; 3-methylbutyl butanoate (isoamyl butyrate) had fruity, floral and acid notes; and 2-methylpropyl acetate (isobutyl acetate) exhibited plastic, rancid and pungent odors (Jordan et al., 2001). Most of these compounds were classified as major compounds in our study.

3.3. Volatile compounds produced after cold storage

3.3.1. Effects of the cold storage on major compounds

The cold storage effect on the major compound abundances were processed using linear regression. In cv. Nanicão, the esters 2-pentanol acetate, 3 methyl-1-butanol acetate, 2-methylpropyl butanoate, 2-methylpropyl 3-methylbutanoate, butyl butanoate, 3-methylbutyl butanoate and 3-methylbutyl 3-methylbutanoate were the most abundant volatiles. The regression analysis (Table 1) revealed that except for the compound 3-methylbutyl 3-methylbutanoate, all the major esters were affected by low temperature ($p < 0.05$) in pulp and whole fruit, with negative estimated values (β coefficients) in all compounds, indicating that during cold storage (13 °C), the abundance of these compounds tended to decrease. The determination coefficient (R^2) gives the proportion of total variation of each compound explained by the regression. The coefficient values were higher as the effect of cold storage on these compounds was more significant.

In cv. Prata, 2-pentanol acetate, 3 methyl-1-butanol acetate, 2-methylpropyl butanoate, 3-methylbutyl 2-methylpropanoate, 1-methylbutyl 2-methylpropanoate, 3-methylbutyl butanoate and 3-methylbutyl 3-methylbutanoate were the most abundant volatile compounds. In Table 2 no compound was significantly affected by cold storage ($p > 0.05$), i.e., even with storage at 10 °C (temperature below which cv. Nanicão was submitted) and the estimated value

negative (which means that the abundance decreased with storage) or positive (which means that the abundance increased with storage temperature) was not shown as statistically significant for this cultivar.

3.3.2. Effects of cold storage on the total volatile profile

In this study, the principal component analysis (PCA) was used to explore interdependencies among the measured volatiles and to identify variant groups with similar behaviors per cultivar. Graphs labeled A represent samples and the graphs labeled B represent compounds; both are positioned in the space defined by the first two components (F1 vs F2).

For the cv. Nanicão (Fig. 4), the first two components could explain 50.95% of the variability among the samples. The first component separated the samples from the beginning of maturation, i.e., before the ethylene peak (on the left side of the graph) and after the ethylene peak (on the right side of the graph). The second component separated the ripe fruits with respect to the predominance of volatiles, with the control group (pulp and whole fruit) situated in the upper and the cold group in the lower quadrant.

In the whole fruits, it was observed that samples at the beginning of the ripening (C1 and L1) started with a high production of terpenes, such as α-pinene, m-xylene, limonene, α-ocimene, α-cedrene and α-caryophyllene. The control group retained that profile until the 12th DAH and then started to produce other compounds, mainly esters and alcohols, i.e., after the ethylene peak (which occurred at 11th DAH), a burst of volatile production began on the 14th and 15th DAH (C14 and C15), which was highly correlated with the compounds 3-methyl-1-butanol, 3-methylbutyl 3-methylbutanoate and butyl hexanoate. The ripe fruits in the control group (C16 to C18) drastically increased the production of esters (2-pentanol acetate, 3-methyl-1-butanol acetate, butyl butanoate, 2-methylpropyl hexanoate, hexyl pentanoate, pentyl butanoate, pentyl hexanoate, 3-methyl-butyl pentanoate, 3-methyl-3-butenyl pentanoate, among others), terpenes (β-cedrene and eugenol), and alcohols (nonanol and 2-heptanol). However, the cold group maintained a predominance of terpenes until the 21st DAH (L21). Ripe fruits (L22 to L24) were near the center of the chart, corresponding to the production of the alcohols (Z)-5-octenol and (Z)-3-octenol and the esters butyl hexanoate and hexyl acetate. It can be seen that the samples (cold group) were situated far away from the control group in the graph.

Analyzing the pulp stored at 19 °C (control group) it was observed that in the early days of storage it showed a high production of aldehydes and alcohols (PC1 to PC7). On the 14th DAH, the production of esters, alcohols and terpenes strongly increases, but the profile of volatiles from the pulp of ripe fruits (PC15 to PC18) showed a lower

Table 1
Parametric regression fit and parameter estimates from linear regression for major volatiles in banana fruits cv. Nanicão.

Data processing	R^2	Model equation	p_{value}
Pulp			
2-Pentanol acetate (Y_1)	0.85	$Y_1 = 1.8E+08 - 1.7E+08x$	0.0001
3-Methyl-1-butanol acetate (Y_2)	0.92	$Y_2 = 5.8E+08 - 5.6E+08x$	<0.0001
2-Methylpropyl butanoate (Y_3)	0.84	$Y_3 = 1.7E+08 - 1.6E+08x$	0.0002
2-Methylpropyl 3-methylbutanoate (Y_4)	0.68	$Y_4 = 1.0E+08 - 9.1E+07x$	0.0031
Butyl butanoate (Y_5)	0.72	$Y_5 = 1.65E+08 - 1.0E+08x$	0.0019
3-Methylbutyl butanoate (Y_6)	0.58	$Y_6 = 5.3E+08 - 4.5E+08x$	0.0100
3-Methylbutyl 3-methylbutanoate (Y_7)	0.39	$Y_7 = 3.7E+08 - 3.2E+08x$	0.0544
Whole fruit			
2-Pentanol acetate (Y_1)	0.78	$Y_1 = 2.4E+08 - 2.1E+08x$	0.0007
3-Methyl-1-butanol acetate (Y_2)	0.65	$Y_2 = 1.8E+08 - 1.7E+08x$	0.0045
2-Methylpropyl butanoate (Y_3)	0.78	$Y_3 = 3.8E+08 - 3.4E+08x$	0.0006
2-Methylpropyl 3-methylbutanoate (Y_4)	0.55	$Y_4 = 3.9E+08 - 3.4E+08x$	0.0143
Butyl butanoate (Y_5)	0.42	$Y_5 = 5.1E+08 - 3.8E+08x$	0.0407
3-methylbutyl butanoate (Y_6)	0.85	$Y_6 = 1.8E+09 - 1.6E+09x$	0.0001
3-Methylbutyl 3-methylbutanoate (Y_7)	0.31	$Y_7 = 1.5E+09 - 1.2E+09x$	0.0936

Table 2
Parametric regression fit and parameter estimates from linear regression for major volatiles in banana fruits cv. Prata.

Data processing	R^2	Model equation	p_{value}
Pulp			
2-Pentanol acetate (Y_1)	0.0004	$Y_1 = 8.9E+07 - 2.0E+06x$	0.9603
3-Methyl-1-butanol acetate (Y_2)	0.25	$Y_2 = 1.8E+08 - 9.1E+07x$	0.2032
2-methylpropyl butanoate (Y_3)	0.21	$Y_3 = 9.6E+07 - 5.1E+07x$	0.2512
3-Methylbutyl 2-methylpropanoate (Y_4)	0.37	$Y_4 = 5.2E+07 - 4.9E+07x$	0.1101
1-Methylbutyl 2-methylpropanoate (Y_5)	0.10	$Y_5 = 1.1E+08 + 9.4E+07x$	0.4347
3-methylbutyl butanoate (Y_6)	0.30	$Y_6 = 7.3E+08 - 3.8E+08x$	0.1595
3-Methylbutyl 3-methylbutanoate (Y_7)	0.47	$Y_7 = 3.5E+08 - 3.3E+08x$	0.0602
Whole fruit			
2-Pentanol acetate (Y_1)	0.008	$Y_1 = 2.6E+08 + 2.0E+07x$	0.8329
3-Methyl-1-butanol acetate (Y_2)	0.24	$Y_2 = 3.1E+08 - 1.9E+08x$	0.2214
2-Methylpropyl butanoate (Y_3)	0.20	$Y_3 = 1.3E+08 - 7.1E+07x$	0.2656
3-Methylbutyl 2-methylpropanoate (Y_4)	0.35	$Y_4 = 7.4E+07 - 6.9E+07x$	0.1239
1-Methylbutyl 2-methylpropanoate (Y_5)	0.28	$Y_5 = 4.8E+08 + 3.0E+08x$	0.1752
3-Methylbutyl butanoate (Y_6)	0.0010	$Y_6 = 4.4E+08 + 2.2E+07x$	0.9408
3-Methylbutyl 3-methylbutanoate (Y_7)	0.29	$Y_7 = 4.1E+08 - 3.8E+08x$	0.1722

concentration of esters than that in the whole fruits (C16 to C18). The samples from the cold group generally started with a predominance of aldehydes and alcohols, but with a lower intensity than that of the bananas from the control group. With maturation, the production of these aldehydes and alcohols was reduced and the production of esters and terpenes increased. The ripe fruits (PL21 to PL24) were very poor in volatiles, without great variation during ripening, and were far from the control group samples (PC15 to PC18).

In Fig. 5 (cv. Prata), the first two components could explain 53.34% of the variability among samples. The first component separated the samples in relation to the maturation stage and the second component separated them with respect to the type of analysis methodologies: pulp and whole fruit, with pulp samples (control and cold groups) situated in the upper quadrant, which is the region of most volatile compounds, indicate that the volatile profile of the pulp had become richer than the profiles of the corresponding samples of whole fruit (located below).

For this cultivar, the behaviors of the volatiles in the control and cold groups were similar. In the whole fruits, the samples (C1 to C9 for the control group and L1 to L20 for the cold group) began with the production of terpenes (α-pinene, β-myrcene, α-ocimene, perillene, α-cedrene, β-cedrene, muurolene, β-farnesene) and alcohol (1-hepten-4-ol) and the pulp (PC1 to PC9 and PL1 to PL20 for the control and cold groups, respectively) with aldehydes (2-hexenal, hexanal, nonanal, decanal) and alcohols (1-hexanol, 3-hexenol, (Z)-6-nonenol). During ripening, an increase in the production of esters and methylated alcohols was observed. At the end of ripening, the control and cold group samples did not differ with respect to the volatile composition, i.e., the samples of the whole fruits C10 to C13, L21 to L23 had very similar profiles and the samples of the pulp PC10 to PC13, PL21 to PL23 were situated close to one another in the graph, indicating a very strong association.

4. Conclusions

The volatile profiles of pulp and whole fruits appear to be different in the green fruits, but in the ripe fruits the ester profiles were similar, with the same major compounds responsible for the banana aroma. In the cold storage conditions, the same behavior was found by analyses made with both methodologies (pulp and whole fruit). By multivariate statistical analysis, this study indicated that it is possible to differentiate between the samples from the control group and the
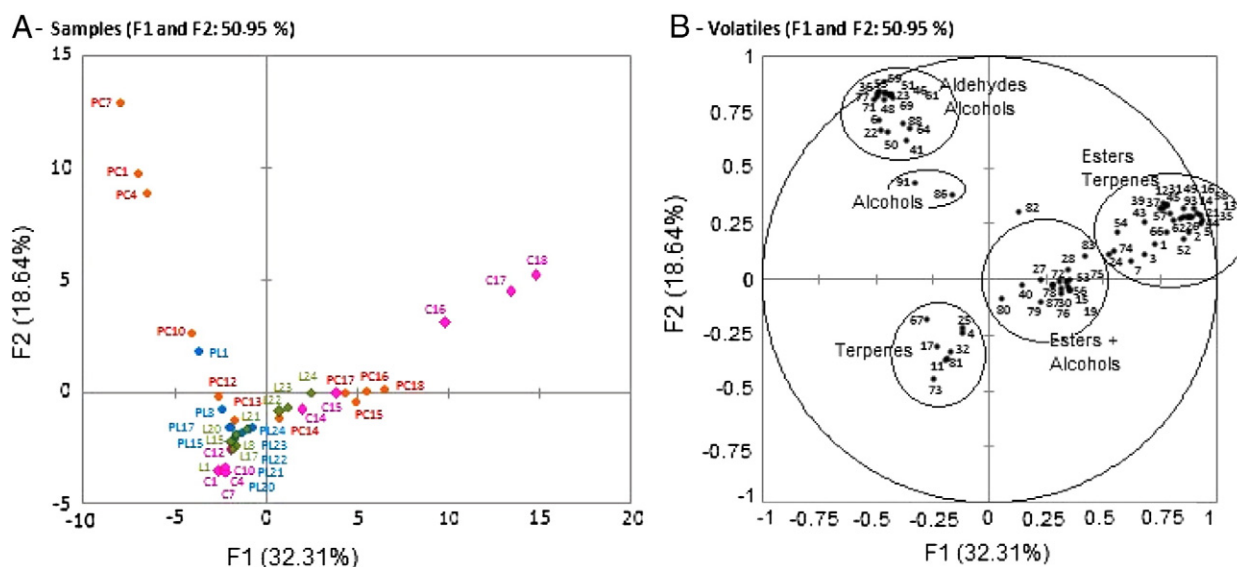

Fig. 4. Principal component analysis of the banana volatile compounds from cv. Nanicão. Sample code: P = pulp from control group (19 °C) in red color; PL = pulp from cold group (low temperature, 13 °C) in blue color. C = whole fruit from control group (19 °C) in pink color; L = whole fruit from cold group (low temperature, 13 °C) in green color. The numbers 1 to 24 refer to day after harvest (DAH). Ripe fruit (19 °C): 16th, 17th and 18th DAH. Ripe fruit (13 °C): 22nd, 23rd and 24th DAH. Volatile numbers refer to compounds in Table A.1 (supplementary data).
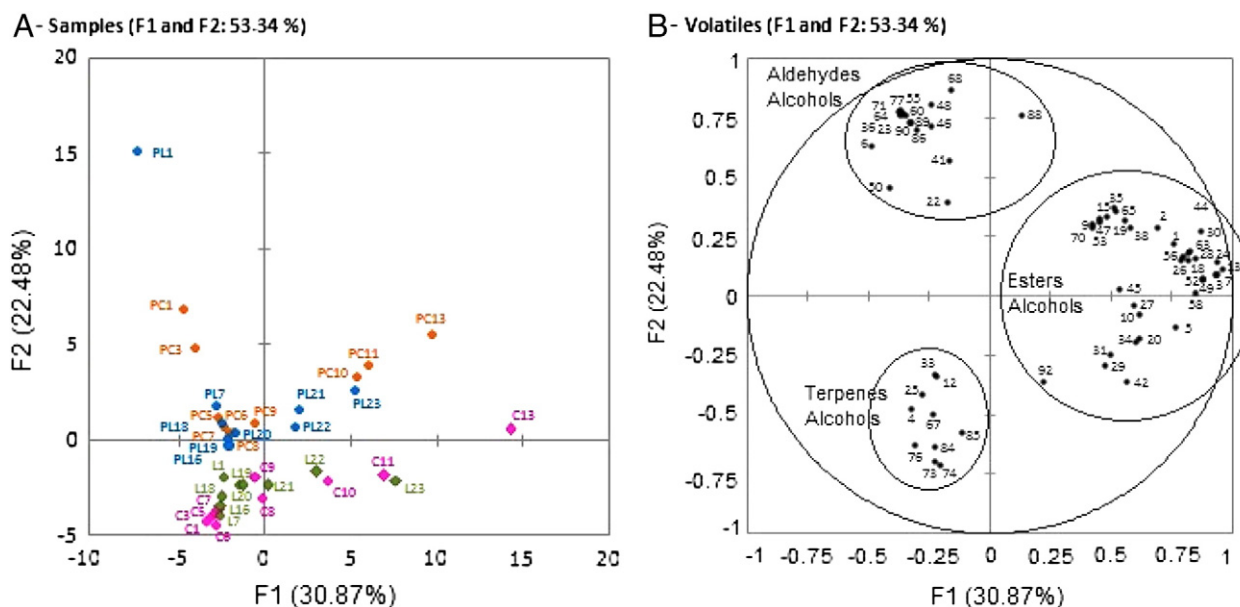
Fig. 5. Principal component analysis of the banana volatile compounds from cv. Prata. Sample code: P = pulp from control group (19 °C) in red color; PL = pulp from cold group (low temperature, 10 °C) in blue color. C = whole fruit from control group (19 °C) in pink color; L = whole fruit from cold group (low temperature, 10 °C) in green color. The numbers 1 to 24 refer to day after harvest (DAH). Ripe fruit (19 °C): 10th, 11th and 13th DAH. Ripe fruit (10 °C): 21st, 22nd and 23rd DAH. Volatile numbers refer to compounds in Table A.1 (supplementary data).

cold storage group with cv. Nanicão drastically affected by storage temperature.

On the other hand, cv. Prata seemed to be tolerant to low temperatures (10 °C), with a lower impact observed with respect to their volatile constituents. Since the volatile compounds are responsible for fruit aroma, this attribute is important in relation to its consumer acceptability. This knowledge can be of interest for the industry and exportation, where transport conditions feature low temperatures, with a view to choose the cultivar with less impact in the aroma composition.

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References

Agopian, R. G. D., Peroni-Okita, F. H. G., Soares, C. A., Mainardi, J. A., Nascimento, J. R. O., Cordenunsi, B. R., et al. (2011). Low temperature induced changes in activity and protein levels of the enzymes associated to conversion of starch to sucrose in banana fruit. *Postharvest Biology and Technology, 62*, 133–140.

Agopian, R. G. D., Purgatto, E., Cordenunsi, B. R., & Lajolo, F. M. (2009). Synthesis of fructooligosaccharides in banana 'Prata' and its relation to invertase activity and sucrose accumulation. *Journal of Agricultural and Food Chemistry, 57*, 10765–10771.

Arthur, C. L., & Pawliszyn, J. (1990). Solid-phase microextraction with thermal desorption using fused silica optical fibers. *Analytical Chemistry, 62*, 2145–2148.

Belitz, H. D., Grosch, W., & Schieberle, P. (2009). *Food chemistry* (4th ed.). Berlin: Springer-Verlag (Chapter 5).

Berger, R. C. (1991). Fruits I. In H. Maarse (Ed.), *Volatile compounds in foods and beverages* (pp. 283–304). New York: Marcel Dekker Inc.

Brereton, R. G. (2007). *Applied chemometrics for scientists* (pp. 145–248). Chinchester: John & Wiley Sons Ltd.

Bugaud, C., Alter, P., Daribo, M. O., & Brillouet, J. M. (2009). Comparison of the physico-chemical characteristics of a new triploid banana hybrid, FLHORBAN 920, and the Cavendish variety. *Journal of the Science of Food and Agriculture, 89*, 407–413.

Cano, M. P., Ancos, B., Matallana, M. C., Cámara, M., Reglero, G., & Tabera, J. (1997). Differences among Spanish and Latin-American banana cultivars: morphological, chemical and sensory characteristics. *Food Chemistry, 59*, 411–419.

Hazzit, M., Baaliouamer, A., Faleiro, M. L., & Miguel, M. G. (2006). Composition of the essential oils of Thymus and Origanum species from Algeria and their antioxidant and antimicrobial activities. *Journal of Agricultural and Food Chemistry, 54*, 6314–6321.

Jiang, Y., Joyce, D. C., Jiang, W., & Lu, W. (2004). Effects of chilling temperatures on ethylene binding by banana fruit. *Journal of Plant Growth Regulation, 43*, 109–115.

Jiang, Y., & Song, J. (2010). Fruits and fruit flavor: Classification and biological characterization. In Y. H. Hui (Ed.), *Handbook of fruit and vegetable flavours* (pp. 3–24). New Jersey: John Wiley & Sons, Inc.

Jordan, M. J., Tandon, K., Shaw, P. E., & Goodner, K. L. (2001). Aromatic profile of aqueous banana essence and banana fruit by gas chromatography–mass spectrometry (GC–MS) and gas chromatography–olfactometry (GC–O). *Journal of Agricultural and Food Chemistry, 49*, 4813–4817.

Kaul, V. K., Gujral, R. K., & Singh, B. (1999). Volatile constituents of the essential oil of flowers of *Rosa brunonii* Lindl. *Flavour and Fragrance Journal, 14*, 9–11.

Kerbauy, G. B. (2008). Frutificação e amadurecimento. In G. B. Kerbauy (Ed.), *Fisiologia vegetal* (pp. 358–383). Rio de Janeiro: Guanabara Koogan.

Lichtemberg, L. A., Malburg, J. L., & Hinz, R. H. (2001). Cold damage in bananas. *Revista Brasileira de Fruticultura, 23*, 568–572.

Lindsay, R. C. (1996). Flavors. In O. W. Fenema (Ed.), *Food chemistry* (pp. 723–765). New York: Marcel Dekker Inc.

Liu, T., & Yang, T. (2002). Optimization of solid-phase microextraction analysis for studying change of headspace flavor compounds of banana during ripening. *Journal of Agricultural and Food Chemistry, 50*, 653–657.

Manly, B. J. F. (2008). *Métodos estatísticos multivariados: uma introdução* (pp. 13–46). Porto Alegre: Artmed-Bookman.

McGorrin, R. G. (2002). Character impact compounds: Flavors and off-flavors in foods. In R. Marsili (Ed.), *Flavor, fragrance and odor analysis* (pp. 375–413). New York: Marcel Dekker Inc.

Mjos, S. A., Meier, S., & Boitsov, S. (2006). Alkylphenol retention indices. *Journal of Chromatography. A, 1123*, 98–105.

Nguyen, T. B., Ketsa, S., & Van Doorn, W. G. (2003). Relationship between browning and the activities of polyphenol oxidase and phenylalanine ammonia lyase in banana peel during low temperature storage. *Postharvest Biology and Technology, 30*, 187–193.

Nogueira, J. M., Fernandes, P. J., & Nascimento, A. M. (2003). Composition of volatiles of banana cultivars from Madeira Island. *Phytochemical Analysis, 14*, 87–90.

Pérez, A. G., Cert, A., Rios, J. J., & Olias, J. M. (1997). Free and glycosidically bound volatile compounds from two banana cultivars: Valery and Pequena Enana. *Journal of Agricultural and Food Chemistry, 45*, 4393–4397.

Rodriguez-Amaya, D. B. (2003). Rotas bioquímicas e químicas para a formação de compostos voláteis em alimentos. In M. R. B. Franco (Ed.), *Aroma e sabor: temas atuais* (pp. 177–194). São Paulo: Livraria Varela.

Salmon, B., Martin, G. J., Remaud, G., & Fourel, F. (1996). Compositional and isotopic studies of fruit flavours. Part I. The banana aroma. *Flavour and Fragrance Journal, 11*, 353–359.

Schrader, J. (2007). Microbial flavour products. In R. G. Berger (Ed.), *Flavours and fragrances: Chemistry, bioprocessing and sustainability* (pp. 507–571). Berlin Heidelberg New York: Springer.

Shiota, H. (1993). New esteric compounds in the volatiles of banana fruit (*Musa sapientum* L.). *Journal of Agricultural and Food Chemistry, 41*, 2056–2062.

Song, J., & Bangerth, F. (2003). Fatty acids as precursors for aroma volatile biosynthesis in pre-climacteric and climacteric apple fruit. *Postharvest Biology and Technology*, *20*, 113–121.

Tayoub, G., Schwob, I., Bessiere, J. M., Masotti, V., Rabier, J., Ruzzier, M., et al. (2006). Composition of volatile oils of Styrax (*Styrax officinalis* L.) leaves at different phenological stages. *Biochemical Systematics and Ecology*, *34*, 705–709.

Tressl, R., & Drawertl, F. (1973). Biogenesis of banana volatiles. *Journal of Agricultural and Food Chemistry*, *21*, 560–565.

Vas, G., & Vékey, K. (2004). Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. *Journal of Mass Spectrometry*, *39*, 233–254.

Vermeir, S., Hertog, M. L. A. T. M., Vankerschaver, K., Swennen, R., Nicolai, B. M., & Lammertyn, J. (2009). Instrumental based flavour characterization of banana fruit. *LWT- Food Science and Technology*, *42*, 1647–1653.

Wendakoon, S. K., Ueda, Y., Imahori, Y., & Ishimaru, M. (2006). Effect of short-term anaerobic conditions on the production of volatiles, activity of alcohol acetyltransferase and other quality traits of ripened bananas. *Journal of the Science of Food and Agriculture*, *86*, 1475–1480.

Wills, R., Mcglasson, B., Graham, D., & Joyce, D. C. (1998). *Postharvest: An introduction to the physiology & handling of fruit, vegetables & ornamentals. (pp 60–76)* (4th ed.). Wallingford: New South Wales University Press.

Yang, F. S., Ashrafuzzaman, M. D., Nakamura, N., & Hayashi, N. (2000). Purification and characterization of polyphenol oxidase from banana. *Journal of Agricultural and Food Chemistry*, *48*, 2732–2735.

Zhang, H., Yang, S., Joyce, D. C., Jiang, Y., Qu, H., & Duan, X. (2010). Physiology and quality response of harvested banana fruit to cold shock. *Postharvest Biology and Technology*, *55*, 154–159.