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Effect of L-Ascorbic Acid and Sodium Metabisulfite in the Inhibition of the Enzymatic Browning of Minimally Processed Apple

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Abstract: The browning of fruits after the harvest, storage or in consequence of damage has great visual impact and diminishes the commercial, sensorial and nutritional quality of these fruits. The enzymatic browning that occurs in the cut surface of eatable tissue minimally processed affects the shelf life of these products. It was analyzed the inhibitory effect of ascorbic acid and sodium metabisulfite on the enzymatic browning of minimally processed apples stored at 5°C, packed or not. The region with stronger enzymatic browning was more intense near to the epidermis, in the central part and around the vascular bundles. The direct application of the sodium metabisulfite solution 10 mM on minimally processed apples packed with PVC film and cooled at 5°C conserved the original color of the minimally processed apples up to seven days.

Key words: Ascorbic acid, sodium metabisulfite, apple

INTRODUCTION

The enzymatic browning of vegetables, mainly fruits, after the harvest, storage or in consequence of injuries has great visual impact and decreases the commercial quality, the sensory acceptance and the nutritional value of these foods. The enzymatic browning that occurs on the cut surface area of the minimally processed vegetables for consumption is one of the main problems that reduce shelf-life of these products (Artes *et al*., 1998). For instance, cuts or damages in the tissues promote intracellular release of nutrients and enzymes that favor the enzymatic activity and the proliferation of microorganisms (Fantuzzi *et al*., 2004). Moreover, they harm the appearance, accelerate the senescence and the release of undesirable odors due the acceleration of the respiration and the ethylene production in the cut places (Mattiuz *et al*., 2003).

The minimum processing includes all the operations of cleaning, washing, selection, peeling, cut, packing and storage that intervene in the physical, chemical and biological factors responsible for the deterioration of the product (Rosa and Carvalho, 2000). However, the minimally processed foods present shelf life inferior to the fruits and entire vegetables due to physiological, physico-chemical and microbiological deteriorations of the tissues exposed by the cut (Fontes, 2005).

The enzymatic browning reactions are related with polyphenol oxidase activity (Janovitz-Klapp *et al*., 1990; Eidhin *et al*., 2006; Rojas-Graü *et al*., 2006; Lu *et al*., 2007). This enzyme was isolated from some sources, as: peach, carrot, avocado, apple, potato, banana, cará, tobacco leaf and others (Selvarajah *et al*., 2000). In some fruits, polyphenol oxidase can be detected in all the parts

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of the same one. In a banana variety (*Muse cavendishii* L.), the internal part of the pulp presents greater enzymatic activity than the external part (Galeazzi *et al*., 1981). In the apple Gala, the biggest polyphenol oxidase activity was detected in the epidermis and pericarp regions (Sataque, 1986).

The enzymatic browning usually modifies not only the appearance, but also the flavor and the nutritional value of foods. In sight of this, it is important to know the mechanisms of these reactions and the methods which can be controlled in the diverse products. The stabilization of products minimally processed has been carried out with the assistance of reducing agents and quelling agents in addition to the temperature reduction. The use of enzymatic browning inhibitors in processed fruits is restricted to non toxic components and that do not harm the flavor and aroma of the product. The ascorbic acid, besides being very effective in the enzymatic browning reduction, is also recognized as safe, cheap and well accepted by the consumers (Miranda, 2001). The ascorbic acid acts kidnapping copper, prosthetic group of the polyphenol oxidase and reducing quinones back to phenols, before forming dark pigments (Sapers and Miller, 1998; Chitarra, 2000). The effect of the ascorbic acid and the metabisulfite have been the most studied for the use in processed foods (Golan-Goldhirsh *et al*., 1984). The present work aims to analyze the effect of L-ascorbic acid and sodium metabisulfite on the enzymatic browning of minimally processed apples, stored at 5°C.

MATERIALS AND METHODS

Experimental Proceedings

The selected apples were from the Fuji cultivar and obtained from the producer. They were washed, peeled and cut in 1.5 cm slices with stainless steel knives. The minimally processed sliced apples were separated in six lots with three slices each. Two of them were not submitted to any treatment with inhibitors (controls) and were conditioned in polystyrene trays. One of the trays was packed with PVC film and hermetically sealed (C2), another one remained without the film (C1). The same procedures described above were repeated with slices previously immersed in metabisulfite sodium solution 10 mM per three minutes with the liquid being immediately drained (C4 and C3). In the same way, it was preceded in relation to the L-ascorbic acid (C6 and C5). All the samples were conditioned under refrigeration at 5°C and examined after 1, 3, 5 and 7 days, counting from the beginning of the experiment.

pH

The pH value of minimally processed apples was measured in aqueous extract (20 g of the sample triturated by homogenization with 100 mL of distilled water), in a potentiometer Marconi Pará 200, according to the AOAC methodology (1992).

Color

The color of the samples was evaluated using a Minolta Colorimeter, model Chroma Meter CR-200b. Readings were done for the three slices of apples of each treatment. The samples were evaluated in the L*, a* and b* systems (Bible and Singha, 1993).

Statistical Analysis

The experimental design utilized was entirely randomized in factorial 6×4, being 6 treatments (control without packing (C1), control with packing (C2), sodium metabisulfite without packing (C3), sodium metabisulfite with packing (C4), L-ascorbic acid without packing (C5) and L-ascorbic acid with packing (C6)) and 4 evaluation periods (1, 3, 5, 7 days storage at 5°C). The obtained results for pH and color evaluations were submitted to variance analysis by the F-test and comparison of averages by the Test of Tukey (5%).

RESULTS AND DISCUSSION

The acidity is a factor of great importance for the flavor and aroma of the fruits. Moreover, the pH influences the enzymatic browning of vegetal tissues. The pH reduction causes the decrease of the enzymatic browning of the fruit. The pH values of the minimally processed apples are presented in Table 1.

The minimally processed apples treated with sodium metabisulfite presented pH values lower in all evaluated days while the biggest values of pH were observed in the slices for control. The pH values differed significantly among the treatments with the storage time. In the seventh day it was observed that the biggest values of pH (4.12) were found in the slices for control, without PVC film. In a study with minimally processed apples from the Royal Gala variety submitted to a treatment with ascorbic acid 1%, Moreira *et al*. (2004) verified a decrease in the pH of the fruits in relation to the control.

Color degradation of apple, as indicated by changes in color parameters, was found to be highly correlated with polyphenol oxidase activity (Rocha and Morais, 2001). The variable L* indicates luminosity, differing light and dark colors. Its value varies from 100 for light colors (white) and zero for dark colors (black). The values of luminosity (L*) of minimally processed apples are presented in Table 2. It was observed, in general, reduction in the L* value of the apple slices minimally processed, suggesting its enzymatic browning during the storage at 5°C, except the ones treated with sodium metabisulfite with or without packing. The higher L* values were observed in the slices submitted to the treatment with sodium metabisulfite with packing, indicating that this treatment was the most efficient to avoid enzymatic browning. The lower values of L* were observed in the control for the apple slices minimally processed without packing, being that these values were statistically inferior to the other treatments, from the first to the seventh day of storage at 5°C.

In present study, the enzymatic browning was found in the pulp of the apple, however not uniformly. The epidermis and pericarp became darker demonstrating, thus, that these regions had more enzymatic browning than the extracarpelar region. This result is in accordance with the obtained by Harel *et al*. (1996), which observed an enzymatic browning not uniform in the interior of the apple, being more intense next to the epidermis, in the central part and around the vascular beams. This

Table 1: pH values from minimally processed apples in function of inhibitors and storage time

	Storage time (days)			
Treatments	1	3	5	7
Control without packing (C1)	3.96^{bA}	3.94^{bA}	4.01^{bA}	4.12^{bB}
Control with packing (C2)	3.95^{bA}	3.93^{bA}	3.98^{bA}	4.11^{bB}
Sodium metabisulfite without packing (C3)	3.65^{cA}	3.63^{cA}	3.62^{cA}	3.64^{cA}
Sodium metabisulfite with packing (C4)	3.60^{cA}	3.61^{cA}	3.61^{cA}	3.61^{cA}
L-ascorbic acid without packing (C5)	3.70^{aA}	3.69^{aA}	3.69^{aA}	3.71^{aA}
L-ascorbic acid with packing (C6)	3.71^{aA}	3.68^{aA}	3.69^{aA}	3.72^{aA}

Averages followed of the same the letter(s) in the column and capital letter(s) in the line do not differ among them, for the test of Tukey, in a level of 5% of probability

Table 2: Luminosity (L*) from minimally processed apples in function of inhibitors and storage time

	Storage time (days)			
Treatments	1	3	5	7
Control without packing (C1)	72.6^{aA}	72.0^{aA}	70.2^{aB}	60.2^{aA}
Control with packing (C2)	73.4^{aA}	72.6^{aA}	71.4^{aA}	64.6^{aB}
Sodium metabisulfite without packing (C3)	80.8^{bA}	79.8^{bA}	80.2^{bA}	80.6^{bA}
Sodium metabisulfite with packing (C4)	81.0^{bA}	80.9^{bA}	81.2^{bA}	81.6^{bA}
L-ascorbic acid without packing (C5)	76.4^{abA}	75.8^{aA}	75.2^{aA}	72.6^{aA}
L-ascorbic acid with packing (C6)	76.9^{abA}	76.0^{abA}	75.4^{abA}	73.2^{aA}

Averages followed of the same small letter(s) in the column and capital letter(s) in the line do not differ among them, for the test of Tukey, in a level of 5% of probability

Table 3: Chroma a* from minimally processed apples in function of inhibitors and storage time

	Storage time (days)			
Treatments	1	3	5	7
Control without packing (C1)	-2.4^{aB}	-1.0^{aB}	1.2^{aA}	2.2^{aA}
Control with packing (C2)	-2.2^{aB}	-1.2^{aB}	1.6^{aA}	2.6^{aA}
Sodium metabisulfite without packing (C3)	-4.6^{bB}	-3.6^{bB}	-2.7^{bAB}	-1.6^{cB}
Sodium metabisulfite with packing (C4)	-4.8^{bB}	-3.8^{bB}	-2.2^{bA}	-1.9^{cA}
L-ascorbic acid without packing (C5)	-4.8^{bB}	-4.0^{bB}	-3.8^{bB}	-3.0^{bcB}
L-ascorbic acid with packing (C6)	-5.0^{bB}	-4.2^{bB}	-3.8^{bB}	-3.4^{bA}

Averages followed of the same letter(s) in the column and capital letter(s) in the line do not differ among them, for the test of Tukey, in a level of 5% of probability

Table 4: Chroma b* from minimally processed apples in function of inhibitors and storage time

	Storage time (days)			
Treatments	1	3	5	7
Control without packing (C1)	37.2^{aA}	35.2^{aB}	34.4^{aB}	33.6^{aB}
Control with packing (C2)	36.9^{aA}	35.8^{aA}	34.8^{aB}	34.2^{aB}
Sodium metabisulfite without packing (C3)	28.6^{bB}	29.2^{bB}	29.9^{bB}	30.8^{bB}
Sodium metabisulfite with packing (C4)	27.7^{bB}	28.2^{bB}	29.0^{bAB}	28.6^{bB}
L-ascorbic acid without packing (C5)	28.4^{bB}	29.8^{bB}	30.1^{bAB}	31.2^{bA}
L-ascorbic acid with packing (C6)	27.9^{bB}	28.8^{bB}	29.6^{bB}	30.6^{bA}

Averages followed of the same letter(s) in the column and capital letter(s) in the line do not differ among them, for the test of Tukey, in a level of 5% of probability

behavior seems to be variety-dependent. Valderrama *et al*. (2001), for example, verified that the highest enzymatic activity of peroxidase is present in the apple rind as much in the cultivar Gala rather in the cultivar Fuji, however higher activity of polyphenol oxidase was observed in the rind extract of the of the cultivar Fuji in relation to the variety Gala.

The chroma a* indicates the intensity of the colors varying from green (-60) to red (+60). The values of chroma a* from the minimally processed apples are presented in Table 3. The biggest values of chrome a* were observed in the slices for control with or without packing. In the seventh day it was observed that the lower values of chrome a* were from the slices of apples treated with ascorbic acid with or without packing. The chroma b* indicates the intensity of the colors varying from blue (-60) to yellow (+60). The values of chroma b* from the minimally processed apples are presented in Table 4. In the control treatment it has controlled, with or without packing, it was observed bigger values of chroma b* since the first day of storage. The lower values of chroma b* were found for the apples treated with sodium metabisulfite. Along the storage time occurred alterations of the chroma b* for all the utilized treatments, showing a tendency to the increase of intensity of the yellow.

Increasing storage time of minimally processed apple results in an increase in polyphenol oxidase activity (Rocha and Morais, 2001). After seven days from the beginning of the experiment, the samples without packing and without treatment with inhibitor showed high withering and a very evident enzymatic browning. The samples packed without inhibitor and the treated with L-ascorbic acid had enzymatic browning similar to the presented ones for three days, without withering. All the samples packed without treatment with inhibitor developed enzymatic browning in lower degree than that observed in the samples without packing. The samples treated with sodium metabisulfite, packed and stored at 5°C, had conserved the color and the original aspect of the slices of fresh apple. The samples treated with sodium metabisulfite, without packing and stored at 5°C had also conserved the original color of the slices of fresh apple, however presented a very evident withering.

Browning of apple cubes during storage was found to be moderately correlated with polyphenol oxidase activity (Rocha and Morais, 2001). Sapers and Ziolkowski (1987) compared the inhibitory affect of erythorbic and ascorbic acids on the enzymatic browning in apple. They found a similar effectiveness of both compounds in apple juice, depending on the system in which they are compared.

Pizzocaro *et al*. (1993) evaluated the inhibitory effect of ascorbic acid, citric acid and sodium chloride on polyphenol oxidase of Golden Delicious apples. Ascorbic acid and sodium chloride solutions increased the polyphenol oxidase activity, however citric acid solutions had little or no inhibition effect.

CONCLUSIONS

Refrigerated storage of minimally processed apple for 7 days at 5°C seemed to cause an increase in enzymatic browning. pH reduction and color degradation of apple during storage, as indicated by changes in pH and color parameters, were found to be quite well correlated with enzymatic browning. The region of higher enzymatic browning of minimally processed apple was the epidermis and pericarp, formed by the exocarp and endocarp. Sodium metabisulfite was more effective than L-ascorbic acid for the conservation of the original color, preventing the enzymatic browning, being that, after seven days, only the combined treatment of sodium metabisulfite with storage at 5°C was efficient for color conservation, independently of the type of packing. On the other hand, the use of PVC film contributed to avoid the withering and wrinkling of the slices of apple.

REFERENCES

AOAC (Association of Official Analytical Chemists), 1992. Official Methods of Analysis. 14th Edn. Washington, pp: 1141.

Artes, F., M. Castaner and M.I. Gil, 1998. Enzymatic browning in minimally processed fruit and vegetables. Food Sci. Technol. Int., 4 (6): 377-389.

Bible, B.B. and S. Singha, 1993. Canopy position influences CIELAB coordinates of peach color. HortScience, 28 (10): 992-993.

Chitarra, M.I.F., 2000. Processamento mínimo de frutos e hortaliças. Lavras: UFLA/FAEPE, pp: 113.

Eidhin, D.M.N., E. Murphy and D. O'Beirne, 2006. Polyphenol oxidase from apple (*Malus domestica* Borkh. cv Bramley's Seedling): Purification strategies and characterization. J. Food Sci., 71 (1): C51-C58.

Fantuzzi, E., R. Puschmann and M.C.D. Vanetti, 2004. Microbiota contaminante em repolho minimamente processado. Ciênc. Tecnol. Aliment., 24 (2): 207-211.

Fontes, L.C.B., 2005. Uso de solução conservadora e de películas comestíveis em maçãs da cultivar Royal Gala minimamente processadas: Efeito na fisiologia e na conservação. M.Sc. Thesis, Escola Superior de Agricultura Luiz de Queiroz. Universidade de São Paulo. São Paulo, pp: 132.

Galeazzi, M.A.M., V.C. Sgarbieri and S.M. Constantides, 1981. Isolation, purification and physicochemical characterization of polyphenoloxidase (PPO) from a dwarf variety of banana (*Musa cavendishii*, L.). J. Food Sci., 46 (1): 150-155.

Golan-Goldhirsh, A., J.R. Whitaker and V. Kahn, 1984. Relation between structure of polyphenol oxidase and prevention of browning. Adv. Exp. Med. Biol., 167: 437-456.

Harel, E., A.M. Mayer and Y. Shain, 1966. Catechol oxidases, endogenous substrates and browning in developing apples. J. Sci. Food Agric., 17 (9): 389-392.

Janovitz-Klapp, A.H., F.C. Richard, P.M. Goupy and J.J. Nicolas, 1990. Inhibition studies on apple polyphenol oxidase. J. Agric. Food Chem., 38 (4): 926-931.

Lu, S., Y. Luo, E. Turner and H. Feng, 2007. Efficacy of sodium chlorite as an inhibitor of enzymatic browning in apple slices. Food Chem., 104 (2): 824-829.

Mattiuz, B., J.F. Durigan and O.D. Rossi Jr., 2003. Processamento mínimo em goiabas Paluma e Pedro Sato 2. Avaliação química, sensorial e microbiológica. Ciên. Tecnol. Alimen., 23 (3): 409-413.

Miranda, R.B., 2001. Avaliação da qualidade do mamão (*Carica papaya* L.) minimamente processado. M.Sc. Thesis, Universidade Federal de Lavras. Lavras.

Moreira, G.C., R.L. Vieites, A.J. Campos, E.R. Damatto Jr. and R.M. Evangelista, 2004. Caracterização fisiológica de maçã Royal Gala minimamente processada, submetida a diferentes concentrações de ácido ascórbico. In: Congresso Sociedade Brasileira de Ciência e Tecnologia de Alimentos. Recife.

Pizzocaro, F., D. Torreggiani and G. Gilardi, 1993. Inhibition of apple polyphenoloxidase (PPO) by ascorbic acid, citric acid and sodium chloride. J. Food Proc. Preserv., 17 (1): 21-30.

Rocha, A.M.C.N. and A.M.M.B. Morais, 2001. Influence of controlled atmosphere storage on polyphenoloxidase activity relation to color changes of minimally processed Jonagored apple. Int. J. Food Sci. Technol., 36 (4): 425-432.

Rojas-Graü, M.A., A. Sobrino-López, M.S. Tapia and O. Martín-Belloso, 2006. Browning inhibition in fresh-cut Fuji apple slices by natural antibrowning agents. J. Food Sci., 71 (1): S59-S65.

Rosa, O.O. and E.P. Carvalho, 2000. Características microbiológicas de frutos e hortaliças minimamente processados. Boletim da SBCTA, 34 (2): 84-92.

Sapers, G.M. and M.A. Ziolkowski, 1987. Comparison of erythorbic and ascorbic acids as inhibitors of enzymatic browning in apple. J. Food Sci., 52 (6): 1732-1733.

Sapers, G.M. and R.L. Miller, 1998. Browning inhibition in fresh-cut pears. J. Food Sci., 63 (2): 342-346.

Sataque, Y.E., 1986. Caracterização da polifenoloxidase e sua influência no suco de maçã (*Malus domestica*, var. Gala). M.Sc. Thesis, Universidade Estadual de Londrina. Londrina.

Selvarajah, S., H.M.W. Herath, D.C. Bandara and I.P. Wicramasinghe, 2000. Physiological and enzymatic changes during post-harvest low temperature storage periods and the manifestation of symptoms associated with internal browning in pineapples. J. Food Sci. Technol., 37 (6): 571-576.

Valderrama, P., F. Marangoni and E. Clemente, 2001. Efeito do tratamento térmico sobre a atividade de peroxidase (POD) e polifenoloxidase (PPO) em maçã (*Mallus communis*). Ciên. Tecnol. Aliment., 21 (3): 321-325.