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The functional potential of immortelle (*Helichrysum italicum*) based edible films reinforced with proteins and hydrogel particles

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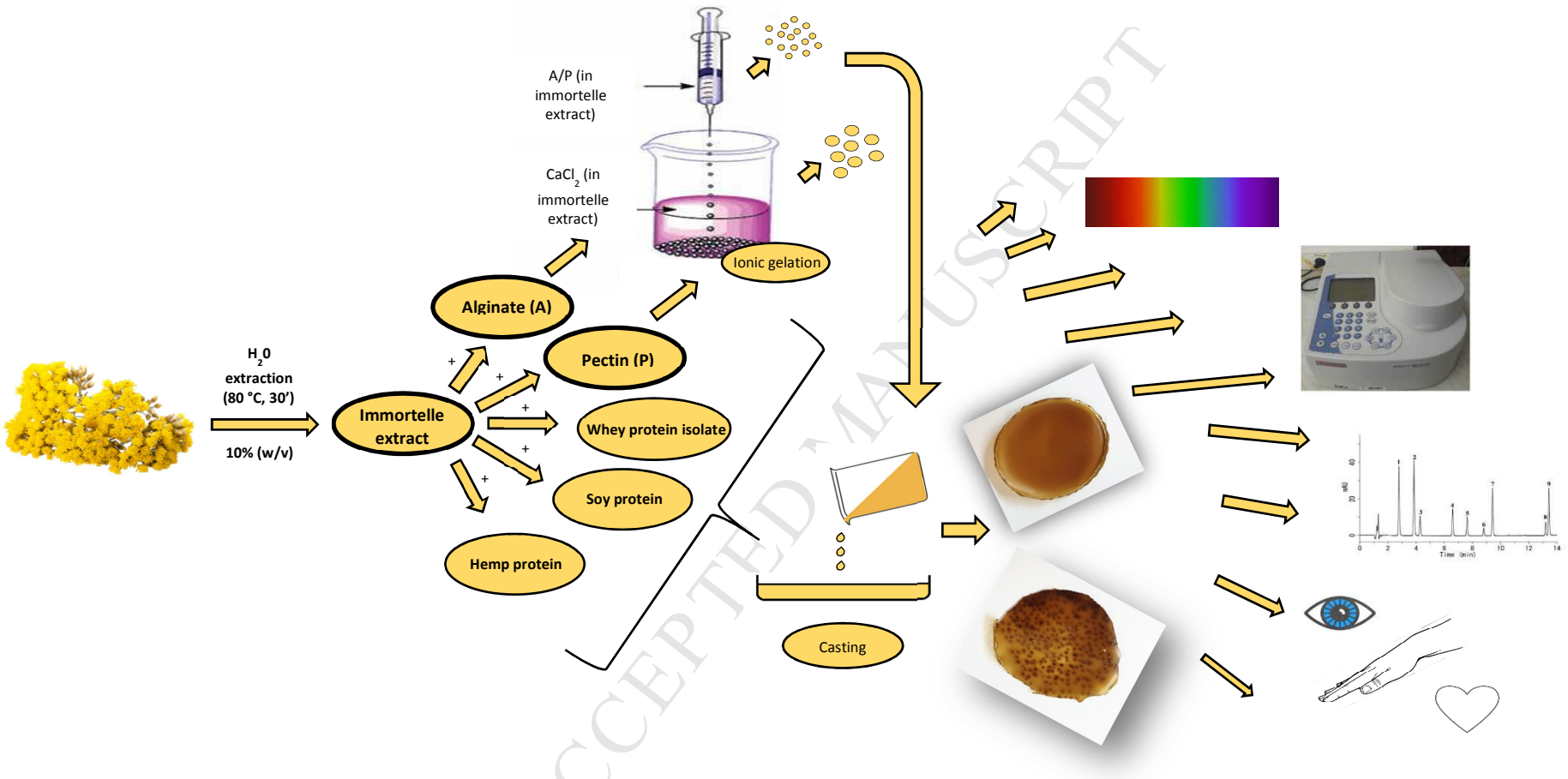
Graphical abstract



ABSTRACT

Since most studies regarding immortelle (*Helichrysum italicum*) are focused on the properties and composition of its essential oil, the aim of this study was to develop edible films based on immortelle water extract. Alginate and pectin combined with various proteins served as carriers for the formulation of biodegradable films and hydrogel particles. Films with different biopolymers and incorporated hydrogel particles were prepared by casting method and hydrogel particles were produced by ionic gelation. The bioactive profile (the content of total (TPC) and specific polyphenols, hydroxycinnamic acids (HCAC) and antioxidant capacity (AC)) of the developed matrices was characterized using spectrophotometric methods and high performance liquid chromatography (HPLC). Zeta-potential and rheological properties of alginate- and pectin-based film-forming solutions and physico-chemical (color, particle size, film thickness, dry matter content) and sensory properties of the developed particles and films were evaluated. The highest TPC (31.31 mg GAE/g sample) and HCAC (27.20 mg CAE/g sample) as well as the highest AC (0.15 mmol TE/g sample) was determined in pectin-based films. The addition of proteins decreased the content of the examined bioactive parameters, while the addition of hydrogel particles altered their physico-chemical properties. The obtained results indicate a great application potential of the developed biodegradable matrices.

Keywords: Antioxidant capacity, Coating films, Immortelle, Ionic gelation, Polyphenols

1. INTRODUCTION

Due to the growing consumer interest in natural, high-quality, and safe foods with prolonged shelf-life, the accelerated increase of the number of innovations in the field of food packaging has been noted in the recent years. The technological advances in the development of biodegradable materials greatly contributed to the deployment of innovative, functional edible films as packaging materials. Edible films are generally defined as thin layers of edible materials on or between food components. A great advantage of this type of food packaging is the ability of its consumption along with food, thus reducing the amount of waste.

Beside the basic barrier properties, edible films produced using natural biopolymers possess many other desirable characteristics, such as the prevention of oxygen, water, carbon dioxide, lipid, and flavor migrations from the ambience into food products, the antimicrobial activity, texture enhancement, prolongation of shelf life, etc. Hydrocolloids (polysaccharides and proteins), lipids, and their combinations are the most commonly used natural biopolymers for the production of edible films. There are several possibilities for using polymers in edible film formulations; they can be produced using single polymers, mixtures of two or more polymers, or multiple polymers by layering them (Garcia, Gomez-Guillen, Lopez-Caballero, & Barbosa-Canovas, 2016). Combining the polymers for the deployment of edible films contributes to the enhancement of their mechanical and barrier properties (Bertuzzi & Slavutsky, 2016).

One of the latest trend in food technology is the production of edible films enriched with various bioactive ingredients in order to improve their functional properties. For the stated purpose, the most commonly used bioactive ingredients are polyphenols due to their strong antioxidant properties, which help to protect the films and, therefore, the components of food, from oxidation and microbial deterioration. The use of polyphenols from herbal extracts as bioactive ingredients in edible film formulations was the focus of several recent

studies, such as the one by Norajit, Kim, & Ryu (2010), who analyzed alginate-based films containing ginseng (*Panax ginseng*) extract.

One of the plant species which represents a rich source of polyphenols (mostly flavonoids and hydroxycinnamic acids) and has a high antioxidant potential (Taglialatela-Scafati et al., 2013) is immortelle (*Helichrysum italicum*), which belongs to the family *Asteraceae*. It is an evergreen plant native to the Mediterranean area, whose leaves and flowers are known to possess many biological properties which include antimicrobial, anti-inflammatory, antioxidant, and antiviral activities (Guinoiseau et al., 2013).

Given the above, the aim of this research was to develop pectin- and alginate-based edible films with and without the incorporated alginate and pectin hydrogel particles containing immortelle extract and examine their physico-chemical, bioactive, and sensory properties. Furthermore, this study was focused on examining the effect of incorporation of hydrogel particles into the film-forming polymer solutions in relation to “plain” films using the Weibull model-fitting method.

2. MATERIALS AND METHODS

2.1. Chemicals

Immortelle (*Helichrysum italicum*) flowers, soy protein, and hemp protein were purchased in a local organic store. Sodium alginate (molecular weight: 80-120 kDa), gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), potassium peroxodisulfate, sodium nitrate, sodium molibdate, and ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) were obtained from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteau reagent, sodium carbonate (anhydrous), ethanol, calcium chloride, and hydrochloric acid were supplied by Kemika (Zagreb, Croatia). Low metoxyl pectin (molecular weight: 70-140 kDa) and whey protein isolate were supplied by Davisco Foods International (Le Seur, MN, USA).

Methanol was retrieved from J.T.Baker (Deventer, Netherlands) and sodium hydroxide from Gram-mol (Zagreb, Croatia). All reagents were of analytical or HPLC grade.

2.2. Preparation of immortelle extract

Immortelle flowers were air-dried at room temperature and ground using a domestic grinder Braun KSM2 (Braun, Kronberg, Germany). The extraction was carried out by pouring 100 mL of distilled water heated to 80 °C over 10 g of ground plant material and stirring on a magnetic stirrer RT 5 Power IKAMAG (Keison Products, Chelmsford, UK) for 30 min. The obtained extract was filtered through a metal strainer and a 4-layer cotton gauze (the plant residue was well pressed to minimize the loss of extract) and filled up to 100 mL.

2.3. Encapsulation in alginate and pectin delivery systems

The preparation of hydrogel particles was carried out by using internal ionotropic gelation of sodium alginate and pectin. Alginate (4 g/100 mL) and pectin (3 g/100 mL) solutions were prepared by dissolving the polymers in the previously prepared immortelle extract by stirring overnight. The prepared encapsulant solutions were dripped through a syringe with a stainless steel needle (18-22 Gauge) into the cross-linking solution consisting of 2 g/100 mL calcium chloride dissolved in the immortelle extract. After the ion-exchange, the hydrogel particles were allowed to stir gently in the solution and then stored in the immortelle extract until analyses.

2.4. Preparation of edible films

Alginate- and pectin-based edible films were prepared by using a casting method. First, alginate - A (4 g/100 mL), pectin – P (3 g/100 mL), whey protein isolate – WPI (5 g/100 mL), soy protein - SP (5 g/100 mL), and hemp protein - HP (5 g/100 mL) solutions were prepared by dissolving the polymers in immortelle extract on a magnetic stirrer at ambient

temperature overnight. The polysaccharide solutions were then mixed with proteins in a ratio of 1:1 on a magnetic stirrer. Edible films with incorporated alginate and pectin hydrogel particles (hp) were prepared with the addition of 2 g formulated particles per 100 g of edible film. All film solutions, 10 g each, were cast evenly into a Petri dish and allowed to dry at room temperature for at least 3 d (whereby the temperature and relative humidity were not controlled), peeled off from the surface of the Petri dish, and stored in a desiccator until the analyses.

Combining alginate and pectin with various proteins and formulating edible films with and without the incorporated hydrogel particles resulted in the development of 16 samples in total: 8 alginate-based ($A_{contr.}$, A_{hp}, A-WPI, A-WPI_{hp}, A-SP, A-SP_{hp}, A-HP, A-HP_{hp}) and 8 pectin-based ($P_{contr.}$, P_{hp}, P-WPI, P-WPI_{hp}, P-SP, P-SP_{hp}, P-HP, P-HP_{hp}).

Edible films developed using formulations containing only alginate and pectin ($A_{contr.}$, $P_{contr.}$) served as control samples in all analyses.

2.4. Determination of zeta-potential and rheological properties of alginate- and pectin-based film-forming solutions

The zeta-potential was measured by the principle of photon-correlation spectroscopy using the Zetasizer Nano Series device (Malvern Instruments Ltd., Malvern, UK). The measurements were performed at room temperature in triplicate and the results were expressed as mean values with the corresponding standard deviations.

The rheological behavior of film forming solutions was investigated by using a Discovery Hybrid Rheometer HR2 (TA Instruments, Newcastle, UK) and non-oscillatory (steady) shear measurements in the parallel plate mode (diameter 25 mm; gap 1000 μm). Steady-shear viscosity of film forming solutions was measured in the shear rate range from 1 to 1000/s at 25 °C. All measurements were performed in triplicate.

2.5. Determination of physico-chemical properties of formulated hydrogel particles and edible films

The dry matter content (DM) of formulated hydrogel particles and edible films was determined using the gravimetric method (by sample mass determination before and after drying at 105 °C to constant mass) according to AOAC (1995).

The size of the particles was determined using the Dino-Lite calibration plate (minimum distance=0.2 mm). The results were expressed as the mean of 10 consecutive measurements with the corresponding standard deviations.

The color of the obtained particles and edible films was evaluated using a colorimeter (Konica Minolta, Sensing, Japan) and the readings of L* (lightness), a* (redness), and b* (yellowness) parameters were recorded on several different locations on each film. The total color difference was calculated according to the equation: $\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$, where subscript "0" refers to the value of immortelle extract (for the hydrogel particles' color evaluation) or the "plain" alginate or pectin edible films (for the edible films' color evaluation). Based on ΔE values, the color deviation from the reference sample was estimated according to the following criteria: ΔE<0.2 (no visible color difference); ΔE=0.2-1 (noticeable color difference); ΔE=1-3 (visible color difference); ΔE=3-6 (well visible color difference); ΔE>6 (apparent color deviation) (Petrović, Milković, & Valdec, 2013). The results for both groups of samples (hydrogel particles and edible films) were expressed as mean values with the corresponding standard deviations.

The thickness of alginate and pectin films was measured using a digital hand-held micrometre Micromar 40 EX (Mahr GmbH, Göttingen, Germany). The measurements were made on at least six random locations on each film and the results were expressed as mean values with the corresponding standard deviations.

142 **2.6. Determination of bioactive profile and encapsulation efficiency**

143 The contents of total polyphenols (TPC), hydroxycinnamic acids (HCAC), and
144 antioxidant capacity (AC) entrapped in the obtained hydrogel particles were estimated by
145 dissolving a known amount of particles in 2 g sodium citrate dissolved in the extract on a
146 magnetic stirrer at ambient temperature until complete dissolution, while the determination of
147 TPC, HCAC, and AC in the developed edible films was carried out by dissolving a known
148 amount of films in diluted ethanol (10 mL ethanol in 100 mL of water) on a magnetic stirrer
149 under the same conditions. TPC was determined using a spectrophotometric Folin-Ciocalteu
150 assay according to Singleton & Rossi (1965), HCAC using the Arnow reagent assay
151 (European Pharmacopoeia, 2002), and AC by the DPPH (Brand-Williams, Cuvelier, &
152 Berset, 1995) and ABTS (Re et al., 1999) radical scavenging assays. The percentage of
153 loading efficiency in the formulated hydrogel particles was calculated as the ratio between
154 TPC or HCAC in the citrate solution of dissolved beads and their respective content in the
155 initial solution.

156 The contents of specific polyphenols were determined by HPLC analysis. The samples
157 prepared for the determination of TPC, HCAC, and AC were diluted with water and filtered
158 through a 0.45 μm filter (Nylon Membranes, Supelco, Bellefonte, USA). 10 μL of sample was
159 injected for the HPLC analysis using the Agilent 1100/1200 Series HPLC device (Agilent,
160 Santa Clara, USA), a Photodiode Array Detector (PAD) (Agilent, Santa Clara, USA), and a
161 reversed-phase column ACE Excel Ultra Robust UHPLC C-18 column (ACE, London, UK)
162 (250 x 4.6 mm i.d.). A modified method by Gonçalves et al. (2016) was used for the analysis,
163 whereas the solvents consisted of 2 g/100 mL re-distilled formic acid (solvent A) and
164 methanol (solvent B) at a flow rate of 1 mL/min, starting with 5% B and using a gradient
165 finishing at 100% B (60 min). The chromatograms were recorded at 320 nm. The detection
166 was performed with a PAD by scanning between 200-400 nm, with a resolution of 2.0 nm.

The specific bioactive compounds were identified by comparing their retention times with those of the standards. The data acquistion was conducted using the Chemstation LC 3D Systems software (Rev. B.04.02). All analyses were performed in a triplicate and the results were expressed as mean values with the corresponding standard deviations.

2.7. Determination of release profiles of polyphenols and antioxidant capacity from the developed hydrogel particles and edible films; The Weibull model-fitting

The release of total polyphenols (TP), hydroxycinnamic acids (HCA), and AC was determined from the developed edible films with incorporated hydrogel particles in simulated gastric (SGF - simulated gastric fluid, pH=1.2) and intestinal (SIF – sodium-phosphate buffer, pH=7.4) conditions. In addition, in order to evaluate the effect of the incorporated hydrogel particles into the films, the release of polyphenols was observed from the films developed without the particles, as well as from plain hydrogel particles. For the analysis, a known amount (0.6 g) of hydrogel particles or edible films was suspended in 30 mL of SGF incubated at 37 °C for 2 h on a magnetic stirrer at 100 rpm. 2 mL of supernatant was taken for the analysis at defined time intervals. After the exposure to simulated gastric conditions, the particles and edible films were recovered by filtration and suspended in 30 mL of SIF under the previously described conditions. The release kinetics was determined by evaluating the TPC and HCAC, as well the retained AC as described in the previous paragraph (2.6.).

The experimentally determined release profiles of HCA were also fitted to the Weibull two-parameter model to the entire polyphenol release curve data given by the equation: $Mt/M_\infty = 1 - \exp(-at^b)$, where M_t is the mass of the polyphenol released at time t, M_∞ is the mass of the released polyphenol at equilibrium, and a and b are constants.

2.8. Sensory evaluation of the developed edible films

The sensory evaluation of edible films was carried out by using a hedonic score scale of 1-9, where 9 indicates a highly desirable quality and 1 signifies a defective product (Ozdemir & Floros, 2008). 30 trained panelists participated in the sensory evaluation. The sensory evaluation of the developed edible films included an assessment of appearance, color, transparency, elasticity, and general acceptability. The results were expressed as mean values of the ratings assigned to each parameter.

2.9. Statistical analysis

The statistical analysis of the obtained results was conducted by using the SPSS Statistics 17.0 software in order to determine the average value and standard error. The variance analysis (Tukey HSD ANOVA test; significance level of α=0.05) was performed in order to examine the effect of different polysaccharide and polysaccharide-protein carriers on physico-chemical and bioactive parameters of the developed edible films.

3. RESULTS AND DISCUSSION

3.1. Zeta-potential and rheological properties

Table 1 displays the zeta-potential of alginate- and pectin-based film-forming solutions. All samples exhibited negative zeta-potentials, indicating that the developed films contained a negative net charge at neutral pH originating from carboxyl groups present in the polysaccharide chains. The zeta-potential values obtained for pectin solutions were a bit less electronegative than those obtained for alginate solutions, which is in accordance with the previous findings (Tello et al., 2015). The results indicate the existence of electrostatic repulsions between the polymers and the absence of phase separation.

Fig. 1 depicts the effect of shear rate on the apparent viscosity for the alginate-protein and pectin-protein dispersions as well as for “plain” alginate and pectin solutions and protein

dispersions. In all cases, typical shear thinning behavior with non-Newtonian (pseudoplastic) features was detected. Additionally, pectin-protein blends exhibited a greater pseudoplastic character (increase in the slope of the curves) than alginate-protein formulations.

3.2. Physico-chemical properties

The formulated alginate and pectin hydrogel particles as well as the developed edible films were examined for their physico-chemical properties: DM, size/thickness, and color (Table 2).

As expected, alginate and pectin particles exhibited low DM inside the matrix. Alginate particles contained 6.88 g DM per 100 g of edible film (g/100 g), while pectin particles contained 8.06 g/100 g DM, which is in accordance with the results by Belščak-Cvitanović et al. (2015) who found that up to 95% of water is retained in the ionic gelation-obtained beads based on alginate and pectin. On the other hand, the developed edible films contained high DM, which varied from 89.55-92.10 g/100 g in alginate-based films and 91.40-92.71 g/100 g in pectin-based ones. The formulated particles exhibited a relatively unimodal particle size distribution. Also, alginate particles were smaller in size compared to the pectin beads, which was previously reported in the study by Tello et al. (2015).

The thinnest films were those developed using “plain” alginate and pectin (98.83 µm and 93.33 µm, respectively), while the reinforcement of biopolymers with proteins resulted in the development of thicker films, ranging from 99.67-325.83 µm in the case of alginate-based films and 137.33-377.67 µm in the case of those based on pectin (Table 2). Alginate-based edible films were generally a lot thinner than the pectin-based ones. As expected, the incorporation of hydrogel particles, whose average size was 1940 µm (alginate particles) and 3080 µm (pectin particles), increased the thickness of the developed films.

Even though both kinds of particles exhibited large color deviations (ΔE values) when compared to the immortelle extract, the color difference between pectin hydrogel particles and

238 the extract is a lot less pronounced than that between alginate particles and the extract.
239 According to the calculated ΔE values, the color difference of alginate-based films in regard
240 to the edible film formulated with "plain" alginate ranged from visible (A_{hp}, A-WPI_{hp}) and
241 well visible (A-WPI, A-SP_{hp}) to apparent color deviations (A-SP, A-HP, A-HP_{hp}). In pectin-
242 based films, the differences in color in comparison to "plain" pectin edible films are more
243 pronounced, varying from well visible (P-SP) to apparent color deviations (P_{hp}, P-WPI, P-
244 WPI_{hp}, P-SP_{hp}, P-HP, P-HP_{hp}).

245 **3.3. Encapsulation efficiency and bioactive profile**

246 Fig. 2 displays the encapsulation efficiencies of TP, HCA, and the retained AC in the
247 formulated alginate and pectin hydrogel particles. As can be seen, pectin particles exhibited a
248 higher encapsulation efficiency of TP and HCA, as well as a higher efficiency of retaining the
249 AC than the alginate ones. Similar results were obtained in the study by Tello et al. (2015),
250 where using pectin as a carrier for the internal ionotropic gelation/emulsification of sunflower
251 oil and model oil increased the encapsulation efficiency of lipids.

252 Since all edible film formulations contained immortelle extract, which represents a
253 rich source of polyphenols, the developed edible films also contained high TPC, HCAC, and a
254 high AC. However, as can be seen from Fig. 3, the reinforcement of alginate and pectin
255 formulations with proteins generally contributed to the reduction of TPC, HCAC, and AC
256 when compared to "plain" alginate or pectin edible films. A possible explanation of such
257 occurrence might lie in the formation of soluble and insoluble protein–polyphenol complexes,
258 which could potentially aggravate the analysis and quantification of polyphenols in the
259 samples containing high concentrations of herbal-origin polyphenols and various proteins.
260 However, the experimental data relating to the structural basis for protein–polyphenol
261 interactions are rather limited. Particularly, studies have often been limited to the interactions
262 of particular proteins or a narrow range of polyphenols. Future research studies need to

address the interactions of a range of proteins and polyphenols in order to furtherly clarify the functioning of the structure–activity relationships, which may also influence the fate of the polyphenols *in vivo* (Papadopoulou & Frazier, 2004). As can be seen from Table 3, the most abundant polyphenolic compounds present in the developed films and immortelle extract were caffeic acid (CA), chlorogenic acid (ChlA), and its derivatives ($ChlA_D$), whereby ChlA, and especially $ChlA_D$, dominated the bioactive composition of the samples. CA was determined in edible films in relatively small concentrations compared to ChlA and $ChlA_D$, ranging between 6.79 mg/L (A-HP) and 54.71 mg/L ($A_{contr.}$), while the immortelle extract contained the highest content of CA (90.36 mg/L). Similarly to the results obtained in this study, de la Garza et al. (2013) also reported on high contents of CA, ChlA, and $ChlA_D$ in immortelle extract obtained by a mixture of water and methanol as extractants, whereby the content of CA was also significantly ($p<0.05$) lower than that of ChlA and $ChlA_D$. Regarding the developed edible films, the content of CA was higher in most pectin-based samples than in those developed using alginate. The films developed using "plain" alginate and pectin were more abundant in CA than the films with incorporated hydrogel particles or those reinforced with proteins. As stated above, the determined contents of ChlA and $ChlA_D$ were much higher in comparison to the contents of CA, with the contents of ChlA ranging from 61.32 mg/L ($A_{contr.}$) to 310.96 mg/L ($P_{contr.}$), and $ChlA_D$ from 679.85 mg/L ($A_{contr.}$) to 2482.17 mg/L ($P_{contr.}$). No significant difference ($p>0.05$) between alginate- and pectin-based edible films was observed regarding the content of $ChlA_D$, while pectin-based films were more abundant in $ChlA_D$ in comparison to those based on alginate. In general, most edible films with incorporated hydrogel particles were more abundant in ChlA than those without the particles, with contents of ChlA being up to 2.7 times higher in the films with than without the particles (among the same group of samples; e.g. alginate- and pectin-based ones), indicating a high potential of incorporation of

hydrogel particles into edible film formulations which, according to the results of this study, furtherly enriches the already notable bioactive composition of such innovative food matrices.

3.4. Release kinetics of polyphenols and antioxidant capacity

As can be seen from Fig. 4, the release of TP and HCA from the majority of the developed alginate- and pectin-based films was markedly prolonged and gradually increasing, both in SGF and in SIF. Only pectin films reinforced with soy and hemp proteins containing hydrogel particles (P-SP_{hp} and P-WPI_{hp}) exhibited a somewhat faster release (in the first 20 and 5 min, respectively) of TP in SGF, and the additional release in SIF (which was also observed in all other samples). Also, the release of HCA from a P-WPI_{hp} film was somewhat faster compared to the other films (a faster release rate was noticed in the first 5 min in SGF, after which the release became slower and more controlled, followed by a continuation in SIF). In the case of AC, an even faster release was observed from all developed films, with the majority of AC being released during the first few minutes in SGF (which is especially noticeable with the ABTS method, while the release of AC determined using the DPPH method was somewhat slower after the first few minutes of fast release in SGF). Furthermore, the release of AC continued in SIF, but with distortions in the release kinetics for most samples. A fast initial release in SGF could be a consequence of the erosion and weakening of the particle matrix structure due to the polysaccharide hydrolysis induced by an acidic pH of SGF (Wang & Copeland, 2012). In addition, the apparent faster release of AC could be attributed to the poor selectivity of DPPH (and ABTS) radicals, whereby the small molecules have better access to the radical site due to high steric accessibility of the radicals, leading to a higher apparent antioxidant activity faster during the release (Cerretani & Bendini, 2010).

3.5. The Weibull model-fitting

Fig. 5 shows the comparisons of the fitting results obtained by the Weibull model of release patterns (lines) to the corresponding experimental results (symbols) obtained for HCA in SGF for all film samples. The kinetic parameters of the fitting curves obtained for the HCA release in SGF are shown in Table 4. The *b* values derived from the fitting of the Weibull model can characterize the release mechanism. According to Papadopoulou, Kosmidis, Vlachou, & Macheras (2006), for $b<0.75$, the release is governed by the Fickian diffusion, which is the case of the HCA release in SGF from alginate-protein and pectin-protein films with incorporated hydrogel particles (with an exception of the P-WPI_{hp} film). The film based on "plain" alginate (A_{hp}) exhibited a *b* value >0.75, which indicates the presence of both kinds of transports - the Fickian diffusion and Case II transport. The hydrogel particles (both alginate and pectin) themselves exhibited the *b* values ≈1, which is compatible with the first-order release, whereby the concentration gradient in the medium drives the release rate (Rinaki, Dokoumetzidis, & Macheras, 2003). On the other hand, the Weibull model applied to the release curves of the films without the incorporated hydrogel particles exhibited $0.75<b\leq1$, with an exception of the film developed using "plain" pectin, which exhibited $b>1$. Both ranges indicate the presence of the combined mass transfer mechanism (Fickian diffusion and Case II transport). According to these results, the incorporated hydrogel particles affect mass transfer by retarding the release and becoming less relaxation-controlled.

3.6. Sensory evaluation

Fig. 6 displays the results of the sensory evaluation of the samples. As can be seen in the case of alginate-based edible films, the sensory panel has shown the highest acceptability towards the plain alginate ($A_{contr.}$) edible film. Furthermore, the appearance and elasticity of the plain alginate ($A_{contr.}$) and alginate films with incorporated hydrogel particles (A_{hp}) were evaluated as the most desirable. On the other hand, the most desirable color was that of alginate film reinforced with hemp protein and with incorporated particles (A-HP_{hp}), while the

transparency of the film developed using alginate reinforced with whey protein isolate containing hydrogel particles (A-WPI$_{hp}$) was evaluated as optimal. The most acceptable pectin-based edible films were those formulated using "plain" pectin (P$_{contr.}$), which also prove to be the most elastic one, and pectin reinforced with whey protein isolate (P-WPI), whose elasticity and appearance were also evaluated as highly desirable. The most desirable color was that of the pectin-hemp protein edible film with (P-HP$_{hp}$) and without (P-HP) the incorporated particles. Alginate films were generally rated as more desirable by the sensory panel.

4. CONCLUSIONS

All developed films exhibited high TPC, HCAC, and a remarkable AC. However, the reinforcement of alginate and pectin with proteins resulted in a decrease of the listed parameters, possibly due to the complex protein–polyphenol interactions, which could have potentially aggravated the analysis and the quantification of polyphenols in the polyphenol-rich samples containing proteins. Chlorogenic acid (ChlA) and its derivatives (ChlA$_D$) dominated the bioactive composition of the samples. Most edible films with incorporated hydrogel particles were more abundant in ChlA than those without the particles, indicating a high potential of hydrogel particle incorporation into edible film formulations which, according to the results of this study, furtherly enriches the already rich bioactive composition of such innovative food matrices. After the exposure to SGF and SIF, most developed edible films exhibited a markedly prolonged and slow release of TP and HCA, while the release kinetics of AC was somewhat faster, especially in the first few minutes in SGF. In terms of sensory characteristics of the developed edible films, the sensory panel evaluated the alginate-based films as generally more desirable compared to the pectin-based ones. The obtained results indicate a great application potential of the developed biodegradable matrices as

359 functional films for active food packaging. However, further studies are needed in order to
360 test the effectiveness of the immortelle-based films on possible food systems.

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Fig. 1. Steady-shear viscosity (η) vs. shear rate of polysaccharide solutions (alginate (4%, w/v) and pectin (3%, w/v)), protein dispersions (whey protein isolate (5% w/v), soy protein (5%, w/v) and hemp protein (5%, w/v)) and polysaccharide-protein blend dispersions used for film formulations (Legend: ■A, ●A-SP, ▲A-WPI, ▼A-HP, ♦P, ◄P-SP, ►P-WPI, ▪P-HP, ★SP, ▪WPI, ●HP)

Fig. 2. Encapsulation efficiencies (%) of total polyphenols (TP), hydroxycinnamic acids (HCA), and the retained antioxidant capacity (AC) by DPPH and ABTS methods in the formulated alginate and pectin hydrogel particles (Legend: ■Alginate particles, ■Pectin particles)

Fig. 3. The contents of a) total polyphenols (TP) and b) hydroxycinnamic acids (HCA); antioxidant capacity determined by c) DPPH and d) ABTS methods in the developed alginate- and pectin-based films *The bars representing the values of the TPC, HCAC and AC with the same letter affixed are not significant ($p>0.05$). (Legend: ■$A_{contr.}$, ▓A_{hp}, ‖A-WPI, ≡A-WPI_{hp}, //A-SP, ♦A-SP_{hp}, ⁘A-HP, \\A-HP_{hp}; ■$P_{contr.}$, ▓P_{hp}, ‖P-WPI, ≡P-WPI_{hp}, //P-SP, ♦P-SP_{hp}, ⁘P-HP, \\P-HP_{hp})

Fig. 4. Release profiles of a) total polyphenols (TP), b) hydroxycinnamic acids (HCA) and antioxidant capacity determined by c) DPPH and d) ABTS methods from the developed edible films and hydrogel particles in simulated gastric (SGF) and intestinal (SIF) fluids (Legend: ▲A_{hp}, ♦A-WPI_{hp}, ●A-SP_{hp}, ■A-HP_{hp}; ▲P_{hp}; ♦P-WPI_{hp}, ●P-SP_{hp}, ■P-HP_{hp}; ▬Alginate particles, ▬Pectin particles)

Fig. 5. Fractional polyphenols release in time *t* obtained for the HCA in SGF for: a) alginate-based films without the incorporated hydrogel particles and b) with the incorporated particles; c) pectin based films without the incorporated hydrogel particles and d) with the incorporated particles; The

symbols (■, ●, ▲, ▼) depict the experimental data, while the lines stand for the fitting of the Weibull

model to the experimental data

(Legend: Fig. 5.a : ■$A_{contr.}$, ●A-WPI, ▲A-SP, ▼A-HP, —$A_{contr.}$, ---A-WPI, · · ·A-SP, – – –A-HP;

Fig. 5.b: ■A_{hp}, ●A-WPI_{hp}, ▲A-SP_{hp}, ▼A-HP_{hp}, —$A_{hp.}$, ---A-WPI_{hp}, · · · A-SP_{hp}, – – – A-HP_{hp};

Fig. 5.c: ■$P_{contr.}$, ●P-WPI, ▲P-SP, ▼P-HP, —$P_{contr.}$, ---P-WPI, · · · P-SP, – – – P-HP;

Fig. 5.d: ■P_{hp}, ●P-WPI_{hp}, ▲P-SP_{hp}, ▼P-HP_{hp}, —$P_{hp.}$, ---P-WPI_{hp}, · · · P-SP_{hp}, – – – P-HP_{hp})

Figure 6. The sensory evaluation of the developed a) alginate- and b) pectin-based edible films

(Legend: Fig. 6.a : ■$A_{contr.}$, □A_{hp}, ♦A-WPI, ◊A-WPI_{hp}, ▲A-SP, △A-SP_{hp}, ×A-HP, ▬A-HP_{hp}

Fig. 6.b. : ■$P_{contr.}$, □P_{hp}, ♦P-WPI, ◊P-WPI_{hp}, ▲P-SP, △P-SP_{hp}, ×P-HP, ▬P-HP_{hp})

Table 1. Zeta-potential of alginate- and pectin-based film-forming dispersions

	Zeta-potential / mV				
A	−42.80 ± 2.12	**P**	−24.40 ± 0.74	**WPI**	−17.80 ± 0.83
A-WPI	−28.20 ± 0.59	**P-WPI**	−24.90 ± 0.21	**SP**	−16.40 ± 0.75
A-SP	−22.90 ± 0.59	**P-SP**	−22.30 ± 1.91	**HP**	−21.90 ± 0.61
A-HP	−28.50 ± 0.50	**P-HP**	−27.70 ± 0.85		

Table 2. Physico-chemical properties of the developed edible films and the formulated hydrogel particles

Sample	Dry matter content / g/100 g	Thickness (size) / µm	Colour deviation / ΔE
$A_{contr.}$	90.34 ± 0.24^{a}	98.83 ± 0.01	-
A_{hp}	90.58 ± 0.13^{ac}	203.50 ± 0.05	1.96 ± 1.22^{a-e}
A-WPI	91.78 ± 0.10^{ab}	121.17 ± 0.02	2.85 ± 1.45^{f-ip}
A-WPI_{hp}	92.10 ± 0.03^{b}	325.83 ± 0.06	1.46 ± 1.06^{j-o}
A-SP	89.55 ± 0.27^{c}	132.67 ± 0.01	10.21 ± 2.01^{aj}
A-SP_{hp}	90.33 ± 0.45^{a}	224.00 ± 0.01	4.72 ± 0.42
A-HP	89.98 ± 0.98^{ac}	99.67 ± 0.00	7.08 ± 3.63
A-HP_{hp}	90.13 ± 0.06^{ac}	231.83 ± 0.04	11.28 ± 3.73^{bfk}
$P_{contr.}$	92.12 ± 0.11^{b}	93.33 ± 0.01	-
P_{hp}	92.71 ± 0.08^{b}	344.00 ± 0.06	7.85 ± 1.84^{dmp}
P-WPI	92.40 ± 0.34^{b}	137.33 ± 0.02	10.56 ± 2.54^{cghil}
P-WPI_{hp}	92.38 ± 0.32^{b}	299.50 ± 0.08	9.43 ± 3.87^{o}
P-SP	92.50 ± 0.76^{b}	272.17 ± 0.09	4.51 ± 2.05
P-SP_{hp}	92.65 ± 0.80^{b}	238.33 ± 0.05	8.33 ± 1.59
P-HP	91.40 ± 0.12^{ab}	157.17 ± 0.06	10.53 ± 2.90^{dmp}
P-HP_{hp}	92.69 ± 0.97^{b}	377.67 ± 0.14	9.90 ± 4.72^{en}
Alginate particles	6.88 ± 0.45	1940 ± 50.08	287.90 ± 14.96
Pectin particles	8.06 ± 0.28	3080 ± 130.12	18.77 ± 0.42

The values for the dry matter content superscripted with the same letter are not significant ($p>0.0$

5), while the ones for ΔE superscripted with the same letter are significant ($p<0.05$).

Table 3. Contents of the specific polyphenols (CA = caffeic acid, ChlA = chlorogenic acid, $ChlA_D$ = chlorogenic acid derivatives) determined in the developed edible films and

Compound /Sample	CA	ChlA	$ChlA_D$
		/ mg L^{-1}	
$A_{contr.}$	54.71±0.91	61.32±0.44	679.85±6.60[a]
A_{hp}	9.18±0.05[ab]	164.60±0.53	832.09±4.48
A-WPI	8.15±0.22[ac]	170.75±0.04	1329.71±8.25
A-WPI_{hp}	8.52±0.18[adef]	190.35±1.08	1252.36±6.99
A-SP	9.20±0.19[ab]	91.56±0.14	959.61±3.84
A-SP_{hp}	8.95±0.05[abe]	193.57±0.16	388.36±4.83
A-HP	6.79±0.02[c]	67.93±0.46	804.65±0.92
A-HP_{hp}	7.53±0.04[cd]	126.80±0.82	769.40±4.44
$P_{contr.}$	12.22±1.37	310.96±0.61	2482.17±4.98
P_{hp}	9.06±0.06[abe]	218.29±0.64	1602.43±7.78[b]
P-WPI	7.93±0.47[ace]	208.74±0.25	1473.84±3.91
P-WPI_{hp}	10.37±0.01[bg]	256.31±1.04	1746.71±7.16
P-SP	9.96±0.12[bg]	177.81±0.27[a]	1440.24±4.52
P-SP_{hp}	9.59±0.04[bfg]	236.93±0.83	1684.24±7.42
P-HP	9.91±0.42[bg]	198.12±0.23	1611.71±4.39[b]
P-HP_{hp}	10.61±0.10[g]	177.13±0.59[a]	876.71±1,80
Extract	90.36±0.10	112.64±0.41	676.98±3.32[a]

immortelle extract

The values superscripted with the same letter are not significant ($p>0.05$)

Table 4. The parameters and coefficients obtained from the Weibull model used for the simulation of release profiles

Sample	*a*	*b*	R^2	RMSE	χ^2
$A_{contr.}$	0.200 ± 0.048^{a}	1.057 ± 0.151^{a}	0.985	0.011	0.0028
A-WPI	$0.303\pm0.065b^{c}$	$0.813\pm0.131b^{c}$	0.973	0.029	0.0048
A-SP	0.143 ± 0.022^{d}	$0.817\pm0.063b^{c}$	0.992	0.009	0.0013
A-HP	0.114 ± 0.013^{cf}	0.953 ± 0.050^{ac}	0.997	0.004	0.0005
A_{hp}	$0.020\pm0.037^{a, b, i\text{-}s}$	0.823 ± 0.090^{bc}	0.986	0.0135	0.0020
A-WPI_{hp}	0.203 ± 0.032^{it}	0.630 ± 0.061^{b}	0.984	0.0153	0.0022
A-SP_{hp}	0.197 ± 0.035^{jh}	0.705 ± 0.075^{b}	0.983	0.0172	0.0025
A-HP_{hp}	0.203 ± 0.039^{kv}	0.683 ± 0.078^{d}	0.980	0.0207	0.0030
$P_{contr.}$	0.208 ± 0.032^{lx}	1.223 ± 0.116^{d}	0.995	0.0053	0.0011
P-WPI	0.231 ± 0.063^{m}	1.080 ± 0.189^{d}	0.977	0.0240	0.0040
P-SP	0.158 ± 0.027^{y}	0.952 ± 0.086^{d}	0.992	0.0083	0.0014
P-HP	0.279 ± 0.059^{fn}	0.995 ± 0.149^{d}	0.982	0.0183	0.0030
P_{hp}	0.229 ± 0.042^{o}	0.628 ± 0.072^{d}	0.977	0.0217	0.0031
P-WPI_{hp}	0.259 ± 0.058^{p}	1.211 ± 0.186^{d}	0.985	0.0185	0.0026
P-SP_{hp}	0.231 ± 0.048^{r}	0.743 ± 0.098^{d}	0.977	0.0245	0.0035
P-HP_{hp}	0.163 ± 0.033^{z}	0.726 ± 0.080^{d}	0.981	0.0194	0.0028
Alginate particles	0.307 ± 0.083^{degh}	1.004 ± 0.205^{d}	0.971	0.0218	0.0054
Pectin Particles	0.378 ± 0.120^{aetuvxyz}	1.074 ± 0.296^{d}	0.966	0.0344	0.0086

The *a* values superscripted with the same letter are not significant ($p>0.05$), while those for *b* superscripted with the same letter are significant ($p<0.05$).

Fig. 1.

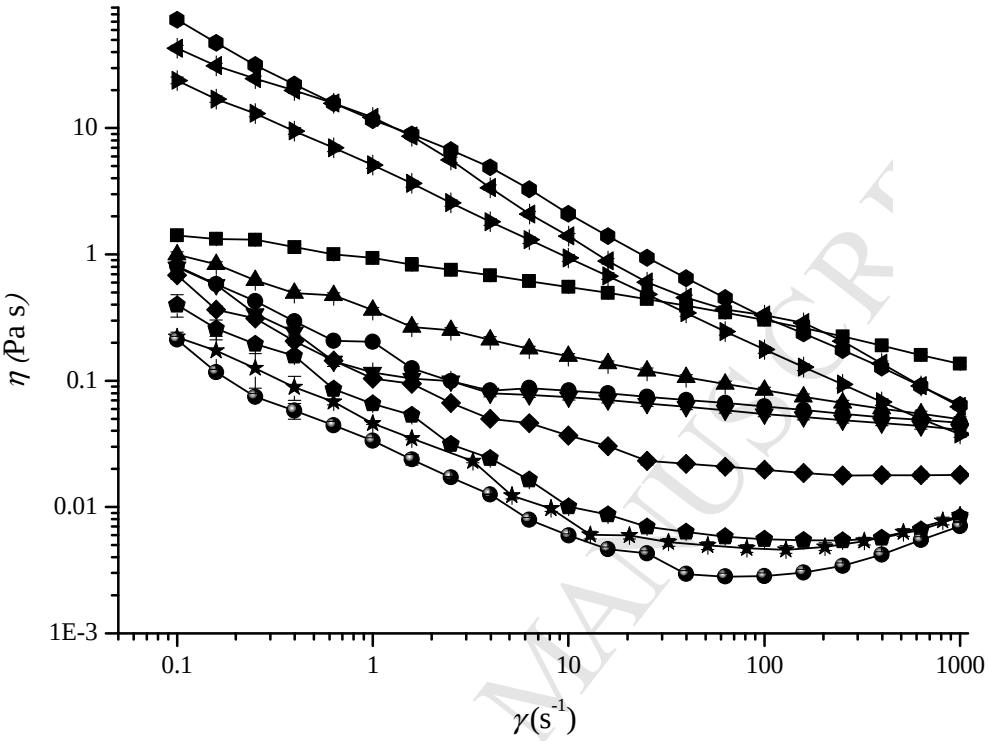

Fig. 2.

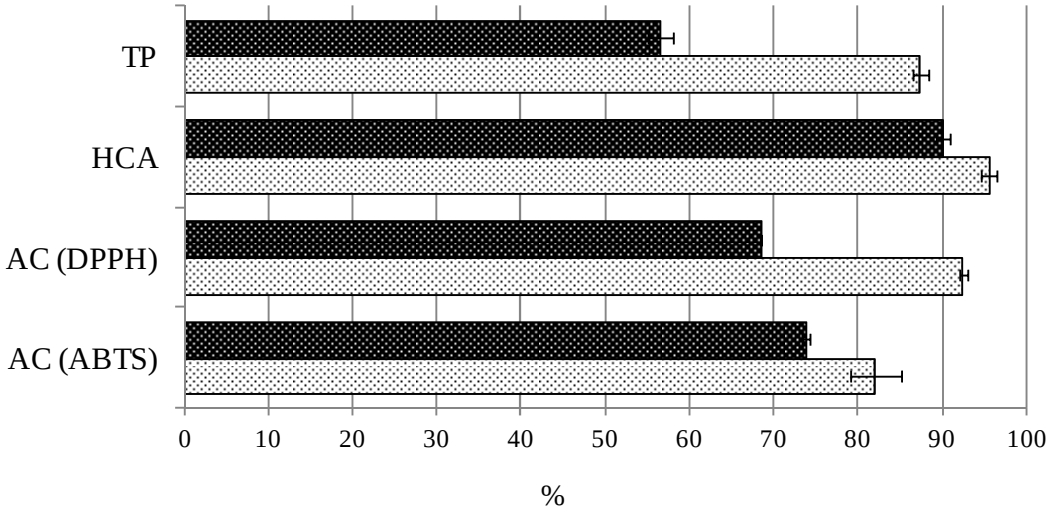

Fig. 3.

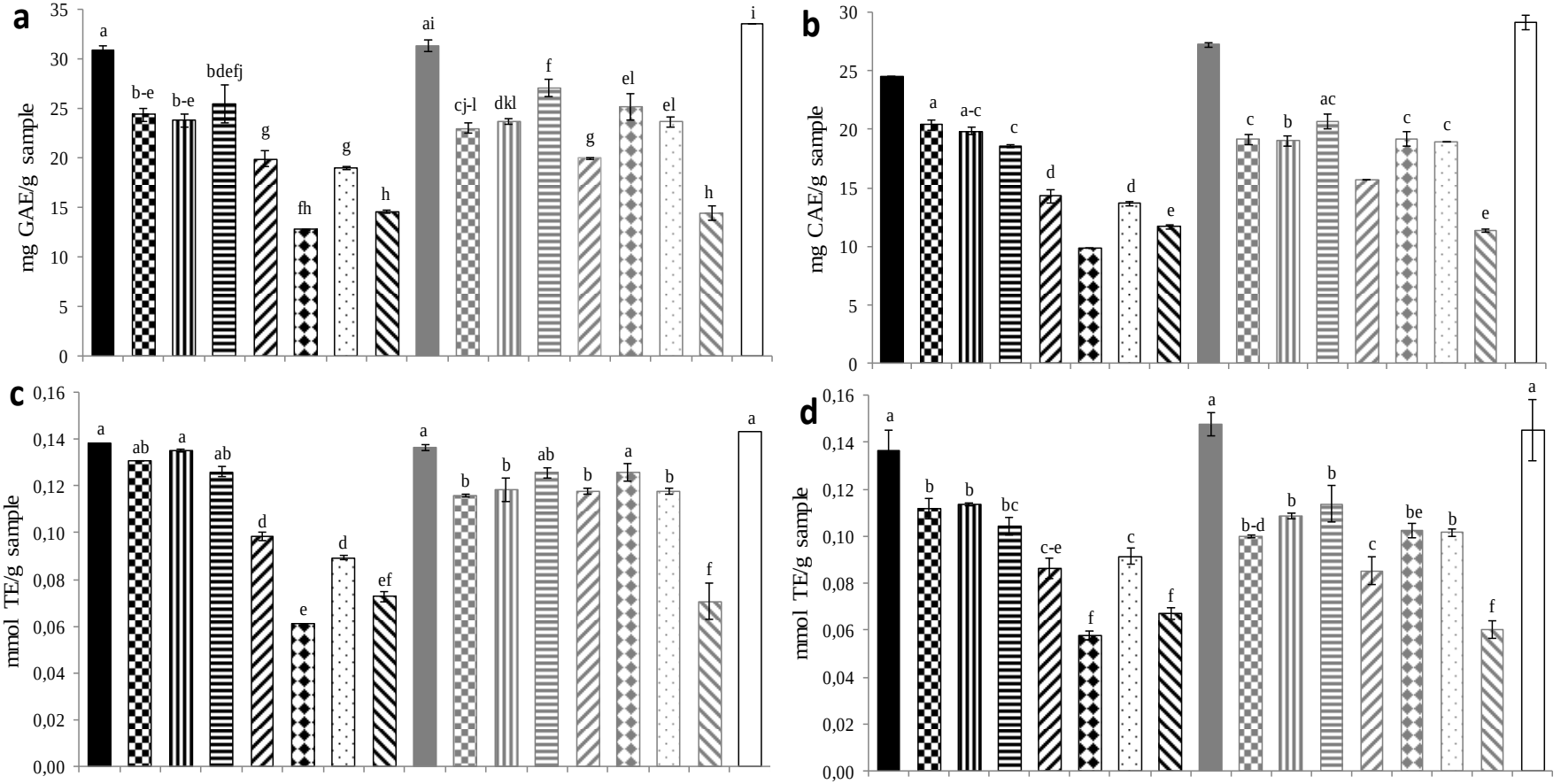
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Fig. 4.

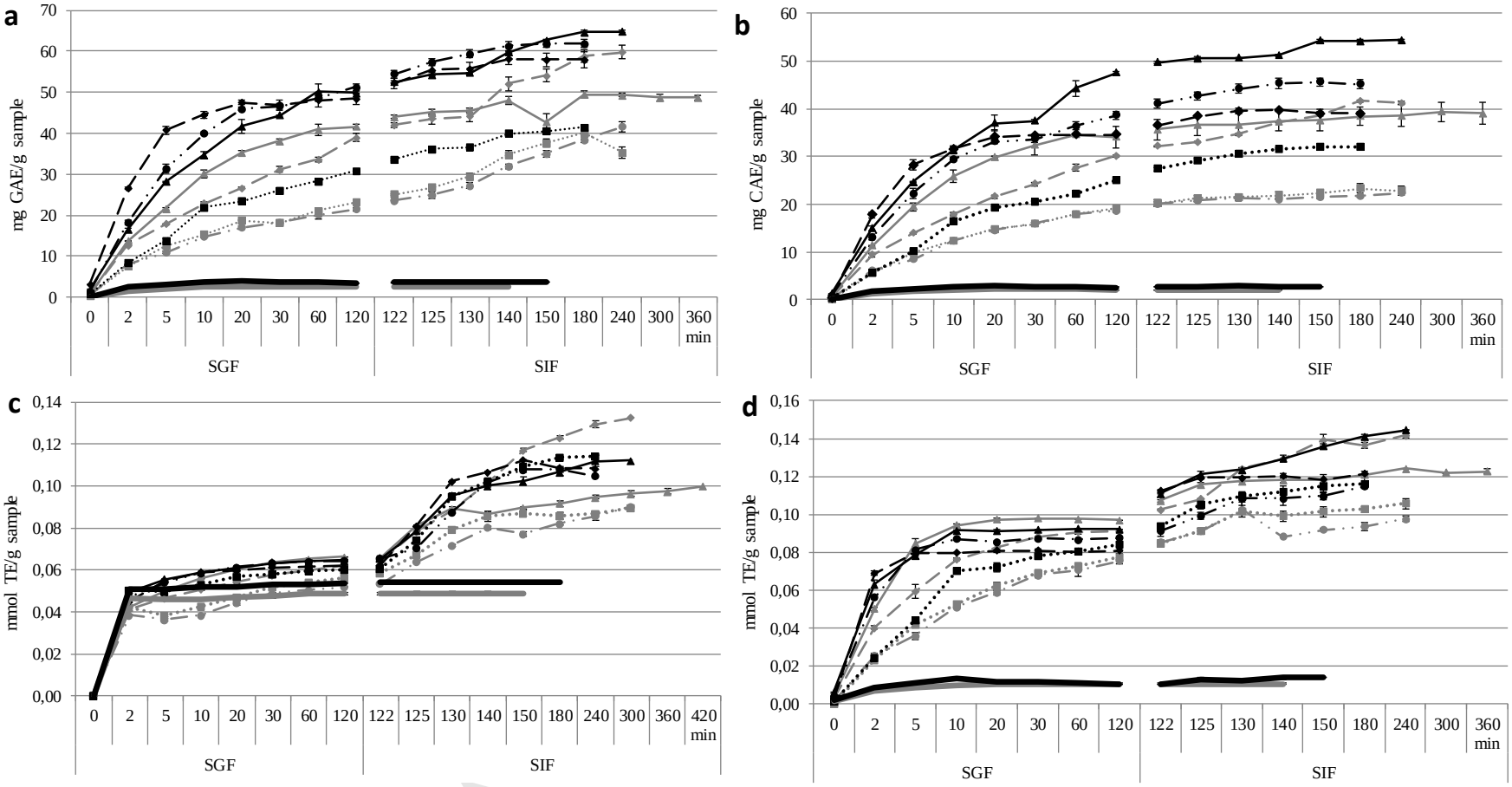
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Fig. 5.

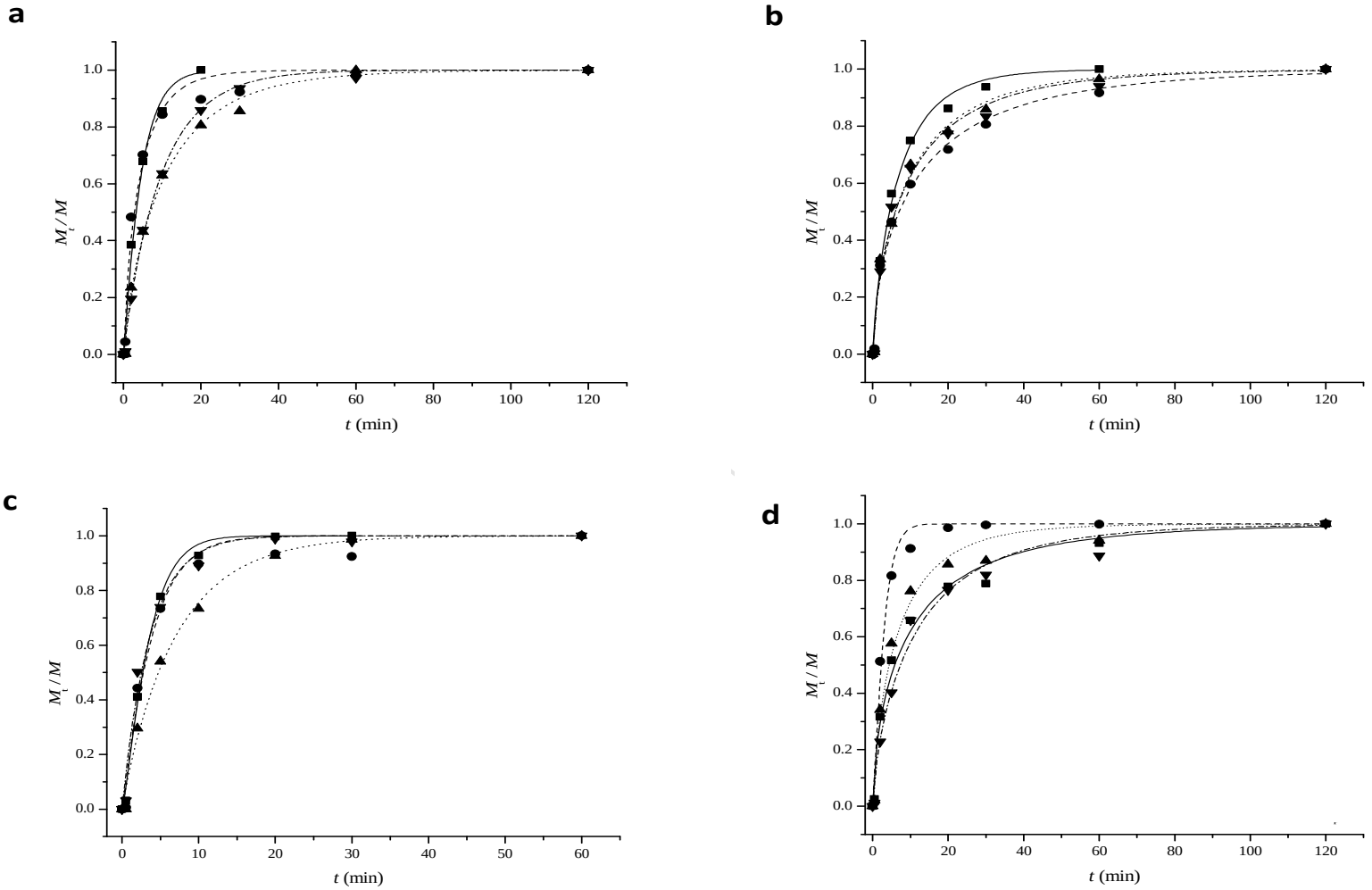
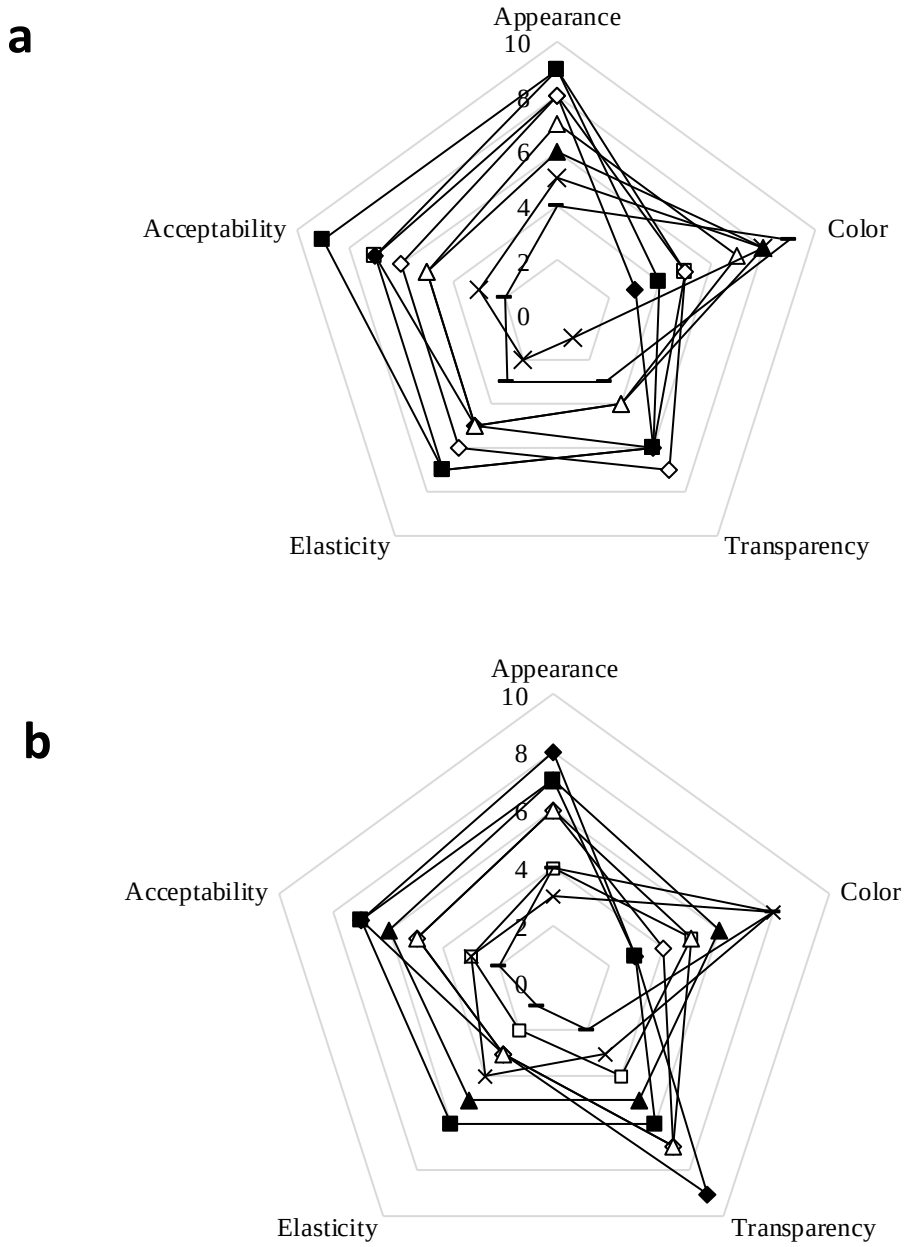


Fig. 6.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Elasticity
Acceptability

Highlights

- Immortelle extract is abundant in polyphenols, resulting in high bioactivity
- Chlorogenic acid derivatives dominate the bioactive profile of immortelle films
- The addition of hydrogels significantly enriched the bioactive profile of the films
- Polyphenols entrapped in films with hydrogels exhibit a prolonged release in SGF
- Alginate-based films have more desirable sensory properties than the pectin-ones