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Modified atmosphere together with refrigeration in the conservation of bananas resistant to black Sigatoka¹

Atmosfera modificada associada à refrigeração na conservação de frutos de bananas resistentes à Sigatoka Negra

Célia Lúcia Siqueira^{2*}, Hilário Junior de Almeida³, Miryan Franciele Pereira Serpa⁴, Paulo Sérgio Cardoso Batista² and Gisele Polete Mizobutsi²

ABSTRACT - The aim of this work was to evaluate the chemical, physical and sensory characteristics of the Caipira and Pakovan Ken cultivars of the banana (*Musa* spp.), resistant to black Sigatoka (*Mycosphaerella fijiensis* Morelet), both with and without low-density polyethylene wrapping and together with refrigeration during the period of post-harvest storage. The experiment was carried out in 2012, in a randomised block design and an arrangement of lots split over time. A 3 x 2 factorial scheme was used for the lots, consisting of fruit with no wrapping and fruit wrapped in 10 and 16 µm polyethylene film, and two cultivars, Caipira and Pakovan Ken. The sub-lots consisted of evaluations made at intervals of six days (0, 6, 12, 18 and 24) and of two days (0, 2, 4, 6 and 8), for fruit stored at temperatures of 12, 15 and 25 °C respectively. The following variables were evaluated: soluble solids, titratable acidity, ratio of soluble solids to titratable acidity, hydrogen potential, total soluble sugars, fruit colouration and sensory analysis. The polyethylene film together with the refrigeration enabled the fruit to be stored for 24 days, maintaining post-harvest characteristics suitable for consumption. The Pakovan Ken cultivar stood out when compared to the Caipira, with higher values for the fruit attributes under evaluation.

Key words: Musa spp.. Mycosphaerella fijiensis Morelet. Post-harvest. Polyethylene wrapping.

RESUMO - O objetivo do trabalho foi avaliar as características químicas, físicas e sensoriais dos frutos de banana (*Musa* spp.) cultivar Caipira e Pacovan Ken, resistentes à Sigatoka Negra (*Mycosphaerella fijiensis* Morelet), com e sem embalagem de polietileno de baixa densidade, associada à refrigeração durante o período de armazenamento pós-colheita. O experimento foi instalado em 2012, no delineamento de blocos casualizados em esquema de parcelas subdivididas no tempo, tendo nas parcelas um fatorial 3 x 2 referente aos frutos sem embalagem e com embalagem de polietileno de 10 e 16 µm e duas cultivares Caipira e Pakovan Ken, e nas subparcelas as avaliações em intervalos de seis dias (0; 6; 12; 18 e 24) e dois dias (0; 2; 4; 6 e 8), para frutos armazenados nas temperaturas de 12; 15 e 25 °C, respectivamente. As variáveis avaliadas foram: sólidos solúveis, acidez titulável, relação sólidos solúveis/ acidez titulável, potencial hidrogeniônico, açúcares solúveis totais, coloração do fruto e análise sensorial. A embalagem de polietileno associada à refrigeração manteve os frutos armazenados por 24 dias, mantendo as características pós-colheita adequadas ao consumo. A cultivar Pakovan Ken destacou-se quando comparada à Caipira, apresentando valores superiores nos atributos dos frutos avaliados.

Palavras-chave: Musa spp.. Mycosphaerella fijiensis Morelet. Pós-colheita. Embalagem de polietileno.

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INTRODUCTION

The banana is the most produced and appreciated fruit in the world, and Brazil is noteworthy in this scenario, producing 6,949,316 tonnes of fruit in in 2015 (IBGE, 2016). The fruit is of great socioeconomic importance for the north of the State of Minas Gerais. According to data from Epamig (2015), the production of 385.97 thousand tonnes of bananas, marketed for BRL 489.8 million, generated more than 38 thousand direct and indirect jobs. The north of Minas Gerais is the third largest banana-producing region in the country, due to suitable soil and climate conditions together with irrigation for banana farming. Despite this advantage, the main cultivars marketed in the country, and produced in the region, 'Prata', 'Prata Anã', 'Pacovan', 'Maçã' and 'Nanica', are susceptible to the Mycosphaerella fijiensis Morelet fungus, which causes black Sigatoka (RODRIGUES; LEITE, 2008). This is considered globally to be the most serious disease in the banana, and results in severe defoliation, compromising the active photosynthetic area and resulting in reduced plant growth and productivity, as well as compromising fruit quality due to early maturation (SANTOS et al., 2006). In an effort to reduce losses in the production and quality of the harvested fruit, one alternative, from an economic point of view and for preserving the environment, would be the strategy of using disease-resistant cultivars to reduce the risks of disease and lower production costs by utilising different inputs (PIMENTEL et al., 2010).

There exists a complete agronomic characterisation for the resistant genotypes, including vigour, productivity, plant height, pseudostem diameter, number of hands per bunch, and fruit weight, among others, but information on the physico-chemical and sensorial characteristics of the fruit are still not common in the literature. The physical and chemical variables, and the quality attributes, contribute to a profile of each variety of banana, and to the selection of less-susceptible genetic material for planting in breeding programs (BEZERRA, 2011).

According to Siqueira *et al.* (2010), some cultivars are already the target of research and analysis, such as 'Thap Maeo', 'Fhia-02', 'Tropical', 'PV-4285', 'Pakovan Ken' and 'Caipira'. In view of the above, it is essential to develop post-harvest technologies for new cultivars that are resistant to black Sigatoka, as planting such cultivars will be one of the main control measures to be adopted by producers.

The study of post-harvest techniques for handling the fruit of tolerant cultivars makes it possible to have a longer shelf life without loss of quality to the consumer, and avoids food waste (HOJO *et al.*, 2007; SCANAVACA JÚNIOR; FONSECA; PEREIRA, 2007; SERPA *et al.*,

2014). Among known postharvest techniques, a modified atmosphere, with the use of low-density polyethylene and edible biofilms, whether together with refrigeration or not, has shown positive results. Both the modified atmosphere and refrigeration reduce metabolic activity and the loss of water by the fruit and vegetables, maintaining their attractive appearance. Santos *et al.* (2011) reported that combining a modified atmosphere, and refrigeration with low temperature and high relative humidity, ensures the conservation of fresh fruit during transportation and storage.

The aim of this study therefore, was to evaluate the effects of a modified atmosphere together with refrigeration during fruit storage, with no loss of postharvest quality, in the banana cultivars Pakovan Ken and Caipira, both resistant to black Sigatoka.

MATERIAL AND METHODS

The fruit was harvested in an experimental area of the town of Jaíba, Minas Gerais (15°05'12.37" S, 44°00'58.35" W) in 2012. The banana cultivars Caipira and Pakovan Ken were used. Hands of bananas from randomly picked bunches were selected when the fruit reached physiological maturity. Colour index 2 (green fruit with traces of yellow) was used, as described in the visual colour scale by Dadzie and Orchard (1997). The hands were later immersed for 5 minutes in 15 L of a suspension of Sportak 450 CE post-harvest fungicide, at a dose of 0.280 mL L⁻¹, and the surfactant Iharaguem, at a concentration of 0.3 mL L⁻¹. The hands were then divided into bunches of three fruits, wrapped in either 10 um or 16µm film, or left unwrapped, placed in expanded polystyrene trays and stored in cold rooms at temperatures of 12, 15 and 25 °C, and at 90% relative humidity for all temperatures. The temperature of 12°C was tested to see whether chilling injury occurred in the bananas; if the fruit suffered no damage from the cold, this temperature would be ideal to prolong the storage period. The temperature of 15 °C is recommended in the literature, and 25 °C was used to simulate the ambient temperature.

The experiment was carried out in a randomised complete block design in lots split over time, with a 3x2 factorial for the lots (10 and 16µm polyethylene film, and no film x the cultivars Caipira and Pakovan Ken) and the five evaluation periods for the sub-lots, in six-day intervals for fruit stored at 12 and 15 °C, and two day intervals for fruit stored at 25 °C, with four replications and three fruits per replication.

The parameters under evaluation were: a) soluble solids (SS), using an Atago field refractometer with the result expressed in ^oBrix; b) titratable acidity (TA),

determined by titration with a 0.1 N NaOH solution and an indicator of 1% phenolphthalein, as per the standards of the Association of Official Analytical Chemists (AOAC, 2005); c) relationship of soluble solids to titratable acidity, obtained from the ratio of the soluble-solid content to the titratable acidity; d) hydrogen potential (pH), using a digital pH meter with a direct reading of the pH value of the extract; e) total soluble sugars (TSS), determined with the methodology described by Somogyi, and adapted by Nelson (1944); f) fruit colouration, determined using the Loesecke scale (1980); g) for the sensory analysis, 30 nontrained tasters were recruited from the State University of Montes Claros, Janaúba campus. The tasters gave their opinion on attributes of aroma, pulp colour, taste and texture, using a hedonic scale of from 1 to 9 ('dislike extremely' and ''like extremely'), similar to that described by Villanueva and Da Silva (2009). The samples were placed in disposable cups and duly coded; the fruit used was from the final day of storage at 12 °C.

The data were submitted to analysis of variance by F-test, and when significant for the quantitative factors, regression analysis was carried out. The mean values for the cultivars, when significant, were compared by F-test at 5% significance. The regression equations were adjusted to find the best model to explain the phenomenon using the SISVAR statistical software (FERREIRA, 2011).

RESULTS AND DISCUSSION

Fruit of Pakovan Ken, with no wrapping and stored at 25 °C, reached 18.5 °Brix on the 8th day of storage (Figure 1A), a value greater than the 13.7 °Brix found in the cultivar Caipira (Figure 1B) stored under the same conditions. Both cultivars, stored at 25°C and wrapped in 16μm, film reached values for SS of 14.5 and 12.2 °Brix, which was less than the unwrapped fruit (Figures 1A and 1B) evaluated at 8 days after harvesting. At a temperature of 12 °C, fruit of the cultivar Pakovan Ken, stored for 24 days wrapped in 10 and 16µm film, achieved lower SS values than those found at 25 °C, reaching values equal to 9 and 8 °Brix respectively (Figure 1C). While the cultivar Caipira under the same conditions, displayed values of 10.1 and 7.0 °Brix respectively (Figure 1D). The lower values for SS show the direct effect of the polyethylene film in delaying ripening in response to the decrease in the metabolic activity of the fruit, which is due to the atmospheric changes inside the wrappings, resulting in lower brix levels (SILVA et al., 2006).

There was a difference for fruit of the banana cultivar Pakovan Ken, wrapped and stored at 15 °C for 24 days, with the lowest SS content of 7 °Brix being recorded for the fruit stored in $16\mu m$ film (Figure 1E). On the other

hand, fruit of the Caipira cultivar had higher values for SS, 13 °Brix (Figure 1F), showing that the fruit matured more quickly, having the effect of a lower shelf life compared to the cultivar Pakovan Ken.

The titratable acidity (TA) of the banana fruit stored at 12, 15 and 25 °C increased for both cultivars (Figures 2A, 2B, 2C and 2D). The fruit stored at 25 °C showed no difference for packaging or cultivar; however, there was an increase in acidity with storage period (Figure 2A). At the end of storage, the unwrapped fruit stored at 12 °C had a higher recorded value for TA of 0.59%, which is higher than that found for the wrapped fruit (Figure 2B). At 12 and 15 °C, fruit of the Caipira banana showed higher values for TA than those of the Pacovan Ken banana (Figures 2C and 2D).

In the banana, unlike in most fruits, increases have been recorded in the titratable acidity of the fruit throughout the period of storage and ripening (ALENCAR et al., 2010). This situation, together with the increase in soluble-solid content, ensures an improvement of the appreciable sweet flavour of the fruit (MATSUURA et al., 2002). Data behaviour agrees with Sarmento et al. (2015), who found an increase in titratable acidity in fruit of the Prata Catarina banana during the storage period, covered in cassava starch or wrapped in polyvinyl chloride film (PVC), with lower values for the fruit wrapped in PVC film. For those authors, wrapping the fruit in PVC film slows the metabolism, delays the synthesis of organic acids, and increases the shelf life of the fruit.

Fruit of Pakovan Ken, stored at 25 °C with no wrapping, and wrapped in 10 and 16µm film (Figure 3A) reached greater values for SS/TA than fruit of the Caipira banana under the same conditions (Figure 3B). At the end of the evaluation, the relationship of SS to TA ranged from 22 to 30 and from 19 to 24 for the Caipira and Pacovan Ken cultivars respectively. The results for the SS/TA ratio showed similar behaviour to the soluble-solid content, increasing with the storage period, except for the fruit of Pacovan Ken and Caipira stored at 12°C with 16 and 10µm film respectively, which recorded constant values throughout the evaluation period (Figure 3C and 3D). These results guarantee an appreciable sweet flavour to the fruit, being one of the main parameters in the qualitative attributes of fruit flavour. Even finding similar results, the taste varies from cultivar to cultivar; such variations in fruit of the same species may occur due to several factors, such as the varieties analysed, soil and climate conditions, and cropping practices (CHITARRA, CHITARRA, 2005). Further, according to these authors, the relationship of soluble solids to titratable acidity is a representative index of the separate measurement of sugars or acidity, since it expresses the sugar/acid ratio, which results in the flavour of the fruit.

Most of the flavours presented by fruit are a result of a mixture of scores attributed to the sweet and acidic taste, where the proportion of sugar to acid can be accompanied naturally by the SS/TA ratio, allowing the fruits to ripen to the point where the sugars have increased and the acids decreased to the desired ratio (BEZERRA; DIAS, 2009).

Figure 1 - Soluble-solid content (SS), in the fruit of banana cultivars (*Musa* spp.) with no wrapping (NW) and wrapped in 10 and 16μm film; stored at 2 °C for 8 days: A) Pacovan Ken, B) Caipira; stored at 12 °C for 24 days: C) Pacovan Ken, D) Caipira; stored at 15 °C for 24 days: E) Pacovan Ken, F) Caipira. ŷ1: NE; ŷ2: 10μm; ŷ3: 16μm

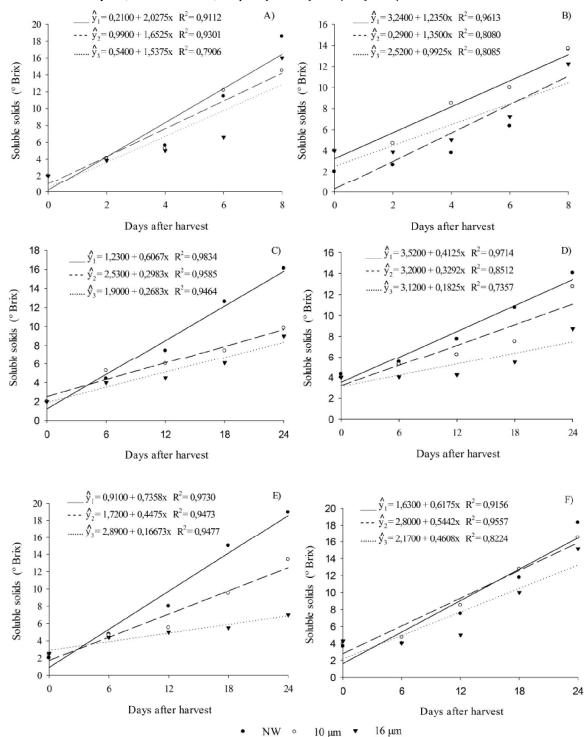
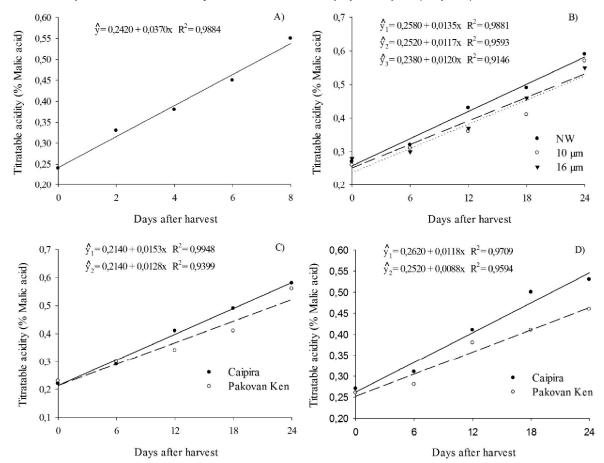


Figure 2 - Titratable acidity (TA), in the fruit of banana cultivars (*Musa* spp.) with no wrapping (NW) and wrapped in 10 and 16μm film. A) Pacovan Ken and Caipira stored at 25 °C for 8 days; B) NW, 10 and 16μm film stored at 12 °C for 24 days; C) Pacovan Ken and Caipira stored at 12 °C for 24 days; D) Pacovan Ken and Caipira stored at 15 °C for 24 days. ŷ1: NW; ŷ2: 10μm; ŷ3: 16μm



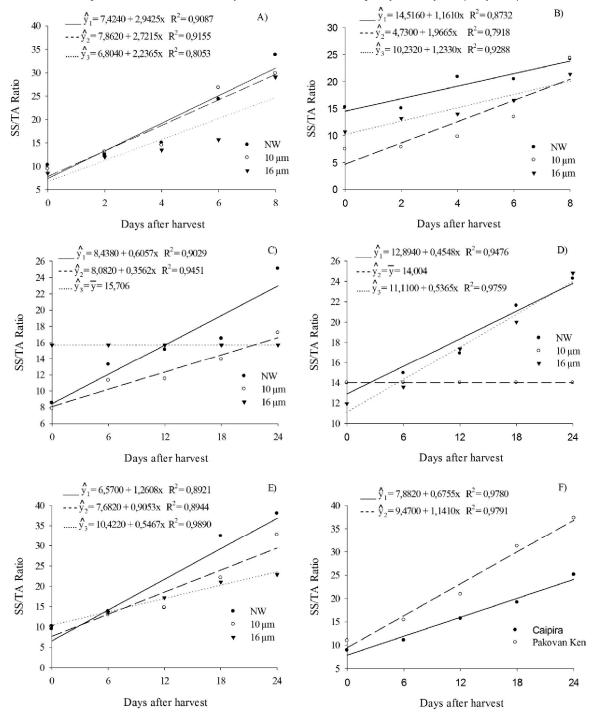
The three types of wrapping had different effects on the fruit that was stored at 15°C (Figure 3E). Furthermore, at 15 °C, higher values for the SS/TA ratio were seen for fruit of the Caipira banana when compared to Pacovan Ken (Figure 3F). A previous study had shown higher values for SS/TA in the same cultivars stored at 25 °C and a humidity of 80% (RIBEIRO *et al.*, 2012). According to the scientific report and to data from the present study, although the percentage of SS to TA of the fruit was different, it still presented values considered ideal for *in natura* consumption.

The pH decreased with fruit maturation at all temperatures tested (Figures 4A, 4B, 4C, 4C, 4D, 4E and 4F), except for the fruit of Pacovan Ken stored at 15 $^{\circ}$ C and wrapped in 10µm film, which remained constant throughout the period of storage (Figures 4E). In the fruit stored at 12 $^{\circ}$ C, the lowest reduction in pH was found for the 16µm film, with values at the end of the evaluation

of 4.90 and 4.84 for the cultivars Pacovan Ken and Caipira respectively (Figures 4C and 4D). These results are similar to those of 4.41 and 5.10 seen in fruit of the Pacovan Ken and Caipira banana by Ribeiro *et al.* (2012) under conventional cultivation in Cruz das Almas, in the State of Bahia.

During the evaluation period, there was an increase in the levels of total soluble sugars (TSS) for all treatments (Figures 5A, 5B, 5C, 5D and 5F). At 25 °C, the highest values for TSS were recorded in fruit of the Pacovan Ken cultivar in relation to the Caipira cultivar (Figures 5A and 5B). Fruit of the Pakovan Ken cultivar at a temperature of 12 °C displayed higher levels of TSS, especially the fruit with no wrapping (Figure 5C). At the same temperature (12 °C), fruit of the cultivar Caipira displayed a value for TSS of 17% for fruit with no wrapping, whereas the wrapping of 10 and 16μm film gave the lowest values for TSS of 13% and 11% respectively (Figure 5D).

Figure 3 - Relationship of soluble solids to titratable acidity (SS/TA), in the fruit of banana cultivars (*Musa* spp.) with no wrapping (NW) and wrapped in 10 and 16μm film; stored at 25 °C for 8 days: A) Pacovan Ken, B) Caipira; stored at 12 °C for 24 days: C) Pacovan Ken, D) Caipira; stored at 15 °C for 24 days: E) Pacovan Ken, F) Caipira. ŷ1: NW; ŷ2: 10μm; ŷ3: 16μm



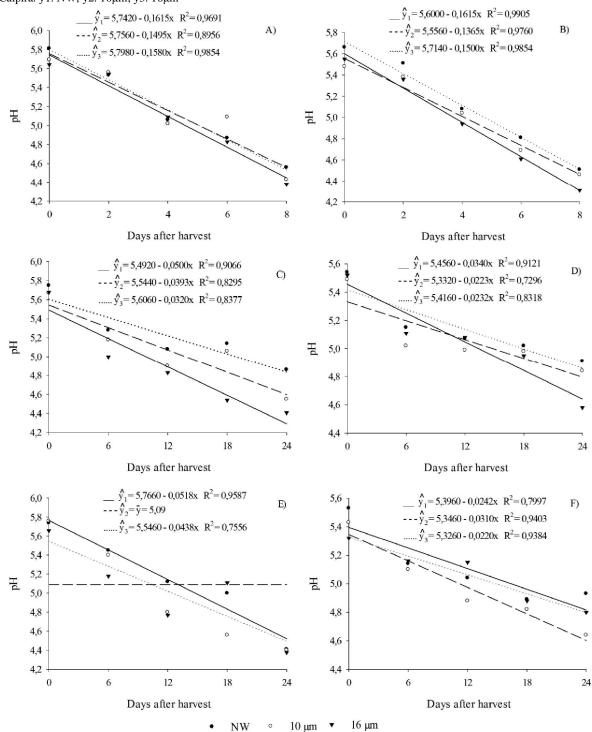
Fruit of the Pakovan Ken cultivar stored at 15 °C showed a TSS value of 19.68%, with a value of 17.85% in fruit of the Caipira cultivar (Figure 5E). The greatest accumulation of TSS found in the fruit from all

the treatments is related to the conversion of starch into sugars. To this effect, Carvalho *et al.* (2011) state that during maturation of the banana, changes take place in the appearance, texture and chemical composition,

characterised by the conversion of starch into sugars, with a consequent increase in soluble solids, sugars and titratable acidity in response to the reduction in pH.

Sugars usually make up 0.8 to 1% of the pulp in green bananas, increasing to 20 to 22% in the mature pulp, due to degradation of the starch (NASCIMENTO JÚNIOR

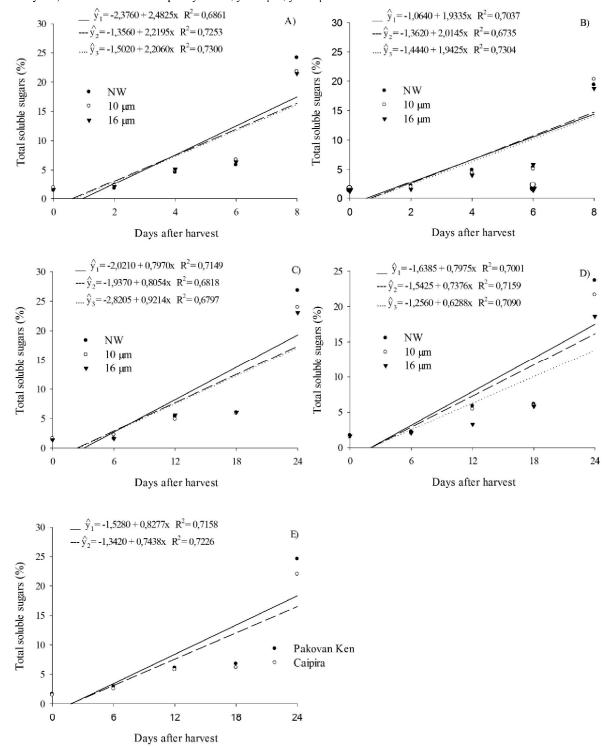
Figure 4 - pH in the fruit of banana cultivars (*Musa* spp.) with no wrapping (NW) and wrapped in 10 and 16μm film; stored at 25 °C for 8 days: A) Pacovan Ken, B) Caipira; stored at 12 °C for 24 days: C) Pacovan Ken, D) Caipira; stored at 15 °C for 24 days: E) Pacovan Ken, F) Caipira. ŷ1: NW; ŷ2: 10μm; ŷ3: 16μm



et al., 2008). Studies point out that evaluation of the same characteristics by different authors, who found differing results, may be associated with the soil and climate

conditions of the crop and to the stage of maturation when the fruits were harvested. The results of the present study reaffirm research conducted with the same cultivars,

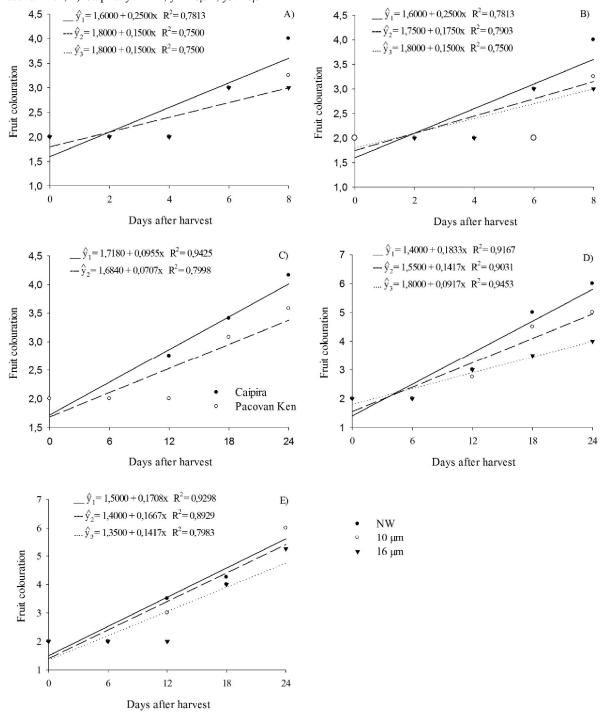
Figure 5 - Total soluble sugars (TSS) in the fruit of banana cultivars (*Musa* spp.) with no wrapping (NW) and wrapped in 10 and 16μm film; stored at 25 °C for 8 days: A) Pacovan Ken, B) Caipira; stored at 12° C for 24 days: C) Pacovan Ken, D) Caipira; stored at 15 °C for 24 days: E) Pacovan Ken and Caipira. ŷ1: NW; ŷ2: 10μm; ŷ3: 16μm



however under different climatic conditions (RAMOS; SARITA; MISCHAN, 2009).

Fruit stored with no wrapping displayed an increase in the yellowish colour of the skin, indicating the advance of maturation irrespective of cultivar or temperature (Figures 6A, 6B, 6C, 6D and 6E). Under such conditions, chlorophyll degradation by the enzyme chlorophyllase occurs in the pericarp, and carotenoid synthesis is reduced due to an increase in ethylene, increasing the speed of metabolism and accelerating maturation (YANG *et al.*, 2009). Fruit of

Figure 6 - Colouration in the fruit of banana cultivars (*Musa* spp.) with no wrapping (NW) and wrapped in 10 and 16μm film; stored at 25 °C for 8 days: A) Pacovan Ken, B) Caipira; stored at 12 °C for 24 days: C) Pacovan Ken and Caipira; stored at 15 °C for 24 days: D) Pacovan Ken, E) Caipira. ŷ1: NW; ŷ2: 10μm; ŷ3: 16μm



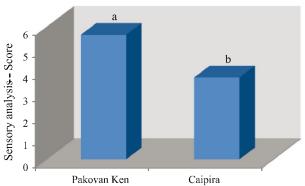
Pacovan Ken stored at 25 °C and wrapped in 10 and 16µm film showed a skin colouration of 3.2 and 3.0 (50% green and 50% yellow) respectively at the end of storage (Figure 6A). While the Caipira, cultivar stored under the same conditions had values of 3.2 (50% green and 50% yellow) and 2.8 (light green) respectively (Figure 6B).

Fruit of the Caipira cultivar stored at a temperature of 12 $^{\circ}$ C displayed more-advanced skin colouration at the end of 24 days when compared to the Pacovan Ken banana (Figure 6C). The cultivars stored at 15 $^{\circ}$ C and wrapped in 10 and 16 μ m film showed less change in skin colouration during the evaluation period in relation to fruit with no wrapping under the same conditions. The Pacovan Ken cultivar displayed the least skin colouration at the end of storage for the 10 and 16 μ m wrapping (Figure 6D and 6E).

This result is positive, as it presupposes that the technique resulted in the fruit having a longer shelf life, increasing the period of marketing, without losing the qualities desired for consumption. According to Silva *et al.* (2006), skin colouration is an important factor in evaluating the quality of the banana to be marketed, serving as a reference to establish the maturation stage of the fruit.

The results of the sensory analysis showed a difference between the cultivars Pacovan Ken 5.63 (neither like nor dislike) and Caipira 3.70 (dislike moderately); the former obtaining better results for acceptability from tasters in Janaúba in the north of the State of Minas Gerais (Figure 7). Previous studies under varying climatic conditions had already demonstrated the low acceptance of the cultivar Caipira when compared to other cultivars (CARVALHO *et al.*, 2011; SOUZA *et al.*, 2013). In sensory analysis, flavour is one of the main attributes, as it reflects consumer preference for the product. The results of the present study therefore reaffirm previous research, in which the cultivar Caipira was the least accepted by the tasters.

Figure 7 - Sensory analysis of fruit of the banana cultivars Pacovan Ken and Caipira



Mean values followed by the same letter do not differ by F-test at 5% probability

CONCLUSIONS

- 1. The modified atmosphere together with the temperatures of 15 and 12 °C delayed ripening in the Pacovan Ken and Caipira bananas;
- 2. The Pacovan Ken cultivar was more responsive to the ripening techniques, and more acceptable to the tasters than the Caipira cultivar.

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