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Effect of different packaging materials on the shelf-life of chestnut (*Castanea sativa* Mill)

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ABSTRACT

Chestnuts are very consumed in Portugal and worldwide; however, it is a perishable product that loses weight and spoils quickly. So, it is crucial to find methods to reduce economic losses. Trying to find answers to this problem, the purpose of the present research work was to evaluate the effect of using different packaging materials in chestnuts storage to increase their shelf-life. Two storage assays were performed, one during six-weeks (short-storage), and other during six months (long-storage). In the first assay, different packagings were tested, namely: polyethene packaging ”POLY”, polyethene packaging with holes “PH”, Modified Atmosphere Packaging ”MAP”, Vacuum bags “VAC-bags” and unpackaged chestnuts (“control”). All samples were stored at room temperature during 0, 2, 4 and 6 weeks, to find out the best solution to reduce the industrial losses of chestnuts during their selling process in supermarkets (retail conditions). In the long-storage assay, micro-perforated bags (MP-bags) were used in chestnuts storage in refrigerated industrial chambers for 1, 2, 3 and 6 months. The weight loss, colour, texture, water activity (a_w), moisture content, titratable acidity, total soluble solids, reducing sugars, starch, amylose, and microbiological analysis were determined.

In the short-storage assay, the colour, texture, moisture content, a_w, titratable acidity and total soluble solids were little affected by the type of bags used. Also, the starch ranged from 35.2 to 50.4% dry matter (d.m.) without a specific trend, as well as amylose expressed on the starch basis (25.7 to 45.0%), suggesting no remarkable changes in starch functionality. On the contrary, significant differences were observed between bags in weight loss, reducing sugars and microbial counts. The VAC-, MAP-, and POLY-bags showed percentages of weight loss lower than 2%, while the control and PH-bags had values equal to 13.2 and 9.2%, respectively. The highest values of reducing sugars were observed in POLY- and PH-bags, followed by the control, suggesting partial hydrolysis of the starch. Furthermore, the application of VAC- and MAP-bags caused a considerable decrease in aerobic mesophils, moulds and yeasts growth compared to the control during two and four weeks of storage. After this period, the counts increased probably due to the appearance of microscopic cracks in the bags, as a result of their excessive expansion.

During long-storage, no significant differences were noted in the colour of chestnut kernel between the MP-bags and the control, in the majority of the situations. Furthermore, the texture was also less affected. After six months, the weight loss was much lower in MP-bags (1.9%) than in control (23%). After six months, a significant difference in moisture content was observed between the control and MP-bags (42.7 and 55.2%, respectively), suggesting that the packaged chestnuts retained more the water. Titratable acidity, total soluble solids and starch did not vary significantly between packages. The reducing sugars were significantly influenced by the packaging type and storage time. The highest values were observed in unpackaged chestnuts (control). Along the storage period, some variability, without a definite trend, in the MP-bags, was observed. In control, the contents of reducing sugars increased from 0.09 to 2.06 g glucose/100 g d.m.. For the control and MP-bags, no significant differences in aerobic mesophilic and moulds and yeasts were observed at the end of storage. MP-bags demonstrated to be a promising solution for extending the shelf-life of chestnuts during long storage (6 months) by preserving the nutritional quality of the fruits.

The results of the present study showed that the preservation of the chestnuts by the use of a specific type of packaging might have a substantial impact on preserving the colour and texture of the fruits, on preventing the weight loss and microbial growth and on maintaining the moisture content of the fruits.

Keywords: Chestnuts, *Castanea sativa*, packaging, physicochemical parameters, microbiological analysis, storage.

RESUMO

A castanha é muito consumida em Portugal e no mundo. Contudo, é um produto muito perecível que perde peso e se estraga facilmente. Assim, é muito importante encontrar métodos que permitam reduzir as perdas económicas. Na tentativa de encontrar respostas a este problema, o objetivo do presente trabalho de investigação foi avaliar o efeito de usar diferentes materiais de embalagem no armazenamento da castanha de modo a aumentar o tempo de prateleira. Foram realizados dois ensaios de armazenamento, um durante seis-semanas (armazenamento de curta duração), e outro durante seis meses (armazenamento de longa duração). No primeiro ensaio foram testadas diferentes embalagens, designadamente: sacos de polietileno "POLY", sacos de polietileno com furos "PH", embalagens em atmosfera modificada "MAP", sacos de vácuo "VAC" e sem embalagem ("controlo"). Todas as amostras foram armazenadas à temperatura ambiente, durante 0, 2, 4 e 6 semanas, de modo a encontrar a melhor solução para reduzir as perdas durante o processo de venda no supermercado (condições de retalho). No armazenamento de longa duração, foram utilizados sacos micro-perfurados (sacos-MP), tendo–se armazenado as amostras em câmaras industriais durante 1, 2, 3 e 6 meses. Os parâmetros determinados foram a perda de peso, cor, textura, atividade da água (a_w), teor de água, acidez titulável, sólidos solúveis totais, açúcares redutores, amido, amilose e análises microbiológicas.

No armazenamento de curta duração, a cor, a textura, o teor de água, a a_w, a acidez titulável, e os sólidos solúveis totais foram pouco afectados pelo tipo de embalagem utilizada. O amido variou entre 35,2 e 50,4%, peso seco (p.s.), sem qualquer tendência específica. A amilose expressa em amido (25,7 a 45,0%), sugeriu a ocorrência de poucas alterações ao nível da funcionalidade do amido. Pelo contrário, diferenças significativas foram observadas entre os diferentes tipos de sacos, no que se refere à perda de peso, açúcares redutores e contagens microbianas. Os sacos VAC, MAP e POLY apresentaram percentagens de perda de peso inferiores a 2%, enquanto o controlo e os sacos PH apresentaram valores de 13,2 e 9,2%, respetivamente. Os maiores valores de açúcares redutores foram observados nos sacos POLY e PH, seguidos do controlo, sugerindo uma hidrólise parcial do amido. Adicionalmente, a aplicação de sacos VAC e MAP causaram um decréscimo considerável no crescimento de mesófilos aeróbios, bolores e leveduras quando comparado com o controlo durante as duas ou quatro semanas de armazenamento. Após este período, as contagens aumentaram possivelmente devido ao aparecimento de fissuras microscópicas nos sacos, resultado da sua excessiva expansão.

Durante o armazenamento de longa duração, não foram observadas diferenças significativas na cor interior da castanha entre os sacos-MP e o controlo, na maioria das situações. Além disso, a textura foi pouco afetada. Após seis meses, a perda de peso nos sacos-MP foi bastante menor (1,9%) do que no controlo (23%). Após 6 meses, uma diferença significativa no teor de água foi observada entre o controlo e os sacos-MP (42,7 e 55,2%, respectivamente), sugerindo que as castanhas embaladas retêm mais a água. A acidez titulável, sólidos solúveis totais e o amido não variaram significativamente entre as embalagens. Os açúcares redutores foram significativamente influenciados pelo tipo de embalagem e o tempo de armazenamento. Os maiores valores foram observados nas castanhas não embaladas (controlo). Ao longo do armazenamento em sacos-MP, alguma variabilidade nos valores foi observada, mas sem qualquer tendência definida. No controlo, os teores de açúcares redutores aumentaram de 0,09 a 2,06 g glucose/100g p.s.. No controlo e sacos-MP não foram observadas diferenças significativas nos mesófilos aeróbios, bem como nos bolores e leveduras no final do armazenamento. Os sacos-MP demonstraram ser uma solução promissora para aumentar o tempo de prateleira de castanhas durante o armazenamento de longa duração (6 meses), preservando a qualidade nutricional dos frutos.

Os resultados do presente estudo demonstraram que a conservação da castanha pelo uso de uma embalagem específica pode ter um impacto substancial na preservação da cor e textura do fruto, em prevenir a perda de peso e o crescimento microbiano, e em manter o teor de água do fruto.

Palavras-chave: Castanha, *Castanea sativa*, embalagem, parâmetros físico-químicos, análises microbiológicas, armazenamento.

1 INTRODUCTION

1.1 World and Portuguese chestnuts productions and main cultivars

Chestnuts are globally popular and valued for their sensory, nutritional and health attributes. According to the Statistical Database of the Food and Agriculture Organization (FAO), the total world production of chestnuts is 2 353 825 tonnes. As presented in Figure 1, China is the leader by a total of 1 965 351 tonnes, followed by Bolivia (96 833 tonnes), Turkey (63 580 tonnes) and Korea (53 384 tonnes) (FAO, 2018).

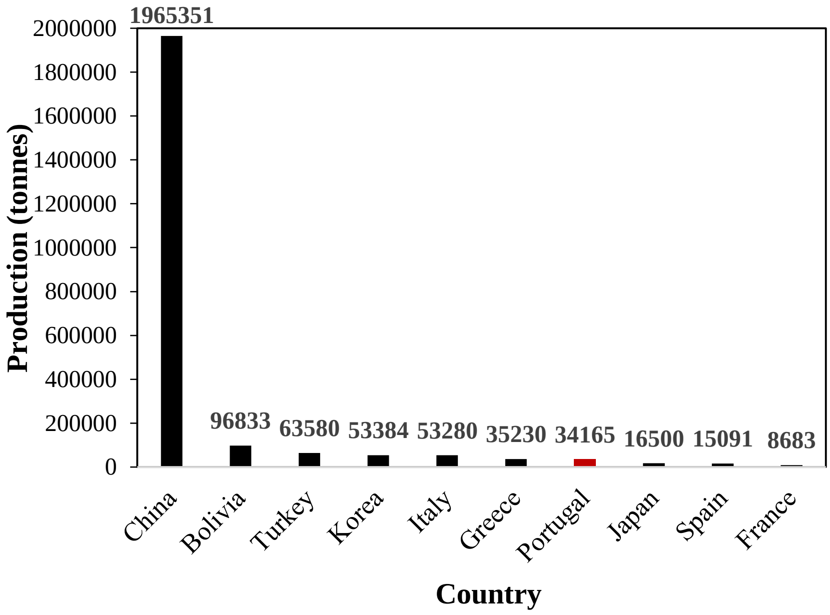

Figure 1: The ten most important countries in chestnut production in the world (Source: FAO, 2018).

Chestnuts are majorly found in Asia, North America and Europe. Several chestnut species exist, being twelve of them described in Table 1. *Castanea sativa* Mill is known as the European chestnut. It is a specie with great importance both economically and ecologically.

Table 1: Different species of chestnuts all over the world
(Source: Mencarelli, 2001).

European	Asian	American
C. sativa	*C. crenata* (Japanese chestnut)	*C. dentata* (Eastern states)
	C. mollissima (Chinese chestnut)	*C. pumila* (Eastern states)
	C. seguinii (China)	*C. ashei* (Southern states)
	C. davidii (China)	*C. floridana* (Southern states)
	C. henryi (China)	*C. alnifolia* (Southern states)
		C. paupispina (Southern states)

In Europe, *C. sativa* is the predominant species and the most consumed. Italy, Greece and Portugal are the head producers of chestnuts in Europe, and this fruit has a relevant role at the socioeconomic scale of these countries. The total production of chestnut in Europe is 154 612 tonnes (FAO, 2018). In Figure 2, the leading European producers are presented.

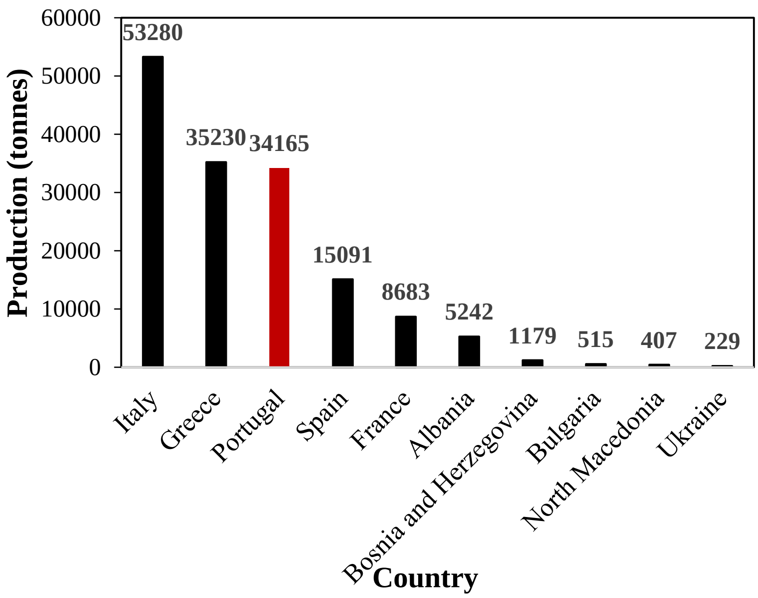

Figure 2: European countries main producers of chestnuts (Source: FAO, 2018).

At the European scale, Portugal is ranking the third place of chestnuts production, representing 22.1% of the total production in Europe.

In Portugal, Trás-os-Montes e Alto Douro is the most important region for chestnut production, generating 85% of the total national output (Borges et al. 2007). In the past, chestnut had an important role in human nutrition in this region; however, the potato substituted it. Nevertheless, in recent years, chestnut consumption has increased due to its nutrient contents and potential beneficial health effects.

1.2 Physicochemical characterization of European chestnuts

Chestnuts differ from other nuts for their low-fat content, which makes them ideally suitable for high complex carbohydrate and low-fat diets. In Table 2, some physicochemical properties of *C. sativa* cultivars from three different European countries are presented.

Chestnuts have a high moisture content (> 45%), explaining the perishability of this nut. Carbohydrates are the main constituent, representing around 90% of the dry matter, followed by proteins (4.5 to 8.2 g/100 g dw). Chestnuts have a low-fat content (<4 g/100g dw) and an energy value around 400 kcal/100g dw.

When comparing chestnuts cultivars (Table 2), some differences between them, mainly in the crude fat and protein contents, can be observed.

Regarding carbohydrates, chestnuts are rich in starch and free sugars, namely monosaccharides (ex. fructose, glucose) and disaccharides (ex. sucrose). In Table 3, the concentrations of sucrose, glucose and fructose are presented for some of the most representative cultivars of chestnuts found in Portugal, showing the existence of heterogeneity between them. In that table, values found in those cultivars but grown in other countries are also indicated.

Table 2: Physicochemical properties of some cultivars of *C. sativa* Mill.

Country	Cultivars	Moisture (%, fw)	Carbohydrates	Crude protein (g/100g d.m.)	Crude Fat	Total Ash	Energy (kcal/100g d.m.)	References
Portugal	Aveleira	49.9±1.6	--	4.87±0.12	2.57±0.06	1.53±0.21	--	Borges et al., 2008
		52.1±1.3	90.2±2.4	6.53±1.02	1.75±0.13	1.52±0.08	402±16	Barreira et al., 2009
	Boa Ventura	54.6±1.0	91.6±2.7	5.04±0.43	1.72±0.16	1.50±0.07	402±13	Barreira et al., 2009
	Demanda	53.3±0.9	--	7.37±0.12	3.10±0.10	1.87±0.21		Borges et al., 2008
	Judia	52.9±0.8	90.1±4.7	5.40±0.20	2.63±0.06	1.90±0.10	--	Borges et al., 2008
		53.3±1.8	91.7±2.6	6.34±0.59	1.73±0.18	1.93±0.11	401±22	Barreira et al., 2009
	Longal	51.6±1.4	--	6.83±0.12	1.97±0.06	1.60±0.00	--	Borges et al., 2008
		51.9±1.0	87.3±0.04[1]	5.14±0.41	1.64±0.15	1.64±0.13	402±12	Barreira et al., 2009
		48.2±0.02	89.9±0.4	5.0±0.07	2.6±0.04	1.9±0.01	--	Correia et al., 2009
	Martaínha	52.3±0.5	--	5.57±0.38	2.50±0.10	1.97±0.35	--	Borges et al., 2008
		47.9±0.05	--	4.3±0.04	3.0±0.04	2.1±0.03	--	Correia et al., 2009
		50.0±0.21	87.1±0.04[1]	4.51±0.04	3.80±0.26	1.76±0.06	412.0±0.7	Gonçalves et al., 2010
	Rebordã	46.3±0.9	--	5.60±0.17	1.73±0.06	2.20±0.20	--	Borges et al., 2008
	Trigueira	47.4±1.7	--	5.30±0.27	2.60±0.00	1.83±0.06	--	Borges et al., 2008
	Zeive	51.6±2.6	--	5.50±0.10	2.73±0.06	2.20±0.20	--	Borges et al., 2008
Spain	15 cultivars	48.37-59.35[2]	--	6.02-8.58	1.26-2.98	1.79-3.10	--	Míguelez et al., 2004
	47 cultivars	40.3-60.1[2]	--	4.5-9.6	1.7-4.0	1.8-3.2	--	Pereira-Lorenzo et al., 2006
	19 cultivars	53.1-57.2[2]	--	5.79-7.87	--	2.00-2.41	--	Peña-Méndez et al.,2008
Italy	Capannaccia	47.86±0.89	--	5.16±0.39	2.93±0.38	2.52±0.03	--	Piccolo et al., 2020
	Carpinese	53.67±0.58	--	4.83±0.58	2.38±0.44	2.59±0.09	--	Piccolo et al., 2020
	Castel del Rio[3]	51.38±0.24	--	5.25±0.04	3.00±0.04	2.38±0.17	--	Neri et al., 2010
	Castel del Rio[4]	52.89±0.18	--	4.55±0.02	3.53±0.06	2.12±0.22	--	Neri et al., 2010
	Marradi[3]	45.37±0.12	--	4.78±0.03	4.64±0.03	1.92±0.14	--	Neri et al., 2010
	Marradi[4]	50.55±0.21	--	4.54±0.00	3.65±0.03	1.88±0.15	--	Neri et al., 2010
	Morona	47.79±1.50	--	4.69±0.32	2.20±0.08	2.25±0.00	--	Piccolo et al., 2020
	Pontecosi	49.71±0.96	--	3.80±0.05	2.86±0.66	2.52±0.10	--	Piccolo et al., 2020
	Valle Castellana[3]	49.40±0.09	--	4.18±0.01	4.38±0.13	1.22±0.16	--	Neri et al., 2010
	Valle Castellana[4]	42.27±0.47	--	4.31±0.00	3.60±0.15	2.44±0.09	--	Neri et al., 2010

d.m. – dry matter.
[1]NFE – Nitrogen free extract (%NFE (d.m.) = 100-(%Protein + %Fat + %Ash + %Fibre)) (Correia et al., 2009). [2]Range of the values. [3]Year 2003. [4]Year 2004.

Table 3: Sugar composition (g/100g d.m.) of the most representative chestnut cultivars found in Portugal

Cultivars (Country)	Fructose	Glucose (g/100g d.m.)	Sucrose	Reference
Aveleira (Portugal)	0.72±0.07	1.10±0.10	22.05±1.48	Barreira et al., 2010
Boa Ventura (Portugal)	5.18±0.39	6.63±0.49	4.03±0.30	Barreira et al., 2010
Judia (Portugal)	0.62±0.05	1.02±0.06	23.30±0.83	Barreira et al., 2010
(Portugal)[1]	0.21±0.25	0.17±0.16	11.32±1.50	de Vasconcelos et al., 2010b
(Portugal)[2]	0.19±0.22	0.22±0.29	11.14±2.33	de Vasconcelos et al., 2010b
Longal (Spain)	0.19±0.11	0.17±0.13	8.96±2.34	Bernárdez et al., 2004
(Spain)	0.19±0.11	0.17±0.13	8.96±2.34	Míguelez et al. 2004
(Portugal)	1.00±0.02	0.73±0.01	27.54±0.00[3]	Correia et al., 2009
(Portugal)	1.81±0.12	2.69±0.23	9.56±0.91	Barreira et al., 2010
(Portugal)[1]	0.25±0.35	0.16±0.17	10.62±1.94	de Vasconcelos et al., 2010b
(Portugal)[2]	0.24±0.35	0.19±0.27	12.98±2.31	de Vasconcelos et al., 2010b
Martaínha (Portugal)[1]	0.15±0.15	0.12±0.10	11.35±1.27	de Vasconcelos et al., 2010b
(Portugal)[2]	0.41±0.59	0.27±0.34	10.18±2.75	de Vasconcelos et al., 2010b

[1]Year 2006 (it includes fruits subjected to different processing steps); [2]Year 2007 (it contains fruits subjected to various processing steps); [3]Sucrose + Maltose (Correia et al., 2009)

1.3 Problems encountered during chestnuts storage and selling

As stated previously, chestnuts present a high moisture content, limiting their shelf-life. Furthermore, chestnuts have high metabolic activity (increased respiration and transpiration rates), causing several problems during their storage and selling.

Chestnut quality is affected by external factors such as surface blemishes, and moulds, which affect consumer acceptance. Also, internal disorders may result from anatomical or physiological changes such as moisture loss, chemical conversion, discolouration, senescence, cell break-down (physiological decay), or microorganism attack and insect injury (Uylaser et al. 2010). Among all these problems, weight loss due to dehydration, infestation by insects and microorganisms development, are the main problems that chestnut producers and industrials face (Cecchini et al. 2011). All these processes decrease the quality of the fruit. Figure 3 shows a rotten chestnut due to fungi proliferation.

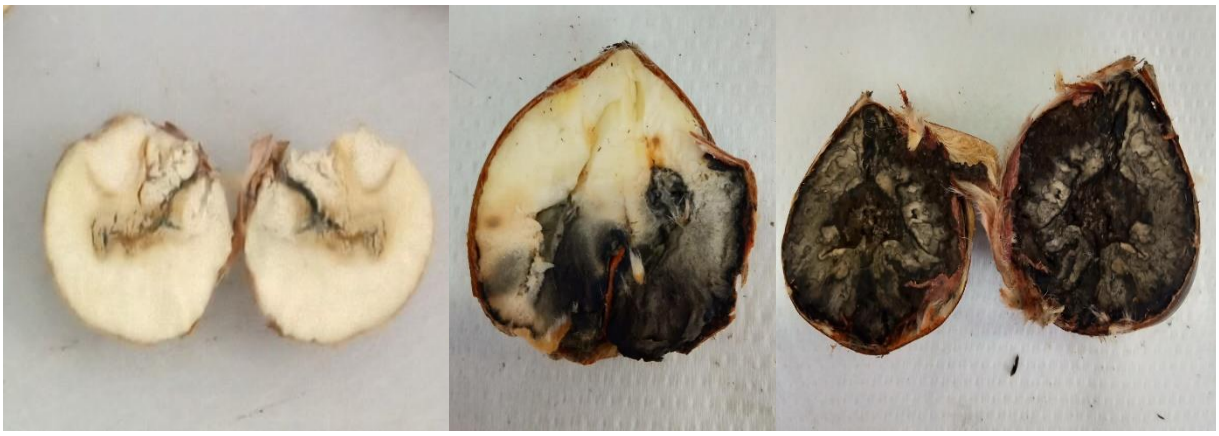

Figure 3: Spoiled chestnuts due to the development of fungi.

By Donis-González et al. (2010), filamentous fungi including *Penicillium expansum*, *P. griseofulvum*, *P. chysogenum*, *Coniophora puteana*, *Acrosperia mirabilis*, *Botryosphaeria ribis*, *Sclerotinia sclerotiorum*, *Botryotinia fuckeliana* and *Gibberella* sp. are the predominant microorganisms that affected fresh chestnuts.

1.4 Chestnuts packaging

Chestnuts are considered a high perishable product because of its high water and sugar contents, and as a consequence, their shelf-life is very limited (Vekiari et al., 2007). One of the most important challenges, after harvesting and processing of chestnuts, is to maintain the quality and limit the infestation of insects and microorganism growth. All these processes will favour the degradation of the flavour and appearance, reduce the shelf-life, and hence the market value (Künsch et al., 2001).

Generally, chestnuts may be submitted to the following preservation techniques: thermohydrotherapy (immersion into the water at 50 ºC for 45 min, and then submitted to drying); drying (to reduce the water content. Afterwards, fruits will be peeled and milled into flour); cold storage (chambers at 0 to 2 ºC and 90-95% of relative humidity); modified atmospheres; and freezing.

Another possible alternative to increase the shelf-life of chestnut is to use suitable packaging to reduce the problems mentioned above. The packaging material will be one of the critical aspects for the preservation of the product as it will have an important role to increase the shelf-life of the chestnuts. The packaging material will affect the fruit's respiration and transpiration rates, as well as the development of microorganisms. In Table 4, the characteristics, properties and applications of several plastic materials are presented.

Among these types of plastics, LDPE is commonly used in food packaging and is found in fruit and vegetable bags used in grocery stores. It is a material already approved for food contact by Food Drug and Administration (FDA).

In Table 5, some previous work done on chestnut packaging are mentioned. The different types of packaging used, the pre-treatments applied on the samples before storage, as well as the storage conditions applied, are described. In general, various packaging materials have been applied to chestnuts, being plastics from polyethene and polyamide the most used. Furthermore, Nogueira and (Correia, 2008) observed that chestnuts stored in paper boxes showed a higher percentage of damage fruits than those kept in plastic bags.

The studies made until now have been performed along with chestnut storage from 19 days to 6 months, being the more extended periods the most analysed. Moreover, more studies on storage at low temperatures have been performed than at high temperatures.

Taking these data into account, the use of other types of bags can be a possibility. Additionally, few works have been done on chestnuts subjected to the retail conditions, namely at room temperature, which is considered high for chestnuts, and low relative humidities. These conditions can be a problem to the chestnut industrials because significant weight losses may occur, originating critical economic losses. Thus, the search for new methods or packagings that minimise these losses is of fundamental importance.

Table 4: Main characteristics and applications of different packaging materials (Source: Hahladakis & Iacovidou, 2018)

Plastic-type	Characteristics and properties	Application
Polyethylene Terephthalate (PET)	Semi-crystalline, high density, very hard, and rigid. High chemical and wear resistance and low moisture absorption.	Widely used in the food and personal care packaging applications as it is an excellent barrier to flavours and is usually transparent.
High-density polyethene (HDPE)	Semi-crystalline, translucent, low density and hardness characteristics. Poor chemical and wear resistance, and very low moisture absorption.	Can be a low barrier for oxygen and other gases, odours and flavours.
Polyvinyl chloride (PVC)	Amorphous, optically transparent, high density, hard. Good chemical and wear resistance, and very low moisture absorption.	The most widely used of the amorphous plastics. It is available in two forms - plasticized (flexible) or un-plasticized (hard, tough) and is used in blister packaging for pharmaceuticals and capsules.
Low-density polyethylene (LDPE)	Semi-crystalline, translucent, with low density and hardness characteristics. Poor chemical and wear resistance, and low moisture absorption.	Squeezable tubes and bottles, wrappers and bags, frozen food containers, coating material for bottle cartons.
Polypropylene (PP)	Semi-crystalline, low density. Good chemical and wear resistance, and low moisture absorption.	It has the lowest density of all thermoplastics. Can make it attractive in many packaging applications, such as closures of all kinds, several boil-in-bag food packages and containers exposed to high levels of thermal and chemical stress
Polystyrene (PS)	Amorphous, optically transparent, high density. Poor chemical and wear resistance to hydrocarbon solvents, low moisture absorption.	PS is available in a range of grades which generally vary in impact strength from brittle to very tough. It is used for low strength structural applications when impact resistance, machinability, and low cost are required, such as in vending cups, yoghurt containers, bottles for pharmaceutical tablets and capsules, and packaging of fragile products.

Table 5: Previous works performed on chestnut packaging.

Species and cultivars	Country	Packages	Pre-treatment	Storage conditions	References
C. sativa Amarelal, Aveleira, Bária, Colarinha, Enxerta, FS1, FS2, Judia, Longal, Martaínha, Negral, Rebordã, Verdeal	Portugal	Plastic bags (Freshmate®) Paper boxes	Fresh samples	5 months of storage time in normal environmental conditions (15 ºC, 65% relative humidity) and at 10 ºC and 70% relative humidity	Nogueira & Correia, 2008
C. sativa	Portugal	Vacuum packaging High barrier packaging (polyamide/polyethene bags) Modified atmosphere packaging (polyester bags) Polyethene packaging	Fresh samples submitted to 48-50 °C hot water treatment for 45 minutes.	6 months of storage at 0 °C and 90% relative humidity	Fernandes et al., 2020
C. sativa Famosa	Spain	Vacuum polyamide/polyethene bags	Unpeeling the whole external shells of chestnuts	10 weeks of storage at room temperature (21°C) or cold chamber (3°C) or freezer (-18 °C)	Chenlo et al., 2010
C. sativa	Greece	Vacuum high-density polyethene bags	Raw samples Peeled samples Heat treated samples	3 months of cold storage (0 or 4 °C)	Vekiari et al., 2007
C. crenata	South Korea	Master packaging system: Low density polyethene (LDPE) + Thick oriented polyethene (OPP) bags with different micro-perforations (5, 10, 15 or 20 of size 110±21 μm)	Fresh samples	19 days storage at 0±1 °C and 90% relative humidity. Additional 4 days storage at 0, 7, 13 and 20°C.	Kim et al., 2012

2 OBJECTIVES OF THE WORK

The objectives of this research try to find answers to two issues at the same time, which are reducing the weight loss and spoilage of chestnuts along the storage period.

The maintenance of chestnuts quality and safety will have a significant impact on the producers' incomes, by reducing the losses of the weight of the chestnuts, as well as to increase consumers' acceptability by proving a better product.

This study intends to apply different types of packaging to chestnuts and to determine the influence of the packaging material on their physic-chemical and biological characteristics. The specific objectives were:

- To test different packaging materials for the short time storage of chestnuts to find out the best solution to reduce the industrial losses of chestnuts during the selling process of the fruit in supermarkets (fruits stored at room temperature and low relative humidity – retail conditions).

- To study the effect of using micro-perforated bags for the long time storage of chestnuts in refrigerated industrial chambers to maintain their quality for a more extended period.

3 MATERIALS AND METHODS

3.1 Samples

The chestnuts (*Castanea sativa*) used in the present work were of the variety Martaínha. They were supplied by the "Cooperativa Agrícola de Penela da Beira" that is a cooperative of farmers of the region "Penela da Beira" (NE Portugal) in November 2019. In the same day of acquisition of the fresh chestnuts, they were taken immediately to the laboratory. There, the fruits were treated in hot water. This step involved the immersion of chestnuts in hot water at a temperature between 48 and 50 °C for 45 minutes, to eliminate the larvae and eggs of insects, namely of *Cydia pomonella*, *Cydia flagiglandana*, *Cydia splendana* and *Curculio elephas*. This procedure is compulsory for all enterprises that want to export chestnuts (DGAV, 2018).

After the hot water treatment, the fruits were immediately immersed in cold water and then left to dry in trays with absorbent paper for approximately two days.

3.2 Packaging

In the present work, different types of packaging were tested to find some solutions to the most problems mentioned by the chestnut industry that are the following:

- The chestnut quickly loses weight, due to its high rates of respiration and transpiration. The weight loss translates into substantial economic losses, primarily when the chestnut is being handled by food retailers and being sold in large stores. In these shops, the chestnut is placed in bulk, subjected to high temperatures, without any protection;

- During long storage in refrigeration chambers, the chestnut also loses weight and fungi development can occur.

Thus, to try to find out methods to decrease the losses mentioned above, two experiments were planned to study the effect of different packaging on chestnut quality during storage.

1) Short-time packaging (0, 2, 4 and 6 weeks) - Four different types of packaging were studied, namely: (*i*) Polyethylene packaging (POLY-bags) (polyethylene bags with the dimensions 30cm/19,8cm); (*ii*) Perforated polyethylene packaging (PH-bags) (the same bags as described in point (*i*) but with four macro holes of 5,5 mm of diameter);

(*iii*) Modified Atmosphere Packaging (MAP-bags) (high barrier bags of polyester (TECNOPACK, Portugal) with the initial gas composition: 32% CO_2, 0.3% O_2 and N_2 as filler); and (*iv*) Vacuum packaging (VAC-bags) (polyamide and polyethylene – high barrier packaging) (Alfa, Spain). A control, unpackaged chestnuts placed on trays, was also performed;

2) Long-time packaging (0, 1, 2, 3 and 6 months) - Micro-perforated packaging (MP-bags with the dimensions 35cm/24.7cm of the brand Simpack and with the initial gas composition: 32% CO_2, 0.3% O_2 and N_2 as filler) was performed. A control, unpackaged chestnuts placed on trays, was also done.

In each package or tray, 310 grams of chestnuts were placed. At each sampling time, triplicates (three individual bags or trays) were taken to perform the physic-chemical and microbiological analyses.

3.3 Storage condition and gas analysis

3.3.1 Storage condition

The samples packed for the short-time storage and the unpacked chestnuts (control) were stored at room temperature, for six weeks. Along this period, daily monitoring of the temperature and relative humidity (RH) was made (HANNA – HI 9564 Thermo Hygrometer).

Concerning the long-time storage samples, after packing the chestnuts in the micro-perforated bags in modified atmosphere, they were taken directly to the refrigerated chamber of the "Cooperativa Agrícola de Penela da Beira". At 1, 2, 3 and 6 months, samples were taken in triplicate and transported immediately to the laboratory. The physic-chemical and microbiological analyses were done to evaluate the quality of chestnuts in each period.

3.3.2 Gas analysis

In both storage conditions (short and long-time), the percentages of O_2 and CO_2 were measured, using a gas analyzer (WITT OXYBABY® M+, Germany) (Figure 4) to monitor their variation in the different times of storage.

Figure 4: O_2 and CO_2 gas analyzer for Modified Atmosphere Packaging.

3.4 Physico-chemical parameters

3.4.1 Colour

The colour of chestnuts was measured both outside (shell colour) and inside (kernel colour) of 10 fruits chosen randomly from each bag or tray. The colour was determined by a colourimeter (Minolta, model CR-400, Japan) according to the CIELab colour space. The following parameters were evaluated: (*i*) *L** that represents the lightness/darkness dimension, varying between 0 (black) and 100 (white); (*ii*) *a** that corresponds to the greenness-redness coordinate, ranging from green (-*a**) to red (+*a**); (*iii*) *b**, the blueness-yellowness coordinate values, extending from blue (-*b**) to yellow (+*b**); (*iv*) h, the hue angle; and (*v*) *C**, the saturation index or Chroma coordinate.

3.4.2 Texture

The texture measurements were conducted on ten randomly chosen chestnuts from each bag or tray with a TA.XT*plus* texture analyser (Stable Micro Systems, Godalming UK), equipped with a cylindrical probe of 2 mm of diameter. The test speed was of 1 mm/sec. The chestnuts were tested without the external shell. The parameters determined were: (*i*) the skin strength equal to the positive peak force (g); (*ii*) the elasticity (mm); and (*iii*) the positive area (g.sec), which represents the work done to penetrate the chestnuts.

3.4.3 Weight loss (WL), Water activity (a_w) and Moisture content

At each sampling time, the weight loss was calculated by the following equation:

$$Weight\ loss\ (\%) = \frac{Chestnut\ mass_{t=0\ days} - Chestnut\ mass_t}{Chestnut\ mass_{t=0\ days}} \times 100 \qquad \textbf{Equation 1}$$

, varying *t* between 0, 2, 4 and 6 weeks for the short-time packaging, and 1, 2, 3 and 6 months for the long-time packaging.

After determining the weight loss, some fruits were peeled and crushed in a universal mill (IKA, M20 Universal Mill). The milled samples were placed in plastic dishes to evaluate the water activity (a_w) that was determined in a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland). The moisture content was measured by the conventional drying method at 105 °C, until achieving constant weight, being the results expressed in %.

3.4.4 Titratable Acidity and Total Soluble Solids

A titrimetric analysis was used to determine the titratable acidity (TA) of the chestnuts. The method consisted of titration with a 0.1 M NaOH solution. Around 10 g of fresh chestnuts, previously ground, was put in a round bottom balloon and mixed with 50 mL of distilled water (previously boiled). After placing a reflux condenser, the solution was heated, on a heating blanket, for approximately 30 minutes. Then, the obtained solution was transferred to a graduate cylinder, and distilled water (recently boiled) was added to reach a total volume of 100 mL. After mixing with a stirring rod, the solution was filtered through gauze (three layers), being 25 mL of the filtered solution (measured by a volumetric pipet) transferred to a beaker. Afterwards, the NaOH solution was added, and the pH of the solution was monitored continuously to obtain the titration curve. The pH at the equivalence point was established as 8.1, as indicated in the Portuguese regulation NP-1421 (1977).

The values were expressed on mg citric acid /100 g dry weight (d.m.). The pH values were evaluated using a Crison-Micro pH 2002 (Crison, Barcelona, Spain) potentiometer.

The solution used in the titratable acidity determination (after filtration), was also used to measure the total soluble solids (TSS) contents (°Brix) at 20 °C. The measurements were performed in an Abbe refractometer (Optic Ivymen System, Madrid, Spain).

3.4.5 Reducing Sugars

This procedure of determination of reducing sugars within the chestnuts was described by Barreira et al. (2010), including some modifications. Dried and defatted powder (0.4 g) was extracted with 5 ml of aqueous ethanol (80%, v/v) at 70 °C for 30 min. The resulting suspension was centrifuged at 4,000 rpm for 15 min. Five millilitres of the supernatant was used to determine the reducing sugars by the dinitrosalicylic acid (DNS) method. The absorbance of the solution at 575 nm was measured in a spectrophotometer (Nanocolor UV/VIS, Machery-Nagel, U.S.A.). A calibration curve was obtained with glucose (0.05 to 0.60 g/L) and the results expressed on mg glucose/100 g dry weight.

3.4.6 Starch and Amylose

The methods used to determine the starch and amylose were the ones described by Correia et al. (2009), based on the method proposed by Garcia and Wolf (1972) and Correia & Beirão-Da-Costa (2011), respectively. For the starch, the process consisted of weighting 0.5 g of chestnut to a beaker. Twenty millilitres of DMSO (90%, v/v) were added, and the solution was mixed for 24 hours. Afterwards, the solution was centrifuged (3500 rpm for 30 minutes). Five millilitres of the supernatant was taken and mixed with 45 mL of DMSO (90%, v/v). The optical rotation of this solution was measured in a Polarimeter. The following equation was used to determine the starch content:

$$Starch\ (\%) = \frac{[\alpha]\times 100\times V_1\times \frac{V_3}{V_2}}{[\alpha]_{546}^{25}\times P\times W\times (100-\%H)} \qquad \textbf{Equation 2}$$

Where, *[α]* = Optical rotation of the solution; V_1 = Volume of the mixture (chestnut + DMSO), equal to 20 mL; V_3/V_2 = Dilution factor, equal to 50/5; $[\alpha]_{546}^{25}$= 220º, equal to the optical rotation of starch in DMSO (90%, v/v); *P* = cell length (dm); *W* = Mass of chestnut flour (g); *%H* = Moisture Content (%).

For the amylose, it was used the colourimetric method proposed by (Juliano, 1971). The amylose content was expressed on the sample dry basis and starch basis. The procedure consisted of weighting 100 mg of each sample into a 50-mL Erlenmeyer flask. One mL of 95% ethanol and 9 mL of NaOH 1N were added, being the mixture heated for 10 min in a boiling water bath to gelatinise the starch. After cooling, the solution was transferred, with several water washings, into a 100-mL volumetric flask. The volume was brought up with water and then mixed well. A total of 5 mL of this solution was pipetted into a 100-mL volumetric flask, and 1 mL of 1 N acetic acid and 2 mL of iodine solution (0.2 g iodine and 2 g potassium iodide in 100 mL of aqueous solution) were added. The solution was made up to volume with distilled water, shaken, and allowed to stand for 20 min. The absorbance of the solution at 620 nm was measured in a UV/VIS spectrophotometer (Lambda 25, PerkinElmer, Mass., U.S.A.). Potato amylose was used as a reference to construct the standard curve.

3.5 Microbial parameters

Control (0 days) and packaged samples for both short-time storage (2, 4 and 6 weeks) and long-time storage (1, 2, 3 and 6 months), in triplicates, were collected to determine the microbial quality of chestnuts. Three chestnuts of each bag or tray were mixed with sterile peptone water (Liofilchem, Italy) solution and homogenized. After shaking, serial dilutions were prepared in the same diluent, and 200 µL aliquots of each dilution were spread in duplicate on an appropriate culture media.

The growth media and incubation conditions for the studied microorganisms were the following:

- Aerobic mesophilic at 30 °C: Plate Count Agar (PCA, Liofilchem, Italy) incubated for 48–72 hours at 30 °C, ISO 4833:2, (2013).

- Yeasts and moulds: Dichloran Rose Bengal Chloramphenicol Agar (DRBC agar, Liofilchem, Italy) incubated at 25 °C for five days, ISO 21527-1, (2008).

The number of microorganisms was calculated as a weighted average of two successive dilutions according to the following equation:

$$N = \frac{\sum C}{V \times \left(N_1 + (N_2 \times 0{,}1)\right) \times D} \qquad \textbf{Equation 3}$$

Where:

N: number of microorganisms in food (colony forming units per gram, CFU/g)

Σ C: the sum of the colonies counted on all the dishes retained from two successive dilutions

V: the volume of inoculum applied to each dish in millilitres.

N_1: the number of dishes retained at first dilution

N_2: the number of dishes retained at the second dilution

D: the dilution factor corresponding to the first dilution retained.

The results were expressed in the logarithm of the number of colony-forming units per gram (log_{10} CFU/g) of fresh sample.

3.6 Statistical analysis

SPSS statistical software version 18.0 (SPSS Inc., Chicago, IL) was used for data analysis. Results were presented as means ± standard deviation (SD) (n = 3). Levene's test verified the homogeneity of the variances, and the Shapiro-Wilk test confirmed the normality of the data. As the data followed a normal distribution, analyses of variance (ANOVA) or ANOVA Welch were carried out to evaluate if there were significant differences ($p < 0.05$) between samples. ANOVA was applied when homogeneity of variances was observed, while ANOVA Welch was used for the other cases. Additionally, significant Post-Hoc analyses were performed, namely: the Tukey HSD test, if variances in the different groups were identical, or the Games-Howell test, if they were not.

4 RESULTS AND DISCUSSION

4.1 Short Storage packaging

4.1.1 Gas analysis

In Figure 5 are represented the concentrations of oxygen (O_2) and carbon dioxide (CO_2) within the inner atmospheres of the several packagings applied – Modified Atmosphere Packaging (MAP), Polyethene (POLY) and Macro Perforated Polyethene bags (with holes) (PH) – of chestnuts subjected to the short-time storage. The gas concentrations of the environment were also measured, corresponding to the control (unpackaged chestnuts).

As expected, the control and PH bags presented normal percentages of O_2 and CO_2 during all the six weeks of storage. This fact is due to their direct exposure to the room atmosphere, corresponding to an O_2% of 21% and CO_2% around 0%.

MAP has shown, during the first two weeks, a negligible level of O_2 and a high level of CO_2, around 77.3%. On the contrary, from 2 to 6 weeks of storage, a significant increase in O_2 concentration (0.6 to 8.0%) and a decrease in the CO_2 level (77.3 to 7.3%) were observed. This could be explained by the cellular respiration of the chestnuts. This process caused an increase in the pressure inside the bags, leading to the creation of micro-cracks in the packaging film, due to its expansion. Thus, the O_2% increased in the bag, while the CO_2 level decreased due to the diffusion of both gases.

POLY bags also showed significant changes in O_2 and CO_2 concentrations during the six weeks of storage. Along the first four weeks, the O_2 level remained almost constant (6.1 to 10.1%), decreasing after that time. During the same period (4 weeks), the CO_2 level remained low (around 6.0%), increasing rapidly afterwards. Based on these results, POLY packaging was also able to be a barrier between the inner and outer atmospheres of the packaging. Therefore, the changes of O_2/CO_2 concentrations observed were due to the cellular respiration of the fresh samples (O_2 decreased, while CO_2 increased), showing these bags specific permeabilities to both gases and some elasticity. This last property made these bags to be able to withstand the internal pressure generated by the release of CO_2, related to the chestnut breathing. These results suggest that the frequency of appearance of micro-cracks in POLY-bags seems to be lower than those in MAP-bags.

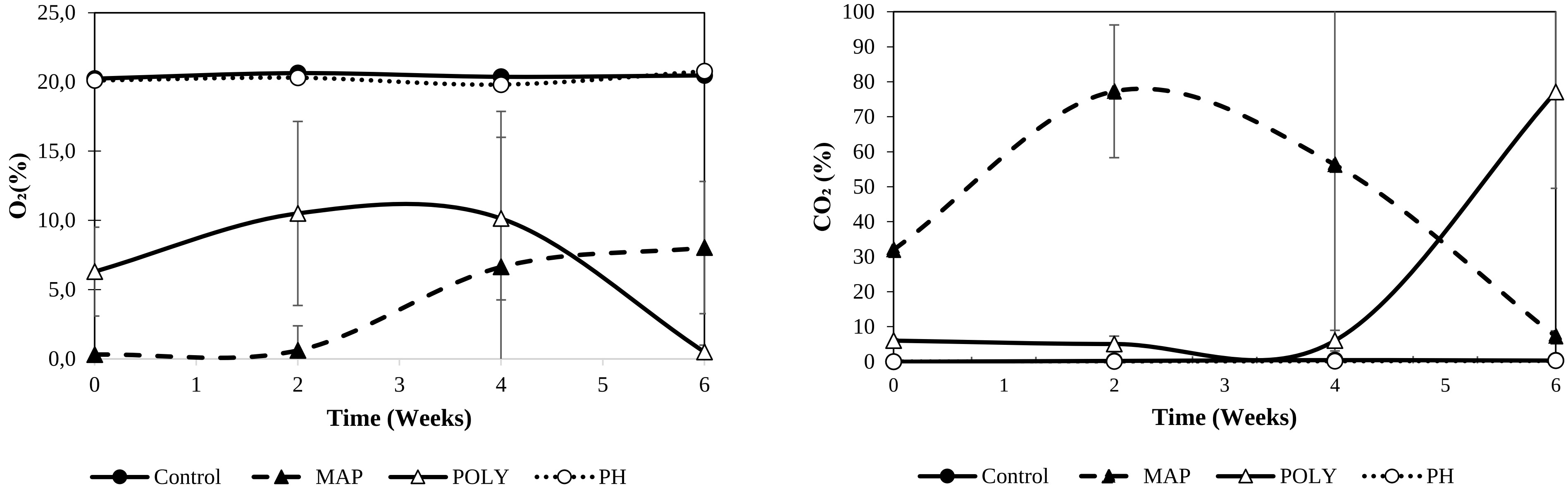

Figure 5: Gas concentrations (O_2 and CO_2) measured in the inner atmospheres of the packaged chestnuts (MAP, POLY and PH bags) and on the environment (control) throughout six weeks of storage.

4.1.2 Colour

The colour parameters (*L**, *a**, *b**, *C** and h), both outside (shell) and inside (kernel) of the samples during the short time storage, are presented in Tables 6 and 7, respectively. In general, the results of unpackaged (control) and packaged chestnuts – VAC, MAP, POLY and PH – stored for six weeks, had shown some variability, and in some situations, significant differences were detected; however, all values presented a similar order of magnitude. Looking in more detail the colour results, the shell of the chestnuts packaged in PH-bags after six weeks of storage showed the lowest values for the *b** and *C** parameters when comparing with the other bags. So, the use of these bags may induce slight changes in the colour of the chestnut shell.

Concerning the kernel, in most situations, no significant differences were observed between the different bags. Along the time of storage, in some parameters, such as *L** in control (6 weeks), *b** and *C** in VAC- and MAP-bags, and h in control and VAC-bags, a slight decrease was observed when compared to the initial time (0 weeks). Nevertheless, all values showed to be of the same order of magnitude.

These results indicate that the packaging of chestnuts combined with adequate storage conditions, may preserve the physical appearance and more specifically the colour of the chestnuts, that it is a parameter considered very important for the customers at the moment of purchase.

4.1.3 Texture

The chestnut texture can be affected by the storage conditions, leading to a loss of firmness of the fruit and by that lowering the product quality. Therefore, a physical analysis of texture was conducted on *C. sativa* fruits, as mentioned in Section 3.4.2. The chestnuts were packaged on different types of bags and the skin strength, elasticity and the work necessary to puncture the chestnut tissue were determined during the short time storage of six weeks.

Table 6: Color parameters of the shell of unpackaged and packaged chestnuts over six weeks of storage.

Color parameters	Storage Time (Weeks)	Outside the fruit				
		Packaging				
		Control	Vacuum bags (VAC)	Modified atmosphere packaging (MAP)	Polyethene bags (POLY)	Poly-hole bags (PH)
L*	0	35.6±2.0^{A}	35.6±2.0^{A}	35.6±2.0^{A}	35.6±2.0^{A}	35.6±2.0^{B}
	2	32.6±1.8^{a,B}	30.5±1.9^{c,B}	30.5±2.1^{c,D}	32.0±1.8^{a,b,B}	30.9±1.9^{b,c,C}
	4	31.2±2.2^{a,C}	31.2±1.8^{a,B}	32.2±2.8^{a,C}	31.5±2.7^{a,B}	30.7±1.7^{a,C}
	6	35.4±1.6^{b,A}	35.1±2.4^{b,A}	34.1±1.4^{b,B}	31.5±1.8^{c,B}	37.5±1.9^{a,A}
a*	0	11.3±1.3^{A}	11.3±1.3^{A}	11.3±1.3^{A}	11.3±1.3^{A}	11.3±1.3^{A}
	2	11.4±2.1^{a,A}	10.5±2.0^{a,b,A,B}	9.7±2.5^{b,B}	10.1±2.0^{a,b,A,B}	9.4±2.0^{b,B}
	4	9.3±2.3^{a,B}	9.4±2.3^{a,B}	9.8±2.3^{a,B}	9.6±2.4^{a,B}	9.5±1.8^{a,B}
	6	10.5±1.5^{a,A,B}	10.7±1.5^{a,A,B}	10.3±1.9^{a,A,B}	10.8±2.4^{a,A,B}	8.6±1.1^{b,B}
b*	0	5.1±2.7^{B}	5.1±2.7^{A,B}	5.1±2.7^{A,B}	5.1±2.7^{A,B}	5.1±2.7^{A}
	2	6.9±3.0^{a,A}	6.1±2.8^{a,A}	6.1±2.9^{a,A}	6.8±2.5^{a,A}	6.2±2.7^{a,A}
	4	5.1±2.4^{a,B}	4.2±2.5^{a,B}	4.4±3.3^{a,A,B}	6.2±3.5^{a,A,B}	4.8±2.2^{a,A}
	6	6.8±2.0^{a,A}	4.4±2.3^{b,A,B}	4.1±2.2^{b,B}	4.1±2.7^{b,B}	2.0±2.0^{c,B}
C*	0	12.5±2.0^{B}	12.5±2.0^{A}	12.5±2.0^{A}	12.5±2.0^{A}	12.5±2.0^{A}
	2	13.4±3.1^{a,B}	12.3±3.0^{a,A,B}	11.5±3.6^{a,A}	12.3±2.9^{a,A}	11.4±3.0^{a,A,B}
	4	10.8±2.8^{a,A}	10.4±2.9^{a,B}	11.1±3.3^{a,A}	11.6±3.8^{a,A}	10.7±2.6^{a,B}
	6	12.6±2.1^{a,B}	11.7±2.1^{aA,B}	11.2±2.5^{a,A}	11.7±3.0^{a,A}	9.0±1.5^{b,C}
h	0	22.9±10.3^{C}	22.9±10.3^{A,B}	22.9±10.3^{B}	22.9±10.3^{A}	22.9±10.3^{B}
	2	29.6±9.1^{a,A,B}	28.3±8.6^{a,A}	30.3±7.8^{a,A}	32.6±7.2^{a,B}	31.6±8.9^{a,A}
	4	26.5±8.8^{a,B,C}	22.9±9.4^{a,A,B}	21.3±12.5^{a,B}	29.6±11.0^{a,B}	25.6±7.2^{a,A,B}
	6	32.6±6.1^{a,A}	21.3±8.9^{a,b,B}	20.5±7.6^{a,b,B}	19.1±9.5^{a,b,A}	13.9±10.7^{b,C}

Values
Values

Values are expressed as Mean ± Standard Deviation. Different lowercase letters on the same line indicate significant differences ($p<0.05$) between packages; Different capital letters on the same column indicate significant differences ($p<0.05$) between storage time.

Table 7: Color parameters of the kernel of unpackaged and packaged chestnuts over six weeks of storage.

Color parameters	Storage Time (Weeks)	Inside the fruit				
		Packaging				
		Control	Vacuum bags (VAC)	Modified atmosphere packaging (MAP)	Polyethene bags (POLY)	Poly-hole bags (PH)
L*	0	85.2±3.8^{A}	85.2±3.8^{A}	85.2±3.8^{A}	85.2±3.8^{A}	85.2±3.8^{A}
	2	86.2±3.1^{a,A}	86.7±2.3^{a,A}	86.4±4.4^{a,A}	85.4±3.2^{a,A}	85.5±3.3^{a,A}
	4	82.1±8.3^{a,A,B}	84.0±7.4^{a,A}	85.1±4.9^{a,A}	84.6±4.7^{a,A}	83.1±7.2^{a,A}
	6	81.2±6.0^{a,B}	83.6±5.7^{a,A}	84.6±3.2^{a,A}	84.4±4.2^{a,A}	84.3±4.0^{a,A}
a*	0	-2.3±0.7^{B}	-2.3±0.7^{B}	-2.3±0.7^{B}	-2.3±0.7^{A}	-2.3±0.7^{A}
	2	-2.7±0.7^{b,B}	-0.8±2.1^{a,A}	-2.4±0.8^{b,B}	-2.2±1.3^{b,A}	-2.6±0.9^{b,A}
	4	-1.8±1.9^{a,A,B}	-1.6±1.3^{a,A,B}	-2.1±0.9^{a,B}	-2.1±1.4^{a,A}	-2.1±1.9^{a,A}
	6	-1.3±1.7^{a,b,A}	-1.2±1.4^{a,A}	-1.5±0.9^{a,b,A}	-1.8±1.4^{a,b,A}	-2.2±1.0^{b,A}
b*	0	21.6±3.2^{A,B}	21.6±3.2^{A}	21.6±3.2^{A}	21.6±3.2^{A}	21.6±3.2^{A}
	2	23.6±3.2^{a,A}	20.9±2.8^{b,A,B}	21.7±3.2^{a,b,A}	21.0±2.3^{b,A}	22.3±2.3^{a,b,A}
	4	21.8±4.0^{a,b,c,A,B}	19.5±2.1^{c,B,C}	20.6±3.3^{b,c,A}	22.8±3.0^{a,b,A}	23.3±3.2^{a,A}
	6	21.2±3.0^{a,B}	18.5±3.8^{b,C}	17.6±1.9^{b,B}	22.3±3.0^{a,A}	22.0±2.5^{a,A}
C*	0	21.7±3.2^{A}	21.7±3.2^{A}	21.7±3.2^{A}	21.7±3.2^{A}	21.7±3.2^{A}
	2	23.5±3.8^{a,A}	21.0±2.8^{b,A,B}	21.8±3.2^{a,b,A}	21.2±2.4^{b,A}	22.4±2.3^{a,b,A}
	4	22.0±4.0^{a,b,A}	19.6±2.1^{c,B,C}	20.7±3.3^{b,c,A}	22.9±3.0^{a,b,A}	23.5±3.2^{a,A}
	6	21.3±3.0^{a,A}	18.6±3.8^{b,C}	17.7±2.0^{b,B}	22.4±3.1^{a,A}	22.1±2.5^{a,A}
h	0	96.1±1.4^{A}	96.1±1.4^{A}	96.1±1.4^{A}	96.1±1.4^{A}	96.1±1.4A
	2	96.5±1.6^{a,A}	95.6±1.7^{a,A}	95.7±3.2^{a,A}	96.5±2.5^{a,A}	96.5±2.1^{a,A}
	4	94.5±5.4^{a,A,B}	94.5±4.2^{a,A,B}	95.7±2.5^{a,A}	95.2±3.3^{a,A}	94.8±5.6^{a,A}
	6	93.4±4.8^{a,B}	93.1±4.2^{a,B}	94.6±2.8^{a,A}	94.5±3.5^{a,A}	95.6±2.8^{a,A}

Values are expressed as Mean ± Standard Deviation. Different lowercase letters on the same line indicate significant differences ($p<0.05$) between packages; Different capital letters on the same column indicate significant differences ($p <0.05$) between storage time.

When comparing the different bags with each other (Table 8), for the three parameters analysed, it was found that the results obtained were quite similar to each other, except for rare situations. Regarding the storage time, it was observed that for the VAC-, MAP- and PE-bags, a slight increase in the area below the curve was observed after six weeks of storage in comparison to the beginning. A higher area indicates the need to do more work to penetrate the fruit. This slight increase in the mechanical work may be related to some stiffening of the fruit, caused by the possible loss of water. However, concerning elasticity and maximum force, there were no substantial differences in values throughout six weeks of storage. These results suggest that chestnuts still maintain the mechanical strength observed at the beginning, as well as a good degree elasticity.

These results are quite promising, suggesting that the bags studied in the present work do not cause negative textural changes in the fruits.

Table 8: Texture parameters determined in unpackaged and packaged chestnuts during six weeks of storage.

Parameters	Storage Time (Weeks)	Packaging				
		Control	Vacuum bags (VAC)	Modified atmosphere packaging (MAP)	Polyethene bags (POLY)	Poly-hole bags (PH)
Maximum force (g)	0	3522±476^{A}	3522±476^{A}	3522±476^{A}	3522±476^{A}	3522±476^{A}
	2	3479±539^{a,A}	3736±488^{a,A}	3597±427^{a,A}	3753±371^{a,A}	3576±514^{a,A}
	4	3264±751^{a,A}	3514±317^{a,A}	3691±447^{a,A}	3615±590^{a,A}	3337±590^{a,A}
	6	3438±787^{a,A}	3690±407^{a,A}	3639±414^{a,A}	3668±298^{a,A}	3435±729^{a,A}
Elasticity (mm)	0	3.5±1.2^{A}	3.5±1.2^{A}	3.5±1.2^{A}	3.5±1.2^{A,B}	3.5±1.2^{A,B}
	2	3.6±1.4^{a,A}	3.5±1.3^{a,A}	3.5±1.4^{a,A}	3.0±1.3^{a,B}	2.7±1.4^{a,B}
	4	3.8±1.3^{a,b,A}	3.2±1.4^{b,A}	3.7±1.3^{a,b,A}	4.3±1.4^{a,A}	3.6±1.2^{a,b,A}
	6	4.0±1.3^{a,A}	3.3±1.4^{a,A}	3.5±1.4^{a,A}	3.8±1.5^{a,A,B}	3.0±1.5^{a,A,B}
Area below the curve (g.s)	0	14089±3690^{A}	14089±3690^{B}	14089±3690^{B}	14089±3690^{B}	14089±3690^{A,B}
	2	14342±3175^{a,A}	17193±1885^{a,A}	16384±2095^{a,A}	17236±1999^{a,A}	16287±2469^{a,A}
	4	13138±3941^{b,A}	16122±1955^{a,A}	16979±2066^{a,A}	16185±3180^{a,A,B}	13550±2988^{b,B}
	6	12646±3723^{c,A}	15950±2542^{a,b,A}	16238±2094^{a,b,A}	17225±2022^{a,A}	14271±3647^{b,c,A,B}

Values are expressed as Mean ± Standard Deviation. Different lowercase letters on the same line indicate significant differences ($p<0.05$) between packages; Different capital letters on the same column indicate significant differences ($p <0.05$) between storage time.

4.1.4 Weight loss, Moisture content, a_w, and Titratable Acidity

The weight loss is a crucial parameter, as it may represent significant economic losses that are the major problem for the producers.

In Figure 6, it was stated that for all packagings, it was observed an increase in weight loss during the period of storage. In more detail, the unpackaged (control) and the PH-bags were those that presented the highest percentages of weight loss, achieving 13.2 and 9.2%, respectively, after six weeks of storage. This can be justified by the direct exposure of chestnuts to environmental changes, such as a high room temperature that may increase chestnut transpiration, without the existence of any barrier that would be able to decrease chestnut dehydration.

On the contrary, for the rest of the packagings, VAC-, MAP- and POLY-bags, the lowest percentages of weight loss were observed, namely 1.8, 1.9 and 1.6%, respectively. These values indicate that these bags may function as a barrier to water loss, being a promising result. All the bags showed weight losses of less than 10%. Acedo (1997) mentioned by Getinet et al. (2008), refers that approximately 10% physiological loss in weight is considered as an index of termination of the shelf-life (threshold level) of commodities. With all bags tested, this value has never been reached except for the control.

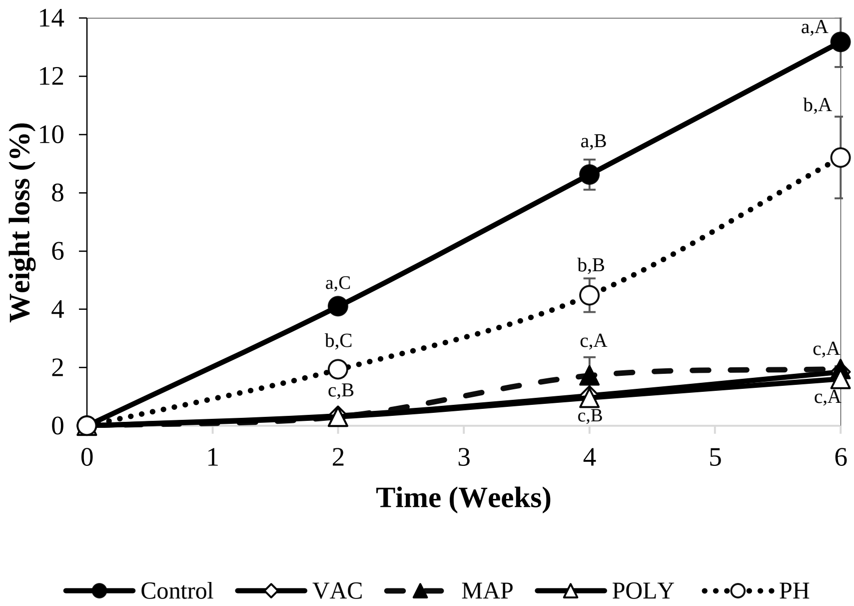

Figure 6: Weight loss (%) among the unpackaged and packaged chestnuts during six weeks of storage.

In all packaged samples, there were no significant differences ($p>0.05$) concerning moisture content all along the storage time (Table 9). Even though the weight losses mentioned above were observed, the values were at maximum equal to 13% for the control, which did not cause significant changes in the moisture content of the chestnuts. During the six weeks of storage, the moisture content varied between 43.2 and 53.9%, in line with the values reported by Correia et al. (2009) for Longal (48.2%) and Martaínha (47.9%) cultivars, and similar to the reference value indicated in the literature for this fruit (51.7%, Holland et al., 1992). The short variation observed in the moisture content showed that the use of packaging in storage conditions at room temperature for a short time (6 weeks) allow the maintenance of the moisture content of the chestnuts. This fact may also explain the non-detection of significant changes in fruits texture during storage, referred before. These results are in line with those reported by Chenlo et al. (2010), who verified that chestnuts stored in vacuum bags kept, approximately, their moisture content independently on the storage temperature (-18, 3 and 21 ºC) during ten weeks.

Regarding the a_w, in most situations, no significant differences were observed between the different types of bags, as well as along the storage time. These results are in line with those of moisture content. In general terms, the a_w values varied between 0.95 and 0.98. This range is similar to the one reported by (Nogueira and Correia, 2008) for Portuguese cultivars, namely 0.973-0.996. Values of a_w between 0.93 and 0.98 inhibit the growth of Gram negative bacteria, which give rise to Gram positive bacteria (*Bacillaceae*, *Lactobacillaceae*, *Microccocaceae*) (Ordónez et al., 2005). With these a_w, the growth of salmonella, *Clostridium botulinum* and other pathogenic bacteria might be inhibited (Ordónez et al., 2005). This point is essential to help ensure the safety of the fruits packaged in the VAC- and MAP-bags since these give rise to anaerobic conditions conducive to the growth of *C. botulinum*. However, these a_w values allow the development of moulds and the risk of mycotoxins to be elaborated (Ordónez et al., 2005). Thus, it is important to inform the chestnut producers that they must always follow good hygiene and handling practices in order to reduce the microbial load.

Table 9: Moisture content, water activity (a_w) and titratable acidity of unpackaged and packaged chestnuts throughout six weeks of storage.

Parameter	Storage time (weeks)	Packaging				
		Control	Vacuum bags (VAC)	Modified atmosphere packaging (MAP)	Polyethene bags (POLY)	Poly-hole bags (PH)
Moisture (%)	0	51.7±3.3^{A}	51.7±3.3^{A}	51.7±3.3^{A}	51.7±3.3^{A}	51.7±3.3^{A}
	2	50.5±5.8^{a,A}	48.7±4.4^{a,A}	52.8±2.3^{a,A}	50.3±1.3^{a,A}	53.9±1.8^{a,A}
	4	43.2±4.0^{a,A}	49.0±1.8^{a,A}	49.8±5.9^{a,A}	50.2±2.9^{a,A}	50.4±4.0^{a,A}
	6	45.5±6.0^{a,A}	48.9±4.4^{a,A}	52.3±0.1^{a,A}	52.8±2.4^{a,A}	53.9±5.4^{a,A}
a_w	0	0.974±0.003^{A}	0.974±0.003^{A}	0.974±0.003^{A}	0.974±0.003^{A,B}	0.974±0.003^{A}
	2	0.970±0.013^{a,A}	0.972±0.003^{a,A}	0.979±0.005^{a,A}	0.968±0.003^{a,B}	0.973±0.004^{a,A}
	4	0.951±0.014^{b,A}	0.974±0.002^{a,A}	0.972±0.010^{a,b,A}	0.973±0.005^{a,b,A,B}	0.971±0.005^{a,b,A}
	6	0.953±0.024^{a,A}	0.976±0.003^{a,A}	0.972±0.003^{a,A}	0.979±0.001^{a,A}	0.979±0.006^{a,A}
Titratable Acidity (g citric acid/100 g d.w.)	0	0.14±0.04^{A}	0.14±0.04^{A}	0.14±0.04^{A}	0.14±0.04^{A}	0.14±0.04^{A}
	2	0.17±0.02^{a,A}	0.14±0.05^{a,A}	0.13±0.03^{a,A}	0.16±0.08^{a,A}	0.13±0.02^{a,A}
	4	0.12±0.02^{a,A}	0.11±0.02^{a,A}	0.09±0.01^{a,A}	0.14±0.02^{a,A}	0.13±0.03^{a,A}
	6	0.24±0.11^{a,A}	0.12±0.02^{a,A}	0.14±0.04^{a,A}	0.14±0.01^{a,A}	0.22±0.05^{a,A}

Values are expressed as Mean ± Standard Deviation. Different lowercase letters on the same line indicate significant differences ($p<0.05$) between packages; Different capital letters on the same column indicate significant differences ($p <0.05$) between storage time.

Concerning the titratable acidity that measures the total acidity and is a better predictor than pH of how organic acids in the food impact flavour (Tyl & Sadler, 2017), the values varied between 0.09 and 0.24 g citric acid/100 g d.w.. Between packagings and along storage time, no significant differences were observed, suggesting that organic acids present in chestnuts were not being used as a substrate in the respiration process. Our values were similar to those determined in 19 Spanish cultivars analysed by Peña-Méndez et al. (2008), namely 0.05-0.16 g citric acid/100 g d.w..

4.1.5 Total soluble solids (TSS), reducing sugars, amylose and starch content

The contents in TSS are presented in Table 10. Among the different packages applied to the chestnut, there were no significant differences between them, except in the second week. In that week, the PH-bags showed higher values than the VAC-bags and the control. However, this behaviour was not observed at 4 and 6 weeks. Regarding the storage time, it was also for the unpackaged chestnuts (control) and VAC-bags that some significant differences were observed; however, none specific trend was perceived. Moreover, from time 0 and 6 weeks, there were no significant differences in this parameter. Our range of TSS (20.8-54.0%, d.m.) was higher than the values determined by Peña-Méndez et al. (2008) in 19 Spanish cultivars (12.2-21.1%, d.m.), suggesting that the Portuguese cultivars were slightly sweeter than the Spanish.

It is known that the main carbohydrates present in chestnuts are starch and sugars. The starch contents are presented in Table 10 and ranged from 35.2 to 50.4% on a dry matter basis, except in control after two weeks of storage (28.6%, d.m.), a value that seems to be underestimated. The range obtained is in line to those determined for *C. sativa* cultivars collected in Portugal (38.6-47.9%, d.m., Borges et al., 2008), Greece (38.9-51.4% d.m., Vekiari et al., 2007), Italy (58.3% d.m., Attanasio et al., 2004; approx. 50% d.m., Piccolo et al., 2020), Spain (42.2-66.5% d.m., Pereira-Lorenzo et al., 2006; 56.0-63.5% d.m., Peña-Méndez et al., 2008), and Switzerland (41.5 to 57.6% d.m., Künsch et al., 2001). On the contrary, our values were lower than those determined by (Míguelez et al. 2004) in 15 varieties collected in Spain (56.7-81.7% d.m.).

Table 10: Total soluble solids (TSS), starch content and amylose content of unpackaged and packaged chestnuts over six weeks of storage.

Parameter	Storage time (weeks)	Packaging				
		Control	Vacuum bags (VAC)	Modified atmosphere packaging (MAP)	Polyethene bags (POLY)	Poly-hole bags (PH)
TSS (%, d.w.)	0	47.2 ± 8.7^{A}	47.2 ± 8.7^{A}	47.2 ± 8.7^{A}	47.2 ± 8.7^{A}	47.2 ± 8.7^{A}
	2	$23.2\pm7.6^{b,B}$	$20.8\pm6.2^{b,B}$	$37.2\pm8.9^{a,b,A}$	$28.5\pm5.0^{a,b,A}$	$41.1\pm3.2^{a,A}$
	4	$40.6\pm5.0^{a,A,B}$	$30.2\pm12.1^{a,A,B}$	$33.0\pm3.2^{a,A}$	$41.4\pm4.1^{a,A}$	$29.5\pm6.8^{a,A}$
	6	$34.5\pm12.2^{a,A,B}$	$32.3\pm3.2^{a,A,B}$	$35.5\pm4.5^{a,A}$	$41.4\pm9.8^{a,A}$	$54.0\pm14.7^{a,A}$
Starch Content (%, dm)	0	35.2 ± 0.3^{B}	35.2 ± 0.3^{C}	35.2 ± 0.3^{B}	35.2 ± 0.3^{B}	35.2 ± 0.3^{B}
	2	$28.6\pm1.0^{c,C}$	$47.7\pm0.3^{a,B}$	$37.9\pm2.3^{a,b,B}$	$37.5\pm1.3^{b,B}$	$45.7\pm4.0^{a,b,A}$
	4	$49.4\pm0.2^{a,A}$	$49.5\pm0.4^{a,A}$	$38.6\pm2.6^{b,B}$	$47.8\pm3.0^{a,A}$	$48.5\pm2.9^{a,A}$
	6	$47.9\pm2.5^{a,b,A}$	$49.8\pm0.2^{a,A}$	$43.7\pm0.3^{c,A}$	$44.8\pm0.3^{b,c,A}$	$50.4\pm0.7^{a,A}$
Amylose Content (%, dm)	0	11.6 ± 0.7^{C}	11.6 ± 0.7^{C}	11.6 ± 0.7^{B}	11.6 ± 0.7^{B}	11.6 ± 0.7^{A}
	2	$24.8\pm0.4^{a,A}$	$16.1\pm0.5^{b,B}$	$14.2\pm0.7^{b,c,A}$	$11.3\pm0.3^{d,B}$	$11.8\pm2.1^{c,d,A}$
	4	$16.4\pm0.7^{b,B}$	$16.6\pm0.4^{b,A,B}$	$14.0\pm1.5^{cA,B}$	$21.0\pm0.6^{a,A}$	$13.8\pm0.7^{c,A}$
	6	$17.4\pm0.8^{b,B}$	$17.4\pm0.4^{b,A}$	$14.1\pm0.3^{c,A}$	$20.2\pm0.3^{a,A}$	$14.0\pm0.1^{c,A}$
Amylose Content expressed on starch basis (%)	0	33.0 ± 1.7^{B}	33.0 ± 1.7^{A}	$33.0\pm1.7^{A,B}$	33.0 ± 1.7^{B}	33.0 ± 1.7^{A}
	2	$86.9\pm2.6^{a,A}$	$33.7\pm0.9^{b,c,A}$	$37.5\pm1.1^{b,A}$	$30.1\pm0.5^{c,d,B}$	$25.7\pm2.4^{d,B}$
	4	$33.1\pm1.6^{b,c,B}$	$33.5\pm0.6^{b,c,A}$	$36.3\pm2.6^{b,A,B}$	$44.1\pm2.1^{a,A}$	$28.5\pm1.8^{c,A,B}$
	6	$36.3\pm0.6^{b,B}$	$35.0\pm0.6^{b,A}$	$32.2\pm0.9^{c,B}$	$45.0\pm0.4^{a,A}$	$27.7\pm0.3^{d,B}$

Values are expressed as Mean ± Standard Deviation. Different lowercase letters on the same line indicate significant differences ($p<0.05$) between packages; Different capital letters on the same column indicate significant differences ($p <0.05$) between storage time.

Even though some significant differences between the packages were observed, the majority of the conditions provided results of the same order of magnitude, with no trend detected. Along time, for each type of packaging, a slight increase in starch contents was stated. Different activities of the enzymes could explain this fact during storage. This behaviour was also reported by Vekiari et al. (2007) in heat-treated chestnuts collected in Volos (Greece). High contents of starch and moisture are important to the fresh market; however, they are responsible for the development of mould, which decreases the storage life (Borges et al., 2008).

For the amylose expressed on the starch basis, the majority of the values varied from 25.7 and 45.0%, except for the control for two weeks (86,9%). This last percentage seems to be overestimated. Probably the percentage of starch determined in these samples was too low, a fact also mentioned in the previous paragraph. The range obtained is in line with the values reported by Attanasio et al. (2004) (32.9±2.2%, d.m.) for Italian chestnuts. In the present work, no particular trend was observed along the storage time, suggesting no remarkable changes in starch functionality. Concerning the bags, the PH-bags were the one that generally showed the lowest percentages of amylose expressed on the starch basis. Furthermore, our results also show that amylose account for 26-45% of the starch content, corresponding the remainder to amylopectin. These two forms of starch are beneficial to human health, providing energy through the catabolism of both amylose and amylopectin into glucose, and by having positive effects on gut functions, related to the bacterial catabolism of amylopectin-derived dextrins into short-chain fatty acids (De Vasconcelos et al., 2010a).

All samples showed an intermediate amylose content (from 11.3 to 24.8% d.m.). This range is in line with the value determined in fresh chestnut fruits (17.0±0.4% d.m.) by Torres et al. (2014). These authors also stated that these values were similar to those in commercial cereal (rice, corn and wheat) starches commonly used in the food industry.

The reducing sugars contents were significantly influenced by the packaging type and storage time (Figure 7). The highest values were observed in POLY- and PH-bags, followed by the control. These results indicate that the presence of oxygen can influence the enzymatic reactions leading to partial hydrolysis of the starch. However, as the concentrations of reducing sugars remained low (<1 g glucose / 100 g d.m.), the observed increase was not felt in a reduction in the percentage of the starch present. The portion of this last component is high, being > 40%. Reducing sugars, such as glucose and fructose, can also be produced by sucrose hydrolysis. Sucrose is a non-reducing sugar, and it was not determined in the present work, not being possible to evaluate its time variation. On the contrary, the reducing sugars contents remained almost constant for the MAP- and VAC-bags. Nevertheless, as stated by Piccolo et al. (2020), the interconversion of starch to soluble sugars in chestnut is not well understood. The factors responsible for affecting the enzymatic processes are not well studied. There are several variables that may affect the carbohydrate interconversion, such as the available substrate, temperature, and activity of enzymes linked to sucrose synthesis/degradation (Piccolo et al., 2020). Concerning the reducing sugars contents determined in the present work, they were slight lower than those determined by Borges et al. (2008) in eight Portuguese chestnut cultivars (1.77-3.67 g/100 g d.m.), suggesting a lower partial hydrolyzation of the starch.

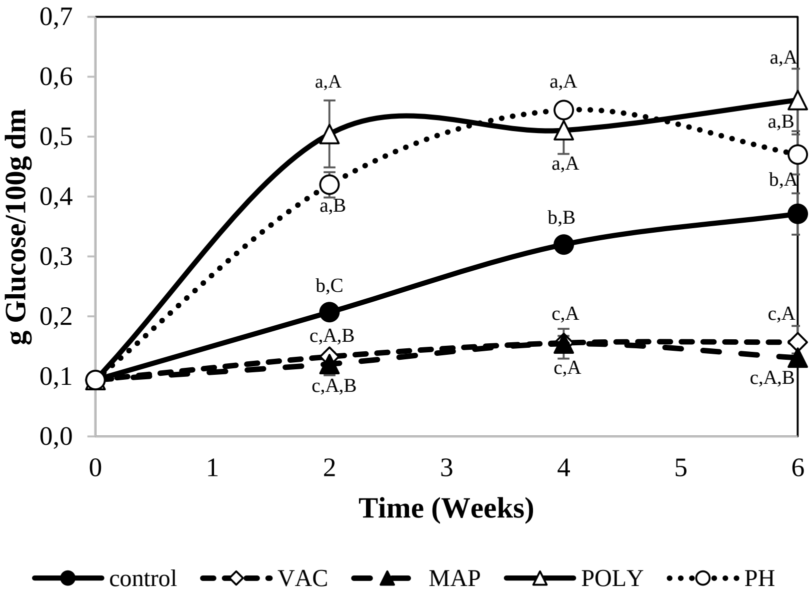

Figure 7: Reducing sugars in unpackaged and packaged chestnuts throughout six weeks of storage.

In summary, all bags tested did not cause significant physic-chemical changes in chestnuts along six weeks of storage, which is crucial to guarantee the excellent quality of Portuguese chestnuts during their storage in retail conditions and in the moment of purchase by the customers.

4.1.6 Microbial analysis

The composition of fresh chestnuts containing high amounts of starch, sugars, and water makes them a preferable medium for microbial growth, which constitutes the main problem during storage (de Vasconcelos et al., 2010).

Table 11 shows the mean counts of total aerobic mesophilic and moulds/yeasts in unpackaged (control) and packaged chestnut samples – Vacuum packaging (VAC), Modified Atmosphere Packaging (MAP), Polyethene Packaging (POLY), and Poly-hole bags (PH) – stored for a short-time (0, 2, 4 and 6 weeks). In general, the counting of aerobic mesophilic, moulds and yeasts microorganisms showed significant differences ($p<0.05$) between the control, the VAC- and MAP-bags over the six weeks of storage.

Table 11: Mean counts (log CFU/g) of total aerobic mesophilic, and moulds/yeasts in unpackaged (control) and packaged chestnut samples, stored for six weeks.

Microbial groups	Time (Weeks)	Control	Vacuum bags (VAC)	Modified atmosphere packaging (MAP)	Polyethene bags (POLY)	Poly-hole bags (PH)
Aerobic mesophilic	**0**	5.58 ± 0.52^{A}	5.58 ± 0.52^{A}	5.58 ± 0.52^{A}	5.58 ± 0.52^{A}	5.58 ± 0.52^{A}
	2	5.17 ± 0.21^{aA}	3.93 ± 0.40^{bB}	2.67 ± 0.25^{cC}	5.37 ± 0.38^{aA}	5.07 ± 0.12^{aA}
	4	5.67 ± 0.15^{aA}	3.13 ± 0.42^{cB}	3.67 ± 1.35^{bcBC}	5.13 ± 0.12^{abA}	5.40 ± 0.20^{aA}
	6	5.50 ± 0.17^{aA}	5.27 ± 0.23^{abA}	5.00 ± 0.17^{bAB}	5.57 ± 0.15^{aA}	5.47 ± 0.15^{abA}
Molds and yeasts	**0**	4.32 ± 0.44^{C}	4.32 ± 0.44^{A}	4.32 ± 0.44^{A}	4.32 ± 0.44^{B}	4.32 ± 0.44^{B}
	2	5.20 ± 0.20^{aAB}	3.13 ± 1.45^{bA}	2.43 ± 0.25^{bB}	5.50 ± 0.10^{aA}	5.40 ± 0.20^{aA}
	4	5.63 ± 0.06^{aA}	3.33 ± 0.74^{cA}	3.70 ± 1.25^{bcAB}	5.30 ± 0.20^{abA}	5.40 ± 0.20^{abA}
	6	4.67 ± 0.15^{abBC}	3.90 ± 0.8^{bA}	4.37 ± 0.51^{abAB}	5.37 ± 0.21^{aA}	5.47 ± 0.15^{aA}

Values are expressed as Mean (log cfu/g) ± standard deviation. Different lowercase letters on the same line indicate significant differences ($p<0.05$) between packages; Different capital letters on the same column indicate significant differences ($p <0.05$) between storage time.

These results indicated that the application of VAC- and MAP-bags on chestnuts had caused a considerable decrease in aerobic mesophilics, moulds and yeasts counts compared to the control during two and four weeks of storage. This could be due to the high concentrations of CO_2 observed in those bags (Figure 5). However, there is a slight increase in the counts after six weeks of storage. On the other hand, the application of both POLY- and PH-bags didn't show significant differences ($p>0.05$) in aerobic mesophilic counts concerning the initial (0 days) samples. On the contrary, for POLY- and PH-bags, there was an increase ($p<0.05$) in the counts of moulds and yeasts during the six weeks storage period. In the end, some microbial growth could be visible to the human eyes in some bags. In Figure 8 are shown the chestnuts packaged in all bags studied in the present work after six weeks.

6 weeks

Control

VAC

MAP

POLY

PH

Figure 8: Chestnuts after six weeks of storage.

The results obtained in POLY- and PH-bags are explained by the atmospheric conditions in these bags remained similar to the control (unpackaged chestnuts) (Figure 5). Mould spoilage can be a food safety problem due to the production of mycotoxins or allergens by these moulds. Of our knowledge, there are no microbiological levels established for fresh chestnut to evaluate their quality and safety. Nevertheless, when considering the limits set for ready-to-eat foods (INSA, 2019), the values to be considered satisfactory (Group 3) for raw fruits and vegetables must be < 6 log CFU/g of total aerobic mesophilic. Concerning moulds, the values should be < 2.7 log CFU/g. Taking into account these values, the counts obtained in the present work were not satisfactory for the moulds. However, the chestnuts are generally cooked (ex. boiled, roasted or fried) before consumption. Nevertheless, a very high microbial count in the shell of the chestnut may pose their safety in risk. So, it is essential to maintain low values of contamination, being essential the compliance of good agriculture practices.

By way of conclusion, and in comparison with previous works on the effect of MAP- and VAC-bags on chestnuts (Fernandes et al., 2020; Ozturk et al., 2016), it was also shown that these type of packagings were the most effective to inhibit the microbial growth. However, this effect was more evident only until four weeks of storage. After this period, the counts may increase due to the appearance of microscopic cracks in the bags, as a result of their excessive expansion, decreasing the CO_2 levels and therefore, its fungistatic properties. This effect was more evident in MAP-bags than in VAC-bags (Figure 5 and Table 10), because the VAC-bags are made from a more resistant material. Thus, these results indicated that the acquisition of the gas mixture used in the MAP might not be worthwhile, as similar and more consistent microbiological results were obtained only with the application of vacuum.

4.2 Long Storage Packaging

4.2.1 Gas analysis

For extended storage packaging (until six months), the application of microperforated bags (MP-bags) was evaluated in comparison to the unpackaged condition (control). In Figure 9, the concentrations of O_2 and CO_2 along the storage are represented. Stable levels of O_2 were measured, close to 21%. This fact suggests that the O_2 permeability was not affected by the existence of the MP-bags. On the contrary, the concentrations of CO_2 were remarkably higher in the MP-bags than the control (unpackaged) during the six months. In MP-bags, some oscillation in CO_2 levels was observed during the first three months (varying between 1.0 and 2.5%), decreasing afterwards to 0.7%, until the end of storage time (6 months).

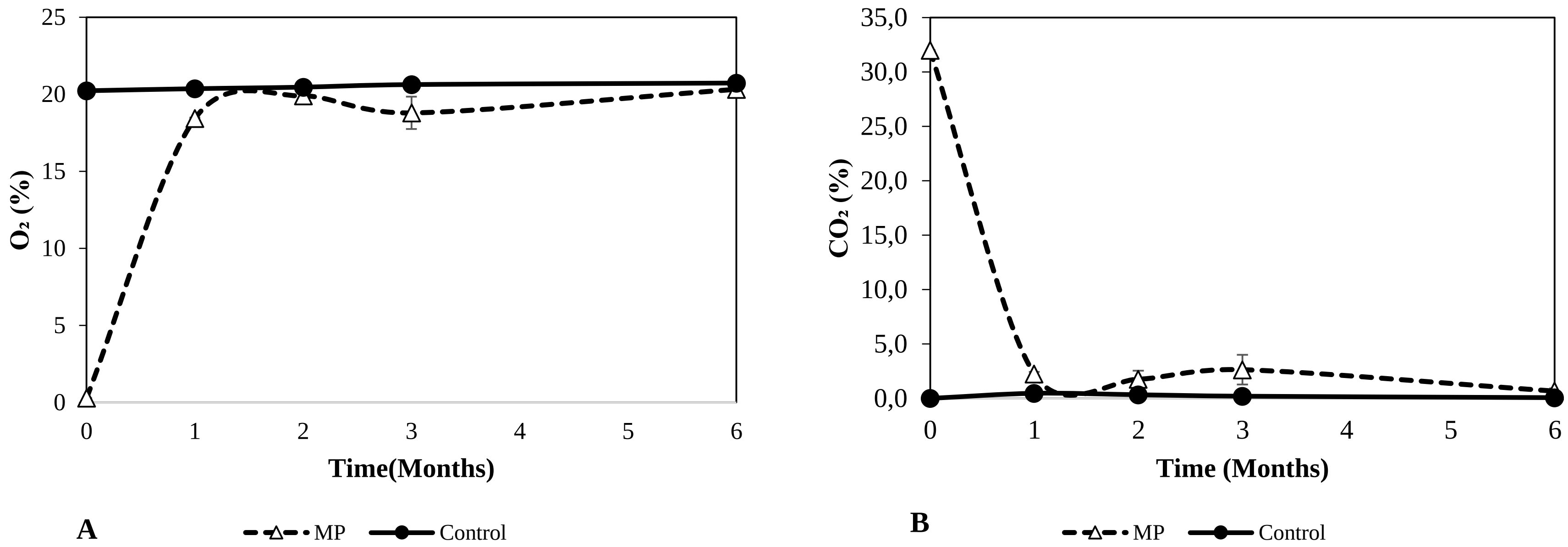

Figure 9: Gas concentrations of O_2 (A) and CO_2 (B) in the inner atmosphere of unpackaged and packaged chestnuts in MP-bags throughout six months of storage.

For the control, no significant changes in the CO_2 percentages were observed for six months. Thus, the type of packaging (micro-perforated) and the cellular respiration of fresh chestnuts have a substantial role in maintaining high levels of CO_2 among the inner atmosphere of the packaging and therefore, probably helping to sustain the good quality of chestnuts.

4.2.2 Colour

The role of micro-perforated bags and MAP on the colour parameters of the chestnuts was evaluated by regular testing of colour during storage, both outside (shell) and inside (kernel). The results are presented in Tables 12 and 13, respectively.

For the outside (shell) and in particular the lightness/darkness dimension (*L**), the chestnuts packaged in MP-bags showed a more significant decrease (35.6 to 28.9) compared to the control (35.6 to 31.3) during storage, suggesting a loss in shells lightness. Also, for the hue angle (h), significant differences between MP-bags and control were found, and the values increased after six months for both situations. For the rest of the parameters - the greenness-redness (*a**), the blueness-yellowness (*b**) coordinate values, and the saturation index or Chroma coordinate (*C**) – in the majority of the situations, no significant differences were found throughout storage. These results were different from those reported by Cecchini et al. (2011) for Italian chestnuts exposed to air or a controlled atmosphere (CA) (15% CO_2 and 3% O_2) during two months storage. These authors indicated colour differences between both storage conditions, namely a lower yellowing for the CA condition.

Table 12: Outside colour parameters of chestnuts (shells) in micro-perforated packaging and control during six months of storage.

Parameters	Packaging	Outside the fruit (Shell)				
		Storage time (Months)				
		0	1	2	3	6
L*	MP-bags	35.6 ± 2.0^{A}	$32.4 \pm 2.1^{a,B}$	$31.7 \pm 2.4^{b,B,C}$	$30.0 \pm 3.0^{b,C,D}$	$28.9 \pm 1.8^{b,D}$
	Control	35.6 ± 2.0^{A}	$31.2 \pm 2.2^{b,C}$	$35.4 \pm 1.6^{a,A}$	$33.1 \pm 1.8^{a,B}$	$31.3 \pm 2.0^{a,C}$
a*	MP-bags	$11.3 \pm 1.3^{A,B}$	$11.0 \pm 2.2^{a,A,B}$	$11.3 \pm 2.6^{a,A}$	$11.4 \pm 2.3^{a,A}$	$9.8 \pm 2.1^{a,B}$
	Control	11.3 ± 1.3^{A}	$9.3 \pm 2.3^{b,B}$	$10.5 \pm 1.5^{a,A,B}$	$9.7 \pm 2.0^{b,B}$	$10.5 \pm 1.4^{a,A,B}$
b*	MP-bags	5.1 ± 2.7^{B}	$7.2 \pm 2.2^{a,A}$	$6.2 \pm 3.3^{a,A,B}$	$6.4 \pm 2.5^{a,A,B}$	$6.2 \pm 2.6^{b,A,B}$
	Control	5.1 ± 2.7^{B}	$5.1 \pm 2.4^{b,B}$	$6.8 \pm 2.0^{a,A}$	$7.6 \pm 2.5^{a,A}$	$7.8 \pm 1.7^{a,A}$
C*	MP-bags	12.5 ± 2.0^{A}	$13.0 \pm 2.8^{a,A}$	$13.1 \pm 3.4^{a,A}$	$13.2 \pm 2.9^{a,A}$	$11.7 \pm 3.0^{b,A}$
	Control	$12.5 \pm 2.0^{A,B}$	$10.8 \pm 2.8^{b,B}$	$12.6 \pm 2.1^{a,A,B}$	$12.4 \pm 2.9^{a,A,B}$	$13.1 \pm 2.0^{a,A}$
h	MP-bags	22.9 ± 10.3^{B}	$33.0 \pm 4.5^{a,A}$	$27.1 \pm 11.0^{b,A,B}$	$29.0 \pm 7.5^{a,A,B}$	$31.5 \pm 7.3^{b,A}$
	Control	22.9 ± 10.3^{C}	$26.5 \pm 8.8^{b,C}$	$32.6 \pm 6.1^{a,B}$	$37.3 \pm 6.1^{b,A}$	$36.2 \pm 3.9^{a,A,B}$

Values are expressed as Mean ± Standard Deviation. Different lowercase letters on the same column indicate significant differences ($p<0.05$) between packages; Different capital letters on the same line indicate significant differences ($p<0.05$) between storage time.

For the chestnuts inside (kernel) and among the colour parameters, no significant differences have been noted between the micro-perforated packaging and the control in the majority of the situations. However, for the control, a decrease in the *L** values, as well as "h", and an increase in *a** values, were observed after six months when compared to the beginning. This fact can be explained by the exposure of the samples directly to the refrigerated environment and storage conditions that makes the fruit more vulnerable to perishability.

Table 13: Inside colour parameters of chestnuts (kernel) in micro-perforated packaging and control during six months of storage.

Parameters	Packaging	Inside the fruit				
		Storage time (Months)				
		0	1	2	3	6
L*	MP-bags	85.2 ± 3.8^{A}	$84.5 \pm 2.0^{a,A}$	$85.2 \pm 4.2^{a,A}$	$79.9 \pm 12.6^{a,A}$	$83.8 \pm 3.0^{a,A}$
	Control	85.2 ± 3.8^{A}	$82.1 \pm 8.3^{a,A,B}$	$81.2 \pm 6.0^{b,B}$	$80.2 \pm 5.5^{a,B}$	$77.6 \pm 11.4^{b,B}$
a*	MP-bags	-2.3 ± 0.7^{A}	$-2.1 \pm 1.1^{a,A}$	$-1.8 \pm 2.0^{a,A}$	$-1.0 \pm 2.6^{a,A}$	$-2.0 \pm 0.8^{b,A}$
	Control	-2.3 ± 0.7^{B}	$-1.8 \pm 1.9^{a,A,B}$	$-1.3 \pm 1.7^{a,A}$	$-1.7 \pm 1.0^{a,A,B}$	$-0.6 \pm 2.6^{a,A}$
b*	MP-bags	21.6 ± 3.2^{A}	$21.2 \pm 3.0^{a,A}$	$23.2 \pm 3.0^{a,A}$	$21.1 \pm 4.5^{a,A}$	$22.2 \pm 2.9^{a,A}$
	Control	21.6 ± 3.2^{A}	$21.8 \pm 4.0^{a,A}$	$21.2 \pm 3.0^{b,A}$	$22.6 \pm 4.1^{a,A}$	$22.6 \pm 4.5^{a,A}$
C*	MP-bags	21.7 ± 3.2^{A}	$21.3 \pm 3.0^{a,A}$	$23.4 \pm 3.0^{a,A}$	$21.2 \pm 4.6^{a,A}$	$22.3 \pm 2.9^{a,A}$
	Control	21.7 ± 3.2^{A}	$22.0 \pm 4.0^{a,A}$	$21.3 \pm 3.0^{b,A}$	$22.7 \pm 4.2^{a,A}$	$22.8 \pm 4.4^{a,A}$
h	MP-bags	96.1 ± 1.4^{A}	$96.2 \pm 2.1^{a,A}$	$94.6 \pm 4.8^{a,A,B}$	$92.5 \pm 6.6^{a,B}$	$95.3 \pm 1.9^{a,A,B}$
	Control	96.1 ± 1.4^{A}	$94.4 \pm 5.4^{a,A,B}$	$93.4 \pm 4.8^{a,B}$	$94.2 \pm 2.7^{a,B}$	$90.3 \pm 8.5^{b,B}$

Values are expressed as Mean ± Standard Deviation. Different lowercase letters on the same column indicate significant differences ($p<0.05$) between packages; Different capital letters on the same line indicate significant differences ($p<0.05$) between storage time.

4.2.3 Texture

Previous works on micro-perforated packaging, namely Kim et al. (2012) and Mistriotis et al. (2016), have achieved promising results for the quality preservation of vegetables and fruits such as chestnuts. So, the present work was performed with the aim to apply micro-perforated packaging with a modified atmosphere to chestnuts, as described in Section 3.4.2. In the current chapter, it was intended to evaluate the differences among texture properties during long time storage of six months.

When observing Table 14, significant differences were detected in the maximum force after six months for unpackaged (control) and MP-packaged chestnuts. The control presented a lower maximum force than MP-bags (3335 *versus* 3810 g, respectively). After 2, 3 and 6 months, the work done to penetrate the chestnuts (area below the curve) was always higher in the MP-bags than in control. This fact suggests some stiffening of the fruit, caused by the possible loss of water. Nevertheless, no significant differences were detected in elasticity, meaning that chestnuts still maintain a good degree elasticity. Cecchini et al. (2011) also verified that CA maintained chestnut firmness along with two months of storage, probably linked to the reduced metabolic activity due to the low oxygen availability.

In summary, the micro-perforated packaging under modified atmosphere showed to be efficient in the preservation of the quality of chestnuts, both in the shell and kernel, when compared to the control, being a promising solution for increasing the shelf-life for six months. The texture and colour were not negatively affected.

Table 14: Texture parameters of chestnuts in micro-perforated packaging during six months of storage.

Parameters	Packaging	Storage time (Months)				
		0	1	2	3	6
Maximum force (g)	MP-bags	3522±476^{A}	3559±550^{a,A}	3523±476^{a,A}	3499±776^{a,A}	3810±462^{a,A}
	Control	3522±476^{A}	3264±751^{a,A}	3438±787^{a,A}	3313±694^{a,A}	3335±664^{b,A}
Elasticity (mm)	MP-bags	3.5±1.2^{A}	3.8±1.4^{a,A}	4.0±1.4^{a,A}	3.7±1.4^{a,A}	3.9±1.5^{a,A}
	Control	3.5±1.2^{A}	3.8±1.3^{a,A}	4.0±1.3^{a,A}	4.1±1.1^{a,A}	4.4±1.1^{a,A}
Area below the curve (g.s)	MP-bags	14089±3690^{B}	14975±3429^{a,B}	15615±2990^{a,A,B}	16034±4257^{a,A,B}	17623±1927^{a,A}
	Control	14089±3690^{A}	13138±3941^{a,A,B}	12646±3723^{b,A,B,C}	11472±2530^{b,B,C}	10513±2906^{b,C}

Values are expressed as Mean ± Standard Deviation. Different lowercase letters on the same column indicate significant differences ($p<0.05$) between packages; Different capital letters on the same line indicate significant differences ($p<0.05$) between storage time.

4.2.4 Weight loss, Moisture content, a_w, and Titratable Acidity

As mentioned before, the weight loss has an economic impact on the profitability of the chestnut producers. Therefore, for preventing this disadvantage, this work intended to apply micro-perforated packaging during long storage, to determine the differences found in weight loss between the packaged and the unpackaged (control) samples. The results are presented in Figure 10.

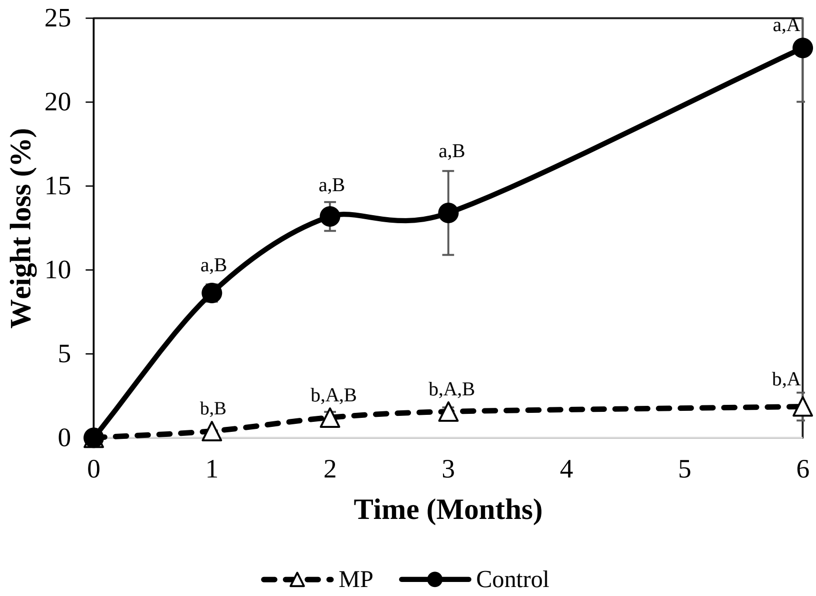

Figure 10: Weight loss (%) of unpackaged and packaged chestnuts in MP-bags during six months of storage.

During the six months storage, the control samples had shown a remarkable increasing pattern in the weight loss (from 0 to 23%), which can be explained with the direct exposure of the nuts to the ambient atmosphere. These results are similar to those reported by Cecchini et al. (2011), who also observed that air-stored fruits during two months, lost an average of 3.05%±0.56 more weight than those stored in CA. Alternatively, in the present study, the modified atmosphere and micro-perforated packaging showed a much lower percentage of weight loss (0 to 1.9%). These results indicated the MP-bags showed to be a promising solution to reduce the water loss and subsequently, the economic losses of chestnut producers. Another point to consider was the fact that in texture, it was suggested that the higher work detected for the MP-bags, could indicate a stiffening of the fruit by the loss of water. However, this stiffening may instead be due to the fruit remaining turgid and less soft when packed in MP-bags when compared to the control.

Furthermore, it was also possible to state that the storage at low temperatures also decreases the water loss. In the short-storage at room temperature, the weight loss was 13.2% after six weeks, while under refrigeration it was equal to 8.6% after one month. Furthermore, a weight loss equal to 10% was never achieved with MP-bags, which is an excellent result. Thus, the use of micro-perforated and modified atmosphere packaging may have a significant impact on retaining the weight of the chestnuts due to the controlling mechanism of gas permeability provided by the packaging film, preventing dehydration, give protection from environmental effects and decrease the perishability of the samples.

In all MP-packaged samples, there were no significant differences ($p>0.05$) concerning the moisture content all along the storage time (Table 15). After six months, a significant difference was observed between the control (42.7%) and MP-bags (55.2%), suggesting that the packaged chestnuts retained more water, drying less.

Regarding the a_w, in most situations, no significant differences were observed between MP-bags and the control, as well as along the storage time. These results are in line with those observed in the short-time storage. Again, the a_w varied between 0.95 and 0.98, which are values that allow the development of moulds and the risk of mycotoxins to be elaborated (Ordónez et al., 2005).

Table 15: Weight loss, moisture content, a_w and titratable acidity of chestnuts in micro-perforated packaging and unpackged throughout six months of storage

Parameters	**Packaging**	**Storage time (Months)**				
		0	**1**	**2**	**3**	**6**
Weight Loss	MP-bags	--	$0.40±0.03^{b,B}$	$1.21±0.34^{b,A,B}$	$1.56±0.24^{b,A,B}$	$1.86±0.83^{b,A}$
	Control	--	$8.62±0.52^{a,B}$	$13.18±0.86^{a,B}$	$13.39±2.51^{a,B}$	$23.22±3.21^{a,A}$
Moisture (%)	MP-bags	$51.7±3.3^{A}$	$51.6±2.2^{a,A}$	$47.9±2.4^{a,A}$	$51.1±1.7^{a,A}$	$55.2±4.1^{a,A}$
	Control	$51.7±3.3^{A}$	$43.2±4.0^{b,A}$	$45.5±6.0^{a,A}$	$46.2±2.9^{a,A}$	$42.7±1.3^{b,A}$
a_w	MP-bags	$0.974±0.003^{A}$	$0.977±0.002^{a,A}$	$0.980±0.004^{a,A}$	$0.979±0.002^{a,A}$	$0.972±0.004^{a,A}$
	Control	$0.974±0.003^{A}$	$0.951±0.014^{b,A}$	$0.953±0.024^{a,A}$	$0.960±0.005^{b,A}$	$0.960±0.007^{a,A}$
Titratable Acidity (g citric acid/100 g d.w.)	MP-bags	$0.14±0.04^{A}$	$0.12±0.02^{a,A}$	$0.20±0.15^{a,A}$	$0.16±0.06^{a,A}$	$0.22±0.01^{a,A}$
	Control	$0.14±0.04^{A}$	$0.12±0.02^{a,A}$	$0.24±0.11^{a,A}$	$0.12±0.01^{a,A}$	$0.16±0.04^{a,A}$

Values are expressed as Mean ± Standard Deviation. Different lowercase letters on the same column indicate significant differences ($p<0.05$) between packages; Different capital letters on the same line indicate significant differences ($p<0.05$) between storage time.

Concerning the titratable acidity, the values varied between 0.12 and 0.24 g citric acid/100 g d.w. Between MP-bags and the control, as well as along storage time, no significant differences were observed, suggesting that organic acids present in chestnuts were not being used as a substrate in the respiration process. These results were different from those mentioned by Bovi et al. (2018), who applied micro-perforated packaging on fruits. These authors reported that the permeability of the packaging permitted the exchange of O_2 and CO_2, which could affect the acidity of the packaged samples directly and increased the value throughout the long storage.

In general, the application of micro-perforated packaging under modified atmosphere caused no changes in the titratable acidity of the samples. It also did not cause damages in the appearance (colour and texture) of chestnuts. Furthermore, no off-odours were felt. All these results suggest that MP-bags might be a packaging type with proved advantages for maintaining crucial properties, such as small weight losses, and by preserving the quality of chestnuts during long storage periods.

4.2.5 Total soluble solids (TSS), reducing sugars, amylose and starch content

The contents in TSS are presented in Table 16. Among the MP-bags and control, there were no significant differences between them, except in the sixth month. In that month, the control showed a lower value than the MP-bags. However, this TSS value seemed to be underestimated. Regarding the storage time, some variation in the values was observed; however, none specif trend was perceived.

The starch contents are also presented in Table 16 and ranged from 35.2 to 48.6% on a dry matter basis, similar to the range obtained in the short-time storage. No significant differences were observed between MP-bags and control. Along time, for MP-bags and control, a slight increase in starch contents was stated on 3 and 6 months in comparison to the beginning. As indicated previously, different activities of the enzymes could explain this fact during storage. These results were in line with those obtained in the assay of short-time storage.

Table 16: Total soluble solids (TSS), reducing sugars, starch and amylose contents of chestnuts packaged in micro-perforated bags and without packaging throughout six months of storage.

Parameters	Packaging	Storage Time (Months)				
		0	1	2	3	6
TSS (%, d.m.)	MP-bags	47.2±8.7^{A,B}	33.7±2.9^{a,B}	28.4±0.8^{a,B}	60.5±17.3^{a,A}	63.1±9.1^{a,A}
	Control	47.2±8.7^{A}	40.6±5.0^{a,A}	34.5±12.2^{a,A}	40.6±14.7^{a,A}	28.5±7.0^{b,A}
Starch Content (%, d.m.)	MP-bags	35.2±0.3^{C}	43.5±0.2^{a,B}	40.9±1.8^{a,B}	48.5±1.2^{a,A}	48.6±1.2^{a,A}
	Control	35.2±0.3^{C}	44.8±1.0^{a,B}	40.2±10.7^{a,A,B,C}	48.2±0.3^{a,A,B}	48.4±0.7^{a,A}
Amylose Content (%, d.m.)	MP-bags	11.6±0.7^{C}	16.2±0.7^{b,B,C}	17.2±3.3^{b,B}	29.4±0.6^{a,A}	28.1±2.3^{a,A}
	Control	11.6±0.7^{C}	21.4±0.6^{a,B}	24.1±1.1^{a,A}	26.0±1.1^{b,A}	26.6±1.1^{a,A}
Amylose Content expressed on starch basis (%)	MP-bags	33.0±1.7^{B}	37.2±1.5^{b,B}	42.2±9.5^{a,B}	60.6±0.5^{a,A}	57.8±6.3^{a,A}
	Control	33.0±1.7^{B}	47.9±0.7^{a,A}	64.1±23.0^{a,A,B}	53.8±2.2^{b,A}	55.0±2.9^{a,A}
Reducing Sugars (g Glucose/100g d.m.)	MP-bags	0.09±0.01^{D}	0.31±0.01^{b,B,C}	0.46±0.01^{b,A}	0.33±0.02^{b,B}	0.26±0.01^{b,C}
	Control	0.09±0.01^{D}	0.64±0.05^{a,B,C}	0.58±0.03^{a,C}	0.86±0.07^{a,B}	2.06±0.09^{a,A}

Values are expressed as Mean ± Standard Deviation. Different lowercase letters on the same column indicate significant differences ($p<0.05$) between packages; Different capital letters on the same line indicate significant differences ($p<0.05$) between storage time.

For the amylose expressed on the starch basis, the majority of the values varied from 33.0 and 60.6%. In the present work, some significant differences were observed between MP-bags and the control (one and three months). Concerning storage time, the lowest percentage of amylose was obtained at the beginning, increasing afterwards.

The reducing sugars contents were significantly influenced by the packaging type and storage time (Table 16). The highest values were observed in the unpackaged chestnuts (control), as showed on Figure 11. These results indicate that the absence of barriers can influence the enzymatic reactions leading to partial hydrolysis of the starch, as well as to sucrose hydrolysis. Along the storage period, some variability, without a definite trend, in the MP-bags, was observed. On the contrary, in control, the contents of reducing sugars increased from 0.09 to 2.06 g glucose/100 g d.m., from the beginning to 6 months of storage, probably due to starch or sucrose hydrolysis. However, as the concentrations of reducing sugars remained low (<2.1 g glucose / 100 g d.m.), the observed increase was not felt in a reduction in the percentage of the starch present. The portion of this last component was high, superior to 35%. This fact was also observed in the short-time storage assay.

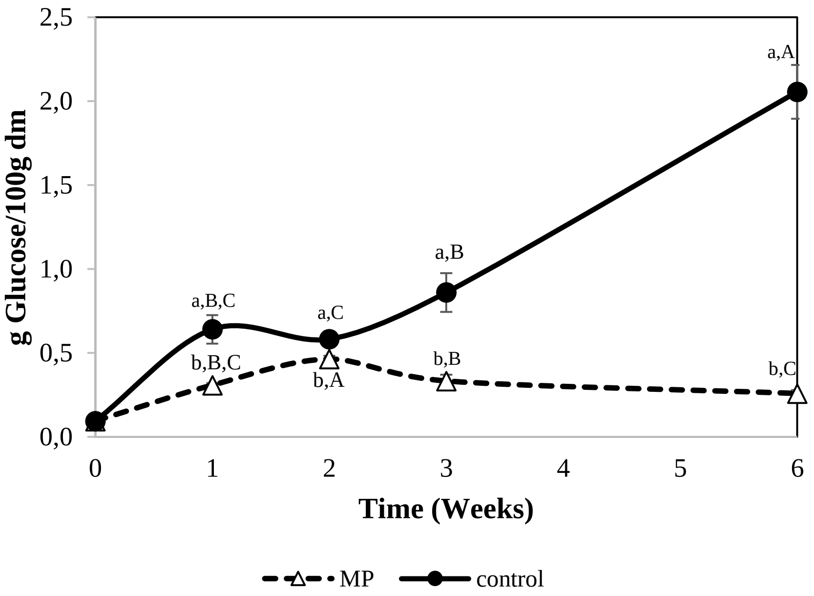

Figure 11: Reducing sugars of unpackaged and packaged chestnuts in MP-bags throughout six months of storage.

4.2.6 Microbial analysis

The impact of using micro-perforated (MP) bags under a modified atmosphere on the spread of aerobic mesophilic, moulds and yeast, in chestnuts was evaluated during six months storage. The results achieved are indicated in Table 17.

Table 17: Mean counts (log CFU/g) of total aerobic mesophilic, and moulds/yeasts in chestnuts stored for six months unpackaged and in micro-perforated bags

Microbial groups	Packaging	Time(Months)				
		0	1	2	3	6
Aerobic mesophilic	**Control**	5.58 ± 0.52^{aA}	5.67 ± 0.15^{aA}	5.67 ± 0.15^{aB}	5.67 ± 0.25^{aA}	5.43 ± 0.31^{aA}
	MP-bags	5.58 ± 0.52^{aA}	5.60 ± 0.17^{aA}	6.20 ± 0.52^{aA}	5.33 ± 0.30^{aA}	5.73 ± 0.64^{aA}
Moulds and yeasts	**Control**	4.32 ± 0.44^{cA}	5.63 ± 0.06^{aA}	5.67 ± 0.15^{bcA}	4.70 ± 0.17^{bcA}	5.30 ± 0.36^{abA}
	MP-bags	4.32 ± 0.44^{bA}	4.37 ± 0.06^{abB}	4.97 ± 0.21^{abA}	4.17 ± 0.45^{bA}	5.10 ± 0.17^{aA}

Values are expressed as Mean (log cfu/g) ± standard deviation. Different lowercase letters on the same line indicate significant differences (p<0.05) between storage time; Different capital letters on the same column indicate significant differences (p <0.05) between packages.

The effect of micro-perforated packaging on the proliferation of aerobic mesophilics showed non-statistical significant differences among the countings during six months (5.58 to 5.73 log CFU/g), as well as for the control samples (5.58 to 5.43 log CFU/g). Furthermore, the population of moulds and yeasts had expressed a minor increase in their numbers (4.32 to 5.10 log CFU/g), showing almost an increase of one logarithmic cycle after six months. For the control, an increase in one logarithmic cycle was stated; however, between both samples (control and MP-bags), no significant differences were observed at the end of storage.

The lowest counts of moulds and yeasts were obtained in MP-bags after one and three months, and it could be due to the highest CO_2 concentrations measured in those bags (Figure 9). This gas has fungistatic properties, inhibiting fungi growth.

In general, the results showed that the use of micro-perforated bags did not significantly reduce microbial development. However, this type of packaging had significantly reduced the weight loss, which means a significant economic gain for the producers and industry of this fruit. In the future, it will be interesting to carry out an economic feasibility study of the process, taking into account the use of modified atmosphere and so the costs of the bags and gas mixture.

5 CONCLUSION

The present work exhibited significant commercial results for the chestnut industry concerning the extension of the shelf-life and maintenance of the quality of this nut, mainly by reducing the weight loss, by the use of different packagings during short and long storage time.

In the short-storage assay, the colour, texture, moisture content, a_w, titratable acidity and total soluble solids were little affected by the type of bags used. The starch contents varied between 35.2 to 50.4% d.m. without a specific trend. Similar results were observed in amylose expressed on the starch basis (25.7 to 45.0%), suggesting no remarkable changes in starch functionality. On the other hand, significant differences were observed between bags in weight loss, reducing sugars and microbial counts. The VAC-, MAP-, and POLY-bags showed percentages of weight loss lower than 2%, while the control and PH-bags had values equal to 13.2 and 9.2%, respectively. The highest values of reducing sugars were observed in POLY- and PH-bags, followed by the control, suggesting partial hydrolysis of the starch. The application of VAC- and MAP-bags caused a considerable decrease in aerobic mesophiles, moulds and yeasts counts compared to the control during two and four weeks of storage. After this period, the counts increased probably due to the appearance of microscopic cracks in the bags, as a result of their excessive expansion. These cracks might explain the decrease in CO_2 levels and therefore, its fungistatic properties. This effect was more evident in MAP-bags than in VAC-bags, because the latter ones are made from a more resistant material. Thus, these results indicated that the acquisition of the gas mixture used in the MAP might not be worthwhile, as similar and more consistent microbiological results were obtained only with the application of vacuum.

During long-storage, stable levels of oxygen were measured, close to 21% in both situations (MP-bags and unpackaged); however, the concentrations of CO_2 were remarkably higher in the MP-bags than the control during the six months. Chestnuts packaged in MP-bags showed a significant decrease in shells lightness and an increase in the hue angle (*h*); however, for the other parameters, no significant differences were found. On the contrary, no significant differences were noted in the colour of chestnut kernel between the MP-bags and the control, in the majority of the situations. Furthermore, the texture was also less affected. Only after 2, 3 and 6 months, the work done to perforate the chestnuts was always higher in the MP-bags than in control. After six months, the weight loss was much lower in MP-bags (1.9%) than in control (23%), and a significant difference in moisture content was observed between the control and MP-bags (42.7 and 55.2%, respectively), suggesting that the packaged chestnuts retained more the water. Titratable acidity, total soluble solids and starch did not vary significantly between packages. The reducing sugars were significantly influenced by the packaging type and storage time. The highest values were observed in unpackaged chestnuts (control). Along the storage period, some variability, without a definite trend, in the MP-bags, was observed. In control, the contents of reducing sugars increased from 0.09 to 2.06 g glucose/100 g d.m.. For the control and MP-bags, no significant differences in aerobic mesophiles and moulds and yeasts were observed at the end of storage. In general, the MP-bags did not significantly reduce microbial development but had significantly reduced weight loss. Thus, the MP-bags demonstrated to be a promising solution for extending the shelf-life of chestnuts during long storage (6 months) by preserving the nutritional quality of the fruits.

In conclusion, the results of the present study showed that the preservation of the chestnuts by the use of a specific type of packaging might have a substantial impact on preserving the colour and texture of the fruits, on preventing the weight loss and microbial growth and on maintaining the moisture content of the fruits.

6 REFERENCES

Acedo, A.L. (1997). Storage life of vegetables in simple evaporative coolers. *Tropical Science*, **37**, 169-175.

Attanasio, G., Cinquanta, L., Albanese, D., Matteo, M.D. (2004). Effects of drying temperatures on physico-chemical properties of dried and rehydrated chestnuts (*Castanea sativa*). *Food Chemistry*, **88**, 583-590.

Barreira, J. C. M., Casal, S., Ferreira, I. C. F. R., Oliveira, M. B. P. P., & Pereira, J. A. (2009). Nutritional, Fatty acid and triacylglycerol profiles of castanea sativa Mill. Cultivars: A Compositional and Chemometric Approach. *Journal of Agricultural and Food Chemistry*, *57*(7), 2836–2842. https://doi.org/10.1021/jf803754u

Barreira, J. C. M., Pereira, J. A., Oliveira, M. B. P. P., & Ferreira, I. C. F. R. (2010). Sugars profiles of different chestnut (Castanea sativa Mill.) and almond (Prunus dulcis) cultivars by HPLC-RI. *Plant Foods for Human Nutrition*, *65*(1), 38–43. https://doi.org/10.1007/s11130-009-0147-7

Bernárdez, M.M., Miguélez, J.M., Queijeiro, J.G. (2004). HPLC determination of sugars in varieties of chestnut fruits from Galicia (Spain). *Journal of Food Composition and Analysis*, **17**, 63-67.

Borges, O., Gonçalves, B., Soeiro de Carvalho, J.L., Correia, P., Silva, A.P. (2008). Nutritional quality of chestnut (*Castanea sativa* Mill.) cultivars from Portugal. *Food Chemistry*, 106, 976-984.

Borges, O. P., Soeiro Carvalho, J., Reis Correia, P., & Paula Silva, A. (2007). Lipid and fatty acid profiles of Castanea sativa Mill. Chestnuts of 17 native Portuguese cultivars. *Journal of Food Composition and Analysis*, *20*(2), 80–89. https://doi.org/10.1016/j.jfca.2006.07.008

Bovi, G. G., Caleb, O. J., Ilte, K., Rauh, C., & Mahajan, P. V. (2018). Impact of modified atmosphere and humidity packaging on the quality, off-odour development and volatiles of ‘Elsanta’ strawberries. *Food Packaging and Shelf Life*, *16*(April), 204–210. https://doi.org/10.1016/j.fpsl.2018.04.002

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*(2–3), 131–136. https://doi.org/10.1016/j.postharvbio.2011.03.001

Chenlo, F., Moreira, R., Chaguri, L., & Torres, M. D. (2010). Effects of storage conditions on sugars and moisture content of whole chestnut fruits. *Journal of Food Processing and Preservation, 34*(4), 609–620. https://doi.org/10.1111/j.1745-4549.2008.00361.x

Correia, P., Leitão, A., & Beirão-da-Costa, M. L. (2009). The effect of drying temperatures on morphological and chemical properties of dried chestnuts flours. *Journal of Food Engineering*, *90*(3), 325–332. https://doi.org/10.1016/j.jfoodeng.2008.06.040

Correia, P. R., & Beirão-Da-Costa, M. L. (2011). Effect of Drying Temperatures on Starch-Related Functional and Thermal Properties of Acorn Flours. *Journal of Food Science*, *76*(2). https://doi.org/10.1111/j.1750-3841.2010.01978.x

De La Montaña Míguelez, J., Míguez Bernárdez, M., & García Queijeiro, J. M. (2004). Composition of varieties of chestnuts from Galicia (Spain). *Food Chemistry*, *84*(3), 401–404. https://doi.org/10.1016/S0308-8146(03)00249-8

de Vasconcelos, M. C. B. M., Bennett, R. N., Rosa, E. A. S., & Ferreira-Cardoso, J. V. (2010). Composition of European chestnut (Castanea sativa Mill.) and association with health effects: Fresh and processed products. *Journal of the Science of Food and Agriculture*, *90*(10), 1578–1589. https://doi.org/10.1002/jsfa.4016

de Vasconcelos, M.C.B.M., Nunes, F.,Viguera, C.G., Bennett, R.N., Rosa, E.A.S., Ferreira-Cardoso, J.V. (2010b). Industrial processing effects on chestnut fruits (*Castanea sativa* Mill.) 3. Minerals, free sugars, carotenoids and antioxidant vitamins. *International Journal of Food Science and Technology*, **45**, 496-505.

Donis-González, I.R., Fulbright, D.W., Ryser, E.T., Guyer, D. (2010). Shell mold and kernel decay of fresh chestnuts in Michigan. *Acta Horticulturae*, **866**, 353-362.

DGAV, 2018. Manual de Procedimentos – Exportação de castanha em fresco submetida a tratamento com água quente em sistema contínuo. Procedimento a adotar nas Centrais de Armazenagem e Embalagem (CAE) de castanha. Versão 01 de 18/04/2018.

Fernandes, L., Pereira, E. L., Fidalgo, M. D. C., Gomes, A., & Ramalhosa, E. (2020). Effect of Modified Atmosphere, Vacuum and Polyethylene Packaging on Physicochemical and Microbial Quality of Chestnuts (Castanea sativa) during Storage. *International Journal* *of Fruit Science*, *00*(00), 1–17. https://doi.org/10.1080/15538362.2020.1768619

Garcia, W.J., Wolf, M.J., 1972. Polarimetric determination of starch in corn with dimethyl sulfoxide as a solvent. *Cereal Chemistry*, **49**, 298–306.

Getinet, H., Seyoum, T., Woldetsadik, K. (2008). The effect of cultivar, maturity stage and storage environment on quality of tomatoes. *Journal of Food Engineering*, **87**, 467-478.

Gonçalves, B., Borges, O., Costa, H. S., Bennett, R., Santos, M., & Silva, A. P. (2010). Metabolite composition of chestnut (Castanea sativa Mill.) upon cooking: Proximate analysis, fibre, organic acids and phenolics. *Food Chemistry*, *122*(1), 154–160. https://doi.org/10.1016/j.foodchem.2010.02.032

Holland, B., Unwin, I.D., Buss, D.H. (1992). Fruit and Nuts. First Supplement to McCance & Widdowson's – The Composition of Foods (Fifth Edition). Royal Society of Chemistry – MAFF, United Kingdom.

Hahladakis, J. N., & Iacovidou, E. (2018). Closing the loop on plastic packaging materials: What is quality and how does it affect their circularity? *Science of the Total Environment*, *630*, 1394–1400. https://doi.org/10.1016/j.scitotenv.2018.02.330

ISO 4833-2. 2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 2: Colony count at 30 degrees C by the surface plating technique.

ISO 21527-1. 2008. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 1: Colony count technique in products with water activity greater than 0,95. 1st edition.

ISO 7218. 2007. Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations. 3rd edition.

Juliano BO. 1971. A simplified assay for milled-rice amylose. *Cereal Sci Today*, **16**, 334–40.

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, Agriculture and Environment*, *10*(3–4), 168–172.

Künsch, U., Schärer, H., Patrian, B., Höhn, E., Conedera, M., Sassella, A., Jermini, M., &

Jelmini, G. (2001). Effects of roasting on chemical composition and quality of different chestnut (Castanea sativa Mill) varieties. *Journal of the Science of Food and Agriculture*, *81*(11), 1106–1112. https://doi.org/10.1002/jsfa.916

Mencarelli, F. (2001). Postharvest handling and storage of chestnuts. Working document of the project: TCP/CPR/8925 "Integrated Pest Management and Storage of Chestnuts in XinXian County, Henan Province, China.". FAO (Ed.)

Míguelez, J.D.M., Bernárdez, M.M., & Queijerio, J.M.G. (2004). Composition of Varieties of Chestnuts from Galicia (Spain), *Food Chemistry*, *84*(3), 401-404. https://doi.org/10.1016/S0308-8146(03)00249-8

Mistriotis, A., Briassoulis, D., Giannoulis, A., & D'Aquino, S. (2016). Design of biodegradable bio-based equilibrium modified atmosphere packaging (EMAP) for fresh fruits and vegetables by using micro-perforated poly-lactic acid (PLA) films. *Postharvest Biology and Technology*, *111*, 380–389. https://doi.org/10.1016/j.postharvbio.2015.09.022

Neri, L., Dimitri, G., & Sacchetti, G. (2010). Chemical composition and antioxidant activity of cured chestnuts from three sweet chestnut (Castanea sativa Mill.) ecotypes from Italy. *Journal of Food Composition and Analysis*, *23*(1), 23–29. https://doi.org/10.1016/j.jfca.2009.03.002

Nogueira, C., & Correia, P. (2008). Conservation of nuts from native Portuguese chestnut cultivars. *Acta Horticulturae*, *784*, 65–69. https://doi.org/10.17660/actahortic.2008.784.8

Ozturk, I., Sagdic, O., Yalcin, H., Capar, T. D., & Asyali, M. H. (2016). The effects of packaging type on the quality characteristics of fresh raw pistachios (Pistacia vera L.) during the storage. *LWT - Food Science and Technology*, *65*, 457–463. https://doi.org/10.1016/j.lwt.2015.08.046

Ordóñez, J.A., Rodríguez, M.I.C., Álvarez, L.F., Sanz, M.L.G., Minguillón, G.D.G.F., Perales, L.H., Cortecero, M.D.S. (2005). Tecnologia de Alimentos, Vol. 1, Componentes os Alimentos e Processos. Juan A. Ordóñez Pereda (Ed.), Artmed Editora, Brasil.

Peña-Méndez, E.M., Hernández-Suárez, M., Díaz-Romero, C., Rodríguez-Rodríguez, E. (2008). Characterization of various chestnut cultivars by means of chemometrics approach. *Food Chemistry*, **107**, 537-544.

Pereira-Lorenzo, S., Ramos-Cabrer, A.M., Díaz-Hernández, M.B., Ciordia-Ara, M., Ríos-Mesa, D. (2006). Chemical composition of chestnut cultivars from Spain. *Scientia Horticulturae*, **107**, 306-314.

Piccolo, E.L., Landi, M., Ceccanti, C., Mininni, A.N., Marchetti, L., Massai, R., Guidi, L., Remorini, D. (2020). Nutritional and nutraceutical properties of raw and traditionally obtained flour from chestnut fruit grown in Tuscany. *European Food Research and Technology*, **246**, 1867-1876.

Torres, M.D., Moreira, R., Chenlo, F., Morel, M.H., Barron, C. (2014). Physicochemical and structural properties of starch isolated from fresh and dried chestnuts and chestnut flour. *Food Technology and Biotechnology*, **52**(1), 135-139.

Tyl, C., Sadler, G.D. (2017). pH and Titratable Acidity. In: Food Analysis. Suzanne Nielsen (ed). Food Science Text Series. Springer, Cham. https://doi.org/10.1007/978-3-319-45776-5_22

Uylaser, V., Incedayi, B. K., Mert, C., & Soylu, A. (2010). A research on suitability of some chestnut cultivars for candied chestnut. *Acta Horticulturae*, *866*, 571–579. https://doi.org/10.17660/ActaHortic.2010.866.77

Vekiari, S. A., Panagou, E. Z., & Mallidis, C. (2007). The effects of cold storage on the quality of peeled, raw or heat-treated greek chestnuts packed under vacuum. *Journal of Horticultural Science and Biotechnology*, *82*(6), 967–973. https://doi.org/10.1080/14620316.2007.11512334