Effects of Citric Acid and Na-Metabisulphite on the Shelf Life of Minimally Processed *Haciomer* cv. Chestnut

Vildan Uylaşer

Bige İncedayı

GökçenYıldız

Department of Food Engineering
Faculty of Agriculture
Uludag University
16059, Bursa, Turkey

Abstract

In this study, the potential use of citric acid and Na-metabisulphite for browning control and quality maintenance of minimally processed Haciömer cv. chestnuts was investigated. Chestnuts were dipped into solutions of 0, 1 and 2 mM citric acid and Na-metabisulphite, placed into trays over-wrapped with plastic film and then stored at 4 °C. The following characteristics were determined: color; polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL) activity; value changes in titratable acidity, antioxidant activity and ascorbic acid accumulation during storage. The results showed that both treatments reduced enzyme activities; however Na-metabisulphite treatment was less effective for PAL activity. The treatment extended the shelf life of minimally processed Haciömer cv. chestnuts by about 15 days.

Key words: Chestnut, Antioxidant activity, Browning, Citric acid, Na-metabisulphite, Shelf life, Peeled

1. Introduction

Turkey is one of the most important chestnut producers, representing nearly 3 % of world production and 55 % of European production. The worldwide chestnut production (2010) was 1.958.547,00 tons. China (1.620.000,00 tons), Turkey (59.171,00 tons) and Italy (42.700,00 tons) are the main producers of chestnuts (FAO, 2010). In Turkey chestnut trees are scattered throughout the forest areas (Soylu, 2004; Topçu, 2009; Uylaşer et al., 2010). In recent years, chestnut has received increasing attention because of its nutritional quality and potential beneficial health effects. The chestnut tree, sometimes called a "bread tree," provides a fundamental nutrient to the human body (Ertürk et al., 2006). Unlike most other tree nuts, chestnuts are low in protein and fat but high in carbohydrates.

The fruit contain (g/100 g dry matter basis) total carbohydrates between 58.18-94.21, total sugar between 10.32-28.84, invert sugar between 0.08-2.00, starch between 34.24-75.25, sucrose between 8.05-27.04, ash between 1.02-3.22, crude cellulose between 3.58-5.96, total fat between 0.49-2.47 and total protein between 4.88-10.87. Ca, Mg, Fe, Mn, Cu, Zn, P, Na and K contents were (mg/100 g) between 43-230, 70-160, 0.4-5.7, 0.7-5.5, 0.6-3.8, 1.8-9.1, 107-191, 6-41 and 761-1271, respectively (Ertürk et al., 2006; Öztürk et al., 2010; Uylaşer et al., 2010). The antioxidant characteristics of chestnut also contribute to its valuable characteristics (Barreira et al., 2008; Wazquez et al., 2008; Wazquez et al., 2009; Almeida et al., 2010). Radical scavenging activity and scavenging activity against superoxide anion and hydroxyl radicals was previously reported for *Castanea sativa* leaves. Such antioxidant activity was suggested to be related to phenolic composition (Calliste et al., 2005). Rutin, hesperidin, quercetin, apigenin, morin, galangin, kaempferol and isoquercitrin have been identified in *C. sativa* leaves (Basile et al., 2000; Calliste et al., 2005).

One of the most important challenges after harvesting and during processing of the seeds is browning control. Cut-surface browning, considered to be mediated by polyphenol oxidase (PPO) and peroxidase (POD), is a major concern with regard to quality deterioration and short shelf life. A major problem of chestnut production is the short storage period (Tan et al., 2007).

Various approaches are now applied to prevent the browning of peeled fruits and vegetables, one of which is the use of different modified atmosphere conditions during low temperature storage.

Other approaches include the use of chemical inhibitors of enzymatic browning (Mayer and Harel, 1991). However, this practice has several deficiencies. It is therefore necessary to develop a commercially suitable technology for shelf-life extension.Browning is associated to changes in color, flavor and softening (probably due to the action of pectic enzymes), with the exception of nutritional properties. These parameters are necessary to select the ideal cultivar for production and elicit consumer preference (Kundakçı and Ergönül, 2004).Deterioration in quality also reduces the market price of chestnuts as a foodstuff (McEvily et al., 1992; Martinez and Whitaker, 1995; Xu, 2005; Uylaşer et al., 2010).

Minimally processed products can meet the ever-increasing consumer demand for high quality, fresh, natural, nutritive and easy-to-prepare fruits and vegetables. Consequently, alternative means for browning control of peeled chestnuts are needed. Non-toxic and enzyme inhibitor chemicals like citric acid or sulphites are suitable for this task; citric acid acts as a chelating agent and acidulant, which functionally inhibits PPO activity (Santerre et al., 1988; Sapers, 1993). Sulphites, which are act as PPO inhibitors, react with intermediates to prevent pigmentation (Sapers, 1993).

However, there is currently an inadequate amount of research regarding the chemical preservation of peeled chestnuts. This research was carried out to investigate the consequence of citric acid and Na-metabisulphite treatment on the browning and shelf life of peeled *Haciömer*cv. chestnuts.

2. Materials and Methods

2.1. Materials

In this research, *Haciömer*cv. chestnuts which were grown in Bursa, Turkey were used. Chestnuts were selected for uniformity size and bruised or diseased fruits were discarded. Fruits were washed and peeled; dipped for 1 min in a solution containing 0 mM (as a control), 1 mM and 2 mM citric acid and Na-metabisulphite; and then air-dried at room temperature for 30 min. After being air-dried, the treated fruits about 225 g were placed into plastic cap (0.02-mm thick polyethylene films, Sekeroglu Corporation, Konya, Turkey) and then stored at 4^{o}C for progressive assessments. The control and treated chestnut samples were stored for 15 days and on the 0, 3, 5, 7 and 15 days analyzed enzymatically, chemically and physically. Three replicates were used for each treatment.Methanol, 2,2-diphenyl-1-picryhydrazyl radical (DPPH), 2,6-dichlorophenol indophenol, sodium ascorbate, Triton X-100, L-phenylalanine, trichloroacetic acid, guaiacol and mercaptoethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyvinylpyrolidone (PVPP), sodium hydroxide and oxalic acid were obtained from E. Merck (Darmstadt, Germany).

2.2. Extractions and Determination of PAL, PPO and POD Activities

PAL (Phenylalanine ammonia lyase) was extracted according to the method of Lister *et al*. (1996) with slight modifications. Tissue (30 g) from whole chestnut was homogenized in 50 mL of 0.05 M phosphate buffer (pH 7.0), which was containing 5% polyvinylpyrolidone, 0.05 M sodium ascorbate, 0.018 M mercaptoethanol and 0.1% (v/v) Triton X-100. The homogenate was filtered through four layers of cotton cloth and then centrifuged for 10 min at 20000 *g* with a Sigma 3K30 model (UK) centrifuge at 4^{o}C. The supernatant was collected as the enzyme extract. PAL activity was assayed by the method of McCallum and Walker (1990) with slight modifications, using a reaction mixture of 0.06 M sodium borate buffer (pH 8.8) containing 11 mM L-phenylalanine and 0.4 mL of crude enzyme, with a final volume of 2.4 mL. The tubes were incubated for 2 h at 30^{o}C, and the reaction was stopped by adding 0.6 mL of 35% (w/v) trichloroacetic acid. After the tubes were centrifuged for 5 min at 5000 *g* to pellet the denatured proteins, the absorbance was measured at 290 nm by a Shimadzu UV⁄ VIS 1800 model (Kyoto, Japan) spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.001 in absorbance per minute (U/g).

To measure PPO and POD activities, tissue (30 g) from whole chestnut was homogenized in 0.2 g PVPP and 70 mL of 0.05 M phosphate buffer (pH 6.8), and the homogenate was filtered through four layers of cotton cloth to remove cellular debris. The clear supernatant after centrifugation at 12720 *g* (Sigma 3K30, UK) for 15 min at 4^{o}C was collected as enzyme extracts. PPO activity was assayed according to the method of Jiang (1999) by measuring the oxidation of 4-methylcatechol. The increase in absorbance at 420 nm was automatically recorded for 3 min using a spectrophotometer.

One unit of enzyme activity was defined as the amount that caused a change of 0.001 in absorbance per minute (U/g). POD activity was measured according to the procedure of Mac-Adam et al. (1992). The assay mixture consisted of 0.05 M sodium phosphate buffer (pH 7.0), 0.012 M H_2O_2, 0.007 M guaiacol and 0.1 mL of enzyme solution in a final volume of 3.0 mL.

The increase in absorbance at 470 nm was recorded for 3 min. One unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.001 in absorbance per minute (U/g).

2.3. Extraction and Determination of Antioxidant Activity

Antioxidant activity was measured using the DPPH radical. The inhibition percentage of the DPPH free radical at 517 nm was determined (Moon and Terao, 1998; Zhang and Hamauzu, 2004). By this method, 1 g of homogenized chestnut was extracted with 4.5 mL methanol (80%) and stirred at 140 rpm for 2 h at room temperature, then centrifuged at 10.000 *g* for 15 min. Clear supernatant was taken out and the residual was extracted again with the same procedure. Supernatants of the two extracts were combined, filtered and collected for antioxidant activity determination. The extracted samples, which were made to react with the radical solution (DPPH) and rest for an hour at room temperature, were measured for absorbance (Abs) at 517 nm by a Shimadzu UV⁄ VIS 1800 model (Kyoto, Japan) spectrophotometer and the inhibition percentage of DPPH free radical was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = [(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{blank}}] \times 100.$$

2.4. Determination of Dry Matter, Titratable acidity, Ascorbic acid and Color Parameters

Dry matter contents of the samples were analyzed with the oven-dry procedure (ED115 Binder, Tuttlingen, Germany) (Uylaşer and Başoğlu, 2011). Titratable acidity was determined as citric acid by titrating samples against 0.1 N NaOHsolution (AOAC, 1990). Potentiometric determination of pH was performed using a Sartorius PB11 doc model pH meter (Goettingen, Germany). The ascorbic acid levels were determined by spectrophotometry (Shimadzu UV⁄ VIS 1800, Kyoto, Japan) using 2,6-dichlorophenol indophenol dye (Cemeroğlu, 2007).The color of the chestnuts (reflectance values) was measured using a Miniscan EZ4500L model Hunterlab colorimeter (Virginia, USA) and expressed in a three-dimensional *L*, *a*, *b* color space, where *L** represents the darkness/lightness of the sample, *a** represents the redness (positive) and greenness (negative), and *b** represents the yellowness (positive) and blueness (negative). The degree of browning was analyzed in terms of changes in *L**and hue angle ($h = \tan^{-1}(b / a)$ values (Karaaslan & Tuncer, 2008; Manolopou & Varzakas, 2011).

2.5. Statistical Analysis

The results were analyzed using the JMP (Version 7.0, SAS Institute Inc., Cary, NC, USA). Mean differences were tested for significance with a least significant difference (LSD) test at a 5% level of significance.

3. Results and Discussion

3.1. PPO, PAL, and POD Activities

The effects of citric acid and Na-metabisulphite on PPO, PAL, and POD activities are shown in Table 1. As mentioned above, the major limitation of the peeled chestnut's shelf life is surface discoloration, which is associated with phenol metabolic enzymes, such as POD, PPO and PAL (Jiang, 1999; Lopez-Serrano and RosBarcelo, 1999; Loaiza-Velarde and Saltveit, 2001; Zhang et al., 2003).

When compared with the control sample, citric acid and Na-metabisulphite inhibited increase in PPO activity and higher concentration of solutions (2mM) displayed more effective inhibition. There was a significant decrease ($P<0.05$) in the PPO activity from 937.895 U/g in the control sample to 430.920 U/g on the 15th storage day with the use of 2 mM Na-metabisulphite. Generally the activity of PPO increased in the early stage of storage and then decreased. This inhibition effect was at its maximum on the 7th storage day with the use of 2 mM solutions. Jiang et al. (2004) reported the same effect of citric acid on the Chinese water chestnut. Na-metabisulphite is an antioxidant agent and can successfully controls enzymatic browning, whereas the inhibiting effect of citric acid could be related to its phenolase Cu-chelating action (Jiang et al., 2004; Peng and Jiang, 2006).

The PAL activity of the samples changed between 595.420 to 3335.395 U/g for citric acid; 1379.380 to 10295.420 U/g for Na-metabisulphite treatments, 3350.735 to 11021.935 U/g for control sample.

Citric acid treatment significantly (P<0.05) inhibited the increase of PAL activity latter of storage associated with a reduced browning index, but Na-metabisulphite did not adequately decreased the activity of this enzyme. The inhibition of PAL activity reduced the biosynthesis of precursors for the formation of brown substances (Loaiza-Velarde et al., 1997; Loaiza-Velarde and Saltveit, 2001).

As can be seen from Table 1, there was a significant decrease (P<0.05) in the POD activity of chestnuts from 4.335 U/g in the control sample to 0.390 on the 5th storage day with the use of 2 mM citric acid. POD activity of control samples was more influenced by citric acid treatment and the significantly (P< 0.05) lowest POD activity (0.900 U/g) was on the 15th storage day was obtained with the use of 2 mM citric acid. The other treatments were less effective compared with the 2 mM citric acid treatment but more effective than that of the control group.

3.2. Antioxidant Activity, Ascorbic Acid and Total Titratable Acidity

The results of the changes in antioxidant activity, ascorbic acid and total titratable activity caused by the treatments of chestnut samples are presented in Table 2.The antioxidant activity of the chestnut changed between 18.475 to 54.815% for citric acid; 37.960 to 75.575% for Na-metabisulphite treatments, 27.740 to 64.210% for control sample. Antioxidant activity of the chestnut sample treated with 2 mM Na-metabisulphite (61.615%) was the highest (P<0.05) whereas control sample (44.510%) had the least antioxidant activity of all chestnut samples.

The content of ascorbic acid of chestnut samples changed between 6.025 to 26.830 mg/100g and generally was reduced by storage. On the 15th storage day, all concentrations of treatment solutions, except 1 mM Na-metabisulphite, had significantly (P< 0.05) higher ascorbic acid content than the control samples. Peng and Jiang (2006) found a similar result for salicylic acid treated fresh-cut Chinese water chestnut after 12 days of storage at 4 °C. Because L-ascorbic acid is a water-soluble, thermolabile vitamin, which is particularly prone to both chemical and enzymatic oxidation, its concentration can be considered as a quality factor for foods and therefore important to monitor it during processing and storage (Serpen et al., 2007; Cortes et al., 2008). Total titratable acidity values were increased by the end of the storage period and it can be related to microbial growth.

3.3. Color Parameters

Table 3 shows the effects of treatments on the L^* and h values of the chestnut samples compared with control sample. The L^* and h values were selected as the most suitable parameters to measure the surface color change of minimally processed chestnut.

According to the Turkish Candied Chestnut Standard (TS 9400), candied chestnuts should not be dark brown (Anonymous, 1991). The L^* and h values of the chestnut changed between 54.265 to 62.090 and 69.115° to 81.687°, respectively. Generally the L^* value of all samples decreased during the storage but this decrease was not significant (P>0.05), except at the 3th storage day. Among the chemical treatments, 2 mM citric acid and 2 mM Na-Metabisulfite inhibitors showed the least change in L^* at the 15th day of storage. Similarly, there was not a significant (P>0.05) difference in the h value of all the chestnut samples. The control sample had descriptively (P>0.05) lower h value of than samples treated with 2 mM citric acid, 1mM and 2 mM Na-Metabisulfite solutions at the 15th day of storage.

In addition, the samples were spoiled microbiologically, and a gummy form on surface was observed at the end of two weeks. This change was higher in the control samples (at the 7th and 10th days of storage). The least spoilage was determined for the 2 mM citric acid-treated samples (at the 15th days of storage).

4. Conclusions

The results show that Na-metabisulphite (2 mM) and citric acid (2 mM) best maintains the color and general acceptance of minimally processed *Haciömer*cv. chestnuts. The 2 mM citric acid application was effective for the inhibition of PPO, POD, and PAL activities. The Na-metabisulphite treatment was not effective for PAL inhibition, which causes phenolic biosynthesis. However, antioxidant activity was higher in the 2 mM Na-metabisulphite treatment group. In conclusion, Na-metabisulphite (2 mM) and especially citric acid (2 mM) applications could be suggested for reducing surface browning and extending the shelf life of chestnuts by 15 days. This time period is sufficient for acceptable marketing practices.

References

Almeida, I.F., Costa, P.C. and Bahia, M.F. 2010. Evaluation of functional stability and batch-to-batch reproducibility of a *Castanea sativa* leaf extract with antioxidant activity. *AAPS Pharm.Sci.Tech.*,11:120-125.

Anonymous, 1991.Candied Chestnut TS 9400.Turkish Standards Institute, Ankara, Turkey.

AOAC, 1990. Official methods of analysis.Edition no. 15th; Washington DC: USA, Association of Official Analytical Chemists.

Barreira, J.C.M., Ferreira, I.C.F.R. Oliveira, M.B.P.P. and Pereira, J.A. 2008. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit.*Food Chemistry*.107:1106-1113.

Basile, A., Sorbo, S. Giordano, S. Ricciardi, L. Ferrara, S. and Montesano, D. 2000. Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves. *Fitoterapia*, 71:110-116.

Calliste, C.A., Trouillas, P. Allais D.P. and Duroux, J.L. 2005.*Castanea sativa*Mill.leaves as new sources of natural antioxidant: an electronic spin resonance study. *Journal of Agricultural & Food Chemistry*, 53:282-288.

Cemeroğlu, B. 2007.Food Analysis. Food Technology Association Publications, No: 34, Ankara,p.657.

Cortes, C., Esteve, M.J. and Frigola, A. 2008.Effect of refrigerated storage on ascorbic acid content of orange juice treated by pulsed electric fields and thermal pasteurization.*European Food Research and Technology*, 227: 629-635.

Ertürk, Ü.,Mert, C. and Soylu, A. 2006. Chemical composition of fruits of some important chestnut cultivars.*Brazilian Archives of Biology and Technology*, 49: 183-188.

FAO, 2010.Food and Agriculture Organization of the United Nations FAOSTAT.Available from: http://faostat.fao.org.

Jiang, Y.M. 1999. Purification & some properties of polyphenol oxidase of longan fruit.*Food Chemistry*, 66:75-79.

Jiang, Y.M., Peng, L.T. and Li, J.R. 2004.Use of citric acid for shelf life and quality maintenance of fresh-cut Chinese water chestnut.*Journal of Food Engineering*, 63: 325-328.

Karaaslan, S.N. and Tuncer, İ.K. 2008. Development of a drying model for combined microwave-fan-assisted convection drying of spinach. *Biosystems Engineering*, 100:44-52.

Kundakçı, A. and Ergönül, B. 2004.Kestaneşekeriüretimteknolojisivesorunları.GelenekselGıdalarSempozyumuBildiriKitabı. 23-24 Eylül,Van-Turkey, 80-84.

Lister, C.E., Lancaster, J.E. and Walker, J.R.L. 1996. Phenylalanine ammonia-lyase activity and its relationship to anthocyanin and flavonoid levels in New Zealand grown apple cultivars.*Journal of the American Society for Horticultural Science*, 121:281-285.

Loaiza-Velarde, J.G., Tomas-Barbera, F.A. and Saltveit, M.E. 1997.Effect of intensity and duration of heat shock treatments on wound-induced phenolic metabolism in iceberg lettuce.*Journal of American Society for Horticultural Science*, 122: 873-877.

Loaiza-Velarde, J.G. and Saltveit, M.E. 2001. Heat shocks applied either before or after wounding reduce browning of lettuce leaf tissue. *Journal of American Society for Horticultural Science*, 126:227-234.

Lopez-Serrano, M. and RosBarcelo, A. 1999.H_2O_2-Mediated pigment decay in strawberry as a model system for color alterations in processed plant foods.*Journal of Agriculture and Food Chemistry*, 47:824-827.

Manolopou, E. and Varzakas, T. 2011. Effect of storage conditions on the sensory quality, colour and texture of fresh-cut minimally processed cabbage with the addition of ascorbic acid, citric acid and calcium chloride.*Food and Nutrition Science*, 2:956-963.

Martinez, M.V. and Whitaker, J.R. 1995.The biochemistry and control of enzymatic browning.*Trends in Food Science and Technology*, 6:195-200.

Mayer, A. and Harel, E. 1991.Phenoloxidases and their significance in fruit and vegetable.In:P. F. Fox (Editor), *Food Enzymology*, Elsevier: London, p. 373-398.

McAdam, J.W., Nelson, C.J. and Sharp, R.E. 1992. Peroxidase activity in the leaf elongation zone of tall fescue.*Plant Physiology*, 99:872-878.

McCallum, J.A. and Walker, J.R.L. 1990. Phenolic biosynthesis during grain development in wheat: change in phenylalanine ammonia-lyase activity and soluble phenolic content. *Journal of Cereal Science*, 11:35-49.

McEvily, A.J., Iyengar, R. and Otwell, W.S. 1992. Inhibition of enzymatic browning in foods and beverages.*Critical Reviews in Food Science and Nutrition*, 32: 253-275.

Moon, J.H. and Terao, J. 1998.Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low-density lipoprotein.*Journal of Agriculture and Food Chemistry*, 46:5062-5065.

Öztürk, S., Çetin, M. and Çetin, M. 2010.Chemical composition and fatty acid contents of chestnuts grown in Bursa province, Turkey.*Asian Journal of Chemistry*, 22:1845-1852.

Peng, L. and Jiang, Y. 2006. Exogenous salicylic acid inhibits browning of fresh-cut Chinese water chestnut. *Food Chemistry*, 94: 535-540.

Santerre, C.R., Cash, J.N. and Vannorman, D.J. 1988. Ascorbic acid/citric acid combinations in the processing of frozen apple slices.*Journal of Food Science*, 53:1713-1716.

Sapers, G.M. 1993. Browning of foods: control by sulfites, antioxidants, and other means. *Food Technology-Chicago*, 47:75-84.

Serpen, A., Gökmen, V., Bahçeci, K.S. and Acar, J. 2007. Reversible degradation kinetics of vitamin C in peas during frozen storage.*European Food Research and Technology*, 224: 749-753.

Soylu, A. 2004.Chestnut growing and specialities.Hasad Publication, Istanbul, Turkey, 64p.

Tan, Z.L., Wu, M.C. Wang, Q.Z. and Wang, C.M. 2007. Effect of calcium chloride on chestnut.*Journal of Food Processing and Preservation*, 31:298-307.

Topçu, K., Serdar, U. Öztürk, A. and Genç, M. 2009. A chestnut monument tree in Sinop: PadişahKestanesi. *ActaHorticultura*, 815:63-170.

Uylaşer, V., Incedayı, B., Mert, C. and Soylu, A. 2010. A research on suitability of some chestnut cultivars for candied chestnut. *ActaHorticultura*, 866:571-579.

Uylaşer, V. and Başoğlu, F. 2011.Fundamental Food Analysis.Dora Publication, Bursa, ISBN 978-605-4485-13-0, p.125.

Vazquez, G., Fontenla, E., Santos, J. Freire, M.S., Gonzalez-Alvarez, J. and Antorrena, G. 2008. Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts. *Industrial Crops and Products*, 28: 279-285.

Vazquez, G., Gonzalez-Alvarez, J. Santos, J., Freire M.S. and Antorrena, G. 2009. Evaluation of potential applications for chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts. *Industrial Crops and Products*, 29: 364-370.

Xu, J. 2005. The effect of low-temperature storage on the activity of polyphenol oxidase in *Castaneahenryi* chestnuts.*Postharvest Biology and Technology*, 38: 91-98.

Zhang, Y., Chen, K.S., Zhang S.L. and Ferguson, I. 2003. The role of salicylic acid in postharvest ripening of kiwifruit.*Postharvest Biology and Technology*, 28:67-74.

Zhang, D. and Hamauzu, Y. 2004. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry*, 88:503-509.

Table 1. Effects of citric acid and Na- metabisulphite on PPO, PAL and POD activities of minimally processed *Haciömer*cv. chestnutduring the storage at 4°C

Solutions	Days	PPO (U/g)	PAL (U/g)	POD (U/g)
	0	$682.610^{a,ABC}$	$3335.395^{b,A}$	$3.480^{a,ABC}$
	3	$872.295^{a,AB}$	$663.605^{c,D}$	$5.815^{a,A}$
Citric Acid (1mM)	5	$1013.310^{a,A}$	$2798.005^{b,AB}$	$3.100^{ab,BC}$
	7	$608.145^{ab,BC}$	$1067.950^{c,BCD}$	$2.740^{c,BCD}$
	15	$630.240^{ab,ABC}$	$966.165^{c,BCD}$	$3.635^{b,AB}$
	0	$598.835^{a,BC}$	$2563.030^{b,ABC}$	$2.660^{a,BCD}$
	3	$601.175^{a,BC}$	$636.475^{c,D}$	$1.215^{b,BCD}$
Citric Acid (2mM)	5	$834.490^{ab,AB}$	$1561.635^{b,ABCD}$	$0.390^{c,D}$
	7	$392.080^{b,C}$	$870.815^{c,CD}$	$1.730^{c,BCD}$
	15	$430.920^{b,C}$	$595.420^{c,D}$	$0.900^{e,CD}$
	0	$580.370^{a,AB}$	$10295.420^{a,A}$	$2.690^{a,BC}$
	3	$932.915^{a,A}$	$5053.335^{ab,BC}$	$1.010^{b,E}$
Na-Metabisulfite (1mM)	5	$595.950^{b,AB}$	$1379.380^{b,D}$	$2.855^{ab,BC}$
	7	$624.625^{ab,AB}$	$6690.135^{b,BC}$	$3.770^{b,A}$
	15	$662.220^{ab,AB}$	$4350.250^{a,CD}$	$2.670^{c,BC}$
	0	$391.780^{a,B}$	$9904.320^{a,A}$	$1.505^{a,DE}$
	3	$597.750^{a,AB}$	$6680.590^{a,BC}$	$1.105^{b,E}$
Na-Metabisulfite (2mM)	5	$550.550^{b,AB}$	$7993.580^{a,AB}$	$1.770^{bc,DE}$
	7	$423.945^{b,B}$	$5777.720^{b,BC}$	$3.460^{b,AB}$
	15	$511.355^{ab,B}$	$3939.950^{a,CD}$	$2.100^{d,CD}$
	0	$621.600^{a,A}$	$5913.280^{b,C}$	$4.455^{a,A}$
	3	$875.225^{a,A}$	$3550.735^{b,D}$	$3.510^{ab,A}$
Control	5	$884.085^{ab,A}$	$11021.935^{a,A}$	$4.335^{a,A}$
	7	$917.675^{a,A}$	$8456.300^{a,B}$	$4.935^{a,A}$
	15	$937.895^{a,A}$	$1945.915^{b,D}$	$4.290^{a,A}$

$^{a-c}$Different letters in the same column concerning the same days indicate significantly different values ($P< 0.05$) [comparison of chemical solutions].

$^{A-E}$Different letters in the same column concerning the same chemical solutions indicate significantly different values ($P < 0.05$) [comparison of days].

Table 2. Effects of citric acid and Na- metabisulphite on antioxidant activity, ascorbic acid and total titratable acidity content of minimally processed *Haciömer* cv. chestnut during the storage at 4°C

Solutions	Days	Antioxidant Activity (%)	Ascorbic Acid (mg/100g)	Titratable Acidity (%)
Citric Acid (1mM)	0	$58.125^{b,A}$	$26.830^{a,A}$	$0.100^{c,D}$
	3	$49.645^{d,B}$	$21.515^{b,C}$	$0.099^{c,D}$
	5	$38.195^{b,C}$	$18.595^{bc,D}$	$0.151^{a,D}$
	7	$34.130^{c,C}$	$6.375^{d,EF}$	$0.455^{b,B}$
	15	$51.285^{c,AB}$	$7.000^{b,EF}$	$0.379^{d,C}$
Citric Acid (2mM)	0	$40.445^{c,C}$	$25.855^{b,AB}$	$0.279^{a,A}$
	3	$49.870^{d,B}$	$25.150^{ab,B}$	$0.113^{bc,D}$
	5	$35.110^{c,C}$	$21.780^{a,C}$	$0.145^{a,D}$
	7	$18.475^{e,D}$	$6.025^{d,F}$	$0.390^{bc,C}$
	15	$54.815^{b,AB}$	$7.510^{b,E}$	$0.567^{c,A}$
Na-Metabisulfite (1mM)	0	$55.255^{b,C}$	$24.085^{c,B}$	$0.102^{c,G}$
	3	$54.905^{b,CD}$	$26.655^{a,A}$	$0.135^{a,FG}$
	5	$40.735^{b,F}$	$20.100^{ab,D}$	$0.139^{a,EFG}$
	7	$37.960^{b,G}$	$22.670^{a,BC}$	$0.447^{b,C}$
	15	$38.260^{e,FG}$	$5.135^{d,G}$	$1.394^{a,A}$
Na-Metabisulfite (2mM)	0	$75.575^{a,A}$	$21.695^{d,C}$	$0.191^{b,E}$
	3	$52.430^{c,DE}$	$17.090^{c,E}$	$0.117^{ab,FG}$
	5	$51.135^{a,E}$	$9.295^{d,F}$	$0.169^{a,EF}$
	7	$56.635^{a,C}$	$8.940^{c,F}$	$0.355^{c,D}$
	15	$61.615^{a,B}$	$8.485^{a,F}$	$0.728^{b,B}$
Control	0	$41.430^{c,B}$	$24.705^{c,A}$	$0.101^{c,B}$
	3	$64.210^{a,A}$	$27.895^{a,A}$	$0.110^{bc,B}$
	5	$29.705^{d,C}$	$16.560^{c,B}$	$0.161^{a,B}$
	7	$27.740^{d,C}$	$19.570^{b,B}$	$0.612^{a,A}$
	15	$44.510^{d,B}$	$6.030^{c,C}$	$0.593^{c,A}$

[a-e]Different letters in the same column concerning the same days indicate significantly different values ($P< 0.05$) [comparison of chemical solutions].

[A-G]Different letters in the same column concerning the same chemical solutions indicate significantly different values ($P< 0.05$) [comparison of days].

Table 3. Effects of citric acid and Na-metabisulphite on color parameters (*L, h) minimally processed *Haciömer* cv. Chestnutduring the storage at 4°C**

Solutions	Days	*L**	*h*
	0	$56.635^{a,AB}$	$76.394^{a,AB}$
	3	$62.090^{a,A}$	$72.401^{a,AB}$
Citric Acid (1mM)	5	$57.085^{a,AB}$	$75.207^{a,AB}$
	7	$55.020^{a,B}$	$75.142^{a,AB}$
	15	$58.950^{a,AB}$	$71.061^{a,B}$
	0	$57.860^{a,AB}$	$78.619^{a,A}$
	3	$58.495^{b,AB}$	$72.976^{a,AB}$
Citric Acid (2mM)	5	$58.075^{a,AB}$	$72.704^{a,AB}$
	7	$56.725^{a,AB}$	$75.144^{a,AB}$
	15	$58.580^{a,AB}$	$74.628^{a,AB}$
	0	$56.440^{a,A}$	$78.486^{a,AB}$
	3	$60.285^{ab,A}$	$72.417^{a,BCD}$
Na-Metabisulfite (1mM)	5	$60.070^{a,A}$	$74.945^{a,BCD}$
	7	$57.810^{a,A}$	$70.119^{a,CD}$
	15	$59.790^{a,A}$	$73.733^{a,BCD}$
	0	$58.690^{a,A}$	$81.687^{a,A}$
	3	$58.715^{b,A}$	$72.727^{a,BCD}$
Na-Metabisulfite (2mM)	5	$57.005^{a,A}$	$69.115^{a,D}$
	7	$58.110^{a,A}$	$75.776^{a,ABC}$
	15	$58.895^{a,A}$	$73.733^{a,BCD}$
	0	$57.020^{a,A}$	$80.021^{a,A}$
	3	$54.620^{c,A}$	$73.825^{a,B}$
Control	5	$54.265^{a,A}$	$74.397^{a,AB}$
	7	$58.580^{a,A}$	$72.719^{a,B}$
	15	$58.490^{a,A}$	$73.580^{a,B}$

$^{a-c}$Different letters in the same column concerning the same days indicate significantly different values ($P< 0.05$) [comparison of chemical solutions].

$^{A-D}$Different letters in the same column concerning the same chemical solutions indicate significantly different values ($P< 0.05$) [comparison of days].