Food and Bioprocess Technology
https://doi.org/10.1007/s11947-019-02378-9

COMMUNICATION

Gaseous Ozone as a Suitable Solution for Postharvest Chestnut Storage: Evaluation of Quality Parameter Trends

Anna Maria Vettraino[1] · Vittorio Vinciguerra[1] · Giulia Pacini[1] · Roberto Forniti[1] · Valentina Goffi[1] · Rinaldo Botondi[1]

Received: 10 July 2019 / Accepted: 12 November 2019

Abstract
The storage of chestnuts is a highly critical phase due to the considerable postharvest losses of the product with relevant commercial impact. The effect on the chemical quality parameters of chestnuts treated with 300 ppb of gaseous ozone in air or in atmospheric air at $T = 2$ °C for 150 days during the storage has been investigated. The ozone treatment increased sucrose and total sugar contents; while tocopherols were reduced. No significant differences in weight loss and fatty acid content were recorded between chestnut treated with ozone and untreated fruits ($P \geq 0.05$). In addition, chestnut ozone exposure for 150 days reduced the total microbial populations associated with the pericarps of about 1.6 logs for mesophilic bacteria and 1.0 logs for fungi. Overall, our results suggested that gaseous ozone treatment is a valid and economic technology to ensure the shelf life quality of chestnut during the long storage periods.

Keywords O_3 · *Castanea sativa* mill · Free sugars · Fatty acids · Tocopherols · Postharvest quality

Introduction

Chestnut fruits have been linked to the traditional European cuisine for centuries. Chestnuts are well appreciated for their taste and chemical characteristics. They are sources of sugars (sucrose, approximately 16%, and glucose and fructose, both around 1% on a dry weight basis) and essential fatty acids (FA), mainly linoleic, oleic, and palmitic acids. They also contain vitamins and minerals, such as magnesium and manganese. These characteristics contribute to their nutritional value and health benefits (Mert and Ertürk 2017; Delgado et al. 2016; Barreira et al. 2012a, b; Fernandes et al. 2011). For instance, the large amounts of FA in nuts (rich in polyunsaturated fatty acids) prevent the related tissue oxidation (Delgado et al. 2016; Kalogeropoulos et al. 2013) while tocopherols are important lipophilic antioxidants which prevent rancidity during storage and have beneficial effects on living organisms, stabilizing the fatty acids contained in nuts (Barreira et al. 2012a, b; Li et al. 2007). Chestnuts reduce cholesterol levels and, due to the content of several minerals and vitamins, fight off free radicals. Besides the nutrition and benefits of chestnuts, these fruits are an important economic value, especially in marginal areas, with an annual worldwide production over two million tons. During storage chestnuts tend to spoil soon, due to the high content of water and sugars and the presence of insects and molds, as *Gnomoniopsis castanea* and *Sclerotinia pseudotuberosa* (Lione et al. 2019; Vettraino et al. 2005). In order to extend their shelf life, it is essential to implement effective postharvest handling practices and treatments, such as storage and packaging systems. Currently, fresh chestnuts are often kept in cold storage (0–4 °C) before or after the "water curing." The water curing consists of treatments in cold water (14–18 °C for 7–9 days) (Migliorini et al. 2010; Botondi et al. 2009) or hot water (46–51 °C for 45 min plus cold water, 15 °C for 8 days) (Cetin et al. 2018) before the cold storage. These methods can extend the shelf-life of chestnut but are not an effective method to prevent fruit decay (Vettraino et al. 2019; Lee et al. 2016, Kim et al., 2012).

To ensure the quality of product, today, it is essential to implement effective suitable technologies in food processing and food safety (Sun 2014, 2012). Several methods have been developed to extend the storage period of nuts, including the use of

Anna Maria Vettraino and Vittorio Vinciguerra contributed equally to this work.

✉ Rinaldo Botondi
rbotondi@unitus.it

[1] Department for Innovation in Biological, Agro-food and Forest systems - DIBAF, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy

Published online: 30 November 2019

atmosphere with high carbon dioxide levels (Cecchini et al. 2011), the fumigation with various chemicals (i.e., carbon sulfide, phosphine and manly, methyl bromide), irradiation with gamma rays or electron beam–drying methods in chestnut slices and biological products added to the water curing (Delgado et al. 2017; Delgado et al. 2016; Ruocco et al. 2016). Recently, ozone treatments have been also showed to be effective in affecting some fruits and vegetables quality traits (Tzortzakis and Chrysargyris, 2017; Botondi et al., 2015a, 2015b; Horvitz and Cantalejo, 2015; Alwi and Ali, 2014) and notably during chestnut storage, e.g., acidity and polyphenols (Vettraino et al. 2019; Donis-González et al. 2010). Ozone is produced by passing oxygen (O_2) gas through a high-voltage electrical discharge or by ultraviolet light irradiation (Mahapatra et al. 2005**)**. For commercial use, ozone is classified as GRAS (generally recognized as safe) because its degradation produces O_2, which leaves no residue on the treated fruit. For this reason, ozone can be used in food contact applications (FDA 2001) and is considered a cost-effective and "eco-friendly treatment." This study investigated the effect of the gaseous ozone treatments during chestnut storage on the analytical evolution of the quality parameters, focusing on characteristics not previously considered.

Material and Methods

Samples Procedure and Ozone Treatment

Castanea sativa cv *Castagna*, harvested in the Cimino-Sabatini area (northern Latium region of Italy), were selected free from visual defects and for uniformity of weight and shape. After dipping the chestnuts in sterile distilled water, floating fruits were eliminated, and the remaining nuts were randomly divided into two groups. For each group, 5 Kg of chestnuts per box were placed in no. 10 plastic perforated trays (100% recyclable polypropylene) (40 × 60 × 10 cm) in a cold chamber ($T = 2.0 \pm 1.0$ °C and 90 ± 5% RH). Control samples (CK) were kept in a normal atmosphere while treated ozone samples were exposed to continuous flow of 300 ppb of gaseous ozone in air. An ozone generator (C32-AG, Industrie De Nora Spa, Milan, Italy) equipped with an oxygen concentrator (nominal production capacity of 32 g ozone h^{-1}) was used to produce gaseous ozone. The ozone concentration in the cold room was continuously monitored using an UV-11 photometric ozone analyzer (BMT 146 Messtechnik Gmbh, DE). Sampling and analyses were performed at 30, 90, and 150 days for the five-month storage period.

Microbial Analysis of the Chestnut Pericarp

Five fragments of pericarps (5 × 5 mm) randomnly selected from 50 peeled chestnuts have been employed for the test according to Toti et al. (2018). After homogenization in peptone water solution, the diluted samples aliquots were plated on a (a) plate count agar (BBL, Difco, Becton, Dickinson and Company, Sparks, MD, USA) (PCA) and incubated at 30 °C for 72 h for detection of mesophilic aerobic bacteria or on a (b) potato dextrose agar (BBL, Difco) and incubated at 25 °C for 5 days for enumeration of yeast and molds.

Physical and Chemical Analysis

The percentage of weight loss (WL) was assessed according to Vettraino et al. (2019). For the chemical analyses, 1 Kg of the chestnuts were freeze-dried and lyophilized. Chemical analysis was performed on healthy chestnuts, visually evaluated for their phytosanitary status according to Vettraino et al. (2019).

Analysis of Free Sugars

The sugars were extracted from 50.0 mg of lyophilized sample according to Barros et al. (2007) with minor modifications. Free sugars were determined by gas chromatography with flame ionization detector (GC-FID) using a Perkin Elmer Autosystem XL instrument equipped with a capillary column (Restek Rxi®-5Sil MS 10 m × 0.15 mm i.d., 0.15 μm film thickness). The silylated sugars were identified by comparing their retention time with that of the silylated standard; quantification was based on the internal standard method using 10 mg of ribitol. The sugar contents are expressed in g Kg^{-1} of dry weight.

Analysis of Fatty Acids

Fatty acid extraction was carried out from 2.0 g of lyophilized sample according to Astorga España et al. (2011) with minor modifications. Fatty acids were converted to methyl ester derivatives, identified by gas chromatography-mass spectrometry (GC-MS), and quantified by gas chromatography with flame ionization detector (GC-FID) using a Shimadzu QP5050 and a Perkin Elmer Autosystem XL instrument respectively, both equipped with a capillary column (Restek Rxi®-5Sil MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness). Fatty acid methyl esters (FAMEs) were obtained by alkaline-catalyzed ultrasound-assisted transmethylation according to Li et al. (2009). The FAMEs were identified by interpreting their mass spectra and by comparing them with those listed in the NIST and Wiley computer libraries. Quantification of the FAMEs was based on the FID response expressed as a percentage of total FAMEs.

Analysis of Tocopherols

Tocopherol contents were determined by reversed phase high-performance liquid chromatography with UV detector (RP-HPLC-UV). The extraction procedure was performed from 0.5 g of lyophilized sample according to Ziegler et al. (2015) with minor modifications. The analysis was performed

Table 1 Total free sugars, fructose, glucose, and sucrose content (g Kg^{-1} dw) of untreated (CK) and ozone samples during chestnuts cold storage at 2 °C for 150 days. Data are reported as the mean ± standard error of the mean (± SEM) of three experiments. Means, in the same group of analysis, followed by the different letter are statistically different ($P < 0.05$)

	d 0	d 30		d 90		d 150	
		CK	Ozone	CK	Ozone	CK	Ozone
Fructose	2.41 ± 0.57^{bc}	2.19 ± 0.63^{bc}	2.29 ± 0.53^{bc}	3.25 ± 0.74^{b}	1.39 ± 0.44^{cd}	4.95 ± 0.80^{a}	2.07 ± 0.61^{c}
Glucose	1.54 ± 0.47^{c}	1.33 ± 0.36^{c}	1.83 ± 0.55^{c}	3.34 ± 0.58^{b}	1.33 ± 0.38^{c}	4.57 ± 0.49^{a}	1.91 ± 0.35^{c}
Sucrose	230.9 ± 1.3^{f}	212.7 ± 1.5^{g}	285.6 ± 1.8^{e}	416.6 ± 1.7^{a}	334.0 ± 1.5^{c}	308.3 ± 2.1^{d}	349.3 ± 1.8^{b}
Total free sugars	234.8 ± 1.8^{f}	216.2 ± 2.0^{g}	289.7 ± 1.7^{e}	423.2 ± 2.3^{a}	336.7 ± 1.6^{c}	317.8 ± 1.5^{d}	353.3 ± 1.9^{b}

according to Górnás et al. (2014) with minor modifications using a reversed phase column (Hypersil GOLD™ PFP 5 μm, 150 × 4.6 mm, Thermo Scientific). The compounds were identified by comparing their chromatographic data with those of authentic standards. Quantification was based on the UV signal response, measured using an external standard calibration curve. The tocopherol contents are expressed in mg Kg^{-1} of dry weight.

Statistical Analysis

All physical and chemical data are the mean of three replicate samples ± SEM (standard error of the mean). Data were analyzed using analysis of variance (ANOVA) and Duncan's test was carried out to identify significant differences between samples at $P < 0.05$. All analyses were carried out using the SPSS software package, Version 20.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Microbial Analysis of the Chestnut Pericarp

Regardless the treatments, the storage at 2 °C did not stop microbial growth of bacteria and fungi (yeasts and molds). Nevertheless, the use of gaseous ozone reduced the microbial populations during postharvest storage. The mean count of mesophilic bacteria and fungi isolated from CK samples increased from 6.12 and 4.31 (at time zero) to 8.28 CFU g^{-1} and 5.58 CFU g^{-1} (after 150 days of storage), respectively. The ozone treatment showed the lowest mean count of bacteria and fungi, 6.67 and 4.60, respectively at the end of storage (data not shown). Several other researchers reported a decrease of the population of fungi and bacteria in the chestnut pericarp rather than in the kernel when nuts were treated with ozone (Donis-González et al. 2017; Carocho et al. 2014; Antonio et al. 2012; Donis-González et al. 2010; Jermini et al. 2006). Further research should focus on the identification of the best treatment condition of temperature, RH, and exposure time to decrease the initial microbial contamination level of the product surface.

Weight Loss

Fruit quality is mainly affected by weight loss which depends on the relative humidity of the surrounding ambient air and the intensity of transpiration and respiration processes of the fruit during storage (Duarte-Molina et al. 2016). In our experiment, the weight loss decreased in all treatments of approximately 1.2 ± 0.5%, on a monthly basis (data not shown). No significant differences were observed between the control and the samples treated with ozone (ANOVA, $P < 0.05$). These data confirm previous findings (Vettraino et al. 2019; Donis-González et al. 2010). Conversely, other studies reported increased weight loss in fruits, e.g., grape berries, strawberries, and chili peppers treated with ozone (Contigiani et al. 2018; Glowacz and Rees 2016; Cayuela et al. 2009). The different

Table 2 Total satured (SFA), monounsatured (MUFA), and polyunsatured PUFA) fatty acid content (%)) of untreated (CK) and ozone samples during chestnuts cold storage at 2 °C for 150 days. Data are reported as the mean ± standard error of the mean (± SEM) of three experiments. Means, in the same group of analysis, followed by the different letter are statistically different ($P < 0.05$)

	d 0	d 30		d 90		d 150	
		CK	Ozone	CK	Ozone	CK	Ozone
Total SFA	22.2 ± 0.32^{a}	20.8 ± 0.27^{ab}	18 ± 0.65^{ab}	17.7 ± 0.32^{b}	18.4 ± 0.23^{ab}	18.2 ± 0.32^{ab}	16.9 ± 0.29^{b}
Total MUFA	32.2 ± 0.64^{c}	39.6 ± 0.73^{a}	38.4 ± 0.63^{ab}	37.2 ± 0.50^{ab}	41.1 ± 0.61^{a}	34.6 ± 0.11^{bc}	38.7 ± 0.26^{ab}
Total PUFA	45.6 ± 1.66^{ab}	39.7 ± 1.07^{b}	43.6 ± 1.12^{ab}	45.2 ± 0.83^{ab}	40.5 ± 0.89^{ab}	47.1 ± 1.13^{a}	44.5 ± 1.14^{ab}

Table 3 Fatty acid content (% of total FAME) of untreated (CK) and ozone samples during chestnuts cold storage at 2 °C for 150 days. Data are reported as the mean ± standard error of the mean (± SEM) of three experiments. Means, in the same group of analysis, followed by the different letter are statistically different ($P < 0.05$)

	d 0	d 30		d 90		d 150	
		CK	Ozone	CK	Ozone	CK	Ozone
C14:0	0.102 ± 0.038^{a}	0.079 ± 0.008^{ab}	0.092 ± 0.006^{ab}	0.1 ± 0.003^{a}	0.068 ± 0.008^{b}	0.0353 ± 0.008^{c}	0.0387 ± 0.006^{c}
C16:0	21 ± 0.10^{ab}	19 ± 0.095^{ac}	16.5 ± 0.064^{c}	16.6 ± 0.014^{bc}	17.2 ± 0.016^{ac}	17.1 ± 0.036^{ac}	15.8 ± 0.043^{c}
C16:1	0.326 ± 0.042^{ab}	0.402 ± 0.043^{ab}	0.32 ± 0.012^{ab}	0.283 ± 0.009^{c}	0.576 ± 0.021^{a}	0.215 ± 0.031^{c}	0.165 ± 0.011^{d}
C18:0	0.766 ± 0.036^{bc}	1.05 ± 0.048^{a}	0.915 ± 0.026^{ab}	0.649 ± 0.013^{c}	0.989 ± 0.009^{ab}	0.927 ± 0.028^{ab}	0.86 ± 0.021^{ac}
C18:1	31.4 ± 0.586^{c}	38.6 ± 1.08^{a}	37.5 ± 0.915^{ab}	36.6 ± 0.33^{ab}	40.1 ± 0.41^{a}	33.9 ± 0.42^{bc}	38 ± 0.27^{a}
C18:2	45.6 ± 1.66^{ab}	39.7 ± 1.7^{b}	43.6 ± 1.12^{ab}	45.2 ± 0.83^{ab}	40.5 ± 1.18^{ab}	47.1 ± 1.03^{a}	44.5 ± 0.98^{ab}
C20:0	0.223 ± 0.041^{bd}	0.346 ± 0.044^{a}	0.279 ± 0.014^{ab}	0.257 ± 0.015^{bc}	0.163 ± 0.019^{d}	0.174 ± 0.021^{d}	0.184 ± 0.014^{cd}
C20:1	0.449 ± 0.013^{ab}	0.621 ± 0.084^{a}	0.559 ± 0.024^{a}	0.284 ± 0.012^{b}	0.473 ± 0.012^{a}	0.529 ± 0.025^{a}	0.53 ± 0.017^{a}
C22:0	0.159 ± 0.010^{b}	0.276 ± 0.051^{a}	0.197 ± 0.019^{b}	0.062 ± 0.016^{c}	0.0425 ± 0.015^{c}	0.0343 ± 0.013^{c}	0.0205 ± 0.017^{c}

effect of ozone treatments on weight loss for each commodity could be due both to the thickness of the product cuticle and the treatment procedures. The presence of hard pericarps of chestnuts does not enhance the ozone penetration at the condition used in this research.

Total Free Sugars

The maximum total free sugars content was measured on chestnuts stored for 90 days under CK conditions. However, the total sugars content tended to increase over time up to 90 days in CK and up to 150 days in ozone samples. The conversion from starch to sugars seems to occur most rapidly in response of cold temperature (CK samples). An increase in the sucrose content of chestnuts after the first few weeks of cold storage at 0–3 °C has been already observed by several authors both in *C. sativa* (Jermini et al. 2006) and in *C. crenata* (Nomura et al. 1995). Ozone treatment differently affected the content of hexoses considered in this study. Sucrose was the main sugar with a concentration of 230.9 g Kg^{-1} in chestnuts at harvesting (Table 1). It is worthy to note that the sucrose content of the CK sample was almost doubled after 90 days of storage at 2 °C (416.6 g Kg^{1}) and then decreased significantly until the end of the test reaching values of 308.3 g Kg^{-1}. Contrastingly, in the ozone samples, the sucrose content increased gradually reaching the maximum value of 349.3 g Kg^{-1} after 150 days of storage. When analyzed after 30 days, sucrose values were higher in the ozone than CK samples (285.6 versus 212.7 g Kg^{-1}). At harvest time, fructose and glucose were present in minimal traces (2.4 g Kg^{-1} and 1.5 g Kg^{-1}, respectively). The fructose and glucose contents of the CK samples gradually increased after 30 days of storage. At the end of the experiment, the values doubled and almost tripled (4.95 and 4.57 g Kg^{-1}, respectively). These results lie with the data from Jermini et al. (2006) and Nomura et al. (1995).

Fatty Acids

Data confirmed that between the fractions of FA, chestnuts had low total saturated fatty acid content (16.9–22.2%) with respect to the monounsatured (32.2–41.1%) and the polyunsatured acids (39.7–47.1%) (Table 2). These results are consistent with those observed by Delgado et al. (2016, 2017), Barreira et al. (2012a, b), and Borges et al. (2007) in *C. sativa*. The SFA values of CK and ozone treatments decreased significantly during storage whereas the MUFA values of the two samples showed an upward trend (ANOVA, $P < 0.05$).

Table 4 Tocopherols content (mg Kg^{-1} of dry weight) of untreated (CK) and ozone samples during chestnuts cold storage at 2 °C for 150 days. Data are reported as the mean ± standard error of the mean (± SEM) of three experiments. Means, in the same group of analysis, followed by the different letter are statistically different ($P < 0.05$)

	d 0	d 30		d 90		d 150	
	CK	CK	Ozone	CK	Ozone	CK	Ozone
δ-Tocopherols	3.18 ± 0.12^{a}	3.04 ± 0.37^{ab}	2.44 ± 0.39^{bc}	1.24 ± 0.02^{d}	1.0 ± 0.01^{d}	2.20 ± 0.113^{c}	1.17 ± 0.346^{d}
γ-Tocopherols	124.0 ± 4.4^{a}	112.0 ± 3.2^{b}	75.4 ± 3.5^{d}	77.0 ± 0.53^{d}	66.8 ± 0.77^{d}	90.4 ± 1.71^{c}	65.3 ± 1.69^{d}
α-Tocopherols	2.60 ± 0.12^{a}	0.42 ± 0.03^{b}	0.25 ± 0.01^{bd}	0.33 ± 0.02^{bc}	0.16 ± 0.01^{cd}	0.18 ± 0.027^{cd}	0.66 ± 0.07^{d}

There were no significant variations in the PUFA content based on storage times or different treatments. The fatty acids C16:0, C18:1, and C18:2 represented more than 97% of the total FA, which is in accordance with Borges et al. (2007) and Barreira et al. (2009). There were significant differences in the FA contents between treatments during 150 days of storage. The C16:0 values at the end of the experiment were significantly lower in the CK sample, but mainly in samples treated with ozone (ANOVA; $P < 0.05$); conversely, ozone samples were characterized by higher values of C18:1 after 150 days than the time zero (ANOVA; $P < 0.05$). The content of C18:2 was not affected by treatment and storage time (ANOVA; $P > 0.05$) (Table 3). Despite the mentioned particular effects of ozone in some individual fatty acids, no linear effects were generally observed for SFA, MUFA, and PUFA contents.

Vitamin E

The data collected in the study showed γ-tocopherol as the most important component with values equal to 124.0 mg Kg^{-1} at the beginning of the experiment. This value decreased significantly during storage in all samples until values equal to 90.4 mg Kg^{-1} in CK and 65.3 mg Kg^{-1} in ozone samples after 150 days of storage (Table 4). It is interesting to note that there is a greater decrease in the γ-tocopherol content in the ozone-treated samples than in CK samples for any of the measurements taken over time. Similarly, δ- and α-tocopherol decreased during storage even if they had lower values at the start of the experiment (d 0). The results obtained are in accordance with those by Delgado et al. (2016). These findings may indicate that tocopherol degradation, clearly evident in the ozone samples, could be due to (i) the oxidation of the tocopherols due to the direct interaction of chestnut and the gaseous oxidants and (ii) by the antioxidant effectiveness of tocopherols on the lipid fraction (Brooks and Csallany 1978).

Conclusions

In the last years, much more attention has been paid to the use of sustainable methods for chestnut storage. This study makes it possible to affirm that the use of gaseous ozone technology (300 ppb in air) prolongs storage at 2 °C of chestnuts, without negative important effects on the qualitative parameters studied. It is effective in controlling the microbial growth, while no undesirable effects occur on weight loss evolution. In samples treated with gaseous ozone, the sucrose content constantly increased during the storage, contrary to control samples. Glucose and fructose were only detectable in traces. No significant differences between treatments of FA content whereas a greater decrease of tocopherols during storage was observed.

These findings are a baseline for developing integrated postharvest strategies for the chestnut chain and contribute to increase the economic benefits for chestnut producers. However, further research is required in order to better define potential ozone technological applications.

References

Alwi, N. A., & Ali, A. (2014). Dose-dependent effect of ozone fumigation on physiological characteristics, ascorbic acid content and disease development on bell pepper (*Capsicum annuum* L.) during storage. *Food and Bioprocess Technology, 8*(3), 558–566.

Antonio, A. L., Carocho, M., Bento, A., Quintana, B., Botelho, M. L., & Ferreira, I. C. F. R. (2012). Effects of gamma radiation on the biological, physic-chemical, nutritional and antioxidant parameters of chestnuts–a review. *Food and Chemical Toxicology, 50*, 3234–3242.

Astorga España, M.S, Rodríguez Galdón, B., Díaz Romero, C., Rodríguez Rodríguez, E., (2011). Fatty acid profile in varieties of chestnut fruits from Tenerife (Spain). *CyTA-Journal of Food,* 77–81.

Barreira, J. C. M., Antonio, A. L., Gunaydi, T., Alkan, H., Bento, A., Botelho, M. L., & Ferreira, I. C. F. R. (2012a). Chemometric characterization of gamma irradiated chestnuts from Turkey. *Radiation Physics and Chemistry, 81*, 1520–1524.

Barreira, J. C. M., Casal, S., Ferreira, I. C. F. R., Peres, A. M., Pereira, J. A., & Oliveira, M. B. P. P. (2012b). Chemical characterization of chestnut cultivars from three consecutive years: chemometrics and contribution for authentication. *Food and Chemical Toxicology, 50*(7), 2311–2317.

Barros, L., Baptista, P., Correia, D. M., Sá Morais, J., & Ferreira, I. C. F. R. (2007). Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. *Journal of Agricultural and Food Chemistry, 55*(12), 4781–4788.

Borges, O. P., Carvalho, J. S., Correia, P. R., & Silva, A. P. (2007). Lipid and fatty acid profiles of *Castanea sativa* mill. Chestnuts of 17 native Portuguese cultivars. *Journal of Food Composition and Analysis, 20*, 80–89.

Botondi, R., Vailati, M., Bellincontro, A., Massantini, R., Forniti, R., & Mencarelli, F. (2009). Technological parameters of water curing affect postharvest physiology and storage of marrons (*Castanea sativa* Mill., *Marrone fiorentino*). *Postharvest Biology and Technology, 51*(1), 97–103. https://doi.org/10.1016/j.postharvbio.2008.06.010.

Botondi, R., Bartoloni, S., Baccelloni, S., & Mencarelli, F. (2015a). Biodegradable PLA (polylactic acid) hinged trays keep quality of fresh-cut and cooked spinach. *Journal of Food Science and Technology, 52*(9), 5938–5945.

Botondi, R., De Sanctis, F., Moscatelli, N., Vettraino, A. M., Catelli, C., & Mencarelli, F. (2015b). Ozone fumigation for safety and quality of wine grapes in postharvest dehydration. *Food Chemistry, 188*, 641–647.

Brooks, R. I., & Csallany, A. S. (1978). Effects of air, ozone, and nitrogen dioxide exposure on the oxidation of corn and soybean lipids. *Journal of Agricultural and Food Chemistry, 26*(6), 1203–1209.

Barreira, J. C. M., Casal, S., Ferreira, I. C. F. R., Beatriz, M., Oliveira, P. P. and Pereira, J. A. (2009). Nutritional, Fatty Acid and Triacylglycerol Profiles of Mill. Cultivars: A Compositional and Chemometric Approach . *Journal of Agricultural and Food Chemistry 57 (7)*: 2836-2842

Carocho, M., Antonio, A. L., Barreira, J. C. M., Rafalski, A., Bento, A., & Ferreira, I. C. F. R. (2014). Validation of gamma and electron beam irradiation as alternative conservation technology for

European chestnuts. *Food and Bioprocess Technology, 7*, 1917–1927.

Cayuela, J. A., Vazquez, A., Perez, A. G., & Garcia, J. M. (2009). Control of table grapes postharvest decay by ozone treatment and resveratrol induction. *Food Science and Technology International, 15*, 495–502.

Cecchini, M., Contini, M., Massantini, R., Monarca, D., & Moscetti, R. (2011). Effects of controlled atmospheres and low temperature on storability of chestnuts manually and mechanically harvested. *Postharvest Biology and Technology, 61*, 131–136.

Cetin, M., Akbudak, B., & Ozer, M. H. (2018). The effects of hot and cold water treatment on quality parameters and enzymatic activity in chestnut. *Tarim Bilimleri Dergisi – Journal of Agricultural Sciences, 24*(3), 403–412.

Contigiani, E. V., Jaramillo-Sánchez, G., Castro, M. A., Gómez, P. L., & Alzamora, S. M. (2018). Postharvest quality of strawberry fruit (*Fragaria x Ananassa Duch* cv. *Albion*) as affected by ozone washing: fungal spoilage, mechanical properties, and structure. *Food and Bioprocess Technology, 11*(9), 1639–1650.

Delgado, T., Pereira, J. A., Ramalhosa, E., & Casal, S. (2016). Effect of hot air convective drying on the fatty acid and vitamin E composition of chestnut (*Castanea sativa* Mill.) slices. *European Food Research and Technology, 242*(8), 1299–1306. https://doi.org/10.1007/s00217-015-2633-5.

Delgado, T., Pereira, J. A., Ramalhosa, E., & Casal, S. (2017). Comparison of different drying methods on the chemical and sensory properties of chestnut (*Castanea sativa* M.) slices. *European Food Research and Technology, 243*(11), 1957–1971.

Donis-González, I. R., Fulbright, D. W., Ryser, E. T., & Guyer, D. (2010). Efficacy of postharvest treatments for reduction of molds and decay in fresh Michigan chestnuts. *Acta Horticulturae, 866*, 563–570. https://doi.org/10.17660/ActaHortic.2010.866.76.

Donis-González, I.R., Jeong, S., Guyer, D.E., Fulbright, D.W., (2017). Microbial contamination in peeled chestnuts and the efficacy of postprocessing treatments for microbial spoilage management *Journal of Food Processing and Preservation*, 41(2), art n. e12874. doi: https://doi.org/10.1111/jfpp.12874.

Duarte-Molina, F., Gómez, P. L., Castro, M. A., & Alzamora, S. M. (2016). Storage quality of strawberry fruit treated by pulsed light: fungal decay, water loss and mechanical properties. *Innovative Food Science & Emerging Technologies, 34*, 267–274.

FDA. (2001). Secondary direct food additives permitted in food for human consumption. *Rules Regulatory, 64*, 44122–44123.

Fernandes, A., Antonio, A. L., Barros, L., Barreira, J. C. M., Bento, A., Botelho, M. L., & Ferreira, I. C. F. R. (2011). Low dose γ-irradiation as a suitable solution for chestnut (*Castanea sativa* Miller) conservation: effects on sugars, fatty acids and tocopherols. *Journal of Agricultural and Food Chemistry, 59*, 10028–10033.

Glowacz, M., & Rees, D. (2016). The practicality of using ozone with fruit and vegetables. *Journal of the Science of Food and Agriculture, 96*, 4637–4643.

Górnaś, P., Siger, A., Polewski, K., Pugajeva, I., and Waśkiewicz, A. (2014). Factors affecting tocopherol contents in coffee brews: NP-HPLC/FLD, RP-UPLC-ESI/MSn and spectroscopic study. *European Food Research and Technology 238 (2)*:259-264

Horvitz, S., & Cantalejo, M. J. (2015). Effects of gaseous O_3 and modified atmosphere packaging on the quality and shelf-life of partially dehydrated ready-to-eat pepper strips. *Food and Bioprocess Technology, 8*(6), 1386–1392.

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. *Journal of the Science of Food and Agriculture, 86*, 877–885. https://doi.org/10.1002/jsfa.2428.

Kalogeropoulos, N., Chiou, A., Ioannou, M. S., & Karathanos, V. T. (2013). Nutritional evaluation and health promoting activities of nuts and seeds cultivated in Greece. *International Journal of Food Sciences and Nutrition, 64*(6), 757–767.

Kim, H. K., An, D. S., Lee, S. J., & Lee, D. S. (2012). Dependence of individual primary package atmosphere on retail display temperature and micro-perforations in a master packaging system for chestnuts. *Journal of Food Agricultural and Environment, 10*, 168–172.

Lee, U. K., Joo, S., Klopfenstein, N. B., & Kim, M. S. (2016). Efficacy of washing treatments in the reduction of postharvest decay of chestnuts (*Castanea crenata* '*Tsukuba*') during storage. *Canadian Journal of Plant Science, 96*, 1–5. https://doi.org/10.1139/cjps-2015-0089.

Li, L., Tsao, R., Yang, R., Kramer, J. K. G., & Hernandez, M. (2007). Fatty acid profiles, tocopherol contents, and antioxidant activities of heartnut (*Juglans ailanthifolia* Var. *cordiformis*) and Persian walnut (*Juglans regia* L.). *Journal of Agricultural and Food Chemistry, 55*, 1164–1169.

Li, J., Yue, Y., Li, T., Hu, X., & Zhong, H. (2009). Gas chromatography-mass spectrometric analysis of bonded long chain fatty acids in a single zebrafish egg by ultrasound-assisted one-step transmethylation and extraction. *Analytica Chimica Acta, 650*(2), 221–226.

Lione, G., Danti, R., Fernandez-Conradi, P., Ferreira-Cardoso, J. V., Lefort, F., Marques, G., & Gonthier, P. (2019). The emerging pathogen of chestnut *Gnomoniopsis castaneae*: the challenge posed by a versatile fungus. *European Journal of Plant Pathology, 153*(3), 671–685. https://doi.org/10.1007/s10658-018-1597-2.

Mahapatra, A. K., Muthukumarappan, K., & Julson, J. L. (2005). Applications of ozone, bacteriocins and irradiation in food processing: a review. *Critical Reviews in Food Science and Nutrition, 45*(6), 447–461.

Mert, C., & Ertürk, U. (2017). Chemical compositions and sugar profiles of consumed chestnut cultivars in the Marmara Region, Turkey. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, Open Access, *45*(I1), 203–207.

Migliorini, M., Funghini, L., Marinelli, C., Turchetti, T., Canuti, S., & Zanoni, B. (2010). Study of water curing for the preservation of marrons (*Castanea sativa* Mill., *Marrone fiorentino* cv). *Postharvest Biology and Technology, 56*, 95–100.

Nomura, K., Ogasawara, Y., Uemukai, H., & Yoshida, M. (1995). Change of sugar content in chestnut during low temperature storage. *Acta Horticulturae, 398*, 265–276.

Ruocco, M., Lanzuise, S., Lombardi, N., Varlese, R., Aliberti, A., Carpenito, S., Woo, S. L., Scala, F., & Lorito, M. (2016). New tools to improve the shelf life of chestnut fruit during storage. *Acta Horticulturae, 1144*, 309–315.

Sun, D.-W., (2012). *Handbook of Food Safety Engineering* (p. 864). Wiley-Blackwell Publishing Ltd DOI:https://doi.org/10.1002/9781444355321.

Sun, D.-W., (2014). *Emerging technologies for food processing* 2nd edition (p.666). Academic Press https://doi.org/10.1016/C2012-0-07021-4.

Toti, M., Carboni, C., & Botondi, R. (2018). Postharvest gaseous ozone treatment enhances quality parameters and delays softening in cantaloupe melon during storage at 6 °C. *Journal of the Science of Food and Agriculture, 98*(2), 487–494. https://doi.org/10.1002/jsfa.8485.

Tzortzakis, N., & Chrysargyris, A. (2017). Postharvest ozone application for the preservation of fruits and vegetables. *Food Reviews International, 33*(3), 270–315. https://doi.org/10.1080/87559129.2016.1175015.

Vettraino, A. M., Bianchini, L., Caradonna, V., Forniti, R., Goffi, V., Zambelli, M., Testa, A., Vinciguerra, V., & Botondi, R. (2019). Ozone gas as a storage treatment to control *Gnomoniopsis castanea*, preserving chestnut quality. *Journal of the Science of Food and Agriculture, 99*, 6060–6065. https://doi.org/10.1002/jsfa.9883.

Vettraino, A. M., Paolacci, A., and Cannini, A. (2005) Endophytism of *Sclerotinia pseudotuberosa:* PCR assay for specific detection in chestnut tissues. *Mycological Research 109 (1)*:96-102

Ziegler, J. U., Schweiggert, R. M., & Carle, R. (2015). A method for the simultaneous extraction and quantitation of lipophilic antioxidants in *Triticum* sp. by HPLC-DAD/FLD-MS^{n}. *Journal of Food Composition and Analysis, 39*, 94–102.