Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 90 (2005) 705–711
Food Chemistry
www.elsevier.com/locate/foodchem

Bioactive amines and carbohydrate changes during ripening of 'Prata' banana (*Musa acuminata* × *M. balbisiana*)

Regina C. Adão, M. Beatriz A. Glória *

Departamento de Alimentos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Olegário Maciel 2360, Belo Horizonte, MG 30180-112, Brazil

Received 18 July 2003; accepted 14 May 2004

Abstract

Green bananas were harvested at the full three-quarter stage, conditioned in polyethylene and stored for 35 days at 16 ± 1 °C and 85% relative humidity. Peel colour changed with time. The yellow colour ideal for consumption was achieved at 21 days and, after 28 days, brown specks started to appear. There was a significant increase in the pulp-to-peel ratio. The green fruit had high starch and low soluble sugars levels. Starch levels decreased significantly throughout ripening. At the seventh day of storage sucrose was prevalent, however, fructose and glucose levels increased while sucrose remained constant, decreasing after 28 days. Starch loss followed a first order reaction. Formation of glucose and fructose followed zero order kinetics with higher rate for fructose. The bioactive amines detected were putrescine, spermidine and serotonin. Serotonin decreased significantly after the 14th day of storage. Putrescine levels were similar up to 21 days and decreased significantly thereafter.

Keywords: Bioactive amines; Serotonin; Starch; Soluble sugars; Banana; Ripening

1. Introduction

Banana (*Musa* spp.) is one of the most important fruit crops in Brazil (FAO, 1999). Brazil is the second worldwide producer, contributing 11% of the total production and the highest per capita consumption (50–60 kg/year). Banana is the most consumed fruit in the world. It constitutes a valuable source of energy, vitamins and minerals and an important food component throughout the world.

Through breeding, several different banana varieties are available worldwide, with well-known agronomic characteristics and organoleptic properties such as colour, size, texture, sweetness and flavour. According to Mota, Lajolo, and Cordenunsi (1997), ripened bananas of different varieties have distinct starch levels and different soluble sugar profiles and contents. However, little is known about the metabolic changes of some varieties during storage and ripening and its consequences in the final product.

During ripening there are changes in appearance, texture and chemical composition of banana. The colour of the peel goes from green to yellow. There is migration of water from the peel to the pulp and degradation of starch, which can soften the pulp. There is also production and accumulation of low molecular weight sugars. These changes contribute to the appearance, desirable sweetness and eating quality of the ripened banana (Marriot, 1980; Mota et al., 1997).

According to Forsyth (1980) and to Hubbard, Pharr, and Huber (1990), the most relevant biochemical change occurring during banana ripening is the conversion of starch into simple sugars. Starch level in banana decrease to very low levels with ripening. Simultaneously, there is an increase in soluble sugars content from 1 to 20 g/100 g. The soluble sugars detected in ripened banana are mainly sucrose, glucose and fructose (Fernandes, Carvalho, & Cal-Vidal, 1979; Forsyth, 1980; Mota et al., 1997; Terra, Garcia, & Lajolo, 1983). The relative proportions of these constituents depends on the

* Corresponding author. Tel.: +55-31-3339-7616; fax: +55-31-3339-7632.
E-mail address: beatriz@farmacia.ufmg.br (M.B.A. Glória).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.05.020

stage of maturity and on the variety (Loeseck, 1950). Investigations by Terra et al. (1983) indicated that, in *Musa acuminata*, as starch was degraded, sucrose content increased and preceded formation of glucose and fructose. The hexoses which appear after sucrose, ultimately exceed sucrose concentration (Hubbard et al., 1990). According to Terra et al. (1983), amylases, glycosidases, phosphorylases, sucrose synthase and invertase can act in the degradation of starch and in the formation and accumulation of soluble sugars.

The presence of bioactive amines has been reported in different edible fruits. According to Marriot (1980), Smith (1980–81) and Coutts, Baker, and Pasutto (1986), dopamine, noradrenaline, octopamine, serotonin, histamine and 2-phenylethylamine have been detected in banana and banana products. Amines such as histamine, phenylethylamine and serotonin can act as protecting substances, deterring insects and molds (Glória, 2003). Dopamine and noradrenaline are susceptible to enzymatic browning, being responsible for such reactions in banana (Marriot, 1980). According to Coutts et al. (1986), serotonin is involved in the regulation of a number of important functions in men, including sleeping, thirst, hunger, mood and sexual activity.

According to Foy and Parrat (1962), the levels of noradrenaline, serotonin and dopamine in plantain and in 'matoke' (*M. sapientum* var. *paradisiaca*) increase with ripening, decreasing in the over-ripe fruit. However, Vettorazi (1974) reported that serotonin levels decreased during ripening of *M. cavendish*.

'Prata' is one of the most consumed banana in Brazil. There is contradictory information on sugars (Fernandes et al., 1979; Mota et al., 1997; Torres & Brandão, 1991) and no information on bioactive amines in the green fruit and changes which occurs during ripening. The objective of this work was to follow changes in colour, pulp-to-peel ratio, starch, soluble sugars and bioactive amines during storage of 'Prata' banana at 16 ± 1 °C and 85% relative humidity for 35 days. Such information will allow understanding on how these changes affect eating quality and shelf life of banana.

2. Materials and methods

2.1. Materials

Samples of 'Prata' banana (*M. acuminata* × *M. balbisiana*) were provided by 'Fazenda Experimental – EPAMIG' in Janaúba, state of Minas Gerais, Brazil. Bananas of the second bunch from the stalk were harvested green at the full three-quarter stage (34 mm diameter). The fruits were conditioned in polyethylene boxes and stored at 16 ± 1 °C and 85% relative humidity. Samples were collected at seven day intervals and analyzed for peel colour, pulp/peel ratio, levels of starch, soluble sugars and bioactive amines. The fruits were harvested at three different times throughout the year.

2.2. Methods of analysis

2.2.1. Peel colour

The colour of the peel was monitored by comparison with a scale developed by Loeseck (1950) for 'Prata' banana ripening. The scale ranged from 1 to 8, with values representing, respectively: Green (1), traces of yellow (2), more green than yellow (3), more yellow than green (4), yellow with green endings (5), completely yellow (6), yellow with slight brown specks (7), and yellow with more brown specks (8).

2.2.2. Pulp-to-peel ratio

The pulp-to-peel ratio was determined by weighing the parts of individual samples in an analytical balance. The results were expressed as percent pulp relative to peel weights.

2.2.3. Starch

The samples were defatted with diethyl ether, and the soluble sugars were removed with 80% ethanol at 80 °C (several successive extractions). After drying the residue at 70 °C, the starch was hydrolyzed with 0.25 M sulfuric acid at 100 °C for 1 h. The hydrolysate was mixed with 9,10-dihydro-9-oxoanthracene (anthrone) under acidic conditions (0.1 g/100 ml of 76% sulfuric acid), heated for 10 min and cooled. A blank was prepared with distilled water instead of sample extract. The absorbance was determined at 620 nm in a Shimadzu UV–Vis 160A spectrophotometer (Kyoto, Japan). The concentration of glucose was calculated from standard curves built periodically ($r^2 \geqslant 0.9937$). Starch levels (g/100 g fresh pulp) were determined by multiplying glucose concentrations by the conversion factor 0.9 (Stevens & Chapman, 1955).

2.2.4. Soluble sugars

The soluble sugars were extracted from samples and analyzed by HPLC according to the procedure described by Conrad and Palmer (1976). The samples were refluxed in 80% ethanol. The extract was filtered and the filtrate was used for HPLC analysis. The HPLC system consisted of a SCL-8A pump, a Rheodyne manual injector with a 20 μl loop, a Shim-pack CLC-NH_2 column (150×4 mm), a differential refractive index detector model RID-6A and an integrator model C-R4A (Shimadzu Corp., Kyoto, Japan). The sugars were separated using a mobile phase of acetonitrile:water (75:25) at a flow rate of 0.7 ml/min. The identification of the sugars was performed by comparison of retention time of peaks in samples with those in the standard solution containing fructose, glucose, sucrose, galactose and maltose. Addition of the suspected sugar to the sample was used

for the confirmation of peak identity. Quantification was performed by means of external standard curves ($r^2 \geqslant 0.9958$). Results were expressed as grams of sugar/100 g of sample. Determination limit for the sugars was 0.10 g/100 g.

2.2.5. Bioactive amines

Samples were analyzed for bioactive amines, among them, spermine, spermidine, agmatine, putrescine, cadaverine, histamine, tyramine, dopamine, tryptamine, 2-phenylethylamine and serotonin, according to the procedure described by Vale and Glória (1997).

The amines were extracted from the samples with 5% trichloroacetic acid and filtered through a 0.45 μm filter prior to HPLC analysis. The HPLC system consisted of two LC-10AD pumps; a Rheodyne manual injector with a 20 μl loop, μBondapak C18 (300 × 3.9 mm) and guard columns (Waters, Milford, MA, USA), a post-column derivatization apparatus, a fluorescence detector model RF-551 at 340 and 445 nm of excitation and emission, respectively; a CBM-10AD controller; and a Pentium PC (Shimadzu, Kyoto, Japan). The separation of amines was achieved by using a gradient elution of solution A in B at a flow rate of 0.8 ml/min. Solution A consisted of 0.1 M acetate buffer with 10 mM octanesulfonic acid sodium salt, pH adjusted to 5.2. Solution B was composed of 0.2 M acetate buffer, with 10 mM octanesulfonic acid sodium salt, pH adjusted to 4.5:acetonitrile, 66:34, v/v. The derivatization solution, prepared by mixing 25 g of boric acid, 22 g of potassium hydroxide, 0.2 g *o*-phthaldialdehyde, 1.5 ml Brij-35 and 1.5 ml mercaptoethanol in 500 ml HPLC water, was pumped at 0.4 ml/min. The identification of amines was possible by comparing retention times with those of standards and by adding the suspected amine to the sample. The concentration of amines was calculated by interpolation from standard curves ($r^2 \geqslant 0.9926$) and expressed in mg of amine/100 g of sample. Determination limits were 0.04 mg/100 g for putrescine, cadaverine, histamine, spermine, spermidine and 2-phenylethylamine; 0.06 mg/100 g for tyramine, dopamine and tryptamine; and 0.08 mg/100 g for serotonin and agmatine (Vale & Glória, 1997).

2.3. Statistical analysis

The data was subjected to analysis of variance and the means were compared by the Duncan test at 5% of probability.

3. Results and discussion

3.1. Peel colour

The results obtained for the colour of the peel are shown in Fig. 1. When harvested, the colour of the peel was green (grade 1). During ripening there was a gradual change to yellow. In 21 days, the colour achieved grade 6 to 7, which is considered the ideal stage for consumption of 'Prata' banana (Vieira, 1995). After that time, brown specks started to appear, increasing with time. The appearance of specks negatively affected the appearance of the banana, indicating an over-ripe product.

3.2. Pulp-to-peel ratio

The pulp-to-peel ratio of the green banana at the beginning of the experiment (34 mm diameter) was 1.49

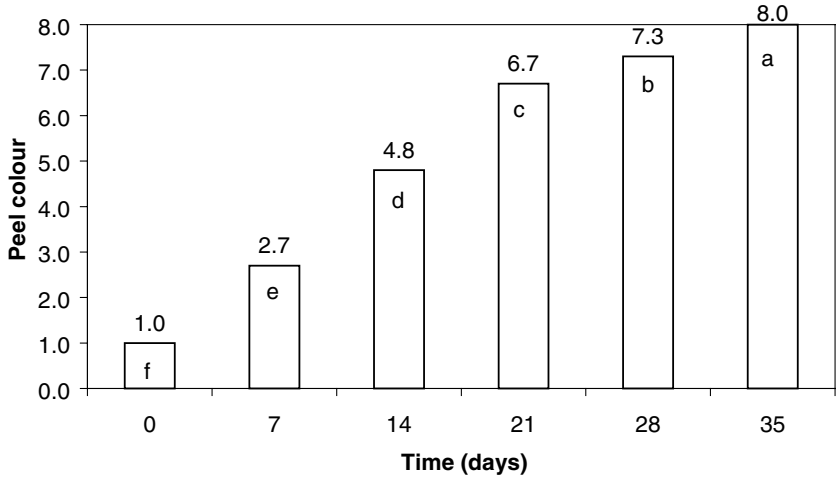

Fig. 1. Values attributed to the colour of the peel of 'Prata' banana during storage at 16 ± 1 °C and 85% relative humidity. Scale according to Loeseck (1950): (1) green, (2) traces of yellow, (3) more green than yellow, (4) more yellow than green, (5) yellow with green endings, (6) completely yellow, (7) yellow with slight brow specks, (8) yellow with more brown specks. Values with the same letter do not differ significantly by the Duncan test at 5% probability. (For interpretation of the references to colour in this figure legend, the reader is referred to the web of this article.)

(Fig. 2). During ripening, there was a significant increase in the pulp-to-peel ratio with time, reaching 2.10 at the ideal stage of consumption (21 days of storage) and 2.29 at 35 days of storage. These results are similar to those reported by Loeseck (1950) and Fernandes et al. (1979). According to Loeseck (1950) and Fernandes et al. (1979), there is an increase in water content of the pulp, derived from carbohydrates utilized in respiration and osmotic transfer from the peel to the pulp. This happens as a marked difference in osmotic pressure between peel and pulp develops during ripening, because sugar content increases more rapidly in the pulp than in the peel.

3.3. Starch levels

The mean level of starch in the green banana was 15.7 g/100 g (Fig. 3). During ripening it decreased significantly to 3.40 g/100 g at 21 days (ideal stage of consumption) and to 2.27 g/100 g at 35 days of storage.

The starch levels detected in this study are lower than those reported by Mota et al. (1997) for 'Prata' banana (17.4–18.7 g/100 g) in green fruits, but similar to the levels found by these investigators for ripened banana, e.g. 2.5–5.2 g/100 g. Furthermore, starch levels are similar to the values reported by Fernandes et al. (1979). According to Loeseck (1950), starch levels can vary with stage of maturity, variety of fruit, cultivation and ripening conditions.

3.4. Soluble sugars

Three types of soluble sugars were detected in 'Prata' banana – fructose, glucose and sucrose. The total soluble sugars in the green fruit (34 mm diameter) was 1.26 g/100 g (Fig. 3). It increased significantly to 14.3 g/100 g in 21 days; however, no significant difference was observed up to 35 days of storage (14.8 g/100 g).

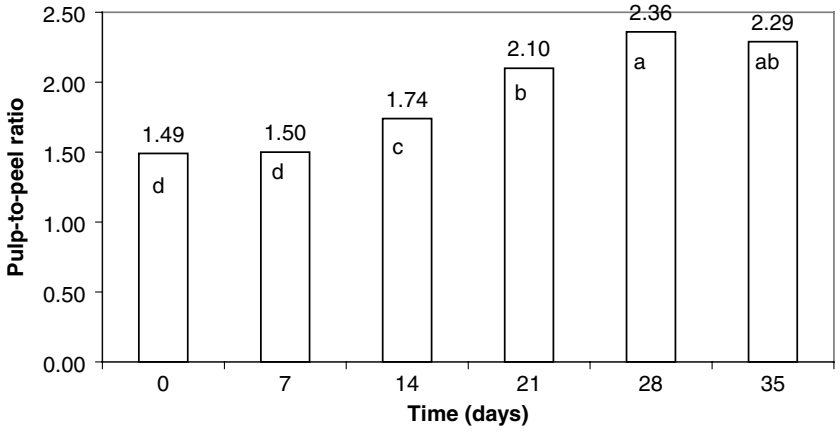

Fig. 2. Pulp-to-peel ratio of 'Prata' banana during storage at 16 ± 1 °C and 85% relative humidity. Values with the same letter do not differ significantly by the Duncan test at 5% probability.

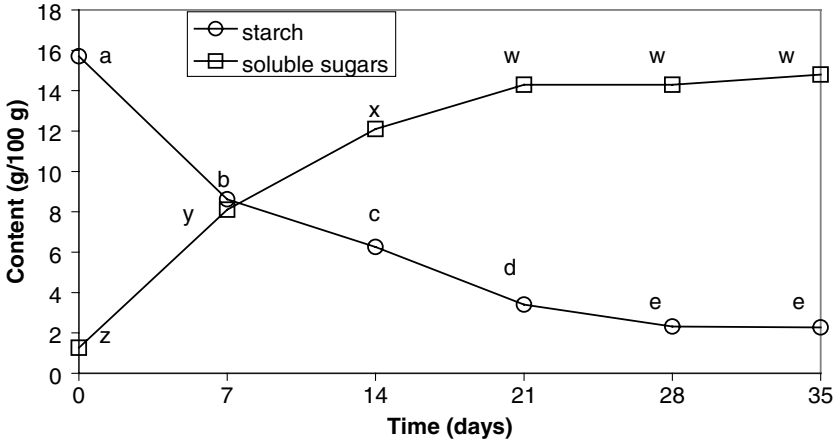

Fig. 3. Levels of starch and total soluble sugars in 'Prata' banana during storage at 16 ± 1 °C and 85% relative humidity. Values with the same letter (abcde for starch and wxyz for soluble sugars) do not differ significantly by the Duncan test at 5% probability.

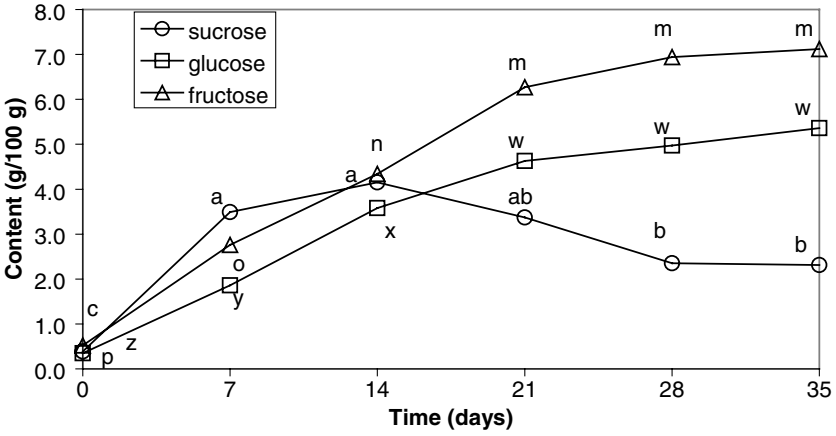

Fig. 4. Levels of sucrose, fructose and glucose in 'Prata' banana during storage at 16 ± 1 °C and 85% relative humidity. Values with the same letter (ab for sucrose, wxyz for glucose and mnop for fructose) do not differ significantly by the Duncan test at 5% probability.

The green banana contained 0.52, 0.35 and 0.39 g/100 g of fructose, glucose and sucrose, respectively (Fig. 4). At seven days of storage, sucrose levels increased significantly to 3.49 g/100 g, however, no significant difference was observed in levels from the 7th to the 21st day, but levels decreased significantly until the 35th day of storage.

Up to 21 days of storage, when peel colour reached 6, which is considered the ideal stage for consumption, there was a significant increase in the levels of fructose and glucose with time, reaching levels of 6.27 and 4.63 g/100 g, respectively. No significant difference was observed in the levels of these sugars from the 21st to the 35th day of storage.

According to these results, up to the first seven days of ripening, sucrose was the prevailing soluble sugar. However, after that time, fructose, followed by glucose, were the predominant soluble sugars. These results are similar to the findings of Terra et al. (1983) and Hubbard et al. (1990) for *M. acuminata* and *M. cavendish*, respectively. According to these authors, sucrose is the predominant sugar during the early stages of banana ripening; however, sucrose levels are lower than other sugars in the ripened fruits. Fernandes et al. (1979) also observed that sucrose levels were lower than glucose and fructose levels in ripened 'Prata' banana.

Kinetic studies (Table 1) of the degradation of starch indicated a first order reaction with a rate constant of −0.058 g/day and a half life ($t_{1/2}$) of 12 days. The formation of glucose and fructose followed zero order kinetics with reaction rates of 0.145 and 0.194 g/day, respectively. Therefore, fructose was formed at a faster rate which is consistent with the higher fructose levels accumulated in the ripened fruit.

3.5. Bioactive amines

Among the 11 bioactive amines investigated, only six were detected in 'Prata' banana, among them putrescine, serotonin, spermidine, spermine, dopamine and tyramine. The last three amines were present at very low levels, below the quantification limit of the analytical technique.

Serotonin, dopamine and tyramine were also detected in different varieties of banana by Udenfriend, Lovenberg, and Sjoerdsma (1959), Foy and Parrat (1962) and Vettorazi (1974). The presence of spermidine, spermine and putrescine was not reported in any of these studies; however, it is well known that these polyamines are naturally present in vegetables. They are implicated in a number of physiological processes, such as cell division regulation, plant growth, flowering, fruit development, response to stress and senescence (Glória, 2003; Ohta, Yoza, Takeda, & Nogata, 1993).

The total level of amines in green banana was 3.52 mg/100 g, decreasing significantly to 3.12 at 21 days (ideal stage for consumption) and to 1.99 mg/100 g at 35 days of storage. According to Fig. 5, the levels of serotonin

Table 1
Kinetic studies of the degradation of starch and formation of glucose and fructose during storage of 'Prata' banana at 16 ± 1 °C and 85% relative humidity

Compound	Reaction order	Kinetic equation[a]	Correlation coefficient
Starch	First order	ln Starch = −0.058t + 2.62	0.9070
Glucose	Zero order	Glucos = 0.145t + 0.93	0.9190
Fructose	Zero order	Fructose = 0.194t + 1.27	0.9280

[a] t = time in days, compound concentration in grams.

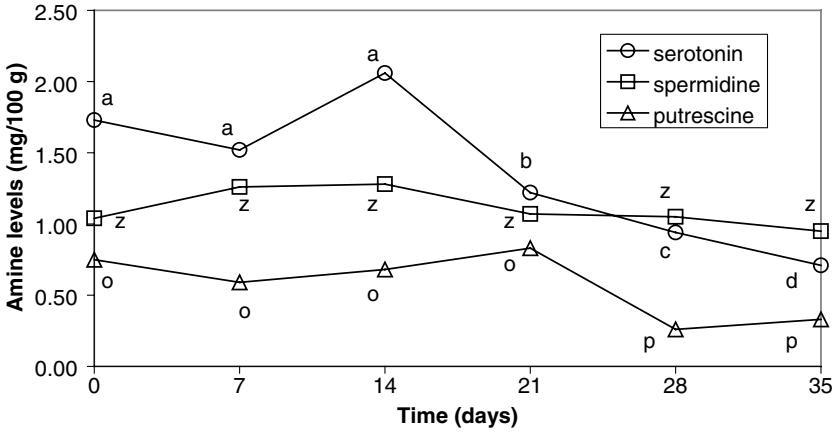

Fig. 5. Levels of the bioactive amines, serotonin, spermidine and putrescine, in 'Prata' banana during storage at 16 ± 1 °C and 85% relative humidity. Values with the same letter (abcd for serotonin, z for spermidine and op for putrescine) do not differ significantly by the Duncan test at 5% probability.

did not differ significantly up to the 14th day of storage, decreasing significantly afterwards. Different results were reported by Udenfriend et al. (1959) and by Vettorazi (1974) who observed a decrease in serotonin levels during ripening of *M. cavendish*, and of plantain, respectively. However, the results obtained by Foy and Parrat (1962) for plantain (*M. sapientum* var. *paradisiaca*), were similar to those obtained in this study, e.g. levels varied from 4.99 to 5.67 mg/100 g during ripening and decreased to 1.20 mg/100 g in the over-ripe fruit.

With respect to the polyamines, the levels of spermidine did not differ significantly throughout the storage time and the levels of putrescine did not differ up to 21 days, decreasing significantly thereafter. Many researchers, studying polyamine levels in fruits, have reported a decrease during fruit maturation (Esti et al., 1998; Ohta et al., 1993; Serrano, Martínez-Madrid, Riquelme, & Romojaro, 1995). According to these studies, polyamine and ethylene biosynthesis have a common intermediate, *S*-adenosylmethionine, thus polyamine concentration is expected to decrease as soon as the fruits begin to synthesize ethylene.

The contribution of each amine to the total level varied with storage time. In the green fruit, serotonin was the prevalent amine (49.2%), followed by spermidine (29.5%) and by putrescine (21.3%). In 21 days of storage, at the ideal consumption stage, there was a decrease in serotonin contribution (39.1%) and an increase in putrescine (26.6%) to total amine levels. At 35 days, spermidine was the prevalent amine (47.7%), followed by serotonin (35.7%) and by putrescine (16.6%).

4. Conclusions

During storage, at 16 ± 1 °C and 85% relative humidity, of 'Prata' banana harvested in the green stage (34 mm diameter), the following changes were observed. Peel colour changed from green to yellow, reaching the colour considered ideal for consumption at 21 days. There was a significant increase in the pulp-to-peel ratio up to 28 days. Starch level in the green fruit was 15.7 g/100 g. At ideal peel colour, starch levels decreased significantly to 3.4 g/100 g. The green fruit contained low levels of fructose, sucrose and glucose. In the first seven days of storage, sucrose was the prevalent sugar. However, after 14 days of storage, fructose and glucose were the predominant soluble sugars. Up to 21 days, the levels of fructose and glucose increased, remaining unchanged thereafter. Bioactive amines were quantified in 'Prata' banana for the first time. Putrescine, serotonin and spermidine were detected throughout storage. Traces of dopamine, tyramine and spermine were also detected. During storage, there was a decrease in serotonin levels after the 14th day and of putrescine after the 21st day of storage.

References

Conrad, C. E., & Palmer, K. J. (1976). Rapid analysis of carbohydrates by high pressure liquid chromatography. *Food Technology, 30*(10), 84–92.

Coutts, R. T., Baker, G. B., & Pasutto, F. M. (1986). Foodstuffs as sources of psychoactive amines and their precursors: Content, significance and identification. *Advances in Drug Research, 15*, 169–232.

Esti, M., Volpe, G., Massignan, L., Compagnone, D., Notte, E., & Palleschi, G. (1998). Determination of amines in fresh and modified atmosphere packaged fruits using electrochemical biosensors. *Journal of Agricultural and Food Chemistry, 46*, 4233–4237.

FAO (1999). Statistics Series 52. Yearbook Production. (Vol. 117, p. 254). Rome.

Fernandes, K. M., Carvalho, V. D., & Cal-Vidal, J. (1979). Physical changes during ripening of silver bananas. *Journal of Food Science, 44*(4), 1254–1255.

Forsyth, W. G. C. (1980). Banana and plantain. In *Tropical and subtropical fruits* (pp. 258–278). Westport: AVI Publishing.

Foy, J. M., & Parratt, J. R. (1962). Noradrenaline and 5-hydroxytryptamine in plantain (*Musa sapientum, var. paradisiaca*). *Journal of Pharmacy and Pharmacology, 13*, 360–364.

Glória, M. B. A. (2003). Amines. In: B. Caballero, L. Trugo, & P. M. Finglas (Eds.), *Encyclopedia of food sciences and nutrition* (2nd ed., pp. 173–181).

Hubbard, N. L., Pharr, D. M., & Huber, S. C. (1990). Role of sucrose phosphate synthase in sucrose biosynthesis in ripening bananas and its relationship to the respiratory climateric. *Plant Physiology, 94*, 201–208.

Loeseck, H. W. (1950). Chemical changes during ripening. *Bananas – chemistry, physiology and technology* (Vol. 4, pp. 67–118). New York: Interscience.

Marriot, J. (1980). Bananas – physiology and biochemistry of storage and ripening for optimum quality. *CRC Critical Reviews in Food Science and Nutrition*, 41–88.

Mota, R. V., Lajolo, F. M., & Cordenunsi, B. R. (1997). Composição em carbohidratos de alguns cultivares de banana (*Musa* spp.) durante o amadurecimento. *Ciência Tecnologia de Alimentes, 17*(2), 94–97.

Ohta, H., Yoza, K.-I., Takeda, Y., & Nogata, Y. (1993). Influence of storage temperature on the polyamine levels and ethylene production in broccoli (*Brassica oleracea*, Itálica group). *Bioscience Biotechnology and Biochemistry, 57*(5), 831–832.

Serrano, M., Martínez-Madrid, M. C., Riquelme, F., & Romojaro, F. (1995). Endogenous levels of polyamines and abscisic acid in pepper fruits during growth and ripening. *Physiologia Plantarum, 95*, 73–76.

Smith, T. A. (1980–81). Amines in foods. *Food Chemistry, 6*, 169–200.

Stevens, F. J., & Chapman, R. A. G. (1955). The determination of starch in meat products with the anthrone reagent. *Journal of AOAC International, 38*(2), 202–210.

Terra, N. N., Garcia, E., & Lajolo, F. M. (1983). Starch–sugar transformation during banana ripening: The behavior of UDP glucose pyrophosphorylase, sucrose synthetase and invertase. *Journal of Food Science, 48*, 1097–1100.

Torres, C. C., & Brandão, S. C. C. (1991). Determinação de açucares em bananas por cromatografia líquida de alta eficiência. *Boletim da Sociedade Brasileira de Cienciae Tecnologia, 25*(1), 1–5.

Udenfriend, S., Lovenberg, W., & Sjoerdsma, A. (1959). Physiologically active amines in common fruits and vegetables. *Archives of Biochemistry and Biophysics, 85*, 487–490.

Vale, S. R., & Glória, M. B. A. (1997). Determination of biogenic amines in cheese. *Journal of AOAC International, 80*(5), 1006–1012.

Vetorazzi, G. (1974). 5-Hydroxytryptamine content of bananas and banana products. *Food and Cosmetics Toxicology, 12*, 107–113.

Vieira, J. O. (1995). Efeitos da radiação gama em banana Prata (*Musa* sp., grupo AAB) irradiada em diferentes graus de maturidade e armazenada em condição ambiente e em câmara fria. Ph.D. Thesis, Piracicaba: USP, p. 112.