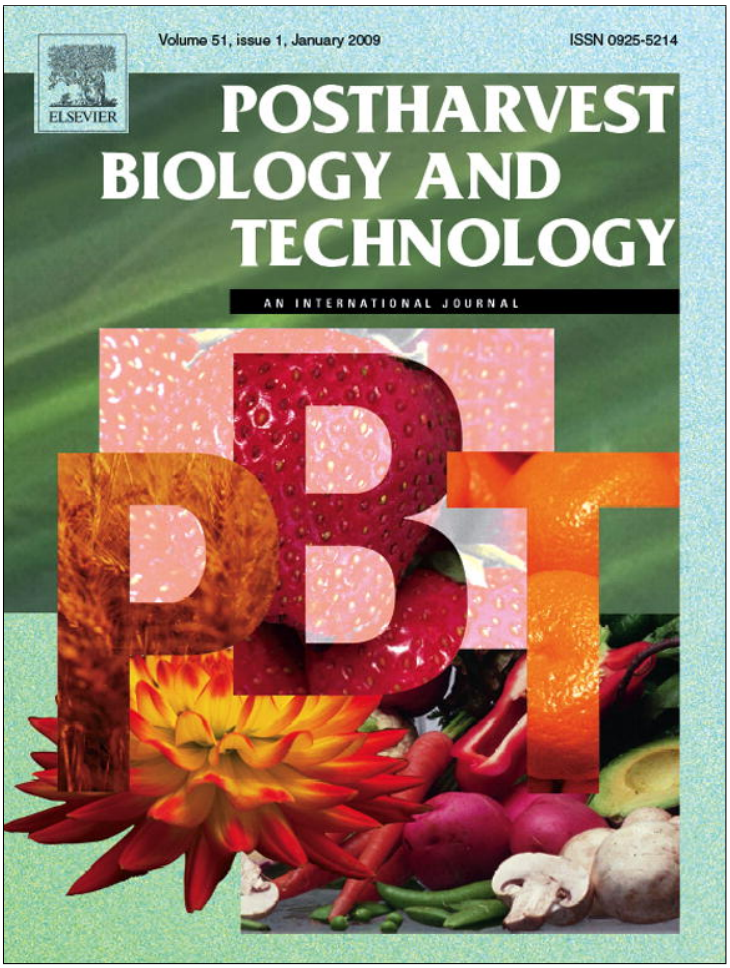

Postharvest Biology and Technology 51 (2009) 97–103

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Technological parameters of water curing affect postharvest physiology and storage of marrons (*Castanea sativa* Mill., Marrone fiorentino)

Rinaldo Botondi, Matteo Vailati, Andrea Bellincontro, Riccardo Massantini, Roberto Forniti, Fabio Mencarelli*

Department of Food Science and Technology, Postharvest Laboratory, University of Viterbo, Viterbo, Italy

ARTICLE INFO

Article history:
Received 9 January 2008
Accepted 14 June 2008

Keywords:
Marrons
Water curing
Ethanol
Acetaldehyde
Polyphenols

ABSTRACT

Little is known about the biochemistry of the marron (*Castanea sativa* cv Marrone fiorentino) when it is soaked in water for the curing process; the immersion treatment time is still empirical and based on traditional knowledge. A study was carried out by keeping marrons in water for 7 d at 14 °C. Tap water and acid water (pH 3) were used in the following water/marron ratios 1:1, 1:2, and 3:2. After curing, the marrons were moved to air storage at room temperature (20 °C and 90% RH) for 1 week (shelf-life), and at 0 °C and 90% RH for 60 d. A large accumulation of carbon dioxide in the head space of the glass jar where the marrons were kept, was observed during water soaking, above all in samples in acid water with a water/marron ratio 1:2. In the first 5 d of water curing, a significant increase in acetaldehyde concentration and phenol content was measured in the marron pulp and subsequently, both compounds declined. Ethanol decreased slightly or remained constant. During the following week of shelf-life, the lowest respiration rate was measured in 1:1 and 1:2 samples while the highest rate was measured in acid water samples. SSC (solid soluble contents) increased in marrons soaked in acid water but remained constant under shelf-life conditions. SSC, ethanol, acetaldehyde and polyphenols did not change following storage at 0 °C for 2 months, but acid water samples showed a higher percentage of decayed marrons. Finally, it is possible to define some technological parameters. The immersion time of marrons in water must be at least 5 and no longer than 7 d, in order to avoid cell destruction. A water/marron ratio of 1:1 is advised. Using acid water, the samples with the ratios of 1:2 and 1:1 showed higher polyphenol and sugar contents. However, the metabolic response of these marrons was assumed to be a "stress response" to acid water.

1. Introduction

The chestnut tree is mainly grown in Europe and Asia. The Asiatic production of chestnuts covers 70% of world production. China is the leader with 445,000 ha, followed by South Korea with 430,000 ha, Turkey with 392,000 ha, Japan with 283,000 ha, and Italy with 275,000 ha. Chestnuts are rich in sugars, mainly monosaccharides and disaccharides such as sucrose, glucose, fructose, and raffinose, as well as starch (Bernardez et al., 2004; De la Montaña et al., 2004). The main storage problems with chestnuts are the presence of insect worms (*Cydia splendana* Hb, *Cydia fagliglandana* Zel. and *Curculio elephas* Gyll), and fungi development, mainly *Cyboria*, which blackens the flesh, but also *Rhizopus*, *Fusarium*, *Collectotrichum*, and *Phomopsis* (Wells and Payne, 1980; Breisch, 1993; Washington et al., 1997). In Italy, the most widespread chestnut treatment implemented before storage or sale is water curing, known in the past as "novena", meaning that the chestnuts were soaked in water for 9 d. The aim of this practice is to reduce fungal development during storage and to aid the killing of worms in chestnuts (Mencarelli, 2004). Polyphenol compounds such as cumarin, scopoletin and esculetin have been considered for the effectiveness of this technique (Bergognoux, 1978). However, no other articles have been published on chestnut metabolism during water curing. Today, fresh chestnuts are often kept in cold storage but problems related to pathogen development still exist (Tian and Bartolini, 1997; Bassi et al., 2001). Controlled atmosphere storage was proposed with a high carbon dioxide concentration (Mignani and Vercesi, 2003) also to avoid flesh hardening which occurs during cold storage, and which is very detrimental to marron glacè production (Estévez et al., 2005). A very high concentration of carbon dioxide has been proposed as an alternative to water curing, but water curing gave better results in controlling fungal decay, if the high concentration of carbon dioxide treatment was not followed by controlled atmosphere storage (Anelli et al.,

* Corresponding author. Tel.: +39 0761 357494; fax: +39 0761 357498.
E-mail address: mencarel@unitus.it (F. Mencarelli).

0925-5214/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2008.06.010

1984). As an alternative to water curing, hot water treatments are commercially applied, and both methods have proved efficient in reducing pathogen development and in maintaining chestnut firmness (Jermini et al., 2006). The aim of this paper was to determine the influence of water curing on the metabolism of fresh marrons during curing, shelf-life, and 2 months of storage at 0 °C, in order to define some technical parameters such as curing time, water/marron ratio, and water pH.

2. Materials and methods

2.1. Experimental procedures

Marrons (*Castanea sativa* cv Marrone fiorentino) harvested in the area surrounding Viterbo, on Giovannelli's organic farm, 800 m altitude, were selected following removal of the burr, for uniform size and external quality. Overall water selection of decayed marrons was carried out by soaking marrons into tap water and by removing the floaters. Subsequently, 1 kg of marrons were cut and the percentage of decayed or injured fruit was calculated. Six different tests were carried out as follows: 2.5 L tap water + 5 kg marrons, water/marron ratio = 1:2; 5.0 L tap water + 5 kg marrons, water/marron ratio = 1:1; 7.5 L tap water + 5 kg marrons, water/marron ratio = 3:2; 2.5 L acid water + 5 kg marrons, water/marron ratio = 1:2; 5.0 L acid water + 5 kg marrons, water/marron ratio = 1:1; 7.5 L acid water + 5 kg marrons, water/marron ratio = 3:2.

Acid water at pH 3 was obtained by electrolysis. The water/marron solutions were kept in open glass jars; when the gas concentration in the headspace had to be measured the jars were tightly sealed with a lid adapted for measuring headspace air. Curing was performed with water at 14 ± 1 °C for 7 d in a cold room. After this period, the marrons were removed from the water and dried superficially by ventilation in a temperature controlled room until their initial weight was reached; subsequently they were kept under shelf-life conditions (20 ± 1 °C and 90 ± 5% RH) for 7 d in order to observe the metabolic changes or in storage at 0 ± 1 °C and 90 ± 5% RH for 60 d. The marrons were analysed during the water treatment period and, subsequently, at shelf-life conditions after 1 week and 60 d of low temperature storage. At each sampling time the marrons were frozen at −20 °C. At the end of the storage period, the marrons were cut to observe the internal quality. To better understand the role of metabolites formed during the anaerobic process occurring during water dipping, one treatment with ethanol solution in water at 5, 10 and 20% (v/v), and tap water as control, for 24 h was performed. After this treatment, the marrons were ventilated and left at room temperature as described previously. The marrons were analysed for their internal ethanol, acetaldehyde, and lactic acid content.

2.2. Analyses

During water treatment, carbon dioxide and oxygen accumulation was measured in the headspace of the curing glass jars. The jars were capped tightly for 3 h, twice a day. During air storage, the marrons of each test were placed in three glass jars and tightly sealed for 2 h to measure the accumulation of carbon dioxide in the headspace. Readings were performed using an OXYCARB 5 analyser [Isolcell, Laives (BZ), Italy] with an infrared CO_2 sensor and an electrochemical oxygen sensor.

SSC was measured using a digital refractometer mod. Palette PR-32 (ATAGO Co., Japan); 5 g of marron pulp were added to 10 mL of distilled water and homogenised with an Ultra-Turrax at 5000 rpm for 1 min. The homogenised sample was poured into an Eppendorf tube and centrifuged. Drops of supernatant were used to read the refractometer and the results were multiplied by two as dilution factor.

The total polyphenol content was determined by a slight modification of the procedure of Montedoro et al. (1993). Eight grams of marron pulp were cut into small pieces and added to 15 mL of 80% methanol and homogenised with an Ultra-Turrax at 8000 rpm for 2 min; they were then filtered and left standing for 30 min. Supernatant (0.25 mL) was added to 1 mL of Folin Ciocalteau reagent (Sigma–Aldrich Co., USA). After 3 min, 4 mL of 10% sodium carbonate (Sigma–Aldrich Co., USA) were added. The solution was taken up to 20 mL with distilled water and left standing for 90 min. The reading was performed using a Lambda 25 UV/VIS spectrophotometer (PerkinElmer Ltd., UK) at 700 nm and the concentration was computed on the basis of a gallic acid standard curve.

Ethanol and acetaldehyde contents were measured adapting the procedure used on peaches by Bonghi et al. (1999). Ten grams of marron pulp were homogenised with 15 mL of distilled water in an Ultra-Turrax at 7000 rpm in an ice bath; the solution was then capped and centrifuged at 4 °C, 5000 rpm for 10 min. Five milliliters of supernatant were poured into a glass miniflask (Supelco, Sigma–Aldrich Co., St. Louis, MO, USA), equipped with a small magnetic stirring bar and capped with a PTFE-faced silicone septum; this was placed in a thermostated bath under continuous stirring at 55 °C for 30 min. Lastly, 1 mL of headspace air was removed with an air-tight syringe and injected into the gas chromatograph FRACTOVAP 4200 (Carlo Erba Spa, Italy), adapted with 5% Carbowax 20M in a Carbograph 1AW column (Supelco, Sigma–Aldrich USA). Injection, oven, and FID temperatures were 100 °C, 120 °C and 200 °C respectively.

Lactic acid was measured by using enzymatic test analysis (R-Biopharm, Darmstadt, Germany).

Water pH was determined with a pH-meter model pH 300 (Hanna Instruments S.r.l, Italy).

2.3. Statistical analysis

All of the chemical and physiological analyses were replicated at least three times, each replication coming from different marrons of the same test. ANOVA was performed for each quality parameter and the least significant difference (LSD) was calculated for the appropriate level of interaction for $P \leq 0.05$.

3. Results and discussion

Water curing of marrons produces a lot of carbon dioxide as a result of aerobic and anaerobic metabolism but no data exist regarding the details of this production. In a closed environment where chestnuts are water-cured, the accumulation of CO_2 may represent a risk for operators. Moreover, monitoring CO_2 and O_2 accumulation in the headspace of curing jars could give indications of the metabolism taking place. In our case, the gas percentage read in the headspace of the jars depended on the water/marron ratio. Just after the first day, 1:1 samples, regardless of the type of water, showed an approximately 50% lower CO_2 concentration than the other samples. Subsequently, all of the samples showed a rising CO_2 concentration in the headspace of the jars during the first 3 d; a new increase was then observed on day 5 with a peak on day 6 (Fig. 1). The highest and the most rapid CO_2 accumulation, 1.1 and 1.4 $\mu mol\ g^{-1}$ fresh weight (f.w.) h^{-1}, in tap water (W) and acid water (AW) samples respectively, had already occurred on the third day in samples 1:2, where there was less water. On the last sampling (day 7), the lowest CO_2 accumulation was found in 1:1 and 1:2 tap water samples (0.6 $\mu mol\ g^{-1}$ f.w. h^{-1}). The O_2 concentration trend was opposite that of CO_2 (data not shown).

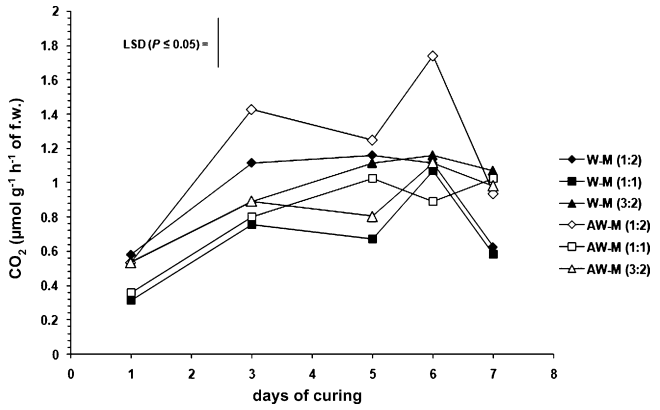

Fig. 1. Concentration (%) of CO_2 measured in the head space during the curing phase in tap water and in acid water. AW = acid water; W = water; M = marron. In parenthesis, the water/marron ratio. Data represent the average of two daily samplings.

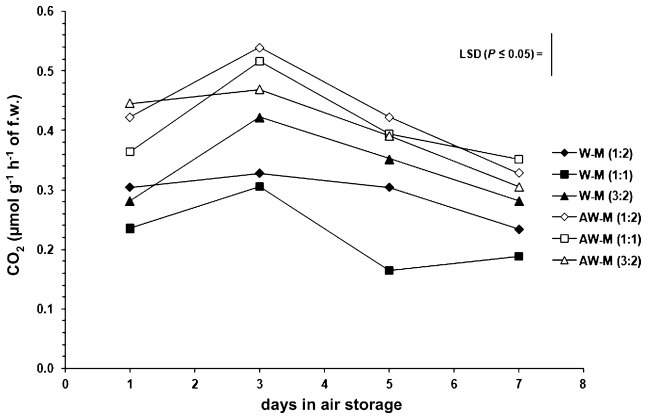

Fig. 2. CO_2 production of marrons during shelf-life (20 °C and 90% RH). AW = acid water; W = water; M = marron. In parenthesis, the water/marron ratio. Data represent the average of three readings from three different jars. Different letters indicate significant differences at $P = 0.05$.

Comparing tap water and acid water samples with the same ratio, we observed that acid water samples accumulated a higher amount of CO_2. This behaviour can be attributed to a stress response of marron cells to acid conditions. Lowering the pH of water may have activated the fermentation by stimulating lactic dehydrogenase, and thus pyruvate decarboxylase. It is known that fermentation acidifies the substrate, and in *Lactobacillus*, low pH is responsible for poor long-term survival under aeration conditions, when compared to the survival of respiring cells (Razaiki et al., 2004). The peak in CO_2 production corresponds with foam formation on the water surface. On the seventh day, the decrease in CO_2 production might indicate that the cells are shifting to a critical survival phase.

During air storage, marrons recovered respiratory activity guaranteeing cell survival. All of the samples showed a peak on day 3, and then a decreasing trend (Fig. 2). Acid water samples had values that were significantly higher than tap water samples. The 1:1 tap water sample showed the lowest rate of CO_2 production (around 0.3 μmol g^{-1} f.w. h^{-1} on day 3), while the highest production was for the samples in acid water (between 0.46 and 0.54 on day 3), confirming what we observed during the curing period relative to CO_2 accumulation. These values are in the respiration rate range found by Anelli et al. (1984) during storage at 0 °C. Taking into account that chestnuts, as seeds, have a dormant phase linked to the availability of stored substances, lower respiratory values are certainly an advantage for chestnuts as they will consume less dry matter (Kays, 1997). Weight loss in samples with a ratio of 1:1 and 1:2 in tap water reached the value of 0.113 g kg^{-1} in 24 h, while that in the sample in acid water at a ratio of 1:1 reached a value of 0.223 g kg^{-1} in 24 h (data not shown). During air storage also, the acid pH of the water affected marron metabolism; indeed, the highest CO_2 production in this phase, and the peak 3 d after the removal of the marrons from the water, lead us to assume a stress response. It is well known that an increase in the hydrogen ion concentration of water causes a strong membrane transfer imbalance thus compromising cell functions (Kang and Saltveit, 2003). Ethanol concentration in marron pulp during curing decreased slightly but significantly only for the water/marron 1:2 sample, especially on the last day; ethanol only increased significantly in the water/marron 3:2 sample, on the second day of curing from 220 up to 242 μmol g^{-1} f.w. (Fig. 3). On the contrary, acetaldehyde immediately increased significantly in all the samples, especially in the tap water samples (Fig. 4). The peak of the rise occurred on day 2 or 3 with the highest concentration in the water/marron 3:2 sample (232 μmol g^{-1} f.w.). At the end of the curing period the concentrations of samples were not significantly different even though samples with a greater water content in curing had a slightly higher acetaldehyde concentration. Comparing ethanol and acetaldehyde

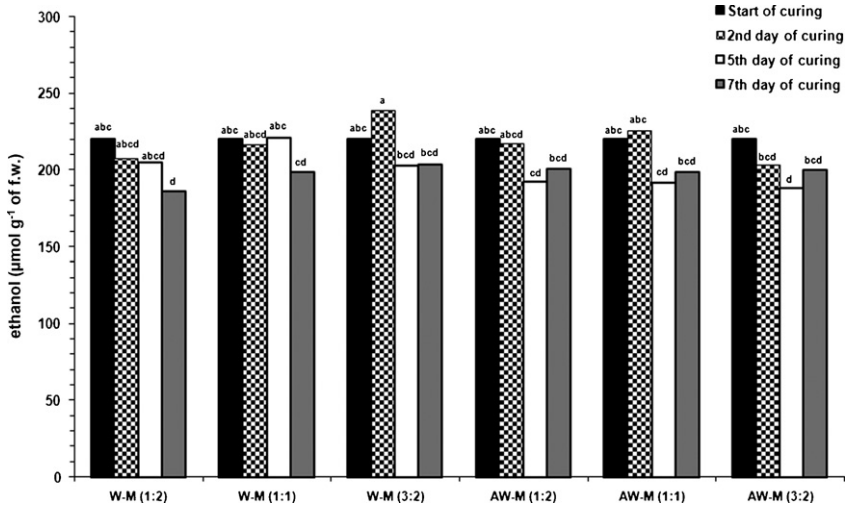

Fig. 3. Ethanol content of marron flesh during the curing phase. AW = acid water; W = water; M = marron. In parenthesis, the water/marron ratio. Data represent the average of three replicated analyses of different marrons. Different letters indicate significant differences at $P = 0.05$.

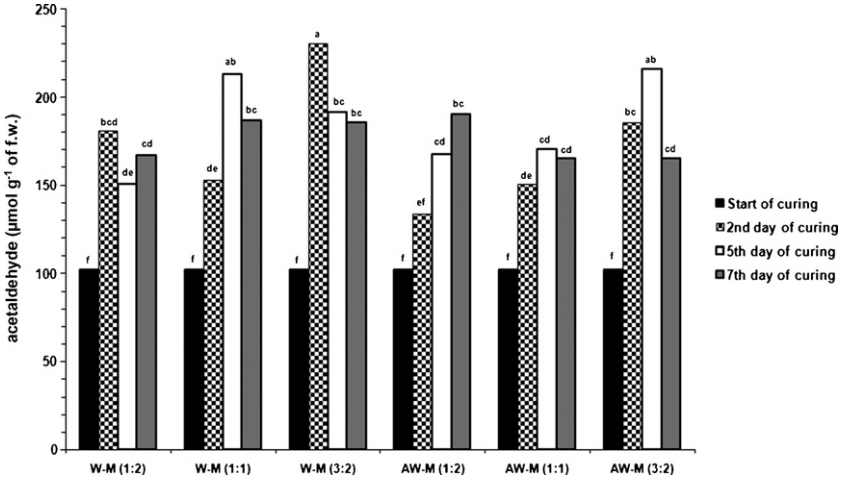

Fig. 4. Acetaldehyde content of marron flesh during the curing phase. AW = acid water; W = water; M = marron. In parenthesis, the water/marron ratio. Data represent the average of three replicated analyses of different marrons. Different letters indicate significant differences at $P = 0.05$.

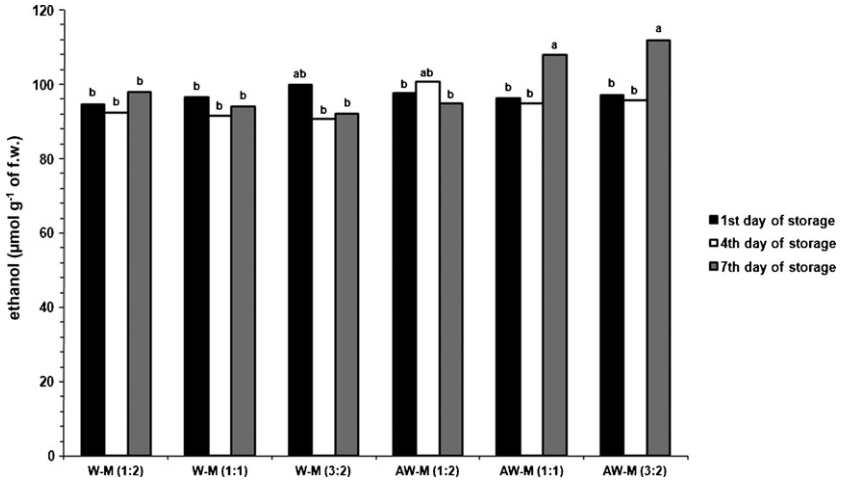

Fig. 5. Ethanol content of marron flesh during shelf-life (20 °C and 90% RH). AW = acid water; W = water; M = marron. In parenthesis, the water/marron ratio. Data represent the average of three replicated analyses of different marrons. Different letters indicate significant differences at $P = 0.05$.

data, a more active fermentative metabolism appears in the first step of acetaldehyde formation from pyruvic acid than in the second one, reduction of acetaldehyde to ethanol. In the anaerobic metabolism, acetaldehyde acts both as a product of the PDC (pyruvate decarboxylase) enzyme activity and as a substrate of the ADH (alcohol dehydrogenase) activity to produce ethanol. It has been seen that in bell peppers kept under O_2 depletion conditions, there is a considerable accumulation of pyruvic acid which, through the action of the PDC, causes an increase in acetaldehyde, and, at the same time, low ADH enzyme activity and low ethanol production (Imahori et al., 2002). Burdon et al. (2007) emphasised the role played by the treatment of CO_2 in avocados stored at 6 °C, where the rise in acetaldehyde content was equal to the increasing concentration of CO_2, while the ethanol level remained very low. In another paper on peaches, Polenta et al. (2005) confirmed a similar tendency in the days following nitrogen treatment. Here, CO_2 accumulation in the headspace (confirmed by the formation of foam on the water surface) during the marron curing phase is an index of intense anaerobic activity which then causes the formation of acetaldehyde but not of ethanol (CO_2 production is the result of PDC activity). This metabolic increase in acetaldehyde can be explained by a slowing down of acetaldehyde oxidation to acetic acid and/or of its reduction to ethanol. Another potential reason for the increase in acetaldehyde is the back oxidation of ethanol to acetaldehyde which occurs when high concentrations of ethanol (feedback effect) occur in the cell. To validate this supposition, the marrons, after being dipped in ethanol solutions for a short time, showed an exponential increase in acetaldehyde from 80 μmol g^{-1} of f.w. for

Table 1
Ethanol, acetaldehyde, and lactic acid content of flesh of marrons treated with different concentrations of ethanol for 24 h and then kept in shelf-life (20 °C and 90% RH)

	Ethanol (μmol g^{-1} of f.w.)	Acetaldehyde (μmol g^{-1} of f.w.)	Ethanol/acetaldehyde ratio	Lactic acid (mg L^{-1})
Water curing	93.3c	80.2d	1.16	19.2b
Ethanol 5%	196.5b	458.6c	0.42	23.4b
Ethanol 10%	254.6b	1462.2b	0.17	41.2a
Ethanol 20%	6958.9a	14102.3a	0.49	41.3a

Data represent the average of three replicated analyses of different marrons. Different letters in the same column indicate significant differences at $P = 0.05$.

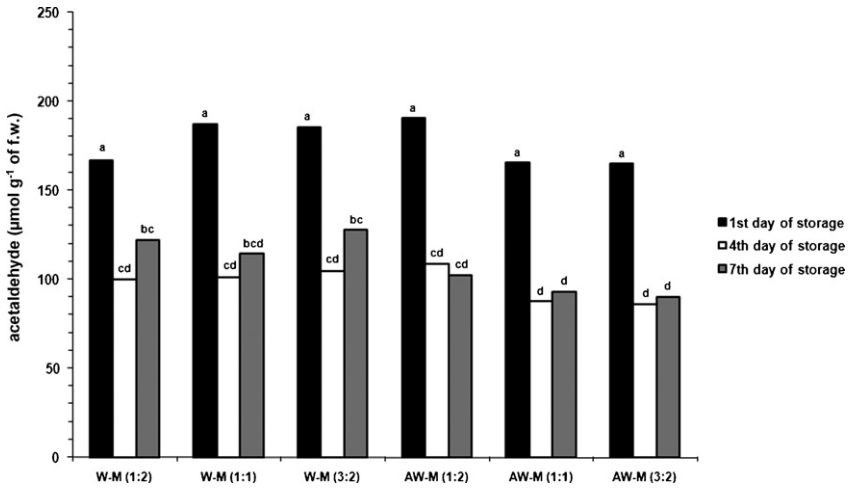

Fig. 6. Acetaldehyde content of marron flesh during shelf-life (20 °C and 90% RH). AW = acid water; W = water; M = marron. In parenthesis, the water/marron ratio. Data represent the average of three replicated analyses of different marrons. Different letters indicate significant differences at $P = 0.05$.

water-cured marrons up to 14,102 for those dipped in a 20% ethanol solution (Table 1). Ethanol increased, but not to the same extent as acetaldehyde. The ethanol/acetaldehyde ratio was 1.16 in water-treated marrons, while in marrons treated with 5%, 10, and 20% of ethanol in water the ratio was 0.42, 0.17 and 0.49 respectively indicating that ethanol was rapidly oxidised to acetaldehyde. This event was observed previously in tomatoes (Botondi et al., 1993). A parallel increase was observed for lactic acid (Table 1) from 19 up to 41 mg L^{-1} in the marron pulp treated with 10 and 20% ethanol. Thus, ethanol was oxidised to acetaldehyde and, at the same time, caused the reduction of pyruvic acid into lactic acid, as observed by Perata and Alpi (1993). Thus acetaldehyde accumulation during the curing phase could be the reason for the antiseptic effect of curing since acetaldehyde is toxic for cells (Perata and Alpi, 1993; Pesis, 2005). Furthermore, the decrease in pH due to the formation of lactic acid which, excreted from the cell acidifies the water as observed in Fig. 7, could further increase the antiseptic effect. In air storage (following the curing treatment) after 1 d, ethanol had already lost half of the concentration measured on the last day of curing (from 220 μM down to around 100 μM), because of rapid evaporation from the tissue. Subsequently, ethanol increased in the sample kept in acid water (1:1 and 3:2), while in the tap water samples and in acid water 1:2, the concentration remained stable during the 7 d of air storage (Fig. 5). On the first day of air storage, acetaldehyde in the marron tissue, remained at the same level as the concentration of the last day in water, between 158 and 190 μM, regardless the samples (Fig. 6). Acetaldehyde decreased in acid water samples.

Comparing the ethanol and acetaldehyde values of marrons during air storage, in tap water samples ethanol decreased greatly on the first day and subsequently remained stable, while acetaldehyde had the same concentration as the last day of curing and, on day 4, decreased significantly. This behaviour could mean a rapid oxidation of ethanol to acetaldehyde as soon as the marrons were removed from water to air; the subsequent decrease in acetaldehyde on day 4 might well be the result of further oxidation of acetaldehyde to acetic acid. In contrast, in acid water samples, ethanol increased during air storage while acetaldehyde diminished as a typical fermentation pattern (reduction of acetaldehyde to ethanol) which might indicate that cells are in a fermentation process as a response to stress effect of acid water. The pH of the tap water decreased on the first day of immersion of the marrons from values of 7 to values of 5.5–6 for the 1:1 and 3:2 samples and to values of 5 for the 1:2 samples, the values remaining constant over the curing period (Fig. 7). The pH of acid water increased from the first day in all the samples and, at the end of the curing phase, the water pH of the 1:1 and 3:2 samples reached values similar to those of the tap water sample with a ratio of 1:2, while the pH of acid water with a ratio of 1:2 reached a lower level. This pH value is optimum for LDH activity which would explain the activation of fermentation in acid water. The increase in the pH of acid water could be due to the release of ethanol as well as NH_4^+ coming from amino acid catabolism following cell stress. Whatever the initial pH value might have been, there is a change (increase or decrease) in the pH values to reach values between 4 and 5.5.

With regard to the polyphenol content in marron pulp which has been postulated to be the reason for the positive effect of water curing on insect control and fungal development during subsequent storage (Bergognoux, 1978), a large increase from 2.65 mg g^{-1} up to 4.76 and 4.14 mg g^{-1} was observed on day 5 in samples 1:2 and 1:1 respectively cured in acid water (Table 2). In all samples, the highest increase occurred on day 5, and then the polyphenol content decreased. Samples in acid water showed higher values than corresponding samples in tap water, and higher water content (3:2) samples had the lowest increase. This response in acid water could be due to a more rapid solubilisation of phenols from the perisperm and/or pericarp towards the pulp which would be confirmed by the fact that the samples dipped in solution with the highest water content (3:2) showed a lower increase. The response in acid water could be due to their major synthesis as stress response. The state

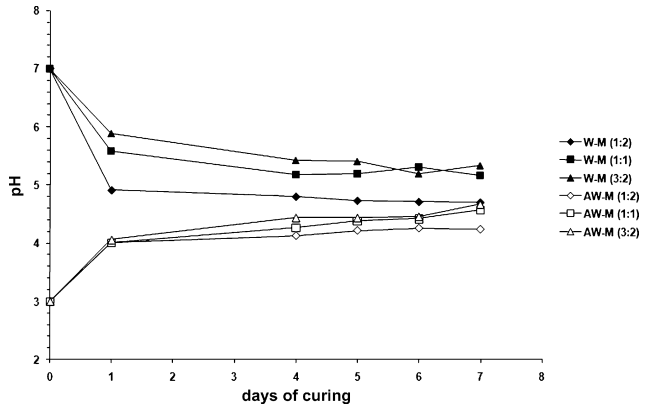

Fig. 7. Variation of the water pH during the curing phase for the different treatments. AW = acid water; W = water; M = marron. In parenthesis, the water/marron ratio.

Table 2
Polyphenol contents of marron pulp during the curing phase and on the seventh day of shelf-life (20 °C and 90% RH)

Curing treatments	Polyphenols (mg g^{-1} of gallic acid)				
	Initial time	2nd day of curing	5th day of curing	7th day of curing	7th day of storage
Initial time	2.64cde				
W–M (1:2)		3.22cd	3.34c	2.69cde	0.92f
W–M (1:1)		2.48de	3.48bc	2.83cd	1.03f
W–M (3:2)		2.51cde	2.88c	2.97cd	0.85f
AW–M (1:2)		1.91e	4.76a	2.79cd	0.63f
AW–M (1:1)		2.61cde	4.14ab	2.84cd	0.91f
AW–M (3:2)		2.25de	3.55bc	2.74cde	0.87f

AW = acid water; W = water; M = marron. In parenthesis, the water/marron ratio. Data represent the average of three replicated analyses of different marrons. Different letters indicate significant differences at $P = 0.05$.

Table 3
Values of SSC of marron flesh during the curing phase and on the seventh day of shelf-life

Curing treatments	SSC (° Brix)				
	Initial time	2nd day of curing	5th day of curing	7th day of curing	7th day of storage
Initial time	8.8g				
W–M (1:2)		10.8de	10.4ef	11.2de	11.4d
W–M (1:1)		11.4d	10.4ef	11.4d	13.4ab
W–M (3:2)		10.4ef	12.4c	12.4c	12.8bc
AW–M (1:2)		10.6def	13.2ab	11.2de	12.6bc
AW–M (1:1)		9.8f	14.0a	11.8cd	11.2de
AW–M (3:2)		11.2de	13.2ab	10.8de	12.6bc

AW = acid water; W = water; M = marron. In parenthesis, the water/marron ratio. Data represent the average of 10 analyses from different marrons. Different letters indicate significant differences at $P = 0.05$.

Table 4
SSC, ethanol, acetaldehyde, and polyphenol contents of marron flesh after 60 d of storage at 0 °C and 90% RH

Curing treatments	SSC (° Brix)	Ethanol (μmol g^{-1} of f.w.)	Acetaldehyde (μmol g^{-1} of f.w.)	Polyphenols (mg g^{-1} of gallic acid)	Internal decay (% of marrons)
W–M (1:2)	13.0b	85.4c	98.2b	0.82ab	13b
W–M (1:1)	14.2a	91.0c	102.1ab	0.91a	15b
W–M (3:2)	14.0a	92.3c	110.3a	0.76bc	20b
AW–M (1:2)	13.4ab	112.4ab	78.4c	0.66d	35a
AW–M (1:1)	13.0b	120.1a	76.1c	0.84ab	38a
AW–M (3:2)	13.2b	105.0b	80.0c	0.74cd	40a

AW = acid water; W = water; M = marron. In parenthesis, the water/marron ratio. Data represent the average of 10 analyses for SSC and the average of 3 replicated analyses of different marrons for the other analyses. Different letters in the same column indicate significant differences at $P = 0.05$.

of oxidation found (redox potential values between +50 and +200) in marrons soaked in acid water might explain the higher synthesis of phenol compounds considered defensive by the plant cell. It is known that strong oxidation activates polyphenol synthesis in fruit rich in phenols such as grapes, as a defense response, especially when the treatment is continuous (Artés-Hernández et al., 2007). This result would confirm the previous hypothesis that marron cells undergoing acid water treatments are subjected to metabolic stress. This hypothesis seems to be supported by data obtained after 2 months of storage, where acid water samples showed a four-fold higher percentage of decayed marrons compared to tap water samples.

A direct comparison of the water colour after curing showed that the water colour intensity increased with a larger quantity of marrons, thus indicating a higher solubilisation–diffusion of the polyphenols in the water, and the consequent lightening of the epicarp (data not shown). This diffusion can take place both inside and outside the pulp, and this could explain the increase in polyphenols during the curing phase. Acid water was clearer due to its state of oxidation. In air storage the polyphenol content decreased significantly from values between 2.7 and 2.9 mg g^{-1} at the end of the curing phase to 0.6 and 1 mg g^{-1} on the seventh day of storage. On this day, the tap water sample with a ratio of 1:1 had the highest value (Table 2). The reason for this drastic decrease in polyphenol content during air storage might be the result of polyphenol oxidation because polyphenols are not strongly bound but free in the tissue due to water diffusion, thus more sensitive to the effect of oxygen.

During the curing treatment a significant increase in soluble solids was observed from an average initial value of 8.8% up to 13–14% in marrons cured in acid water (Table 3). In acid water, SSC increased until day 5 and then decreased slightly, while in tap water SSC remained constant. This SSC trend is parallel to that of CO_2 accumulation which would indicate an active starch catabolism, in order to obtain greater availability of reducing sugars, above all in acid water. It is well known that a drop in pH favours starch hydrolysis (Yahia and Vazquez-Moreno, 1993; Tian et al., 2001). After 1 week of air storage, SSC remained stable or increased significantly in tap water sample 1:1 and in acid water samples 1:2 and 3:2 (Table 3). Analyses of the above chemical characteristics after 2 months of storage (Table 4) showed that sugars increased slightly in all samples and ethanol remained stable, with the highest concentration in acid water-treated samples; acetaldehyde remained stable with the highest content in water-treated samples, and, finally, phenols decreased in all the samples.

4. Conclusions

Curing marrons in water has a strong effect on their metabolism causing significant changes. An increase in acetaldehyde was

observed probably leading to an increase in lactic acid as well. An increase in CO_2, acetaldehyde and polyphenols, together with a presumed increase in lactic acid could be the reason for the antiseptic effect of water curing. Yet, this can happen within certain time limits and with specific water/marron ratios. It is therefore possible to define some technological parameters. Polyphenols increased until day 5 of water curing above all in acid water and then decreased on day 7.The marrons must be immersed in water for a maximum of 5 d to maintain cells survival. A water/marron ratio of 1:1 is advised. Acid water seems to stimulate the anaerobic metabolism, increasing the acetaldehyde concentration during curing and even the polyphenol content above all in the water/marron 1:1 ratio, but this response appears to be a stress response, which does not guarantee the antiseptic effect during the subsequent air storage where ethanol increase was observed.

Acknowledgement

The authors would like to thank Giovannelli farm for having provided the marrons for the experimental work.

References

Anelli, G., Mencarelli, F., Massignan, L., 1984. Trattamenti preconservativi per la conservazione delle castagne. (High CO_2 treatments for the storage of chestnuts). Industrie Alimentari 216, 397–401.

Artés-Hernández, F., Aguayo, E., Artés, F., Tomás-Barberán, F.A., 2007. Enriched ozone atmosphere enhances bioactive phenolics in seedless table grapes after prolonged shelf life. J. Sci. Food Agric. 87, 824–831.

Bassi, D., Casiraghi, M.C., Mignani, I., Vercesi, A., Delaidelli, G., 2001. Effetto dei trattamenti postraccolta e dei metodi di conservazione sulla qualità delle castagne. In: Bellini E. (Ed.), Atti del Convegno Nazionale sul Castagno 2001, Marradi, October 25–27, 2001, University of Florence, pp. 244–249.

Bergognoux, F., 1978. Conservation, transformation et utilization des chataignes et marrons. Invuflec, Paris, pp. 13–56.

Bernardez, M., Miguelez, J., Queijeiro, J., Queijeiro, J., 2004. HPLC determination of sugars in varieties of chestnut fruits from Galicia (Spain). J. Food Comp. Anal. 17, 63–67.

Bonghi, C., Ramina, A., Ruperti, B., Vidrih, R., Tonutti, P., 1999. Peach fruit ripening and quality in relation to picking time, and hypoxic and high CO_2 short-term postharvest treatments. Postharvest Biol. Technol. 16, 213–222.

Botondi, R., Gobattoni, E., Massantini, R., Mencarelli, F., 1993. ADH activity, via ethanol, affects ethylene production in tomato pericarp discs. In: Proceedings of International Symposium on Cellular and Molecular Aspect of Biosynthesis and Action of the Plant Hormone Ethylene, Agen, France, August 31–September 4, 1992, pp. 154–155.

Breisch, H., 1993. Harvest, storage and processing of chestnuts in France and Italy. In: Antognozzi, E. (Ed.), International Congress on Chestnuts, Spoleto, Italy, October 20–23, 1993, University of Perugia, pp. 429–436.

Burdon, J., Lallu, N., Yearsley, C., Burmeister, D., Billing, D., 2007. The kinetics of acetaldehyde and ethanol accumulation in 'Hass' avocado fruit during induction and recovery from low oxygen and high carbon dioxide conditions. Postharvest Biol. Technol. 43, 207–214.

De la Montaña, J., Miguel-Bernardez, M., Garcia, J.M., 2004. Composition of varieties of chestnuts from Galicia (Spain). Food Chem. 84, 401–404.

Estévez, A.M., Lizana, L.A., González, M.X., 2005. Marron glacè quality affected by storage conditions of the nut. Acta Hort. 693, 71–78.

Imahori, Y., Kota, M., Ueda, Y., Ishimaru, M., Cachin, K., 2002. Regulation of ethanolic fermentation in bell pepper fruit under low oxygen stress. Postharvest Biol. Technol. 25, 159–167.

Jermini, M., Conedera, M., Sieber, T.N., Sassella, A., Schärer, H., Jelmini, G., Höhn, E., 2006. Influence of fruit treatments on perishability during cold storage of sweet chestnuts. J. Sci. Food Agric. 86, 877–885.

Kang, H.M., Saltveit, M.E., 2003. Wound-induced increases in phenolic content of fresh-cut lettuce is reduced by a short immersion in aqueous hypertonic solution. Postharvest Biol. Technol. 29, 271–277.

Kays, S.J., 1997. Postharvest Physiology of Perishable Plant Products. Chapter 3: Metabolic Processes in Harvested Products. Exon Press Athens, GA 101.

Mencarelli, F., 2004. Postharvest handling and storage of chestnuts – Compendium, FAO, InPHO. http://www.fao.org/inpho/.

Mignani, I., Vercesi, A., 2003. Effects of postharvest treatments and storage conditions on chestnut quality. Acta Hort. 600, 781–785.

Montedoro, G.F., Servili, M., Baldioli, M., Selvaggini, R., Miniati, E., Macchioni, A., 1993. Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterizations of the secoiridoids derivatives. J. Agric. Food Chem. 41, 2228–2234.

Perata, P., Alpi, A., 1993. Plant responses to anaerobiosis. Plant Sci. 93, 1–17.

Pesis, E., 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. Postharvest Biol. Technol. 37, 1–19.

Polenta, G., Budde, C., Murray, R., 2005. Effects of different pre-storage anoxic treatments on ethanol and acetaldehyde content in peaches. Posthavest Biol. Technol. 38, 247–253.

Razaiki, L., Cesselin, B., Yamamoto, Y., Vido, K., van West, E., Gaudu, P., Grauss, A., 2004. Respiration metabolism reduces oxidative and acid stress to improve long-term survival of *Lactococcus lactis*. Mol. Microbiol. 53, 1331–1342.

Tian, S.P., Bartolini, P., 1997. Biology and pathogenicity of *Rhacodiella castaneae* in chestnuts stored at low temperatures. J. Plant Dis. Prot. 104, 23–28.

Tian, S., Fan, Q., Xu, Y., Wang, Y., Jiang, A., 2001. Evaluation of the use of high CO_2 concentration and cold storage to control *Monilinia fructicola* on sweet cherries. Postharvest Biol. Technol. 22, 53–60.

Washington, W.S., Allen, A.D., Dooley, L.B., 1997. Preliminary studies on *Phomopsis castanea* and other organisms associated with healthy and rotted chestnut fruit in storage. Aust. Plant Pathol. 26, 37–43.

Wells, J.M., Payne, J.A., 1980. Mycoflora and market quality of chestnuts treated with hot water to control the chestnut weevil. Plant Dis. 64, 999–1001.

Yahia, E.M., Vazquez-Moreno, L., 1993. Responses of mango to insecticidal oxygen and carbon dioxide atmospheres. Food Sci. Technol. 26, 42–48.