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Medicinal plant essential oils associated with biofilm to protect papaya fruits

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Abstract

The objective of this work was to associate the use of biofilms to *Lippia sidoides* (Lippia) and *Morinda citrifolia* (Noni) essential oils and their respective major constituents in post-harvest quality components. The evaluations in this study were chromatographic analysis the essential oils, fruit mass reduction effect, total soluble solids, peel color and fruit firmness. Regarding the adjustment and viability of the essential oil concentrations to be used in the treatments, a phytotoxicity test was performed. The main constituent found in Noni essential oil was octanoic acid, while for Lippia essential oil was thymol. The concentration of 3% of Noni and Lippia essential oils was the maximum to reach an acceptable level of phytotoxicity on papaya fruit peel. The paraffin + *L. sidoides* and paraffin + *M. citrifolia* treatments achieved the lowest reduction in pulp mass. In relation to total soluble solids, treatments did not show a significant difference. The best result for firmness was found in sunflower oil + noni coating. Sunflower oil + noni and sunflower oil + octanoic acid were the treatments that maintained normal yellow color in fruits for longer time.

Keywords: *Carica papaya* L.; fruit quality; post-harvest.

Practical Application: Biofilms associated with essential oils reduced the loss of mass, firmness and the color of the fruit for a longer time.

1 Introduction

Papaya (*Carica papaya* L.) is a tropical species belonging to the Caricaceae family, of great worldwide importance. It is grown mainly in tropical countries. Much appreciated by consumers (Santos et al., 2009). Brazil is o the second largest producer of papaya in the world according to the Brazilian Fruit Farming Yearbook of 2017, considered the third largest world's producer of fruit (Food and Agriculture Organization of the United Nations, 2018; Editora Gazeta, 2017). Among the states with the highest production, Bahia and Espirito Santo together account for 64.32% of Brazilian production (Instituto Brasileiro de Geografia e Estatística, 2017). However, it is known that papaya has a relatively short shelf-life after harvesting, with losses near to 40%, depending on storage conditions. Other factors that occur in pre- and post-harvest conditions can also reduce or accelerate fruit metabolism, resulting in quantitative and qualitative losses in the different commercialization phases (Zamperlini et al., 2007). Tropical fruits typically have high moisture content, easily damaged soft-textured pulp and high respiratory rates. Those characteristics combined with heat-generating packaging, as well as the lack of auxiliary treatments, such as the use of plant regulators that slow down maturation-related processes may represent the rapid senescence of fruits (Chitarra & Chitarra, 2005; Godoy et al., 2010). According to Brady (1987) and Tucker & Grierson (1987), degradation and synthesis of pigments, conversion of starch to sugars, reduction in the firmness, degradation of pectins and alteration in enzymatic activity, all occur over maturation processes.

Considering these factors, in the minimally processed products industry, several methods have been used, such as using edible coatings, plastic films or solutions with waxes that can contribute to increase the useful life of fruits, minimally processed, reducing gas exchange, water loss, respiration and oxidation reaction, as well as helping to reduce physiological disorders (Zambolim et al., 2002; Ali et al., 2011; Rojas-Graü et al., 2009). Edible coatings can be prepared from proteins, polysaccharides, lipids or a mixture of these components (Cao et al., 2007). In papaya, gelling agents such as cassava starch, alginate and rice amino have been used as coatings (Pereira et al., 2006; Tapia et al., 2008; Trigo et al., 2012). In addition, it is possible to reconcile edible coatings with preservative agents to prolong their useful life. Among the different groups of plant products, one option found was the use of essential oils, especially because they have antimicrobial properties, antioxidants, antiseptic activity, that is, bactericide, fungicide and virucide, they are used in food preservation (Chevalier et al., 2016). A study has shown that the mixture of cassava starch, glycerol and clove essential oil helps to maintain the quality of papaya (Holsbach et al., 2019).

Among other conservation methods, cold storage has reduced efficiency due to the problems with cold injury (Fagundes et al., 2006) and the use of controlled atmosphere (Vidrih et al., 1990) has a

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very high implantation cost. The inclusion of essential oils (EOs) to different biofilms becomes an alternative in the development of active films in the protection of fruits, also providing antimicrobial and antioxidant action. According to the consulted literature, there is no work trying to associate biofilms to noni and Lippia essential oil with their major constituents (octanoic acid and Timol, respectively) and their effects on the conservation qualities of tropical fruits. In this sense, the use of biofilms associated with essential oils is due to the fact that they represent an interesting possibility for the preservation of food, prolong the shelf life of food and avoid or reduce contamination by microorganisms. Thus, the objective of this work was to evaluate the use of edible coating based on essential oils associated with different biofilms, on the quality and conservation of papaya fruits.

2 Materials and methods

2.1 Essential oil achievement

Ripe noni (*Morinda citrifolia* L.) fruits and leaves of *alecrim-pimenta* (*Lippia sidoides* Cham.). Were collected in the afternoon in the dry season, in the region of Gurupi, in the state of Tocantins, with coordinates Latitute 11° 43'30" South, longitude 49° 4'34" West. The ripe noni fruits were washed and cut into small pieces and the leaves of lippia were dehydrated at room temperature 25 ± 2 °C for seven days, after drying they were cut into small pieces. The essential oil was extracted by means of hydrodistillation method in Clevenger apparatus, which consisted of putting 200g of the plant material in 500 mL of water in a 1-L round bottom flask and boiling for two hours. After extraction, the essential oil was collected as a supernatant, stored in an amber-colored flask, identified and kept at 4 °C until bioassay implantation (Seixas et al., 2012, adapted).

2.2 Chemical composition of lippia and noni essential oil

The chemical composition of the essential oils (lippia and noni oil) was determined through gas chromatography mass spectrometry (GC-ME). The chromatograph used in the study was the Shimadzu GC-210 model equipped with a QP2010 Plus selective mass detector. The equipment was operated under the following conditions: RTX-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 µm film thickness) with the following column temperature setting: 60-240 °C (3 °C/min); injector temperature: 220 °C; helium carrier gas; splitless injection with injected volume of 1 µL of a 1:1000 hexane solution. For the mass spectrometer (MS), the following conditions were used: 70 eV impact energy; ion source and interface temperature: 200 °C. A homologous series of n-alkanes (C9H20 ..... C26H54) was injected under the same conditions as the samples. The obtained spectra were compared with the Nist and Wiley 229 library database and the retention index calculated for each constituent was compared with the tabulated one, according to Adams (2007).

The quantification of the compound contents was expressed as a percentage based on area normalization, which were obtained through gas chromatograph equipped with a flame ionization detector (FID), using the Shimadzu GC-210 apparatus, under the following conditions: capillary column RTX-5MS (30 m × 0.25 mm × 0.25 µm film thickness); injector temperature: 220 °C; FID temperature: 300 °C; with column programming: initial temperature 60 °C with a heating rate of 3 °C min^{-1} to 240 °C, then moving to a heating rate of 10 °C min^{-1} up to 300 °C, remaining at this temperature for 10 min ; nitrogen carrier gas (1.18 mL min^{-1}); Split rate 1:50; 115 kPa column pressure and 1 µL injected volume of a 1: 1000 hexane solution.

2.3 Phytotoxicity in papaya fruits as a function of essential oil concentrations

Phytotoxicity tests were performed by applying the essential oils of lippia and noni on papaya fruits at the concentrations of 1; 2; 3; 4 and 5% and distilled water as a control. Aliquots of essential oil were added in a Tween 80 (1%) solution to obtain the different concentrations. They were spread on the fruit surface with the aid of flexible cotton rods and 200 µL of the essential oil solution. After 48 hours, the phytotoxicity was evaluated by means of a grade scale, proposed by Goes et al. (2004), in percentage of damages occurred in the fruit peel, in which: 0 (zero) - fruits with no symptoms of phytotoxicity; 1-fruits with mild symptoms (fruits with slight, barely noticeable tiny spots, with no restriction on the fresh fruit market 1-10%); 2-fruits with moderate symptoms (fruits with small, visible, localized, sometimes confluent spots, but may be accepted with restriction to the fresh fruit market 11-20%); 3-fruits with severe symptoms (dark visible spots, occupying variable spaces in the fruit, rejected for fresh fruit market> 20%).

2.4 Effect of essential oils associated with different biofilms on weight loss and shelf life of papaya fruits

Formosa cultivar fruits were used in the tests. They were purchased from the local commerce of Gurupi, Tocantins, produced in orchards of the region. All fruits were uniform in in color and appearance "once and for all", that is, before the maturation phase. They were washed with neutral soap and rinsed in sterile distilled water (ADE). Nine treatments were performed: Control (sterile water), Carnauba Wax (30%) + *L. sidoides* (2%), Gelatin (30%) + *L. sidoides* (2%), Paraffin (30%) + *L. sidoides* (2%), Sunflower oil (2%) + *L. sidoides* (2%), Carnauba wax (30%) + *M. citrifolia* (2%), Gelatin (30%) + *M. citrifolia* (2%), Paraffin (30%) + *M. citrifolia* (2%) and Sunflower oil (2%)+ *M. citrifolia* (2%). Using 2% of each essential oil for not causing phytotoxicity. To prepare the coatings, 100 mL of solution was prepared for each treatment. The solutions were prepared by slowly dissolving the carnauba wax, gelatin and paraffin in distilled water, under stirring and heating, until complete dissolution. When the solution temperature reached 45 °C, essential oil was added, and under stirring, it was subsequently cooled for application to the fruits. The solutions of the treatments were applied to the fruits with the aid of a manual sprayer. Immediately after the treatments, the fruits were placed in plastic trays and stored at a temperature (T °C) of 28 ± 1 °C and relative humidity (RH%) ranging from 72 to 85%.

The fruits were weighed on a digital scale (Balmak®-ELP 25) to determine the loss of fresh mass during eight days at two-day

intervals. Three best performing biofilms were selected and noni (*M. citrifolia*) and lippia (*L. sidoides*) essential oils and their major compounds, octanoic acid and thymol, respectively, were added. A completely randomized design with three replications in a 3 × 4 + 1 factorial scheme was used, where factor A = three biofilms and factor B = two essential oils and two major compounds, and water as the control. The postharvest shelf-life of the fruits was defined by three variables: mass loss (dehydration), the change in peel color and the shelf-life that the fruit presented from the day of installation until it was no longer in good conditions to be marketed and/or consumed (onset of senescence). Mass loss was performed every two days during the test with the aid of a digital scale. Peel color was determined by comparing with the Frutiséries 7 color scale (Brasil, 2000), whose maturity stages range from 0 to 5, where: 0 (100% green); 1 (up to 15% of surface is yellow); 2 (up to 25% of the surface is yellow); 3 (up to 50% of the surface is yellow); 4 (50% to 75% of the surface is yellow), and 5 (76% to 100% of the surface is yellow). The concentration of total soluble solids (°Brix) was determined in fruit juice using a manual Atago refractometer, with a scale from 0 to 32 °Brix (Carvalho et al., 1990). The firmness of the pulp was determined by making an insertion in the middle of the fruits, with a SoilControl/USA bench penetrometer, model PDBF-200, with an 8-mm tip, expressed in Newton.

Statistical analyzes were performed using the SISVAR software (Ferreira, 2014). Data means related to the fruit characterization and the physical and chemical characteristics were compared using the test of Tukey at 5% significance level. Storage period data were compared through the Scott Knott cluster test at 5 and 1%.

3 Results and discussion

3.1 Phytotoxicity in papaya fruits as a function of essential oil concentration

Based on the 3% concentration, the essential oils caused degree 1 (1-10%) phytotoxicity as shown in Figure 1. However, at this concentration, the damage to the fruits is minimal, where slight injuries represented by small spots with no restriction on the fresh fruit market. On the other hand, the concentrations of 4 and 5% were considered unsuitable for use in papaya fruits, as they provided the phytotoxicity grades 2 and 3 respectively, impairing the natural appearance of the fruit peel and therefore, although not affecting the internal pulp of the fruits, the negative visual aspect, makes them unsuitable for commercialization. Other reports of essential oils that caused fruit phytotoxicity have also been reported. Oliveira et al. (2013) used *Schinus terebinthifolius* essential oil at a concentration level 0.5% in papayas to test protection against *Colletotrichum gloeosporioides*. Despite the promising "*in vitro* response", the oil could not be recommended due to the high levels of fruit phytotoxicity that make it unsuitable for commercialization. Oliveira et al. (2016) also found that Indian clove essential oil causes complete darkening of the peel, possibly due to a phytotoxic activity of this oil.

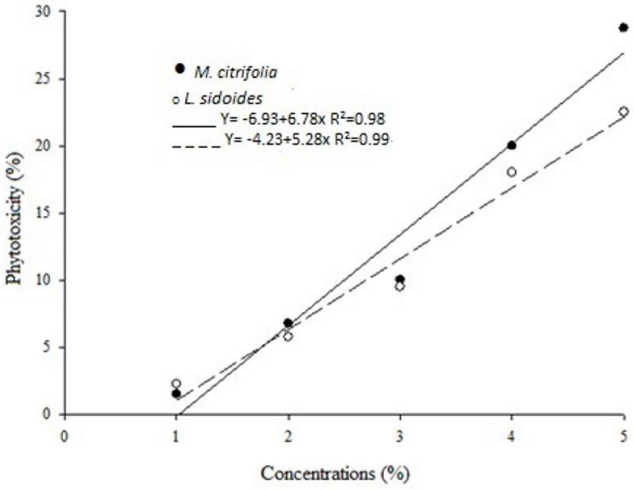

Figure 1. Phytotoxicity of noni (*Morinda citrifolia*) and lippia (*Lippia sidoides*) essential oils, due to increasing concentrations applied to papaya fruits.

3.2 Chemical constituents of lippia and noni essential oils

The chemical constituents found in lippia and noni essential oils are shown in Table 1. Chromato graphic analyses revealed that thymol was the major constituent (92, 68%) for lippia essential oil. Regarding noni essential oil, the octanoic acid was its major constituent (82.24%), followed by hexanoic acid (8.26%). Similar results to this work have also been obtained by other authors. Veras et al. (2014) and Fontenelle et al. (2007) reported the presence of 84.9 and 59.65% of thymol, respectively, as a major constituent in essential oils of *L. sidoides*. Different values were reported by Osorio et al. (2018), where they obtained 64.03% concentration of octanoic acid (caprylic acid) and 8.64% hexanoic acid (caproic acid) in *M. citrifolia* essential oil. Pino et al. (2010) identified 96 compounds, of which octanoic acid (70%) and hexanoic acid (8%) were the major components. Variation in the content of major oils compounds can be influenced by several factors, such as the degree of fruit ripeness, cultivation conditions and development (soil type and climate) harvest time (Pino et al., 2010; Fontenelle et al., 2007).

3.3 Dehydration of biofilm-coated fruits incorporated with essential oils

Over the eight-storage days, a reduction in the fruit mass was observed in all treatments. The results of the mass reduction percentage are shown in Table 2. The fruits treated with paraffin + *L. sidoides*, paraffin + *M. citrifolia* and sunflower oil + *M. citrifolia* presented the lowest mass loss, especially paraffin + *L. sidoides* where the loss was 7.8% at the end of the storage period (eight days), differing statistically from the control.

Fruits in paraffin + *L. sidoides* treatment did not differ statistically from the coating with sunflower oil + *M. citrifolia*, during four and six days of storage. Regarding the control treatment, there was the largest mass loss, reaching 23% of the initial mass in just eight days of storage. Moura et al. (2016), showed that the essential oil at 0.1% lemon grass allowed a smaller reduction in fruit mass of (4.28%), probably because

Table 1. Chemical constituents of lippia (*Lippia sidoides*) and noni (*Morinda citrifolia*) extracted from leaves and fruit, identified by GC/MS and their contents expressed as percentage. Gurupi, Tocantins, 2017.

Lipia sidoides essential oil			
Constituents	RT (min)	IR	(%)
α-tujeno	5.915	924	0.05
α-terpinene	8.680	1014	0.09
ρ-cimene	8.944	1020	1.16
γ-terpinene	10.176	1054	0.25
cis-sabinene hidrate	10.656	1065	0.10
4-terpineol	15.19	1174	0.45
Thymol methyl ether	17.264	1232	0.43
Thymol	20.075	1289	92.68
(E)-caryophylene	25.369	1417	2.23
α-humulene	26.849	1452	0.13
Caryphylene	31.878	1582	0.61
Total	-	-	98.18
Morinda citrifolia essential oil			
Constituents	RT (min)	IR	(%)
3-methyl-3-butenyl-1-acetate	4.583	888	-*
2-heptanone	4.992	897	-
Methyl Hexanoate	5.774	922	-
Hexanoic Acid	7.634	987	8.26
Ethyl Hexanoate	7.974	999	2.48
Methyl Octanoate	12.713	1123	-
Octanoic Acid	15.603	1177	82.24
Ethyl Octanoate	15.803	1196	-
Isopentyl hexanoate	18.537	1259	1.60
Methyl hexanoate	19.983	1292	-
3-methylbutyl octanoate	26.897	1457	4.25
3-methylbut-2-enyl octanoate	28.226	1489	-
Total	-	-	98.77

*Not quantified (values <0.0). RT: retention time; IR: calculated retention index.

it restricted transpiration by minimizing the vapor pressure gradient. The application of biofilms generates CO_2 accumulation and decrease of O_2 available to fruits, thus reducing respiratory rates and ethylene production, which results in delaying the fruit maturation process (Chitarra & Chitarra, 2005). Fakhouri et al. (2015) found that the use of starch/gelatin-based biofilm (1:1) resulted in the mass loss for grapes was 9%. Jacomino et al. (2003), when evaluating the effects of five carnauba-based commercial waxes on postharvest preservation of Pedro Sato guavas, found that waxes were effective in retarding ripening, reducing mass loss and rot incidence.

3.4 Effect of essential oils and their major compounds associated with different biofilms on the quality of papaya fruits

It can be seen in Table 3 that all treatments had similar total soluble solids (TSS) values, with no significant difference. Carnelossi et al. (2009) found that fruits treated and inoculated 24 hours after treatments did not present significant differences among them for the values of (TSS). Guava (*Psidium guajava* L.) fruits coated with chitosan + cassava starch or chitosan + cassava starch + mixed essential oils of the genus of *Lippia gracilies* Schaver showed no significant changes in (TSS) content (Aquino et al., 2015). Perdones et al. (2012) obtained an increase in TSS content in strawberries coated with chitosan and lemon essential oil, however, the values were lower than those obtained for fruits uncoated or coated only with chitosan.

Regarding fruit firmness results (Table 3), a statistical difference was found between treatments. The treatments sunflower oil + octanoic acid and sunflower oil + thymol provided greater firmness of pulp, in which the mixture of sunflower oil and noni essential oil stood out. It is believed that a positive synergism occurred between the fixed sunflower oil associated with noni essential oil as the lipids and oil constituents reduced the metabolism and the permeability of the fruit, therefore reducing the respiratory rate and the delay of ripening.

Cissé et al. (2015) showed that chitosan-coating on mango fruits maintained more initial firmness of the pulp.

Table 2. Water loss in papaya fruits submitted to treatments with different biofilms incorporated to lippia and noni essential oils.

Treatments	Days after application			
	2	4	6	8
	(%)	(%)	(%)	(%)
Witness	5.3 ± 0.17 a*	8.1 ± 0.11 a	13.6 ± 0.4 a	22.6 ± 0.2 a
Carnauba Wax 30% + *L. sidoides* 2%	4.0 ± 0.21ab	6.1 ± 0.08 ab	11.6 ± 0.04 ab	19.7 ± 0.11ab
Gelatin 30%+ *L. sidoides* 2%	4.9 ± 0.15 a	7.9 ± 0.31 a	11.5 ± 0.22 ab	19.5 ± 0.03 ab
Paraffin 30% + *L. sidoides* 2%	3.1 ± 0.18 b	4.4 ± 0.45 b	7.3 ± 0.4 b	7.8 ± 0.30d
Sunflower oil 2% + *L. sidoides* 2%	3.9 ± 0.26 ab	5.7 ± 0.43 ab	9.7 ± 0.1ab	17.4 ± 0.19 ab
Carnauba Wax 30%+ *M. citrifolia* 2%	3.7 ± 0.14 ab	6.0 ± 0.48 ab	11.6 ± 0.02 ab	20.2 ± 0.26 ab
Gelatin 30% + *M. citrifolia*2%	3.6 ± 0.15 ab	5.7 ± 0.49 ab	9.9 ± 0.52 ab	16.6 ± 0.67 ab
Paraffin 30% + *M. citrifolia*2%	3.6 ± 0.15 ab	5.3 ± 0.41 ab	8.8 ± 0.52 ab	9.8 ± 0.51cd
Sunflower oil 2% + *M. citrifolia*2%	3.5 ± 0.3ab	4.1 ± 0.19 b	7.4 ± 0.21 b	14.3 ± 0.88 bc

*Means followed by the same letter in the column do not differ statistically from each other by the Tukey test at the 5% probability level. (±) standard deviation.

Table 3. Content of Total Soluble Solids (°Brix) and pulp firmness in papaya fruits under different treatments applied to the skin after 12 days of storage.

Treatments	TSS °Brix	Firmness (Newton)
Witness	9.67 ± 0.02 ^{ns}	2.7 ± 0.15 b
Sunflower oil + *M. citrifolia*	8.2 ± 0.13	7.9 ± 0.1a
Sunflower oil + *L. sidoides*	9.7 ± 0.19	4.0 ± 0.25 ab
Sunflower oil + O. Ácid	10.3 ± 0.34	6.2 ± 0.45 ab
Sunflower oil + Thymol	9.0 ± 0.75	6.0 ± 0.47 ab
Gelatin + *M. citrifolia*	8.3 ± 0.31	3.1 ± 0.50 b
Gelatin + *L. sidoides*	9.7 ± 0.40	5.1 ± 0.36 ab
Gelatin + O. Ácid	9.3 ± 0.13	4.1 ± 0.35 ab
Gelatin + Thymol	9.7 ± 0.1	4.6 ± 0.55 ab
Paraffin + *M. citrifolia*	7.3 ± 0.3	2.5 ± 0.15 b
Paraffin + *L. sidoides*	8.3 ± 0.25	3.7 ± 0.17 b
Paraffin + O. Ácid	8.7 ± 0.15	2.7 ± 0.55 b
Paraffin + Thymol	8.7 ± 0.21	5.0 ± 0.60 ab
CV %	14.56	30.47

Equal lowercase letters in the column do not differ from each other by the Tukey 5% test. ^{ns}not significant. (±) standard deviation. Firmness: Data transformed by square root. C.V= Coefficient of variation.

Khaliq et al. (2015) using Arabic gum (GA) 10% and calcium chloride 3% + (AG) 10% retained the firmness of fruits for longer. Reduction in respiration and water loss may be responsible for retaining firmness. The use of a suitable coating may delay texture changes and reduce the ripening process as it has been shown by several authors in different fruit varieties (Valero et al., 2013; Maqbool et al., 2011; Ahmed et al., 2009). Weight loss associated with fruit ripening also reflects as a progressive decline in pulp firmness (Forato et al., 2015).

In relation to peel color, sunflower oil associated with noni essential oil and sunflower oil with octanoic acid were the treatments that maintained fruit color and ripeness during the 10-day storage period in which the fruits had not reached full maturity. This result is important for postharvest conservation of fruits, since it was found that from the sixth day, the control was already fully mature, and the fruits were unsuitable for commercialization (Figure 2). It can be verified that the sunflower oil treatments maintained the greenish color of the peel until the sixth day after the treatment. Forato et al. (2015) evaluating cashew gum (CG) and carboxymethylcellulose (CMC) -based coatings both reduced mass loss, preserving firmness and delaying peel color changes in guava fruits. Ripeness of papaya fruits started before harvest, and their ripening progressively increases after harvest, due to physiological processes, which increases transpiration and respiration, thus accelerating their physiological ripening, where the color change is a natural indicator of maturity. During the ripening process, chlorophyll degrades, exposing carotenoids, the main pigment responsible for most of the yellowish shade (Yao et al., 2014).

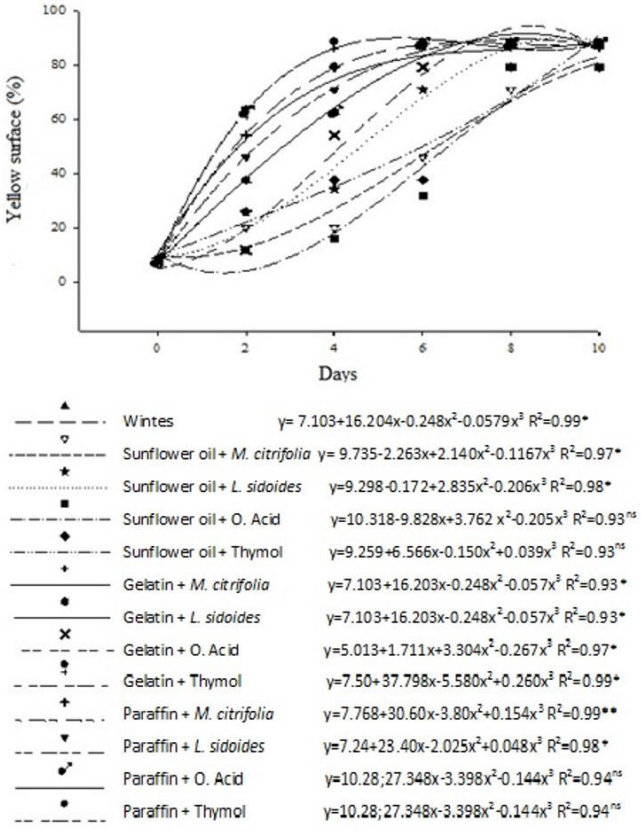

Figure 2. Ripening of papaya fruits as a function of storage time and different treatments with sunflower and lippia oils associated with different biofilms. ^{ns}not significant, *5% and **1% probability by Scott-Knott test.

It was observed in this work that the use of essential oil-incorporated biofilm may help maintain the moisture of the fruits, the firmness of the pulp, and delay the ripening of the fruits, thus increasing shelf-life.

4 Conclusions

This work proved that the application of oils to the fruit must be less than 3% due to the phytotoxicity caused by the papaya peel. The coating based on paraffin 30% + *Lippia sidoides* 2% and paraffin 30% + *Morinda citrifolia* 2% on papaya fruits reduces the loss of mass. Sunflower oil 2% + noni 2% provides greater mechanical resistance of the fruits (greater firmness). The films composed of sunflower oil + noni and sunflower oil + octanoic acid have been shown to be effective in the formation of coatings that prolong the appearance, reducing the color change of the fruits. These results suggest that the paraffin coatings 30% with the mixture of essential oils *Lippia sidoides* and *Morinda citrifolia* at 2% are of interest in the use in the form of biofilms of papayas, which are very perishable, and thus extend their useful life at room temperature.

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