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Effect of *Ascophyllum nodosum* seaweed extract on post-harvest 'Tommy Atkins' mangoes

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Abstract - The aim of this study was to determine the effect of *Ascophyllum nodosum* extract (AN), applied in different doses on the physicochemical characteristics of post-harvest 'Tommy Atkins' mangoes. The selected mango fruits were treated with different extract concentrations (0%, 0.1%, 0.3%, 0.5% and 1.0%) and stored for 12 days at 25±2 °C and 75±5% RH. Every 3 days, fruit mass loss (PM), color angle (Hue) (AH), firmness, pH, titratable acidity (AT), content of total soluble solids in pulp (°Brix) and content of carbohydrates in shell were assessed. The experiments were carried out in CRD in a 5 x 5 factorial arrangement (concentrations x days of storage) with four replications. AN was effective in preventing PM, with a reduction of up to 40.2%, as observed at concentration 0.1% compared to control. There were no differences among concentrations on AH, however, all of them differed significantly from control with higher AH, especially on the 12nd day. Fruit firmness was lower in control, the pH increased with the storage time and AT decreased. For all these parameters, mangoes treated with the extract differed from control. The pulp sample °Brix increased with the storage time as well as the content of reducing sugars, with consequent reduction of non-reducing sugars in fruit shell. These tests indicate the possibility of using AN as an alternative management in preserving mangoes in the post-harvest stage.

Index Terms: *Mangifera indica*, fruit preservation, shelf life.

Efeito do extrato da alga marinha *Ascophyllum nodosum* sobre mangas 'Tommy Atkins' na pós-colheita

Resumo – O objetivo deste trabalho foi verificar o efeito do extrato de *Ascophyllum nodosum* (AN), aplicado em diferentes doses, sobre características físico-químicas de mangas 'Tommy Atkins', na fase de pós-colheita. As mangas selecionadas foram tratadas com diferentes concentrações do extrato (0%, 0,1%, 0,3%, 0,5% e 1,0%) e armazenadas por 12 dias, a 25±2 °C e 75±5% de UR. Foram avaliados, a cada 3 dias, a perda de massa dos frutos (PM), o ângulo de cor (Hue) (AH), a firmeza, o pH, a acidez titulável (AT), o teor de sólidos solúveis da polpa (°Brix) e o teor de carboidratos da casca. Os experimentos foram conduzidos em DIC, em arranjo fatorial 5 x 5 (concentrações x dias de armazenamento), com quatro repetições. O AN foi eficiente em evitar a PM dos frutos, registrando redução de até 40,2%, como observado na concentração de 0,1% em relação ao controle. Não houve diferença entre as concentrações quanto ao AH, contudo todas diferiram significativamente do controle com AHs maiores, especialmente no 12º dia. A firmeza do fruto foi menor no controle, o pH, aumentou com o tempo de armazenamento e a AT diminuiu, sendo que, para todos esses parâmetros, mangas tratadas com o extrato diferiram do controle. O °Brix da polpa das amostras aumentou com o tempo de armazenamento, assim como o teor de açúcares redutores, com consequente redução de açúcares não redutores na casca do fruto. Estes testes apontam para a possibilidade de uso do AN como alternativa de manejo na preservação de mangas na fase de pós-colheita.

Termos para indexação: *Mangifera indica*, preservação de frutos, tempo de prateleira.

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Introduction

Mango (*Mangifera indica* L.) is a typically tropical fruit and can be found throughout Brazil. It has important participation in the Brazilian market of fresh fruits, especially when these are destined for export. According to the Brazilian Yearbook of Fruticulture published in 2015, the export market for fresh fruit in Brazil ended the year of 2014 with a reduction of 5.46% in volume and 3.21% in revenue. However, the mango activity presented the second largest volume of exports, with an increase of 9.04% over 2013, only behind melon and generating revenue of approximately 164 million dollars.

In order to guarantee the growth of this market, producers should keep in mind the appropriate times for fruit production and future commercialization, as well as the techniques of correct management of fruits, aiming, therefore, the quality that meets the international export standards and minimizing losses. With regard to losses, Alves et al. (2010) listed the eight fruits with the highest losses in Brazil in the post-harvest phase, and mango appears in fifth place. These losses are the result of infections acquired in the field, pests and places unsuitable for storage and application of products that interfere with fruit physiology.

Some studies have documented the potential of certain commercially available substances and products in the protection of mangoes and in the improvement of the physiological quality of fruits (AMARIZ et al., 2010; PLOTTO et al., 2010; SOUZA et al., 2011). Many other products are available; however, few have been studied. In addition, most studies do not target the post-harvest life of vegetables. This is the case of the *Ascophyllum nodosum* (L.) Le Jolis seaweed extract, which has been widely used for the most varied purposes and has presented excellent results. According to tests already carried out, its extract is an excellent promoter of plant growth and nourishment of plants (CARVALHO, 1990), accelerates seed germination and favors the establishment of seedlings (JAYRAMAN; NORRIE; PUNJA, 2011), increases the resistance of the plant treated to biotic and abiotic stresses (KHAN et al., 2009) and activates plant resistance mechanisms (CRAIGIE, 2011).

Seaweed extracts are natural compounds, sources rich of nutrients and bioactive compounds. An expressive number of brown seaweeds is already used in agriculture and sold as plant fertilizers (CRAIGIE, 2011). Many works have already been developed with seaweeds for disease control, but few have focused on the post-harvest phase of fruits. Studies aimed at verifying the effect of these new management possibilities on the physicochemical and organoleptic characteristics of fruits are even scarcer.

The market for agricultural inputs with biomolecules that have antimicrobial properties and increase food safety is growing in Brazil. If these molecules are able to increase the useful life of fruits without compromising the environment, especially humans, they will certainly reach market niches, thus generating a chain of benefits from producer to consumer.

Thus, the aim of this work was to verify the effect of *Ascophyllum nodosum* seaweed extract applied at different doses on the physicochemical characteristics of post-harvest 'Tommy Atkins' mangoes.

Material and methods

The work was divided into two stages, the first one developed at the Laboratory of Post-Harvest of the State University of Maranhão, UEMA, in São Luís, MA, and the second at the Laboratory of Physiology and Phytopathological Biochemistry - ESALQ / USP, Piracicaba, SP.

'Tommy Atkins' mangoes were purchased from the Central of Supply of the municipality of São Luís, CEASA and those presenting standardization as to size, physiological stage of maturation and bark coloring corresponding to grade 3 of the GTZ scale were selected (GZT, 1992).

Afterwards, mangoes were taken to the UEMA Laboratory of Post-Harvest and peduncles were standardized to 20 mm in length, and then washed with neutral detergent and drinking water, sanitized in 0.05 % sodium hypochlorite solution, washed once more in drinking water, placed to dry in the environment and separated in trays lined with paper towels, with two fruits each tray.

Commercial *A. nodosum* extract, Acadian®, which was used in tests, presents 5.3% of K_2O and 6.0% of total organic carbon in its constitution. Five concentrations of the product in water were prepared: 0, 0.1%, 0.3%, 0.5% and 1.0% v / v. In addition, at the different concentrations prepared, Tween 20® was added at concentration of 0.5%. Treatments were applied by immersing mangoes at each concentration for 5 min. After treatment, fruits were stored at 25 ± 2°C, 12 h photoperiod and 75 ± 5% RH, for 12 days.

Every 3 days, the total fresh mass of fruits was measured with the aid of a digital scale with maximum capacity of 2,000 g, accuracy of 0.1 g and expressed as percentage of fresh mass lost. Pulp coloration was measured using Minolta colorimeter model Chroma Corp CR-400, expressed by the color angle (Hue). Pulp firmness was expressed in Newton and was evaluated with TA-XT2 texturometer, 8 mm tip, with distance and penetration rate of 9 mm and 1 mm s^{-1}, respectively. Pulp acidity was determined in pH meter and, in addition, titratable total acidity (TA), the latter being expressed in g of citric acid 100 g^{-1} of pulp. In addition, the content of soluble solids was measured in refractometer and expressed in °Brix.

In addition to the tests described above, biochemical analysis of shell was carried out to verify the content of total carbohydrates, expressed by the content of total soluble sugars (TSS), reducing sugars (RS) and non-reducing sugars (NRS). For this, at the end of each day of analysis, 1 g of bark from each replicate was removed and macerated in the presence of liquid nitrogen and PVPP, followed by the addition of 5 mL of 100 mM sodium phosphate buffer (pH 6.0) and centrifuged at 20,000 *g* for 30 min at 4 °C. Then, 500 µL of supernatants (extract) obtained were added of 500 µL of phenol (5.0%) in distilled water and 2.5 mL of concentrated sulfuric acid. After 30 min of incubation at room temperature, absorbance was determined at 490 nm in Hitachi spectrophotometer, model U-1900, based on a standard glucose curve (DUBOIS et al., 1956). The content of reducing sugars was determined by the addition of 1.5 mL of p-hydroxybenzoic acid hydrazide (1 g dissolved in 20 mL of 0.5 M HCl with addition of 80 mL of 0.5 M NaOH) in 500 µl of extract, which was incubated in water bath at 100 ° C for 5 min. After cooling, the absorbance of the sample at 410 nm was determined in Hitachi spectrophotometer, model U-1900, and the concentration of RS was determined based on the standard glucose curve (LEVER, 1972). The concentration of non-reducing sugars (NRS) was indirectly determined through the following calculation: NRS = TSS - RS (RIBEIRO, 2006). The results of these three groups of sugars were expressed in mg g^{-1} glucose of shell.

The experimental design was completely randomized, consisting of two factors: *A. nodosum* extract (0, 0.1%, 0.3%, 0.5% and 1.0%) and storage days (0, 3, 6, 9 and 12 days). The zero dose (0) corresponded to the control of tests. Four replicates were used per treatment, and the experimental plot was composed of two fruits / tray. Data were submitted to analysis of variance and means were compared by the Tukey test (p = 0.05). In addition, for better understanding of data, these were correlated by linear regression analysis.

Results and discussion

The fresh mass results showed that the *A. nodosum* extract, at the different doses applied in fruits, positively interfered in this parameter. A cumulative loss of 8.25% of fresh mass in fruits composing the control treatment was observed at the end of the storage period. However, fruits treated with 0.1%, 0.3%, 0.5% and 1.0% of AN showed losses of 4.93%, 5.20%, 5.69% and 5.62% respectively, at the end of 12 days of storage. That is, mangoes treated with AN had reductions in fresh mass losses of 40.2%, 36.9%, 31% and 31.9%, respectively, for the doses used, in relation to fruits of the control treatment. It is noteworthy that, when the results obtained by treatment of mangoes with the product, excluding control (null concentration), the difference between treatment that most avoided mass loss and treatment with 0.1% of extract (40.2%), and treatment that avoided the least mass loss, treatment with 0.5% of extract (31%), is of 9.2%, a value that, when seen from the commercial perspective, is quite significant and therefore suggests further studies on the recommended concentrations for use of the extract (Figure 1).

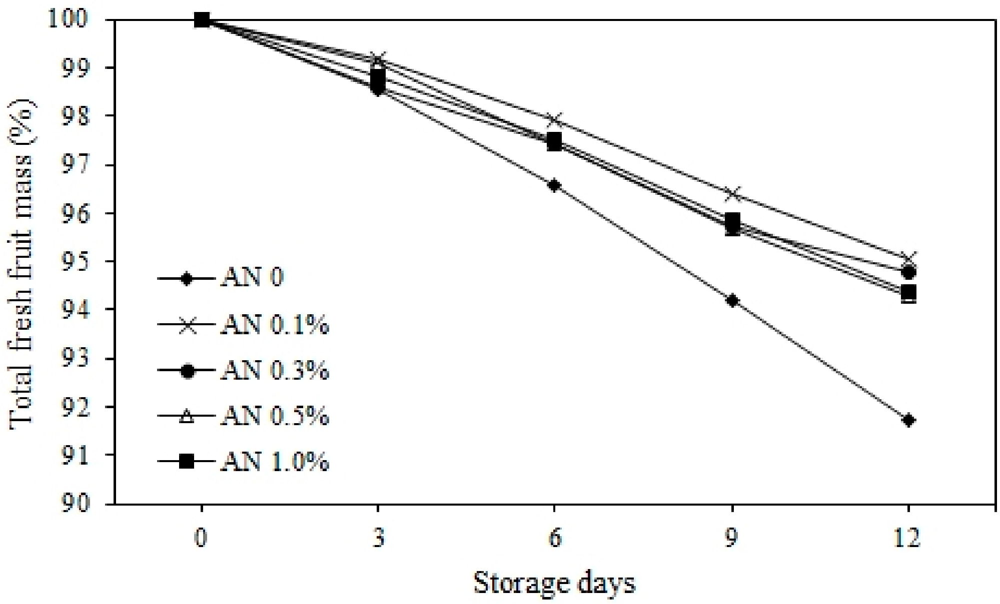

Figure 1. Fresh mass loss percentage of 'Tommy Atkins' mangoes treated with different *Ascophyllum nodosum* (AN) extract doses and stored at 25 ° C, photoperiod of 12 h.

Mass loss during fruit storage is quite common and is linked to factors such as the increased respiratory activity of the plant in the period, increased enzymatic activity in the fruit and consequent change in phenological stage. According to Chitarra and Chitarra (2005), some products are still tradable with moisture loss around 10%; however, mass losses of 3 to 6% are enough to cause a significant decline in fruit quality. There are no records in literature on the use of *A. nodosum* extract for the post-harvest treatment of fruits; however, the use of the extract under field conditions increased productivity and improved the quality of bell pepper, 'Thompson' grape and strawberry fruits, and, for the latter, an increase in size and average weight of fruits was also reported (AMOROS et. al., 2004; ROUSSOS; DENAXA; DAMVAKARIS, 2009; NORRIE; KEATHLEY, 2006). In mangoes, the effects of Biovita® biofertilizer based on *A. nodosum* were observed in mangoes of the Dashehari cultivar, which presented higher productivity than untreated mangoes (REDDY et al., 2011).

Based on colorimetric analysis, it was possible to verify that fruits at the 0 (zero) storage time presented high uniformity regarding color angle (Hue), and there were no significant differences among treatments, which presented, on average, color angle around 90 °, that is, in the yellow band. However, over time, the decrease of these color angles in the first quadrant (0 ° to 90°) was observed, indicating that the color of fruits migrated from yellow to red. Thus, the reduction of the color angle in the control treatment, from 91° to 74.5°, indicated fruit maturation that, visually, presented more yellowish pulp throughout the days.

Comparing this reduction with the other treatments, it was observed that, although there was also a reduction in the color angle, this reduction was less pronounced, from 91.4 ° to 83.7 ° at 0.1% dose, from 91.3 ° to 81.1 ° at 0.3% dose, from 90.3 ° to 80.3 ° at 0.5% dose and from 91.8 ° to 80.9 ° at 1.0% dose of *A. nodosum* extract. Analysis of variance showed that the treatment of fruits with different doses of the extract generated significantly different responses compared to control fruits.

AN treated fruits presented higher color angles; therefore, less mature fruits on all storage days (Figure 2A). However, linear regression analysis showed that the different concentrations used in these tests have low correlation with the Hue angle (R^2 = 0.19) (Figure 2B). In contrast, the storage time presented strong correlation with this parameter (R^2 = 0.98), since the longer the storage time, the lower the color angle observed, i.e., fruits were more mature (Figure 2C). Thus, the experiments demonstrated that the storage time of mangoes under the conditions in which this experiment was conducted, directly influenced fruit ripening (lower Hue angle); however, the treatment of fruits before storage with the *A. nodosum* extract provided a delay in the decrease of the color angle, that is, a probable delay of organ senescence, regardless of dose used.

Silva et al. (2014) demonstrated that the application of *A. nodosum* extract in strawberry leaves resulted in less intense red fruits, that is, with a more open color angle when compared to control. This is probably related to the high capacity of the extract to increase plant resistance to diverse abiotic stresses, as already reported for vines (MANCUSO et al., 2006), olive trees (CHOULIARAS et al., 2009) and *Arabidopsis thaliana* (RAYIRATH et al., 2009).

Regarding pulp firmness, it was verified that the longer the storage time of mangoes, the lower pulp firmness recorded in texturometer (Figure 3A). This was confirmed by both the means test and by linear regression analysis (R^2 = 0.85), which demonstrated the high correlation of the firmness parameter with the fruit storage time under the conditions performed in these trials (Figure 3C). By the analysis of variance, it was observed that, on all evaluation days, fruits treated with the seaweed extract presented mean firmness values higher than control, regardless of dose applied (R^2 = 0.46) (Figure 3B).

Seaweed extracts have a direct effect on fruit firmness in the post-harvest phase, as observed in strawberries fertigated with 4.5 L / ha of *A. nodosum* extract, which presented up to 45% firmer than untreated fruits (SILVA et al., 2014).

The evaluation of the pH of the fruit pulp showed that there were no significant differences among mangoes evaluated at time 0 (zero) of storage (Figure 4A). However, during the evaluation days, it was verified that some fruits presented lower pH values and significantly different from control. On the last day of evaluation, day 12, analysis of variance showed that 0.1% and 0.3% doses had pH values significantly lower than dose 0 (Figure 4A). However, even if there were significant differences among concentrations used, the low correlation presented by the linear regression analysis of concentration *versus* pH (R^2 = 0.21) indicates that these differences have little to do with the increase in the extract concentration (Figure 4B).

Differently, the linear regression analysis that verified correlation of storage days with pH, showed that these parameters are highly correlated (R^2 = 0.90); thus, the longer the storage time, the higher the fruit pH, which indicates that, physiologically, fruits matured over time (Figure 4C). Morais et al. (2002) reported that during ripening, mangoes tend to increase pH and decrease acidity, as observed in this study.

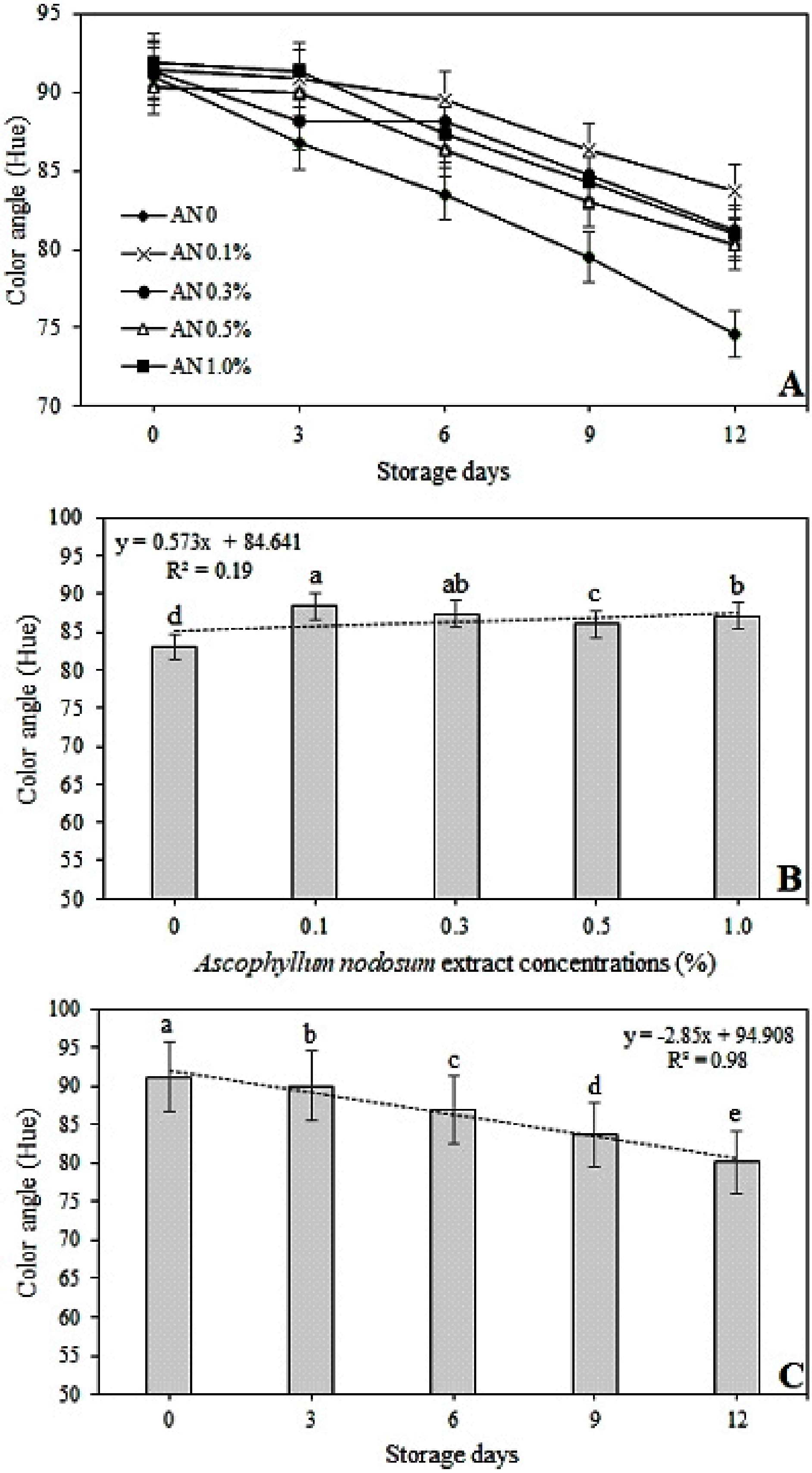

Figure 2. Mean values for the color angle (hue) of 'Tommy Atkins' mango pulps treated with different *Ascophyllum nodosum* (AN) extract concentrations (A) stored at 25°C. Linear regression analysis of extract concentrations (B) and storage days (C) with color angle. Columns followed by the same letter do not differ by the Tukey test (p = 0.05). Bars represent the standard error of the mean.

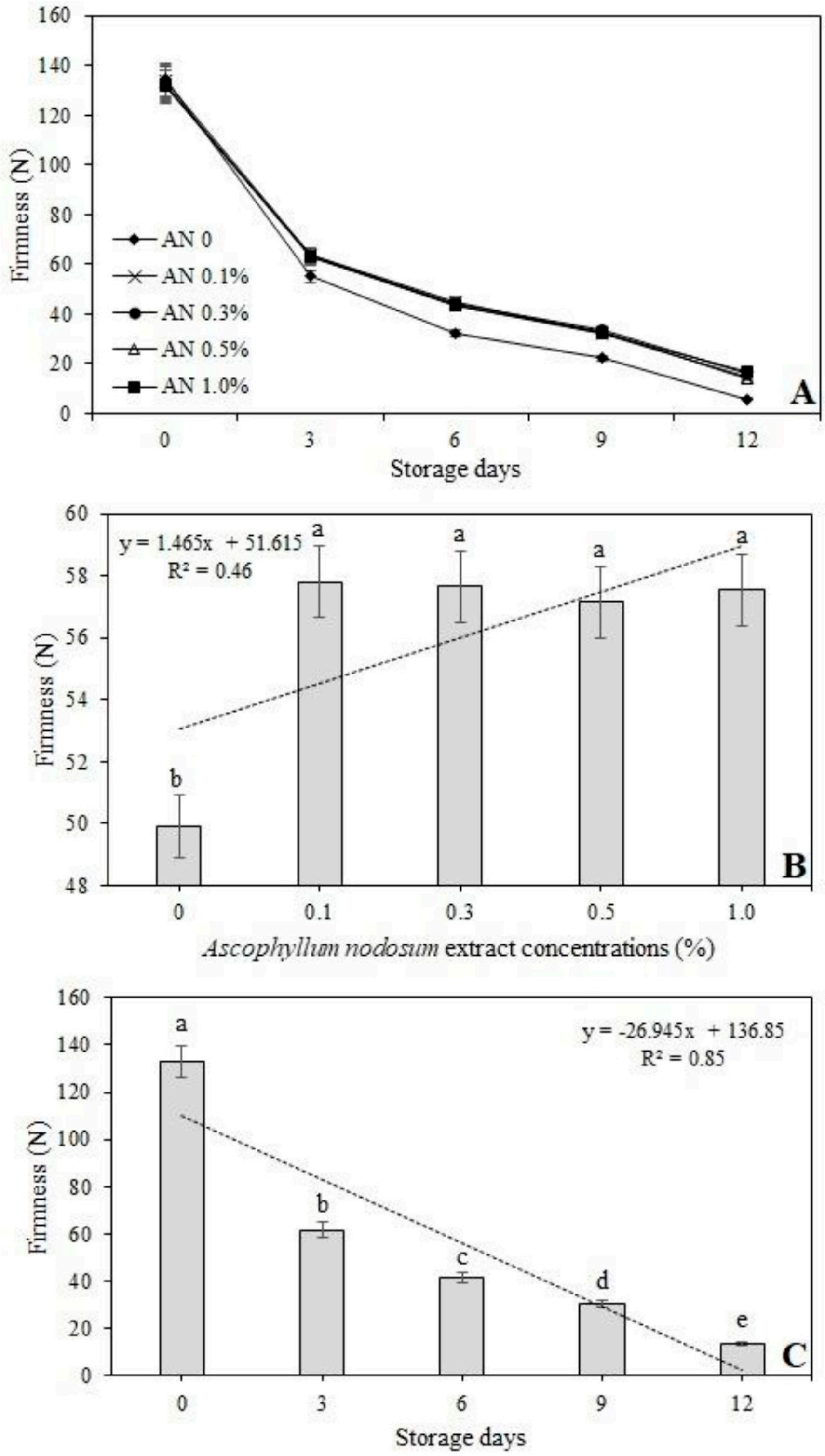

Figure 3. Average pulp firmness (N) values of 'Tommy Atkins' mangoes treated with different of *Ascophyllum nodosum* (AN) extract concentrations (A) and stored at 25 ° C. Linear regression analysis of extract concentrations (B) and storage days (C) with firmness. Columns followed by the same letter do not differ by the Tukey test (p = 0.05). Bars represent the standard error of the mean.

Analysis of the titratable acidity showed that there was a progressive reduction in the contents throughout the storage period in all treatments (Figure 4D). In addition, it was verified that the lowest contents occurred in mangoes not treated with the extract (control), with significant differences from treatments with *A. nodosum* (Figure 4D). This is an indication that treatments with seaweed extract decelerate the normal process of senescence of mangoes, since the decrease in fruit acidity is associated to the consumption of acids in the respiratory process, due to maturation (CHITARRA; CHITARRA, 2005; SOUZA et al., 2011). As in previous trials, linear regression analysis showed that the titratable acidity is much more related to the storage time and consequent fruit ripening (R^2 = 0.98), than to concentrations used in the tests (R^2 = 0.36) (Figures 4E and 4F).

There were significant differences regarding the content of soluble solids among the different samples, especially on days 9 and 12 of evaluation, in which all treatments differed significantly from control, regardless of concentration used and without differing from each other, presenting lower ° Brix values (Figure 5A). In addition, linear regression analysis showed that the use of different *A. nodosum* extract concentrations had little influence on this parameter (R^2 = 0.44), differently from the storage time that was highly correlated with the content of soluble solids in fruits (R^2 = 0.98) (Figures 5B and 5C). That is, the longer the fruit storage time, the greater the content of soluble solids in mangoes.

The application of *A. nodosum* extract in strawberries, both by fertigation and foliar spraying, decreased the content of soluble solids in relation to control (SILVA et al., 2014). This decrease suggests the deceleration of fruit metabolism during storage, with lower sucrose hydrolysis and consequent use of reducing sugars in respiratory processes (MISHRA; KAR, 2014).

Finally, the evaluation of the carbohydrate content in the shell of mangoes during the storage period showed that there was no interference of the seaweed extract on this parameter (Figure 6). The content of total sugars of fruits varied very little among collection times. However, even though there were no differences among treatments, a progressive increase in the content of reducing sugars in mangoes was observed in all treatments and, consequently, reduction in the content of non-reducing sugars (Figure 6).

The majority of reducing sugars are represented by glucose and fructose, non-reducing sugars by sucrose, and total soluble sugars by the sum of these three saccharides (RIBEIRO, 2006). Thus, it is clear that the consumption of sucrose increases with fruit maturation, resulting from the respiration process. In contrast, the degradation of sucrose by the invertase enzyme is accompanied by the increase of glucose and fructose, products of this catabolism, which have their levels increased with the maturation of plant organs.

Results similar to those observed in this study were also verified by Lall et al. (2017) in a study that evaluated the application of *A. nodosum* extract in guavas. The authors highlight the beneficial effects of the product on the productivity of guava fruits, which were heavier and with lower concentrations of soluble solids and ascorbic acid, as well as total sugars, reducing and non-reducing sugars, similar to results presented here. However, there is no finding in literature on direct application of *A. nodosum* in the post-harvest treatment of fruits aiming to improve the physiological quality of fruits. Thus, these data are unprecedented in literature.

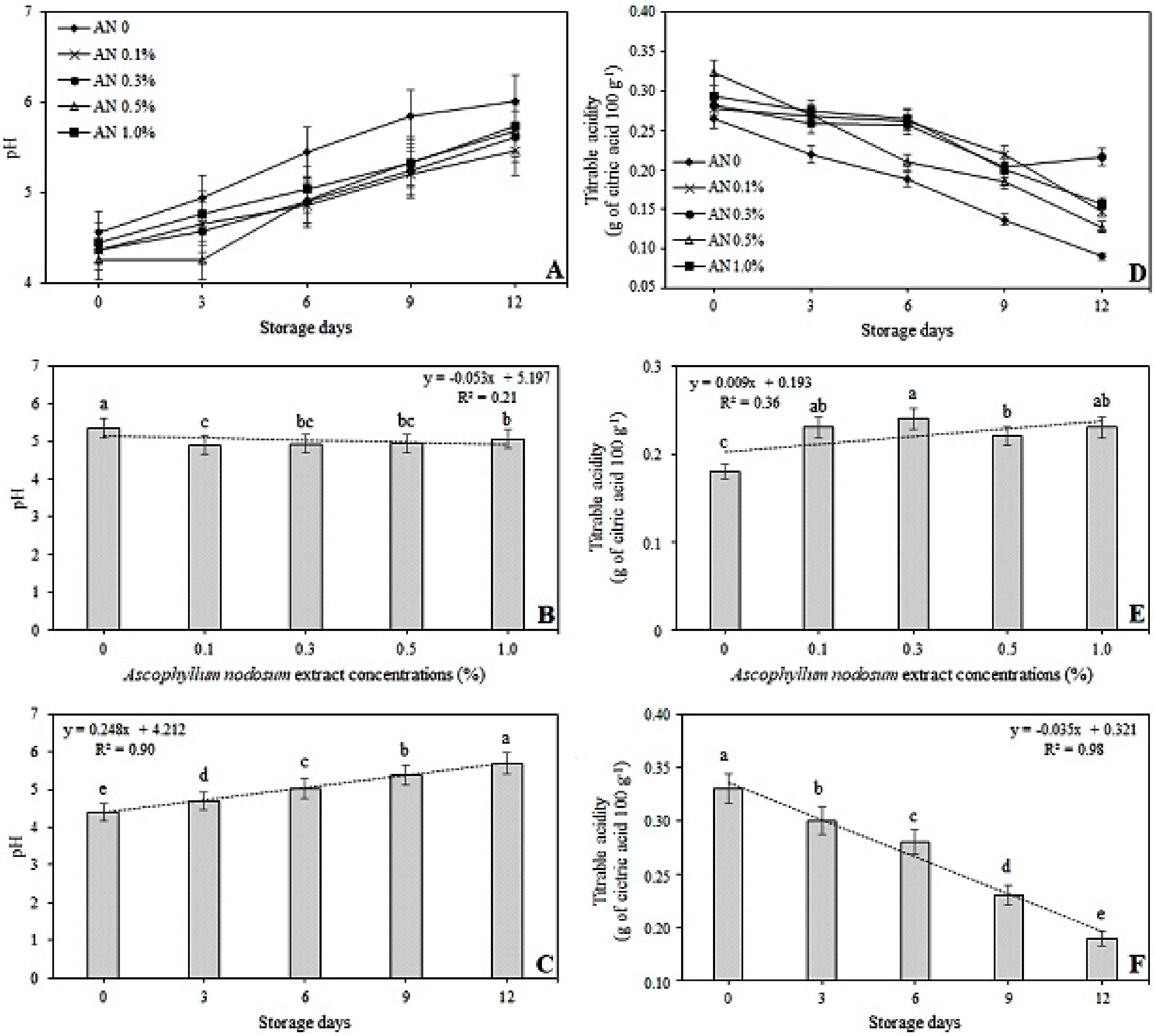

Figure 4. Mean pH and titratable acidity values (g of citric acid 100 g^{-1}) (A and D, respectively) of 'Tommy Atkins' mangoes treated with *Ascophyllum nodosum* extract (AN) and stored at 25°C. Linear regression analysis of extract concentrations (B and E) and storage days (C and F) *versus* pH (B and C) and titratable acidity (E and F). Columns followed by the same letter do not differ by the Tukey test (p = 0.05). Bars represent the standard error of the mean.

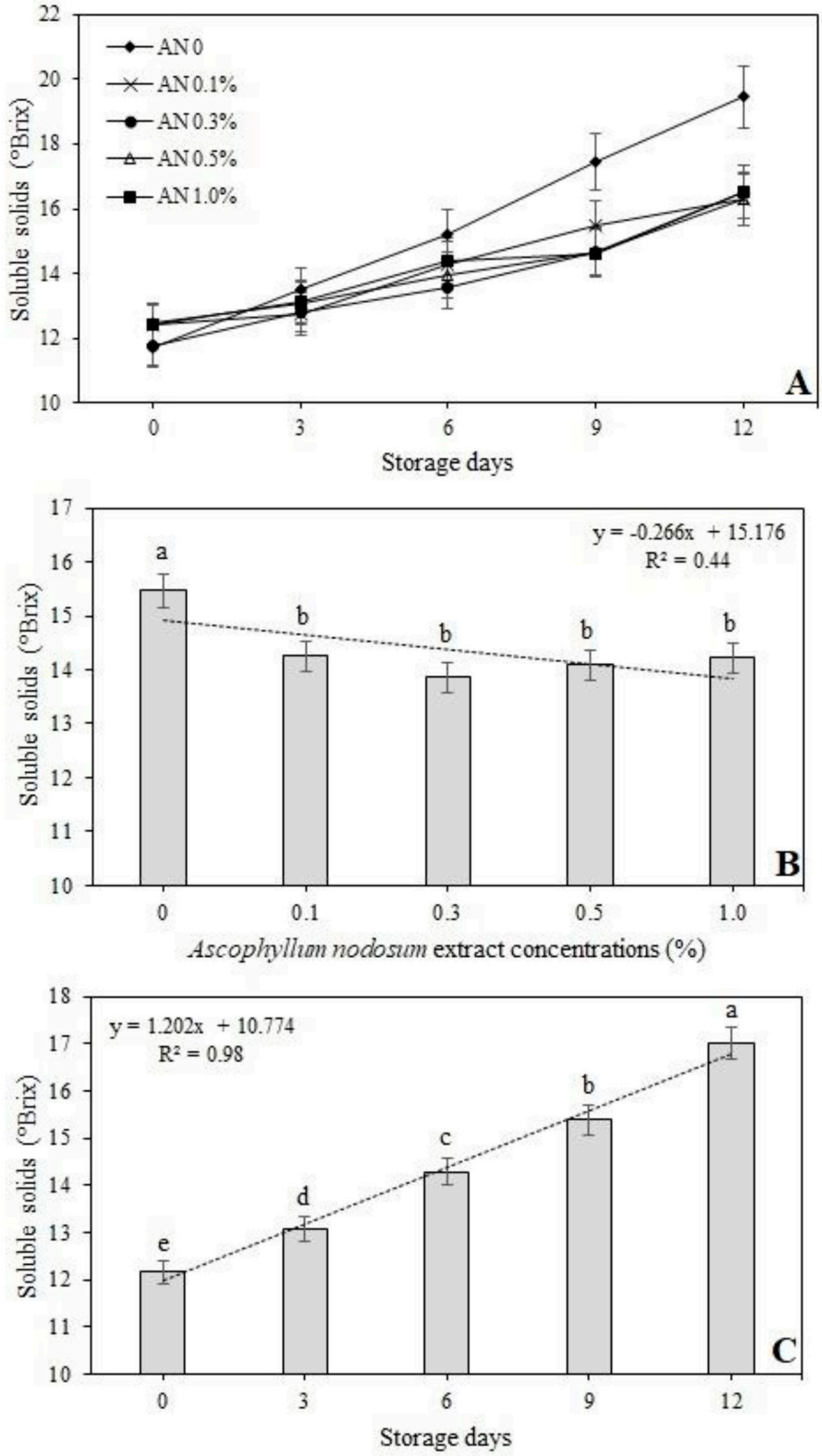

Figure 5. Mean values of the content of soluble solids (° Brix) of 'Tommy Atkins' mangoes, treated with different *Ascophyllum nodosum* (AN) extract concentrations (A) and stored at 25°C. Linear regression analysis of extract concentrations (B) and storage days (C) with ° Brix. Columns followed by the same letter do not differ by the Tukey test (p = 0.05). Bars represent the standard error of the mean.

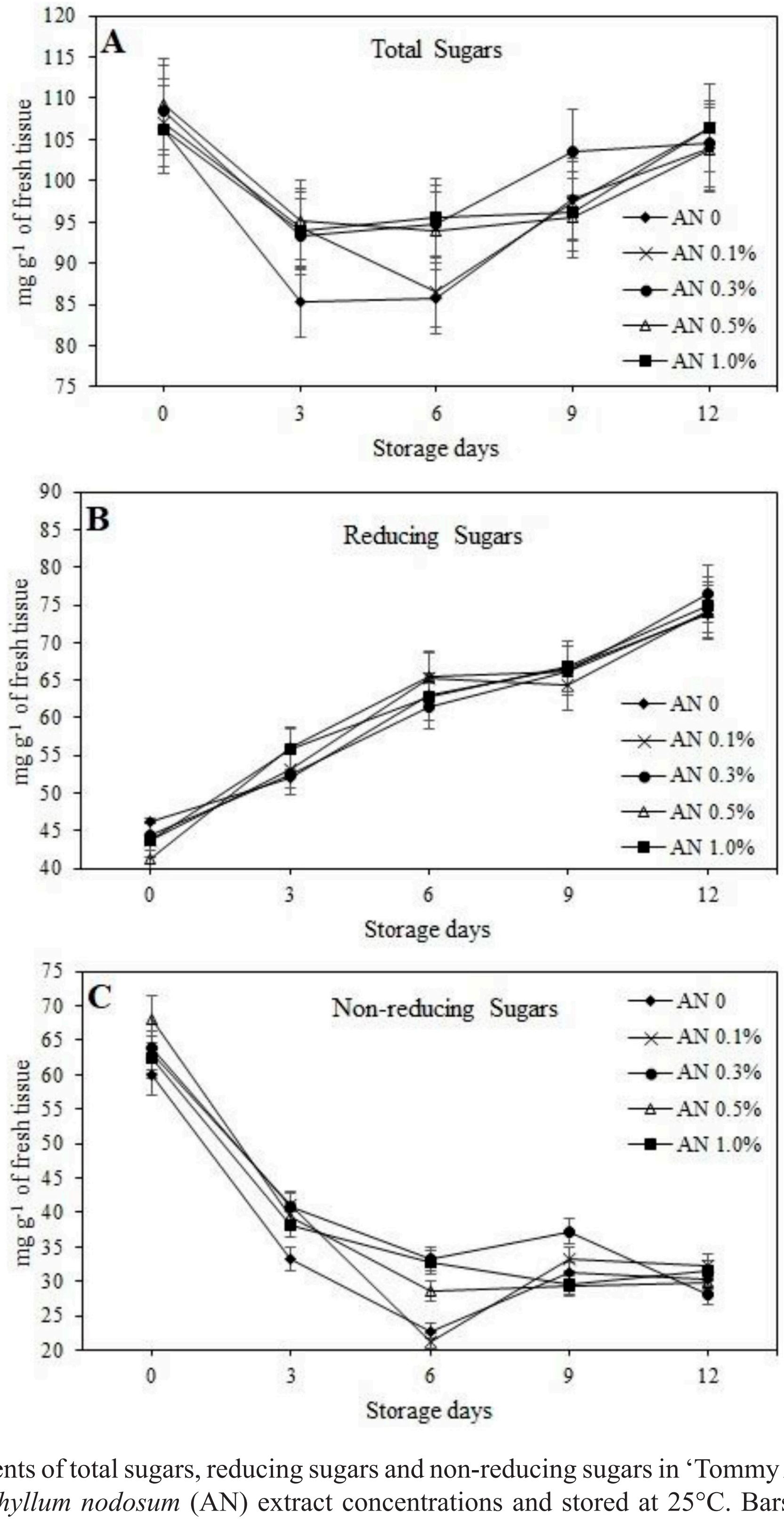

Figure 6. Mean contents of total sugars, reducing sugars and non-reducing sugars in 'Tommy Atkins' mangoes treated with different *Ascophyllum nodosum* (AN) extract concentrations and stored at 25°C. Bars represent the standard error of the mean.

Conclusions

Ascophyllum nodosum seaweed extract has direct effect on the physicochemical parameters of 'Tommy Atkins' mangoes when treated prior to storage. Its effects are observed in the reduction of fruit mass loss, in the delay in the regression of the pulp color angle, in the maintenance of pulp firmness and consequent increase in the shelf life of fruits, in the maintenance of pH and acidity, content of soluble solids and sugars, the latter being parameters strictly related to organ respiration. The extract doses used did not present marked differences among each other, being necessary future studies with larger doses that perhaps will present more significant effects on the product.

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