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The Effects of Hot and Cold Water Treatment on Quality Parameters and Enzymatic Activity in Chestnut

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ABSTRACT

This study was carried out in order to limit the biochemical and enzymatic changes occurred after harvested in a variety of (*Castanea sativa* Mill. cv. 'Sarıaşlama') chestnuts to prolong the stored period by reduce the loss of quality. Before samples were stored, hot (46±2 °C, 45 minutes) and cold water (15±2 °C, 8 days) treatments were performed. After the hot and cold water treatments the fruits were stored in normal (NA) and controlled atmosphere (CA). In CA storage the fruits were kept in three different atmosphere combinations: (10% CO_2, 2% O_2; 15% CO_2, 2% O_2; 20% CO_2, 2% O_2). In both storage methods, chestnuts were stored for 5 months in 0±1 °C temperature and 90±5% relative humidity conditions. During the storage, parameters such as weight loss (%), content of relative water (%), polyphenol oxidase (PPO) (units mg^{-1} protein), soluble solids (°brix), starch (mg mL^{-1}), total sugar (mg mL^{-1}), vitamin C (mg 100 mL^{-1}), macro (potassium, phosphorus, calcium, sodium) and micro (iron, magnesium) elements (mg 100 g^{-1}) were examined in the fruit samples taken. Maximum weight loss was examined in the fruit kept in NA whereas total sugar and starch showed less of a change in CA in this present study. PPO enzymatic activity can successfully control with hot water treatment in 15% CO_2, 2% O_2 gas combination. When soluble solids was examined, a smaller degree of change was observed in the fruit stored in CA. Promising results were achieved with the fruit that was stored using hot water treatment and 15% CO_2, 2% O_2 combination in this study, which was conducted on the 'Sarıaşlama'chestnut.

Keywords: Chestnut; Cold water treatment; Controlled atmosphere; Hot water treatment; Polyphenol oxidase; Quality

1. Introduction

Chestnut (*Castanea sativa* Mill.) is a kind of fruit that is grown in the coastal regions of Turkey. It has been a part of the culture since ancient times and its cultivation and breeding is at an advanced level, especially in different parts of the Marmara Region. 59,789 tonnes of chestnuts are grown on average and 15,417 tonnes are exported (Soylu 2004b; FAO 2012).

Although chestnuts are achene fruit, unlike walnuts, hazelnuts etc., it is a fruit that is rich in terms of carbohydrates, especially starch, but poor in terms of oil (1.5-2.0%), and protein (2.5-3.0%) (Dassler & Heitmann 1991; Holland et al 1992; Ozturk et al 2010). In the fruit, reducing sugars like glucose and fructose are scarce, whereas sucrose is more plentiful (Holland et al 1992). The level of

water is high in chestnuts (45-50%) while it is low in hard-shelled oily fruits (5-10%) (Jaynes 1979; Holland et al 1992; Ozturk et al 2010). Chestnuts contain high levels of potassium and magnesium, as well as vitamin B, C, E and folic acid (Ozturk et al 2010). The pericarp in chestnuts wraps the seed in a ligninased, dry, hardened and browned layers of different tones. The pericarp is dry, crispy and grain-rich in structure. The embryo forms the part that is consumed along with the bilamellate, wavy-indented carpel (Dassler & Heitmann 1991).

Fresh, bright, vibrant, plump chestnut fruit is edible (Ryall & Pentzer 1982). Fruit stored by drying is used in the candy industry. For this reason, chestnuts are stored fresh or dried. However, studies conducted about chestnut storage are not at an adequate level.

Dried chestnuts are stored easier and last longer. While chestnuts with water levels reduced to 10% at 4.5 °C can be stored for only one year, fruit with a 50% water level at 4.5 °C can only be stored for 8 weeks (Westwood 1978; Cetin & Akbudak 2012). The aim of fresh storage is to prevent water loss and molding. There is an embryo in the fruit ready to grow under appropriate conditions. It is essential that it remains fresh during the storage period but that its growth remains suppressed. Dead embryos cause bad smell in the fruit (Ryall & Pentzer 1982). The growth of the embryo, especially offshoot end's coming out of the fruit damages the quality of the fruit (TSE 1982).

Chestnuts are considered a fresh fruit as they contain 45-50% water. For this reason, it should have a storage temperature of 0 °C, or even -1 °C and 85-90% humidity level. In order to decrease water loss, polyethylene (PE) bags and tin-cans with holes are used (Woodroof 1967; Troyan et al 1975; Ryall & Pentzer 1982; Cetin & Akbudak 2012). For this reason, antitranspirant materials were tried in the Bursa region chestnuts (Ayfer et al 1989). Fresh chestnuts can be kept for 4-5 months under appropriate conditions (Westwood 1978; Ryall & Pentzer 1982; Ayfer et al 1989; Bilginer & Serdar 1997; Karacalı 2004). In order to prevent molding, various fungicides were experimented with the chestnuts. Succesful results were seen with tiabendazol (Ayfer et al 1989).

This study intends to determine the preservability, hot and cold water treatments, storage duration, and the quality loss under different storage conditions of the 'Sarıaşlama' variety of chestnut from the Bursa region.

2. Material and Methods

2.1. Material

This research was conducted at Uludag University, Faculty of Agriculture, Department of Horticulture, Cold Storage Research and Treatment Unit and Postharvest Physiology Laboratory. 'Sarıaşlama' chestnut, harvested in September 2013 from a private single-estate field in the Cumalıkızık Village of Bursa. 'Sarıaşlama', is an Anatolian based fruit supplied by a selection process. Its tree grows broadly at a moderate level. It is high yielding and its fruit is medium sized, wide and oval shaped. The fruit shell is medium-thick, typical chestnut colour and bright. The flesh of the fruit is coloured and very good quality. This early and edible type can also be used in candied chestnut production (Soylu 2004a).

2.2. Methods

Chestnuts harvested at commercial maturity abouth 50 kg were brought to Uludag University, Faculty of Agriculture, Department of Horticulture, Cold Storage Research and Treatment Unit a couple of hours after classification and the treatments below were carried out within the scope of experiments.

Normal atmosphere (NA) storage: Chestnuts were stored in both sides perforated plastic box, at 0±1 °C temperature and 90±5% relative humidity in NA with only adjustments made for temperature and humidity.

Hot water treatment+NA: The fruits were stored after waiting 45 minutes in 3 units of 45-48 °C hot water per 1 unit of chestnut.

Cold water treatment+NA: The fruits were stored after waiting 8 days in 3 units of cold water

at 15 °C per 1 unit of chestnuts which was changed every two days (Jermini et al 2006).

Controlled atmosphere (CA) Storage: In this process, the fruits were stored in both sides perforated plastic box, at 0±1 °C temperature and 90±5% humidity level, at 10% CO_2, 2% O_2; 15% CO_2, 2% O_2 and 20% CO_2, 2% O_2 atmosphere combinations (Jermini et al 2006; Wang et al 2008).

Hot water treatment+CA: Fruits were stored at 10% CO_2, 2% O_2; 15% CO_2, 2% O_2 and 20% CO_2, 2% O_2 CA combinations after waiting 45 minutes in 3 units of water at 45-48 °C hot water per 1 unit of chestnuts (Jermini et al 2006).

Cold water treatment+CA: Fruits were stored at 10% CO_2, 2% O_2; 15% CO_2, 2% O_2 and 20% CO_2, 2% O_2 CA combinations after waiting 8 days in 3 units of water at 15 °C per 1 unit of chestnuts that was changed every two days (Jermini et al 2006).

In the study, the fruit samples that were taken during the storage process were examined according to the quality parameters below.

Determination weight loss: Weight loss was calculated in percents (%) by weighing the wet weight of each month's samples' with a high-precision scale (precision of 0.01 g, Radwag PS 3600/C/1, Radom, Poland).

Determination content of relative water: The content of relative water was calculated in percents (%) each month after samples were measured by a with a high-precision scale (precision of 0.01 g, kept in a vacuum furnace at 80 °C (Binder, ED 53 E2, Germany) for 24 hours and measured again.

Determination Polyphenol Oxidase (PPO): PPO activity was assayed according to the method of Jiang (1999), by measuring the oxidation of 4-methylcatechol. The increase in absorbance at 410 nm was automatically recorded for 3 min, using a spectrophotometer (Thermo Spectronic, Nicolet evolution 100, England). The enzym activity was expressed as units per mg protein (units mg^{-1} protein).

Total soluble protein was determined according to Bradford (1976) using bovine serum albimin as the standart.

Determination soluble solids: Fruits grinding with the help of a blender (Moulinex, DJ750, France) and ground using a mortar and a pestle (HM Mellert, M8 S, Germany) were homogenized by adding pure water until took on the consistency of mud. The soluble solids content of the solution obtained was calculated in percents (°brix) with the help of a refractometer (0-32 °brix) (Atago, R 500, Atago Co., Ltd, Tokyo, Japan).

Determination starch: The analysis of the samples was calculated (mg mL^{-1}) at the Marmara Research Centre of the Scientific and Technological Research Council of Turkey via the polarimetric kit method (TSE 2000; EC 2009).

Determination total sugar: The total amount of sugar in the fruit was calculated **(**mg mL^{-1}**)**, after diluted samples went through various processes and measured with a spectrophotometer (Thermo Spectronic, Nicolet evolution 100, England) at a wavelength of 600 nm.

Determination vitamin C: 350 mL of a 0.04% oxalic acid solution was poured into 50 g of fruit puree and it was stirred for 2 minutes and then filtered with vatman paper. 1 mL was taken from the filtered puree and 9 mL of 2,6 Dichlorophenol Indophenole dye solution was addedand it was read at 520 nm absorbance (Hısıl & Otles 1989). Vitamin C in chestnut samples were calculated as mg 100 mL^{-1}.

Determination macro (potassium, phosphorus, calcium, sodium) and micro (iron, magnesium) elements: The macro and micro element determination of the samples was conducted at Yalova Atatürk Horticultural Central Research Institute by the wet decomposition method was calculated with sulfuric acid and hydrogen peroxide (Lott et al 1956; Cottenie 1980; Kacar & Inal 2008). Macro and micro elements in chestnut samples were calculated as mg 100 g^{-1}.

2.3. Statistical analysis

The study was designed to be replicated 3 times with 150 g of fruit for each one according to random blocks test pattern and the results obtained were evaluated using Minitab-14 (2004). The evaluation of the differences between the results was determined with LSD test ($P<0.05$).

3. Results and Discussion

In this study, depending on the storage period of the chestnuts, it was observed that there were some weight losses. While these losses were between 40.21 and 39.45% at NA conditions, highest loss was 7.90% at 10% CO_2, 2% O_2 cold water treatment and the lowest loss was 3.67 and 4.00% at 15% CO_2, 2% O_2 control and 20% CO_2, 2% O_2 hot water treatment (Table 1). In addition, it was also found that there were some significant differences in terms of weight losses among the treatments. According to our results, CA had positive effects on the storage time of the fruits. The effects of atmospheric composition on the physical and chemical structure of the fruit were investigated in this study. As a result of our experiments, it was found that the practical way to reduce water loss without changing the physical and chemical structure of the fruit is to store the fruit in CA. In Ryall & Pentzer (1982), the weight loss was 6-7% between treatments and the weight loss during the storage period was 15-18%. However our studies were similar to Cecchinia et al (2011) and Cetin & Akbudak (2014) studies and the loss rate between treatments in CA were 3.67-7.90%, and the weight loss during the storage period remained within a range of 3.67-40.21%.

While more biochemical changes occurred under NA conditions, there were fewer changes in CA. Under NA slowing the metabolic activity is acheved by reducing the ambient temperature, and under CA, in addition to a decrease in temperature, metabolic activities were slowed down even further by altering the gas compositions of the environment. Metabolic activities increased water consumption. For example, breaking off one mole of glucose from a starch chain is carried out with the consumption of 1 mole of water. There are many other metabolic activities that cause water loss (Table 1). While the water percentage was initially 47.21%, at the end of the storage it varied between 15.17% and 23.28% under NA, between 34.88% and 40.21% in CA in our study. In Tzortzakis & Metzidakis (2012) study the rate of water loss rate in chestnut fruits was 3.57% after 90 day storage period under CA conditions, whereas in our study it was 7.00% at the end of 150 days under CA conditions. This shows that there is a similarity between the relative water loss results of the two studies.

Figure 1 shows changes in PPO activity of chestnut treated with hot and cold water in NA and CA atmosphere conditions in low-temperature for 150 days. PPO activity decreased from 1167.70 units mg^{-1} protein to 507.36 units mg^{-1} protein during storage at NA conditions at 0±1 °C (Figure 1). In contrast, it changed more prominently at CA, by decreasing to 124.28 units mg^{-1} protein in hot water treated gas combination at 15% CO_2, 2% O_2 (Figure 1) this suggests that CA conditions can control enzymatic browning successfully. Xu (2005) were stored fresh chestnuts at 4 °C in a refrigerator, fully covered with pre-sterilized wet sand (sieved through diameter 2 mm, 40% humidity) in boxes. The changes of the PPO specific activity during 6 months low-temperature storage decreased dramatically 1180 units mg^{-1} protein to 340 units mg^{-1} protein for 4 °C at the end of storage. Jiang et al (2004) reported similar effect of low-temperature storage on the chestnut. This might also indicate heavy loss of PPO activity during the separation process for chestnuts stored in NA much more than CA for 150 days at low temperature.

The soluble solids level change in the fruit stored under CA and NA increased during the storage period (Table 1). This increase can be explained by the conversion of some starch to glucose depending on the intrinsic biochemical changes in the fruit. The soluble solids of the fruit stored at various atmospheric compositions under CA did not show significant changes during the storage periods. The soluble solids changes under NA and CA were found to be significant. While the soluble solids

Table 1- Changes in weight loss, content of relative water, the soluble solids, total sugar and vitamin C during the storage of the 'Sarıaşlama' type of chestnut under NA and CA

Storage time (day)	*Treatment 1*	*Treatment 2*	*Weigt loss (%)*	*Content of relative water (%)*	*Soluble solids (°brix)*	*Total sugar (mg mL^{-1})*	*Vitamin C (mg 100 mL^{-1})*
0	NA[a]	Control	0.00	47.21	5.50	8.10	10.60
		Hot water	0.00	47.21	5.50	7.62	10.91
		Cold water	0.00	47.21	5.50	5.70	11.63
	LSD		-	-	-	-	-
30	NA	Control	9.81 a[b]	31.36 b	5.13 b	8.89 a	27.74 a
		Hot water	11.68 a	34.16 b	5.40 a	8.18 a	25.61 a
		Cold water	12.64 a	36.13 b	5.40 a	8.42 a	27.52 a
	10:2	Control	4.36 b	45.50 a	5.37 a	7.39 b	11.94 b
		Hot water	3.88 b	46.52 a	5.47 a	7.18 b	12.38 b
		Cold water	3.03 b	46.01 a	5.47 a	7.62 b	11.84 b
	15:2	Control	1.52 b	45.43 a	5.37 a	7.52 b	11.69 b
		Hot water	1.22 b	46.00 a	5.47 a	7.48 b	11.31 b
		Cold water	1.25 b	46.37 a	5.47 a	7.45 b	11.91 b
	20:2	Control	1.70 b	45.30 a	5.33 a	7.10 b	11.56 b
		Hot water	0.74 b	46.41 a	5.47 a	7.13 b	12.54 b
		Cold water	0.47 b	46.09 a	5.40 a	7.77 ab	10.76 b
	LSD		5.34	4.38	0.16	0.63	2.39
60	NA	Control	27.61 a	28.76 b	5.63 b	9.20 a	16.84 a
		Hot water	27.84 a	32.17 b	6.07 a	9.22 a	15.83 a
		Cold water	32.80 a	34.29 b	6.00 a	9.48 a	15.68 a
	10:2	Control	5.31 b	43.79 a	6.00 a	8.89 ab	9.35 b
		Hot water	4.99 b	44.10 a	6.10 a	8.28 b	8.89 b
		Cold water	4.58 b	45.24 a	6.07 a	8.23 b	8.51 b
	15:2	Control	3.73 b	43.74 a	6.00 a	7.90 bc	5.25 c
		Hot water	2.17 b	46.01 a	6.13 a	7.32 c	4.44 c
		Cold water	2.44 b	46.01 a	6.10 a	7.22 c	4.66 c
	20:2	Control	2.99 b	43.96 a	6.03 a	8.76 ab	8.60 b
		Hot water	2.03 b	45.36 a	6.13 a	8.34 b	7.97 b
		Cold water	4.08 b	45.32 a	6.13 a	8.63 ab	7.54 b
	LSD		8.45	5.27	0.18	0.95	1.25
90	NA	Control	38.37 a	25.02 d	6.17 b	10.93 a	1.55 b
		Hot water	38.04 a	27.98 d	6.50 a	10.84 a	1.68 b
		Cold water	38.98 a	32.87 c	6.67 a	10.45 a	1.80 b
	10:2	Control	5.59 b	39.83 a	6.03 b	9.87 b	4.67 a
		Hot water	6.72 b	42.10 a	6.10 b	9.52 b	3.47 a
		Cold water	5.61 b	42.17 a	6.07 b	9.58 b	3.73 a
	15:2	Control	4.32 b	41.45 a	6.03 b	9.66 b	0.78 c
		Hot water	2.61 b	43.81 a	6.10 b	8.53 c	1.48 b
		Cold water	4.65 b	42.76 a	6.07 b	8.70 c	1.24 bc
	20:2	Control	3.32 b	40.79 a	6.03 b	9.72 b	0.36 c
		Hot water	3.14 b	42.55 a	6.10 b	8.67 c	0.77 c
		Cold water	5.77 b	42.91 a	6.03 b	8.83 c	1.95 b
	LSD		6.83	4.16	0.25	0.51	1.28

Table 1 (Continue)- Changes in weight loss, content of relative water, the soluble solids, total sugar and vitamin C during the storage of the 'Sarıaşlama' type of chestnut under NA and CA

Storage time (day)	*Treatment 1*	*Treatment 2*	*Weight loss (%)*	*Content of relative water (%)*	*Soluble solids (°brix)*	*Total sugar (mg mL^{-1})*	*Vitamin C (mg 100 mL^{-1})*
120	NA	Control	39.27 a	20.20 d	8.10 a	11.83 a	1.27 a
		Hot water	39.07 a	24.30 d	7.17 b	11.12 a	1.04 a
		Cold water	39.65 a	26.00 d	7.33 b	11.48 a	1.38 a
	10:2	Control	5.94 b	37.90 a	6.83 c	10.65 b	4.78 b
		Hot water	7.52 b	39.69 a	7.00 c	10.33 b	5.89 b
		Cold water	7.46 b	39.82 a	6.93 c	10.47 b	4.70 b
	15:2	Control	5.00 b	39.20 a	6.90 c	10.14 b	4.21 b
		Hot water	3.06 b	41.57 a	6.50 d	9.17 c	3.84 b
		Cold water	6.72 b	41.76 a	6.90 c	9.56 c	4.78 b
	20:2	Control	4.45 b	38.90 a	6.93 c	10.59 b	4.56 b
		Hot water	3.80 b	41.65 a	7.00 c	10.26 b	3.84 b
		Cold water	6.58 b	40.73 a	6.97 c	10.33 b	3.77 b
	LSD		8.78	4.69	0.18	0.83	1.87
150	NA	Control	39.93 a	15.17 e	8.43 a	12.66 a	5.59 ab
		Hot water	39.45 a	19.88 d	8.53 a	12.37 a	7.51 a
		Cold water	40.21 a	23.28 d	8.37 a	12.48 a	7.58 a
	10:2	Control	6.34 b	34.88 b	7.90 b	11.83 ab	6.73 a
		Hot water	7.85 b	36.92 a	7.63 b	11.36 b	6.29 a
		Cold water	7.90 b	37.24 a	7.83 b	11.42 b	7.32 a
	15:2	Control	5.97 b	37.60 a	7.73 b	11.05 b	4.49 b
		Hot water	3.67 b	40.17 a	7.23 c	10.23 c	2.59 d
		Cold water	7.44 b	40.21 a	7.35 c	10.25 c	3.60 c
	20:2	Control	4.82 b	36.42 a	7.87 b	11.70 ab	4.66 b
		Hot water	4.00 b	38.53 a	7.71 b	11.00 b	3.47 c
		Cold water	6.95 b	38.14 a	7.69 b	11.33 b	3.65 c
	LSD		8.89	3.80	0.26	0.45	1.35

[a]NA, normal atmosphere; [b]There is a 5% difference between the averages represented by different letters in the same column

rates increased from 5.50 °brix to 8.63 °brix at the end of 5 months of storage under NA, it increased from 5.50 °brix to 7.23 °brix under CA. Kim et al (2006) found the rate of increase in the soluble solids to be 8.00 °brix in the study they conducted on the different varieties of chestnuts grown in Korea during a 16 week storage period. The fact that the storage time was longer and the rate of increase of the brix is low indicates the importance of the findings of this study.

Changes in the proportion of starch in the study are shown in Figure 2. Our results showed that the difference between the treatments is significant. The changes in the starch proportion in 15% CO_2, 2% O_2 and 20% CO_2, 2% O_2 treatments were lower than other treatments. The main reason for the decrease in the starch levels can be the breakdown of starch into sugar through enzymatic activities. Sugar molecules enter the Kreps cycle and get into a reaction with the oxygen in the environment and turn into carbondioxide. Figure 2 shows that the percentages of starch in 15% CO_2, 2% O_2 and 20% CO_2, 2% O_2 treatments were the highest. This is because the CO_2 levels were high in these treatments. The respiration

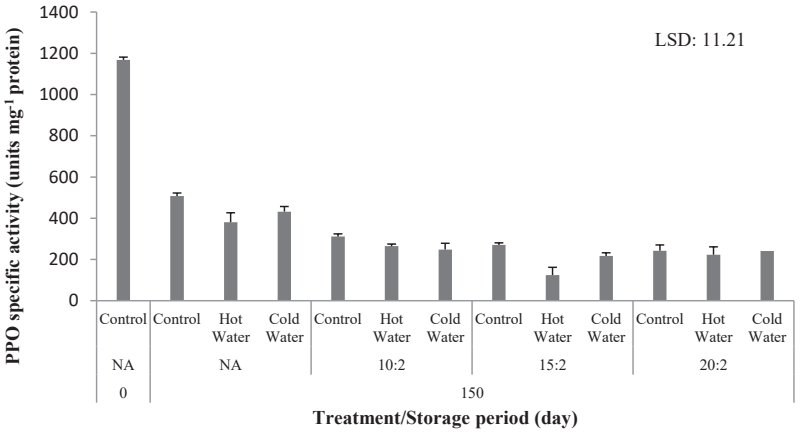

Figure 1- The PPO specific activity (units mg^{-1} protein) changes in the 'Sarıaşlama' type of chestnut at the beginning, during and at the end of the storage periods under NA and CA

rate in the hot water treatment was lower than other treatments. Tzortzakis & Metzidakis (2012) reported a 25% decrease in the rate of starch in the research, conducted on the storage conditions of chestnuts under CA conditions. In our study, decrease in the starch under CA conditions was 17.9% at the end of the 150 day storage period. This show that starch substitution in our study was relatively low.

The total sugar amount in the chestnuts is one of the important quality parameters. Changes in the total amount of sugar in the fruits were significantly effected by the storage process and gas mixtures (Table 1). Chestnut cultivar, 'Sarıaşlama' showed a linear increase in total sugar during the storage period. Under CA, 15% CO_2, 2% O_2 and 20% CO_2, 2% O_2 treatments were identified as better ones. These results were similar to those of Bounous et al (2000).

Vitamin C content had significant differences under NA and CA conditions. Under NA control group, the Vitamin C content rose from 10.60 mg 100 mL^{-1} to 27.74 mg 100 mL^{-1} in the first month,

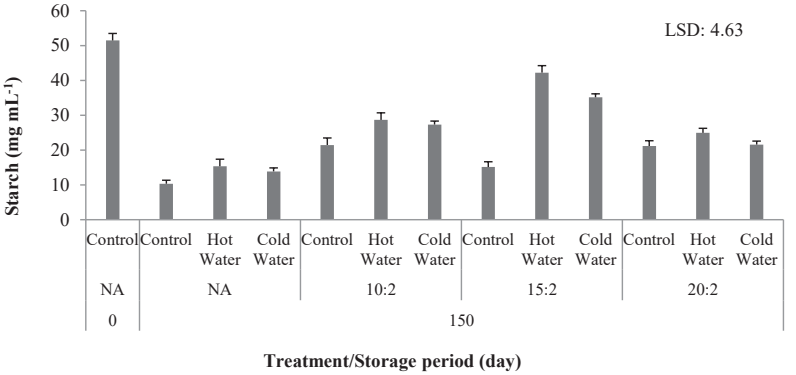

Figure 2- Starch percentages of the fruit at the beginning and at the end of the storage periods of the 'Sarıaşlama' type of chestnut under NA and CA

in the second month, began to fall and at the end of the fourth month fell to 1.27 mg 100 mL^{-1}. Then, it rose again to 5.59 mg 100 mL^{-1} at the end of the fifth month. Under CA, while the Vitamin C content consistently fell from 10.60 mg 100 mL^{-1} to 0.36 mg 100 mL^{-1} but there was an increase in the fourth month. Our results were consistent with the results of Kalt et al (1999) on juicy fruits (Table 1).

Macro and micro element contents at the beginning and the end of the storage period are shown in Figure 3. There were significant differences between the treatments. The amount of potassium increased from 760.12 mg 100 g^{-1} to 810.56 mg 100 g^{-1}, phosphate ions from 138.40 to 146.79 mg 100 g^{-1}, and calcium from 59.63 mg 100 g^{-1} to 60.00 mg 100 g^{-1}in hot water 15% CO_2, 2% O_2 gas combinations. In a similar study conducted by Ho Jin (2012), there was an increase in the amount of potassium, from 263.00 mg 100 g^{-1} to 420.60 mg 100 g^{-1}, and in phosphate ions, from 45.80 mg 100 g^{-1} to 69.60 mg 100 g^{-1}.

As a result, best results were obtained from this study conducted on the 'Sarıaşlama' type of chestnut using hot water treatment and 15% CO_2, 2% O_2 gas combinations in terms of the duration of storage of the stored chestnuts and fruit quality.

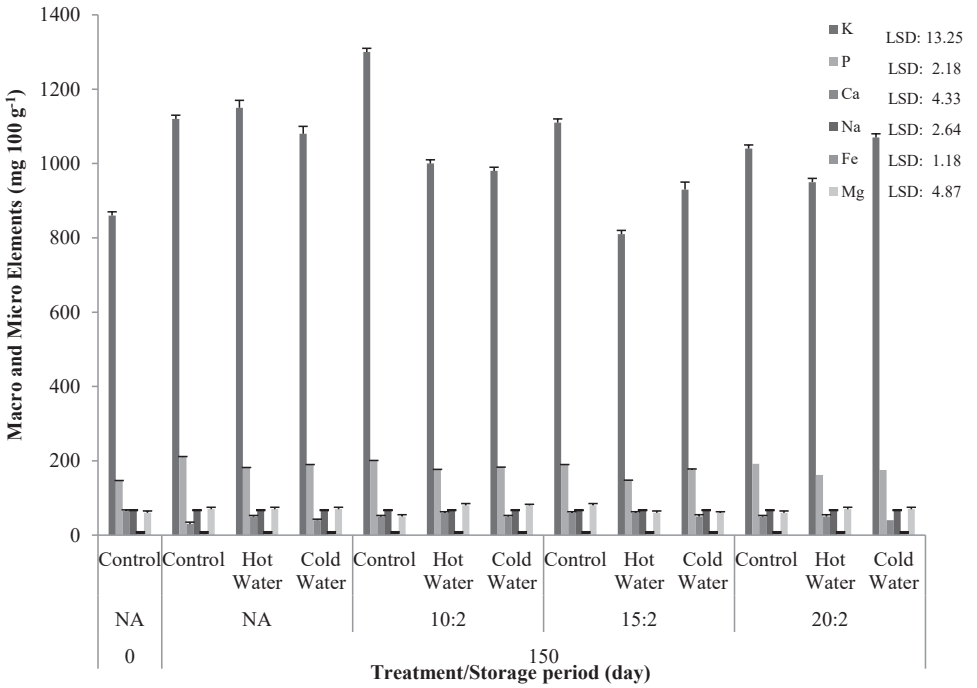

Figure 3- The macro and micro elements in the 'Sarıaşlama' type of chestnut at the beginning, during and at the end of the storage periods under NA and CA

4. Conclusions

The results show that CA conditions at gives on some quality parameters better results than NA conditions in storage of 'Sarıaşlama' chestnuts. The hot water treatment in 15% CO_2, 2% O_2 gas combination at 0±1 °C was effective for the inhibition of PPO activities. In conclusion, hot water treatment with 15% CO_2, 2% O_2 gas combination at 0±1 °C storage temperature could be suggested for reducing surface browning and biochemical changes in chestnut for 150 days.

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