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Original article

Active chitosan–chestnut extract films used for packaging and storage of fresh pasta

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Summary A tannins-rich chestnut extract was used to enhance the antioxidant and antibacterial properties of chitosan-based film materials. The favourable mechanical properties of the novel material enabled its application in the preparation of the sachets that were used for packing and storage of filled fresh pasta. The fresh pasta ageing progressed in conventional refrigerated storage conditions of 8 °C with 60 ± 2% relative humidity in the absence of light for 60 days. The rapid moisture mobility between a starchy food and sachets during the first 9 days of storage induced retrogradation of the fresh pasta, whereby total phenolic content show dependency on moisture throughout the shelf life. Active components within the sachet prevented microbial growth on the food surface during the entire 60 days.

Keywords Active sachet, antibacterial and antioxidant activity, chestnut extract, chitosan-based film, fresh pasta, shelf life.

Introduction

Active biodegradable materials have recently emerged as an alternative capable of replacing conventional fossil fuel-based plastic packaging in the food industry (Moustafa *et al*., 2019; Rodríguez-Rojas *et al*., 2019; Sabbah *et al*., 2019). In addition, antimicrobial properties of such materials can be enhanced by the incorporation of various antimicrobial agents. Providing slow but constant migration of the active agents from the packaging material is an advantage in favour of the control of microbial growth over a longer period of time (Quintavalla & Vicini, 2002; Sung *et al*., 2013; Jideani & Vogt, 2016).

With global production of 14.3 million tons, pasta is one of the most consumed food products worldwide (International Pasta Organisation, 2017). Fresh pasta is classified as intermediate moisture food with moisture content (MC) of 35% and a reported shelf life 37–60 days (Del Nobile *et al*., 2009; de Camargo Andrade-Molina *et al*., 2013). Several studies rely on modified atmosphere to enhance shelf life of perishable foods packed in conventional (fossil fuel-based) plastic packaging (Sanguinetti *et al*., 2011; Kõrge & Laos, 2019), but notably, scarce literature covers packaging of fresh pasta into biopolymer-based sachets (de Camargo Andrade-Molina *et al*., 2013). However, replacing this pliable and easily shaped material is quite challenging because novel biodegradable materials have high water affinity and sometimes do not have sufficient mechanical properties or water vapour barrier capabilities. Therefore, mastering the structural modifications of the current biodegradable materials is an essential step for their practical use in everyday life.

Chitosan is a biocompatible, biodegradable, nontoxic and partly deacetylated, derivative of chitin, whereby its characteristics highly depend on its degree of deacetylation and molecular weight (Borić *et al*., 2018). Chitosan is also endowed with a good film-forming capacity (Chillo *et al*., 2009) that makes it one of the most favourable biopolymers for the preparation of environmentally friendly films intended for food packaging and protection (Divya *et al*., 2018; Wang *et al*., 2018). The inherent antioxidant and antimicrobial properties of chitosan-based films can be significantly improved with the incorporation of various plant-based active components and, therefore, help in the extension of the shelf life of perishable foods (Priyadarshi *et al*., 2018b; Wang *et al*., 2018). Among others, it has been shown that essential oils of citrus (Randazzo *et al*., 2016), caraway (Hromiš *et al*., 2015) and *Litsea cubeba* (Zheng *et al*., 2018) as well as different extract like turmeric (Kalaycıoğlu *et al*., 2017), spirulina (Balti *et al*., 2017), hop and oak (Bajić *et al*., 2019b) can be used for this purpose. In this context, industrial tannin extracts obtained from the chestnut

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wood (*Castanea sativa*) have prominent antioxidant activity (Squillaci *et al*., 2018; Molino *et al*., 2020) and as such could be very interesting candidates as active components in chitosan-based films.

The aim of this study was to explore the possibility to use chestnut extract (CE) as an active component in chitosan-based film materials, which can be further implemented for the preparation of bio-based packaging. Specifically, the sachets prepared from chitosan-based films with incorporated CE were used for packing of fresh pasta and a comparative analysis was conducted to assess the sachet's influence on microbial growth, moisture mobility (in relation to total phenolic content) and microstructural alterations of packed material.

Materials and methods

Materials

High molecular weight chitosan (CH) (acetylation degree ≥75%, 310–375 kDa), lactic acid (LA) (purity ≥85%), gallic acid (GA) and Folin–Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (Steinhem, Germany), while methanol and acetone were from Avantor Performance Materials (Gliwice, Poland) and Honeywell (Hannover, Germany), respectively. Sodium carbonate was obtained from Merck (Darmstadt, Germany) and glycerol (GLY) from Pharmachem Sušnik (Ljubljana, Slovenia). Commercially available CE (≥75% tannins; <4% of ash) was provided by the company Tanin Sevnica (Sevnica, Slovenia). All chemicals except LA were of analytical grade. Milli-Q® water was used throughout all experiments.

Preparation and characterisation of active film packaging

Preparation of film-forming solutions

Film-forming solutions (FFSs) were prepared according to a slightly modified protocol described in Bajić *et al*. (2019a). In short, CH (1.5% w/v) and GLY (plasticiser; 30% w/w based on the mass of CH) were dissolved in an aqueous solution of LA (1.0% v/v). The mixture was agitated overnight on a magnetic stirrer IKA® RCT (IKA, Staufen, Germany) at 1000 r.p.m. and room temperature (24 °C) until homogenisation and then vacuum-filtered through two sheets of medical gauze. Afterwards, the mixtures were supplemented with 0.0% (reference sample), 0.5%, 1.0% and 1.5% w/v of CE in order to study its effect on the active properties of film materials, homogenised (at 6000 r.p.m. during 2 min) with Ultra-Turrax® T50 (IKA, Staufen, Germany) and left overnight. Finally, a formed sticky foam and remaining bubbles were carefully removed from the mixtures using a laboratory spatula, and the mixtures were used for the film preparation.

Preparation of chitosan-based film material

Prepared FFS was cast on rectangular polyurethane 12 cm × 12 cm Petri dishes (~0.32 mL of FFS per cm^2 of a Petri dish) and left to dry in a drying oven Kambič (Semič, Slovenia) with a continues ventilation (24 °C, RH 40% for 48 h) (Bajić *et al*., 2019a). Obtained films (mean diameter 120 ± 2 μm; measured by ABS Digital Thickness Gauge, Mitutoyo, Aurora, IL, USA) were peeled off from the Petri dishes, separated with baking paper to prevent direct contact from each other and stored in an airtight container (24 °C, no exposure to light) prior the analysis.

Total phenolic content of chitosan-based film material

Total phenolic content (TPC) in chitosan-based film material was determined using Folin–Ciocalteu's (FC) phenol reagent, according to the protocol outlined in our previous study (Bajić *et al*., 2019a). Briefly, small rectangular film samples were added in water (~5 mg of film per mL of water), followed by the successive addition of FC phenol reagent and aqueous solution of Na_2CO_3 (10% w/v) in the amount of 10 vol% and 20 vol% based on the volume of water, respectively. After the incubation of samples (2 h in dark, 24 °C), the absorbance was measured at 765 nm using Synergy™ 2 Multi-Detection Microplate Reader (BioTek, Winooski, VT, USA). Gallic acid was used as the standard, and the results were expressed as the mass of gallic acid equivalent (GAE) per mass of the films.

Radical scavenging activity of chitosan-based film material

Determination of the antioxidant activity of chitosan-based films was done using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The rectangular film samples were added in a freshly prepared 60 μM DPPH methanolic solution (~5 mg of film per mL of the solution) and incubated for 2 h in a dark place and room temperature. The absorbance for each methanolic extract was measured at 517 nm on Lambda 40 UV/Vis spectrophotometer (Perkin Elmer, Waltham, MA, USA). The inhibition activity (%I) of each sample was calculated as $\%I = ((A_{control} - A_{sample})/A_{control}) \times 100$ (Kaya *et al*., 2018), where $A_{control}$ and A_{sample} denote the absorbance of DPPH solution and the absorbance of solutions containing the films samples, respectively.

Antibacterial properties of chitosan-based film material

Antibacterial properties of chitosan-based films were tested against *Escherichia coli* K12 (*E. coli*) and *Bacillus subtilis* DSM 402 (*B. subtilis*). A fresh bacterial culture suspension in the exponential growth phase (OD_{600} = 0.5) was spread over the Luria-Bertani agar

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culture medium, and the UV light-sterilised (at 254 nm for 15 min; both sides) rectangular film samples (~1 cm^2) were placed on the plate surfaces and incubated (37 °C, 24 h) (Bajić *et al*., 2019a). Clear areas that appeared around the films were considered as the inhibition zones (I_z).

Optical properties of chitosan-based film material
Rectangular film samples were cut to fit the size of a cuvette and carefully placed in it using tweezers. The samples were always kept to the same wall of the cuvette to provide equal distance from the light source in each measurement. The absorbance (A) was recorded at room temperature using Lambda 40 UV/Vis spectrophotometer under the wavelengths region ranging from 250 to 800 nm, whereby an empty cuvette (i.e. air) was used as the referent. The opacity (O) was calculated as $O = A_{600}$/film thickness (mm) (Sun *et al*., 2017), while the per cent of transmittance ($\%T$) was calculated as $\%T = 10^{-A} \times 100$ (Bajić *et al*., 2019a).

Preparation of sachets

Each sachet was prepared from two sheets of chitosan-based film material (prepared from the FFS containing 1% w/v of CE according to protocols explained in section Preparation and characterisation of active film packaging) by heat sealing (165 °C, 700 Pa, 7 s) on HST-H6 heat seal tester PARAM® (LabThink, Jinan, China). The first set (sachet in contact with food) was used to pack fresh pasta, and the other was heat-sealed without the pasta (reference sachet).

Preparation and packing of fresh pasta

Preparation of fresh pasta
Fresh pasta (from here onwards pasta) was provided by Mlinotest (Ajdovščina, Slovenia). In short, the production of pasta involves filling/dough preparation, shaping, pasteurisation (98 °C), drying/cooling and packaging. The pasta ingredients were as follows: wheat meals, eggs with the filling (cottage cheese and spinach) with nutritional values calculated on the label: carbohydrates 45% (sugars 1.4%), protein 11%, fat 5.1% (saturated fat 2.5%), dietary fibres 2.1% and salt 0.7%. Material that was used to pack pasta is a combination of polyethylene (PE), biaxially oriented polypropylene (BOPP) and ethylene vinyl alcohol (EVOH) (thickness 70 μm).

Packing of fresh pasta
The factory-packed pasta in modified atmosphere (MAP) conditioned PE-BOPP-EVOH plastic packaging (reference pasta) was re-packed into chitosan-chestnut extract (CH-CE) sachets (eight pieces) and heat sealed from the open end. Received sachets were inserted into paper envelopes to prevent their contact with each other. The entire process was conducted under laminar flow hood conditions (constant ventilation, 24 °C, 40% RH, ultraviolet light) to prevent microbial contamination. Samples were stored in a refrigerator at 8 °C and RH of 60 ± 2% during 60 days.

Characterisation of the CH-CE sachet-pasta system
All the samples (reference sachets, reference pasta, sachets with pasta – eventually analysed as a separate sample, i.e. pasta and CH-CE sachet) were tested for water activity, moisture, TPC, microbiology (pasta only), Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) at days 0, 3, 6, 9, 12, 30 and 60. Final CH-CE sachet-pasta systems were analysed in three biological replicas.

Water activity
LabMaster-aw neo (Novasina, Lachen, Germany) was applied to measure reference samples, pasta's and CH-CE sachets' water activity (a_w). After the packaging was removed from storage, it was opened under ventilating laminar flow hood. A pasta piece was cut and immediately placed into a measuring cup to prevent moisture loss. For the film analysis, a sample was cut from the sachets' middle section.

Moisture content
Moisture analyzer HE 53 (Mettler Toledo, Wien, Austria) was used to measure the moisture content of reference samples, pasta and CH-CE sachets. Pasta samples were analysed by receiving a pasta piece from cold storage, cut into smaller particles and immediately placed onto a measuring plate for analysis. Sachet samples were handled in a similar manner, using scissors for cutting.

Total phenolic content

Total phenolic content of sachets
A TPC of active sachets (~5 mg) was determined by Folin–Ciocalteu's (FC) phenol reagent according to protocol as described in section Total phenolic content of chitosan-based film material.

Total phenolic content of fresh pasta
To measure TPC in pasta samples, extraction of phenols was done prior to analysis according to the protocol outlined in a study of Vignola *et al*. (2018). The samples (200 mg) were pre-powdered in liquid nitrogen, weighted on an analytical balance (Kern, Balingen, Germany) and covered with 1 mL extraction solution of methanol:acetone:water (30:30:40 mL). The prepared mixture was agitated on Tehtnica Vibromix

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10 (Domel, Železniki, Slovenia) vortex during 5 min and centrifuged (3 293 ***g***; 10 min) using MiniSpin centrifuge (Eppendorf, Hamburg, Germany). Green-coloured supernatant was pipetted into separate e-tube, and the extraction was repeated. After the double extraction process, supernatants were mixed, and 20 μL of the sample (supernatant) added to 120 μL water, followed by successive addition of FC phenol reagent and Na_2CO_3 (10% w/v) aqueous solution 10% w/v and 20% w/v based on the volume of the sample, respectively. Received samples were diluted with water up to 1 mL. After the incubation of samples (2 h in dark, 24 °C), the absorbance was measured at 765 nm using Synergy™ 2 Multi-Detection Microplate Reader (BioTek, Winooski, VT, USA). Gallic acid was used as the standard, and the results were expressed as the mass of gallic acid equivalent (GAE) per mass of the pasta.

Microbiological analysis of fresh pasta

For the microbiological analysis, 4 ± 0.5 g of pasta sample was aseptically transferred into a sterile plastic homogeniser bag and blended with 36 ± 4.5 mL of 0.1% (w/v) sterile buffer Peptone water (BPW) using Masticator Basic blender (IUL Instruments, Barcelona, Spain) for 30 s, 1500 r.p.m. and at 24 °C. Thereon, dilutions were prepared with 0.1% BPW and 100 μL of each dilution was inoculated into plate count agar growth medium. Dichloran Rose Bengale Chloramphenicol Agar (Biokar diagnostics, Allonne, France) was used for a total count of yeast and moulds, Tryptic Glucose Yeast agar was used for a total bacterial count determination and Violet Red Bile Lactose Agar (Merck KGaA, Darmstadt, Germany) was used for total count of bacterial group *Enterobacteriaceae*.

Scanning electron microscopy

Scanning electron microscopy SUPRA 35VP (Carl Zeiss, Jena, Germany) was used to visualise morphological changes in sachets and pasta dough cross section during shelf life. All the samples were mounted on metal stubs with carbon tape and observed using a voltage of 1.00 kV and magnifications 1.00 K, 500 for the sachet film and pasta, respectively.

Fourier-transform infrared spectroscopy

The Fourier-transform infrared spectra of prepared films and pasta were recorded at the wavenumbers ranging from 4000 to 700 cm^{-1} and resolution of 4 cm^{-1}, using Spectrum Two FT-IR spectrometer (PerkinElmer). The sample (cheese and film) spectres were normalised before carrying out the statistical analysis by averaging each value of the spectrum values. Each sample was scanned in triplicate.

Statistical analysis

The data were subjected to the one-way analysis of variance (ANOVA) with a confidence level of 95% ($P \leq 0.05$), followed by Tukey's test. All the results in triplicate are expressed as the mean ± standard deviation.

Results and discussion

Preparation and characterisation of active film packaging

The FFSs containing 0.0% (control sample), 0.1%, 0.5% and 1.0% (blended samples) were successfully used for the preparation of chitosan-based film material. The prepared films were preliminary characterised regarding the effect of the incorporated extract on their active (antioxidant-, antibacterial- and optical-related) properties (Table 1).

The films exhibited favourable antioxidant properties, whereby the TPC values (up to 18.7 $mg_{GAE}\ g_{film}^{-1}$) were in a positive correlation with films' radical scavenging activities (up to 81.8%). Besides, the incorporation of CE endowed the films by antibacterial activity against *E. coli* (I_z was up to 17.8 mm) and *B. subtilis* (I_z was up to 15.5 mm) (Fig. 1).

Finally, evaluation of the films' optical properties has revealed that samples prepared from the FFSs containing CE had opacity values of up to 5.71 mm^{-1} (Table 1), while the per cent of transmittance in the UV and visible wavelength range (from 250 to 800 nm) can be seen in Fig. 2.

The evaluated properties are important in providing sufficient protective capabilities of chitosan-based film materials. Besides, the trends of antioxidant-, antibacterial- and optical-related properties observed after increasing the concentration of CE were in line with trends reported for other chitosan-based films blended with different types of plant-originating extracts (Sun *et al*., 2017; Kaya *et al*., 2018; Priyadarshi *et al*., 2018a; Bajić *et al*., 2019b; Rambabu *et al*., 2019). Therefore, the incorporation of CE notably improved properties of chitosan-based films, making them appropriate for further use as active packaging materials, as discussed in the following chapters.

Characterisation of the CH-CE sachet-pasta system during shelf life

Moisture mobility

Mobility of the moisture was evaluated under conventional refrigerated storage conditions at 8 °C with 60 ± 2% relative humidity in the absence of light for

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Table 1 The effect of the chestnut extract concentration on the properties of chitosan-based films

	Antioxidant-related properties		Antibacterial-related properties		Optical-related properties	
Film sample*	**TPC† [mg_{GAE} g_{film}^{-1}]**	**$I^{\dagger}$ [%]**	**I_Z (*E. coli*)† [mm]**	**I_Z (*B. subtilis*)† [mm]**	**A_{350}† [%]**	**$O^{\dagger}$ [mm^{-1}]**
CE_0.0	0.4 ± 0.1^{a}	9.0 ± 6.0^{a}	0.01 ± 0.1^{a}	0.01 ± 0.1^{a}	59.9 ± 5.0^{a}	0.85 ± 0.2^{a}
CE_0.1	3.4 ± 0.2^{b}	35.3 ± 11.0^{b}	2.50 ± 0.5^{a}	1.90 ± 1.0^{a}	5.50 ± 1.0^{b}	1.53 ± 0.3^{a}
CE_0.5	11.2 ± 2.0^{c}	76.2 ± 5.0^{c}	2.50 ± 2.0^{a}	7.50 ± 1.0^{a}	0.01 ± 0.1^{b}	3.73 ± 0.4^{b}
CE_1.0	18.7 ± 3.0^{d}	81.8 ± 3.0^{c}	17.8 ± 2.0^{b}	15.5 ± 5.0^{b}	0.01 ± 0.1^{b}	5.71 ± 0.4^{c}

*Labels CE_0.0, CE_0.1, CE_0.5 and CE_1.0 indicate chitosan-based films prepared from the FFSs containing 0.0% (control sample), 0.1%, 0.5% and 1.0% (w/v) of CE, respectively.

†Different subscript letters in each separate column indicate the samples with significantly different mean values ($P < 0.05$).

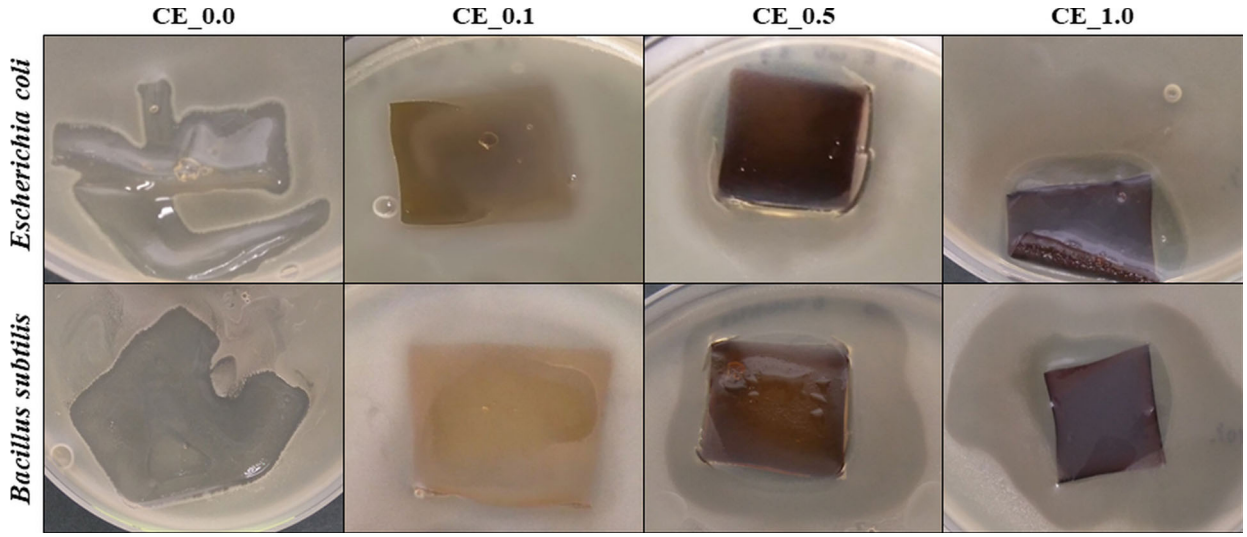

Figure 1 The appearance of inhibition zones visible around chitosan-based films after the incubation in a medium containing *E. coli* (upper row) and *B. subtilis* (lower row). Labels CE_0.0, CE_0.1, CE_0.5 and CE_1.0 indicate chitosan-based films prepared from the FFSs containing 0.0% (control sample), 0.1%, 0.5% and 1.0% (w/v) of CE, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

60 days. Figure 3 depicts results of moisture desorption from pasta to CH-CE sachet and adsorption of the sachet *vice versa*. Reference pasta and reference sachet results are visualised for comparison.

According to the moisture mobility in CH-CE sachet-pasta system, pasta bestowed 9% of its moisture when sachet reached to its moisture equilibrium. From this point on, CH-CE sachet-pasta system depleted its capability to maintain the shelf life of pasta accordance to commercial expectations. A rapid MC desorption of pasta was observed during first 3 days (8%), slowing down over next three (1%) and then a strong decrease (19%) over the remaining time. Tested pasta MC results represented the described behaviour of Bressani (2014) where pasta, during its shelf life goes through three stages: (i) formation of natural crust, (ii) drying and (iii) stabilisation. A retrogradation process took place. A similar outcome has been reported in other studies with a starchy food, in particular with bread staling. Accordingly, moisture starts to move from a crumb to crust and does not move back from crust to crumb because of inability to form new hydrogen bonds in tightened structure (Kerch *et al.*, 2010).

At the same time, Fig. 3 depicts that CH-CE sachet acted as expected regards to pasta changes. During the first 3 days, 6% MC increase in CH-CE sachet was observed. In comparison, bread has been shown to lose 3% of moisture to carboxymethyl cellulose-chitosan sachet after 3 days, but unfortunately, they do not show moisture intake of the sachet (Noshirvani *et al.*, 2017). This is attributed to two theories: (ii) plastification properties of moisture, that is water from the food that relaxes the matrix of the sachet making absorption easier, (ii) due to CE that elevates solubility with its hydrophilic groups through interactions with water, also plasticiser (GLY) (Alves *et al.*, 2018; Sun *et al.*, 2018). The slowing down of moisture mobility during 3–6 days apprised that the turnover point of the sachet moisture intake was approaching. Pereda *et al.* (2010)

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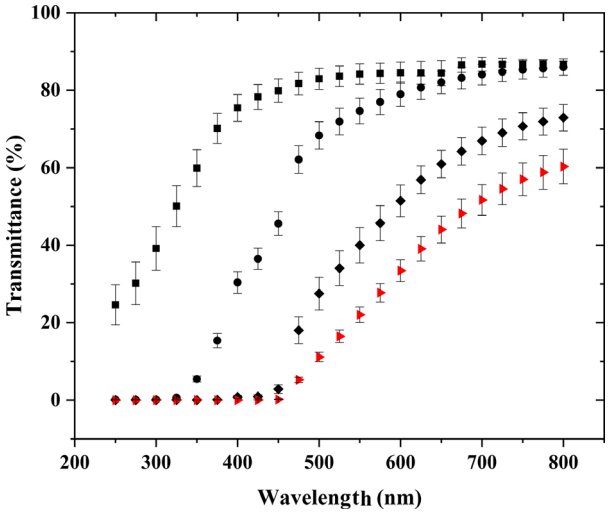

Figure 2 Optical properties of chitosan-based films of CE 0% (■), 0.1% (●), 0.5% (♦) and 1% (►) in the UV-Vis wavelength range. Labels CE_0.0, CE_0.1, CE_0.5 and CE_1.0 indicate chitosan-based films prepared from the FFSs containing 0.0% (control sample), 0.1%, 0.5% and 1.0% (w/v) of CE, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

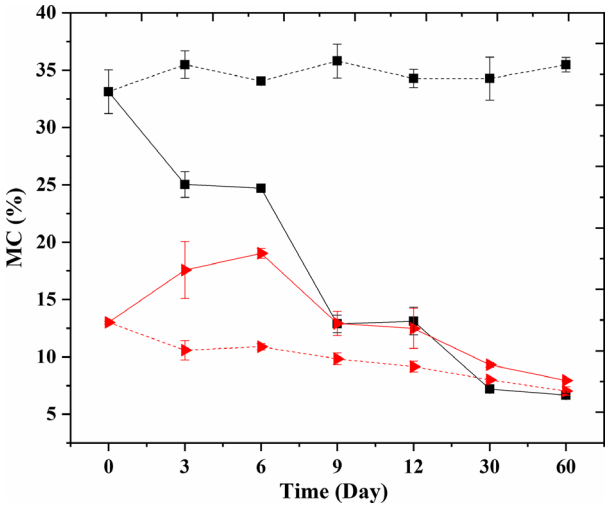

Figure 3 Moisture mobility of the pasta (■) and CH-CE sachet (►) during storage where dashed lines represent reference sample values. [Colour figure can be viewed at wileyonlinelibrary.com]

showed moisture equilibrium of the chitosan film at 20% with 1 day (25 °C, 75% RH). In our study, CH-CE sachet matrix reached its highest MC capacity of 19% (4 °C, 60% RH) on the sixth day when started to reveal properties that allowed easy water molecule passage. Subsequent to the turnover point, pasta MC drop gets steeper and reaches from 25% to 10% by the ninth day compared with CH-CE sachet that lost its MC with slower pace from 19% to 12%.

Total phenolic content relation to water activity

Total phenolic content of bio-based films has been established as a reliable indicator of antioxidant capacity. By observation of moisture mobility and its effect as an intrinsic factor on phenolic content change, its dependence on MC was noted in both matrices. Moisture loss highlighted the concentration of already existing phenolic content in pasta and at the same time dilution in CH-CE sachet. Figure 4 depicts a TPC relation to bound water while the retrogradation process was in progress.

Phenolic content of pasta was measured 0.14 $mg_{GAE}\ g_{pasta}^{-1}$ with a_w 0.950 and 0.18 $mg_{GAE}\ g_{pasta}^{-1}$ with a_w 0.606 and MC 5.5% initially and after storage, respectively. Our results were in the range of 0.09–0.67 $mg_{GAE}\ g_{pasta}^{-1}$ previously stated by Gull *et al.* (2018) who worked with pasta that had 9% of moisture. Moreover, phenolic content in reference pasta (data not shown) remained stable (0.14 $mg_{GAE}\ g_{pasta}^{-1}$, average a_w 0.961) during the entire shelf life indicating, when a_w is stable TPC is unchanged. In CH-CE sachets, the TPC was measured 21.8 $mg_{GAE}\ g_{film}^{-1}$ at the beginning of shelf life. A peculiar operation to marine biopolymer sachet, regards to a_w, describes decrease and after a while an increase in TPC near to initial value. This phenomenon indicates that there is likely no migration of phenolic content to the pasta surface.

Microbiology

The CH-CE sachet met the antimicrobial requirements and pasta, conditioned with 8 °C, 60% RH, did not develop microbial growth during 60 days of storage. Total count of yeast, moulds, bacteria and *Enterobacteriacea* were all under detection limits (data not shown).

A short impact time (before retrogradation) for microbial activity was out ruled by a_w measurement in pasta. Figure 3 depicts that CH-CE sachet reaches a_w 0.730, making it a good surface for microbiology and if with heightened vapour permeability to an unprotective barrier against the hazard. But in opposite, even if a_w decrease in pasta was statistically significant accordance to a_w sorption isotherm graph (Roos *et al.*, 2018), it does not leave the range of moderate mould activity. In light of these results, it can be concluded that (under given conditions) antioxidant CH-CE sachet prevented microbial hazard and with high a_w.

Scanning electron microscopy

Scanning electron microscopy images of pasta and CH-CE sachet cross sections are shown in Fig. 5 to assess their visible change during storage. The protein–starch matrix in the pasta samples is well formed throughout the shelf life with strong and continuous

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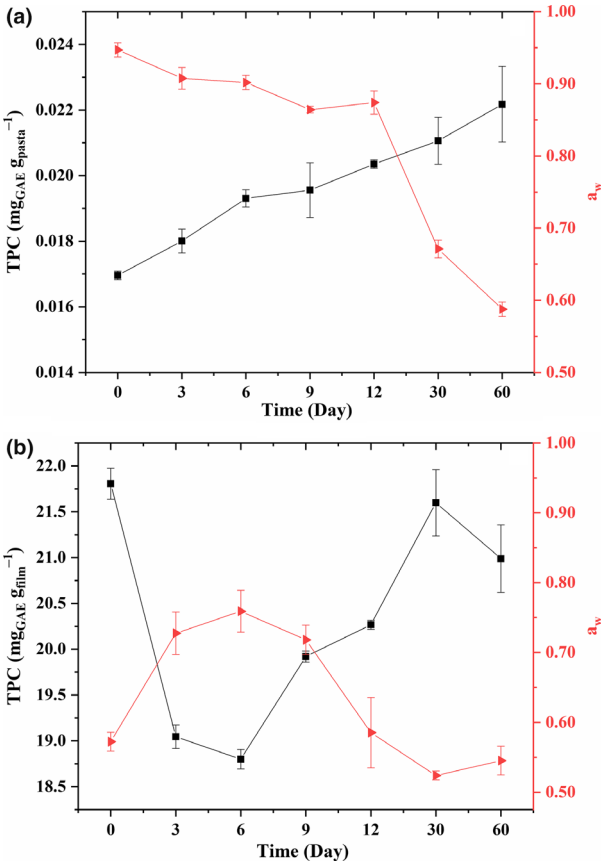

Figure 4 Total phenolic content and water activity changes in the a) pasta and b) CH-CE sachet in contact with one another during storage where the ■- marks represent TPC values and ►- water activity (a_w). [Colour figure can be viewed at wileyonlinelibrary.com]

protein matrix entrapping uniform starch granules (Fig. 5b). Focus on single starch granule showed that in the beginning of shelf life lenticular starch granules were slightly covered with amorphous network-type coating giving the indication of gelatinised starch via pasteurisation during manufacture and presence of moisture (Tudorică *et al.*, 2002; Sicignano *et al.*, 2015). Although the observation of amorphous coating was not seen in the end of the shelf life, starch granules within the CH-CE sachet packed samples showed no signs of swelling and deformation (which could lead to additional gelatinisation), indicating that they have been affected by moisture loss. Moreover, the protein–fibre matrix within reference pasta after 60 days appears to be have less tightened than in the samples from CH-CE packaging, resulting distinguishable A and B-type granules (Sicignano *et al.*, 2015). SEM microphotographs of different size granules and open spaces in between the matrix refer to more fresh-like texture of pasta.

The CH-CE sachet show a homogenous and evenly distributed structure on the third day when its moisture capacity was highest compared with the initial texture at day 0 during shelf life (Fig. 5a). When moisture is evaporating from CH-CE sachet, it starts to show uneven cross section, indicating weakened intermolecular interactions, as reported by Sogut & Seydim (2018). The CH-CE sachets did not develop any pores or visible thinning.

Fourier-transform infrared spectroscopy

It is known that the active packaging is capable of releasing its antioxidants or other macrocomponents onto food surface and *vice versa* (Noshirvani *et al.*, 2017; Sogut & Seydim, 2018; Sun *et al.*, 2018). Figure 6 describes FT-IR spectres used to determine specific absorption bands of pasta and CH-CE sachet. In overall, peaks (Fig. 6a) on pasta spectra in the region 800–1150 cm^{-1} refer to residual starch–protein interactions. The IR region from 1175 to 1450 cm^{-1} provides information about sugars and organic acids. Presented are amide I band at 1650 cm^{-1} that results from C=O stretching and amidic band II from vibrations of N-H group with IR 1550 cm^{-1}. C–H band vibrations at 1700 cm^{-1} till 3000 cm^{-1} refer to ester carbonyl functional groups of the triglycerides. Peak-wise, our results incline with the results of Priyadarshi *et al.* (2018a) and Durazzo *et al.* (2018) groups. The analysed CH-CE sachets produced same characteristic absorption bands to pasta.

All the spectres with CH-CE sachets showed O–H stretching vibrations in accordance with moisture mobility. Pasta, being more heterogeneous, received stronger O–H band intensity during the sixth day, but otherwise showed a similar trend to MC results. No peak shifts or new peaks were detected on FT-IR spectres generated for the reference pasta and sachet during shelf life. Changes were detected on transmittance intensities. The IR regions of 800–1150 cm^{-1} where residual starch is detected do not influence the same region on CH-CE sachet spectra. We could not differentiate CH-CE sachet components influence on pasta surface.

Conclusions

In this study, chitosan-based active film materials incorporated with CE were used to prepare sachets that were further used to pack fresh pasta. The antimicrobial activity of the film and its interactions within an active CH-CE sachet-pasta system during shelf life were evaluated during the shelf life. The results have shown that a retrogradation process of pasta has occurred, giving a hard-like texture after 9 days of preservation in CH-CE sachet. Changes in

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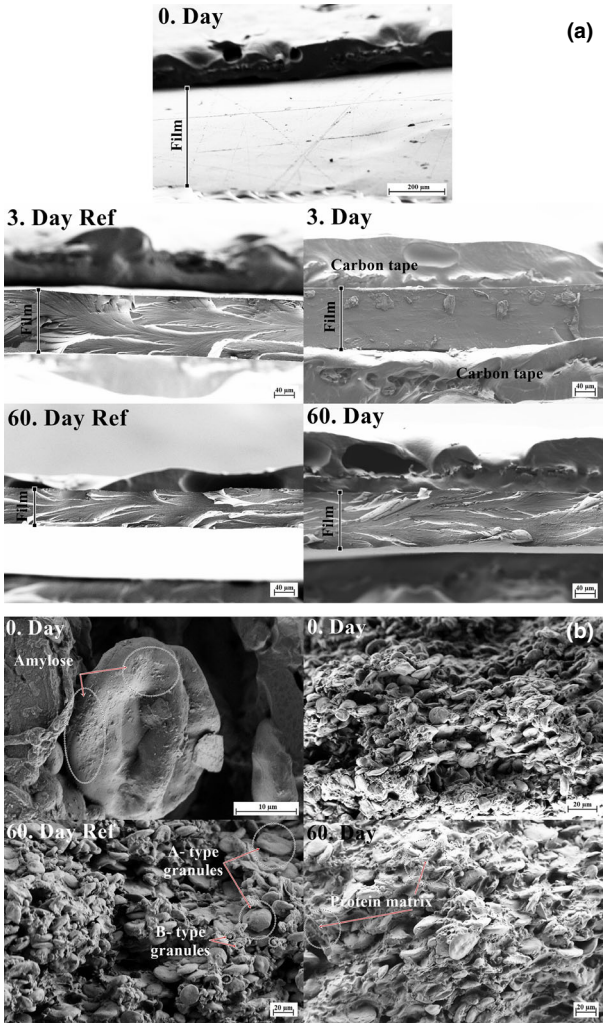

Figure 5 SEM images of (a) CH-CE sachet and (b) pasta on days 0, 3 and 60. From left to right, pasta pictures visualise amylose amorphous phase covering single starch granule and cross-sectional structure on days 0 and 60. Reference sample pictures are sided for comparison. [Colour figure can be viewed at wileyonlinelibrary.com]

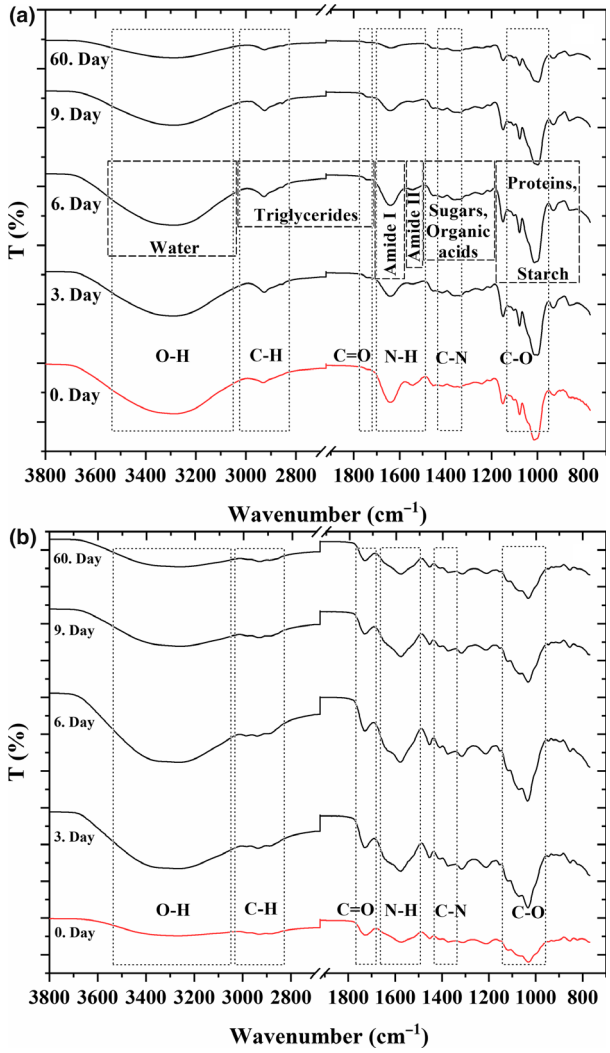

Figure 6 FT-IR results of the (a) pasta and (b) CH-CE sachet in contact during shelf life. Dashed boxes represent an approximate area of macrocomponents. [Colour figure can be viewed at wileyonlinelibrary.com]

a_w show total phenolic content concentration or dilution, respectively, to pasta and CH-CE sachet in contact. Regardless, pasta remained free of microbial spoilage in CH-CE sachet during the entire 60-day shelf life. According to FT-IR and SEM results, active packaging ingredients did not affect food surface and its microstructure. In spite of the antimicrobial properties, fresh pasta shelf life was not realised during the 60 days because of the unacceptable texture. Therefore, further research is required to improve the chitosan-based film permeability properties and/or to investigate alternative food products with a higher natural moisture barrier. Moreover, the possible colouring effect of the films (extracts) onto fresh pasta, which will require additional sensory analysis that relates directly to customers acceptance, was not part of our current studies. The sensory analysis studies will be investigated in the future.

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Conflict of interest

The authors declare no competing interests.

Ethics approval

Ethics approval was not required for this research.

Data availability statement

Research data are not shared.

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