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Ethylene inhibitor, 1-methylcyclopropene, delays macauba fruit ripening and preserves oil quality[1]

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ABSTRACT

The macauba palm produces high-quality oil, with oil content in the fruit potentially increasing after harvest. However, storage limitations due to microorganism and metabolism deterioration restrict the fruit's shelf life. This study evaluated the effects of the ethylene inhibitor 1-MCP on the physiological, physical, and chemical characteristics of macauba palm fruits, focusing on oil quality preservation during storage. Various concentrations of 1-MCP (0, 1000, 2000, and 3000 nL L^{-1}) were applied over two exposures (12 and 24 hours) times during fruit storage, assessed at six intervals. Untreated fruits exhibited damaged mesocarp and spoilage by the end of storage, while 1-MCP treatment notably suppressed spoilage. The inhibitor reduced respiration and ethylene production throughout storage, although it did not completely prevent climacteric peaks of CO_2 and ethylene, which lessened with higher 1-MCP concentrations. Additionally, 1-MCP delayed ripening, reflected in slower accumulation of total soluble solids and reduced mesocarp firmness loss. Treated fruits showed smaller increases in mesocarp oil content during storage, and the inhibitor also slowed oil acidification. Overall, the responses in all parameters improved with higher 1-MCP concentrations, indicating that 1-MCP could be an effective post-harvest strategy for maintaining the quality of macauba palm fruits and their oil.

Keywords: *Acrocomia aculeata*, deterioration, post-harvest, storage.

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INTRODUCTION

The macauba, *Acrocomia aculeata* (Jacq.) Lodd., a palm tree native to Brazil, represents a promising source of vegetable oil. Its annual production can reach up to 5 tons of mesocarp oil per hectare, an amount higher than that of annual oilseed species.[1] Moreover, the fruits offer full usability, minimizing the negative environmental impact of solid waste.

The fleshy-fibrous mesocarp is made of a large portion of oil, around 85%, that continues to increase even after the abscission of the mature fruit during post-harvest storage.[2,3] The mesocarp oil puts the macauba palm tree as a high-value crop for the production of edible oil, similar to olive oil, and fuel, as biodiesel. However, the oil can undergo undesirable changes in quality, mainly resulting from hydrolytic rancidity and oxidative reactions during the post-harvest process.[4] The high water content of freshly harvested fruits enables microorganisms to grow and causes the degradation of oil and other components, yet there is limited understanding of the physiological and physicochemical changes that occur during this period. In many cases, applying plant growth regulators can effectively control the quality of the fruit and its biochemical constituents during storage.[5]

Efficient post-harvest management is possible with the application of 1-Methylcyclopropene (1-MCP), a synthetic plant growth regulator known for inhibiting the action of ethylene.[6,7] In general, 1-MCP delays ripening and hinders deterioration processes in stored fruits.[8-11] Additionally, 1-MCP can slow down the intensification of acidity and lipid oxidation.[12] Aiming to improve the quality of macauba fruit during the post-harvest, the present study evaluated the effect of 1-MCP on the physiological, physical, and chemical characteristics of the mesocarp, as well as its potential for preserving oil quality during storage. To the best of our knowledge, this is the first study addressing postharvest preservation and shelf-life assessment of Acrocomia aculeata fruits. Considering that 1-methylcyclopropene (1-MCP) effectively inhibits ethylene perception across a wide range of plant tissues, it is hypothesized that its application may contribute to maintaining fruit quality and delaying senescence in macauba.

MATERIAL AND METHODS

Plant Material

Bunches containing mature macauba fruits were collected at Fazenda Capela, located in Acaiaca, Minas Gerais, Brazil, at 20.76° S - 42.86° W and 481 meters above sea level. The selection and harvesting time of the bunches were determined by the beginning of the natural abscission of the fruit, which overlaps with the highest oil content in the mesocarp[13]. At present, there are no definitive harvest indices defined for macauba fruits. Nonetheless, some practical indicators are commonly employed. The number of days after flowering and the onset of natural fruit abscission serve as useful references for estimating the stage of maturity.[13,14] At the Biotechnology and Post-harvest Laboratory of the Federal University of Viçosa, the fruits were removed from the bunches and stored in mesh bags.

Treatments - Application of 1-MCP

The 1-MCP treatments comprised of two independent trials, as described:

Trial I: 1-MCP was applied by fumigation at concentrations of 0, 1000, 2000, and 3000 nL L^{-1} using the commercial product SmartFresh™ (Agrofresh Inc., EUA) 0.14%. The mesh bags containing the fruits were placed into airtight plastic containers of 0.103 m^3 and the volatilization and desired concentration of the active ingredient were attained following the manufacturer's instructions. After the planned exposure time to 1-MCP, either 12 or 24 hours at 25 °C, the fruits were transferred to plastic fruit crates and stored under room conditions (24,0 ± 1,5 °C) for up to 50 days.

Trial II: 1-MCP was applied by fumigation at a concentration of 2000 nL L^{-1} for 12 hours, similar to Trial I, but with variations in the number of applications of the inhibitor. The treatments were: Treatment 0 (T0 – no application of 1-MCP), Treatment 1 (T1 – single application: one day after harvest), Treatment 2 (T2 – 2 applications: one day and 30 days after harvest), Treatment 3 (T3 – 3 applications: one, 15 and 30 days after harvest), and Treatment 4 (T4 – 4 applications: one, 7, 15 and 30 days after harvest). This trail setup, due to the increased frequency of exposure to 1-MCP, is ideal for observing the effect of the inhibitor on the fruit ripening progression.

Evaluations

The evaluations differed between trials. In trial I, the following variables were assessed: CO_2 and ethylene production, total soluble solids content, titratable acidity, and mesocarp oil content and acidity index, and these variables were measured at 0, 10, 20, 30, 40, 50 days of storage. At 50 days, photographs were taken of three fruits that represented the visual quality of the treatment, based on the presence and intensity of mesocarp darkening and

spoilage. In trial II, mesocarp firmness was evaluated as an indicator of fruit ripening.

Respiration rate and ethylene production

Three fruits were placed inside 1.3 L glass flasks for 2 hours to allow the accumulation of released gases. The flasks were hermetically sealed with plastic lids that had rubberized centers through which samples of the internal atmosphere were collected using a sterile syringe for gas analysis. The analysis of ethylene and CO_2 increase during storage was conducted by gas chromatography (GC), using a Shimadzu GC 2010 Plus chromatograph (Shimadzu, Japan) equipped with a 2 m packed column Porapak-Q (50/80 mesh), Wide Bore Injector and thermal conductivity detector (TCD) - inorganic gas detector. The working temperatures used for the oven, injector and detector were 85, 100 and 120 °C, respectively. The analysis of ethylene (C_2H_4) evolution was performed together with the analysis of CO_2 evolution by connecting the TCD detector in series to a flame ionization detector (FID), responsible for detecting ethylene. Gas quantification was done by integrating the peak areas generated in the chromatogram and comparing them with samples of standard gases (0,998% CO_2 and 785 ppb ethylene).

Total soluble solids content

The total soluble solids content was assessed on 10-gram samples of mesocarp homogenized in 90 mL of distilled water. After elution, an aliquot of approximately 0.5 mL was placed in a portable refractometer model RT-30ATC (Instrutherm, Brazil), and the results expressed in ºBrix.

Titratable Acidity

To assess acidity, 10-gram samples of mesocarp were homogenized in 90 mL of distilled water, and the homogenate was then titrated with a standardized 0.1 M NaOH solution, using 4% phenolphthalein as an indicator.

Mesocarp oil content

The oil content was determined according to method 032/IV of the Adolf Lutz Institute.[15] The mesocarp samples were dried at 65 °C for 24 hours to remove excess moisture. Then, the samples were crushed and returned to the oven at 65 °C for another 24 hours to remove residual water. The dry samples were placed in filter paper cartridges and the oil was extracted using a Soxhlet extractor (Marconi 044/8/50, Brazil), with n-hexane as the organic solvent. During extraction, the samples were immersed in the solvent for 2 hours at 80 °C. Afterward, the samples were washed with hexane accumulated in the extractor's condenser at 110 °C, a process repeated six times. Following extraction, the cartridges were placed in an oven at 65 °C for 24 hours to evaporate excess of n-hexane. The cartridges containing the sample were weighed on an analytical balance and the oil content was calculated using the equation:

$$\mathrm{TO}(\%) = (P_1 - P_2) \,/\, (P_1 - P)^{-1} \times 100$$

where:
TO (%) = oil content percentage,
P = weight of the cartridge in g,
P1 = weight of the cartridge + dry sample before oil extraction in g,
P2 = weight of the cartridge + sample after oil extraction in g.

Oil acidity index

The acidity index was determined using the Ca 5a – 40 method proposed by the American Oil Chemists' Society.[16] The method is based on the amount of base required to neutralize the free fatty acids present in oils and fats. Approximately 2.00 ± 0.10 g of oil samples were weighed into 125.0 mL Erlenmeyer flasks, and then 25.00 mL of neutral ether-alcohol solution in a 2:1 ratio and two drops of 0.4% phenolphthalein indicator were added. Titration was performed with a standardized 0.1 M NaOH solution, and the acidity index was calculated as:

$$AI = \frac{v \times f \times M \times 28.2}{P}$$

where:
AI = acid value in mg NaOH.g^{-1}; (result expressed as a percentage of oleic acid)
v = volume of the 0.1 mol.L^{-1} NaOH solution used in the titration, in mL,
f = correction factor of the 0.1 mol.L^{-1} sodium hydroxide solution, determined by standardization, dimensionless,
M = molarity of the NaOH solution used,
P = mass of the sample in g.

Mesocarp firmness

Mesocarp firmness (expressed in Newtons) was measured using a hand-pushed PDF 200 digital penetrometer (Soil Control, Brazil) equipped with an 8 mm probe. After removal of the epicarp, two measurements were made at opposite positions on the equatorial diameter of the fruit, and the average of these measurements was used as the result.

Experimental Design and Statistical Analysis

Trials I and II were conducted in a completely randomized design with four replications, and the sample unit consisted of 15 fruits. The treatments in trial I were arranged in a 4 x 2 x 6 triple factorial design, with four different concentrations of 1-MCP (0, 1000, 2000, and 3000 nL L^{-1}), two exposure periods to 1-MCP (12 or 24 hours), and 6 evaluation intervals during storage (0, 10, 20, 30, 40, and 50 days). Meanwhile, the treatments in trial II were carried out in a 4 x 6 factorial design, consisting of four applications (1, 2, 3, and 4 applications) and 6 storage periods (0, 10, 20, 30, 40, and 50 days). The data were subjected to variance and regression analyses ($p < 0,05$) using the Statistical and Genetic Analysis System (SAEG) software, version 9.1[17], except for the evolution of CO_2 and ethylene.

RESULTS AND DISCUSSION

1-MCP delays mesocarp degradation

Macauba palm fruits underwent significant physiological, physical, and chemical changes during storage. In general, these changes were delayed or reduced in fruits treated with 1-MCP, and the intensity of these responses was dose-dependent. Visual damages were delayed in fruits exposed to the inhibitor, while untreated fruits showed a high level of decay, with visible symptoms of microbial infestation and spoilage 50 days after storage (Figure 1). High exposure time (24h) to 1-MCP at 1000 nL L^{-1} resulted in better fruit quality, with preservation of color, less visual darkening, and spoilage.

Therefore, the better quality of fruits treated with 1-MCP is related to reduced pathogen numbers and post-harvest disease severity. Post-harvest disease control has also been reported in other fruits such as peach, apple, citrus, and star fruit treated with 1-MCP.[18-21] In these, fruit resistance to damage was associated with mechanisms such as firmness, senescence delay, oxidative stress reduction, antioxidant activity and defense enzyme stimulation, protein accumulation, among others.[22]

1-MCP reduces ethylene and respiration rate

Untreated fruits exhibited characteristic behavior of climacteric fruits: an abrupt increase in the respiration rate and ethylene emission, which coincided with fruit ripening, followed by a subsequent drop and maintenance of these processes at basal levels (Figure 2a and 2b). The occurrence of the respiratory and ethylene peak during storage, characteristic of climacteric fruits, was previously verified by Goulart[23]. This ripening pattern is also observed in other palm species, such as date palms (*Phoenix dactylifera*) and oil palms (*Elaeis guineensis*), in which ripening is also marked by a burst of ethylene production and a peak in respiration.[24,25]

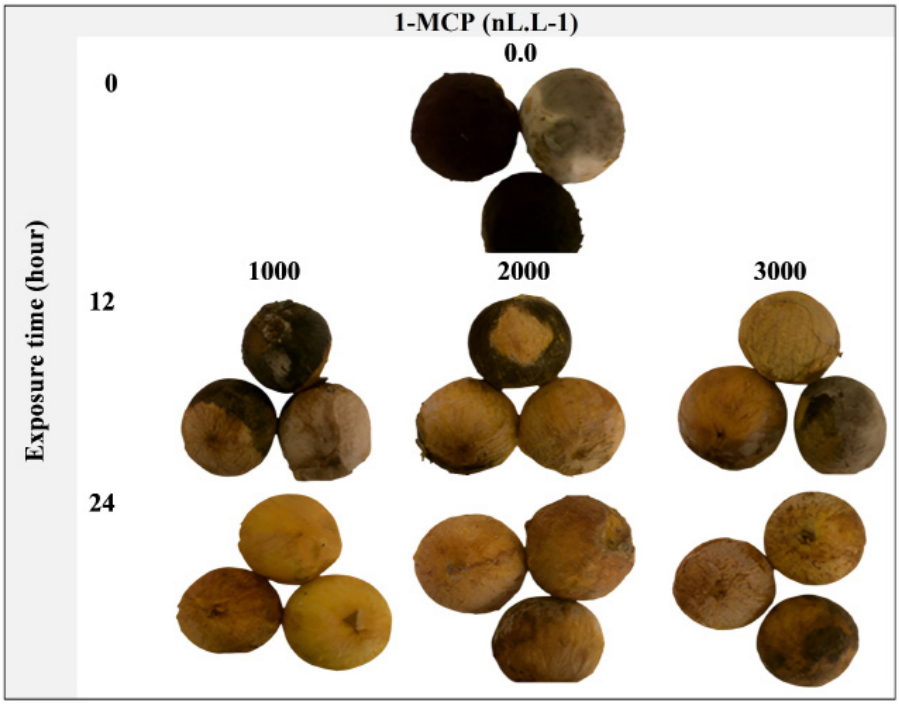

Figure 1. Visual appearance of macauba palm fruits subjected to different concentrations and exposure times of 1-MCP treatment after 50 days of storage.

The reductions of respiratory and ethylene peaks in relation to the inhibitor were dose-dependent, with greater suppression of CO_2 and ethylene emission by the fruits happening as 1-MCP concentrations increased. However, no effect from the exposure time (12 h or 24 h) was observed, and the climacteric peak occurred after 10 days of storage, regardless of the anti-ethylene presence (Figure 2a and 2b). This response in macauba palm is similar to known climacteric species such as guava and mango, where inhibition occurs partially without delaying the climacteric rise.[26,27] Nonetheless, it is noteworthy that the response to 1-MCP application is often multifactorial, including genotype, application method, and stage of fruit maturation.[28] In this study, 1-MCP concentrations affected the intensity of the fruit's response, with higher concentrations delaying the release of CO_2 and ethylene gases.

According to the Kader[29] classification, macauba palm fruits ranked in the category of high-respiration-rate fruits (> 20 mL $CO_2.kg^{-1}.h^{-1}$). Without anti-ethylene treatment (control), the high metabolic rate of the fruits, combined with pathogenic activity, makes them susceptible to rapid deterioration. Therefore, the reduction in respiration rate and ethylene production promoted by 1-MCP contributes to maintaining the quality of macauba fruits.

1-MCP affects characteristics associated with fruit ripening

Although the exposure time to 1-MCP (12 and 24 h) influenced the appearance of the fruits, no substantial difference was observed in ripening indices (TSS, TA, and firmness). However, these variables were affected by the concentration of the ethylene inhibitor and the storage duration.

TSS increased over the storage period in all treatments, regardless of the concentration and exposure time to 1-MCP (Figure 3). This response was expected during climacteric fruit ripening, mainly due to the breakdown of starch and the formation of soluble sugars.[30] Starch degradation during the ripening of macauba palm fruits suggests its contribution to the increase in TSS.[13] In fruits treated with 1-MCP, the increase in TSS occurred more slowly and with less intensity than in untreated fruits, demonstrating the ripening delay effect of this growth regulator (Figure 3). Additionally, the rate of TSS accumulation was inversely related to 1-MCP concentrations. While fruits not exposed to the inhibitor reached 4.8 °Brix, fruits treated with 1000, 2000, and 3000 nL L^{-1} 1-MCP reached 4 °Brix, 3.7 °Brix, and 3.3 °Brix, respectively (Figure 3).

Similar to TSS, the acidity of the mesocarp increased during storage, independent of the application of 1-MCP. This response differentiates macauba palm from most fleshy fruits, where titratable acidity generally decreases during ripening.[31] This difference occurs because the acidity in mesocarp is represented by the volume of a base (NaOH) needed to neutralize the acidity from oleic acid, which increases post-harvest storage efficiency, along with other free fatty acids. Other oil-rich fruits, such as Hass avocado, also undergo this acidification process.[32] Although the mesocarp acidification rate increased during storage, a relatively lower rate was noted in treated fruits, especially at the 3000 nL L^{-1} 1-MCP concentration (Figure 4).

During the storage period, post-harvest ripening in fruits not exposed to 1-MCP was also marked by a loss of mesocarp firmness. However, exposure to the ethylene inhibitor delayed this firmness loss, with higher firmness values observed in fruits that underwent more applications

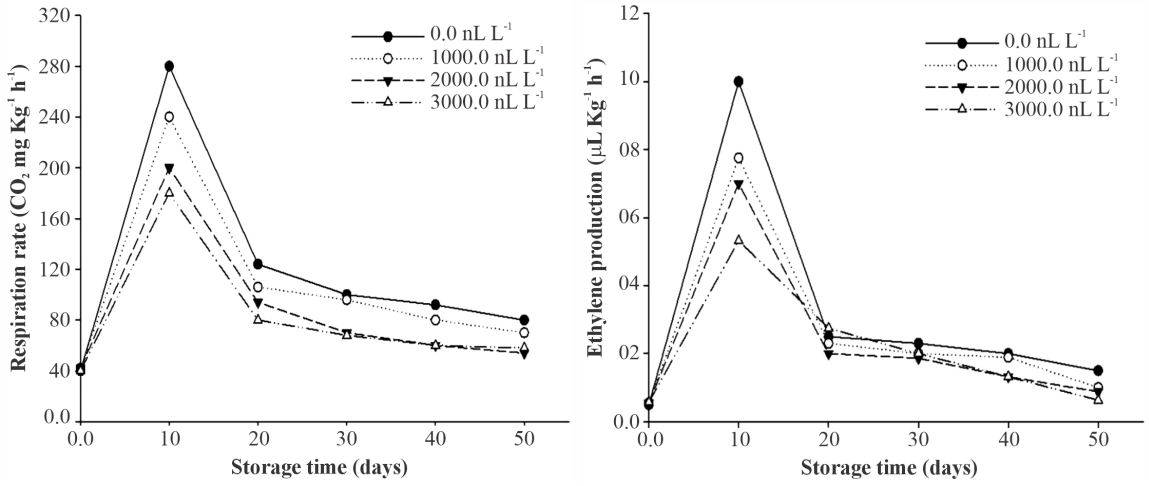

Figure 2. Evolution of CO_2 (a) and ethylene production (b) in the control group and macauba palm fruits treated with 1000, 2000 and 3000 nL L^{-1} 1-MCP during storage for 50 days at room temperature.

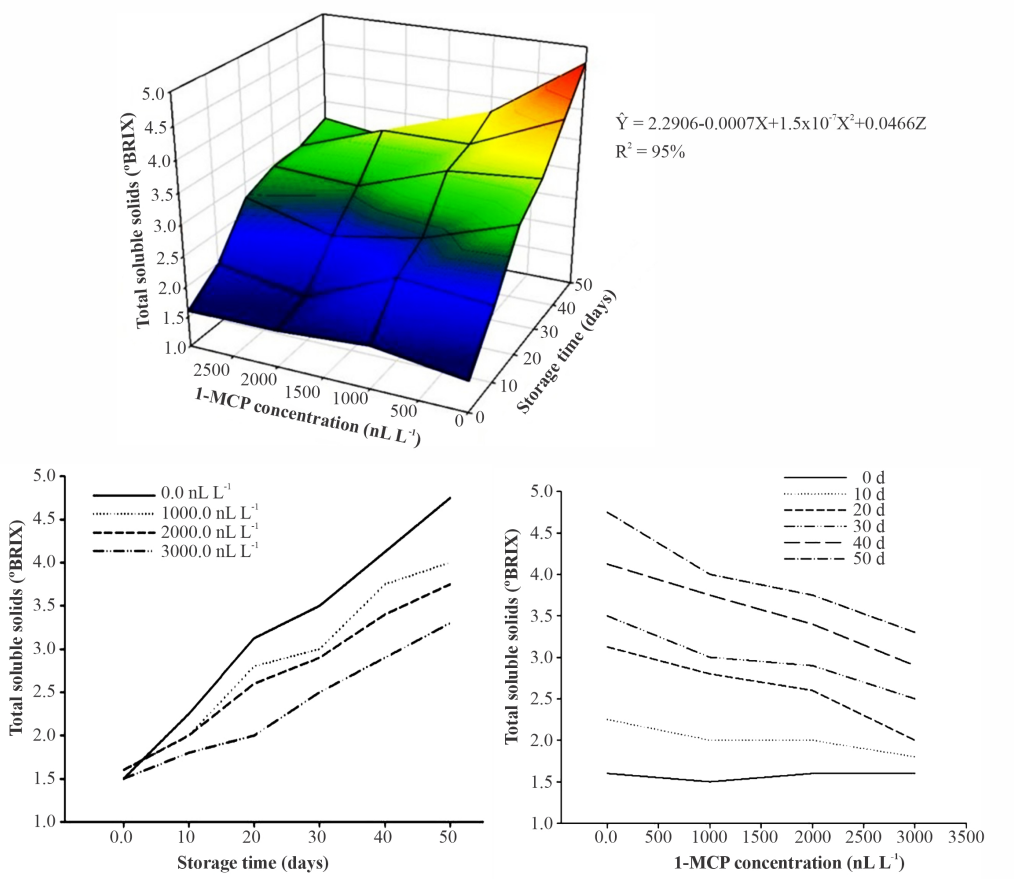

Figure 3. Total soluble solids (°BRIX) value of macauba palm fruit treated with 0, 1000, 2000 and 3000 nL L^{-1} 1-MCP during storage for 50 days at room temperature.

throughout storage. At the end of the evaluation, after 50 days of storage, mesocarp firmness was 5%, 7%, 8%, and 10% higher in fruits treated with 1, 2, 3, and 4 applications of 1-MCP, respectively, compared with untreated fruits (Figure 5). These results confirm the inhibitory action of 1-MCP on the process of cell wall degradation and the activity of hydrolases in macauba palm fruits. Similar results were observed in apple, avocado, persimmon, and kiwi fruits.[21,33-35]

1-MCP impacts oil content and improves oil quality

During storage, there was a substantial increase in oil content in the mesocarp (Figure 6). The progression of oil accumulation highlights the importance of post-harvest storage for optimizing the oil yield of macauba palm. This increase in oil content in macauba palm fruits has also been reported in previous studies.[22,36]

The oil content in the mesocarp of untreated fruits increased from 40% to 57% in just 10 days of storage, while fruits fumigated with 1-MCP showed lower oil accumulation during the same period, a trend that continued throughout the evaluation period. This response varied with the concentration of 1-MCP used, with increases of 13%, 9%, and 7% occurring at 1000, 2000, and 3000 nL L^{-1}, respectively. After 50 days, the oil content reached 60% of the dried mesocarp in fruits exposed to 3,000 nL L^{-1} of 1-MCP, which is 10% lower than in fruits not exposed to the regulator. These findings suggest a possible role of ethylene in oil accumulation during post-harvest storage of macauba palm fruits, as the presence of the inhibitor suppressed oil production and accumulation.

The rancidity of mesocarp oil increased with storage time (Figure 7). However, the acidification process was delayed by the application of 1-MCP. Applying 3000 nL L^{-1} of 1-MCP preserved the initial oil quality (0.4 g oleic acid per 100 g) until the 10th day, reaching 11.1 g oleic acid per 100 g after 50 days of storage. During the same period, the acidity level of the control sample reached 31.5 g oleic acid per 100 g, which was 64.8% higher. A delay in oil deterioration in response to 1-MCP has also been described in oil palm fruits and pecan nuts, indicated by a reduction in free fatty acid production.[12,37] However, the mechanism of 1-MCP's action in maintaining oil quality is still unclear.

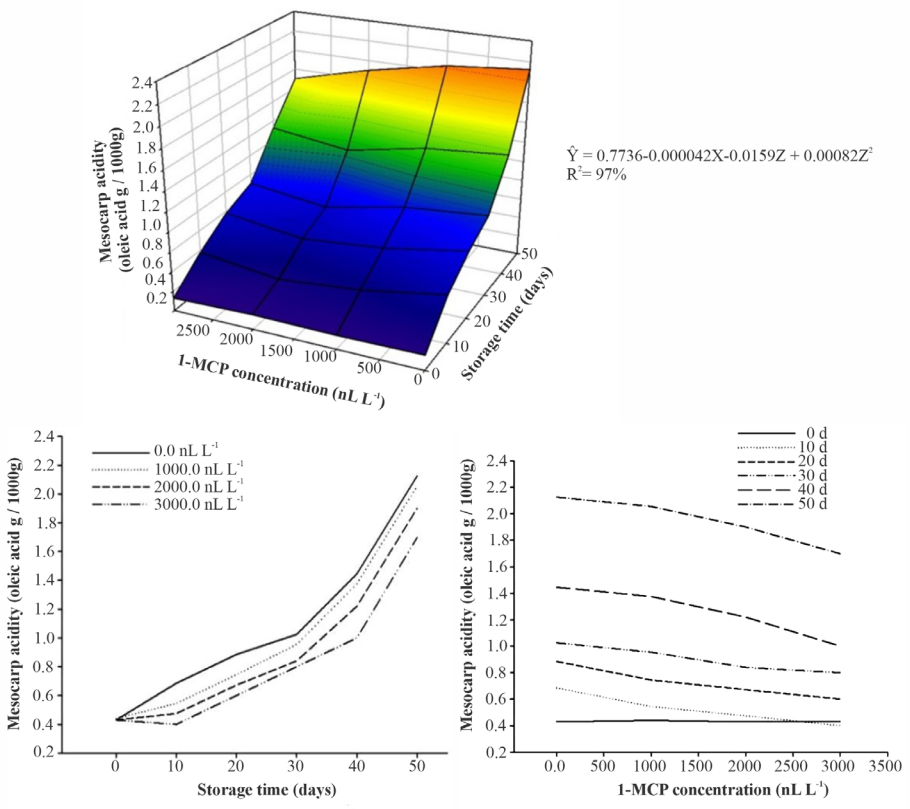

Figure 4. Mesocarp acidity index (oleic acid g/100g) value of macauba palm fruit treated with 0, 1000, 2000 and 3000 nL L^{-1} 1-MCP during storage for 50 days at room temperature.

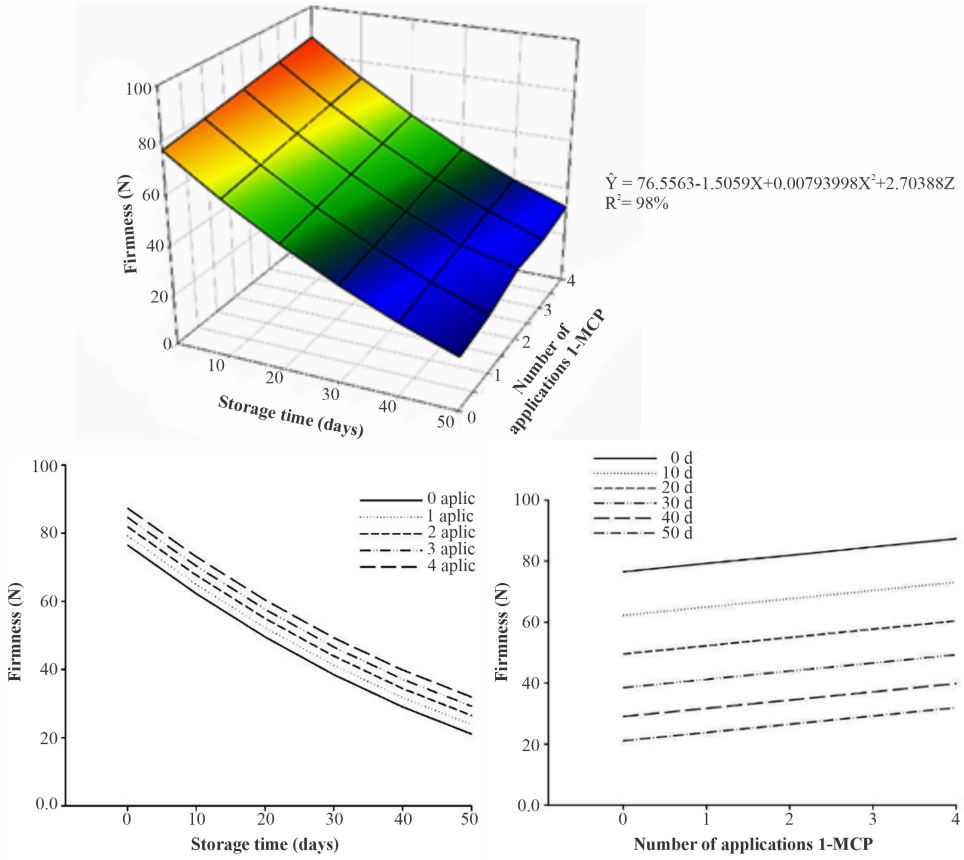

Figure 5. Firmness (Newton) value of macauba palm fruit treated with 2,000 nL L^{-1} 1-MCP for 12 h and 1, 2, 3, and 4 applications during storage for 50 days at room temperature.

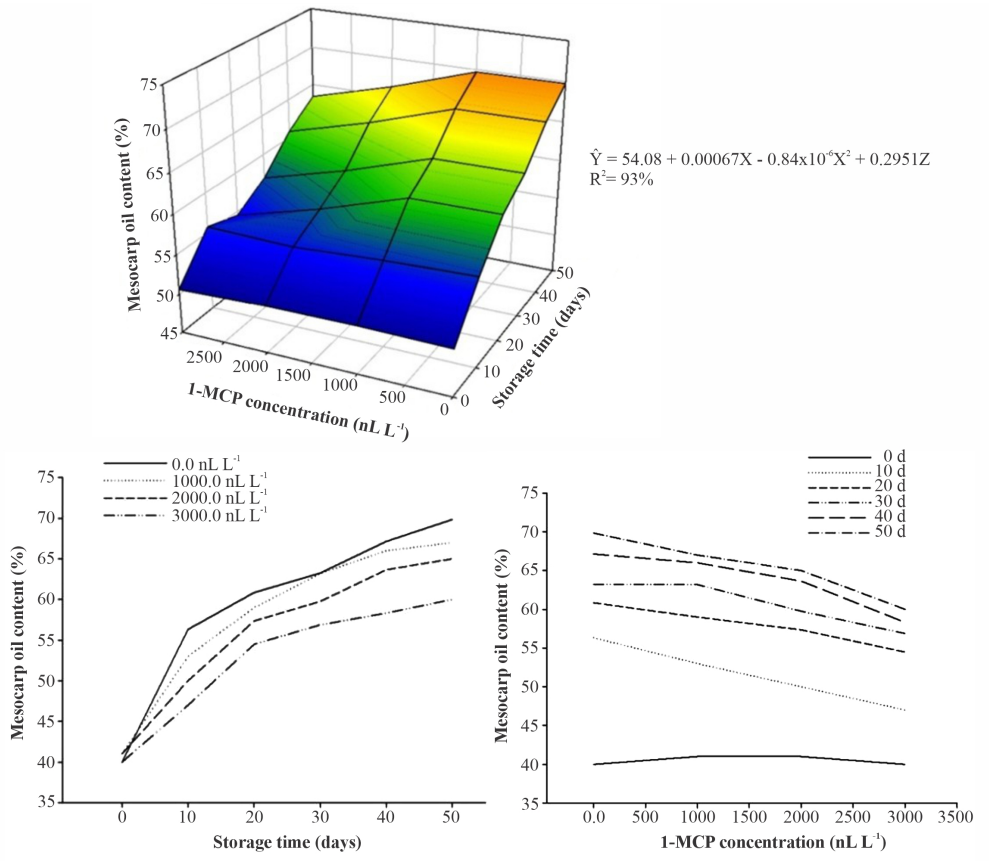

Figure 6. Mesocarp oil content (%) value of macauba palm fruit treated with 0, 1000, 2000 and 3000 nL L^{-1} 1-MCP during storage for 50 days at room temperature.

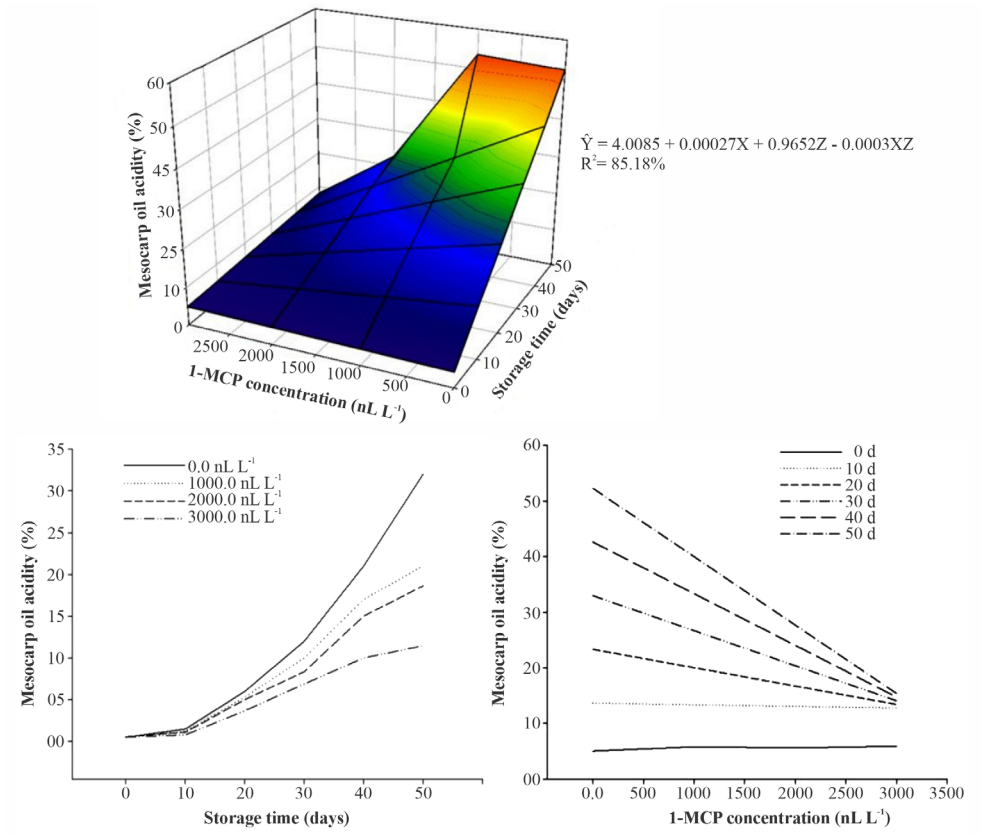

Figure 7. Mesocarp oil acidity (%) value of macauba palm fruit treated with 0, 1000, 2000 and 3000 nL L^{-1} 1-MCP during storage for 50 days at room temperature.

Most processes for converting vegetable oil into biofuel are based on alkaline transesterification, which is limited by the oil's acidity. Alkaline transesterification is not just more efficient but also more economically viable than acid, enzymatic, or non-catalytic transesterification.[38-40] Furthermore, high oil acidity (above 20%) can cause corrosion in the equipment used for oil extraction and refining. Therefore, the use of 1-MCP at concentrations of 2000 or 3000 nL L^{-1} enables fruit processing after 50 days or more of storage.

CONCLUSIONS

The application of 1-MCP interferes with the physicochemical characteristics and physiological processes during the post-harvest storage of macauba palm fruits. The physical damage and loss of color and aroma of the mesocarp were delayed in fruits treated with 1-MCP. The treatment with 3000 nL L-1 1-MCP for 24 h yielded the best quality fruits. The climacteric pattern of the fruits was maintained; however, 1-MCP reduced the respiration rate and ethylene production during both the climacteric and post-climacteric phases. As a result, fruit ripening was delayed in the presence of the ethylene inhibitor, as observed by delays in the accumulation of soluble solids and the loss of mesocarp firmness. Oil content in the mesocarp increased throughout the post-harvest period. However, at increasing concentrations of 1-MCP, the mesocarp oil gain was reduced compared to fruits not exposed to 1-MCP, and at the same time, it improved the preservation of oil quality.

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The authors declare that they have no known competing financial interest or personal relationships that could have influenced the work reported in this study.

DATA AVAILABILITY STATEMENT

The entire data set supporting the results of this study was used in this article.

AUTHOR CONTRIBUTIONS

Conceptualization: José Antônio Saraiva Grossi, Leonardo Duarte Pimentel.

Data curation: Osdneia Pereira Lopes, Samuel de Melo Goulart.

Formal analysis: Osdneia Pereira Lopes, José Antônio Saraiva Grossi, Leonardo Duarte Pimentel.

Funding acquisition: Osdneia Pereira Lopes, Leonardo Duarte Pimentel.

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Methodology: José Antônio Saraiva Grossi.

Project administration: José Antônio Saraiva Grossi, Leonardo Duarte Pimentel.

Writing – original draft: Lucilene Silva de Oliveira, Osdneia Pereira Lopes.

Writing – review & editing: Lucilene Silva de Oliveira, Kacilda Naomi Kuki.

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