Effect of commercial edible coatings on the shelf life of chestnut (Castanea sativa) subjected to long-term storage

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Abstract

The chestnut fruit is very consumed worldwide. However, three main types of problems can be stated in the postharvest and during chestnut storage, namely insect worms and the development of filamentous fungi, and weight loss. Thus, the search for methodologies that minimize these problems is fundamental. The purpose of this present work was to test different commercial edible coatings, namely Proallium, Foodcoat and the two combined, to apply to chestnuts to increase their long-term storage. Thus, the main objective of this work was to check the effect of these edible coatings on the physic-chemical and microbiological properties of the fruit along its storage to evaluate the food quality and safety.

All coated and uncoated samples were stored in refrigerated industrial chambers for four months. The weight loss, color, texture, water activity (a_w), moisture content, titratable acidity, total soluble solids, reducing sugars, individual sugars, starch contents, and microbiological analysis were determined. During the four months of storage, the color parameters of the shell and kernel showed no significant differences between the treatments, with some exceptions. For the texture parameters (skin strength, elasticity and work required to penetrate the chestnut tissue), no significant differences were found between the treatments for a specific storage time. Nevertheless, a decrease in the maximum force and the area below the curve were detected along the storage period for each treatment. For the control, Proallium, Foodcoat, and Proallium + Foodcoat, a decrease in the area under the curve was observed after four months of storage compared to the beginning. Concerning the weight loss, after storage, Foodcoat was the coating with the best performance with the lowest weight loss (5 to 6%). On the contrary, chestnuts with Proallium and Proallium+Foodcoat achieved weight losses of around 14%. The application of the edible coatings had no significant effect on the moisture content and water activity, while the coatings had increased the titratable acidity. The total soluble solids increased in the first month for both coated and uncoated (control) fruit; however, no significant differences were observed between the treatments, suggesting that the different coatings applied did not change the TSS of the chestnuts significantly, with some exceptions. For the reducing sugars, significant differences were only observed after four months. The highest content was determined in control (553 ± 48 mg/100 g d.m.). The starch contents were determined only at the beginning and after four months. The values varied between 48.9 and 51.2%, with no significant differences between treatments and both times. For the individual sugars, the sacharose+maltose were the main free sugars present in the chestnuts. No significant differences were observed between treatments and storage time for raffinose and sacharose+maltose. On the contrary, a decrease in glucose and fructose contents was observed in Foodcoat after four months. In Proallium, this decrease was only determined on fructose. For aerobic mesophiles and the moulds and yeasts, the Proallium was the most efficient during two months. On the contrary, in the control and other treatments, the counts increased along storage time.

In conclusion, the application of commercial edible coatings may significantly prevent weight loss and microbial growth.

Keywords

Chestnut; edible coatings; physic-chemical parameters; microbial counts; storage life.

Resumo

A castanha é um fruto muito consumido no mundo inteiro. No entanto, três principais tipos de problemas podem ser mencionados na pós-colheita e durante o armazenamento da castanha, designadamente a presença de insetos e o desenvolvimento de fungos filamentosos, e a perda de peso. Assim, a procura de metodologias que minimizem estes problemas é fundamental. O objetivo do presente trabalho foi testar diferentes revestimentos comestíveis comerciais, designadamente, Proallium, Foodcoat e a sua mistura (Proallium+Foodcoat), para aplicar às castanhas para aumentar o seu armazenamento a longo prazo. Assim, o objetivo principal deste trabalho foi verificar o efeito destes revestimentos comestíveis nas propriedades físico-químicas e microbiológicas na castanha ao longo do seu armazenamento, a fim de avaliar a qualidade e segurança alimentar.

Todas as amostras revestidas e não revestidas (controlo) foram armazenadas em câmaras industriais refrigeradas por 4 meses. Os parâmetros determinados foram a perda de peso, cor, textura, atividade da água (a_w), teor de humidade, acidez titulável, sólidos solúveis totais, açúcares redutores, açúcares individuais, amido e análise microbiológica. Durante esses quatro meses de armazenamento, a cor da casca e do miolo não mostraram diferenças significativas entre os tratamentos, salvo raras exceções. Para a textura (dureza da casca, elasticidade e trabalho necessário para penetrar a castanha), não foram encontradas diferenças significativas entre os tratamentos para um tempo de armazenamento específico. No entanto, uma diminuição da força máxima e da área abaixo da curva foram detectadas ao longo do período de armazenamento para cada tratamento. Para o controlo, Proallium, Foodcoat e Proallium + Foodcoat, uma diminuição na área sob a curva foi observada após quatro meses de armazenamento quando comparado com o início. Em relação à perda de peso, após o armazenamento, o Foodcoat foi o revestimento que mostrou o melhor desempenho com a menor perda de peso (5 a 6%). Pelo contrário, as castanhas revestidas com Proallium e Proallium+Foodcoat alcançaram perdas de peso de cerca de 14%. A aplicação dos revestimentos comestíveis não teve efeito significativo sobre o teor de humidade e a atividade da água, mas a aplicação de revestimentos aumentaram a acidez titulável. Os sólidos solúveis totais aumentaram no primeiro mês tanto para a fruta revestida quanto para a não revestida (controlo). Entretanto, não foram observadas diferenças significativas entre tratamentos, sugerindo que os diferentes revestimentos aplicados não alteraram significativamente os TSS, salvo algumas exceções. Em relação aos açúcares redutores, diferenças significativas só foram observadas após quatro meses. O valor mais alto foi determinado no controle (553 ± 48 mg/100 g d.m.). Quanto ao teor de amido, os valores só foram determinados somente no início e após quatro meses. Os valores variaram entre 48,9 e 51,2%, sem diferenças significativas entre os tratamentos e ambos os tempos. Para os açúcares individuais, a sacarose+maltose foram os principais açúcares livres presentes nas castanhas. Não foram observadas diferenças significativas entre tratamentos e tempo de armazenamento para a rafinose e sacarose+maltose. Pelo contrário, uma diminuição nos teores de glicose e frutose foi observada no Foodcoat após quatro meses. No Proallium, esta diminuição foi só observada na frutose. Para os microrganismos mesófilos aeróbios e os bolores e leveduras, o Proallium foi o mais eficiente durante dois meses. Pelo contrário, ascontagens aumentaram ao longo do tempo de armazenamento para o controlo e outros tratamentos.

Em conclusão, a aplicação de revestimentos comestíveis comerciais pode prevenir de forma significativa a perda de peso e o crescimento microbiano.

Palavras-chave

Castanha; revestimentos comestíveis; parâmetros físico-químicos; contagens microbiológicas; tempo de prateleira.

1 Introduction

The chestnut (genus *Castanea*) is an important tree that is used for different purposes. It belongs to the beech family (Fagaceae), including beech (*Fagus*), oak (*Quercus*) and Castanopsis (Koyuncu et al., 2004). As indicated in **Table 1**, there are 12 species of chestnuts native to the temperate zone of the Northern Hemisphere. Five species are from East Asia, six from North America, and one from Europe.

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

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

1.1 Chestnut world production, in Europe, Portugal and Tunisia

1.1.1 Chestnut world production

The harvested area of chestnuts in the world was 612 877 hectares, corresponding to 2 353 825 tonnes in 2018 (FAO, 2020). In the following figure, the main producers of chestnuts worldwide are represented. China is the first producer of chestnuts with 1 965 351 tonnes in 2018, followed by Bolivia (84 010 tonnes), Turkey (63 580 tonnes).

Korea (66207 tonnes), Italy (53 280 tonnes), Greece (35 230 tonnes) and then Portugal with a total production of 34 165 tonnes.

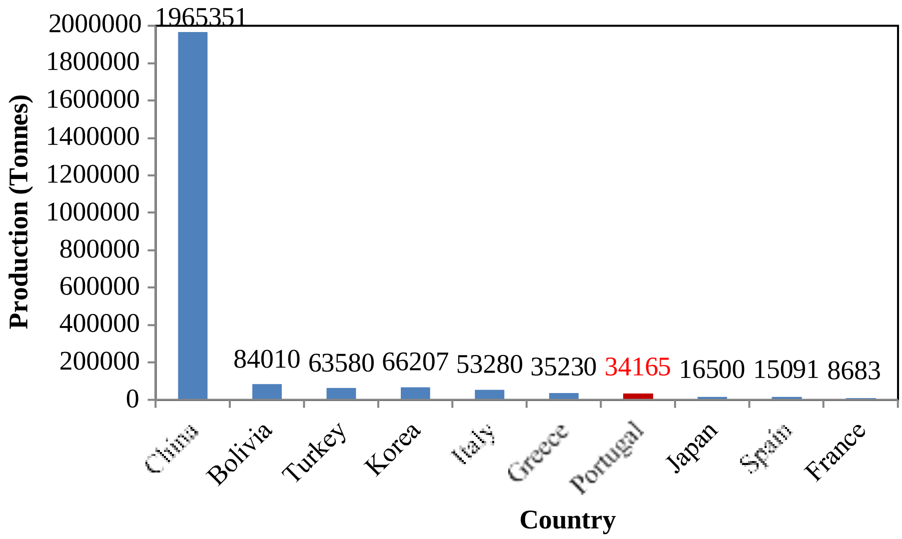

Figure 1: The main producers of chestnuts in 2018 (FAO, 2020).

1.1.2 Chestnut production in Europe

The European harvest area was 119 494 hectares, corresponding to a production of 154 612 tonnes in 2018 (FAO, 2020). **Figure 2** represents the main producers of chestnuts in Europe. In the first place, Italy appears with the highest production in 2018 with 53 280 tonnes, followed by Greece with 35 230 tonnes, and Portugal came in third place with 34 165 tonnes.

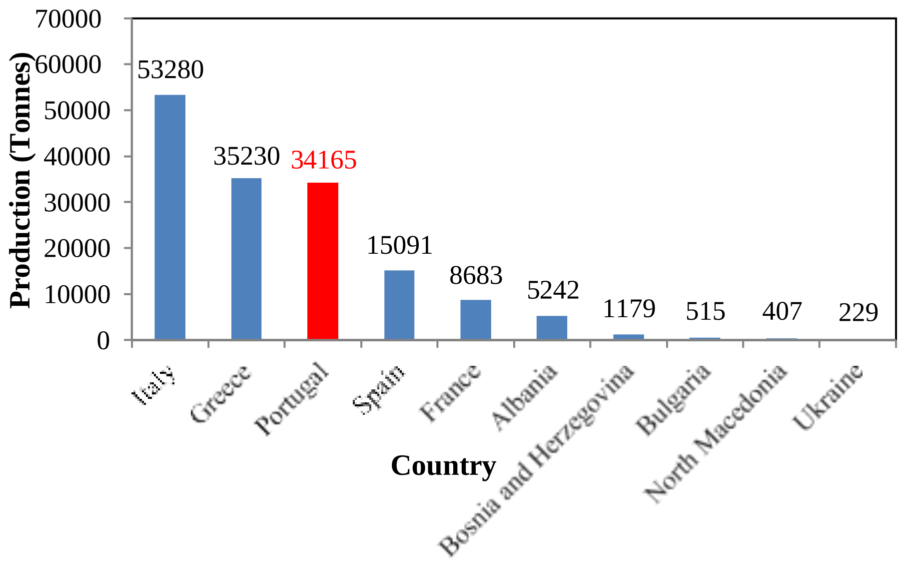

Figure 2: Main European producers of chestnuts (*C. sativa* Mill.) (FAO, 2020).

1.1.3 Chestnuts production in Portugal and Tunisia

As mentioned before, the production in Portugal in 2018 was around 34 165 tonnes for 38 874 hectares of the harvested area of chestnuts. For Tunisia, no information about the production or harvested area was found. As represented in **Figure 3**, the Portuguese chestnut production and harvested area in chestnuts have developed significantly during the last ten years.

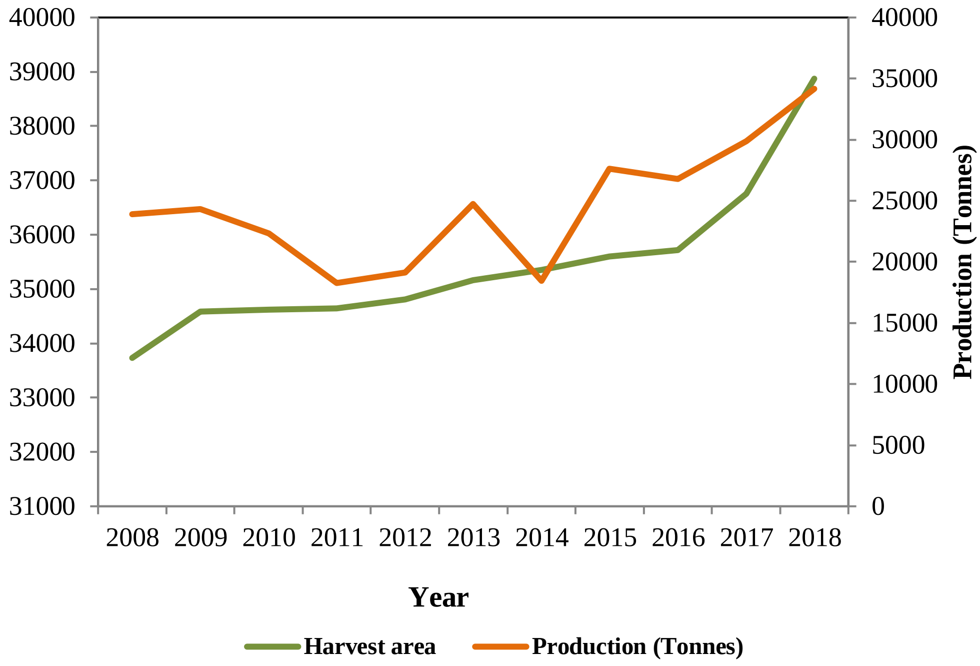

Figure 3: The Portuguese chestnut production and harvested area between 2008 and 2018.

In 2020, a chestnut production is expected close to the registered in the last campaign of 2019 (INE, 2020); however, the actual number is unknown. The producers reported some problems, such as chestnut groves with fewer chestnuts per bur or fruits of smaller calibre and trees with some phytosanitary problems (ex. attacks of the chestnut gall wasp, *Dryocosmus kuriphilus*, an insect pest of sweet chestnut trees) (INE, 2020). Nevertheless, the precipitation and the decreased temperatures in September and October have benefited the chestnut production (INE, 2020).

In the region of Trás-os-Montes (Northeast of Portugal), the chestnut tree finds the edaphic and climatic conditions favourable to its vegetative development and fruiting, producing about 26 000 tonnes of chestnut (Borges et al., 2007). This value corresponds to 76% of the total national production. So, this is the most important Portuguese area of chestnut.

1.2 Main problems stated in postharvest and during chestnut storage

Two main types of problems can be stated in the postharvest and during chestnut storage. The first problem is linked to the presence of insect worms (*Cydia pomonella*, *Cydia flagiglandana*, *Cydia splendana* e *Curculio elephas*) and the development of filamentous fungi, including *Penicillium* spp. (*P. expansum*, *P. griseofulvum*, *P. chrysogenum*), *Coniophora puteana*, *Acrospeira mirabilis*, *Botryosphaeria ribis*, *Sclerotinia sclerotiorum*, *Botryotinia fuckeliana* (anamorph *Botrytis cinerea*) and *Gibberella* sp. (anamorph *Fusarium* sp.) (Donis-González et al., 2010).

On the other hand, during the storage, the chestnuts dry quickly, resulting in flavour decrease and weight loss, contributing to significant economic losses. So, it is of great importance to find methods that can reduce these problems. Some technologies have been applied to chestnuts storage, namely refrigeration (cold industrial chambers) (Fernandes et al., 2020a), modified atmospheres (Fernandes et al., 2020b; Peano et al., 2014), and ozone for the Chinese chestnut (Li et al., 2012). The application of edible coatings is also a possibility that has been applied to chestnuts. This topic will be further discussed in the following issue.

1.3 Edible coatings

Edible coatings and edible films, known as edible packaging, are considered a potential alternative to the previous section's methodologies to protect food quality and extend shelf life by delaying microbial deterioration and providing moisture and gas barrier properties. The development of edible coatings and films has shown promising results in increasing the shelf-life of food products. These methodologies can extend the shelf life by directly applying the coating to the food or using pre-formed film wrapped in food, respectively (Suhag et al., 2020). In the present work, only edible coatings will be studied.

Concerning edible coatings, different methods can be used to perform their deposition on food surfaces, such as dipping, spraying, fluidized-bed and elutriation (Suhag et al., 2020). The dipping method is easy to use and is preferred on a lab scale (Suhag et al., 2020). On the contrary, spraying is the preferred method for coating deposition on a commercial scale (Suhag et al., 2020).

1.3.1 Edible coatings applied to chestnuts

Some edible coatings have been applied to chestnuts, as mentioned in **Table 2**; however, only three works have been performed on this topic. In that table, the coatings used, the storage conditions and the parameters evaluated are mentioned. The coatings applied to chestnuts have been alginate, chitosan, whey protein isolate, carrageenan and whey protein isolate-pullulan. However, the last two have been applied to the Chinese chestnut.

Table 2: Studies in edible coatings applied to chestnuts

Nut	Edible coating used	Storage conditions	Parameters evaluated	Reference
European chestnut (*Castanea sativa* Mill.)	- Alginate - Chitosan - Whey protein isolate	T=0.03 ± 0.57 °C RH=90.7 ± 3.1% (6 months)	Visual appearance, water activity, colour, texture, pH, titratable acidity and microbial quality	Fernandes et al. (2020a)
Chinese chestnuts (*Castanea molissima*)	- Carrageenan - Whey protein concentrate	PE bags without sealing T=5 °C (80 days)	reducing sugars, solublesugars and amylase activity	Tian et al. (2009)
Chinese chestnuts (*Castanea molissima*)	Whey-protein isolate-pullulan	Room temperature for 24h (15 to 120 days)	Sensory attributes using a trained sensory panel (sweetness appearance and colour texture / mouthfeel, flavour, acceptance).	Gounga et al. (2008)

Concerning the three studies, the studied storage conditions have been around 0 ºC and room temperature. No changes were observed in the interior colour and texture parameters during storage; however, the total soluble solids (TSS) increased in the coated samples compared to the control (uncoated chestnuts) (Fernandes et al., 2020a). Furthermore, in the alginate coated chestnuts, an increase in reducing sugars was observed after six months of storage. The chitosan-coated chestnuts showed the lowest weight loss values during storage and the lowest growth of microorganisms (Fernandes et al., 2020a).

Concerning the Chinese chestnut, Tian et al. (2009) observed that the coatings decreased less the starch content and reduced the amylase activity generally observed during the physiological metabolism of the fruit. Furthermore, the chitosan-coated chestnuts presented respiratory rates lower than those determined in the uncoated samples or the coated ones with carrageenan and whey protein. The whey protein isolate-pullulan coating caused positive effects on Chinese chestnut's sensory attributes (Gounga et al., 2008). However, no other parameters (chemical or microbiological)were measured.

In general terms, few works have been done on this subject. So, it will be necessary to try other coatings to check their role in chestnut properties, mainly in the European species. In the following section, a revision in edible coatings applied to nuts has been performed.

1.3.2 Edible coatings applied to nuts

For other types of nuts, such as pecans, walnuts, pine nuts and pistachio, some edible coatings have been applied to see their role on the shelf-life and other parameters mentioned in **Table 3**.

In terms of colour and texture, coatings with carboxymethyl cellulose (CMC) imparted a shine to coated pecan kernels, but did not generally affect their texture (Baldwin & Wood, 2006). On the other hand, the pea starch:whey protein isolate:carnauba wax (PS:WPI:CW) (1:1:2) coating applied to walnuts **(Table 3)** caused an unacceptable yellowish colour (Mehyar et al., 2012). In pistachios, the gelatin-coated samples presented higher hardness than other samples at 20 and 35 °C (Khoshnoudi-Nia & Sedaghat, 2019).

Regarding sensory analysis, the coated pecan kernels generally scored lower for off- flavour and higher for overall flavour (Baldwin & Wood, 2006). In walnuts and pine nuts (**Table 3**), the PS:WPI:CW (1:1:1) coating improved the fruits' smoothness and taste (Mehyar et al., 2012).

Table 3: Edible coatings applied to nuts

Nut	Edible coating used	Storage conditions	Parameters evaluated	Reference
Pecans	Preliminary: Methylcellulose. hydroxypropyl cellulose (HPC) or carboxymethyl cellulose (CMC) + propylene glycol (PG), sorbitol, lecithin, Δ-tocopherol, BHA, BHT and citric acid; 2nd Experiment: [CMC or HPC] + lecithin + citric acid + PG 3rd Experiment: CMC + lecithin + [citric acid and PG or PG or PG + sorbitol or PG + Δ-tocopherol or PG + BHT]	Open air or perforated zip-lock plastic bags at 20 to 25 °C (4 and 8 months)	Shine, off-flavour or overall flavour, and texture	(Baldwin & Wood, 2006)
Walnuts and **Pine Nuts**	- Pea starch (PS) - Whey protein isolate (WPI) - PS:Carnauba wax (CW) (1:1) - WPI:CW (1:1) - PS:WPI:CW (1:1:1) - PS:WPI:CW (1:1:2)	25 ºC (normal reaction conditions, sampled every 2 months) 50 ºC (accelerated reaction conditions, sampled every 2 days)	Peroxide value, acidity, sensory properties	(Mehyar et al., 2012)
Pistachio nuts	- Propyl gallate + Ascorbic acid - Gelatin + Propyl gallate - Gelatin + Ascorbic acid - Gelatin + Propyl gallate + Ascorbic acid	Polyethylene / polyamide plastic bags at 20 ºC (normal condition); 35 and 50 ºC (accelerated condition) 3 months of storage	Chemical (peroxide value, anisidine value and free fatty acid), instrumental texture (hardness), and sensory properties (texture, rancidity, taste, colour, and overall acceptability)	(Khoshnoudi-Nia & Sedaghat, 2019)

Concerning rancidity, pecan kernels coated with CMC presented lower hexanal concentrations than the uncoated ones (Baldwin & Wood, 2006). Hexanal accumulation is a good indicator of rancidity. The CMC coatings with Δ-tocopherol were the most effective (Baldwin & Wood, 2006). All coatings tested in walnuts and pine nuts (Mehyar et al., 2012) (**Table 3**) showed to be effective in preventing oxidative and hydrolytic rancidity in both nuts stored at 25 °C during twelve days. Nevertheless, the coatings were less effective at 50 °C. The gelatin edible coating with ascorbic acid and/or propyl gallate also showed potential for reducing the rate of lipid oxidation, increasing the shelf-life of pistachios, mainly stored at room temperature (Khoshnoudi- Nia & Sedaghat, 2019).

These results show that the application of edible coatings may affect the properties ofthe nuts and must be studied for each fruit in particular.

1.3.3 Commercial edible coatings

Some enterprises commercialize edible coatings for all types of fruits and vegetables through various products and ways to apply them. **Table 4** shows a compilation of enterprises, the products they sell and for which food products they will be used, the methods of application, and the country of the enterprise.

As indicated in **Table 4**, five enterprises are commercializing edible coatings. Three of them are based in Spain, namely AgroFresh, Domca and Fomesa Fruitech. Apeel is in USA, and Sufresca is from Israel. AgroFresh is also in Brasil. These enterprises edible coatings are for vegetables like potato, tomato and pepper, and fruits like mango, avocado, and banana. The main objectives are to prolong the product's shelf life, reduce the weight loss throughout storage, maintain the optimal flavour and keep the outer look of the product fresh for the consumer just like it has been just postharvest.

The edible coatings may include organic acids, fatty acids, proteins, hydrocolloids, emulsifiers and ethanol, and other compounds applied by spraying or immersion. Many of the compounds used in their formulations are plant-based.

Table 4: Edible coatings commercially available: trademark, sources/main components, recommended commodities, way to use, country of the commercializing company.

Enterprise (Product name)	Edible coatings	For what	How to use	Country
AgroFresh				Brasil/Spain
1) VitaFresh Botanicals - Protect	1) Plant source: sugar plants	1) Citrus, pome fruit and stone fruit	1) Spraying, drenching or dipping	
2) VitaFresh Botanicals - Life	2) Plant source: carnauba tree	2) Citrus, apples, avocados, mangos	2) Spraying or dipping	
3) VitaFresh Botanicals - Life Ultra	3) Plant source: carnauba tree	3) Avocados, citrus, apples, papayas, mangoes	3) Spraying or dipping	
4) VitaFresh Botanicals - SoftFruit	4) Plant source: sunflower (*Helianthus* sp.)	4) Fruits with sensitive skin (ex. stone fruit – plums and nectarines)	4) Spraying or dipping	
Domca				Spain
1) Fruit Coat	1) Organic acids	1) Fruits and vegetables	NG	
2) Proallium FRD	2) Organic acids and aromas	2) Minimally processed plant products		
3) Agroallium Lact	3) Food-grade additives + ingredients	3) Citrus		
4) Proallium Brill	4) Food-grade additives + ingredients	4) Fruits and vegetables		
5) Food Coat	5) Fatty acids	5) Fruits and vegetables		
Fomesa Fruitech				Spain
1) Foodwax spray	1) Proteins, hydrocolloids, fatty acids, ethanol	1) Citrus, other fruits and vegetables	1) Suitable spray devices	
2) Greengard coat	2) Hydrocolloids and edible emulsifiers	2) Fruits and vegetables	2) Spray	
3) Greengard scald	3) Carnauba wax, emulsifiers and food antioxidants	3) Apples and pears	3) Apply by drencher	
4) Greengard-LE	4) Hydrocolloids and edible emulsifiers	4) Fruits and vegetables	4) Spraying online	
5) Greengard-PA	5) Food antioxidants and food emulsifiers	5) Pineapples	5) Applied by shower or immersion	
6) Greenseal(VG. coat.spray)	6) Proteins, hydrocolloids, food fatty acids, ethanol, water	6) Fruits and vegetables	6) Through a special applicator that sprays directly on the fruit with nozzles or rotors installed on a brush machine	
7) Lemon spray FG	7) Food products, ethanol and water	7) Lemons and grapefruits	7) Spray apparatus	

Apeel	Made of plant-derived materials - lipids and glycerolipids - that exist in the peel, seeds and pulp of all fruits and vegetables	Fruits and vegetables	Spray, dip, or brush	USA
SUFRESCA	The products include water, glazing agents, emulsifiers, stabilizers, acidity regulators	Peppers, cucumber, tomato, garlic, pomegranate	NG	Israel

NG: not given

2 Materials and Methods

2.1 Samples

The chestnuts (*Castanea sativa* Mill.) used in the current work were supplied by a local industry of Carrazedo de Montenegro (Northeast of Portugal) and belonged to the Martaínha variety. The fruits were treated in hot water, following the mandatory industrial treatment applied by all companies that want to export chestnuts (DGAV, 2018). This step involved the soaking of chestnuts in hot water at 48 to 50 °C for 45 minutes to eliminate insect larvae and eggs, namely from *Cydia pomonella*, *Cydia flagiglandana*, *Cydia splendana* and *Curculio elephas*. After the hot water treatment, the fruit was immediately immersed in cold water and dried with fresh air. On the same day that the fresh chestnuts were collected in the industry, they were taken to the laboratory immediately.

2.2 Edible coatings

Two edible coatings, Proallium FRD-N and Foodcoat P of DOMCA (**Figure 4**), and the mixture of these two edible coatings were applied. A total of 51 portions of 250 g of chestnuts/portion were divided into three groups that were immersed in the following solutions, prepared according to the manufacture instructions (**Figures 5A**, **5B**):

- Proallium solution: 70.779 g of Proallium were weighted and diluted in 7 litresof distilled water;
- Foodcoat solution: 210.729g of Food Coat were weighted and diluted in 7 litresof distilled water;
- Proallium + Food Coat solution: 69.999 of Proallium and 210.469 of Food Coat were weighted, mixed and then diluted in 7 litres of distilled water.

All samples were immersed for 2 min in the edible coating solutions and then dried for 2 min. At the same, uncoated chestnuts were left without any treatment to be the control. The uncoated and coated chestnuts were subjected to the storage conditions described in the following section.

Figure 4: The commercial products used in the present work: Foodcoat P (on the left) and Proallium FRD-N (on the right) of DOMCA®.

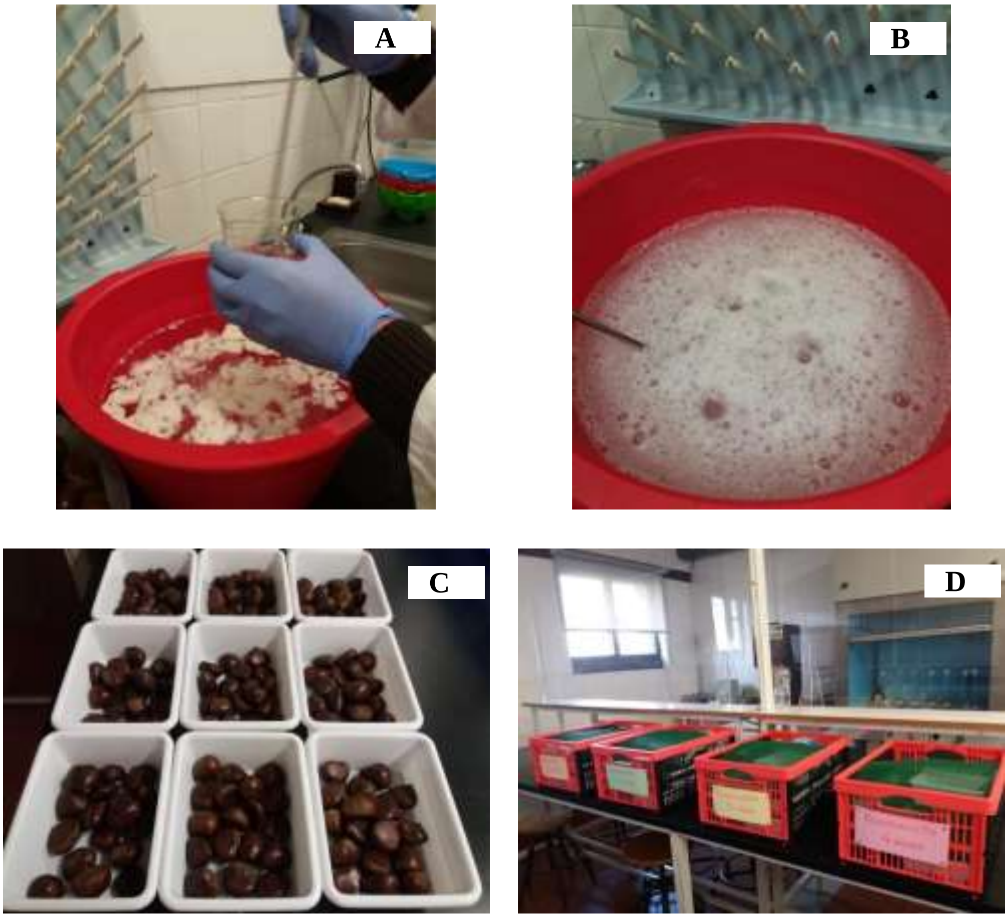

Figure 5: Preparation of the edible coat solution (Foodcoat P®) (A, B), chestnuts in thetrays (C), and the perforated plastic boxes (D).

2.3 Storage conditions

The coated and uncoated chestnuts (control) were put in trays (**Figure 5C**) that were placed in perforated plastic boxes exposed to air (**Figure 5D**). These were taken directly to the industry, described in Section 2.1. The boxes were put in the refrigerated chambers at a temperature between 0 - 2ºC, without relative humidity control. At 1, 2, 3 and 4 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.

2.4 Physic-chemical parameters

2.4.1 Colour

The outer (shell) and inner (kernel) colour of the chestnuts were evaluated in 10 fruits randomly selected from each 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*) $L*$ represents the brightness, varying from 0 (black) to 100 (white); (*ii*) $a*$ corresponds to the green$^-$-red$^+$ coordinate; (*iii*) $b*$ is the blue-yellow coordinate, varying between blue ($^-b*$) and yellow ($^+b*$); (*iv*) h is the hue angle that defines the pure colour but also defines mixtures of two pure colours; and (*v*) $C*$ that corresponds to the chromaticity coordinate, corresponding to a higher value, a higher purity or colour intensity.

2.4.2 Texture

A TA.XTplus texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a cylindrical probe with a diameter of 2 mm was used to measure the texture of ten randomly selected chestnuts in each tray. The test speed was 1mm/sec. The chestnuts were tested without the shell. The parameters determined were: (*i*) skin strength equal tothe positive peak force (g); (*ii*) elasticity (mm); and (*iii*) positive area (g.sec), which represents the work of penetrating the chestnuts.

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

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

$$WL\ (\%) = \frac{m(chestnuts)_{t=0} - m(chestnuts)_t}{m(chestnuts)_{t=0}} \times 100 \qquad \textbf{(Eq. 1)}$$

where *t* was equal to 1, 2, 3 and 4 months.

After determining the WL, some fruits were peeled and crushed in a universal grinder (IKA, M20, Staufen, Germany). The ground samples were placed in a plastic dish to evaluate the water activity (a_w) measured in a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland). The equipment was previously calibrated with the following standards (Nalgene, Olivais Sul, Portugal): SAL_T 11 (11% - LiCl >25%), SAL_T 84 (84% - KCl >25%), and SAL_T 58 (58% - NaBr >25%).

The moisture content was measured at 105 °C by the conventional drying method (weight of loss) until a constant weight was reached. The results were expressed in %, fresh weight (f.w.).

2.4.4 Titratable Acidity and Total Soluble Solids

The titratable acidity (TA) values of chestnuts were determined by titration with 0.1 M NaOH solution. About 2.5 g of pre-ground lyophilised chestnuts were put in a round- bottomed balloon and mixed with 50 mL of distilled water (pre-boiled). After placing the reflux condenser, the solution was heated on a heating blanket for about 30 minutes. Then, the obtained solution was transferred to a graduated cylinder, and distilled water (recently boiling) was added to reach a total volume of 100 mL. After mixing with a stir bar, the solution was filtered through gauze (three layers). Twenty-five mL of the filtered solution (measured with a volumetric pipette) was placed in a beaker. After that, the NaOH solution was added, being the pH of the solution continuously monitored (Crison-Micro pH 2002, Barcelona, Spain). As indicated in the Portuguese Regulation NP-1421 (1977), the volume of NaOH necessary to achieve a pH of 8.1 was determined. The TA was expressed in mg citric acid/100 g dry weight (d.m.).

The solution used in the TA determination (after filtration) was also used to measure the total soluble solids (TSS) content (°Brix) at 20°C. The measurement was performed in an Abbe refractometer (Optical Ivymen System, Madrid, Spain). The TSS was expressed in g TSS/100 g dry weight (d.m.).

2.4.5 Reducing Sugars Content

The extraction procedure followed to determine the reducing sugars was described by Barreira & Pereira (2010), including some modifications. Dried and defatted powder (0.3 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 prepared with glucose (0.05 to 0.60 g/L), and the results were expressed on mg glucose/100 g dry weight (d.w.).

2.4.6 Total starch content

The method used to determine starch was carried out with the Megazyme Kit "Total Starch (Amyloglucosidase / α-Amylase Method) K-TSTA-100A". The process involved milling the dried chestnut sample then passing it through a 500 µm screen. One hundred milligrams of freeze-dried chestnut sample was mixed with 5.0 mL of aqueous ethanol (80%, v/v). The mixture was incubated at 80-85 ºC for 5 min. The contents were mixed on a vortex stirrer, and another 5 mL of 80% v/v aqueous ethanol was added. After that, the solution was centrifuged (3000 rpm, 10 min). The pellet was resuspended in 10 mL of 80% v/v aqueous ethanol and stirred on a vortex mixer. The solution was centrifuged as above, and the supernatant was carefully poured off. After that, a magnetic stirrer bar and 2 mL of 2M KOH to each tube with the pellet were added. This solution was stirred for approximately 20 min in an ice/water bath over a magnetic stirrer. Then, 8 mL of 1.2 M sodium acetate buffer (pH 3.8) was added to each tube withstirring on the magnetic stirrer. Immediately 0.1 mL of thermostable Δ-amylase and 0.1 mL of amyloglucosidase (AMG) enzymes were added. The solutions were mixed well, and the tubes were placed in a water bath at 50 ºC. The tubes were incubated for 30 minutes with intermittent mixing on a vortex mixer. The contents of the tubes were quantitatively transferred to 100 mL volumetric flasks. The volume was adjusted with distilled water and mixed well. An aliquot of the solutions was centrifuged (3000 rpm, 10 min). Then duplicate aliquots were incubated with glucose oxidase/peroxidase reagent (GOPOD) (3 mL) and incubated at 50 °C for 20 min. Finally, the absorbance of each solution against the blank was measured at 510 nm. The blank solution was prepared with 0.1 mL of water and 3 mL of GOPOD reagent. This solution was incubated at 50 °C for 20 min. In parallel, a D-glucose control was prepared. It consisted of 0.1 mL of D-glucose standard (1 mg/mL) and 3 mL of GOPOD reagent. This solution was also incubated at 50 °C for 20 min. The absorbance of this solution was measured at 510 nm against the blank.

The following equation was used to determine the starch content in the samples:

$$Starch\ (\%, d.w.) = Abs_{Sample\ against\ the\ blank} \times \frac{F}{mass\ of\ the\ sample\ in\ milligrams} \times 90$$

where $F = \frac{100\ \mu g\ D-Glucose}{Abs_{D-Glucose}}$, which was determined through the D-glucose control.

2.4.7 Individual sugars

In an Erlenmeyer of 50 mL, 0.15 g of ground freeze-dried chestnut were weighted. Ten mL of boiled ultra-pure water and 200 µL of arabinose (15 mg/mL, as internal standard) were added. All reagents used were pro-analysis (p.a.) of purity grade. The solution was placed in a hot water bath (96 °C) with agitation for 5 minutes. The resulting suspension was centrifuged at 10 000 rpm for 5 min. The supernatant was collected and filtered through a Nylon filter of 0.2 µm. The filtrates were placed in glass vials of 1.5 mL. Afterwards, high-performance liquid chromatography and a refractive index detector (HPLC-RI) determined the individual sugar profiles.

The conditions of HPLC-RI system were the following:

- Mobile phase - Sulfuric acid at 0.005 mol/L
- Pump (Varian, ProStar, Model 220) with a flow rate of 0.6 mL/min
- Column Aminex HPX-87H (300 mm υ7.8 mm) (BioRad)
- Manual injector (Rheodyne, Model 7725i) with a loop of 20 µL
- Oven of the column (Jones Chromatography, Model 7981) at 30 ºC
- Refractive Index Detector (Varian, Model RI-4)

The quantification was done by internal standard and through calibration curves prepared for the following sugars: glucose, fructose, raffinose, arabinose, saccharose and maltose. The results were expressed on g sugar/100 g d.w..

2.5 Microbial parameters

Three chestnuts from each tray were placed in a sterile stomacher bag, diluted 1:10, in sterile buffered peptone water (Liofilchem, Roseto Degli Abruzzi, Italy) and then shaken vigorously for 2 min. After serial dilutions, 200 µL aliquots of each dilution were spread on Plate Count Agar (PCA, Liofilchem, Italy) for enumeration of aerobic mesophilic microorganisms (ISO 4833:2, 2013), and Dichloran-rose bengal chloramphenicol (DRBC Agar, Liofilchem, Roseto Degli Abruzzi, Italy) forquantification of yeasts and moulds (ISO 21527-1:2008). PCA and DRBC plates were incubated for 72 h at 30 °C and 25 °C for 5 days, respectively.

The number of microorganisms was calculated as the weighted average of two successive dilutions (ISO 7218:2007). The results were expressed in the logarithm of the number of colony-forming units per gram (log10 CFU/g) of fresh sample.

2.6 Statistical analysis

The data analysis was performed using the SPSS statistical software version 18.0 (SPSS Inc., Chicago, Illinois). The results were expressed as mean ± standard deviation (SD) (n=3). Levene's test proved the uniformity of the variance (called homogeneity of variance or homoscedasticity), while the Shapiro-Wilk test confirmed the normality of the data. Since the data followed a normal distribution, Analysis of Variance (ANOVA) or ANOVA Welch was performed to assess whether there were significant differences between samples or not. When uniformity of variance was observed, ANOVA wasused, while ANOVA Welch was used in other cases. In addition, when significant differences between samples ($p<0.05$) were observed, a post hoc analysis was performed, namely the Tukey HSD test (if the variances in different groups were the same) or Games-Howell test (if the variances in different groups were not the same).

3. RESULTS AND DISCUSSION

3.1 Colour

The colour parameters (*L**, *a**, *b**, C*, and *h*) of the exterior (shell) and interior (core)of the chestnuts during storage are listed in **Tables 5** and **6**, respectively. In general, the results of uncoated (control) and coated chestnuts ±Proallium, Foodcoat and Proallium + Foodcoat - stored for four months presented similar values with no significant differences between the four treatments with some exceptions. Nevertheless, significant differences were detected when comparing the *L** in time zero with the following months. For example, in control and Foodcoat, significant differences were already detected between zero and one month; in Proallium, it was between zero and three months; and Foodcoat + Proallium, it was between zero and four months. The lowest *L**values were detected initially, suggesting that the lightness slightly increased along withstorage. For the other parameters, even though some significant differences were detected in some samples, no specific trend was seen, indicating that the treatments and storage time did not influence the *a**, *b**, C* and h of the outer skin of the chestnuts.

Regarding the kernel, in some cases, significant differences were observed. Concerning *L**, even though some significant differences were detected, no specific trend was observed between treatments and storage time. On the contrary, for the *a**, *b**, C* and h, significant differences between the beginning and after four months were detected in control, Proallium, and Foodcoat + Proallium samples. So, applying these edible coatings seemed to slightly originate a reddish and yellowish colour in the chestnut kernel, causing a slight increase in the chroma (C*) and a decrease in the hue (h) of these fruits. Nevertheless, all colour parameters of the exterior shell and kernel showed the same order of magnitude.

These results showed that applying these commercial edible coatings on chestnuts can maintain the colour of chestnuts, and in this way, their appearance. This property is quite important to consumers when purchasing this fruit.

Table 5: Colour parameters of the outside of the fruit of the control and coated chestnuts during four months of storage.

Colour parameters	Storage Time (months)	Outside the fruit treatment			
		Control	Proallium	Foodcoat	Foodcoat+Proallium
L*	0	30.8 ± 1.7^{A}	30.8 ± 1.7^{A}	30.8 ± 1.7^{A}	30.8 ± 1.7^{A}
	1	32.4 ± 1.6^{bB}	32.1 ± 1.9^{abABC}	32.3 ± 1.4^{bB}	31.1 ± 2.0^{aA}
	2	31.8 ± 2.0^{abAB}	31.4 ± 1.5^{aAB}	32.6 ± 1.8^{bB}	31.7 ± 1.7^{abAB}
	3	32.6 ± 1.9^{aB}	32.9 ± 1.7^{aC}	32.5 ± 2.1^{aB}	32.0 ± 2.0^{aAB}
	4	32.8 ± 1.5^{aB}	32.4 ± 2.0^{aBC}	32.4 ± 1.8^{aB}	32.7 ± 2.3^{aB}
a*	0	8.3 ± 1.6^{A}	8.3 ± 1.6^{A}	8.3 ± 1.6^{AB}	8.2 ± 1.6^{A}
	1	7.7 ± 1.7^{aA}	8.7 ± 1.7^{aA}	8.4 ± 1.4^{aAB}	8.5 ± 1.5^{aA}
	2	7.4 ± 1.7^{aA}	8.0 ± 1.5^{abA}	8.5 ± 1.8^{abB}	8.5 ± 1.3^{bA}
	3	7.6 ± 1.5^{aA}	8.7 ± 1.5^{bA}	7.4 ± 1.3^{aA}	8.0 ± 1.4^{abA}
	4	7.3 ± 1.4^{aA}	8.1 ± 1.4^{abA}	8.0 ± 1.7^{abAB}	8.6 ± 1.6^{bA}
b*	0	10.0 ± 2.0^{A}	10.0 ± 2.0^{A}	10.0 ± 2.0^{A}	10.0 ± 2.0^{A}
	1	11.1 ± 2.2^{aA}	11.7 ± 2.7^{aB}	12.1 ± 2.0^{aB}	10.5 ± 2.8^{aA}
	2	10.2 ± 2.2^{aA}	11.3 ± 2.0^{abAB}	11.6 ± 2.2^{bB}	11.5 ± 2.1^{abAB}
	3	11.2 ± 2.3^{aA}	12.1 ± 1.7^{aB}	10.8 ± 2.2^{aAB}	11.2 ± 2.2^{aAB}
	4	11.4 ± 1.8^{aA}	11.6 ± 2.2^{aB}	11.2 ± 2.1^{aAB}	12.4 ± 2.4^{aB}
C*	0	13.1 ± 2.1^{A}	13.1 ± 2.1^{A}	13.1 ± 2.1^{A}	13.1 ± 2.1^{A}
	1	13.6 ± 2.5^{aA}	14.6 ± 2.9^{aAB}	14.8 ± 2.2^{aB}	13.6 ± 2.8^{aAB}
	2	12.7 ± 2.4^{aA}	13.9 ± 2.2^{abAB}	14.4 ± 2.6^{bAB}	14.4 ± 2.2^{bAB}
	3	13.6 ± 2.6^{abA}	15.0 ± 2.0^{bB}	13.2 ± 2.2^{aAB}	13.8 ± 2.2^{abAB}
	4	13.6 ± 2.2^{aA}	14.2 ± 2.4^{aAB}	13.8 ± 2.5^{aAB}	15.1 ± 2.6^{aB}
h	0	50.2 ± 6.1^{A}	50.2 ± 6.1^{A}	50.2 ± 6.1^{A}	50.2 ± 6.1^{A}
	1	55.3 ± 5.8^{bB}	53.1 ± 5.3^{abAB}	55.1 ± 3.8^{bB}	50.5 ± 6.1^{aA}
	2	53.8 ± 7.1^{aAB}	54.6 ± 5.1^{aB}	53.9 ± 4.6^{aB}	53.2 ± 4.9^{aAB}
	3	55.6 ± 3.8^{aB}	54.3 ± 4.4^{aB}	55.5 ± 5.2^{aB}	54.4 ± 5.5^{aB}
	4	57.3 ± 3.8^{bB}	55.0 ± 4.5^{abB}	54.4 ± 4.8^{aB}	55.2 ± 4.1^{abB}

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

Table 6: Colour parameters of the kernel of control and coated chestnuts during four months of storage.

Colour parameters	Storage Time (months)	Inside the fruit (kernel) – Treatment: Control	Proallium	Food coat	Foodcoat+Proallium
L*	0	86.3±2.6^{AB}	86.3±2.6^{A}	86.3±2.6^{AB}	86.3±2.6^{A}
	1	87.0±3.3^{abAB}	88.0±1.3^{abA}	88.2±1.4^{bB}	86.7±2.4^{aAB}
	2	87.6±2.0^{aB}	86.9±4.4^{aA}	87.8±1.2^{aB}	88.2±1.3^{aB}
	3	87.4±1.8^{aAB}	86.5±2.7^{aA}	86.5±2.5^{aAB}	87.0±1.8^{aAB}
	4	85.6±3.5^{aA}	86.8±1.9^{aA}	85.4±1.9^{aA}	85.5±2.6^{aA}
a*	0	-3.0±0.5^{A}	-3.0±0.5^{A}	-3.0±0.5^{A}	-3.0±0.5^{A}
	1	-2.6±0.7^{aAB}	-2.8±0.7^{aAB}	-2.7±0.6^{aAB}	-2.6±0.7^{aAB}
	2	-2.7±0.7^{aAB}	-2.5±0.9^{aAB}	-2.7±0.6^{aAB}	-2.7±0.5^{aAB}
	3	-2.6±0.8^{aAB}	-2.3±0.6^{aB}	-2.6±1.1^{aAB}	-2.4±0.7^{aB}
	4	-2.3±1.0^{aB}	-2.4±0.7^{aB}	-2.3±1.1^{aB}	-2.2±0.9^{aB}
b*	0	21.9±2.4^{A}	21.9±2.4^{A}	21.9±2.4^{A}	21.9±2.4^{A}
	1	22.4±2.0^{aAB}	23.7±3.0^{aA}	22.7±2.4^{aAB}	23.3±2.6^{aA}
	2	23.8±2.1^{aBC}	23.0±2.6^{aA}	23.7±3.0^{aABC}	22.3±2.6^{aA}
	3	23.4±2.8^{aABC}	23.5±3.2^{aA}	24.4±3.4^{aBC}	23.2±2.3^{aA}
	4	24.1±2.4^{aC}	23.8±2.9^{aA}	24.8±3.4^{aC}	25.2±2.8^{aB}
C*	0	22.1±2.4^{A}	22.1±2.4^{A}	22.1±2.4^{A}	22.1±2.4^{A}
	1	22.5±2.0^{aAB}	23.9±3.0^{aA}	22.8±2.4^{aAB}	23.4±2.6^{aA}
	2	24.0±2.1^{aB}	23.1±2.6^{aA}	23.9±3.0^{aAB}	22.5±2.6^{aA}
	3	23.6±2.8^{aAB}	23.6±3.2^{aA}	24.6±3.4^{aB}	23.4±2.4^{aA}
	4	24.2±2.4^{aB}	24.0±2.9^{aA}	25.0±3.4^{aB}	25.3±2.9^{aB}
h	0	97.9±0.8^{C}	97.9±0.8^{C}	97.9±0.8^{C}	97.9±0.8^{D}
	1	96.7±1.6^{aB}	96.7±1.3^{aB}	96.7±1.3^{aBC}	96.4±1.7^{aBC}
	2	96.5±1.6^{aAB}	96.3±2.1^{aAB}	96.6±1.1^{aB}	96.8±1.0^{aC}
	3	96.3±1.5^{aAB}	95.6±1.2^{aA}	96.1±2.0^{aAB}	95.8±1.6^{aAB}
	4	95.4±2.1^{aA}	95.6±1.5^{aA}	95.3±2.8^{aA}	94.9±1.9^{aA}

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

3.2 Texture

The texture of chestnuts may be affected by the storage conditions, resulting in amodification in fruit hardness. This fact will reduce the product quality. Therefore, the analysis of the texture of the chestnut fruit was carried out. The skin strength, elasticity and work required to penetrate the chestnut tissue were determined during four months' storage.

When comparing the different coatings for the three parameters analyzed and represented in **Table 7**, no significant differences were found between the treatments for a specific storage time. On the contrary, a decrease in the maximum force and the area below the curve were detected along the storage period for each treatment. For the control, Proallium, Foodcoat, and Proallium + Foodcoat, a decrease in the area under the curve was observed after four months of storage compared to the beginning. A lower area indicates that less work is needed to be done to penetrate the fruit. This slight decrease in mechanical work may be related to the softening of the fruit, suggesting that the water loss was not significant. These results were in line with the ones observed for the maximum force, where a decrease in the values was perceived when comparing the beginning with four months when the edible coatings were applied. Regarding elasticity, there were no substantial differences in the values during the 4-month storage period. These results indicated that the chestnuts still retain the mechanical strength observed initially, as well as a degree of elasticity.

Table 7: Texture parameters determined in the control and coated chestnuts during four months of storage.

Parameters	Storage Time (months)	Treatment			
		Control	Proallium	Foodcoat	Foodcoat+Proallium
Maximum force (g)	0	3510±342^{A}	3510±342^{B}	3510±342^{B}	3510±342^{B}
	1	3283±441^{aA}	3288±492^{aAB}	3263±438^{aAB}	3348±454^{aAB}
	2	3191±497^{aA}	3447±394^{aAB}	3337±504^{aAB}	3327±449^{aAB}
	3	3208±346^{aA}	3202±452^{aAB}	3260±526^{aAB}	3245±498^{aAB}
	4	3303±571^{aA}	3186±513^{aA}	2993±682^{aA}	3070±581^{aA}
Elasticity (mm)	0	3.9±1.5^{B}	3.9±1.5^{C}	3.9±1.5^{B}	3.9±1.5^{A}
	1	2.9±1.3^{aA}	2.7±1.1^{aA}	2.8±1.1^{aA}	3.1±1.1^{aA}
	2	3.1±1.3^{aAB}	3.0±1.1^{aABC}	3.2±0.9^{aA}	3.1±1.3^{aA}
	3	3.2±1.2^{aAB}	3.2±0.9^{aABC}	3.3±1.0^{aAB}	3.5±1.3^{aA}
	4	3.4±1.0^{aAB}	3.5±1.0^{aBC}	3.4±0.7^{aAB}	3.7±0.9^{aA}
Area below the curve (g.s)	0	16423±1794^{C}	16423±1794^{C}	16423±1794^{D}	16423±1794^{C}
	1	13988±2390^{aB}	13810±2126^{aB}	14066±2090^{aC}	13624±2380^{aB}
	2	12484±2720^{aAB}	13675±1906^{aB}	12644±2383^{aBC}	13545±2028^{aB}
	3	11388±2831^{aA}	11459±2504^{aA}	12043±2736^{aB}	12457±2660^{aB}
	4	11644±2643^{aA}	11621±2583^{aA}	10207±2524^{aA}	10452±2219^{aA}

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

3.3 Weight loss, moisture content, water activity (a_w), and titratable acidity

Weight loss is a crucial parameter, as it may represent significant economic losses that are the major problem for the producers. It was stated that an increase in weight loss during storage was observed (**Figure 6**). In more detail, approximately a 6% increase in the weight loss was observed after one month for all four treatments. After this period, even though with the Foodcoat some variability was observed, the lowest weight losses were determined at 3 and 4 months. The values were still near 5 and 6%. On the contrary, after four months, the uncoated and coated chestnuts with Proallium and Proallium+Foodcoat achieved weight losses of around 14%, suggesting that Foodcoat was the coating with the best performance for this parameter.

There were no significant differences in the moisture content during the entire storage time (**Table 8**) except the chestnuts coated with Proallium+Foodcoat. Even though some weight loss was observed, as mentioned before, no significant changes in the chestnuts' moisture contents were observed.

During the four-month storage period, the moisture content varied between 45.1% and 55.2%, consistent with the values reported by (Correia et al., 2009) for Longal (48.2%) and Martaínha (47.9%) cultivars, as well as the reference value of this fruit indicated in the literature (51.7%, Holland et al., 1992). Thus, no significant decrease in the moisture content along time was observed, suggesting that the storage conditions did not cause any substantial water losses, in line with the previously observed for the texture.

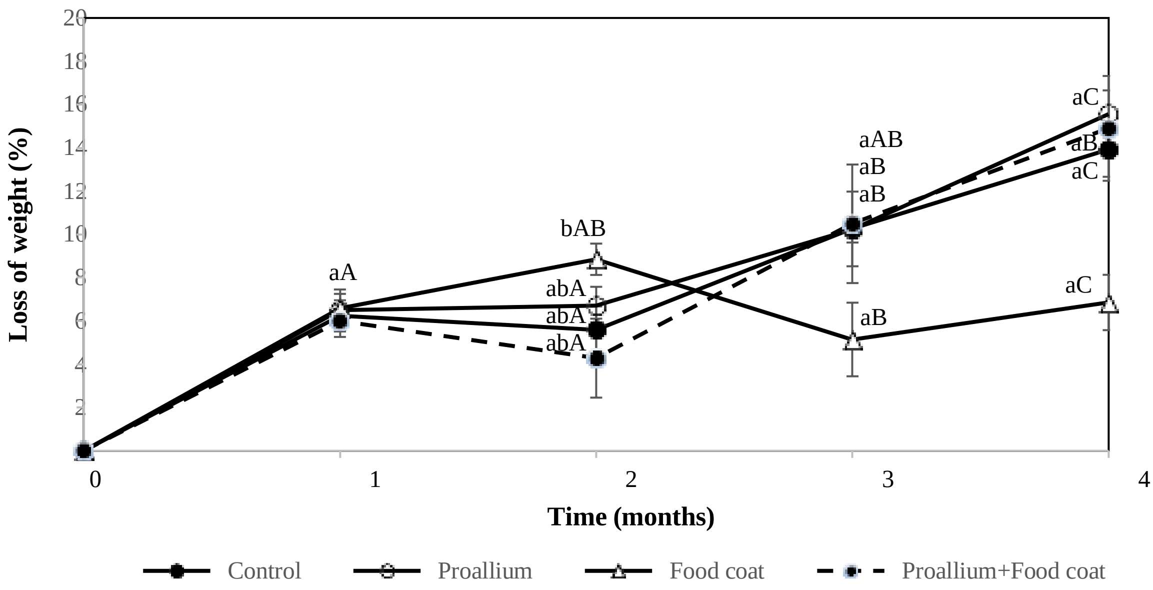

Figure 6: Weight loss (%) of control and coated chestnuts during four months of storage.

Regarding the a_w, as indicated in **Table 8**, the values varied between 0.958 and 0.937. No significant differences were observed between the different types of edible coatings. Concerning the storage time, significant differences were only observed between the beginning and the four months, being the lowest values determined in this last condition. However, this variation on a_w was not perceivable in the moisture content.

Regarding the titratable acidity, the values varied between 172 and 336 mg citric acid/100 g d.w. Only at the four months, some significant differences were observed between the coatings. The lowest value was observed in control, 196 mg citric acid/100 g d.w, while the highest value was observed in the mixture of Proallium+Foodcoat, with 336 mg citric acid/100 g d.w. The Proallium and Foodcoat treatments also caused a significant increase in the acidity of the chestnuts, indicating that after four months of storage, these treatments may induce some acidity to the fruits. The combination of both products had increased the titratable acidity even more. However, this effect was not observable over three months of storage.

Table 8: Moisture content, a_w and titratable acidity of the control and coated chestnuts throughout the four months of storage.

Parameter	Storage time (months)	Treatment			
		Control	Proallium	Foodcoat	Proallium + Foodcoat
Moisture (%)	0	55.2±5.1^{A}	55.2±5.1^{A}	55.2±5.1^{A}	55.2±5.1^{B}
	1	50.3±1.7^{aA}	48.6±4.8^{aA}	50.9±2.0^{aA}	48.4±3.1^{aAB}
	2	50.1±3.3^{aA}	49.1±1.3^{aA}	49.1±2.0^{aA}	51.8±0.9^{aAB}
	3	48.8±1.3^{aA}	47.0±1.7^{aA}	49.9±6.1^{aA}	50.1±2.3^{aAB}
	4	49.9±3.7^{aA}	47.7±3.9^{aA}	45.1±2.5^{aA}	45.3±2.7^{aA}
a_w	0	0.958±0.006^{B}	0.958±0.006^{B}	0.958±0.006^{B}	0.958±0.006^{B}
	1	0.954±0.009^{aAB}	0.951±0.007^{aAB}	0.945±0.007^{aB}	0.955±0.006^{aB}
	2	0.946±0.002^{aAB}	0.944±0.007^{aAB}	0.945±0.006^{aAB}	0.949±0.003^{aAB}
	3	0.948±0.003^{aAB}	0.951±0.002^{aAB}	0.953±0.003^{aAB}	0.949±0.006^{aAB}
	4	0.943±0.005^{aA}	0.940±0.009^{aA}	0.937±0.012^{aA}	0.938±0.010^{aA}
Titratable Acidity (mg citric acid/100 g d.w.)	0	172±25^{A}	172±25^{A}	172±25^{A}	172±25^{A}
	1	186±8^{aA}	189±5^{aA}	218±34^{aA}	210±8^{aA}
	2	210±21^{aAB}	201±29^{aAB}	204±8^{aA}	196±22^{aA}
	3	247±21^{aB}	207±22^{aAB}	224±22^{aAB}	210±30^{aA}
	4	196±29^{aAB}	252±12^{bB}	280±30^{bB}	336±23^{cB}

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

3.4 Total soluble solids (TSS), reducing sugars, starch content and individual sugars contents

In some situations, the TSS contents of the control and the coated chestnuts increased in the first and second months (**Table 9**). For example, the TSS values of the control, Foodcoat, and Proallium+Foodcoat rose from 49.28 to 67.95, 75.81 and 73.25% d.m., respectively, after one month of storage. However, after this period, the values remained identical to those determined initially, suggesting that this increase did not continue in the following months, not being observed any remarkable trend. Regarding the treatments applied, except at three months and between the control and the Proallium+Foodcoat coating, no significant differences were observed between the uncoated and coated treatments, suggesting that the different coatings applied did not change the TSS of the chestnuts significantly.

Regarding the reducing sugars (**Table 9**), these represented less than 1% of the dry matter of the chestnuts. When comparing treatments, significant differences were only observed after four months. The lowest values were determined in the Proallium and Foodcoat treatments, followed by Proallium+Foodcoat. The highest content was determined in control (553 ± 48 mg/100 g d.m.). Significant increases in the reducing sugars contents were observed after four months of storage compared with the beginning for the control and Proallium+Foodcoat treatments. These higher values may be due to some starch or sucrose hydrolysis that may have occurred.

It is known that the main carbohydrate present in chestnuts is starch. The starch contents are listed in **Table 9** and were determined only at the beginning and after four months. The values varied between 48.9 and 51.2%, with no significant differences between treatments and both times. The range obtained was consistent with the contents determined for *C. sativa* cultivars collected in Portugal (38.6-47.9%, d.m. by Borges et al., 2008), Greece (38.9-51.4% d.m. by Vekiari et al., 2015), Italy (58.3% d.m. by Attanasio et al., 2004; approximately 50% d.m., Lo et al., 2020), Spain (42.2-66.5% d.m. by Pereira- Lorenzo et al., 2006; 56.0-63.5% d.m. by Peña-Méndez et al., 2008) and Switzerland (41.5 to 57.6% d.m. by Künsch et al. 2001).

Concerning the individual sugars, sucrose is the main free sugar in chestnuts, followed by glucose and fructose. Nevertheless, other sugars such as maltose and raffinose can also be present in chestnut fruits in smaller amounts. **Table 10** represents the sugars determined in the uncoated and coated chestnuts.

Table 9: Total soluble solids (TSS), reducing sugars and starch contents of the control and coated chestnuts throughout four months of storage.

Parameter	Storage time (months)	Treatment			
		Control	Proallium	Foodcoat	Proallium + Foodcoat
TSS (%. d.m.)	0	49.28 ± 9.20^{AB}	49.28 ± 9.20^{A}	49.28 ± 9.20^{AB}	49.28 ± 9.20^{A}
	1	67.95 ± 0.06^{abC}	52.00 ± 13.86^{aA}	75.81 ± 0.09^{bC}	73.25 ± 4.57^{bB}
	2	54.93 ± 2.46^{aAB}	53.56 ± 6.11^{aA}	58.66 ± 5.09^{aB}	72.60 ± 12.24^{aB}
	3	59.88 ± 0.06^{bBC}	54.56 ± 4.46^{abA}	55.93 ± 0.06^{abB}	47.91 ± 6.87^{aA}
	4	43.92 ± 3.99^{aA}	43.96 ± 0.07^{aA}	42.64 ± 2.29^{aA}	43.85 ± 0.01^{aA}
Reducing Sugars (mg Glucose/100g d.m.)	0	288 ± 22^{A}	288 ± 22^{A}	288 ± 22^{A}	288 ± 22^{A}
	1	405 ± 31^{aAB}	442 ± 108^{aA}	462 ± 108^{aAB}	350 ± 39^{aAB}
	2	425 ± 106^{aAB}	426 ± 82^{aA}	410 ± 48^{aAB}	421 ± 24^{aBC}
	3	427 ± 81^{aAB}	422 ± 85^{aA}	562 ± 48^{aB}	446 ± 30^{aC}
	4	553 ± 48^{cC}	392 ± 12^{aA}	430 ± 7^{abAB}	484 ± 12^{bC}
Starch Content (%. d.m.)	0	51.2 ± 6.3^{A}	51.2 ± 6.3^{A}	51.2 ± 6.3^{A}	51.2 ± 6.3^{A}
	4	48.9 ± 2.9^{aA}	50.1 ± 7.4^{aA}	50.3 ± 5.6^{aA}	50.5 ± 2.2^{aA}

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

Table 10: Individual sugars determined on the control and coated chestnuts throughout four months of storage.

Sugar	Storage time (months)	Individual Sugars (g/100 g d.w) Control	Proallium	Foodcoat	Proallium + Foodcoat
Raffinose	0	0.11 ± 0.03^{A}	0.11 ± 0.03^{A}	0.11 ± 0.03^{A}	0.11 ± 0.03^{A}
	4	0.17 ± 0.05^{aA}	0.13 ± 0.04^{aA}	0.17 ± 0.04^{aA}	0.11 ± 0.03^{aA}
Saccharose+Maltose	0	18 ± 1^{A}	18 ± 1^{A}	18 ± 1^{A}	18 ± 1^{A}
	4	20 ± 2^{aA}	20 ± 2^{aA}	19 ± 1^{aA}	22 ± 7^{aA}
Glucose	0	0.18 ± 0.01^{A}	0.18 ± 0.01^{A}	0.18 ± 0.01^{B}	0.18 ± 0.01^{A}
	4	0.24 ± 0.06^{A}	0.14 ± 0.06^{A}	0.14 ± 0.01^{A}	0.23 ± 0.06^{A}
Fructose	0	0.57 ± 0.02^{A}	0.57 ± 0.02^{B}	0.57 ± 0.02^{B}	0.57 ± 0.02^{A}
	4	0.43 ± 0.11^{aA}	0.39 ± 0.08^{aA}	0.43 ± 0.07^{aA}	0.46 ± 0.09^{aA}

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

It was impossible to distinguish the saccharose from maltose because they coeluted; however, the sacharose+maltose were the main free sugars present in the chestnuts. The others, such as raffinose, glucose or fructose, represented less than 1% of the dry matter. No significant differences were observed between treatments and storage time for raffinose and sacharose+maltose. On the contrary, a decrease in glucose and fructose contents was observed in Foodcoat after four months. In Proallium, this decrease was only determined on fructose. These results showed again that the hydrolysis of starch or sucrose was not significant because no increase in the glucose and fructose contents wasobserved.

3.5 Microbial analysis

The effect of edible coatings on the proliferation of aerobic mesophiles showed some statistically significant differences in the four months. The results achieved are represented in **Table 11**. A significant increase was observed after four months of storage in the control and samples coated with Foodcoat and Proallium+Foodcoat. On the contrary, the Proallium was the most efficient, and no significant increase was observed in that period. The highest values were always determined in control along the storage period, closely followed by Foodcoat.

Regarding the moulds and yeasts, their counts also increased mainly in the control and Foodcoat. **Figure 7** shows the chestnuts after the treatments and storage times. The most efficient treatment was Proallium, followed by Proallium+Foodcoat. Proallium was quite efficient during two months, with no significant differences compared with the beginning.

In general, the results showed that using some edible coatings may significantly reduce microbial growth. Proallium was the coating that showed to be more efficient to minimize microbial development. For moulds and yeasts, its role was significant until two months. Thus, Proallium may be recommended in the future, being its effect more significant for moulds and yeasts mainly in two months of storage.

Table 11: Mean counts (log CFU/g) of total aerobic mesophilic and moulds/yeasts determined in the uncoated and coated chestnuts stored for four months

Microbial groups	Treatment	Time (Months)				
		0	1	2	3	4
Aerobic mesophilic	**Control**	2.40±0.61 ^{aA}	4.43±0.15 ^{bBC}	5.23±0.58 ^{bcB}	5.67±0.58 ^{cB}	4.43±0.06 ^{bB}
	Proallium	2.40±0.61 ^{aA}	2.60±1.56 ^{aAB}	1.63±0.05 ^{aA}	2.70±0.89 ^{aA}	3.63±0.06 ^{aA}
	Foodcoat	2.40±0.61 ^{aA}	4.70±0.26 ^{bC}	4.90±0.00 ^{bB}	4.67±0.11 ^{bB}	4.13±0.38 ^{bAB}
	Proallium+ Foodcoat	2.40±0.61 ^{aA}	2.10±0.00 ^{aA}	2.00±0.69 ^{aA}	4.43± 0.45 ^{bB}	3.97±0.25 ^{bAB}
Moulds and yeasts	**Control**	2.96±0.49 ^{aA}	4.60±0.20 ^{bB}	5.50±1.10 ^{bB}	4.97±0.50 ^{bA}	4.67±0.38 ^{bB}
	Proallium	2.96±0.49 ^{aA}	2.63±0.20 ^{aA}	2.77±0.35 ^{aA}	4.47±0.25 ^{bA}	3.97±0.15 ^{bA}
	Foodcoat	2.96±0.49 ^{aA}	5.23±0.55 ^{bB}	5.57±0.11 ^{bB}	5.50±0.44 ^{bA}	4.57±0.15 ^{bB}
	Proallium+ Foodcoat	2.96±0.49 ^{abA}	2.47±0.17 ^{aA}	3.63±0.19 ^{abcA}	4.67±0.45 ^{cA}	4.50±0.00 ^{bcAB}

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 times; Different capitalletters on the same column indicate significant differences (p <0.05) between treatments.

Figure 7: Visual appearance of the chestnuts after the treatments and along the storage time.

4. Conclusion

The present work is intended to improve the performance of the chestnut industry. The main objectives were to reduce weight loss and minimize the microorganisms' growth during long-term storage through edible coatings. The different commercial edible coatings studied, namely, Proallium, Foodcoat and their mixture, showed distinctive effects on chestnuts' physicochemical and microbial parameters throughout four months of storage. In colour and texture parameters, slight changes were observed during storage for coated samples. Concerning the colour, it was observed that applying these commercial edible coatings on chestnuts can maintain the colour of the fruits, and in this way, their appearance. In what concerns the texture, no significant differences were found between the treatments for a specific storage time. For the weight loss, after four months, the uncoated and coated chestnuts with Proallium and Proallium+Foodcoat achieved weight losses of around 14%, while with Foodcoat lower values were achieved(5 to 6%).

All coated samples, as well as the control, had similar behaviours for moisture content, water activity, total soluble solids, and starch content throughout storage. On the contrary, titrable acidity increased in coated chestnuts compared to control at the four months. The Proallium and Foodcoat treatments also caused a significant increase in the acidity of the chestnuts, indicating that after four months of storage, these treatments may induce some acidity to the fruits. The combination of both products had increased the titratable acidity even more. Regarding the reducing sugars, the lowest values were determined in the Proallium and Foodcoat treatments, followed by Proallium+Foodcoat. The highest content was determined in control. Regarding individual sugars, no significant differences were observed between treatments and storage time for raffinose and sacharose+maltose. On the contrary, a decrease in glucose and fructose contents was observed in Foodcoat after four months. In Proallium, this decrease was only determined on fructose. Proallium coating exhibited the lowest growth of microorganisms. In particularly, Proallium coating controlled the growth of microorganisms during two months of storage, appearing to be a promising preservation technique.

In conclusion, the application of selected commercial edible coatings can significantly prevent weight loss and microbial growth.

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