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Korrespondenzadresse:
vionor@yahoo.com

[1]) Dunarea de Jos University of Galati, Faculty of Food Science and Engineering, Domneasca Street 111, 800201, Galati, Romania; [2]) University of Craiova, Faculty of Horticulture, A.I. Cuza Street 13, 200585, Craiova, Romania

Effect of chitosan based edible coating enriched with extracts from walnut leaves and sweet cherry stems on the quality of precooked pork patties

Wirkung eines essbaren Überzugs auf Chitosan-Basis, angereichert mit Extrakten aus Walnussblättern und Süßkirschstängeln, auf die Qualität von vorgekochten Schweinefleischpasteten

Andrei Iulian Boruzi[1]), Violeta Nour[1,2])

Summary

The effects of chitosan-based edible coating (CH) with and without the addition of either walnut leaf extract (CWL) or cherry stem extract (CCS) on lipid oxidation, color preservation, water losses, and pH of precooked pork patties were evaluated over a 15-day refrigerated storage. The antioxidant activity of meat samples was also investigated. The chitosan-based coatings decreased lipid oxidation of the meat compared to the control. After 15 days of refrigerated storage, TBARS of the coated samples were 12.7 %, 44.53 % and 37.91 % lower than those of control samples for CH, CWL and CCS respectively. Meat samples coated with chitosan showed higher antioxidant activity ($p<0.05$) than control samples probably due to the antioxidant activity of chitosan itself and the incorporation of walnut leaf and cherry stem extracts into chitosan coatings further increased the antioxidant activity of the coated patties, significantly. The coatings significantly decreased relative weight loss compared to the control. This study shows that a chitosan-based coating incorporating walnut leaf and cherry stem extracts could be effective at preventing lipid oxidation and improving the shelf life in meat products during refrigerated storage.

Keywords: antioxidant activity, color, cherry stems, lipid oxidation, meat products, walnut leaves

Introduction

Because of the increasing consumer demand for safe, high quality ready-to-eat products, recent research in the food industry has turned to the development of new processing and packaging technologies that can increase the shelf life and retain the sensory properties and chemical composition of these products (Kokoszka and Lenart, 2007).

Moisture loss and lipid oxidation are major causes of deterioration and reduced shelf life of precooked meat products during refrigerated storage (Wu et al., 2000). Myoglobin and lipid oxidation generate products that modify color and cause off-flavors and odors in meat, which negatively affect the acceptability and overall quality of meat products (Vital et al., 2016).

Consequently, several synthetic antioxidants have been added to meat products in order to decrease the oxidation rate and to enhance their shelf life. However, public concern about the safety of synthetic antioxidants has led to an increasing interest for bioactive compounds with antioxidant activity from natural sources as alternatives (Ponce et al., 2008).

Application of an edible coating is a new technology that has been developed into food processing to prolong the shelf life and preserve the quality of food products. Edible coatings made of polysaccharides, proteins, and lipids reduce the migration of water vapor, oxygen, carbon dioxide, aromatic compounds and lipids, but they may also serve as carriers for antioxidants, preservatives, aromatic substances, colorants etc. (Kokoszka and Lenart, 2007).

Various types of coatings with added food additives (i.e. antimicrobials, antioxidants) have been tested in an attempt to extend the shelf life of meat products by reducing the risk of pathogen growth and by retarding dehydration, oxidative rancidity and surface browning (Fan et al., 2008; Chidanandaiah et al., 2009; Haque et al., 2009; Kang et al., 2007; Vásconez et al., 2009; Song et al., 2011; Quirós-Sauceda et al., 2014).

Polysaccharide-based films and coatings can be made using cellulose, native or modified starch, pectin derivatives, seaweed extracts (e.g., alginates, carrageenan and agar), exudate gums (e.g., acacia, tragacanth and guar) and chitosan. As polysaccharides are hydrophilic, they are poor barriers to moisture, but they present low oxygen permeability, resist lipid migration and retain product aroma (Soliva-Fortuny et al., 2012; Dehghani et al., 2018).

Chitosan is a natural bioactive polysaccharide derived from the partial deacetylation of chitin, a major component of the exoskeletons of crustaceans, fungi and insects (Ahmadi et al., 2015; van den Broek et al., 2015). Chitosan has good film-forming ability and excellent carrier properties for various additives.

Chitosan-based films have good mechanical properties and selective permeabilities for CO_2 and O_2, while the high sensitivity to moisture limits their application (Ruban, 2009; Nouri et al., 2018). It was demonstrated that chitosan exhibits intrinsic antibacterial and antifungal activity that was linked to the presence of its positively charged amino groups in the polymer backbone and their ionic interactions with negatively charged microbial cell membrane constituents (Ziani et al. 2009; Goy et al., 2016). Chitosan has been extensively evaluated as a food preservative either by in vitro trials or through direct application on real foods, and the results confirmed its potential in food conservation. As a result, chitosan was extensively used to protect, improve quality and extend the shelf life of fresh and processed foods (Campos et al., 2010; Duran and Kahve, 2019). Several studies developed chitosan coatings enriched with plant essential oils or extracts with the aim of increasing the antimicrobial and antioxidant efficacy of the coatings (Ponce et al., 2008; Ojagh et al., 2010; Mannozzi et al., 2018). However, only a few research reports are available on application of chitosan active coatings to meat products (Siripatrawan and Noipha, 2012; Kanatt et al., 2013; Bonilla et al., 2014; Abdallah et al., 2017).

This study was conducted with the objective of evaluating the effectiveness of chitosan-based edible coatings containing walnut leaf and sweet cherry stem extracts against moisture loss and lipid oxidation in precooked pork patties under refrigerated storage.

Materials and methods

Materials

Chitosan from BiOrigins (Fordingbridge, UK) was used for the coating formulations and food-grade glycerol (Fluka, Madrid, Spain) was used as a plasticizer. Thiobarbituric acid and potassium persulfate were from Sigma-Aldrich (St Louis, MO, USA), trichloracetic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and malondialdehyde were from Merck (Darmstadt, Germany) and 2,2‘-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) from Alfa Aesar (Karlsruhe, Germany).

Preparation of walnut leaf and cherry stem extracts

Fresh walnut (*Juglans regia* L.) leaves and sweet cherry (*Prunus avium* L.) stems were collected randomly from four different mature trees growing in the experimental orchard of the University of Craiova located at Râmnicu Vâlcea (Romania) research station (45°07‘N/24°22‘E). After collection, the leaves were immediately transferred to the laboratory, removed from the stems, cleaned and dried in the shade (final moisture content = 5.9%). The dried walnut leaves and cherry stems were ground in a coffee grinder (Bosch MKM6000, Germany), mixed to obtain homogenous samples and stored in the dark at room temperature in high density polyethylene bags for further use. For preparation of the extracts, 10 g of dried leaf or stem powder were mixed with 100 mL boiling distilled water and left for 1 h. The extracts obtained by filtration were used in further experiments.

Preparation of pork patties

Fresh pork meat and back fat were purchased from a local supermarket immediately following cutting and processed after storage for 24 hours at 4 °C. The excessive fat and connective tissue were removed from the meat. The pork patties were made according to the following recipe: 73.5% lean pork meat, 20% pork back fat, 5% ice and 1.5% salt. The meat and fat were minced through a 3 mm plate, then the ice and salt were added. The mixture was blended by hand for 10 min and shaped by hand into 50 g patties using 180 × 565 mm Petri dishes. The samples were cooked in electric oven (Beko, BIM24300GPS, Istanbul, Turkey) preheated for 15 minutes at 180 °C till the internal temperature reached 75 ± 1 °C. After cooling to room temperature, a total of 240 patties were randomly and equally divided into four groups: uncoated (C); coated with chitosan (CH); coated with chitosan containing walnut leaf extract (CWL) and coated with chitosan containing cherry

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stem extract (CCS). Experiments were independently conducted twice.

Preparation of coatings

Chitosan solution was prepared by dissolving 3 g chitosan in 100 mL of 1% acetic acid aqueous solution with 1 g glycerine. The mixture was heated to boiling (about 100 °C) on a magnetic stirrer/hot plate until the solution became clear, agitated in an ultrasonic bath for 60 min to eliminate bubbles and then kept at room temperature until use for coating. Active edible coatings were made in the same way as above but using infusions of sweet cherry stems and walnut leaves, respectively, as the solvent of the 1% acetic acid solution.

Application of edible coatings

The patties were individually dipped in the coating solutions for 10 s at room temperature and allowed to drain (to remove coating excess) for 10 s. This dipping procedure was repeated three times, then the patties were dried for 2 h in a laminar flow hood. Patties, with or without coating, were individually packed into small polyethylene bags and stored at 2 °C.

Relative weight loss, pH, instrumental colour, lipid oxidation and antioxidant activity were determined in pork patties at the end of the coating application process and after 5, 10, and 15 days of storage. Lipid oxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS) while antioxidant activity was measured using the ABTS method. All the assays were performed in triplicate.

Relative weight loss (RWL)

The water loss was evaluated gravimetrically. Samples were weighed before and after the respective storage period at 4 °C. The percentage weight loss relative to the initial weight was calculated as: RWL (%) = [(initial weight – final weight)/initial weight] x 100

pH measurements

10 g sample of pork patties was dispersed in 50 mL of distilled water and stirred for 1 min. The pH of the dispersion was measured using a multiparameter instrument Hanna HI255 (Hanna Instruments, Padova, Italy).

Color

Color was measured in the middle part of the patties at 0, 5, 10 and 15 days of storage, using a PCE-CSM1 reflectance colorimeter (PCE Instruments, Southampton, UK) calibrated against a white standard. Color was expressed as L* (lightness), a* (redness), and b* (yellowness) reflectance values of the CIELab system. Chroma and hue values were calculated as follows:

Chroma = $(a^{*2} + b^{*2})^{1/2}$ and hue angle (h) = arctan $(-b^*/a^*)$

The analysis was performed on three samples from each treatment with four readings in each sample.

Thiobarbituric acid reactive substances

TBARS values (mg malondialdehyde/kg) were determined as described by Witte et al. (1970) with slight modifications. The sample (5 g) was homogenised with 20% TCA solution (12.5 mL) in a vortex, then transferred to a 25-mL volumetric flask and dilluted up to the volume with cold distilled water. The mixture was centrifuged for 10 min at 6,000 rpm and the supernatant was filtered through Whatman 0.45-mm filter paper (Whatman International Ltd., Maidstone, UK). Five milliliters of the filtrate were transferred to a capped test tube and then 5 mL of 0.02 M 2-thiobarbituric acid solution was added.

Five mL extract was mixed with 5 mL of 0.02 M 2-thiobarbituric acid and heated at 100 °C for 35 minutes. The mixture was incubated in a water bath at 100 °C for 35 min and then cooled in cold water. The absorbance was measured at 532 nm with a Varian Cary 50 UV spectrophotometer (Varian Co., Palo Alto, USA). The standard curve was prepared using malondialdehyde (MDA) and TBARS values were expressed as mg MDA/kg sample.

Total phenolic content

Total phenolics were estimated colorimetrically by Folin-Ciocalteu assay as proposed by Singleton et al. (1999). Aliquots of extracts (0.1 mL) were mixed with 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. After 30 sec to 8 min of reaction, 1.5 mL of Na2CO3 (20%) and 2.9 mL of distilled water were added. The absorbance was measured at 765 nm in a Varian Cary 50 UV spectrophotometer (Varian Co., Palo Alto, USA) after 30 minutes of incubation at 40 °C. Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g.

Total flavonoid content

Total flavonoids were measured by the aluminum chloride colorimetric method developed by Zhishen et al. (1999). An aliquot (1 mL) of appropriately diluted sample or standard solutions of quercetin (20–100 mg/L) was added to a 10 mL volumetric flask containing 4 mL H_2O. At zero time, 0.3 mL 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 mL 10% $AlCl_3$ was added. Finally, at 6 min, 2 mL 1 M NaOH was added to the mixture and the volume was made up to 10 mL with H_2O. Absorbance of the mixture was determined at 510 nm against reagent blank prepared with water. Total flavonoid content of plant extracts was expressed as mg quercetin (QE) per 100 g.

ABTS antioxidant activity

The ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assay was conducted according to the procedure described by Re et al. (1999). The ABTS cation radical solution (ABTS +) was prepared by mixing 5 mL of a 7.0 mM ABTS solution and 88 µL of a 145 mM potassium persulfate solution. The mixture was incubated in the dark at room temperature for 16 h. The ABTS+ solution was then diluted with 80% ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Samples (120 µL) were mixed with ABTS+ solution (12 mL) and absorbance was recorded after 6 min against ethanol as blank. The standard curve was constructed using Trolox and the results were expressed in µM Trolox per 100 g of sample.

Sensory evaluation

Samples were evaluated immediately after processing (day 0) and during 15 days of refrigerated storage period, at 5-day intervals, with regard to appearance, color, flavor and overall acceptability, using a 10-point hedonic scale (1 = extremely dislike, 10 = extremely like). Patties were warmed in a microwave oven for 20 s just before sensory evaluation and coded samples were served at room temperature. Water was served to clear the taste between samples. The panel consisted of twelve members from the University of Craiova staff and postgraduate students in food science and technology.

Statistical analysis

All analyses were run in triplicate and results are reported as mean ± standard deviation. In order to assess the effects of treatments and storage time, data were subjected to the analysis of variance (ANOVA) using Statgraphics Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA). The Duncan's multiple-range test was used to test for difference between means with the significance defined at $p<0.05$.

TABLE 1: *Total phenolic content, total flavonoid content and antioxidant activity in walnut leaf and cherry stem extracts*.*

Sample	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg QE/100 g)	ABTS antioxidant activity (mmol Trolox/100 g)
Walnut leaf extract	497.67 ± 23.34	353.66 ± 16.45	3.67 ± 0.21
Cherry stem extract	685.66 ± 31.15	145.35 ± 11.19	2.84 ± 0.14

*: Values are expressed as mean ± standard deviation.

Results and discussion

Walnut leaf and cherry stem extracts were tested for total phenolic content, total flavonoid content and ABTS antioxidant activity (Table 1). In the walnut leaf extract, both total flavonoid content and antioxidant activity were higher than in the cherry stem extract. Walnut leaf was previously reported to be a rich source of flavonoids (Martinez et al., 2009; Carvalho et al., 2010). Total phenolic content, however, was higher in cherry stem extracts than in walnut leaf extracts.

Weight loss significantly ($p<0.05$) increased for all samples during the 15 days storage period (Table 2). The edible coating significantly decreased relative weight loss in the pork patties during refrigerated storage. Chitosan coating determined a reduction of relative weight loss by 44.9%, 29.3%, and 20.5% over control patties after 5, 10 and 15 days of refrigerated storage, respectively.

A reduction of the relative moisture loss by 66% has been reported in chitosan coated beef patties over unpackaged patties by Wu et al. (2000). Several researchers have emphasized that certain polysaccharides, including chitosan, applied in the form of high moisture gelatinous coatings, can retard moisture loss from coated foods by functioning as sacrificing agents rather than moisture barriers (Bourlieu et al., 2008; Jiménez et al., 2015; Cardoso et al., 2016). The enrichment of the chitosan coating with plant extracts did not significantly affect the relative moisture loss of the meat samples.

Thiobarbituric acid reactive substances (TBARS) of control and coated pork patties were determined and compared as presented in Table 3. The edible coatings decreased lipid oxidation of the meat samples compared to the control. The coatings with plant extracts were most effective (44.53% and 37.91% decrease in lipid oxidation as compared with the control for CWL and CCS respectively) and also showed the highest antioxidant activity.

After 15 days of refrigerated storage, TBARS of CH-coated samples were 12.7% lower ($p<0.05$) than those of control samples. This may be due to the antioxidant properties of chitosan and to its low oxygen permeability (Xu et al., 2005). In addition, the oxidation decreased because the coating prevented the interaction between air and the surface of meat. Cardoso et al. (2016) found that an edible chitosan gelatin-based coating limited lipid oxidation of beef and attributed this effect to chitosan and its antioxidant property. Jeon et al. (2002) found also lower contents of TBARS in chitosan-coated herring and cod samples than in uncoated samples throughout the storage time.

The composition of the CH coating might also contribute to its lower O_2 permeability. Chitosan is water-insoluble but is readily soluble in dilute organic acid (Rodríguez-Sánchez and Rha, 1981). Acetic acid was used as solvent to form CH coating in our study. Acetic CH film has been reported to have lower O_2 permeating coefficient than lactic acid and formic acid CH films (Caner et al., 1998) but was less resistant against water than other acid CH films (Rhim et al., 1998). Compared to CH, the TBARS values of CWL and CCS were lower due to the presence of antioxidant extracts and CWL had the best effect. Other previous studies concluded also that an edible coating incorporating a natural antioxidant may improve the shelf life of meat products by preventing lipid oxidation (Kang et al., 2007; Song et al., 2011; Vital et al., 2016).

Lipid oxidation increased significantly ($p<0.05$) during storage, particularly in the control patties, which showed the highest increase. This may be attributed to the partial dehydration of pork paties and the increased oxidation of unsaturated fatty acids. According to Campo et al. (2006), a TBARS value of 2 mg MDA/kg was regarded as threshold for the sensory detection of rancid flavours in beef. In this study, the initial TBARS values of cooked patties were in the range 0.72-0.96 mg MDA/kg and TBARS values exceeded 2 mg MDA/kg on day 10 of storage for C and CH respectively. However, the TBARS values of CWL and CCS exceeded 2 mg MDA/kg on day 15. Some previous studies reported also that chitosan coatings enriched with plant extracts or essential oils prevented oxidation in beef burgers (Georgantelis et al., 2007), pork salamis (Kanatt et al., 2008) and poultry products (Giatrakou et al., 2010).

TABLE 2: *Relative weight loss (%) of the pork patties during refrigerated storage for 15 days*.*

Treatment[1]	Storage period (days)		
	5	10	15
C	1.67 ± 0.28^{bA}	3.44 ± 0.25^{bB}	4.63 ± 0.22^{bC}
CH	0.92 ± 0.10^{aA}	2.43 ± 0.18^{aB}	3.68 ± 0.32^{aC}
CWL	0.98 ± 0.13^{aA}	2.51 ± 0.26^{aB}	3.80 ± 0.22^{aC}
CCS	1.11 ± 0.14^{aA}	2.63 ± 0.19^{aB}	3.92 ± 0.24^{aC}

*: Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment ($p<0.05$), while different uppercase letters are indicative of significant differences due to storage period ($p<0.05$). [1]: C: uncoated; CH: coated with chitosan; CWL: coated with chitosan containing walnut leaf extract; CCS: coated with chitosan containing cherry stem extract.

TABLE 3: *TBARS values (mg MDA/kg) of the pork patties during refrigerated storage for 15 days*.*

Treatment[1]	Storage period (days)			
	0	5	10	15
C	0.72 ± 0.03^{aA}	1.42 ± 0.09^{bB}	2.39 ± 0.14^{cC}	3.93 ± 0.20^{cD}
CH	0.96 ± 0.05^{cA}	1.30 ± 0.08^{bB}	2.12 ± 0.11^{bC}	3.43 ± 0.16^{bD}
CWL	0.82 ± 0.03^{bA}	1.10 ± 0.06^{aB}	1.55 ± 0.08^{aC}	2.18 ± 0.09^{aD}
CCS	0.78 ± 0.04^{abA}	1.02 ± 0.04^{aB}	1.48 ± 0.07^{aC}	2.44 ± 0.12^{aD}

*: Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment ($p<0.05$), while different uppercase letters are indicative of significant differences due to storage period ($p<0.05$). [1]: C: uncoated; CH: coated with chitosan; CWL: coated with chitosan containing walnut leaf extract; CCS: coated with chitosan containing cherry stem extract.

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TABLE 4: *ABTS antioxidant activity (µM Trolox/100 g) of the pork patties during refrigerated storage for 15 days*.*

Treatment[1]	Storage period (days)			
	0	5	10	15
C	0.44 ± 0.02^{aD}	0.40 ± 0.02^{aC}	0.36 ± 0.02^{aB}	0.29 ± 0.02^{aA}
CH	0.51 ± 0.03^{bC}	0.46 ± 0.02^{bBC}	0.42 ± 0.03^{bAB}	0.38 ± 0.03^{bA}
CWL	0.75 ± 0.04^{cB}	0.73 ± 0.03^{cB}	0.63 ± 0.03^{cA}	0.61 ± 0.03^{cA}
CCS	0.72 ± 0.03^{cC}	0.68 ± 0.03^{dBC}	0.64 ± 0.02^{cB}	0.58 ± 0.03^{cA}

*: Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment (p<0.05), while different uppercase letters are indicative of significant differences due to storage period (p<0.05). [1]: C: uncoated; CH: coated with chitosan; CWL: coated with chitosan containing walnut leaf extract; CCS: coated with chitosan containing cherry stem extract.

TABLE 5: *pH of the pork patties during refrigerated storage for 15 days*.*

Treatment[1]	Storage period (days)			
	0	5	10	15
C	6.07 ± 0.03^{B}	6.22 ± 0.03^{abC}	5.97 ± 0.06^{A}	5.89 ± 0.05^{A}
CH	6.05 ± 0.03^{B}	6.27 ± 0.02^{bC}	5.93 ± 0.03^{A}	5.87 ± 0.05^{A}
CWL	6.02 ± 0.07^{B}	6.19 ± 0.05^{aC}	5.91 ± 0.03^{A}	5.88 ± 0.07^{A}
CCS	6.00 ± 0.06^{B}	6.17 ± 0.02^{aC}	5.92 ± 0.05^{AB}	5.91 ± 0.04^{A}

*: Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment (p<0.05), while different uppercase letters are indicative of significant differences due to storage period (p<0.05). [1]: C: uncoated; CH: coated with chitosan; CWL: coated with chitosan containing walnut leaf extract; CCS: coated with chitosan containing cherry stem extract.

Meat samples coated with chitosan showed higher antioxidant activity (p<0.05) than control samples probably due to the antioxidant activity of chitosan (Yen et al., 2008). Other previous studies indicated that chitosan, as a coating, interacts with components in the food surface, enhancing its antioxidant properties (Ponce et al., 2008).

Furthermore, results of this study showed that incorporation of walnut leaf and cherry stem extracts into chitosan coatings significantly increased the antioxidant activity of the coated meat samples (Table 4).

Radical scavenging activity significantly decreased (p<0.05) throughout the 15 days storage period. After 15 days of storage, antioxidant activity was significantly higher in samples coated with chitosan containing phenolic-rich extracts than in samples coated with chitosan and uncoated samples. No significant difference was found between CWL and CCS with respect to the antioxidant activity of pork patties at the end of the storage period.

The pH values of patty samples are presented in Table 5. The coated samples had lower pH values than controls because of the low pH of the chitosan coating solution, resulting from acetic acid incorporation into the formulation. However, significant differences were found between chitosan coated patties and the other treatments only after 5 days of refrigerated storage. This lower pH values in samples were maintained throughout the storage period and they could be attributed to chitosan's ability to inhibit growth of bacteria that might cause alterations in pH value (Aşik and Candoğan, 2018).

The evolution of color values (L^*, a^* and b^*) for all treatments as a function of storage time is presented in Table 6. In the present study lightness was significantly (p<0.05) higher in chitosan coated than uncoated samples. Similarly, Petrou et al. (2012) reported that chicken breast meat samples coated with chitosan and oregano oil had higher L^* values than control samples during the storage period. However, no significant differences in lightness were found between the control samples and those coated with chitosan containing plant extracts.

The lightness values increased during the storage period for all treatments.

The a^* values of CWL and CCS treated patties were lower than those of the control and CH patties at day 0 and this trend was kept throughout the storage period. A significant decrease in redness (a^* values) was registered in all samples, indicating the change of color from red to brown. A similar variation has been reported in previous studies (Park et al., 2007; Devatkal et al., 2011) and it was attributed to the formation of metmyoglobin as a result of pigment oxidation (Faustman et al., 2010). Several authors have linked the loss of redness in meat samples during refrigerated storage to the ocurrence of oxidative reactions (Haak et al., 2009; Yu et al., 2010).

CWL and CCS patties had significantly lower (p<0.05) b^* values than control and CH coated patties. Mean Hue values were significantly (p<0.05) higher in C and CH samples and lowest in CWL treated samples. Hue values increased with storage intervals in all the treatments. Chroma value (color intensity) was significantly higher in CWL and CCS samples than control and CH treatments throughout the storage period. Chroma showed an increasing trend with storage interval in all the treatments.

The sensory attributes of uncoated and coated pork patties during 15 days of refrigerated storage were evaluated and shown in Table 7. The CH coated samples showed higher sensory scores than the control samples but the differences were significant (p<0.05) only regarding appearance and general acceptability on day 15th.

As expected, progressive quality deteriorations of samples were observed with extended storage period, with slower evolution for the coated samples. The sensory evaluati-

TABLE 6: *Color parameters of the pork patties during refrigerated storage for 15 days.*

Color parameters	Treatment[1]	Storage period (days)			
		0	5	10	15
L*	C	60.95 ± 0.38^{aA}	63.20 ± 1.17^{abB}	66.23 ± 1.56^{bC}	67.20 ± 0.93^{cC}
	CH	64.31 ± 0.91^{bA}	64.73 ± 1.57^{bcA}	65.93 ± 2.49^{bAB}	67.64 ± 1.51^{cB}
	CWL	59.93 ± 2.00^{aA}	62.63 ± 0.16^{aB}	62.57 ± 0.54^{aB}	62.59 ± 0.57^{aB}
	CCS	61.98 ± 1.69^{aA}	65.91 ± 0.94^{cB}	69.98 ± 0.56^{cC}	65.41 ± 0.52^{bB}
a*	C	4.34 ± 0.32^{abC}	3.49 ± 0.23^{aB}	3.22 ± 0.07^{aAB}	3.12 ± 0.03^{bA}
	CH	4.56 ± 0.27^{bD}	3.93 ± 0.08^{bC}	3.53 ± 0.17^{abB}	3.08 ± 0.13^{abA}
	CWL	3.85 ± 0.42^{aB}	3.74 ± 0.18^{abB}	3.71 ± 0.84^{abB}	2.76 ± 0.41^{aA}
	CCS	3.87 ± 0.25^{aB}	3.72 ± 0.27^{abB}	3.95 ± 0.12^{bB}	2.77 ± 0.42^{abA}
b*	C	14.91 ± 0.78^{b}	14.75 ± 0.61^{ab}	15.31 ± 0.56^{b}	15.55 ± 0.29^{b}
	CH	15.00 ± 0.85^{b}	15.58 ± 0.34^{c}	15.43 ± 0.76^{b}	15.15 ± 0.54^{b}
	CWL	13.80 ± 0.66^{aA}	14.39 ± 0.34^{aAB}	14.25 ± 0.39^{aAB}	14.85 ± 0.65^{abB}
	CCS	13.75 ± 0.55^{aA}	15.15 ± 0.50^{bcB}	16.76 ± 0.22^{cC}	13.80 ± 0.66^{aA}
C	C	15.53 ± 0.83^{b}	15.16 ± 0.65^{ab}	15.64 ± 0.56^{b}	15.86 ± 0.28^{b}
	CH	15.68 ± 0.89^{b}	16.07 ± 0.34^{c}	15.83 ± 0.78^{b}	15.46 ± 0.55^{b}
	CWL	14.33 ± 0.73^{a}	14.87 ± 0.29^{a}	14.74 ± 0.25^{a}	15.12 ± 0.75^{ab}
	CCS	14.29 ± 0.60^{aA}	15.60 ± 0.54^{bcB}	17.22 ± 0.24^{cC}	14.33 ± 0.73^{aA}
H	C	73.79 ± 0.50^{abA}	76.72 ± 0.37^{bB}	78.11 ± 0.32^{bC}	78.67 ± 0.27^{bC}
	CH	73.09 ± 0.09^{aA}	75.84 ± 0.35^{abB}	77.11 ± 0.34^{abC}	78.53 ± 0.21^{bD}
	CWL	74.45 ± 1.05^{bA}	75.43 ± 0.94^{aA}	75.41 ± 3.46^{aA}	79.54 ± 1.03^{aB}
	CCS	74.29 ± 0.42^{bA}	76.20 ± 0.66^{abB}	76.75 ± 0.23^{abB}	79.07 ± 1.05^{aC}

*: Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment (p<0.05), while different uppercase letters are indicative of significant differences due to storage period (p<0.05). [1]: C: uncoated; CH: coated with chitosan; CWL: coated with chitosan containing walnut leaf extract; CCS: coated with chitosan containing cherry stem extract.

on results are associated with the chemical properties. Due to a high lipid oxidation and microbial growth, the control samples showed deterioration, appearing as off-odor as well as discoloration after 15 days of storage. Addition of plant extracts to chitosan coating enhanced the beneficial effects on sensory attributes and overall acceptability of pork patties significantly ($p<0.05$) during the 15 days storage period. Thus, antioxidant and antimicrobial effects of chitosan coating incorporating plant extracts may minimize the oxidative reactions, and as a result extending the products' shelf life. These results are in good agreement with those reported in previous studies (Kanatt et al., 2008; Giatrakou et al., 2010). The active coating made of chitosan + cherry stems extract led to the highest scores among other treatments in appearance, color and overall acceptability throughout storage time. The samples coated with chitosan + walnut leaf extract had a lower score in color because of the dark brown shade but the highest score in flavor due to the interesting aroma of walnut leaves.

TABLE 7: *Sensory evaluation of pork patties during refrigerated storage for 15 days*[*].

Color parameters	Treatment[1]	Storage period (days) 0	5	10	15
Appearance	C	7.42 ± 0.90^{aB}	7.25 ± 0.75^{aB}	6.83 ± 0.58^{aAB}	6.33 ± 0.65^{aA}
	CH	7.67 ± 0.78^{abB}	7.42 ± 0.67^{aB}	7.17 ± 0.58^{aAB}	6.83 ± 0.39^{bA}
	CWL	8.17 ± 0.72^{bcC}	8.00 ± 0.74^{bBC}	7.67 ± 0.49^{bB}	7.17 ± 0.39^{bA}
	CCS	8.58 ± 0.51^{cB}	8.42 ± 0.51^{bB}	8.25 ± 0.45^{cB}	7.75 ± 0.45^{cA}
Color	C	7.17 ± 0.83^{aB}	6.83 ± 0.72^{aAB}	6.50 ± 0.52^{aA}	6.33 ± 0.49^{aA}
	CH	7.08 ± 0.79^{aA}	6.92 ± 0.67^{aA}	6.67 ± 0.49^{aA}	6.58 ± 0.51^{aA}
	CWL	7.33 ± 0.98^{aB}	7.08 ± 0.79^{aAB}	6.92 ± 0.67^{abAB}	6.67 ± 0.65^{abA}
	CCS	7.42 ± 0.51^{aA}	7.25 ± 0.45^{aA}	7.17 ± 0.58^{bA}	7.08 ± 0.51^{bA}
Flavor	C	7.58 ± 0.79^{aB}	7.33 ± 0.78^{aAB}	7.08 ± 0.67^{aAB}	6.92 ± 0.51^{aA}
	CH	7.83 ± 0.72^{abA}	7.67 ± 0.65^{abA}	7.58 ± 0.51^{abA}	7.42 ± 0.51^{abA}
	CWL	8.75 ± 0.45^{cB}	8.58 ± 0.51^{cAB}	8.42 ± 0.51^{cAB}	8.25 ± 0.62^{cA}
	CCS	8.33 ± 0.78^{bcA}	8.17 ± 0.83^{bcA}	8.00 ± 0.85^{bcA}	7.83 ± 0.83^{bcA}
General acceptability	C	7.50 ± 0.80^{aB}	7.33 ± 0.78^{aB}	6.92 ± 0.67^{aAB}	6.33 ± 0.65^{aA}
	CH	7.75 ± 0.75^{abB}	7.50 ± 0.67^{aB}	7.25 ± 0.62^{aAB}	6.83 ± 0.39^{bA}
	CWL	8.25 ± 0.75^{bcB}	8.08 ± 0.79^{bB}	7.75 ± 0.62^{bB}	7.17 ± 0.39^{bA}
	CCS	8.67 ± 0.49^{cB}	8.50 ± 0.52^{bB}	8.33 ± 0.49^{cB}	7.83 ± 0.39^{cA}

[*]: Different lowercase letters indicate significant differences due to treatment ($p<0.05$), while different uppercase letters are indicative of significant differences due to storage period ($p<0.05$). [1]: C: uncoated; CH: coated with chitosan; CWL: coated with chitosan containing walnut leaf extract; CCS: coated with chitosan containing cherry stem extract.

Conclusions

Results of this study showed that chitosan-based coatings were effective in controlling lipid oxidation in pork patties. The coatings retarded water loss and maintained acceptable values of pH throughout the period studied. The incorporation of walnut leaf and cherry stem extracts in chitosan coatings improved the antioxidant protection, offering an advantage in the prevention of lipid oxidation in meat products. The meat samples with chitosan-based coatings incorporating antioxidant extracts showed lower TBARS values, which remained below the limits of acceptability for 10 days of refrigerated storage. However, further improvements are necessary to develop a more successful application of edible coatings enriched with plant extracts.

Authors' contributions

V.N. planned and supervised the work, A.I.B. collected plant material, conducted the experiments, performed the measurements and the analysis. A.I.B. and V.N. processed the experimental data, V.N. drafted the manuscript. Both authors discussed the results and commented on the manuscript.

Conflict of interest

No potential conflict of interest was reported by the authors.

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Address of corresponding author:
Violeta Nour
University of Craiova
Faculty of Horticulture
Department of Horticulture & Food Science
A.I.Cuza Street 13
200585, Craiova
Romania
vionor@yahoo.com