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Harvest and post harvest

Ethanol effect associated with hydrothermal treatment on 'Tommy Atkins' mango's quality

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Abstract- One of the great challenges of the mango production chain is the maintenance of post-harvest quality of this fruit for export with a focus on food safety. This study evaluated the effect of the hydrothermal treatment associated to ethanol on mango's quality. Two tests were performed, in both were used 'Tommy Atkins' mangoes. In the first one, the heat treatment consisted of immersing the fruits in a bath at 46.1 °C / 75 minutes with ethanol concentrations of 0, 5, 10 and 20% v / v, then a cooling bath at 21.1 °C / 30 minutes and stored at 25 °C. In the second, fruits were submitted to the baths, but with ethanol concentrations of 0, 3, 5 and 7% v/v, and stored at 25°C and 10°C. Physical-chemical and phytopathological analysis and percentage of mango rottenness were evaluated. Mangoes treated with more than 10% ethanol (stored at 25°C), or those treated at ethanol concentrations below 7% (stored at 10°C) did not show good external appearance. It was concluded that ethanol at the concentrations of 3, 5 and 7% v/v associated to hydrothermal treatment did not affect the quality parameters of the fruits stored at 25ºC, but these treatments decreased the mangoes shelf life, since these fruits matured faster and are not recommended for exportation.
Index terms: Alcohol, *Mangifera indica*, Postharvest, Thermotherapy.

Efeito do etanol associado ao tratamento hidrotérmico na qualidade de manga cv. Tommy atkins

Resumo- Um grande desafio da cadeia produtiva da manga é a manutenção da qualidade pós-colheita desta fruta para exportação com o foco na segurança de alimentos. Este trabalho avaliou o efeito do tratamento hidrotérmico associado ao etanol sobre a qualidade da manga. Realizaram-se dois testes, e em ambos se utilizaram mangas 'Tommy Atkins'. No primeiro, o tratamento térmico consistiu na imersão dos frutos em banho a 46,1°C/75 minutos, com concentrações de etanol a 0; 5; 10 e 20% v/v, resfriamento em banho (21,1°C/30 minutos) e armazenados (25°C). No segundo, os frutos foram submetidos aos banhos, porém utilizaram-se concentrações de etanol a 0; 3; 5 e 7% v/v e armazenamento a 25°C e 10 ºC. Realizaram-se análises físico-químicas, fitopatológicas e porcentagem de podridão das mangas. Mangas tratadas com mais de 10% de etanol (armazenamento a 25°C), ou aquelas tratadas a concentrações de etanol inferiores a 7% (armazenados a 10ºC) não apresentaram boa aparência externa. Concluiu-se que o etanol, nas concentrações de 3; 5 e 7% v/v, associado à hidrotermia, não afetou os parâmetros de qualidade dos frutos armazenados a 25ºC, porém esses tratamentos diminuíram a vida de prateleira das mangas, uma vez que esses frutos amadureceram mais rápido, não sendo recomendados para tratamento das mangas para exportação.
Termos para indexação: Álcool, *Mangífera índica,* Pós-colheita, Termoterapia.

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Introduction

Mango (*Mangifera indica*), an important tropical fruit, with increasing export volume in Brazil, is considered a fruit of excellent sensorial characteristics. According to the Brazilian Yearbook of Fruticulture (2016), in 2015 were exported 122 000 tons of mango and in 2016, exports reached values in the order of US $137,588,916. However, the requirements of importers countries of *in natura* fruits have been increasing in relation to the sanitary quality of the product.

Fruits that are destined for the export market must pass through the post-harvest treatments that vary according to the requirements of the importing country. The use of hydrothermal treatment has been required to avoid problems with fruit fly; however, its good efficiency in fungus control and the prohibition of post-harvest fungicide use by the Food and Drug Administration (USDA-APHIS, 2002) caused it to be established as quarantine method for mangoes exported to the United States and Japan.

According to Lima et al. (2007) the appearance of the mango fruit is the most important factor of the success in its international commercialization, being the defects in the peel little tolerated. The peel should be perfect until reaching the final consumer, which is the biggest challenge in the export of this fruit, since it is fragile and in the lenticels usually shelter spores of Colletotrichum gloeosporioides Penz, causal agent of anthracnose as well as other pathogens.

There are indications that ethanol has potent antimicrobial activity, including positive effects on post-harvest disease control in several crops (PESIS, 2005).

Research indicates that ethanol may be an alternative for the elimination of phytopathogenic agents (KARABULUT et al., 2004; CHERVIN et al., 2005; MARGOSAN et al., 1994; MARGOSAN et al., 1997; GUTIÉRREZ-MARTÍNEZ et al., 2012).

This study aimed to evaluate the effect of hydrothermal treatment associated with ethanol on postharvest quality of 'Tommy Atkins' mango.

Material and methods

We used 380 mangoes 'Tommy Atkins' cv. from the city of Petrolina, Pernambuco at maturity stage 2, according to the scale proposed by Protrade (1992), considered as reference for the harvest of this cultivar.

The experiments were conducted in the Post-Harvest Engineering Group (GEPC) of the Institute of Food Technology (ITAL, Campinas-SP). In the laboratory, the fruits were divided into two trials: Test I - Laboratory Scale and Test II - Pilot Scale.

Evaluation of the ethanol evaporation during the hydrothermal treatment

In order to verify the ethanol loss during the mangoes hydrothermal treatment (46.1 ºC) experiments were carried out using a solution containing 20% of ethanol. Regular intervals of 18 minutes were chosen and to evaluate the concentration of ethanol in the solution we used an alcoholometer, the measurements were performed up to a total time of 90 minutes and the experiment was repeated in triplicate.

Effects of ethanol addition in hydrothermal treatment on mangoes' quality

Test I - Laboratory Scale-In this test, 60 'Tommy Atkins' mangoes were used. The heat treatment consisted of immersing the fruits in a bath (capacity for 12 liters of water) at 46.1 °C for 75 minutes, always using treated water from Campinas - SP municipal system. The water temperature was measured with a graduated mercury thermometer, capable of measuring temperatures up to 100 °C. The ethanol concentrations used in the bath were 0, 5, 10 and 20% v/v. After the hydrothermal treatment, cooling was carried out in another bath at 21.1 °C for 30 minutes. After the treatments the fruits were dried with paper towel and stored at 25 °C and 75% RH for 7 days. The ethanol effects on mango physico-chemical characteristics were evaluated, the analyzes were performed the day they were submitted to the treatments and on the seventh day of storage.

Test II – Pilot Scale-In this experiment 320 'Tommy Atkins' mangoes (10 fruits per plot) from Petrolina - Pernambuco at maturation stage 2 were used. The experiments were conducted at GEPC - ITAL. The heat treatment consisted of immersing the fruits in a pilot scale bath (capacity for 250 liters of water) at 46.1 °C for 75 minutes, the water temperature was measured with a graduated mercury thermometer, capable of measuring temperatures of up to 100 ° C. The ethanol concentrations used in the bath were 0, 3, 5 and 7% v/v. After the hydrothermal treatment, cooling was carried out in another bath at 21.1 °C for 30 minutes. We evaluated the ethanol effect on the mango physico-chemical characteristics. After the treatments, the fruits were dried and subdivided for storage at 25 °C and 75% RH for 8 days and at 10 °C and 90% RH for 14 days with addition of six (6) days storage under ambient conditions (25 °C and 75% RH), simulating shelf life. These conditions were chosen to simulate the way the mangoes are commercialized, ambient temperature and maritime export of the fruits, respectively. Fruits stored at 25 °C and 75% RH were analyzed on days 0, 3, 6 and 8. However, those stored at 10 °C and 90% RH were analyzed on days 0, 7 and 14 (after that time they were immediately transferred for

storage at 25 °C and 75% RH), these fruits were then analyzed on the 17th and 20th days after hydrotherapy. We evaluated the content of soluble solids, titratable acidity, SS/TA ratio, pH and firmness.

Physicochemical analysis -The soluble solids content (SS) was determined in a portable refractometer Atago with a scale of 0 to 32 °Brix. The titratable acidity (g of citric acid equivalent per 100 g^{-1}) was determined by titration with NaOH 0.1 N to pH 8.1 (AOAC, 2005). The pH value was obtained by the potentiometric method, with a pH meter Tecnopon brand. The SS/TA ratio is the ratio between the values of soluble solids and titratable acidity.

The pulp firmness, determined by the reading of two points in the equatorial region of the fruits, after the removal of the peel, we used TAXT2i texturometer, with the 8mm flat-end stainless steel cylindrical tip, with pre-test, test and post-test velocities of 2.0, 0.6, and 2.0 mm/s, respectively, with 4 mm of pulp penetration, the results were expressed in Newton (N).

Fungi identification -The identification of the main fungi causing post-harvest rottenness was also evaluated by the symptoms presence. Rotten fruits were disinfected and small fragments of damaged tissue were transferred to Petri dishes containing potato dextrose agar (PDA) with oxytetracycline (50 µg ml^{-1}) and incubated in a chamber for 8 days at 25 °C, with alternation of 12 h lightening (PONZO, 2014). The results were expressed in percentage (%), according to the presence of the respective characteristics in the mangoes stored at 10ºC that were submitted to the concentrations of 3, 5 and 7% of ethanol.

Experimental design and statistical analysis Test I was installed in a completely randomized design. The data were submitted to analysis of variance (ANOVA), checking for the F test significance and the averages were compared by the Tukey test at 5% probability.

Test II was installed in a completely randomized design. The data were submitted to analysis of variance and regression, and the linear and nonlinear models were chosen based on the potential to explain the biological phenomenon in question, in the coefficient of determination and the in significance of the regression coefficients. For the analysis, we used the computer program Sisvar (FERREIRA 2000).

Results and discusion

Evaluation of the ethanol evaporation during the hydrothermal treatment- Regarding to the ethanol evaporation in the hydrothermal bath, there was an average reduction of 0.02% of ethanol per minute, starting from an initial concentration of 20%, up to 90 minutes of heating. These data should be considered in the mangoes treatment, since the alcohol evaporation can mischaracterize the treatment. Table 1 presents these results, and the linear regression of the tabulated data can be described by equation 1:

$$A = -0.0204*t + 19.9690 \quad r^2 = 0.9609 \qquad (1)$$

where:
A - alcohol concentration (%v/v);
t - time in minutes.

Test I - Laboratory Scale -The results of the mangoes physico-chemical analyzes used in the experiments are presented in Table 2. The mean values found in this study were higher than those detected by Souza (2002) and Cocozza (2004) in studies with 'Tommy Atkins' mango at maturity stadium 2, this difference can be understandable, because the centesimal composition of a fruit is a function of several factors, such as: time of the year, irrigation technique used for planting, region from which the fruit is derived, among others.

In Table 2, we observed that after 7 days of application of the treatments (0, 5, 10 and 20% v/v of ethanol) the mangoes treated with alcohol presented significant changes to pH, titratable acidity and firmness.

On the seventh day of storage at 25 °C and 75% RH the mangoes matured according to their natural physiology. However, it was verified that the control fruits (0% of ethanol) matured more slowly. These results corroborate with the findings of Itamura et al. (1991) and Park, Lee (2005), the same ones showed that the ethanol can help in the maturation, since it stimulates the ethylene production, consequently inducing a greater respiration of the fruits.

The SS/TA ratio was influenced by the combined action of soluble solids and titratable acidity (Table 2). Among the concentrations, the difference was significant with 20% for soluble solids and from 5% of ethanol, when fruits stored after 7 days had lower values of titratable acidity. It was observed higher soluble solids contents and lower acidity in the treatments with higher ethanol concentrations, this fact may indicate that the alcohol caused an increase in the respiratory rate and, consequently, in the sugar content.

The pH differed statistically ($p < 0.05$) among treatments. The mangoes pH ranged from 5.0 to 5.6. The lowest values were verified in the control fruits (0% ethanol).

The fruits from the control treatment showed greater firmness, and this difference was statistically significant, reinforcing the statement that ethanol may have accelerated fruit metabolism. According to Oshida (1996) the ethanol, when absorbed by the fruit, is transformed into acetaldehyde by the action of the enzyme alcohol

dehydrogenase. The acetaldehyde is formed during fruit ripening, but is also responsible for the production of typical aromas during this process (PESIS, 2005).

According to Taira (1997), the firmness reduction during maturation processes is due to the increased activity of cell wall degradation enzymes, such as cellulase, pectinamethylesterase and polygalacturonase. The firmness reduction occurs during the mango maturation, being important from the economic point of view, since it affects the quality and resistance to transport (DANTAS et al., 2017).

No damage was observed on the mangoes' outer surface until the seventh day of storage after the treatment with 5%. However, in the fruits treated with 20% of ethanol, on the second day of storage, dark spots and depression on the surface were observed.

Less markedly, in the treated fruits with 10% of ethanol, small spots were also observed from the second day (Figure 1).

These results indicated a physiological limitation of the ethanol use in the mangoes hydrothermal treatment. Thus, tests with ethanol concentrations below 10% were carried out to verify the influence of these concentrations on fruit quality.

Test II - Pilot Scale

Storage at 25 ° C / 75% RH -Soluble solids content was significantly influenced by ethanol concentration and storage time (Figure 2). We observed a linear increase with the storage time in the control fruits (0%), and in fruits treated with 3, 5 and 7% of ethanol the data were adjusted to the quadratic model, and that there was an increase of the soluble solids content during the storage. The increase in soluble solids is an indicative of pulp maturation, however, we observed that fruit treated with 7% of ethanol showed less SS content at the end of storage, indicating that such treatment probably contributed to the delaying of fruit ripening.

The titratable acidity showed no significant effect. We observed a reduction of the acid content during the storage, getting at the end of the storage with an average value of 0.12% of citric acid. As the fruit matures simultaneously the acid value is reduced and the content of soluble solids increased due to the consumption of organic acids during the ripening process by the respiratory process.

A significant effect was observed for the interaction of ethanol concentration (%) and storage time for the SS/TA ratio. We observed that there was a linear increase in this variable as a function of the storage time.

The ratio values clearly indicate the increase in sugar ratio compared to acid ratio with storage time and alcohol concentration, especially on the eighth day of storage. However, concerning to the treatments, we observed that the treatment with 3% of ethanol had a higher average SS/TA ratio than the other treatments (Figure 3).

In the control fruits, the ratio was lower than in the fruits treated with ethanol. On the last day of evaluation, the average values found for the ethanol percentage of 0, 3, 5 and 7% were, respectively, 96.60; 144.09; 132.74 and 99.12.

The pH had a significant interaction between storage time and ethanol concentrations. During storage there was an increase in pH, and the ethanol concentration of 3% had a higher pH value at the end of storage (Figure 4). We noted that during the storage the control (0% ethanol) had lower pH values compared to the treatments.

The firmness was affected by both storage time and ethanol concentrations. During the storage period, there was a reduction in firmness values, with the mean value initially being 120.74, 120.74, 123.15 and 120.23 N, for the concentrations of 0, 3, 5 and 7%, respectively. And on the eighth day this value was reduced to 6.17, 3.6, 0.12 and 4.54 N for the concentrations of 0, 3, 5, 7%, respectively (Figure 5).

During the storage at 25 °C, neither the rottenness of the mangoes at the ethanol concentrations studied nor the presence of deep black spots were observed.

Storage at 10 °C / 75% RH -There was an increase in the content of soluble solids, with the advancement of the storage time, and there was no statistical difference among the treatments. At 14 days of refrigeration, plus 6 days at room temperature, soluble solids content was above 14%, similar findings were found by Liu et al. (2012) in studies with melons treated with ethanol, in which the authors verified increase of the sugars metabolism of these fruits during storage.

Figure 6 shows that the acid content had a significant effect on the ethanol concentrations used and for the storage time. The content of organic acids decreased with the storage time, this is notable in most tropical fruits, due to the use of these acids in the Krebs cycle as a source of energy during the respiratory process and even as a source of carbon for the sugars' synthesis (BERNARDES-SILVA, 2003).

For the SS/TA ratio and pH it was observed that there were no statistically significant differences among the treatments during the storage period.

There was a decrease in fruit firmness during the storage time, but there was also no statistical difference. The fruits texture characterized by firmness, fibrosity, strength and elasticity (FIGUEIREDO NETO et al, 2015) also varies during major chemical components of the tissues, responsible for changes in the texture of fruits and vegetables.

In mangoes stored at 10 °C there was presence of rottenness and deep black spots in the fruits that were subjected to hydrothermal treatment with ethanol (Figures

7, 8 and 9), and this rottenness was proportional to the increase of the ethanol concentration. One hypothesis for this behavior is that the storage at 10 °C left the mango more sensitive to physiological disturbances that when added to the effects of the hydrothermal bath with ethanol caused a deleterious effect to the fruit, since the control fruits (0% ethanol) presented a lower incidence of rottenness (14% of the fruits) when compared with the other treatments.

Regarding the external appearance of the fruits, we observed that those treated with ethanol and submitted to refrigerated storage, presented darkening of the peel, thus compromising their commercialization.

Table 1. Ethanol concentration in the hydrothermal bath at 46.1 °C after 0, 18, 36, 54, 72 and 90 minutes

Time (min)	Ethanol concentration (%)
0	20.00
18	19.73±0.15
36	19.03±0.23
54	18.9±0.26
72	18.37±0.25
90	18.27±0.15

* Results obtained by the mean and standard deviation of the three tests.

Table 2. Mean values and standard deviation of the physico-chemical analyzes performed on 'Tommy Atkins' mango submitted to the hydrothermal treatment (46.1 ºC /75 minutes) with different ethanol concentrations at the beginning of the experiments and after 7 days of storage at 25 ºC and 75% RH.

Experiments	Characteristics evaluated				
	SS (ºBrix)	TA (%)	SS/TA Ratio	pH	Firmness (N)
0 day	11.46± 0.59	1.03± 0.13	11.1	3.47± 0.22	93.32± 3.78
0%	14.8±0.91 b	0.17±0.03 a	87.1	5.0±0.11 c	21.4±9.57 a
5%	15.1±1.20 ab	0.12±0.01 b	125.8	5.3±0.12b c	10.5±2.08 b
10%	15.4±0.64 ab	0.11±0.03 b	140.0	5.4±0.38 ab	9.8±1.19 b
20%	16.01±0.56 a	0.11±0.02 b	145.5	5.6±0.12 a	8.9±2.01 b

Means followed by different letters in the same column differ from each other by the Tukey test at 5%.
SS - Soluble Solids; TA - Titratable Acidity.

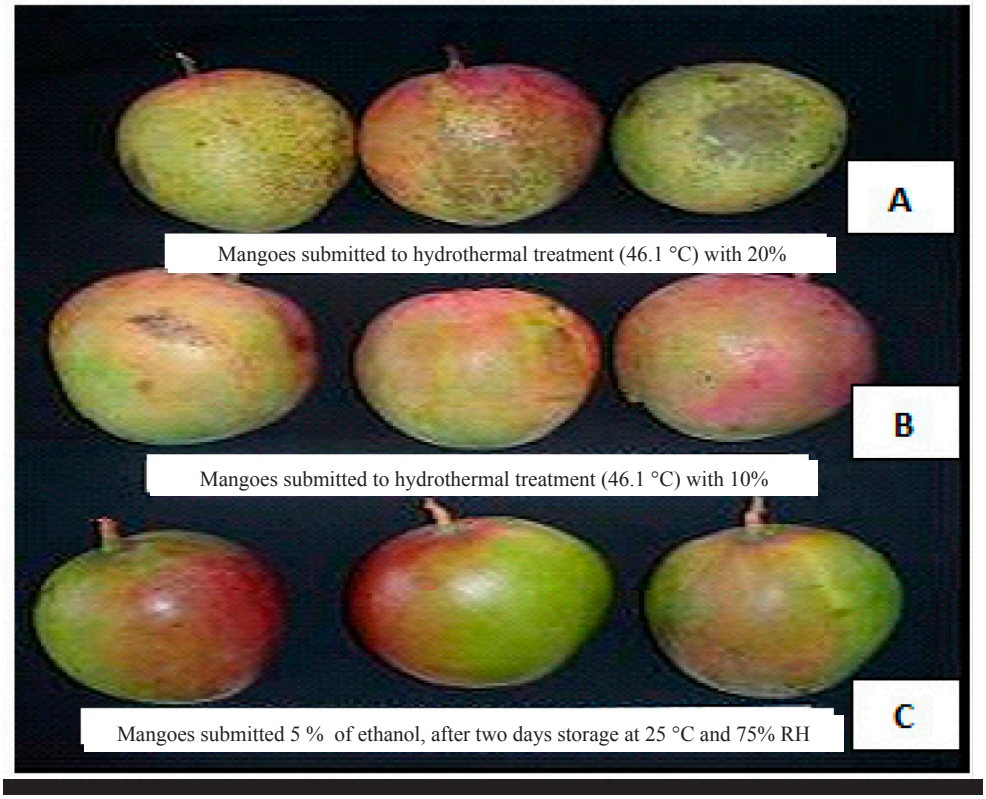
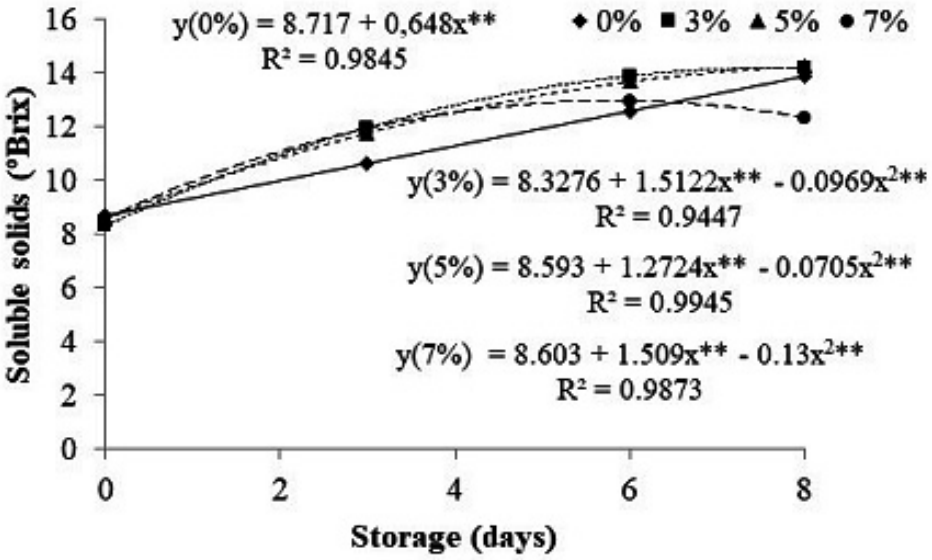
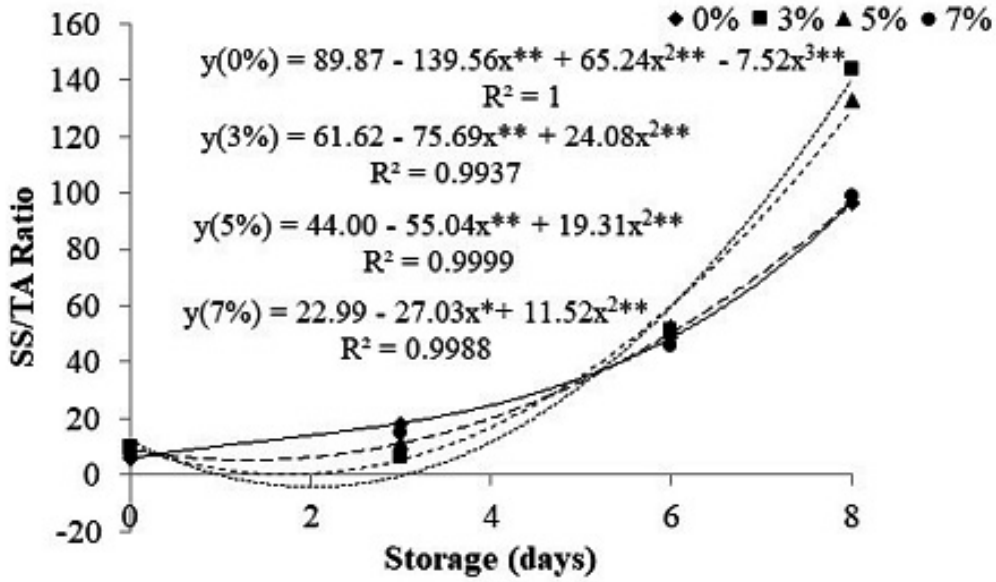


Figure 1. 'Tommy Atkins' mangoes submitted to hydrothermal treatment (46.1 °C) with 20% (A), 10% (B) and 5% (C) of ethanol, after two days storage at 25 °C and 75% RH.



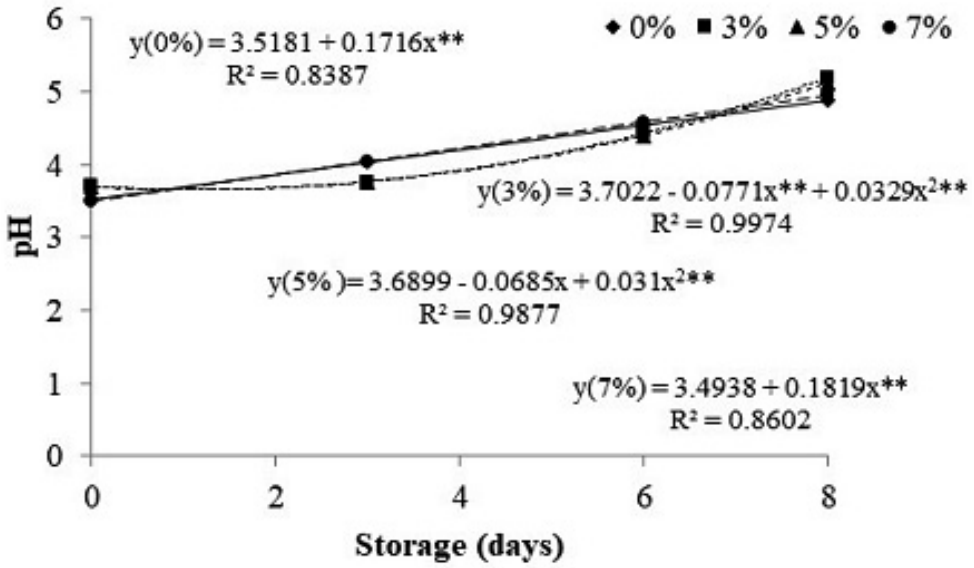
**Significant at the level of probability p <0.01.

Figure 2. Soluble solids content in 'Tommy Atkins' mangoes treated with different ethanol concentrations and stored at 25 °C and 75% RH for 8 days.



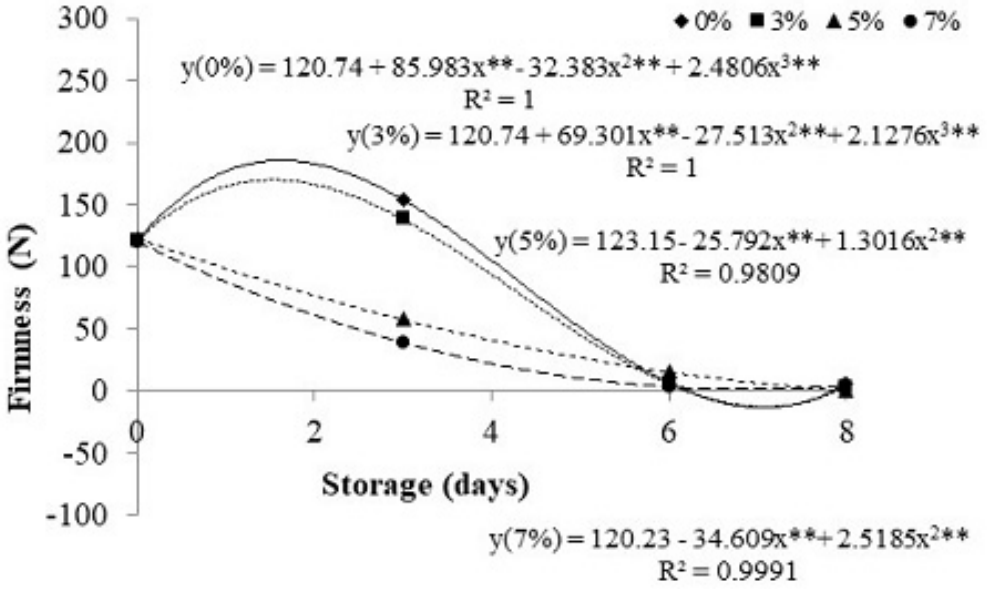
* Significant at the level of probability p <0.05 and * p <0.01.

Figure 3. SS/TA ratio in 'Tommy Atkins' mangoes treated with different ethanol concentrations and stored at 25.0 °C and 75% RH for 8 days.



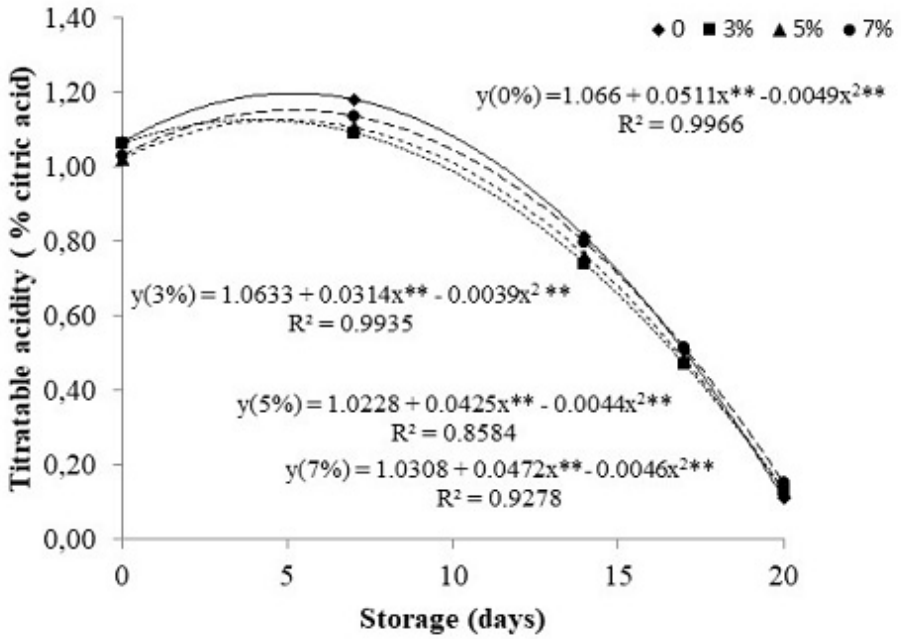
**Significant at the level of probability p <0.01.

Figure 4. pH in 'Tommy Atkins' mangoes treated with different ethanol concentrations and stored at 25.0 °C and 75% RH for 8 days.



**Significant at the level of probability p <0.01.

Figure 5. Firmness (N) in 'Tommy Atkins' mangoes treated with different ethanol concentrations and stored at 25.0 °C and 75% RH for 8 days.



**Significant at the level of probability p <0.01.

Figure 6. Titratable acidity in 'Tommy Atkins' mango submitted to hydrothermal treatment (46.1 °C and 75 minutes) stored for 14 days with addition of six days of storage under ambient conditions (25 °C and 75% RH), simulating shelf life.

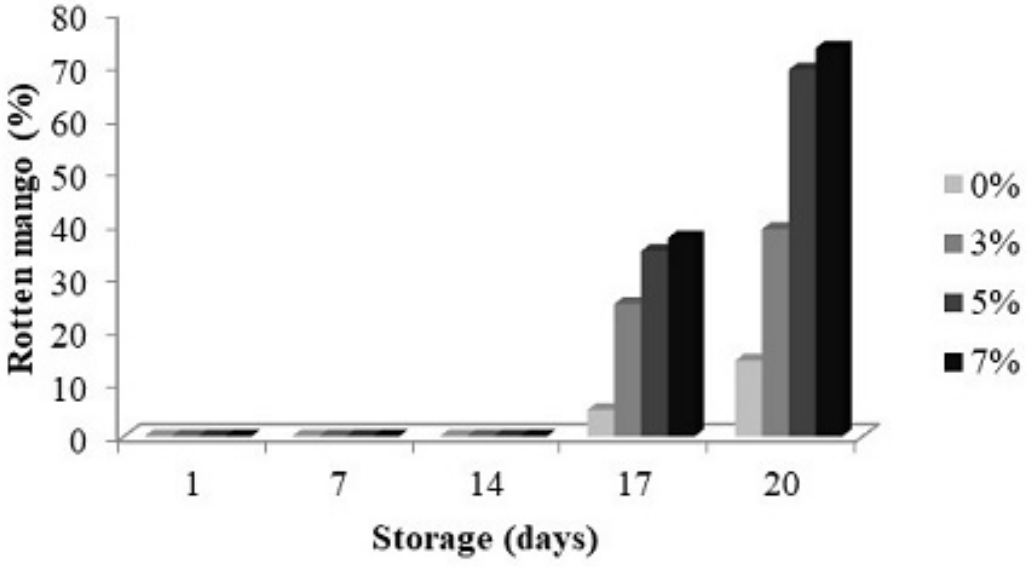


Figure 7. Percentage of rotten mangoes submitted to hydrothermal treatment (46.1 °C / 75 min) with different ethanol concentrations during storage at 10 °C and 90% RH with addition of six days of storage under ambient conditions (25 °C and 75% RH), simulating shelf life.

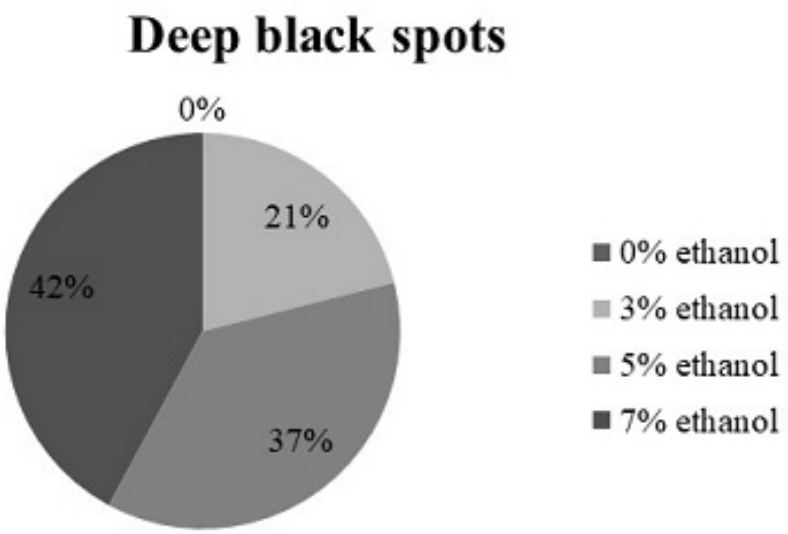


Figure 8. Amount of mangoes with deep black spots that were submitted to hydrothermal treatment (46.1 °C for 75 minutes) with different ethanol concentrations during storage at 10 °C and 90% RH with addition of six days of storage under ambient conditions (25 °C and 75% RH), simulating shelf life.

Figure 9. Mangoes submitted to hydrothermal treatment (46.1 °C for 75 minutes) with 7% of ethanol after 17 days storage at 10 °C and 90% RH with addition of six days of storage under ambient conditions (25 °C and 75% RH), simulating shelf life.

Conclusions

The mangoes treated with ethanol matured faster than the mangoes of the control treatment.

Treatments with dosages lower than 10% did not cause physiological damage to the fruits, when they were stored at 25 °C and 75% RH.

In storage at 10 °C and 90% RH, we observed a higher rate of rottenness and deep black spots in the mangoes that were subjected to hydrothermal treatment with ethanol.

The use of hydrothermia associated with ethanol decreased the mangoes shelf life, since these fruits matured faster and are not recommended for mangoes treatment for exportation.

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