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Physiology and postharvest conservation of 'Paluma' guava under coatings using Jack fruit seed-based starch[1]

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Abstract - The aim of this work was to evaluate the effect of jackfruit seed starch-based (S) coatings, added to chitosan and alginate on the physiology and maintenance of quality of cold stored 'Paluma' guavas, followed by transfer to the room condition. The design was the completely randomized, in a 4x2 factorial scheme, in 4 replications, with 4 coatings (dispersion of S - 4%; S 2% + chitosan - 2% (SC); S - 2% + alginate - 2% (SA); and the uncoated control), in 2 environments (refrigerated (10±2 °C e 80±2% RH) with transfer to room condition (25±3 °C e 75±4% HR), on the 16th and 20th day of cold storage. The SC and SA coatings were efficient in reducing the respiratory rate in fruits during 10 days at room condition. The SC coating delayed fruit ripening, and maintained firmness and color, with intention of purchasing and appearance higher than the limit of acceptance for another 6 days, following transferring to room condition, at the 16th day of refrigeration.

Index terms: *Artocarpus heterophyllus*, fruit waste; chitosan, sodium alginate, fruit quality.

Fisiologia e conservação pós-colheita de goiaba 'Paluma' sob recobrimentos à base de amido de sementes de Jaca

Resumo –O objetivo deste trabalho foi avaliar o efeito de recobrimentos à base de amido de sementes de jaca (A), adicionado à quitosana e ao alginato, na fisiologia e manutenção da qualidade de goiabas 'Paluma', no armazenamento refrigerado, seguido da transferência para a condição ambiente. O delineamento foi o inteiramente casualizado, em 4 repetições, e esquema fatorial 4x2, sendo 4 recobrimentos (dispersão de A - 4%; A - 2% + quitosana - 2% (AQ), A - 2% + alginato - 2% (AA) e o controle - sem recobrimento), em 2 ambientes (refrigerado (10±2 °C e 80±2% U.R) com transferência para o ambiente (25±3 °C e 75±4%, RU) no 16° e 20º dias de refrigeração. Os recobrimentos AQ e AA foram eficientes em reduzir a taxa respiratória em frutos durante 10 dias ao ambiente. O recobrimento AQ retardou o amadurecimento dos frutos, manteve a firmeza e a coloração, com intenção de compra e aparência superiores ao limite de aceitação por mais 6 dias, após a transferência para o ambiente, aos 16 dias de refrigeração.

Termos para indexação: *Artocarpus heterophyllus*, descarte de frutos, quitosana, alginato de sódio, qualidade.

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Introduction

The functional value, flavor, aroma and appearance, as well as the dual aptitude for fresh consumption and processing place guava as a tropical fruit of great importance (FLORES et al., 2015). However, postharvest quality conservation of guava is still a challenge in the production chain due to the reduced postharvest life from the high respiratory rate, fast loss of firmness and incidence of decay during storage (FORATO et al., 2015). Thus, high levels of postharvest losses of guavas require efficient and sustainable technologies to maintain quality (CISSÉ et al., 2015), such as biodegradable coatings obtained from starch sources from discarded fruit parts.

A coating should allow adequate gaseous exchanges between the fruit and the environment, thereby avoiding the risk of fermentation but satisfactorily (LIANG et al., 2015) reducing metabolic rates, and therefore respiration in order to increase its shelf life (AQUINO et al., 2015). Cassava starch is a biodegradable source with high potential for use (GUIMARÃES et al., 2017). However, unconventional sources of starch such as yams and sweet potatoes have been exploited with respect to technological properties (ALCÁZAR-ALAY; MEIRELES, 2015). Jackfruit (*Artocarpus heterophyllus* Lam.) is an abundant fruit in Northeast Brazil, where 15 to 25% of seeds are considered as waste. Both soft and hard types of jackfruit have seeds with high starch content (92.8% and 94.5%, respectively (MADRUGA et al, 2014) with 24 and 32% amylose (ZHANG et al, 2016). This seed is widely consumed directly or as an ingredient in Asian cuisine, with potential for starch extraction to be used in food formulations (RENGSUTTHI; CHAROENREIN, 2011)

Chitosan coatings and combinations have been used in the postharvest conservation of cherries to increase shelf life (XIN et al., 2017). Cassava starch associated to chitosan delayed ripening of 'Tommy Atkins' mangoes followed refrigeration at 12°C, in addition to providing better fruit appearance (AZERÊDO et al., 2016). Coating with 1% alginate kept the quality attributes of *Arbutus unedo* fruits in cold storage (GUERREIRO et al., 2015).

Although refrigeration is the most practical and efficient tool for postharvest fruit conservation, an evaluation of the influence of the exposure time to cold when exposed to higher temperatures at markets is fundamental in determining the quality. Thus, it is necessary to demonstrate the effectiveness of postharvest treatments in reducing the impacts of stress caused by the sudden alteration of temperature on fruit quality and shelf life (AZERÊDO et al., 2016).

In this context, the objective of this study was to evaluate the physiology and postharvest conservation of 'Paluma' guava coated with biodegradable films based on jackfruit seed starch added to chitosan and sodium alginate, and to verify the changes that occurred during cold storage and following transfer to room conditions.

Material and Methods

'Paluma' guavas (*Psidium guajava*) were harvested in the early morning at commercial maturity (predominant light green coloration) from an orchard in Nova Floresta-PB municipality, and soon transported to the laboratory in isothermal boxes.

The starch from jackfruit (*Artocarpus heterophyllus* Lam.) seeds was prepared following the harvest of ripe fruits and the seeds were removed from the pulp and placed to dry in the shade. After drying, the seed coat was manually removed from the seeds and sanitized with 50ppm of free chlorine for 3 min. The seeds were ground three times with bidistilled water followed by filtration, and the residue was crushed again in a multiprocessor for 3 minutes. After the filtrations the collected product was decanted for 12 hours. At the end of the decantation, the water was discarded and the starch was oven dried at 40°C for 10 hours until reaching a residual moisture of 17%.

To prepare the coatings, 2% chitosan was diluted in 1% acetic acid and the solution stirred for 2 hours. The 2% sodium alginate was diluted in distilled water heated to 60°C under stirring. The jackfruit seed starch (2 and 4%) was gelled with distilled water at 70°C (LIMA et al., 2012). Each dispersion was combined with that of 2% starch, in addition to the starch dispersion alone, such that after homogenization, the concentration of the components in the matrices was 4%. From preliminary tests, 1% of glycerol and 0.5% of Tween 20® were added to the matrices with ultrasonic aid (LIANG et al., 2015).

The fruits were sanitized with sodium hypochlorite solution at 50 ppm and dried in the air. The coatings were then applied by immersion for 60 seconds with subsequent drying in the air, and 6 fruits were packed in trays of expanded polystyrene and stored under refrigeration (10±2 °C and 80±2% RH), being transferred to room condition (25±3 °C and 75±4% RH) at the 16th day to simulate marketing.

The design was completely randomized, in a 4x2 factorial scheme, with 4 coatings (4% acetic acid starch (S), S 2% + 2% chitosan (SC), S 2% + sodium alginate a 2% (SA), and witness (W), fruits without coatings) and 2 environments (refrigerated and room condition), in 4 replications. Part of the experimental units kept under refrigeration were transferred to room condition at 16 (sensory) or 20 days to simulate marketing. The evaluations were carried out at intervals of 2 days for both storage conditions.

The fruits were evaluated for weight loss, by daily weighing using a semi-analytic scale, by the percentage difference in relation to the initial weight; firmness of the intact fruit using a Magness Taylor Pressure Tester penetrometer, with two readings in the equatorial region on opposite sides of the fruit; the color index (CI) of the peel, calculated from the values of *L*, a* and b*, using an equation proposed by Camelo and Gomes (2004), IC = 2000 * (a*) / *L** (√ (a*) + (b*)²); Soluble Solids (SS), with Abbe type refractometer at 20°C; titratable acidity (TA g citric acid/100 g^{-1} pulp) by titration with 0.1M NaOH solution; SS/TA ratio, by dividing SS index by TA (LIMA et al., 2012). The respiratory activity was evaluated 12 hours after the application of the coatings at room condition in 4 replications (≅650g/replication) in 2000 mL hermetically sealed jars during 10 days. The samples were continuously ventilated with a supply of dehumidified and CO_2-free air, with a flow rate of 10 mL.min^{-1}. The system was closed for one hour to collect CO_2 samples with an insulin syringe and 1 mL was injected into CO_2 analyzer (CA-10, Sable Systems-USA) coupled to the integrator. CO_2 production (mL CO_2 .kg^{-1}.h^{-1}) was obtained by comparing with the 2.5% CO_2 standard (DANTAS et al., 2016).

Sensory evaluations were performed to describe fruit quality during storage, using 24 judges previously trained according to Ellendersen e Wosiack (2010). The judgment of the coded samples was done using 10 cm unstructured scale, in which the panelist marked the intensity of the characteristics evaluated with a vertical trace. The samples were composed of 9 fruits of each coating, being evaluated the coloration (Green to Yellow), serious damages (Absent to Serious), purchase intention (I would not buy/would certainly buy) and global acceptance (I greatly disliked /I liked it very much), with 4 as the limiting score.

Data were submitted to analysis of variance by the F test ($p \leq 0.05$). For the factor storage days and their interaction with the coatings, polynomial regression was applied until the second degree, and Tukey's test ($p \leq 0.05$) was applied for the coating factor. Based on Azerêdo et al. (2016), the nested comparison of storage days within each environment and the interaction between coatings with the two environments was considered. It was performed a correlation and analysis of principal components, considering as significant variables, those above the limit of inclusion of variables (result of the multiplication of the largest auto vector by 0.7), using software SAS® 9.3 (2012).

Results and Discussion

The coating effect on the 'Paluma' guava respiratory activity harvested in the predominant light green color during storage at room conditions (25±3 °C and 75±4% RH) during 10 days was evaluated (Figure 1A). The respiratory peak in control fruits occurred at 7 days postharvest (~ 65 mL of CO_2.Kg^{-1}.h^{-1}). In coated guavas, the respiratory rate was about 50% lower, especially in jackfruit seed starch (2%) and chitosan (2%) (SC), and starch (2%) + alginate (2%) (SA). In this way, the coatings formed a barrier around the fruits, modifying the internal atmosphere, reducing metabolic rates and delaying ripening; this has also been reported (but with less impact) by Vishwasrao and Ananthanarayan (2016) on guavas coated with hydroxypropyl methyl cellulose (HPMC).

After 20 days of storage at 10°C, guavas coated with SC and SA presented lower weight loss (Figure 1B) of 2.5% on average (~ 22% less than S and W), certainly due to the lower metabolic rate. This level of mass loss is well acceptable, since this should be less than 5% for fresh produce (PAREEK et al., 2016). The weight loss did not differ among coatings following the transfer of fruits to the room condition on the 20th day at 10±2°C.

The firmness of the guavas declined independently of the coatings and storage conditions. However, fruits covered with SC were much firmer in refrigeration, followed by SA. After transfer to the room condition at 20 days, fruits coated only with jackfruit seed starch (S) lost more firmness, but they were firmer when associated with chitosan (Figure 1C). Therefore, it is noted that the jackfruit seed starch alone was not efficient in delaying the loss of mass, nor in maintaining the firmness in both the refrigeration and after the transfer to the room conditions, although it did reduce the respiratory rate in relation to control when evaluating the effect of the different coatings during 10 days at room conditions. As it has amylose as the major component (ZHANG et al., 2016), it appears that the starch composition of the jackfruit seed makes coatings more permeable to gases and moisture, since there are few hydrogen bonds and covalent bonds between starch chains. Thus, the SC followed by SA associations formed more stable and less permeable coatings, thereby reducing the guava's mass and firmness losses during storage. Cherries coated with combinations of chitosan with nano-SiOx showed inhibition of pectin breakdown (XIN et al., 2017). Guava firmness is an important indicative of quality and of longer shelf life, being relevant in guava acceptance by the consumer (AQUINO et al., 2015).

The soluble solids (SS) did not differ in relation to the cold storage periods and following the transfer to the room conditions. However, following 20 days of storage at 10°C, the highest SS contents were of guavas coated with SC (14.18%) and SA (14.41%) (Figure 1D), pointing out the lower use of substrates for the respiratory flux, and clearly indicating the reduction of metabolic rate (PAREEK et al., 2016), as also shown for the respiratory rate. One of the responses to the use of coatings in fruits is the reduction of respiratory rate and loss of mass due to the reduced permeability to moisture and gases (CO_2 and O_2), reducing the metabolic rate and therefore maintaining the SS content since the hydrolysis of starch to soluble sugars is much lower (VERSINO et al., 2016).

The titratable acidity (TA) of 'Paluma' guava increased (Figure 1E) and the SS/TA ratio decreased (Figure 1F) by the 10^{th} day, then declined until the 20^{th} day at 10±2°C. However, the transfer to the room condition on day 20 caused an increase in TA and reduction in the SS/TA ratio in fruits of all the coatings. In general, TA was higher in fruits coated with isolated starch (S) with 0.98

g citric acid.100g^{-1}, and lower in SC-coated fruits, with a higher SS/TA ratio (17.31) during storage. Vishwasrao and Ananthanarayan (2016) also reported an increase in TA in 'Lalit' guavas kept at room conditions under HPMC-based coatings. In this study, it was verified that the SC combination reduced the acidity contents in guavas both under refrigeration and room conditions, as also reported by Aquino et al. (2015) in guavas coated with chitosan.

The reducing sugar (RS) in guavas did not differ among coats at 10°C, but declined after the transfer on the 20th day to room conditions, being higher in those coated with SC (Figure 2A). The decline of RS after withdrawal to the room conditions was probably due to increased respiratory activity, being an indication that the ripening evolved after the temperature change. The highest RS contents in guavas are generally observed around the climacteric peak, then decreasing as the fruit loses its firmness (VISHWASRAO; ANANTHANARAYAN, 2016).

The color index of the peel (CI) also indicated a clear delay in the guava ripening with the fruits coated with SC, followed by those with SA, those with the highest inhibition, and the green color was maintained after transfer on the 20th day to the room condition (Figure 2B). The abrupt change in coloration after refrigeration transfer is a physiological response to temperature shock, leading to changes in chlorophyll content and altering fluorescence (AZÊREDO et al., 2016). This not only results in abrupt changes in the peel color, but also in the pulp, which rapidly loses its characteristic color (FORATO et al., 2015). Thus, the role of a coating is to soften the effect of the higher temperature in the post-cooling. Therefore, jackfruit seed starch alone did not delay the maturation of guavas in both storage conditions and presented behavior similar to the control; unlike the other coatings that maintained the external (green) coloration for a longer time.

The effect of the starch-based coatings of jackfruit seed was also clearly detected by the sensory analysis. Guavas coated with S and control showed a loss in green color and development of yellow from the 4th day, with predominance of the latter from day 8 at 10ºC (Figure 2C). However, in guavas coated with SC followed by SA, the green color predominated until the 20th day (note 4), maintaining the yellowish green coloration after the transfer to room condition for a further 6 days. Thus, the addition of chitosan potentiated the ability of the jackfruit seed starch-based coating to retard the evolution of yellow coloration, and based on the Q10 concept (PAREEK, 2016) in the cold storage resulted in reduced respiratory activity, consequently delaying fruit ripening also at room condition.

Serious damage in 'Paluma' guava (0= absent, 2.5= low, 5= moderate, 7.5 = moderate and 10 = strong) were barely perceptible until the 8th day at 10ºC, guavas with SC followed by SA remained acceptable until the 16th day (score>4). After transfer to the room condition, SC guavas showed low incidence of serious damages for a further 6 days; this is in contrast to the control and S fruits which already exceeded the acceptance limit on the 16th day at 10ºC (Figure 2D). The serious damages described by the panellists for guavas during storage were the incidence of stains and rot, and the occurrence of the latter already leading to rejection (score >4).

For purchase intention (0 = would not buy; 5 = maybe would buy and 10 = surely would buy), the panellists rejected fruits coated with S and those of the control from the 14th day, which reached scores below 2.6. On the other hand, scores higher than 5 were obtained for SC fruit for 16 days at 10ºC, remaining above the acceptance level until the 22nd day after transfer to room condition (Figures 2E and 2F), indicating the superiority of this coating in maintaining the quality of the fruits in market displays.

On the overall acceptance, only guavas coated with SC followed by SA (Figure 2G) obtained scores above the acceptance limit (four) for 20 days at 10°C. However, when they were transferred to room condition at 16 days at 10°C, only those coated with SC remained above the acceptance limit for a further 6 days (Figure 2H). As the incidence of serious damages was also considered in the overall acceptance, which takes into account all evaluated sensory attributes, SA guavas exceeded the limit of acceptance due to the presence of fungi, which was not observed in the SC. One of the main benefits of using biodegradable coatings in fruits is the maintenance of appearance, the absence of fungi, and the reduced metabolism that may delay ripening (VERSINO et al., 2016). By reducing the loss of mass during storage, the coating maintains its turgidity, in addition to the fruit's brightness (AZÊREDO et al., 2016; GUIMARÃES et al., 2017). In this study, there was a clear indication of the greater efficiency of SC in delaying guava ripening.

In comparing the color index (CI) of the peel with the studied variables (Figure 3), a strong and negative correlation (r = -0.74) was observed with the firmness of the fruits, indicating that the CI in guava declines as yellow color evolves during maturation. At the same time, TA increased with a weak and positive correlation (r = 0.34), and the SS/TA ratio decreased with a weak and negative correlation (r = -0.29). For appearance, there was a strong and positive correlation with the CI of the peel and severe damage, indicating that the fruit tended to present higher rates of serious damage with the evolution of the yellow coloration, independently of the coating and even in different intensity. The intent to purchase and overall acceptance declined as storage advanced, thus showing strong and negative correlations with the rate of serious damage. Taken together, the chitosan addition of jackfruit seed starch appears to have improved the physical properties of the coating, thus reducing metabolic rates in order to delay maturation, maintain appearance and extend the postharvest life of 'Paluma' guavas in 6 days at room condition. These results are particularly important considering that the jackfruit seed is a food processing waste, and therefore of low cost.

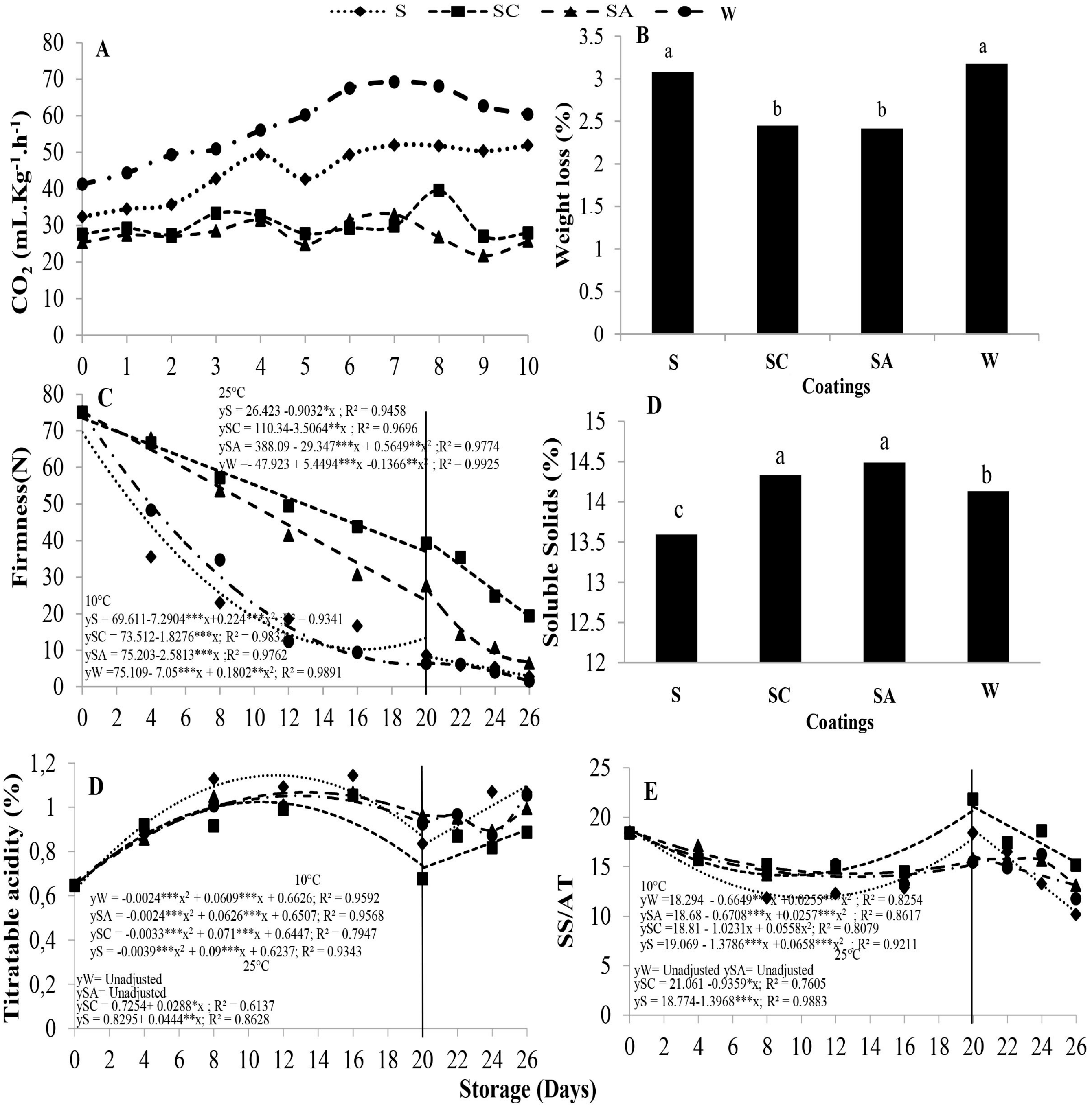


Figure 1- Respiratoty rate (mL CO_2.Kg^{-1}.h^{-1}) during 10 days at room condition (25±3 °C and 72 ± 4% RH) (A); firmness (N) (C), titratable acidity (g citric acid.100g^{-1} pulp) (E) and SS/TA ratio (F) during cold storage at 10±2 °C and 80 ± 2% RH and after transfer to room condition at the 20th day; and the mean values of weight loss (%) (B) and soluble solids (%) (D) during the cold storage, for 'Paluma' guava under coatings based on jackfruit seed starch at 4% (S); S at 2% + chitosan 2% (SC); S at 2% + sodium alginate at 2% (SA); and Control (W).

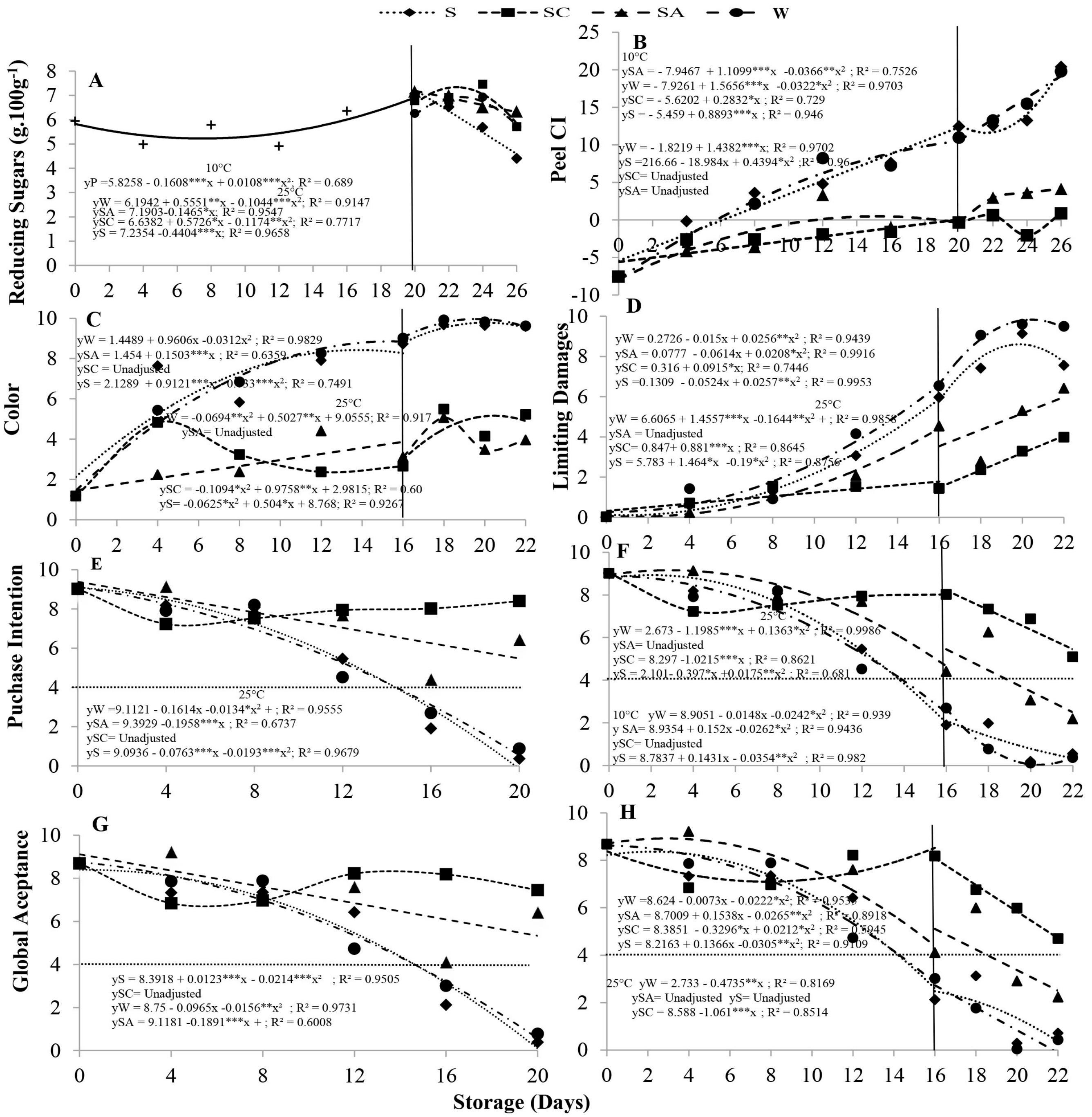
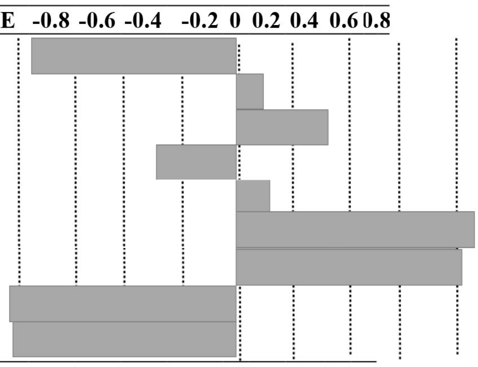


Figure 2- Reducing sugars (g glucose.100g-1 pulp) (A), peel color index (CI) (B), color (C), limiting damages (D), purchase intention (E and F) and global acceptance (G and H) of 'Paluma' guava during cold storage during cold storage at 10±2 °C and 80 ± 2% RH and after transfer to room conditions at the 20th day for 'Paluma' guava under coatings based on jackfruit seed starch at 4% (S); S at 2% + chitosan 2% (SC); S at 2% + sodium alginate at 2% (SA); and Control (W). n=4.

VARIABLE	BY VARIABLE	CORRELATION	SIGNIFICANCE
PEEL CI	Firmness	-0.7446	<.0001*
	SS	0.0988	0.1729
	Titratable Acidity	0.3367	<.0001*
	SS/AT	-0.2942	<.0001*
	Reducing Sugars	0.1179	0.1033
	Color	0.8769	<.0001*
	Limiting Damages	0.8291	<.0001*
	Purchase Intention	-0.8235	<.0001*
	Global Acceptance	-0.819	<.0001*



n= 192

Figure 3 - Simple correlation among the color index (CI) of de skin, and characteristics related to quality and appearance of 'Paluma' guava during cold storage at 10±2 °C and 80 ± 2% RH and after transfer to room condition (25±3 °C and 72±4% RH) at 16 e 20 days, under coatings based on jackfruit seed starch at 4%; jackfruit seed starch at 2% + chitosan 2%; jackfruit seed starch at 2% + sodium alginate a 2%; control.

Conclusions

The use of the coating associated with 2% jackfruit seed starch + 2% chitosan (SC) and + 2% alginate (SA) were efficient in reducing the respiratory rate of 'Paluma' guava maintained at room condition for 10 days. Coating fruits with SC delayed ripening, maintaining guava quality and acceptance during cold storage and also after transfer to room condition. Fruits coated with SC maintained firmness and color index, with intention to purchase and overall acceptance above the acceptance limit for a further 6 days after transfer to room condition at 16 days of refrigeration, thus constituting a promising alternative for postharvest conservation of 'Paluma' guava.

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