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Original research paper

PEA PROTEIN COATINGS INCORPORATED WITH PHENOLIC COMPOUNDS REDUCE OXIDATIVE RANCIDITY IN RAW HAZELNUTS (*CORYLUS AVELLANA* L.)

Camila A. Perussello[1#], Ivan Lj. Milovanović[2], Miona M. Belović[2*], Dragana V. Plavšić[2], Milica M. Pojić[2], Brijesh K. Tiwari[1]

[1]Teagasc Food Research Centre, Department of Food Chemistry and Technology, Ashtown, Dublin 15, Ireland.
[2]University of Novi Sad, Institute of Food Technology Novi Sad, Bulevar cara Lazara 1, 21000, Novi Sad, Serbia.

Abstract: With the aim of producing a functional edible coating to delay rancidity in fat-rich foods, phenolics were extracted from apple pomace and incorporated into 10%-aqueous solutions of pea protein at mass percentages ranging from 0% to 5%. Hazelnuts were coated with these solutions, dried under laminar air flow, vacuum-packed in polypropylene bags and stored at room temperature for 14 days. The 5%-extract coatings increased the antioxidant activity of the hazelnuts by 30 times, while maintaining a texture akin to the uncoated product. The fatty acid profile of all samples remained unchanged during storage. Uncoated hazelnuts and those coated with the 1%-formulation showed detectable levels of hexanal (0.65 mg/kg and 0.31 mg/kg, respectively), a marker of lipid oxidation. In contrast, hexanal was not detected in the samples coated with the 2% and 5%-formulations, indicating a protective effect against lipid oxidation.

Key words: *functional coating, antioxidant activity, hazelnuts, shelf life, lipid oxidation, fatty acids*

INTRODUCTION

Films and coatings have been used throughout history to protect various food products from external factors, such as moisture, oxygen and microbial growth. In recent years, there has been a growing trend towards the development of bio-based packaging and films as environmentally friendly alternatives to replace conventional polymer-based packaging materials (Kadam, Pankaj, Tiwari, Cullen & O'Donnell, 2015; Gupta, Lall, Kumar, Patil & Gaikwad, 2024). Packaging technology increasingly requires innovative materials that not only protect food products from surrounding factors, but also preserve essential qualities such as aroma, taste, and moisture, thus extending the shelf life of perishable products. Edible films are defined as structures being formed separate of any potential intended use, whereas edible coatings are thin layers of material formed directly on the surface of the food product, thereby becoming an integral part of food (Kowalczyk & Baraniak, 2011). Edible films and coatings are usually classified according to the main structural material used for their production: proteins, polysaccharides, lipids or composites (Galus & Kadzińska, 2015). Protein-based films and coatings of various sources are widely used in the food and pharmaceutical industry, with

Corresponding author: Phone: +381214853751, Fax: +38121450725
E-mail address: miona.belovic@fins.uns.ac.rs
#Current affiliation (C.A.P.): University College Dublin, School of Biosystems and Food Engineering, Belfield, Dublin 4, Ireland.

examples such as collagen, gelatin, caseins, whey protein, zein, wheat gluten, egg white protein and others (Dangaran, Tomasula & Qi, 2009; Mihalca et al., 2021). While the film-forming properties of the listed proteins have been extensively researched, properties of proteins from other sources, such as legumes and pseudocereals, are becoming the focus of recent investigations (Shevkani & Singh, 2015; Linares-Castañeda et al., 2023). These sources offer high protein content, reduced cost and a lower environmental impact than animal-based proteins conventionally used in edible films (Shevkani & Singh, 2015; Zhang, Boateng, Xu & Zhang, 2024). Moreover, these proteins do not exhibit the high allergenic potential of commercially available protein films derived from wheat, whey and soybean (Boye et al., 2010).

Peas are various cultivars of *Pisum sativum* L., belonging to the *Leguminosae* family of plants. Peas are the second most produced legume crop in Europe, after soybean (FAOSTAT, 2022). Main nutritional constituents of dried peas are carbohydrates (35%), proteins (27%), fibres (27%) and a low amount of lipids. Compared to other pulses, peas are richer in carbohydrates and fibres, and have lower lipid content (Choi & Han, 2001). Peas also contain 5-20% fewer trypsin inhibitors than soybeans (Shevkani et al., 2015). Peas are a valuable source of proteins, including globulins and albumins, which provide essential amino acids and support various dietary and functional applications (Zhang, Boateng, Xu & Zhang, 2024). For instance, pea proteins are excellent components for edible coatings due to their functional properties, such as film-forming ability and bio-degradability (Zioga, Papantonopoulou, & Evageliou, 2023; Zhang, Boateng, Xu & Zhang, 2024). As with any other protein, the formation of films from pea proteins and coadjuvant materials, such as glycerol, sorbitol and pectin, requires thermal treatment and adjustment of pH values in the film-forming solution to achieve protein denaturation, thereby improving the overall physical and mechanical properties of the films (Zioga, Papantonopoulou, & Evageliou, 2023). In general, pea protein films exhibit physical properties similar to those of other vegetable proteins in terms of elasticity, water vapour permeability (WVP), tensile strength (TS), moisture content and water solubility (Choi & Han, 2002; Shevkani & Singh, 2015; Kowalczyk & Baraniak, 2011; Dangaran, Tomasula & Qi, 2009). Additionally, when isolated pea proteins are combined with phenolic extracts from fruits and vegetables, they have the potential to form sustainable and functional coatings that exhibit both antioxidant and antimicrobial properties, enhancing their usability in various applications (Chong & Brooks, 2022). The use of phenolics rich agrifood by-products for this type of application is particularly valuable in the context of sustainability, as it promotes the repurposing of waste materials. There is a significant gap in current research regarding the synergistic effects of pea protein and phenolic compounds from agricultural waste, which warrants further investigation.

Polyphenols have been suggested to be responsible for the health benefits associated with fruits and vegetables consumption, with apples being recognised as one of the main sources of dietary phenolics worldwide (Hyson, 2011). Apple (*Malus x domestica* Borkh.) is the second most produced fruit in Europe (FAOSTAT, 2014). Apple pomace represents the solid waste resulting from the industrial processing of apple juice or cider production, which consists of peel, seeds, core, stems and exhausted soft tissue (García, Valles, & Lobo, 2009). The amount of apple pomace generated during juice or cider production represent 20-30% of the weight of processed apples (Pingret, Fabiano-Tixier, Le Bourvellec, Renard & Chemat, 2012). Apple pomace is a valuable by-product of the apple processing industry, rich in nutrients such as carbohydrates, proteins, phenolic compounds, dietary fibres and vitamins, making it suitable for various food applications (Ćetković et al., 2008; Perussello, Zhang, Marzocchella & Tiwari, 2017). It is abundant in various polyphenols, including flavonoids such as quercetin and catechins, phenolic acids (e.g., chlorogenic acid and cinnamic acid), as well as dihydrochalcones such as phloridzin and various tannins, all of which contribute to its antioxidant properties and health benefits (Perussello, Zhang, Marzocchella & Tiwari, 2017).

Procyanidins are considered to be the major contributors to the antioxidant activity of apples, accompanied by the hydroxycinnamic and benzoic acids, as well as flavonols (Schieber et al., 2003; García et al., 2009). A number of epidemiological studies have shown that consumption of apple phenolics leads to decreased

risk of chronic diseases such as cardiovascular disease, cancer and asthma (Boyer & Liu, 2004). Additionally, the coexistence of pectin and polyphenols in apple pomace may enhance the functional and technological properties of pea protein coatings that incorporate apple pomace extract (Perussello, Zhang, Marzocchella & Tiwari, 2017).

The aim of this work was to develop an edible coating based on pea protein and apple pomace extract in order to increase the shelf life of hazelnuts. Although protein-based coatings are known to act as a barrier to atmospheric oxygen, the addition of phenolic compounds should considerably improve their antioxidant capacity. This could, in turn, provide greater protection to perishable products such as nuts against the onset of rancidity without impairing the sensory properties of the coated product.

MATERIALS AND METHODS

Preparation of apple pomace extracts

Dried and ground apple pomace was used as the extraction substrate, provided in this form by a cider manufacturing company in Dublin, Ireland. A mixture of apple pomace and solvent (300 g apple pomace in 1.5 L of ethanol:water solution 1:1 v/v) was treated in an ultrasound water bath (Transsonic TI-H-5, Elma, Germany) for 15 min at 25 kHz and then set aside at 50^{o}C for around 60 min prior to being filtered using a Büchner funnel. The apple pomace extract was evaporated using a rotary evaporator until approximately 25% of moisture content was reached. Benzoic acid (0.05% m/m) was added to prevent fermentation of the extract, which was kept refrigerated (4 °C) during the experiments.

Addition of phenolics to protein-based edible coatings

Pea protein isolate from the brand Pulsin (Gloucester, UK), with a protein content of 80%, was utilised in this study. Aqueous solutions consisting of 10% pea protein isolate (film-forming substance), 4% of glycerol (plasticiser) and 1, 2 and 5% apple pomace phenolic extract were prepared and cast into Petri dishes. A control sample (without apple pomace) was also prepared for comparison purposes. Ultrasound treatment was applied in an ultrasonic bath (Branson 3510, USA) at 42 kHz for 5 minutes to ensure homogeneity and eliminate air bubbles. After drying in a fume hood at room temperature and under laminar air flow for 24 h, the resulting films were analysed visually for the presence of air bubbles, formation of a continuous film, colour and transparency. Ten grams of raw whole hazelnuts, bought at the local market in Dublin, Ireland, were coated with 2 mL of protein-apple pomace solutions in small aluminium round trays and dried in the same manner as the films.

Basic chemical analyses

The total dry matter of the hazelnuts was determined using a moisture analyser balance (Sartorius MA150, Germany). The total soluble solids content was assessed using a portable refractometer (Fisher Scientific™, UK, 0-32°Brix). The water activity (Aw) was determined using a portable Aw meter (Testo 205, Testo AG, USA) equipped with a combined penetration tip with temperature probe, according to the ISO standard method (ISO, 2004). The dry coatings were removed from the Petri dishes and analysed for colour using a LAB colorimeter (D25A DP-9000, Hunter Lab, USA).

Total phenolic content

The total phenolic content (TPC) was determined according to the method by Singleton, Orthofer, & Lamuela-Raventos (1999). Basically, 100 μL of 0.1M Folin-Ciocalteu reagent were added to 5 μL of extract and 195 μL of methanol. After 5 min, 700 μL of 20% w/v sodium carbonate solution were added and the reaction mixture was subsequently incubated for 30 min in the dark. The absorbance of the measuring solution was read at 735 nm with a spectrophotometer (Shimadzu UV-1700, Shimadzu Corporation, Kyoto, Japan). The blank was prepared by mixing 200 μL of methanol, 100 μL of Folin-Ciocalteu reagent and 700 μL of sodium carbonate solution. A gallic acid curve was built as standard curve, and the TPC was expressed as mg gallic acid equivalents (GAE)/g of dry weight. The experiments were performed in triplicate.

DPPH radical scavenging activity

The spectrophotometric determination of free radical scavenging activity was based on the DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich) radical transformation in the presence of antioxidants according to Espín, Soler-Rivas, & Wichers (2000). The reaction mixture in the test tubes consisted of 50 μL of sample, 300 μL of DPPH solution and 900 μL of ethanol. The control contained 300 μL of DPPH solution and

900 µL of ethanol. After 60 min of incubation in the dark at room temperature, the absorbance was measured at 515 nm. Each sample was tested at five different concentrations to obtain the IC_{50}, and the experiments were performed in triplicate.

ABTS radical scavenging activity

The antioxidant activity was also assessed by the ABTS radical scavenging. Trolox (6-hydroxy -2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma Aldrich) was used as an antioxidant standard. A 2.0 mM Trolox solution was prepared in methanol for use as a stock solution. Trolox standards (concentration 0-10 mM) were prepared upon dilution of the stock with ethanol. ABTS (2,2'-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid)) diammonium salt, potassium persulfate and ethanol were also obtained from Sigma-Aldrich. ABTS and potassium persulfate were dissolved in distilled water to a final concentration of 7 mM and 140 mM, respectively. These two solutions were mixed and the mixture was allowed to stand in the dark at room temperature for 16 h before use in order to produce the ABTS radical ($ABTS^{\bullet+}$). Then, the ABTS radical solution was diluted with distilled water to an absorbance of 0.70 ± 0.05 at 734 nm. Trolox standards and ABTS solution were mixed and readings were taken at 734 nm 6 min afterwards, using ethanol as blank. The results were expressed in µM Trolox/g product and the experiments were performed in triplicate.

Shelf life tests

A two-week shelf life study was undertaken in order to assess the protective properties of the investigated coatings against lipid oxidation during the storage of hazelnuts. The shelf life tests of control and coated hazelnuts were conducted by modification of the method by Zacheo, Cappello, Perrone and Gnoni (2002). Hazelnuts were removed from the vacuum packaging, spread onto open Petri dishes and placed into a plant growing chamber (Thermo Fisher Scientific, USA) that enabled precise control over environmental conditions. The operation conditions used were temperature of 30 °C, relative humidity 60% and medium light flux. One batch (approximately 10 g) of each sample was removed at the beginning of the experiment and after two weeks and tested for signs of oxidative rancidity.

Fatty acid analysis

Total lipids were extracted from hazelnuts using a chloroform-methanol solution (3×5 mL, with a 2:1 chloroform-to-methanol ratio). After evaporating the solvents under a stream of nitrogen, the residue was weighed (Colla, Reinehr, Reichert & Costa, 2007).

Fatty acid methyl esters were prepared from the extracted lipids by transesterification using 14% boron (III)-fluoride in methanol (Karlović & Andrić, 1996). The obtained samples were analysed using the gas chromatograph Agilent 7890A with flame-ionization detector (FID), auto injection module for liquid samples and a fused silica capillary column (DB WAX 30 m, 0.25 mm, 0.50 um). Helium was used as a carrier gas (purity > 99.9997 vol %, flow rate of 1.26 mL/min). The fatty acids peaks were identified by comparison of the retention times in relation to standards from the Supelco 37 Component Fatty Acid Methyl Ester Mix (Sigma-Aldrich). The results in the chromatograms were expressed as the percentage of individual fatty acids or fatty acid groups in relation to the total fatty acids (%).

Determination of hexanal content

Hexanal was extracted from the hazelnut samples using acetone (HPLC gradient grade, Macron chemicals, Poland). Two grams of homogenised samples were extracted with 10 mL of acetone, facilitated by an orbital shaker (Boeco, Germany) at 400 rpm for 1 hour. The acetone extract obtained was directly analysed using the Agilent Technologies GC-MS Model 7890 A Series gas chromatograph coupled to a 5975 C mass selective detector. An HP 5 MS (30 m × 0.25 mm i.d.) (J & W Scientific, USA) fused silica capillary column with a 0.25 µm film thickness was used with helium as carrier gas (purity > 99.9997 vol %, flow rate of 1.0 mL/min). The chromatographic analysis was modified from the method by Jiménez, Beltrán, & Aguilera (2004), adapted for the analysis of liquid extracts and mass spectrometric means of detection. The oven temperature programme was started at 40 ^{o}C (held for 10 min) and a linear temperature gradient was applied at a rate of 4 ^{o}C/min to a final temperature of 220 ^{o}C and then held for 10 min (total run time of 112 min). The ion source temperature was kept at 230 ^{o}C and the quadrupole at 150 ^{o}C. The mass spectra were obtained in the mass-to-charge ratio (m/z) of 30-400 at electron energy of 70 eV. One µL

of sample was injected in splitless mode and the inlet temperature was set to 220 ^{o}C.

The ChemStation software (Agilent Technologies) was employed for data analysis. Hexanal was identified by analysing the mass spectra of its peak using the NIST05 database. The abundance of the ionized fragment with a m/z of 44 was utilised for quantification. Hexanal standard (Sigma Aldrich, USA) dilutions in acetone were used to build a 5 point-calibration curve ranging from 0.2 to 10 µg/mL). The extraction method using acetone was compared to the previously validated headspace-GC-FID method by Mandić, Sedej, Sakač and Mišan (2013), demonstrating satisfactory hexanal recovery (86%).

Textural properties of hazelnuts

Texture analysis of both uncoated and coated hazelnuts was carried out using a TA.XT Plus Texture Analyser (Stable Micro Systems, England, UK). This analysis aimed to determine their exterior and interior hardness following two weeks of storage. A shearing test was performed on three randomly selected whole hazelnuts from each sample, utilising an Extended Craft Knife (A/CKB) probe and a 5 kg-load cell. Instrumental settings were derived from the sample project (NUT1_CKB.PRJ) within the Texture Exponent Software TEE32 (version 6.1.6.0., Stable Micro Systems, England, UK) and adjusted according to the characteristics of the samples being analysed. Prior to the experiment, the instrument underwent calibration to minimise measurement errors. The instrument was operated at a pre-test speed of 1.5 mm/s, a test speed of 1.0 mm/s and a post-test speed of 10.0 mm/s. The probe travelled a distance of 6 mm, and the trigger force was set to 15 g. All tests were performed in triplicate.

Mould identification

The mycological tests comprised the determination of the total number of moulds (CFU) and their identification. The total mould count in hazelnut samples was determined by the Koch's method of dilutions. Dilutions were prepared using a 0.1% sterile solution of peptone water. The isolation and quantification of moulds were conducted on two surfaces:

1. Dichloran 18% glycerol agar (DG18) (Biokar Diagnostics, France) for isolation of xerotolerant moulds, which grow below 0.9 A_w;

2. Malt yeast 50% glucose agar (MY50G) (malt extract 10 g, yeast extract 2.5 g, agar 10 g, glucose 500 g, distilled water 500 mL, pH 5.3 ± 0.2) for isolation of extremely xerophile moulds, which grow below 0.7 A_w (Samson, Hoekstra, & Frisvad, 2004; Pitt & Hocking, 2009).

The seeded media were incubated at 25 °C. Results were obtained after 5 and 7 days and the samples were analysed in triplicate.

Monocultivation of moulds was conducted as conidia and fragments of hypha from mould colonies, which were translated on Czapek yeast (autolisate) extract agar (CYA) [$NaNO_3$ 3 g, K_2HPO_4 1 g, KCl 0.5 g, $MgSO_4x7H_2O$ 0.5 g, $FeSO4x7H_2O$ 0.01 g, yeast extract 5 g, sucrose 30 g, solution of microelements 1 mL ($ZnSO_4x7H_2O$ 1 g, $CuSO_4x7H_2O$ 0.5 g, distilled water 100 ml), agar 20 g, distilled water 1000 mL, pH 6.0-6.5] or Malt extract agar (MEA) (malt extract 20 g, peptone 1 g, glucose 20 g, agar 20 g, distilled water 1000 mL, pH 5.6 ± 0.2) or Potato dextrose agar (PDA, pH 5.6 ± 0.2) (Himedia, India). In accordance with the macromorphological characteristics, the colonies presumed to belong to the genera *Penicillium* and *Aspergillus* were seeded on CYA, and the others on MEA and PDA. Seeded media were incubated for 7 days at 25 °C. The criteria described by Samson et al. (2004), Samson & Frisvad (2004) and Pitt et al. (2009) were used for species identification.

Statistical analysis

The experimental results were evaluated statistically by the Tukey test at 95% significance level ($p < 0.05$), using the software Statistica (Tibco Inc., Palo Alto, CA, USA) version 14.0.

RESULTS AND DISCUSSION

Total phenolics content and antioxidant activity of hazelnuts

The apple pomace extract had a total soluble solids content of 24.4°Brix and a TPC of 2.63 mg GAE/mL extract (or 11.60 mg GAE/ g extract DW). All pea protein coating formulations showed low viscosity (as per sensory evaluation) and smooth colour (Fig. 1). When applied as coatings, they demonstrated desirable properties, including the formation of a continuous film around each hazelnut, good adherence to the product, transparency, and shininess.

All hazelnut samples – *in natura*, coated with the control solution, 1%-extract solution, 2%-

extract solution and-5% extract solution – were assessed for TPC by the Folin-Ciocalteu method and for antioxidant activity by both the DPPH and the ABTS free radical scavenging assays. The 5%-coatings provided the hazelnuts with the highest TPC and antioxidant activity amongst all samples added of phenolic extract, except the samples coated with the control coating (no extract), which showed the highest TPC among the tested samples and an unusually high antioxidant activity (Tab. 1). The high antioxidant activity of hazelnuts coated with the control film may be explained by sample inhomogeneity: as the skin around the hazelnut kernel is a very rich source of phenolic compounds (Shahidi, Alasalvar & Liyana-Pathirana, 2007), remaining portions of skin on the products' surface may explain the unexpected high values of TPC and antioxidant activity of this particular sample.

The TPC had a three-fold increase between *in natura* and 5%-coated hazelnuts: 8.1 mg GAE/g extract DW versus 24.8 mg GAE/g extract DW. In accordance with the TPC results, the antioxidant activity was higher for the 5%-coated hazelnuts compared to the *in natura*. For instance, hazelnuts coated with the 5%-extract solutions exhibited an antioxidant activity of 244.8 TE/g dry matter (ABTS method), about 2.5 times higher than that of uncoated (*in natura)* hazelnuts (97.7 TE/g dry matter). The most notable increase in antioxidant activity is observed within the results of the DPPH assay: hazelnuts coated with the 5%-extract solutions had IC_{50} values (31.4 μg/mL) 30 times lower than hazelnuts *in natura* (955 μg/mL).

It can be supposed that the observed higher sensitivity of DPPH test in comparison with ABTS test can be attributed to the difference in the calculation (IC_{50} value versus mg Trolox equivalents) and solvent used (ethanol versus water).

Since apple pomace was extracted by a mixture of ethanol and water, it can be supposed that most of the extracted phenolic compounds were soluble in ethanol and therefore more active in DPPH test reaction system.

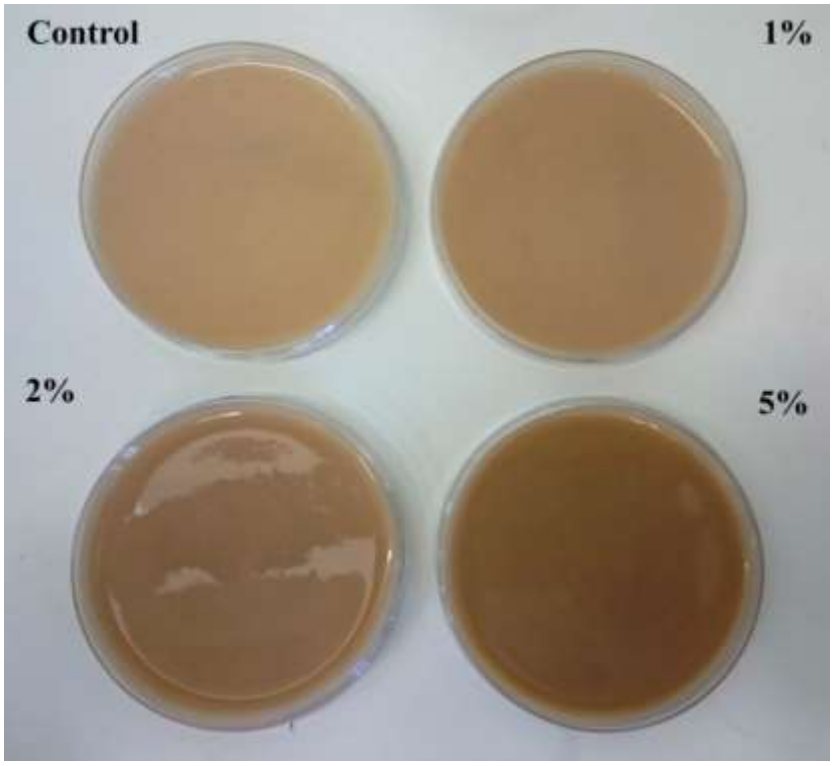

Figure 1. Pea protein coatings with different percentages of apple pomace extract

Colour of coatings

The addition of apple pomace extract influenced colour (Tab. 2). As expected, lightness (L*) decreased with increasing extract concentration, while both the red tone (+a*) and yellow tone (+b*) increased in the films with the added 5%-extract, due to the higher amount of products of phenolic compounds oxidation which are characterized by brownish colour (Fernandes et al., 2019). Namely, polyphenol oxidase was active in apple pomace since none of the inhibitors (such as ascorbic acid) was used during its processing.

Shelf life studies of coated and uncoated hazelnuts

Microbiological tests

The shelf-life results were collected during only two weeks of storage since afterwards all coated samples began to show signs of mould growth, which led to termination of the experiment. This occurrence may be explained by the high A_w values found in coated hazelnut samples (Tab. 3), since high water activity is the main factor enabling microbial growth. The moulds identified on hazelnut surfaces belonged to various *Penicilium* species (*P. digitatum*, *P. corylophilum*, *P. aurantiogriseum*, *P. brevicompactum*, *P. solitum*) and *Eurotium* (*E. herbariorum*, *E. amstelodami*). Additionally, two species of *Aspergillus* moulds (*A. niger* and *A. vesicolor*) were identified on uncoated hazelnuts, which may indicate the possibility of contamination with aflatoxins. These results are in accordance with previous findings regarding microbial contamination of various nut species (Gürses, 2006; Mexis & Kontominas, 2009). The total number of moulds in the samples showed that the uncoated hazelnuts were the least contaminated with $2.2x10^2$ CFU/g. Samples coated with the control coating and coating with 1%-phenolic extract showed the highest total number of moulds ($1.4x10^4$ and $1.2x10^4$ CFU/g, respectively), while samples coated with 2% and 5%-extract coatings showed significantly lower counts ($3.0x10^3$ CFU/g in both samples). These results can be ascribed to the higher concentration of pectic compounds in coatings with higher percentage of apple pomace extract

Table 1.
Total phenolic content and antioxidant activity of uncoated and coated hazelnuts.

Sample	TPC (mg GAE/g extract DW)	DPPH IC_{50} (µg/mL)	ABTS (mg TE/g DW)
Hazelnut *in natura*	8.10 ± 0.33^{c}	955 ± 38.2^{a}	97.7 ± 3.7^{c}
Control coating	42.14 ± 2.51^{a}	$56.7 \pm 7.4_{d}$	182.4 ± 3.9^{b}
1%-extract	5.30 ± 0.32^{c}	648.0 ± 34.4^{b}	80.3 ± 4.8^{d}
2%-extract	6.36 ± 0.38^{c}	272.0 ± 16.5^{c}	87.3 ± 4.7^{cd}
5%-extract	24.84 ± 0.35^{b}	31.4 ± 2.1^{d}	244.8 ± 4.4^{a}

Data are expressed as means (n = 3)

Table 2.
Colour of casted films.

Film	L*	a*	b*
Control coating	44.63 ± 0.95^{a}	10.78 ± 0.66^{b}	32.27 ± 2.53^{b}
1%-extract	40.47 ± 0.75^{b}	10.94 ± 0.78^{b}	34.60 ± 0.86^{b}
2%-extract	37.43 ± 1.36^{c}	13.68 ± 1.49^{a}	34.60 ± 1.36^{b}
5%-extract	32.77 ± 0.49^{d}	15.64 ± 0.48^{a}	41.10 ± 2.20^{a}

Data are expressed as mean ± standard deviation (n = 6)

Table 3.
Texture and water activity (A_w) of hazelnuts after 14 days of storage.

Hazelnut sample	Hardness of Exterior (g)	Fracturability (mm)	Shear Work - Exterior (g.s)	Shear Work - Interior (g.s)	Aw
in natura	627.9 ± 275.1^{a}	0.80 ± 0.46^{a}	900.8 ± 198.9^{a}	1013.8 ± 707.6^{b}	0.454 ± 0.004^{e}
control coating	273.4 ± 88.18^{ab}	0.13 ± 0.04^{b}	1442.7 ± 158.7^{a}	2670.6 ± 244.8^{a}	0.798 ± 0.003^{a}
1%-extract	239.4 ± 17.62^{b}	0.20 ± 0.04^{b}	1272.4 ± 403.3^{a}	2670.1 ± 794.6^{a}	0.715 ± 0.002^{b}
2%-extract	325.8 ± 36.04^{ab}	0.16 ± 0.01^{b}	1397.0 ± 70.0^{a}	2557.5 ± 246.8^{ab}	0.684 ± 0.004^{c}
5%-extract	223.8 ± 72.08^{b}	0.16 ± 0.06^{b}	1169.0 ± 283.0^{a}	1740.8 ± 289.3^{ab}	0.602 ± 0.003^{d}

Data are expressed as means ± standard deviation (n = 3)

(2% and 5%), which are known to bind free water and lower the water activity of the samples (Table 3). Coated samples did not show growth of *Aspergillus* mould species, which is significant since these moulds are toxicologically important as potential producers of aflatoxins.

The coating process involved spreading pea protein suspensions over the hazelnuts, followed by drying at room temperature. This process likely led to moisture absorption by the hazelnut kernels. Subsequent drying of the thin protein coatings resulted in the retention of water within the kernels, as the dry coatings formed a more effective barrier against moisture migration.

Moreover, proteins provide an ideal environment for microbial growth due to their high nutritional value. While spoilage may not be immediately evident during shorter shelf life tests, as in the experiment conducted by Mehyar, Al-Ismail, Han and Chee (2012), or in cases of accelerated shelf life investigations, it can become problematic in situations where coated products are exposed to high relative humidity conditions.

Texture

After two weeks of storage, three texture parameters—hardness, fracturability, and shear work—were assessed to evaluate the impact of different coatings on the hardness and crunchiness of hazelnuts, which are key sensory properties. The hardness of the hazelnut exterior was determined as the mean force of the first peak on the force-distance curve, a measure often associated with consumer perceptions of "first bite" hardness. Fracturability, representing the product's brittleness, was quantified by the mean distance of the first peak on the force-distance curve: lower values indicating higher brittleness.

Shear work was evaluated by integrating the area under the force-distance curve, with the mean area integrated to 2 mm representing the work needed to cut through the hazelnut exterior, and the mean area integrated from 2 mm to 4 mm representing the work on the hazelnut interior. This measure may reflect the energy required to break up the hazelnut in the mouth until it is sufficiently palatable for swallowing.

The results from Tab. 3 indicate that, overall, uncoated hazelnuts showed higher exterior hardness and greater fracturability, indicating that they are harder and less brittle on the first bite than coated hazelnuts.

The sample coated with the film containing 5%-extract (which demonstrated higher antioxidant activity) exhibited lower exterior hardness and fracturability than uncoated hazelnuts. However, the energy required to break it up, represented by the shear work, remained unchanged.

Fatty acid profile and lipid oxidation

The fatty acid composition and hexanal content of hazelnut samples are presented in Table 4.

Table 4.
Fatty acid composition and hexanal content of hazelnuts during storage

Sample/storage time	**Fatty acids (% of the total fatty acids)**							**Hexanal content (mg/kg)**
0 day	16:0	16:1	18:0	18:1n9 c	18:2n6 c	18:3n3	20:1	
uncoated hazelnuts	5.28^{a}	0.22^{a}	2.23^{a}	80.70^{a}	11.27^{a}	0.113^{ab}	0.187^{ab}	ND
control coating	5.22^{a}	0.23^{a}	2.13^{a}	81.04^{a}	11.15^{a}	0.117^{ab}	0.150^{cd}	ND
1%-extract	5.25^{a}	0.21^{a}	2.35^{a}	80.68^{a}	11.20^{a}	0.123^{a}	0.180^{abc}	ND
2%-extract	5.29^{a}	0.22^{a}	2.28^{a}	80.81^{a}	11.20^{a}	0.110^{ab}	0.173^{abcd}	ND
5%-extract	5.31^{a}	0.21^{a}	2.20^{a}	80.80^{a}	11.20^{a}	0.117^{ab}	0.163^{bcd}	ND
14 days	16:0	16:1	18:0	18:1n9 c	18:2n6 c	18:3n3	20:1	
uncoated hazelnuts	5.35^{a}	0.20^{a}	2.15^{a}	80.80^{a}	11.20^{a}	0.100^{b}	0.203^{a}	0.65^{a}
control coating	5.30^{a}	0.22^{a}	2.18^{a}	80.90^{a}	11.15^{a}	0.107^{ab}	0.147^{d}	ND
1%-extract	5.28^{a}	0.19^{a}	2.21^{a}	80.85^{a}	11.20^{a}	0.117^{ab}	0.150^{cd}	0.31^{b}
2%-extract	5.31^{a}	0.20^{a}	2.18^{a}	80.86^{a}	11.16^{a}	0.127^{a}	0.157^{bcd}	ND
5%-extract	5.29^{a}	0.20^{a}	2.21^{a}	80.86^{a}	11.17^{a}	0.107^{ab}	0.163^{bcd}	ND

Data are expressed as means (n = 3). ND = not detected

Monounsaturated oleic acid (18:1n9c) was found to be the predominant fatty acid (80.68-81.04%) in all samples, with significant amounts of polyunsaturated linoleic acid (18:2n6c) also present. Among the saturated fatty acids, palmitic acid (16:0) and stearic acid (18:0) were the most abundant. These findings align with previous studies (Parcerisa, Richardson, Rafecas, Codony & Boatella, 1998; Amaral et al., 2006).

Notably, there was no significant difference observed in the fatty acid profiles of the samples between the beginning of the experiment and after two weeks of storage (Table 4). These results suggest that, under the applied storage conditions, the shelf life experiment could have been extended further. However, this was not feasible due to the pronounced mould growth.

Hexanal is one of the volatile compounds formed during the oxidation of unsaturated fatty acids. As its olfactory threshold value is very low in humans (5 ng/g) (Buttery, Turnbaugh, & Ling, 1988), it can be used as a good indicator of fat rancidity. Hexanal levels have also been used in the scientific literature to assess lipid oxidation and sensory quality – aroma, flavour and colour – of hazelnuts and their coproducts, such as pastry and chocolate (Ghirardello et al., 2016; Nicolotti et al., 2013; Mexis et al., 2010; Fallico, Arena & Zappala, 2003). Hexanal is formed mainly by oxidation of linoleic acid (Boonprab et al., 2003). As linoleic acid is one of the main polyunsaturated (PUFA) components in the lipid fraction of hazelnuts, after oleic acid only (Ebrahem. Richardson, Tetley & Mehlenbacher, 1994; Amaral et al., 2006), the increase in hexanal levels is an excellent indicator of oxidative changes. Nevertheless, there remains a paucity of data regarding hexanal levels during the storage of hazelnuts.

The presence of hexanal was not detected in any of the samples at the beginning of the shelf life experiments (day 0). After two weeks of storage, hexanal was detected in the uncoated sample at the level of 0.65 mg/kg and in samples coated with 1%-film at 0.31 mg/kg. In addition, these two hazelnut samples featured the highest IC_{50} values in the DPPH assay (955 µg/mL and 648 µg/mL for uncoated and 1%-coating samples, respectively), which could be related to hexanal production. As the other samples did not show a detectable increase in hexanal levels, it can be concluded that pea protein coatings exhibit a protective effect toward lipid oxidation in hazelnuts.

Hexanal levels are indeed very low in fresh raw hazelnuts, as reported by Ghirardello et al. (2016), who found 0.0619-0.0633 mg/kg for shelled kernels. Grosso & Resurreccion (2002) reported that the limit hexanal level in roasted peanuts in terms of sensory overall acceptance was 7.40 mg/kg, which is nearly 24 times higher than the hazelnut samples coated with the 1%-formulation. These results may not be fully conclusive as the experiment was shortened due to microbial spoilage of samples however they clearly indicate the high antioxidant potential of the coatings. In a study by Kinderlerer and Johnson (1992), the hexanal levels in shelled hazelnut kernels ten-folded over 3 years of storage at ambient temperature in the dark, accompanied by a substantial reduction in the fatty acid content. Also in this investigation, the unchanged fatty acid profiles throughout the shelf life period were correlated to very low amounts of generated hexanal, which indicates that no significant lipid oxidation occurred.

CONCLUSIONS

An innovative food coating with functional properties, utilising pea protein and apple pomace extract rich in phenolics, was developed and applied to fresh hazelnuts. Notably, hazelnuts coated with 5%-extract solutions exhibited significantly higher antioxidant activity and TPC compared to uncoated hazelnuts. For instance, the IC_{50} values for hazelnuts coated with 5%-extract solutions were 30 times lower (31.4 µg/mL) than those for uncoated hazelnuts (955 µg/mL). In addition, TPC showed a three-fold increase between uncoated and 5%-coated hazelnuts: 24.8 mg GAE/g extract DW versus 8.1 mg GAE/g extract DW.

After two weeks of storage, hexanal levels increased from 0 to 0.65 mg/kg in uncoated hazelnuts. In the same period, hexanal was also detected in samples coated with the 1%-extract solution (0.31 mg/kg), while no increase was observed in samples coated with 2% and 5%-extract. Moreover, the fatty acid profile of the samples remained unchanged during storage.

Texture determinations conducted after 14 days of storage indicated that uncoated hazelnuts are harder and less brittle at the first bite, but require less energy to break up than coated hazelnuts, which is associated with crunchiness.

Corresponding author: Phone: +381214853751, Fax: +38121450725
E-mail address: miona.belovic@fins.uns.ac.rs
#Current affiliation (C.A.P.): University College Dublin, School of Biosystems and Food Engineering, Belfield, Dublin 4, Ireland.

These texture differences may be attributed to the higher water activity of coated hazelnuts. The high water activity of coated samples led to microbial spoilage and the growth of several species of *Penicillium* and *Eurotium* moulds during the shelf life tests, highlighting the need for further experiments to optimise drying conditions. Future studies should aim to investigate the antifungal properties of edible pea protein coatings, building upon the promising findings of this work.

AUTHOR CONTRIBUTIONS

Conceptualization, C.A.P., B.K.T.; Methodology, C.A.P., I.Lj.M., M.M.B, D.V.P.; Investigation, formal analysis, validation, writing-original draft preparation, C.A.P., I.Lj.M., M.M.B; Writing-review and editing, C.A.P., M.M.B, M.M.P.; Supervision, B.K.T.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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PROTEINSKI PREMAZI OD GRAŠKA SA FENOLNIM JEDINJENJIMA SMANJUJU OKSIDATIVNU UŽEGLOST SIROVIH LEŠNIKA (*Corylus avellana* L.)

Camila A. Perussello[1#], Ivan Lj. Milovanović[2], Miona M. Belović[2*], Dragana V. Plavšić[2], Milica M. Pojić[2], Brijesh K. Tiwari[1]

[1]Teagasc Centar za istraživanje hrane, Departman za hemiju i tehnologiju hrane, Eštaun, Dablin 15, Irska
[2]Univerzitet u Novom Sadu, Naučni institut za prehrambene tehnologije u Novom Sadu, 21000 Novi Sad, Bulevar cara Lazara br. 1, Srbija

Sažetak: U cilju proizvodnje funkcionalnog jestivog premaza za odlaganje užeglosti u hrani bogatoj mastima, fenoli su ekstrahovani iz tropa jabuke i ugrađeni u 10% vodene rastvore proteina graška u masenim procentima u rasponu od 0% do 5%. Ovim rastvorima su premazani lešnici, sušeni pod laminarnom strujom vazduha, vakuumirani u polipropilenske kese i čuvani na sobnoj temperaturi 14 dana. Premazi sa 5% ekstrakta povećali su antioksidativnu aktivnost lešnika 30 puta, koji su zadržali teksturu sličnu nepremazanom proizvodu. Profil masnih kiselina svih uzoraka ostao je nepromenjen tokom skladištenja. Nivoi heksanala, kao markera oksidacije lipida, detektovani su kod nepremazanih lešnika i lešnika premazanih formulacijom sa 1% ekstrakta (0,65 mg/kg i 0,31 mg/kg, respektivno). Nasuprot tome, heksanal nije detektovan u uzorcima premazanim formulacijama sa 2% i 5% ekstrakta, što ukazuje na zaštitni efekat protiv oksidacije lipida.

Ključne reči: *funkcionalni premaz, antioksidativna aktivnost, lešnici, rok trajanja, oksidacija lipida, masne kiseline*

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