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Storage of strawberries (*Fragaria ananassa* L.) cv. 'Oso Grande', subjected to 1-MCP

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ABSTRACT. This study aimed to investigate the influence of 1 - methylcyclopropene (1-MCP) on the substances involved in the softening of strawberry, cultivar Oso Grande, stored at room temperature, from the municipality of Itutinga, State of Minas Gerais. The parameters evaluated were: firmness, total pectin, percentage of solubilization, activities of the enzymes pectinmethylesterase (PME) (EC 3.1.1.11) and polygalacturonase (PG) (EC 3.2.1.15). It was concluded that 1-MCP delayed the softening of the fruits, since the fruits that were treated with 1-MCP after storage, have shown greater firmness, lower activities of PG and PME, and lower contents of total pectin, soluble pectin and percentage of solubilization.

Keywords: softening, postharvest, pectic substances, temperature.

Armazenamento de morangos (*Fragaria ananassa* L.) da cv. Oso-Grande, submetidos ao 1-MCP

RESUMO. Este trabalho teve como objetivo investigar a influência do 1- metilciclopropeno (1-MCP) nas substâncias envolvidas com o amaciamento de morangos, da cultivar Oso-Grande, armazenados em temperatura ambiente, provenientes do município de Itutinga, Estado de Minas Gerais. Os parâmetros estudados foram: firmeza, pectina total e solúvel, porcentagem de solubilização, atividades das enzimas pectinametilesterase (PME) (EC 3.1.1.11) e atividade de poligalacturonase (PG) (EC 3.2.1.15). Concluiu-se que o 1-MCP retardou o amaciamento dos frutos, pois os frutos que receberam tratamento com 1-MCP, apresentando após o armazenamento maior firmeza, menores atividades de PG e de PME e menores teores de pectina total, pectina solúvel e porcentagem de solubilização.

Palavras-chave: amaciamento, pós-colheita, substâncias pécticas, temperatura ambiente.

Introduction

Fruits are essential components in human feeding, playing important nutritional role by supplying vitamins, minerals, fibers and energy. Also have no cholesterol and present low fat content, besides being products that most closely match to sensory expectations of the consumers (FRANÇOSO et al., 2008).

The commercial cultivation of strawberry in Brazil is relatively new. Currently it is cultivated in much of the country, highlighting the States of Minas Gerais, São Paulo and Rio Grande do Sul as leading producers (RIGON et al., 2005).

The strawberry is subjected to important physiological processes during post-harvest, such as respiration and transpiration that cause changes in the fruits. Some of these changes are desirable, since they contribute to improving the appearance, flavor and aroma. Nevertheless, most is undesirable because contributes to the loss of quality.

The strawberry is a very perishable fruit, with high respiratory rate and short shelf-life. Mechanical damages, injuries and crashes during harvesting, transportation and commercialization make the fruit susceptible to the action of microorganisms, causing nutritional and economic losses.

Studies have shown the efficiency of 1 – methylcyclopropene (1-MCP) to extend the shelf life of fruits, inhibiting the action of ethylene, a key component involved in the metabolic processes of ripening, and thus, extending the life of these products. Therefore, this study examined the influence of the 1-MCP on the substances involved with the softening of strawberries, stored at room temperature.

Material and methods

Raw material

Fruits of the cultivar 'Oso Grande' were harvested in the afternoon, in a commercial orchard of the region of Itutinga municipality, Minas Gerais

State, located at 910 m altitude and geographic coordinates of 21°18'45" of latitude South, and 44°41'15" of longitude W. Gr. (IBGE, 1959), and taken to the Laboratory of Biochemistry of the Chemistry Department of UFLA, in Lavras municipality, Minas Gerais State, where 320 fruits were selected regarding the size, maturity stage and absence of defects.

Experimental design

It was used the completely randomized design (CRD) being the treatments assigned in a factorial scheme (2 x 4), being two treatments (with 1-MCP and control), four days of analyses, corresponding to the days 0, 2, 4 and 6, with four replicates of ten fruits for each treatment.

Preparation of the samples and experiment installation

The fruits (n = 320) were immersed in a dichloroisocyanurate solution at a concentration of 200 μL L^{-1} for 15 min. for disinfection, placed to dry at room temperature and randomly separated into two lots of 160 fruits each.

The fruits of the first lot received the treatment with 1-MCP at a concentration of 100 nL L^{-1} for 2h, in sealed chamber. Those of the second lot received no treatment and were used as control. However, to accomplish the experiment under the same conditions, the fruits of the second lot were kept in another sealed chamber for 2h.

At the end of this period, the fruits were removed from the chambers, encoded, weighed and stored at room temperature, 21.0 ± 1.4ºC and 55 ± 5.7% of relative air humidity, for six days. The analyses have started soon after the application of 1-MCP (day 0) and, at every two days, until the end of storage period. The same was undertaken for the control fruits.

After the physical analysis of firmness, the fruits were cut into pieces and the pulp was frozen in liquid nitrogen and stored in a freezer at –18.0°C, for further analysis.

Physical analysis

Firmness

The firmness was determined with the aid of a McCormick penetrometer (model FT 327), through four measurements per fruit, in the equatorial region. The results obtained were multiplied by 4.4482 and expressed in Newtons (N).

Chemical analyses

Total and soluble pectin

The extraction of pectic substances was performed following the technique described by McCready and McComb (1952). It was determined the colorimetry by means of the carbazole reaction, according to Bitter and Muir (1962). The results were expressed in mg of galacturonic acid per 100 g of pulp.

Percentage of solubilization

The percentage of solubilization was calculated from data of total and soluble pectin, using the equation: % of solubilization = (soluble pectin/ total pectin) x 100.

Biochemical analyses

Pectinmethylesterase activity (PME)

The PME was determined according to Jen and Robinson (1984). One unit of PME was defined as the amount of enzyme able to catalyze the demethytlation of pectin corresponding to the consumption of 1nmol of NaOH $min.^{-1}$ g^{-1} of pulp.

Polygalacturonase activity (PG)

The enzymatic activity of the PG was determined by measuring the release of reductive groups using the dinitrosalicylic acid method (DNS) (MILLER, 1959). One unit of PG activity was defined as the amount of enzyme able to catalyze the formation of 1 nmol of reductive sugar $min.^{-1}g^{-1}$ of pulp.

Statistical analysis

The results were subjected to an analysis of variance (Anova), using the statistical package Sisvar (FERREIRA, 2003). The mean values of the treatments were analyzed by polynomial regression.

Results and discussion

There was significant interaction between treatment and storage days for all studied parameters.

Firmness

In both treatments, there is a decrease in firmness of the fruits along the storage period, arising from changes undergone during the ripening process (Figure 1), but in the fruits treated with 1-MCP, the loss of firmness was lower.

The fruits treated with 1-MCP presented on average on the first storage day (day 0) a firmness of 0.66 N and at the end (day 6) 0.46 N. The fruits that did not receive the 1-MCP had mean values of firmness of 0.62 N and 0.29 N in the day 0 and 6, respectively. The loss of firmness for the fruits without 1-MCP was more pronounced (53.05%) in relation to the fruits with 1-MCP

(29.83%), at the end of storage period. This difference indicates that the 1-MCP had delayed the senescence of the fruits, extending the shelf life.

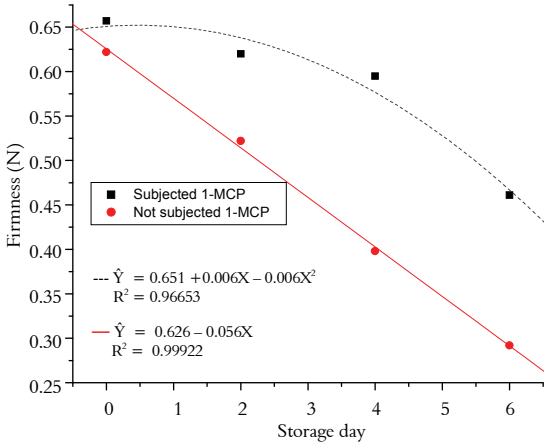

Figure 1. Curves and regression equations of the firmness of 'Oso-Grande' strawberries subjected or not to 1-MCP and stored at room temperature for six days.

The decrease in firmness during the storage was also observed by Hojo et al. (2006), studying the softening of mangoes treated with 1-MCP. Mangoes that received the treatment with 1-MCP were more firm, whereas the fruits that did not receive the treatment were softer.

The fruit softening, during the ripening, is frequently ascribed to enzymatic breakdown of middle lamella of the cell wall (AWAD, 1993; FISHER et al., 1994). There are evidences that this softening is accompanied by the increase in solubilization of pectic substances in the cell wall and middle lamella and increase in the content of water-soluble pectin.

The increase in the solubilization of pectins and in the content of soluble pectin was also observed in the present study.

Enzymatic activity of Polygalacturonase (PG) and Pectinmethylesterase (PME)

The activity of the PME (EC 3.1.1.11) increased over the storage, for both treatments, and the fruits without 1-MCP had the highest enzymatic activity (60.22 nmol min.$^{-1}$ g^{-1} of pulp) when compared to the fruits treated with 1-MCP (30.0 nmol min.$^{-1}$ g^{-1} of pulp). At the end of the storage, the PME activity of the fruits without 1-MCP was twice the activity of the fruits treated with 1-MCP (Figure 2).

From the fourth storage day, the PME activity tended to decrease in the fruits without 1-MCP, and in the fruits with 1-MCP, an increase was observed until the sixth day. This trend points out that this enzyme diesterified almost all pectic compounds that constitute the fruit without 1-MCP, while the fruits treated with 1-MCP still presented substrate for the enzyme to desmethyl.

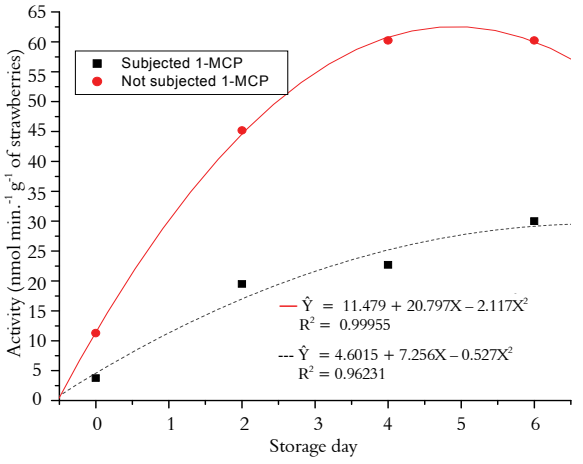

Figure 2. Curves and regression equations of the PME activity of 'Oso-Grande' strawberries subjected or not to 1-MCP and stored at room temperature for six days.

The PG (EC 3.2.1.15) had similar trend in the storage period for both treatments. The fruits without 1-MCP presented higher enzymatic activity throughout the storage. On the sixth storage day, the PG activity for the fruits without 1-MCP was of 6240 nmol min.$^{-1}$ g^{-1} of pulp, and that of the fruits treated with 1-MCP, 4672 nmol min.$^{-1}$ g^{-1} of pulp (Figure 3).

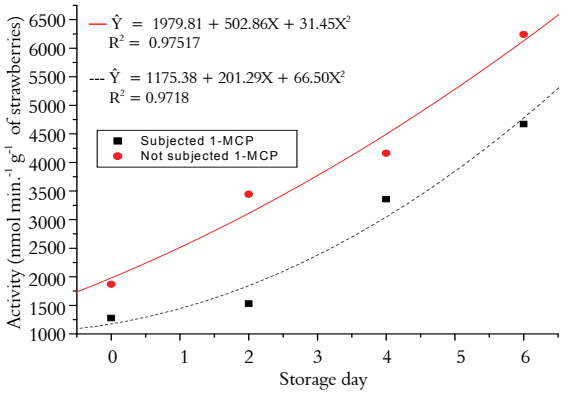

Figure 3. Curves and regression equations of the PG activity of 'Oso-Grande' strawberries subjected or not to 1-MCP and stored at room temperature for six days.

Considering that the pectin solubilization, caused by the action of the enzymes such as PG and PME, is one of the main causes for firmness loss in plant tissue, and that the 1-MCP has been associated

with the prevention of fruit softening, these results suggest the effectiveness of 1-MCP in preventing the softening and maintenance of firmness.

The results found for PG and PME are in agreement with Camargo et al. (2000) and Silva et al. (2009) that studying the softening of strawberries observed that the activity of polygalacturonase (PG), of pectinmethylesterase (PME) and the solubilization of pectins increased in the control fruits during their ripening.

Other studies have reported that in most fruits the activity of the PG increases over the ripening, along with increased softness of the fruit (HOBSON; GRIERSON, 1993; HUBER, 1984; cited by BICALHO et al., 2000). Moreover, usually the degradation of cell wall polysaccharides is accompanied by an increase in the activity of polygalacturonase (enzyme responsible for pectin solubilization) and pectinmethylesterase (enzyme that catalyze the diesterification of free carboxylic groups) (GONÇALVES et al., 2000). The data of the activities of PG and PME are consistent to firmness data (Figure 1). As the activities of the enzymes increase, the firmness decreases.

Total pectin, soluble pectin and percentage of solubilization

During the storage, there was increase in the content of total pectin, of 50.0% in strawberries treated with 1-MCP and 42.9% in those without 1-MCP. In both treatments, the values for the content of total pectin were close (0.78 and 0.80 g of galacturonic acid 100 g^{-1} of pulp, respectively), (Figura 4).

Along the ripening, there is transformation of protopectin into total pectin that, by enzymatic action, undergoes demethylation and simplification of the chains, causing the solubilization until the total degradation, when the fruit is very ripe (SILVA et al., 2009).

Abreu et al. (1998) and Vilas Boas et al. (1996) also registered increase in total and soluble pectin during the ripening of pineapple and banana "prata", respectively, and therefore with the same trend found in the studied fruit.

The increase in total pectin can be related to the efficiency of the extraction methodology when the fruit is unripe, which suggests that the pectin inside the wall is in a form non-accessible by the pectinase (EC 3.2.1.15), and the extraction efficiency can perhaps increase with the ripening, since most polysaccharides is hydrolyzed with advancing ripening (OLIVEIRA JUNIOR et al., 2004).

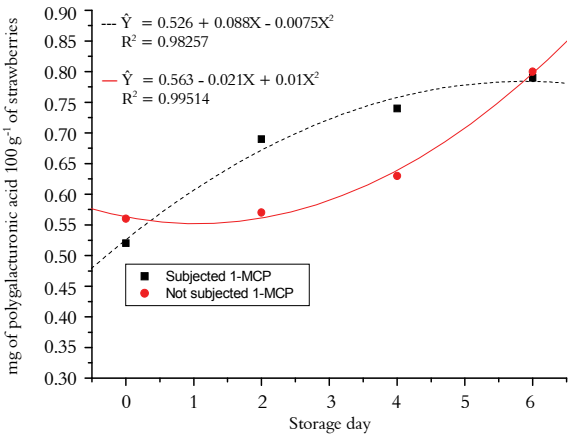

Figure 4. Curves and regression equations of the total pectin of 'Oso-Grande' strawberries subjected or not to 1-MCP and stored at room temperature for six days.

There was increase in the content of soluble pectin along the storage (Figure 5). This trend was due to the increased activities of the enzymes PME (Figure 2) and PG (Figure 3).

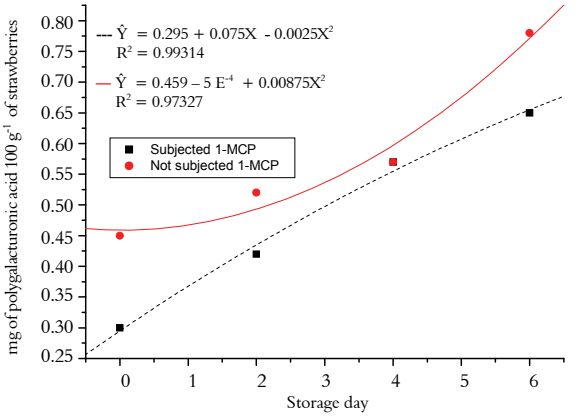

Figure 5. Curves and regression equations of the soluble pectin of 'Oso-Grande' strawberries subjected or not to 1-MCP and stored at room temperature for six days.

The trend observed for the soluble pectin during the storage was similar for both treatments, but the fruits without 1-MCP presented higher solubilization of pectin in relation to treated fruits (Figure 5). This is due to the higher activity of pectinolytic enzymes, presented by these fruits (Figures 2 and 3) and also due to greater loss in firmness (Figure 1).

Corrêa et al. (2000), analyzing the chemical compounds of the 'fruta de lobo' (*Solanum lycocarpum* St. Hil.), found an increase in total and soluble pectin during ripening.

There was increase in percentage of pectin solubilization during the storage in the strawberries subjected to both treatments, and at

the end of the storage, the fruits without 1-MCP had greater solubilization than those treated with 1-MCP (97.5 and 82.28%, respectively) (Figure 6). This result is in accordance with the Figure 1, in which the same fruits presented lower firmness, at the end of the storage.

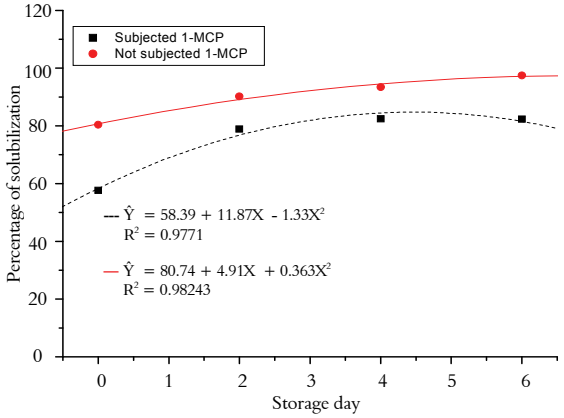

Figure 6. Curves and regression equations of the percentage of solubilization of'Oso-Grande' strawberries subjected or not to 1-MCP and stored at room temperature for six days.

Oliveira et al. (2005) verified that in most fruits, the soluble fraction of pectic substances increases during the ripening, in a process attributed to the action of pectinolytic enzymes, and that the solubilization of pectic substances is a natural trend during the fruits ripening.

Silva et al. (2009) also registered a trend of increase in the percentage of solubilization of voucher fruits, in a study with strawberries.

It is noteworthy that from the fourth storage day, the control fruits had no longer good aspect.

Conclusion

At last, the treatment with 1-MCP, at a concentration of 100 nL L^{-1} applied to strawberries, for 2h in sealed chamber, followed by 6 days-storage at room temperature, was efficient to delay the softening of the fruits, once the strawberries presented greater firmness, lower activity of the enzymes polygalacturonase and pectinmethylesterase, higher content of total pectin, lower content of soluble pectin and percentage of solubilization, when compared to untreated fruits, extending by two days the shelf life of these products.

Acknoledgements

To CNPq, Capes, and Fapemig.

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