PREVENTION OF CHILLING INJURY IN 'TOMMY ATKINS' MANGOES PREVIOUSLY STORED AT 5 °C, USING HEAT TREATMENT AND RADIATION UV (UV-C)¹

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ABSTRACT – The objective of this study was to evaluate the effect of heat treatment and ultraviolet radiation (UV-C) in the prevention of chilling injury in mangoes cv. Tommy Atkins previously stored or not under injury condition after their transference to ambient condition. Fruits were divided into groups: two were hydrothermally treated (46.1 °C/90 min; 55 °C/5 min) and two were exposed to UV-C radiation (1.14 kJ m⁻²; 2.28 kJ m⁻²). These groups were stored under chilling injury conditions (5 °C for 14 days), as established in preliminary tests. Other untreated groups were stored at 12 °C or 5 °C. After the storage period, they were transferred to ambient conditions (21.9 °C; 55% RH) and the quality was evaluated. All the data were submitted to multivariate analysis as the tool to verify the simultaneous effect of the treatments under the quality parameters. The multivariate analysis indicated that the hydrothermal treatments at 46.1 °C/90 min and 55 °C/5 min and the UV-C radiation at doses of 1.14 kJ m⁻² and 2.28 kJ m⁻² were effective in minimized the symptoms of chilling injury in mangoes 'Tommy Atkins' stored at 5 °C for 14 days. However, after their transference to environmental condition at 21.9 °C, only the UV-C kept this control, especially at a dose of 2.28 kJ m⁻². This treatment did not prevent the development of the characteristic color or affected the normal ripening and allowed the conservation of fruit for a period of 14 days at 5 °C, plus seven days of storage at environmental condition, which corresponds to the shipping transportation plus the time for sale. **Index terms:** Mangifera indica, ultraviolet radiation, thermal treatment, factor analysis, cluster analysis.

PREVENÇÃO DE INJÚRIAS PELO FRIO EM MANGAS 'TOMMY ATKINS' PREVIAMENTE ARMAZENADAS A 5 °C, USANDO-SE TRATAMENTO TÉRMICO E RADIAÇÃO ULTRAVIOLETA (UV-C)

RESUMO – O objetivo deste trabalho foi avaliar o efeito do uso de tratamento térmico e da radiação ultravioleta (UV-C) na prevenção de danos pelo frio em mangas cv. Tommy Atkins previamente armazenadas ou não sob condição injuriante, depois de transferidas para condição de ambiente. Frutos foram divididos em grupos: dois foram tratados hidrotermicamente (46,1 °C/90 min; 55 °C/5 min) e dois foram expostos a radiação UV-C (1,14 kJ m²; 2,28 kJ m²). Esses grupos foram armazenados em condição injuriante (5 °C por 14 dias), conforme estabelecido em testes preliminares. Outros grupos não tratados foram armazenados a 12 °C ou 5 °C. Após o período de armazenamento, foram transferidos para condição ambiente (21,9 °C; 55% HR) e avaliados quanto à qualidade. Todos os dados foram submetidos à análise multivariada como ferramenta para verificar o efeito simultâneo dos tratamentos sobre os parâmetros de qualidade. A análise multivariada indicou que o tratamento térmico afetou negativamente o desenvolvimento da cor normal da casca das mangas, reduziu a capacidade antioxidante e injuriou o fruto, como indicado pela elevada atividade da PAL. O dano pelo frio relacionou-se com a diminuição da atividade antioxidante e com o comprometimento da síntese de pigmentos na casca. A radiação UV-C minimizou o aparecimento dos sintomas de injúria pelo frio, especialmente a dose de 2,28 kJ m². Este tratamento não impediu o desenvolvimento da coloração característica ou afetou negativamente o amadurecimento.

Termos para indexação: *Mangifera indica*, radiação ultravioleta, tratamento térmico, análise de fatores, análise de agrupamento.

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INTRODUCTION

Mango (Mangifera indica L.) is a popular and economically important tropical fruit throughout the world, due to its excellent eating quality and nutritional composition (KIM et al., 2009). Although Brazil has great potential for mango cultivation, quality problems have prevented it to achieve desired levels of exports.

Refrigeration is the primary technology used to preserve the quality of mangoes which can be susceptible to chilling injury when exposed to temperatures below 7-13 °C. These injuries are the most common and troubling physiological disorders of mangoes chain, especially in fruit transported by sea (SIVAKUMAR et al., 2011).

Currently, no methods are available to completely prevent it. Notably, hydrothermal treatment prior to cooling is a mandatory quarantine method for exported mangoes. Although this technique controls diseases and increases cold tolerance, it may also accelerate or inhibit the ripening and reduce the antioxidant capacity, depending on the variety, harvest time, treatment duration and temperature (KIM et al., 2009).

A promising technique is the UV-C radiation (190-280 nm), which does not leave residues; is a simple, cold, dry and inexpensive process (RIVERA-PASTRANA et al., 2007). This technique slows the processes associated with ripening (COTE et al., 2013) and acts as an abiotic stressor by activating defense mechanisms in plant tissues (CHARLES et al., 2008), including the synthesis and accumulation of antimicrobial compounds and increased antioxidant-enzyme activity (ALOTHMAN et al., 2009). However, little information is available, in the literature, about the effect of postharvest UV-C irradiation on chilling injury of cold-stored mango fruit.

Besides the technologies described above, recent studies have indicated that controlled atmosphere (SUDHAKAR; GOPALAKRISHNA, 2009), pre-fumigated with nitric oxide (ZAHARAH; SINGH, 2011), the treatment with ${\rm H_2O_2}$ (ZHAO et al., 2010) and the application of vegetal regulators, such as, methyl jasmonate, diphenylamine (TASNEEM et al., 2004) and salicylic acid (BARMAN; ASREY, 2014) are able to give the mangoes higher tolerance to low temperature.

Univariate analyses are usually employed to identify the differences between treatments, but do not consider the simultaneous effects of the various parameters. Multivariate analyses, such as principal component factor analysis, can condense the

information contained in the original variables into a smaller set of variables, called factors by using linear combinations. Cluster analysis is another technique that allows separating or classifying individuals observed in a group or in a specific number of subgroups mutually exclusive so that the subgroups have formed features of large internal similarity and large external dissimilarity (HAIR Jr. et al., 2009).

The objective of this study aimed to evaluate the effect of heat treatment, corresponding to the usual treatment and the ultraviolet radiation (UV-C), a clean technology that fits the current trend of sustainability, in the prevention of chilling injury in mangoes cv. Tommy Atkins previously stored or not under injury condition after their transference to ambient condition.

MATERIALS AND METHODS

Handling, Selection and Fungicide Treatment

Tommy Atkins mangoes picked at mature green from an orchard in Monte Alto (State of São Paulo, Brazil) were immediately and carefully transported to the laboratory, where they were washed with neutral detergent based on benzene alkyl sulphonate sodium linear, rinsed with potable water, selected to eliminate non-uniform ones and their peduncles were standardized to 10-20 mm. All mangoes used in this study were treated with fungicide Magnate 500 EC® 2 mL L-1, where imazalil is the active ingredient.

Fruit lots

We treated mango lots under six different conditions. Two fruit lots were subjected to hydrothermal treatment, by immersion in water at 46.1 °C for 90 minutes or 55 °C for 5 minutes, and then stored under chilling injury condition, at 5 °C and 80% RH for 14 days (MIGUEL et al., 2011). Two other lots were exposed to ultraviolet radiation (UV-C) with energy of 5.44 J s⁻¹ m⁻² for 3.5 or 7.0 minutes (resulting in 1.14 kJ m⁻² and 2.28 kJ m⁻², respectively), and then stored under chilling injury condition. In addition, one control lot was stored under chilling injury conditions without pretreatment in heated water or with UV-C. Lastly, one fruit lot was stored at the recommended temperature for mango storage (12 °C, NUNES et al., 2007) for fourteen days. All fruit lots subjected or not to chilled storage were subsequently kept under environmental conditions (21.9±0.6 °C, with 55±2.3% RH) for up to one week and evaluated every two days. During the refrigerated storage fruits were evaluated on day 0 and day 14. Each treatment had six fruits distributed

in three replications.

Hydrothermal treatment

Mangoes were subjected to hydrothermal treatment by immersion in water at 46.1 °C for 90 minutes (KIM et al., 2009) or 55 °C for 5 minutes (LIMA et al., 2007), followed by cooling at 10 °C in a solution containing fungicide as described above.

UV-C treatment

Ultraviolet radiation treatment of mangoes was performed in a specially constructed box of $0.32~\text{m}^3$, where eight 15W G15T8 lamps (λ = 250-280 nm) coated with aluminum foil were arranged in parallel, four at the top and four at the bottom. Fruit were arranged on a nylon net positioned 15 cm from the radiation source. The radiation dose intensities were established adjusting the distance between the light source and the nylon net and exposure duration, as determined with a radiometer (Instrutherm RS-232, model MRUR-203). Before UV-C radiation, mangoes were treated with fungicide as described above.

Assessments

Chilling injury

The occurrence of chilling injury to the peel was determined by comparing images of the fruit using the 'Paint.net' image analysis software (PAINT. NET, v.3.5.10). Photos taken of the fruit on each day of analysis were used for manually selecting the areas with symptoms. Each photo contained a square of known dimensions (0.6 cm x 0.6 cm) used as a reference for conversion of the area indicated according to the program's scale in the entire affected area (cm²). The percentage of affected area per fruit was calculated as the ratio between the affected area and the total fruit area. These data were related to the scale proposed by Whangchai et al. (2000), where damage was categorized as: 1= no damage; 2= light (2-5% of the peel surface injured); 3= bland (5.1-15% of the peel surface injured); 4= mild (15.1-25% of the peel surface injured); 5= moderate (25.1-35% of the peel surface injured); 6= moderate/strong (35.1-45% of the peel surface injured); 7= strong (45.1-55% of the peel surface injured); 8= severe (>55% of the peel surface injured). Fruits with grades above 4 were considered unacceptable for selling due to reduced visual quality. It was considered as chilling injury symptoms the formation of necrotic spots and depressed regions in peel surface.

Rot

Mangoes were visually assessed for rot by detecting the appearance of lesions with more than 0.5 cm in diameter, and mango lots were considered rotten when exhibiting this symptom.

Color and Firmness

The peel and pulp color were determined using a Minolta CR 400b colorimeter and the results were expressed in luminosity (L*), hue angle (Hue), and chromaticity (Chroma). Pulp firmness was determined on opposite peeled sides of the fruit using a penetrometer. Mean pulp firmness was expressed in Newtons (N).

Chemical Analysis

Several parameters of the fruit pulp were quantified, including the levels of soluble solids (SSol) using a digital refractometer, titratable acidity (TA) based on the AOAC method (2005), ascorbic acid (AA) (STROHECKER; HENNING, 1967), soluble sugars (SSug) (DUBOIS et al., 1956), and reducing sugars (RS) (MILLER, 1959). The total antioxidant activity of the pulp was also determined by measuring ABTS free-radical scavenging activity and by using the beta carotene/linoleic acid system (RUFINO, 2008). Moreover, the level of total extractable polyphenols (TEPP) (OBANDA; OWUOR, 1997) and the specific activities of the enzymes polyphenol oxidase (PPO) and peroxidase (POD) were measured in the peel and pulp using an extraction method adapted from Allain et al. (1974). A method modified by Teixeira et al. (2007) was used to measure the PPO level, and the method of Lima et al. (1999) was used to measure the POD level. To measure the activity of phenylalanine ammonia-lyase (PAL), a method adapted from Cahill and McComb (1992) was used.

Statistical Analysis

For the multivariate analyses, the data sets were standardized so that each variable had a mean of zero and a variance of one. The original data were initially subjected to a factor analysis to identify factors representing the relationships among a set of interrelated variables. It was considered significant, the coefficients with rates higher or equal to 0.70 (SOUNIS, 1975). The hierarchical cluster analysis employed the Euclidean distance as the similarity coefficient. Ward's clustering method was used to identify the similarities between groups. A nonhierarchical cluster analysis was performed using the k-means algorithm, in which k corresponds to the number of groups indicated in the hierarchical cluster analysis (HAIR Jr. et al., 2009). This method complements the results obtained in the hierarchical analysis, since it minimizes the variance within each group. The standardized averages of variables that have values close to 0 indicate that they were not representative for group's differentiation. The principle-components and cluster analyses were performed using the program Statistica version 7.0.

RESULTS AND DISCUSSION

Before presenting the results, it is important to note that chilling injury was not included as a variable in the cluster analysis because it is a categorical variable (i.e., a non-numerical trait).

Refrigerated Storage

The hydrothermal and UV-C treatments were effective in controlling chilling injury. After 14 days of storage, 15.1-25.0% of the surface of untreated fruits showed chilling injury (grade 4). Effective control of chilling injury has been previously reported by González-Aguilar et al. (2001) for Tommy Atkins mangoes exposed to UV-C radiation and by Shao et al. (2013) for loquat fruit heat treated.

The principal component factor analysis of the treated and chilled fruits indicated that the variables could be summarized by two main factors that accounted for 86.64% of the total variance. Of the 22 variables analyzed, only 8 were highly correlated with the two principal factors (Table 1).

CP1 explained 63.30% of the total variance in the analysis and was linked to the variables L_peel, Hue_peel, L_pulp, firmness, and PAL activity in the peel and pulp, which were directly correlated with each other. These results indicated that fruits with higher L* values for the peel and pulp (i.e., brighter-colored fruits) had firmer flesh and higher PAL activity in both the peel and pulp, characteristics of immature mangoes.

CP2 explained 23.34% of the total variance and was related to chilling injury and to POD activity in the peel, which were directly correlated with each other. These results indicated that chilling injury symptoms are aggravated as the intensity of POD activity increases, in contrast to the findings of Trejo-Márquez et al. (2010) for Keitt mangoes.

The dendrogram generated from the cluster analysis (Figure 1A) showed two distinct groups. Group 1 (G1) consisted of fruits sampled at the beginning of the storage period (day 0), which exhibited high values of L_peel, Hue_peel, L_pulp, Hue_pulp, and firmness; low chroma values in the peel and pulp; low SSug levels; and high PAL activity in the peel and pulp. These traits indicate that the mangoes were in the late stages of maturation, as evidenced by their light coloration (low chroma), peels with light-red coloration (high hue and L*), light-yellow pulp (high hue and L*), high pulp firmness, and lack of soluble sugars (Figure 1B).

Group 2 (G2) consisted of fruits from all

treatment groups after 14 days of storage. These fruits were characterized by intense red coloration of the peel (low L* and hue and high chroma), intensely yellow-tinted pulp, decreased firmness (low L*, hue, and firmness and high chroma), low PAL activity in the peel and pulp, and high SSug levels (Figure 1B). These results demonstrate that the observed changes were due to the effect of the storage time alone and were linked to events associated with the normal ripening of mangoes, consistent with the ripening-induced changes reported by Balloch and Bibi (2012).

It is important to note that although the cluster analysis did not reveal the effects of the pre-storage treatments on the physical and chemical attributes of the fruit, the chilling injury control fruits differed from those subjected to hydrothermal or UV-C treatment. The latter groups exhibited greater efficiency in curbing the onset of symptoms (showing no visible symptoms of injury) compared to the control fruits, in which up to 25% of the peel was injured (grade 4) after the 14-day storage period.

Storage under ambient conditions

Chilling injury symptoms appeared rapidly after the transfer to ambient conditions (Table 2). In the control fruits, 25.1-35% of the surface was injured (grade 5) on day 3, corresponding to the limit for commercialization. In contrast, the fruits treated with UV-C had injury percentages of approximately 7.5% (UV-C/2.28 kJ m⁻²) to 13.6% (UV-C/1.14 kJ m⁻²) and remained marketable over the seven days of storage at ambient temperature (grades 3 and 4, respectively). Hydrothermally treated fruit were not suitable for commercialization as early as the first day after transferring to ambient temperature due to the presence of blanching symptoms on more than 55% of the fruit surface (grade 8). González-Aguilar et al. (2001) have also reported that chilling injury symptoms are minimized in Tommy Atkins mangoes exposed to ultraviolet radiation at 4.11 kJ m⁻² and that more prolonged exposures are more efficient.

Principal component factor analysis was used to analyze the data collected after the fruits were transferred to ambient temperature. Two factors accounted for 77.22% of the total variance in the 24 variables analyzed, and only 12 variables showed strong correlations (Table 3).

CP1 explained 54.71% of the total variance in the analysis. The variables associated with this factor included L_pulp, Hue_pulp, firmness, AA, and TA, which were directly correlated with each other and inversely related to the variables Chroma_pulp, SSol, SSug, and RS. These relationships indicate that the

increase in sugar (SSug and RS) and soluble solids occurs in parallel with increased pigment synthesis in the pulp and that these changes are inversely related to the development of orange-tinted coloration. Furthermore, these events are accompanied by a loss of firmness and decreased ascorbic-acid levels and acidity in the pulp. The trends indicated by these variables reflect the changes that occur during fruit ripening (RAZZAQ et al., 2013).

CP2 accounted for 22.51% of the total variance. The variables Chroma_peel and antioxidant activity (b-carotene) in the pulp were correlated with each other and inversely related to chilling injury. These results indicate that pigment synthesis in the peel is directly related to the antioxidant protective capacity of b-carotene in the pulp (VÁSQUEZ-CAICEDO et al., 2005). As chilling injury worsens, the antioxidant activity in the pulp is also impaired. These symptoms are also involved in the impairment of the peel pigmentation, consistent with the findings of Chidtragool and Ketsa (2013) for 'Nam Dok Mai' mangoes stored at 4 °C and transferred to ambient temperature (25 °C).

The dendrogram generated from the cluster analysis is shown in Figure 2. In this analysis, four groups emerged.

Group 1 (G1) consisted of the control fruits (5 °C and 12 °C) stored at ambient temperature for three days, and the UV-C-exposed fruit after three to five days at ambient temperature. This group was characterized by low PPO and POD activity in the pulp and by the red-tinted coloration of the peel (low L* and hue). Thus, these fruits had not entered the advanced stages of ripening or senescence, especially given the low oxidative activity in the pulp (PÉREZ-TELLO et al., 2009).

Group 2 (G2) consisted of fruits that were hydrothermally treated at 46.1 °C or 55 °C and stored at ambient temperature for three, five, or seven days. This group was characterized by peels with yellow-greyish colouration (high hue and low chroma), high PAL activity in the peel and pulp, low polyphenol levels in the peel (TEPP), and low antioxidant activity in the pulp (based on ABTS activity and the b-carotene/linoleic acid system). These results suggest that the thermal treatment impaired the development of the normal peel coloration of the mangoes (i.e., intense yellow-reddish coloration), reduced the antioxidant capacity, and injured the fruit, as expressed by the higher activity of PAL, which is a stress-related signaling enzyme.

Kim et al. (2009) noted that thermal treatment can reduce antioxidant capacity and cause nutritional loss in mangoes by triggering oxidative processes. Sudhakar Rao and Gopalakrishna Rao (2009) reported that this treatment may negatively affect pigment synthesis in the peel, consistent with the results of the present study.

Group 3 (G3) consisted of the control fruits (5 °C and 12 °C) stored at ambient temperature for five to seven days, and the fruits treated with UV-C radiation and stored at ambient temperature for seven days. These fruits exhibited light-colored peels (high L*), orange-tinted pulp (low L* and hue), and low firmness. Additionally, these fruits had low TA and AA levels and high SSol, SSug, and RS levels. The fruits in this group also exhibited a high incidence of rot, reduced polyphenol content in the pulp, and high inhibition of b-carotene oxidation. The parameter's behaviors indicate that the fruits were senescent as described by Chitarra; Chitarra (2005) and González-Aguilar et al. (2007).

The characteristics noted above indicate that storage under injurious conditions (5 °C) for 14 days following exposure to ultraviolet radiation does not negatively affect fruit ripening, given that the UV-treated mangoes were similar to those stored at the recommended temperature (12 °C). Notably, however, the preservation of the control mangoes (5 °C) was limited by the worsening of their chilling injury symptoms, which negatively affected the visual appearance of the fruits as indicated in Table 2.

Group 4 (G4) consisted of fruits from all treatments that were stored for 14 days under chilling injury or non-injurious conditions (5 °C or 12 °C) and sampled after one day at ambient temperature. This group showed a low incidence of rot; low SSol, SSug, and RS levels; high TA and AA levels; light, slightly intense yellow coloration (high L* and hue and low chroma); and firm pulp. The fruits in this group were also characterized by high PPO and POD activity and low PAL activity in the pulp and peel, elevated TEPP levels in the pulp, and elevated antioxidant activity (ABTS). These characteristics are consistent with fruit in the late stages of maturation.

TABLE 1 – Correlation coefficients of the variables associated with the first two principal component factors for Tommy Atkins mangoes subjected to different treatments prior to storage at 5 °C (80% RH) or 12 °C (82% RH) for 14 days.

Variable	CP1	CP2
L_peel	0.925015	0.170042
Hue_peel	0.977632	-0.034795
L_pulp	0.801157	0.052204
Firmness	0.934527	0.020663
Chilling Injury	0.003501	-0.932527
PAL_peel	0.921762	0.305430
POD peel	-0.265474	-0.884195
PAL_pulp	0.903916	0.299052
Exploratory variance	5.063810	1.867404
Proportion of the total variance (%)	63.30	23.34

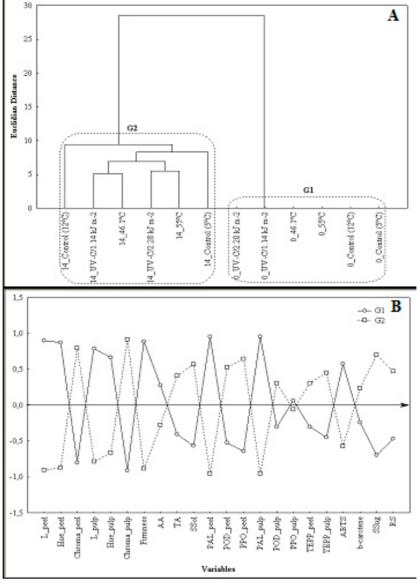


FIGURE 1 – Hierarchical (A) and non-hierarchical (B) cluster analyses grouped Tommy Atkins mangoes subjected to different treatments and storage conditions at 5 °C (80% RH) or 12 °C (82% RH) according to storage duration (0 or 14 days). In panel A, the numerals 0 and 14 in the group names correspond to the storage duration.

TABLE 2 – Chilling injury* in Tommy Atkins mangoes stored at ambient temperature (21.9 °C, 55% RH) for seven days, after being subjected to different treatments and stored at 5 °C (80% RH) or 12 °C (82% RH) for 14 days.

Treatments —	Time (days)				
	1	3	5	7	
Control (5 °C)	4	5	5	6	
UV-C/1.14 kJ m ⁻²	3	3	4	4	
UV-C/2.28 kJ m ⁻²	2	3	3	3	
46.1 °C/90 min	8	8	8	8	
55 °C/5 min	8	8	8	8	
Control (12 °C)	1	1	1	1	

^{*}Grades: 1= no damage; 2= light (2-5% of the peel surface injured); 3= bland (5.1-15% of the peel surface injured); 4= mild (15.1-25% of the peel surface injured); 5= moderate (25.1-35% of the peel surface injured); 6= moderate/strong (35.1-45% of the peel surface injured); 7= strong (45.1-55% of the peel surface injured); 8= severe (>55% of the peel surface injured).

TABLE 3 – Correlation coefficients of the variables for Tommy Atkins mangoes kept at ambient temperature (21.9 °C, 55% RH) for seven days, after being subjected to different treatments and stored at 5 °C (80% RH) or 12 °C (82% RH) for 14 days.

Variable	CP1	CP2
Chroma_peel	0.365589	0.829314
L_pulp	-0.873217	0.037193
Hue_pulp	-0.893765	-0.225143
Chroma_pulp	0.758008	0.060938
Firmness	-0.924983	-0.004326
AA	-0.901188	-0.041773
TA	-0.808018	-0.102163
SSol	0.889884	0.228252
Chilling Injury	0.046081	-0.953931
b-carotene	-0.003037	0.727819
SSug	0.837946	0.319959
RS	0.691976	0.592404
Exploratory variance	6.565191	2.700868
Proportion of the total variance (%)	54.71	22.51

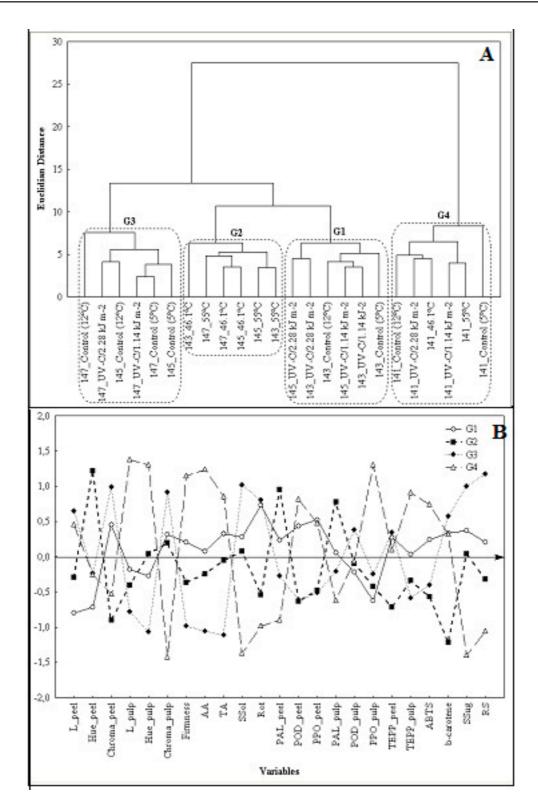


FIGURE 2 – Hierarchical (A) and non-hierarchical (B) clustering of 'Tommy Atkins' mangoes subjected to different treatments and storage conditions at 5 °C (80% RH) or 12 °C (82% RH) for 14 days and then kept at ambient temperature (21.9 °C, 55% RH) for seven days. In panel A, the numerals 1, 3, 5, and 7 in the group names correspond to the number of days at ambient temperature.

CONCLUSIONS

The hydrothermal treatments at 46.1 °C/90 min and 55 °C/5 min and the UV-C radiation at doses of 1.14 kJ m⁻² and 2.28 kJ m⁻² were effective in minimized the symptoms of chilling injury in mangoes 'Tommy Atkins' stored at 5 °C for 14 days. However, after their transference to environmental condition 21.9 °C, only the UV-C kept this control, especially at a dose of 2.28 kJ m⁻². This treatment did not prevent the development of the characteristic color or affect the normal ripening and allowed the conservation of fruit for a period of 14 days at 5 °C, plus seven days of storage at environmental condition, which corresponds to the shipping transportation plus the time for sale.

The UV-C radiation showed to be an alternative technology in controlling of chilling injury symptoms in fruits cv. Tommy Atkins stored at low temperatures. It has the advantages to be a cold treatment, dry, simple, of low cost, no residue and that can be used on a commercial scale.

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