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ORIGINAL RESEARCH

Effects of Moderate Electric Fields on the Post-harvest Preservation of Chestnuts

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Abstract

Ohmic heating (OH) was evaluated as a post-harvest technology to improve chestnuts' shelf-life (*Castanea sativa* Mill.) by controlling molds and insect larvae proliferation. Chestnuts were processed by OH at 35, 45, and 55 °C and compared with untreated fruits and the conventional hydrothermal technology (HT - 50 °C for 45 min), which is the process currently used by the chestnut industry. Shelf-life studies were carried out at different atmospheric conditions for 60 days: (i) 25 °C and 40% relative humidity (RH); (ii) 5 °C and 70% RH. The results show that the OH conducted at 55 °C (OH-55 °C), combined with storage at 5 °C, was more effective in controlling molds and larvae growth than the other treatments. Moreover, under these conditions, chestnuts' shelf-life could be extended for 60 days without substantial changes in the fruits' color and texture. After the OH-55 °C treatment, lower losses of some nutrients and vitamin C were registered compared to HT. This study demonstrates for the first time that OH has the potential to be used by the chestnut industry for the post-harvest disinfestation of this fruit.

Keywords Ohmic heating · Martaínha chestnut · Shelf-life · Fungi · Weight loss

Introduction

Chestnuts are the fruit of the genus *Castanea* (Zhu, 2017). Data collected in 2018 show that the worldwide production of chestnuts was around 2.35 million tons, with China being the primary contributor with a share of 83% (FAOSTAT, 2020). In the European Union, chestnut production represents only 6% of the global market. Italy, Greece, Portugal, Spain, and France are the primary producers in Europe. According to FAOSTAT (2020), Portuguese chestnut production was 34,165 tons in 2018.

The consumption of chestnut fruits has increased in recent years because of their interesting nutritional properties, unique flavor, and potential as a functional food (Bounous et al., 2002; FAOSTAT, 2020; Wang et al., 2020; Zhu, 2017). The chestnut kernel is low in protein and fat, contains several essential fatty acids, and is rich in starch—its primary component (Hou et al., 2018; Zhang et al., 2018; Zhu, 2016). Chestnuts are also an excellent source of vitamin C and are gluten-free (Ribeiro et al., 2007; Wang et al., 2020; Zhu, 2017). Nevertheless, unlike other edible nuts, chestnuts are fruits with a relatively high moisture content and metabolic activity (Blaiotta et al., 2014). Therefore, chestnuts have a limited shelf-life and are prone to post-harvest decay, suffering weight loss due to dehydration, undesired changes in color and texture, and growth of insect larvae and fungi, if not adequately processed or stored (Vettraino et al., 2020; Zhao et al., 2018; Zhu, 2016).

Fumigation with methyl bromide (MeBr) has been used in the past to extend chestnuts' shelf-life (UNEP, 2014). Because of its ozone-depleting properties, MeBr production and use were banned worldwide after the Montreal Protocol (UNEP, 2014). Since then, other preservation methods such as the immersion in cold water (water curing at 15–20 °C for 3–9 days) and the immersion in hot water (hydrothermal process at 47–50 °C for 30–45 min) have been used by the chestnut industry (Bounous et al., 2002). Nonetheless, the long processing times of these methods contribute to raising the moisture content of chestnuts (Neri et al., 2010; Silva et al., 2011), thus increasing the risk of deterioration during the storage period due to fungal growth (Vettraino et al., 2020).

The existing drawbacks in current chestnuts' post-harvest treatments together with the increasing demand for high-

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quality food, the industrial concerns about food preservation, safety and shelf-stable life of the products, and the existing environmental requirements have triggered the need for new post-harvest processing technologies for chestnuts. Several alternative treatments have been studied with more or less success and reviewed by Zhu (2016). Some of the reported strategies are described as follows: (i) hot air (55–62 °C) assisted by radiofrequency (6 kW, 27.12 MHz) followed by storage at 4–35 °C and 95% relative humidity (RH) (Hou et al., 2014, 2015, 2018); (ii) microwaves at 420–900 W during 1.5–15 min (Wani et al., 2017; Zhang et al., 2018); (iii) natural air drying at 15–25 °C and RH of 20–40% during ≥ 4 days (Wang et al., 2020; Zhang et al., 2018; Zhao et al., 2018); (iv) electron beam and gamma irradiation at 0–10 kGy followed of storage at 4 °C and 90–95% RH (Barreira et al., 2012; Carocho et al., 2012, 2014; Fernandes et al., 2011a, b); (v) controlled atmosphere (150–300 ppb of O_3 or 15.20 kPa CO_2 and 3.04 kPa O_2) and storage temperature at − 1 °C and 95% RH (Vettraino et al., 2019, 2020); and (vi) packaging in a CO_2-enriched and O_2-impoverished atmosphere (10–80% CO_2, 0.3–10.5% O_2 and N_2 as filler) followed by storage at 0 and 8 °C (Fernandes et al., 2020a, b; Panagou et al., 2006; Peano et al., 2014). However, many of these studies have not evaluated the treatment efficacy in controlling the fungal load.

During the last decade, ohmic heating (OH) has emerged as a high-potential technology for food processing. OH is an environment-friendly technology that relies on the application of external moderate electric fields to create fast and uniform heating (Kaur & Singh, 2016; Pereira & Vicente, 2010). The process involves the application of a voltage on two electrodes located at the extremities of a container. The alternating current is forced to pass through the food material existing in the container, resulting in internal heat generation due to food electrical resistance—Joule effect (Pereira & Vicente, 2010). OH has gained considerable interest because it can be used in a wide diversity of food processes such as blanching, evaporation, dehydration, thawing, extraction, peeling, fermentation, sterilization, and pasteurization (Kaur & Singh, 2016; Pereira & Vicente, 2010). OH has a rapid and uniform heating capacity, and the combined electrical and thermal effects can enhance the inactivation of microbes and enzymes with minimal thermal damage (Kaur & Singh, 2016; Machado et al., 2010). Thus, foods with low nutritional losses and highly stable shelf-life are obtained (Kaur & Singh, 2016).

The advantages of OH lie fundamentally on (i) its ability to quickly reach higher pasteurization temperatures; (ii) its low environmental impact due to its low energy consumption profile, which also helps to reduce processing costs; (iii) its short heating times, which allows small food cooking values (*C*-value) and low processing-dependent changes in quality (Jaeger et al., 2016); and (iv) its capacity to better preserve the nutritional, functional, structural, and sensory properties of food products than conventional thermal technologies.

However, the OH method is not devoid of disadvantages since the food's electrical conductivity is a critical property that influences the heating process uniformity. Therefore, food products with multi-phase components of different electrical conductivity may heat at different rates giving rise to non-uniform heating of the product and possible underprocessing or overprocessing (Pereira & Vicente, 2010). On the other hand, the high investment costs and lack of regulatory framework have delayed the widespread use of this technology at an industrial scale (Pereira & Vicente, 2010). Nonetheless, OH equipment suitable for operating in the industry's production lines is currently commercially available.

Considering the OH advantages and the demand for processing technologies that can maintain chestnuts' quality as similar to fresh as possible, this study aimed at evaluating the use of OH technology for the post-harvest treatment of chestnuts. The impact of OH on the chestnuts' shelf-life and physicochemical and nutritional properties was evaluated and compared with untreated fruits and chestnuts treated by the conventional hydrothermal treatment (HT), currently used in the chestnut industry.

Material and Methods

Chestnuts Sampling

Chestnuts (*Castanea sativa* Miller) from the Portuguese variety Martaínha were obtained in a local wholesaler unit (Frusantos, SA) from Sernancelhe, PDO of Soutos da Lapa, north of Portugal, in October 2018. Chestnuts with no apparent defects were harvested manually, packed in net bags by the wholesaler, and transported fresh to the laboratory.

Post-harvest Processing of Chestnuts

The fresh chestnuts, with an approximate average weight of 11.2 g, were submerged in water, and the floating fruits were then discarded since it meant they were rotten, dehydrated, or immature, and thus unsuitable for use in this study. Subsequently, chestnuts were dried for 5 min with pressurized air at room temperature and divided into lots to be processed differently. One lot was used without any treatment and is described as "untreated" control. A second lot was then submitted to HT, which implied dipping the chestnuts in a 200 ppm sodium hypochlorite solution at a temperature of 50 °C for 45 min with magnetic agitation. The fruits were then immersed in cold water for 15 min and dried for 5 min with pressurized air at room temperature, which allowed to remove excess water from the chestnut's shell. Finally, a third lot of chestnuts was submitted to OH at different temperatures (35, 45, and 55 °C). The treatments were conducted in an ohmic reactor (8 cm diameter and

25 cm height) containing 500 mL of 0.02 M NaCl and 200 ppm of sodium hypochlorite under constant magnetic stirring (Fig. 1). The electrical conductivity of chestnuts and NaCl solution was calculated according to Tulsiyan et al. (2008), being approximately 2.02 mS/cm and 2.23 mS/cm at 25 °C, respectively. OH was performed using a digital function generator (1 Hz–25 MHz and 1–10 V; Agilent 33220A, Penang, Malaysia) to produce a sinusoidal electric wave of small peak voltage. The generated electric wave was then amplified (Peavey CS3000, Meridian, MS, USA) and delivered to the ohmic reactor. A portable oscilloscope (ScopeMeter 125/S, Fluke, WA, USA) was used to measure the electrical frequency, voltage, and current intensity during the OH treatments. The applied moderate electric field was 9 V/cm with an electrical frequency of 25 kHz. Two temperature probes (Type-K thermocouple ±1 °C, Omega Engineering, Inc., Stamford, CT, USA) were used during the process. One was placed in the geometric center of one chestnut, while the other was put in the surrounding water (Fig. 1). Both probes were connected to a data logger (USB-9161, National Instruments Corporation, Austin, TX, USA) to record the treatments' temperature using Lab View 7 Express software (National Instruments, NI Data logger). The OH process was stopped as soon as the target temperature was reached in the fruits' geometric center. At that point, the water temperature exceeded the temperature registered in the chestnut center in ± 10.5 °C. On average, the times taken to achieve target temperatures of 35 °C, 45 °C, and 55 °C were 2.00, 2.83, and 3.33 min, respectively. After the OH treatment, the chestnuts were cooled with cold water and dried for 5 min with pressurized air at room temperature to remove excess water. For both treatments, batches of 5 chestnuts were processed until 10 kg of treated fruits were obtained for each condition.

Analysis Performed Before and Immediately After Each Treatment

Weight Gain Determination

Weight gain of treated chestnuts was evaluated by weighing the chestnut fruits before and immediately after the treatments. Weight gain was expressed as a percentage of the initial chestnuts' weight and represents the percentage of water incorporated into the chestnuts during each treatment.

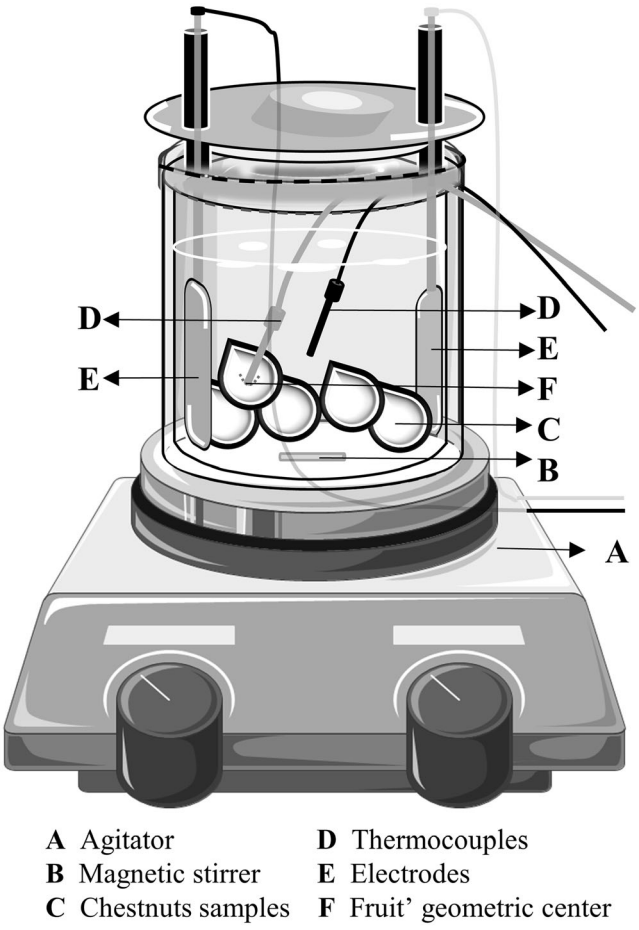

Fig. 1 Schematic view of the ohmic heating reactor

Proximate, Starch, Sugars, and Ascorbic Acid Analysis

Sample Preparation

Before and immediately after each treatment, 1 kg of chestnuts was taken randomly from each lot. Then, the chestnuts were peeled, placed on trays, frozen at − 80 °C (Thermo Scientific, Forma 8600, USA), and lyophilized for 30 h (Christ alpha 1-4 LDplus, Germany). The lyophilized chestnuts were pulverized using a grinder (Taurus, Aromatic II, Portugal) and were sifted to obtain 40 mesh powder. Finally, the chestnut flours were vacuum packaged and stored at room temperature for subsequent analysis. All the analyses were carried out in triplicate.

Proximate Composition

The samples were analyzed for proximate composition (moisture, fat, ash, and protein) using the procedures previously described by Gonçalves et al. (2010). Moisture content was evaluated by drying 2.5 g of chestnut kernels in an oven at 105 °C (Thermo Electron, T6 Heraeus) until constant weight. The total content of lipids was obtained by Soxhlet extraction (Soxtec 8000, Foss) using petroleum ether (ChemLab, CL00.1608). Ash content was determined after incineration of 1.5 g of dried samples in a muffle furnace at 550 °C (Nabertherm, D2804). Total protein content was determined by the Kjeldahl method (Kjeltec 8400, Foss) using the conversion factor of 5.30, which is specific for chestnut fruit (Pereira-Lorenzo et al., 2006).

Total and Resistant Starch Determination

Total starch was determined using the assay kit amyloglucosidase/α-amylase method (AMG/AA) from Megazyme (Ireland). The analysis was performed according to the instructions supplied by the manufacturer. The calculations were done using the Mega-Calc™ Excel® based calculator (AMG/AA), also provided by Megazyme. Resistant starch, defined as the starch which is not digested within 4 h, was determined using the assay kit digestible starch/resistant starch (K-DSTRS) from Megazyme (Ireland). The analysis was performed according to the manufacturers' instructions and the calculations using the Megazyme Mega-Calc™ Excel® based calculator (K-DSTRS). Both types of starch content were expressed as a mass percentage of lyophilized material.

Soluble Sugars Determination

The sample preparation for soluble sugars determination was performed according to the method described by Hou et al. (2014) with some modifications. Briefly, a sample of lyophilized chestnut powder (1.0 g) was dissolved in 10 mL of 80% (v/v) aqueous ethanol and heated at 80 °C in a water-bath with magnetic agitation for 30 min. The resulting suspension was cooled, centrifuged at 2500*g*, 4 °C, 20 min (Heraeus, Multifuge X3R, Portugal), and the supernatant filtered through 0.22 μm cellulose membrane and then refrigerated until analysis. After that, soluble sugar was performed through the anthrone-sulfuric acid assay, as reported by Leyva et al. (2008). The absorbance was determined at 620 nm in a spectrophotometer microplate reader (Synergy HT, Biotek, USA) using distilled water as blank. A six-point calibration curve was prepared using a standard glucose solution (0.020 to 0.40 g/L), and the content of total soluble sugars was expressed as g glucose/100 g dry weight.

Ascorbic Acid Determination

The analysis of vitamin C was performed using the ascorbic acid assay kit (K-ASCO) from Megazyme (Ireland). The calculation of L-ascorbic acid content was performed using the Megazyme Mega-Calc™ Excel® based calculator (K-ASCO). The L-ascorbic acid content was expressed as mg/kg of lyophilized material.

Nutrients and Vitamin C Retention

The nutrient and vitamin C retention based on the fruit's edible part after each treatment were calculated using Eq. (1) (Bogna & Piekarski, 2000).

$$Retention\% = \frac{nc/100g\,ts}{nc/100g\,fs} \times \frac{swap\,(g)}{swbp\,(g)} \times 100 \quad (1)$$

where *nc* is the nutrient content; *ts* is the treated sample; *fs* is the untreated sample; *swap* is the sample weight after processing; *swbp* is the sample weight before processing.

Cooking Value

The degree of cooking, expressed in terms of the cooking value (*C*) induced by each treatment, was calculated according to Ling et al. (2015) using Eq. (2).

$$C^{Z}_{Tref} = \int_0^t 10^{(T-Tref)/z_Q}\, dt \quad (2)$$

where *dt* is the differential time used in the processing of chestnuts, *Tref* the cooking reference temperature (100 °C), Z_Q the reference cooking temperature increase (33 °C) that induces a 10-fold increase of the overall rate of the involved chemical reactions. The processing time and temperature were measured in the chestnut center by the thermocouple, as described before.

Shelf-Life Studies

Before and immediately after the chestnuts' post-harvest processing, the obtained lots (identified as untreated, HT, OH-35 °C, OH-45 °C, and OH-55 °C) were divided into two and stored separately under different atmospheric conditions: (i) at 5 °C and 70% RH (to simulate the wholesaler refrigerated storage chambers) and (ii) at 25 °C and 40% RH (to simulate the wholesaler storage at room temperature). Chestnuts were placed in open plastic boxes and stored for 60 days in those conditions using incubators (Binder, KBF 115, Germany) with automatic temperature and RH control. The temperature and RH of the different storage conditions were monitored along with the shelf-life using an iButton data logger. The microbiological (bacteria and molds) and weight loss analyses were done before and immediately after the chestnuts treatments (0 days of storage) and within 8-day intervals of storage. The visual quality of chestnuts was assessed at 0, 30, and 60 days of storage, while their color and texture were measured on days 0 and 60. The analyses were performed as explained below.

Microbiological Analyses

The microbial load of five chestnuts was determined as follows: in a laminar flow chamber (Scanlaf Mars EB7, Denmark), using sterilized instruments, the whole fruits were cut into small pieces and added to 350 mL of sterile buffered peptone water (1.07228, Merck). The mixture was shaken for about 30 min and serially diluted up to 10^5 using the same peptone solution. Adequate dilutions were plated on Plate Count Agar (PCA, PanReac AppliChem) and Rose-Bengal (RBC, PanReac AppliChem) agar plates in triplicate and incubated for 2–8 days in the dark at 30 °C and 25 °C, respectively (Blaiotta et al., 2014). The colonies counted were expressed as log CFU per gram of chestnut.

Weight Loss Determination

Weight loss was monitored along the storage period using a technical balance (Kern, ABS 320-4N, Germany) and expressed as a percentage variation relative to the fruits' weight at the beginning of the storage. The weight loss (*WL*) was determined according to Eq. 3:

$$WL = (1 - W_t/W_0) \tag{3}$$

where W_0 is the initial sample weight, and W_t is the sample weight at time t.

Color Determination

Color parameters were measured using a colorimeter (Chroma meter CR-400, Konica Minolta, Japan) with a D65 light source. The measurements were made on the external (shell) and internal (kernel) parts of the fruits at three different points. Color images of six chestnuts per treatment were captured. The results were expressed according to CIE L^* a^* b^* color space definition. In the CIE $L^*a^*b^*$ color space, L^* is brightness (varying from 0 = black to 100 = white), a^* varies from green ($-a^*$) to red ($+a^*$), and b^* varies from blue ($-b^*$) to yellow ($+b^*$). Also, the color difference (ΔE^*) CIE76 was calculated according to Eq. 4.

$$\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \tag{4}$$

where ΔL^*, Δa^*, and Δb^* are the differences between the color parameter of the samples processed over the storage and the color parameter of the control sample at 0 days of storage.

Texture Determination

Measurements of chestnut texture were done at room temperature using a Texture Analyzer (TA-XT. PLUS/50, Stable Micro System Ltd., UK) and the Exponent software (version 6.0.7.0, Stable Micro Systems Ltd., UK) to record the force-time graphs.

A penetration force test to determine the puncture characteristic on the whole chestnut (kernel with shell) was performed using a 2-mm diameter probe and a load cell of 20 kg. The puncture force was measured in *N* from the penetration curve. The testing velocity was 2.0 mm/s. Tests were conducted on ten chestnuts per treatment.

A compression test was also performed on the chestnut kernel. The texture analyzer was equipped with a probe of 75 mm and a load cell of 50 kg; the height was set to 20 mm, the reserved height to 3 mm, the test speed to 1 mm/s, the hold time to 2 s, the retraction speed to 5 mm/s, the trigger force to 20 g, and the compression percentage to 80%. The maximum compression force (height of the first peak), defined as the firmness parameter, was recorded, and the measures were reported in *N*.

Visual Quality Evaluation

During the storage period, the chestnuts were visually inspected for insect larvae and mold growth, dehydration, and sprouting. At each sampling time, fourteen fruits were cut in half and examined, both externally and internally. The degree of these occurrences was determined using a qualitative scale (Fig. 2). This scale covered the absence (-), some occurrence (±), or the heavy occurrence (+) of each referred

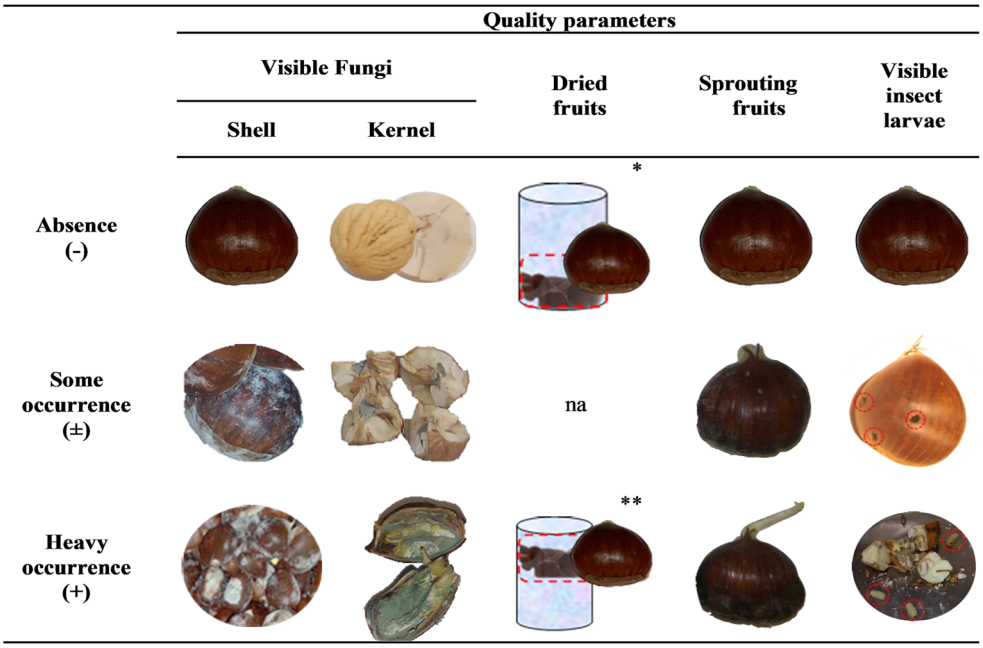

Fig. 2 Qualitative parameters of visual inspection of chestnuts' shell and kernel. *Chestnuts that went to the bottom after being submerged in water were not dried; **chestnuts that floated after being submerged in water were dried; na – not applicable

parameter (presence of insect larvae, visible molds, fruits dehydration, and sprouting). The dehydration was assessed by immersing chestnuts in water, as already explained previously.

Statistical Analysis

The results were compared by analysis of variance (ANOVA) and Tukey's test at a significance level of 5% using the GraphPad Prism v6. Ink software. The data reported are expressed as the average of triplicate observations ± standard deviation.

Results and Discussion

Effects of Treatments on Chestnuts' Chemical Properties and Cooking Value

The proximate chemical composition, resistant starch, and vitamin C contents of untreated and treated chestnuts' kernels are shown in Table 1. Moisture (46.1%) and carbohydrates (88.2 g/100 g) are the most abundant components of the edible part of Martaínha chestnuts. The crude protein, fat, and ash represent less than 11.8 g/100 g of the fruit. Among carbohydrates, starch accounted for 71.0 g/100 g and soluble sugars

Table 1 Proximate composition (g/100 g in dry weight) of chestnuts' kernel before and immediately after the treatments

Parameters	Treatments				
	Untreated	Hydrothermal	OH-35 °C	OH-45 °C	OH-55 °C
Moisture*	46.1 ± 0.7^{a}	51.6 ± 0.4^{b}	46.2 ± 0.9^{a}	47.0 ± 0.3^{a}	49.8 ± 0.2^{c}
Carbohydrates					
Starch	71.0 ± 0.5^{a}	67.0 ± 0.2^{b}	68.3 ± 0.7^{c}	68.1 ± 0.2^{cd}	67.3 ± 0.1^{bd}
Soluble sugars	17.2 ± 0.1^{a}	22.3 ± 0.3^{b}	20.2 ± 0.2^{c}	20.1 ± 0.40^{c}	21.4 ± 0.1^{d}
Resistant starch	24.6 ± 0.3^{a}	24.7 ± 0.5^{a}	28.1 ± 0.2^{b}	23.4 ± 0.5^{a}	24.0 ± 0.6^{a}
Crude protein	5.5 ± 0.1^{a}	4.7 ± 0.1^{b}	5.1 ± 0.0^{ab}	5.1 ± 0.0^{ab}	5.0 ± 0.1^{ab}
Crude fat	4.6 ± 0.4^{a}	4.0 ± 0.2^{b}	4.6 ± 0.1^{a}	4.8 ± 0.2^{a}	4.6 ± 0.1^{a}
Ash	1.7 ± 0.1^{a}	2.0 ± 0.2^{a}	1.8 ± 0.1^{a}	1.9 ± 0.1^{a}	1.7 ± 0.1^{a}
Vitamin C**	45.1 ± 0.1^{a}	39.9 ± 0.2^{b}	44.2 ± 0.1^{ac}	43.6 ± 0.3^{c}	41.5 ± 0.1^{d}

Values are expressed as means ± standard deviation (SD)

* Value is presented as a percentage on a wet weight base

** Data is presented in mg/kg on a dry weight base

Means with different superscript letters in a row are significantly different ($p < 0.05$). Untreated – not processed, Hydrothermal – conventional treatment, OH – ohmic heating

for 17.2 g/100 g. Additionally, 24.6 g/100 g of the edible part was resistant starch. This proximate composition agrees with data previously reported by Gonçalves et al. (2010) for Martaínha chestnuts, who found moisture contents of 50.0%, and quantities of ash, protein, and fat of 1.7, 4.5, and 3.8 g/100 g, respectively. The results obtained in this study also agree with data previously reported by Wang et al. (2020) and Barreira et al. (2012), who found values of moisture between 44.7 and 50.0% for Chinese and Turkish chestnuts, respectively. As already mentioned, the fruit's high moisture content is a determinant parameter for its post-harvest storage because it can potentiate molds' proliferation and decay. On the other hand, the carbohydrates content of Turkish and Portuguese chestnuts have been estimated in 91.0–94.0 g/100 g by Barreira et al. (2012) and Cruz et al. (2013); and the total starch content in 71.1–93.2 g/100 g by Li et al. (2016), Cruz et al. (2013), and Neri et al. (2010). In what concerns the resistant starch, values of 17.2–30.6 g/100 g were reported by Cruz et al. (2013) and Correia et al. (2012). Regarding the other components, values of crude protein (4.8–6.2 g/100 g), fat (2.5–5.9 g/100 g), and ash (1.9–2.3 g/100 g) have been previously reported (Barreira et al., 2012; Gonçalves et al., 2010; Hou et al., 2014; Neri et al., 2010). As for vitamin C, the contents found in this study (i.e., 39.9–45.1 mg/kg) are similar to values (12.8–47.5 mg/kg) obtained previously by Ribeiro et al. (2007).

In regard to changes that the processing methods may have induced, significant differences ($p < 0.05$) were observed in moisture, crude protein, soluble sugars, and vitamin C after some treatments. In particular, it was found that chestnuts' moisture content increased significantly by 5.5% after the HT treatment. This increase can be attributed to the long immersion time in hot water (45 min) that is necessary to treat the fruits. The OH treatments also influenced the final moisture percentages of the fruits but to a lesser extent; increases of 0.1, 0.9, and 3.7% for OH-35 °C, OH-45 °C, and OH-55 °C were respectively observed. The differences were only significant in OH-55 °C treatment. Additionally, regarding the HT samples, the increase of moisture content after the treatment was significantly higher ($p < 0.05$) than those registered after the OH treatments. The obtained moisture values agree with data of Gonçalves et al. (2010), Neri et al. (2010), Silva et al. (2011), and Hou et al. (2018), who reported moisture values between 45 and 58% for chestnuts that were treated with boiling water, cold water, and radiofrequency, respectively.

The total protein content of chestnuts decreased significantly ($p < 0.05$) after the HT treatment at 50 °C for 45 min, suggesting some protein denaturation. The results agree with Gonçalves et al. (2010), who reported a 3.5% decrease in chestnuts' protein content after being boiled for 20 min. On the other hand, the OH treatments did not show a statistical difference ($p > 0.05$) in crude protein compared to untreated and HT samples. The crude fat content also showed a significant difference ($p < 0.05$) after the HT processing, decreasing 0.6% compared to untreated samples. The fat content of OH samples was also significantly different ($p < 0.05$) from the HT samples, but not from the untreated ones. This behavior differs from the decreasing trend observed by Hou et al. (2014) in Chinese chestnuts when radiofrequency treatments were applied at 55 °C for 5 min with an electrical frequency of 27.12 MHz.

In what concerns carbohydrates, a significant decrease ($p < 0.05$) of starch content and a proportional increase of soluble sugars was observed in the treated chestnuts compared with untreated ones. This change was more pronounced in the HT- and OH-55 °C–treated chestnuts. The observed differences after the treatments can be attributed to starch degradation due to the temperature and treatment time conditions (Kan et al., 2016). These results agree with Correia et al. (2009), who found that sugar content increased with the processing temperature (40–60 °C), being this behavior more evident for temperatures of 50–60 °C.

In this study, the treated chestnuts had a starch content of 67.0–68.3 g/100 g, while untreated chestnuts had 71.0 g/100 g. On the other hand, the soluble sugars were 20.1–22.3 g/100 g in processed fruits, while the original value was 17.2 g/100 g. The soluble sugar contents found in this study are similar to data (19–22 g/100 g) previously reported by Carocho et al. (2012) and Fernandes et al. (2011a) after using electron beam and gamma radiation as processing methods. On the other hand, the sugar contents obtained were higher than the 10.0 g/100 g obtained by Hou et al. (2014) when using radiofrequency on Chinese chestnuts. It should also be highlighted that 23.4–24.7 g/100 g of the chestnut's edible part is resistant starch. The registered values for treated and untreated samples were not statistically different ($p > 0.05$).

The vitamin C content was also affected by the temperature and processing time. The highest decrease of 5.2% ($p < 0.05$) was observed in chestnuts treated by HT. Regarding OH treatments, a significant decrease ($p < 0.05$) was also registered for the OH-45 °C (1.5%) and OH-55 °C samples (3.6%). However, the treatment OH-35 °C had a decline of only 0.9%, which was not statistically significant ($p > 0.05$) compared to untreated samples. This loss can be correlated with the well-known vitamin C degradation susceptibility to temperature (Castro et al., 2004; Vikram et al., 2005). Ribeiro et al. (2007) reported substantial losses of ascorbic acid when chestnuts were roasted (46.6–66.0%), boiled (42.3–55.1%), or fried (58.4–59.5%), but these processes use higher heating temperatures and thermal loads (100–200 °C for 7–40 min). On the other hand, Vikram et al. (2005) and Castro et al. (2004) reported that OH treatments between 50 and 100 °C did not affect ascorbic acid levels of orange juice and strawberry products.

The retention of chestnuts' nutrients after OH processing was, overall, higher when compared to the HT treatment. The

OH-treated chestnuts suffered minimum damage and retained almost all their nutrients (92.4% for OH-35 °C, 90.7% for OH-45 °C, and 90% for OH-55 °C). The nutrients retention results are also supported by the calculated cooking values, which were substantially lower for OH-treated chestnuts (C = 0.9 for OH-35 °C, 1.8 for OH-45 °C, and 2.9 for OH-55 °C) than for the HT-treated fruits (C = 3.5). Thus, in general, one can conclude that OH did not substantially change the original nutritional characteristics of the chestnuts. These minimal changes may be due to the rapid, uniform, and internal heating generated by the moderate electric field technology. Other technologies as gamma and electron beam irradiation, when applied at 1 kGy, did not cause substantial changes in chestnut composition either (Carocho et al., 2014). However, according to Kwon et al. (2004), irradiation doses below 3 kGy do not have an immediate effect on the elimination of chestnut insects, which may lead to microbiological safety problems during storage.

Shelf-Life Studies

Fungi growth and weight losses due to dehydration are two of the major concerns in chestnut's post-harvest preservation since they may affect their shelf-life and, consequently, lead to severe economic losses.

Microbial Analysis

In chestnut samples, bacteria CFU were not detected before or immediately after treatments. Moreover, the samples did not show any bacteria CFU during the shelf-life study in both storage conditions (data not shown). Similar results were found by Blaiotta et al. (2014), who reported the absence of bacteria on Italian chestnut samples processed by water curing for 6 days. Several authors also consider that fungi rather than bacteria are the most significant chestnut post-harvest problem (Botondi et al., 2009; Fernandes et al., 2011a; Prencipe et al., 2018).

The fungal growth trend of untreated and treated chestnuts along the 60 days of storage under room and refrigerated temperatures is shown in Fig. 3. The results show significant differences ($p < 0.05$) in fungi counts in all the treatments compared with untreated samples.

At the beginning of the chestnut shelf-life study (day 0), untreated samples revealed fungi (3.8 Log CFU/g), although visual fungal growth was not observed. The obtained values agree with the 3.7 Log CFU/g found by Fernandes et al. (2020a, b) in untreated chestnuts. The chestnut microbiota is acquired in the field, especially during the harvesting period since farmers gather the chestnuts from the soil after they fall naturally off the trees. Additionally, chestnuts' microbiota may suffer considerable changes if fruits are not rapid and adequately processed and stored.

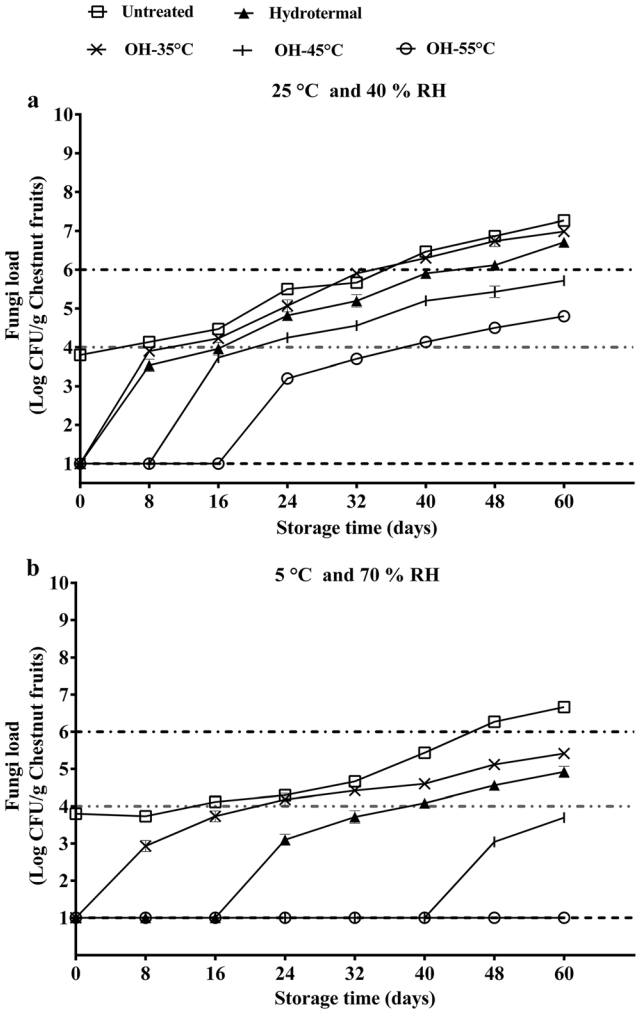

Fig. 3 Chestnuts' fungal load over the 60 days of storage under the different atmospheric conditions tested. Values = 1 correspond undetectable fungi (- - -). Values < 4 correspond to the absence of visible molds (– · · –). Values between 4 and 6 correspond to some occurrence of visible molds. Values ≥ 6 correspond to the heavy occurrence of visible molds (– · –). Untreated – not processed, Hydrothermal – conventional treatment, OH – ohmic heating

In Fig. 3, it can also be observed that HT and OH treatments can reduce the initial fungal load of chestnuts, as no fungal counts were detected immediately after processing (day 0). In OH samples, the CFU decreases cannot just be only explained by the Joule effect because the temperature used in the OH-35 °C treatment is non-lethal for fungi. According to Dix & Webster (1995), most fungi have optimal growth temperatures between 25 and 30 °C and can tolerate temperatures from 5 to 35 °C. However, previous studies have shown that moderate electric fields (< 1000 V/cm) can inactivate some vegetative cells of microorganisms at room temperature (Machado et al., 2010). From a practical point of view, it may not be interesting to treat chestnuts by OH-35 °C, but the results are useful to demonstrate that even mild OH treatments promote fungal inactivation due to the presence of moderate electric fields.

Although no CFU could be detected on day 0, fungi have been detected later on during the fruits' storage. This means that some fungal structures (most likely, spores) could withstand the treatments and developed during the storage period. Therefore, the treatments' efficacy can be correlated with the number of storage days during which the chestnuts remained without CFU. Thus, the less effective treatment was OH-35 °C since fungi could be detected in chestnuts right after 8 days of storage, whether at 25 °C or 5 °C (3.9 and 2.9 Log CFU/g, respectively). On the other hand, the most effective treatment was OH-55 °C, because no CFU were detected in chestnuts preserved at 5 °C during the 60 days' storage period; and because at 25 °C, CFU (3.2 Log CFU/g) started to appear only after 24 days. Chestnuts treated by OH-45 °C showed a microbiological evolution in between those registered for OH-35 °C and OH-55 °C treatments. When stored at 5 °C, CFU were only detected after 48 days (3.0 Log CFU/g), while when stored at 25 °C, they were detected right after 16 days (3.7 Log CFU/g). In what concerns HT, CFU of fungi were detected right after 8 days (3.5 Log CFU/g) when chestnuts storage was done at 25 °C, and only after 24 days (3.1 Log CFU/g) when they were stored at 5 °C. In this sense, HT registered a CFU profile similar to OH-35 °C when the chestnuts were stored at room temperature, but at 5 °C, HT performed better than OH-35 °C.

In summary, chestnuts treated by OH-55 °C and stored at 5 °C and 70% RH maintained no detectable fungi for more 36 days than HT-treated fruits, increasing their shelf-life considerably. Other technologies did not perform so well. For example, Vettraino et al. (2020) observed that chestnuts' fungal population was not fully controlled by gaseous ozone treatments even when the fruits were stored at 2 °C. Also, despite γ-rays and electron beam irradiation capability to eliminate microbes on chestnuts (Zhu, 2016), the total elimination of fungi is only obtained with doses between 3 and 5 kGy (Antonio et al., 2012), and according to Carocho et al. (2014), undesirable physicochemical changes are observed on chestnuts when irradiation doses >1 kGy are used.

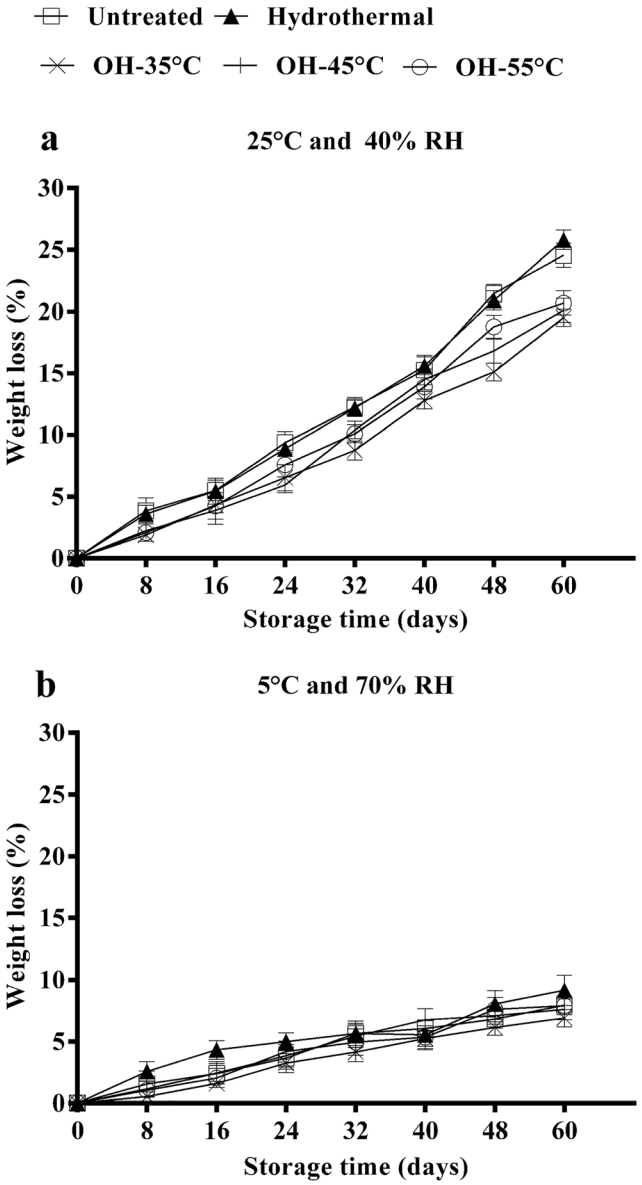

Fig. 4 Weight loss of the chestnuts over the 60 days of storage under different atmospheric conditions. Untreated – not processed, Hydrothermal – conventional treatment, OH – ohmic heating

Weight Loss

The weight loss of untreated and treated chestnuts along the 60 days of storage under room and refrigeration temperature conditions is shown in Fig. 4. The weight loss showed significant differences ($p < 0.05$) between some treatments when the samples were stored at 25 °C, while no significant differences were observed when the storage was done at 5 °C. Also, a gradual weight decrease was observed during storage regardless of the treatment or storage conditions, which means the fruits have dehydrated. However, the weight loss rate was more pronounced in chestnuts stored at 25 °C than at 5 °C. This is due to the combined effects of respiration and transpiration processes of chestnuts, and the temperature and RH of surrounding air. The constant balance between those parameters influences the fruits' moisture content and, consequently, their weight. The loss of weight and dehydration of chestnuts over the storage period was also observed by Zhao et al. (2018) when using 15–25 °C and RH of 20–40%, and by Hou et al. (2014) at 35 °C and 95% RH. Therefore, chestnuts require particular storage conditions with minimal equilibrium RH of 85% (GDV, 1999). However, although high RH can be more efficient in reducing weight loss, it can generate a considerable risk of mold development (GDV, 1999). Still, Vettraino et al. (2019, 2020) found weight losses around 2% per month in Italian chestnuts stored at 2 °C and 95% RH whether treated or not with ozone.

In this study, untreated chestnut samples lost approximately 12% of their weight during the first 30 days of storage at 25 °C and 40% RH, while at the end of 60 days, the weight loss was 24%. It is essential to highlight that weight loss as high as 55% and 82% have been previously reported for Chinese chestnuts when using temperatures between 15 and 25 °C and RH around 20–40% (Wang et al., 2020; Zhao et al., 2018). On the other hand, when chestnuts were stored at 5 °C and 70% RH, the weight loss was around 6% in the first 30 days and 8% after the full 60 days of storage.

The weight loss of HT samples was higher than that of chestnuts treated by OH. Nonetheless, there were no significant differences in weight among the fruits processed with OH (35, 45, and 55 °C). The HT chestnuts suffered weight losses around 12 and 26% after 30 and 60 days of storage at 25 °C, respectively, in a similar trend to that observed for untreated chestnuts. OH-treated chestnuts stored at 25 °C recorded weight losses around 10 and 21% after 30 and 60 days, respectively. In refrigerated conditions, the weight losses were around 6–4% after 30 days and 9–8% after 60 days for HT and OH treatments.

The observed losses of weight indicate that the industry should avoid chestnuts storage at room temperature and, instead, favor their storage on refrigerated conditions with high RH in order to keep the product's characteristics as similar as possible to fresh chestnuts.

Color Measurements

At the beginning of the shelf-life study (0 days), untreated and treated chestnut kernels showed predominantly light and

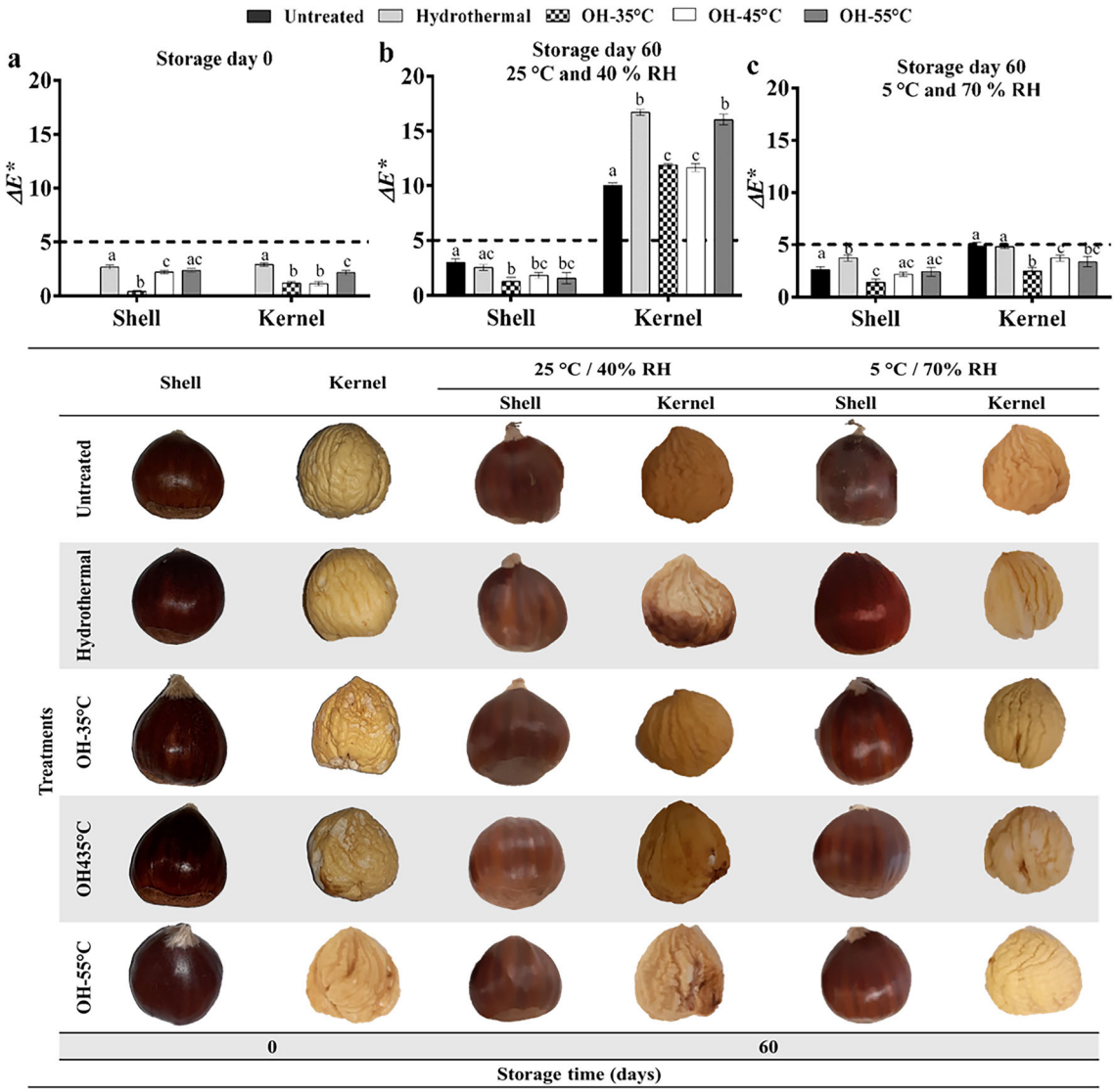

Fig. 5 (a–c) Color difference (ΔE^*) and images of the chestnuts after 0 and 60 days of storage under the different atmospheric conditions. Value $\Delta E^* \geq 5$ indicates substantial color differences that can be perceived by the human eye (Cecchini et al., 2011). Untreated – not processed, Hydrothermal – conventional treatment, OH – ohmic heating. Bars with different letters on the same data set indicate a significant difference between the samples ($p < 0.05$)

bright yellowish color, while the shell showed a bright dark brown color. Due to the difficulty of interpreting the parameters that define color, it was chosen to express the color changes as a color difference (ΔE^*) (Fig. 5). Pictures of untreated and treated chestnuts taken at 0 and 60 days of storage at 5 and 25 °C are also shown. The values of ΔE^* registered immediately after the processing of chestnuts (day 0) were all below 5 (Fig. 5a), regardless of the treatment process, which means that differences were not perceptible to the human eye, according to Cecchini et al. (2011). This indicates that none of the treatments has significantly affected the visual appearance of chestnuts' kernel and shell.

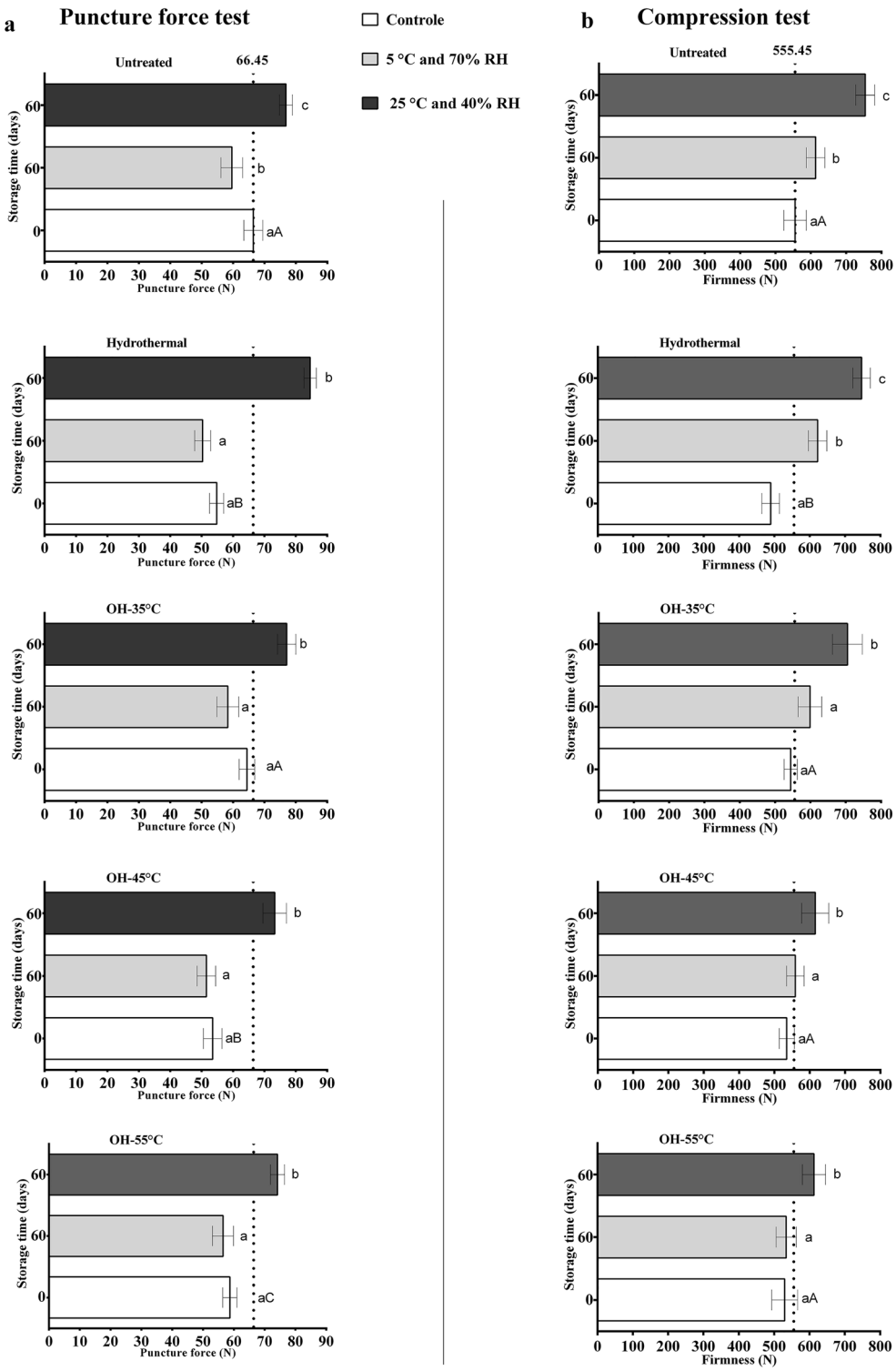

Fig. 6 Texture characterization of the chestnuts after 0 and 60 days of storage under the different atmospheric conditions. The dotted line in the graphs represents the reference value for the untreated chestnut on day 0. (a) The test was performed on the whole chestnut (kernel with shell). (b) The test was performed on the chestnut' kernel. Untreated – not processed, Hydrothermal – conventional treatment, OH – ohmic heating, Control – day 0 for each treatment. Bars with different small letters on the same graph indicate a significant difference between the samples ($p < 0.05$). Bars with different capital letters indicate a significant difference between treatments at 0 days ($p < 0.05$)

On the other hand, the ΔE^* values obtained at the end of the 60 days of storage showed an increasing trend, regardless of treatments and storage conditions (Fig. 5b and c). ΔE^* values > 5 were registered on the chestnuts' kernels stored at 25 °C and 40% RH. Specifically, the kernel became dark yellow without brightness. The results also show that the kernels' color changes were much more perceptible visually than those observed on the chestnuts' shell. Zhao et al. (2018) reported a similar behavior on Chinese chestnuts stored at 15–25 °C and 20–40% RH, with a color change from bright to dark yellow. Additionally, if we compare Fig. 5b and c, it is clear that storage under refrigerated conditions prevents changes of color on chestnuts' kernels, as the ΔE^* values of chestnuts stored at cold conditions were below 5, independently of treatment done, while those stored at 25 °C were all beyond 10.

Texture Measurements

Despite the hardness of the shell and kernel of fresh chestnuts, this fruit needs careful handling during harvesting and post-harvesting stages to avoid damage and future fruit decay. The hardness of the fruits is an essential quality parameter for consumers.

At the beginning of the shelf-life study (0 days), untreated chestnuts showed a high turgidity, registering high values of puncture force (66.5 N) and compression (555.5 N). On the other hand, immediately after the treatments, the puncture force applied to the chestnut samples showed lower values than in untreated samples (Fig. 6a), which implies that chestnuts suffered some kind of softening. The more significant change was recorded for HT samples, while the slightest change was recorded for OH-35 °C. Thus, the fruit's softening increased with the temperature and processing time, probably due to increased cooking value.

In what concerns the compression tests applied immediately after the treatments (Fig. 6b), no significant differences ($p > 0.05$) were observed between OH-treated chestnuts and untreated ones, but some softening was registered for HT-treated samples. These changes could also be attributed to the fruits' cooking value in both situations since they may have changed chestnut starch properties. Kan et al. (2016) also reported the softening of chestnuts' kernels treated with hot water. The kernel's hardness decreased as the cooking degree increased; thus, texture parameters could be measured as a function of the moisture and total starch contents.

The values recorded for the puncture and compression tests for all the treatments studied shown significant differences ($p < 0.05$) and an increasing trend when the samples were stored at room temperature conditions. Visually, the chestnut kernels shrank and hardened due to dehydration phenomena, which justifies the higher puncture and compression forces. The results obtained agree with Kan et al. (2016), who reported that chestnuts' hardness increases as the moisture content decreases; and with Zhao et al. (2018), who observed that chestnuts rapidly lost moisture at room temperature, causing the drying and stiffness of kernels. On the other hand, when the chestnuts were stored in cold conditions, they could maintain their turgescence and texture characteristics more similar to untreated fruits. This behavior could be attributed to the delay of the fruit maturation process due to the low temperature and the higher RH at storage.

Table 2 Result of chestnuts' visual inspection after 0, 30, and 60 days of storage under the different atmospheric conditions tested

Storage conditions	Treatments	Parameters/storage time (days)														
		Visual molds						Dried			Sprouting			Insect larvae		
		Shell			Kernel											
		0	30	60	0	30	60	0	30	60	0	30	60	0	30	60
25 °C and 40% RH	Untreated	-	±	±	-	±	+	-	±	+	-	±	+	-	+	-
	Hydrothermal	-	±	±	-	±	+	-	±	+	-	-	-	-	+	-
	OH-35 °C	-	±	±	-	±	+	-	±	+	-	-	-	-	+	-
	OH-45 °C	-	-	±	-	±	±	-	±	+	-	-	-	-	-	-
	OH-55 °C	-	-	±	-	-	±	-	±	+	-	-	-	-	-	-
5 °C and 70% RH	Untreated	-	-	±	-	±	+	-	-	±	-	-	±	-	±	-
	Hydrothermal	-	-	±	-	-	±	-	-	±	-	-	-	-	±	-
	OH-35 °C	-	-	±	-	±	±	-	-	±	-	-	-	-	±	-
	OH-45 °C	-	-	±	-	-	±	-	-	±	-	-	-	-	-	-
	OH-55 °C	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-

(-) absence; (±) some occurrence; (+) heavy occurrence. Untreated – not processed, Hydrothermal – conventional treatment, OH – ohmic heating

Visual Quality Assessment

The visual quality assessment of chestnut samples at 0, 30, and 60 days of storage under room and refrigeration temperature conditions are shown in Table 2. In general, the visual defects of chestnut quality increased over the storage period. Before and immediately after the treatments (0 days), the chestnuts were devoid of visible molds, both on shells and kernels, and any insect larvae, sprouting, and dehydration. Similar results were obtained by Hou et al. (2015), who did not found insect larvae immediately after applying radiofrequency treatments to Chinese chestnuts (46–50 °C for 2–8 min).

However, control and chestnuts treated with HT and OH-35 °C showed insect larvae at the end of 30 days regardless of the storage conditions. Migliorini et al. (2010) also detected insect larvae in untreated chestnuts after 30 days of storage. HT- and OH-35 °C–treated samples also evidenced a substantial occurrence of visible molds in the chestnut kernel after 60 days of storage. Vettraino et al. (2019) reported a similar development of fungi on chestnuts during their storage for 150 days at 2 °C after being treated with ozone. Concerning insect larvae and visible molds, untreated chestnuts, HT, and OH-35 °C–treated chestnuts stored at 25 °C were the most affected. In the control treatment, it was also observed some occurrence of sprouting during the first 30 days of storage.

The visual evaluation confirmed that the most effective treatment was OH-55 °C when combined with storage at 5 °C and 70% RH, since no visible molds, sprouting, and insect larvae were observed during the 60 days of storage. Other conservation technologies as γ-rays irradiation can also eliminate insects on chestnuts. According to Kwon et al. (2004), chestnuts' irradiation at 0.5 kGy showed 100% of insect mortality after 3 to 4 weeks of storage. However, immediate insect mortality of 100% is only achievable with 3 or more kGy, and stored chestnuts suffer a significant change of color when the irradiation doses were superior to 1 kGy.

Conclusion

This study concludes that the microbiological quality and the shelf-life of chestnuts are strongly dependent on the post-harvest technology used in their treatment and clearly shows the importance of the processing parameters and storage conditions such as temperature and RH. OH is an alternative processing technology where heat is generated directly within the chestnut samples (rapid volumetric heating), resulting in the elimination of the problems associated with heat transfer. The microbiological results of OH treatments showed that the application of moderate electric fields for a few minutes at mild temperatures can reduce the fungal burden in chestnuts. The OH treatment performed at 55 °C in combination with storage at 5 °C and 70% RH proved to be an effective alternative to the conventional HT, currently used by the chestnut industry. With the OH-55 °C treatment, no fungal decay was observed during the 60-day storage period, and the shelf-life of chestnuts was extended by 36 days when compared to HT. This method also presents clear advantages at the industrial level compared to HT since the chestnuts' treatment time can be reduced to 3.3 min, allowing considerable energy savings. Additionally, the physicochemical characteristics of chestnuts treated by OH-55 °C and stored for 60 days showed that fresh fruits' quality can be preserved. Quality parameters such as retention of nutrients, texture, and fruits' color immediately after the treatment were not significantly different from untreated chestnuts. Moreover, fungi, insect larvae, and sprouting were absent. These results encourage further studies to assess the industrial application of OH in the post-harvest treatment of chestnuts.

Author Contribution Enrique Pino-Hernández: investigation, formal analysis, visualization, writing – original draft.
Ricardo N. Pereira: conceptualization, methodology, writing – review and editing.
Lina F. Ballesteros: investigation, writing – review and editing.
António A. Vicente: conceptualization, resources, writing – review and editing.
Luís Abrunhosa: conceptualization, methodology, writing – review and editing, supervision.
José A. Teixeira: funding acquisition, writing – review and editing, supervision.

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Declarations

Conflict of Interest The authors declare no competing interests.

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