Current Research in Food Science 5 (2022) 243–250

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Research Paper

Tannin-rich extracts improve the performance of amidated pectin as an alternative microencapsulation matrix to alginate

Silvia Molino [a], José Ángel Rufián Henares [a,b,**], Laura G. Gómez-Mascaraque [c,*]

[a] Departamento de Nutrición y Bromatología, Instituto de Nutrición y Tecnología de Alimentos, Centro de Investigación Biomédica, Universidad de Granada, Granada, Spain
[b] Instituto de Investigación Biosanitaria Ibs.GRANADA, Universidad de Granada, Granada, Spain
[c] Food Chemistry and Technology Department, Teagasc Moorepark Food Research Centre, Fermoy, Co. Cork, P61 C996, Ireland

ARTICLE INFO

Keywords:
Alginate
Pectin
Microencapsulation
Tannins
Quebracho
Chestnut

ABSTRACT

Microencapsulation of tannin extracts through extrusion-gelation method was performed comparing two alternative encapsulation matrices: alginate and amidated pectin. The microstructure of the generated microbeads was studied, as well as their microencapsulation efficiency and release properties. Overall, pectin-based beads performed better than their alginate-based counterparts. This, combined with a greater incorporation of tannins in the feed formulations led to a higher tannin load in the final beads. The best microencapsulation efficiency was given by pectin microbeads loaded with 10% tannin extract (w/w), but the final tannin content could be further increased by adding a 20% (w/w) concentration of the extracts. During a 14-days storage, only a marginal loss of tannins was recorded for pectin-based microbeads. The results reveal that great potential exists in producing pectin-based microbeads in presence of tannins, which allow better loading capacities and improving structural properties, thanks to the interactions between the tannins and the amidated polysaccharide.

1. Introduction

Tannins are bioactive compounds present in nature in many different families of the higher plants all over the world. The three main categories of tannins are hydrolysable, non-hydrolysable, followed by complex tannins, according to their structural characteristics and reactivity (Khanbabaee et al., 2001). It is well accepted that the biological role of these compounds is related to plant-environmental interactions. Thus, tannins are responsible for the plant growth regulation, as well as the preservation against external threats, such as infection, insects, or animal herbivory. The capacity to protect against these external hazards is associated to their property of precipitating proteins and the capability to form complexes also with polysaccharides and metals (Khanbabaee et al., 2001; Molino et al., 2019).

Many authors reported a plethora of positive health effects for tannins, especially those from quebracho and chestnut, related to their beneficial properties as antioxidant and radical scavenger agents and modulators of the gut microbiota, among others (Molino et al., 2018; Redondo et al., 2014). These properties make these products interesting additives for supplementing dietary products that benefit the human body. However, unfortunately the chemical mechanisms that give them some of their positive health effect (i.e. binding proteins) are also responsible for consumer rejection because of their bitter and astringent taste (Soares et al., 2018). On the other hand, the interaction of tannins with proteins and carbohydrates could also determine effects on the food matrix to which they are added (Molino et al., 2019). Thus, the addition of excessive amounts of tannins to foods could lead to alterations in their structure. In this context, microencapsulation represents an excellent solution to overcome these limitations. The coating or embedding of quebracho and chestnut tannins within a protective matrix would act as physical barrier, masking the chemical interactions with food and the salivary proteins.

In recent years, microencapsulation has been successfully used for the production of improved drug delivery systems in the food, cosmetics and pharmaceutical industries (Mohammadi et al., 2018). Indeed, this technique allows to stabilize biologically active ingredients in food systems (Santhanam et al., 2015), preserve or improve their bioactivity (Aguirre-Calvo, Molino, Perullini, Rufián-Henares and Santagapita,

* Corresponding author.
** Corresponding author. Departamento de Nutrición y Bromatología, Instituto de Nutrición y Tecnología de Alimentos, Centro de Investigación Biomédica, Universidad de Granada, Granada, Spain.
E-mail addresses: silviamolino@correo.ugr.es (S. Molino), jarufian@ugr.es (J.Á. Rufián Henares), Laura.Mascaraque@teagasc.ie (L.G. Gómez-Mascaraque).

https://doi.org/10.1016/j.crfs.2022.01.014
Received 6 October 2021; Received in revised form 11 January 2022; Accepted 17 January 2022
Available online 21 January 2022

2020b), avoid undesired flavors (Khor et al., 2017), add hydrophobic compounds to aqueous food matrices (Braithwaite et al., 2014), and improve their bioavailability (Paolino et al., 2016). Among various encapsulating methods used for food applications, hydrogel beads represent an interesting candidate, because the biopolymers are formulated in aqueous solutions and in mild conditions, but their structures made up of crosslinks ensure the integrity of the gelled beads once they are formed (Gómez-Mascaraque et al., 2019a). Extrusion is by far one the most widely techniques used to yield hydrogel microbeads, and consists of extruding a solution of the encapsulating matrix containing the bioactive ingredients through an orifice. The formation of droplets at the discharge point of the nozzle is followed by their dripping into a gelling bath and subsequent external gelation (Whelehan and Marison, 2011).

The most explored and exploited matrix for hydrogel microbeads formation is alginate, as it shows good gelling performance and easy handling. This polysaccharide obtained from different species of brown algae represents an excellent candidate for food applications because it is a biocompatible, biodegradable, non-toxic biomaterial, and is generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA) (Mohammadi et al., 2018). Alginate is an anionic copolymer of α-L-guluronic acid (G) and β-D-mannuronic acid (M), and can form hydrogels in the presence of di- and trivalent cations (e.g. calcium), which crosslink the G blocks, yielding an egg-box structure (Stokke et al., 2000). However, beads prepared with alginate present some drawbacks, such as high biomolecular leakage rate, low mechanical strength and large pore size (Krasaekoopt and Bhandari, 2012). Due to the pore size, probiotics (Gómez-Mascaraque et al., 2018) or cells (Qiao et al., 2014) are better encapsulated than smaller molecules, such as some polyphenols, whose encapsulation efficiency has been reported to be low (Goh et al., 2012).

Pectin is a biological processing by-product employed as gelling and thickening agent, and stabilizer in the food industry for a wide range of products such as dairy products, jams or bakery products, among others (Chan et al., 2017). Recently, this hydrocolloid is gaining more attention, as it can be employed also as dietary fibre, prebiotics and fat replacer (Gunness et al., 2021). Chemically, pectins are a group of anionic polysaccharides which have in common a backbone of α-1, 4-linked D-galacturonic acids (Chan et al., 2017). Depending on the different degree of esterification, pectins are divided into two different classes: high methoxyl pectin (more than 50% degree of esterification) and low methoxyl pectin (less than 50% degree of esterification). The different degree of esterification of the polymers plays a critical role in the mechanisms of gelation (Axelos and Thibault, 1991), in addition to their macromolecular properties (e.g. size, conformation and composition). Moreover, pectin could be also modified to improve their yield by replacing methyl-ester groups on carboxyl groups in high methoxyl pectin, obtaining primary amide groups. The aminolysis lead to the alteration of the properties of pectin, increasing the gelling ability of low methoxyl pectin, as less calcium and no elevated amount of sugars are need for the gelation. The degree of amidation also characterizes pectin (Chan et al., 2017), indicating the molar ratio of primary amide group present to galacturonic acids units (comprising both free and replaced).

The binding of pectins to cross-linking agents is weak, resulting in very porous networks. This has been attributed to the random distribution of binding sides in pectin chains, which result in a greater extent of defects during the formation of egg-box structures, as compared to alginates in which these binding sites are found in blocks along their chains (Fang et al., 2008). For this reason, pectins alone are not commonly used for microencapsulation, while blends with other biopolymers to improve their encapsulation properties have been previously proposed (Aguirre-Calvo, Molino, Perullini, Rufián-Henares and Santagapita, 2020a). Addition of tannins has been reported to enhance significantly the strength of both low and high methoxyl pectin gels, increasing also their gelling abilities (Mamet et al., 2017). The hydrogen bonds and hydrophobic interactions led to a new aggregated network formed by the gels containing tannins, enhancing the pectin hydrogel properties (Mamet et al., 2017). This could potentially enhance their application as encapsulation matrices. In view of that, our hypothesis was that tannins could be used not only as carried bioactive molecules, but at the same time they could act as enhancers for the formation of microbeads with improved microstructural characteristics.

The objective of our research was to compare the performance of two different polysaccharides, alginate and an amidated pectin, as encapsulation matrices for tannins through the extrusion-gelation method. Two different tannin extracts with distinct composition and structure were considered as representative of hydrolysable tannins (chestnut tannin extract) and condensed tannins (quebracho tannin extract). The microstructure of the various encapsulation systems developed was studied, as well as their microencapsulation efficiency and their release properties.

2. Materials and methods

2.1. Materials

Pure (>99%) food grade sodium alginate grade Manugel GHB (60–65% G, 50–100 mPa s) was kindly donated by DuPont Nutrition & Health (Norway) and its detailed characterization was previously reported by Gómez-Mascaraque et al. (2019b). Pectin AGLUPECTIN LA-20 P (degree of amidation 22–25, degree of methoxylation 20–25, protein content 0.5% (w/w)) was supplied by JRS Silvateam Food Ingredients S.r.l. Tannin extracts from quebracho (QUE) and chestnut (CHE) were provided by Silvateam Spa as powder. Both QUE and CHE were obtained by hot water extraction from wood and bark, respectively. The detailed composition of QUE was previously reported by Pasch et al. (2001) and it is a phytocomplex constituted by profisetinidin condensed tannins with a degree of polymerization (DP) up to 6.25 (Pasch et al., 2001). The composition of CHE, consisting of a mix of hydrolysable ellagitannins, with 30% of isomers castalagin and vescalagin as representative substances, was previously studied by Pasch et al. (2001). Reagents calcium chloride dihydrate, sodium citrate and potassium bromide were obtained from Sigma-Aldrich (Ireland).

2.2. Preparation of the feed formulations

Alginate 1.5% (w/v) and pectin 2% (w/v) aqueous solutions were prepared by magnetic stirring at room temperature. These concentrations were selected according to preliminary trials, achieving the maximum concentration which could be pumped through a 100 μm nozzle as described in Section 2.4. Lower concentrations were not sufficient to form uniform spheres. Increasing concentrations of QUE and CHE with respect to the mass of the different hydrocolloids (0, 10 and 20% w/w) were subsequently added to the solutions and mixed until a homogeneous dispersion was obtained. For alginate, the ratio of 20% w/w of tannins did not allow to obtain microbeads due to obstruction of the nozzle. Table 1 summarises the coding used to indicate the different systems developed.

2.3. Rheological properties of the formulations

The viscosity of the prepared aqueous solutions was analysed with an AR-2000 rheometer (TA Instruments, USA) with a parallel plate geometry (60 mm diameter and 500 μm gap) following a procedure adapted from Gómez-Mascaraque et al. (2015). Continuous shear rate ramps were performed from 0.1 to 200 s^{-1} over 12 min at a controlled temperature of 20 °C, following pre-shear treatment at 1 s^{-1} for 10 s and equilibration for 1 min.

2.4. Preparation of hydrogel beads

All solutions were filtered through 0.8 μm pore syringe filters for

Table 1
Coding used to indicate the different systems developed. Aqueous solution of alginate was prepared at 1.5% (w/v), while that of pectin at 2% (w/v). The concentrations of tannin extracts are referred with respect to the mass of the different hydrocolloids used (w/w). QUE, quebracho tannin extract; CHE, chestnut tannin extract.

Initial feed formulations		Freeze-dried microbeads	
AI	Alginate	**A**	Alginate
AC10I	Alginate + CHE 10% w/w	**AC10**	Alginate + CHE 10% w/w
AQ10I	Alginate + QUE 10% w/w	**AQ10**	Alginate + QUE 10% w/w
AC20I	Alginate + CHE 20% w/w		
AQ20I	Alginate + QUE 20% w/w		
PI	Pectin	**P**	Pectin
PC10I	Pectin + CHE 10% w/w	**PC10**	Pectin + CHE 10% w/w
PQ10I	Pectin + QUE 10% w/w	**PQ10**	Pectin + QUE 10% w/w
PC20I	Pectin + CHE 20% w/w	**PC20**	Pectin + CHE 20% w/w
PQ20I	Pectin + QUE 20% w/w	**PQ20**	Pectin + QUE 20% w/w

aqueous media (Sartorius, Germany). Microbeads were produced according to Gómez-Mascaraque et al. (2019b) with slight modifications. We used an InotechEncapsulator IER-50 (Inotech Biosystems Intl. Inc., Switzerland), to generate alginate/pectin microbeads by extrusion of the solutions through a 100 μm nozzle at a flow rate of 2.5 mL/min into the gelling bath (140 mm diameter) containing 250 mL of 0.1 M $CaCl_2$ solution.

The gelling bath was located at a distance of 16 cm from the nozzle and maintained under constant agitation (stirrer speed on the encapsulator fixed at 5). Alginate/pectin droplet formation and break up was aided by a nozzle vibration frequency of 1240 Hz and an applied voltage of 1.3 kV, as optimized in preliminary trials. The collection time was set at 4 min for each batch, and the microbeads were cured within the gelling solution for 90 min before being filtered and thoroughly washed with deionized water. The produced microbeads were stored at −80 °C and freeze-dried with a FreeZone benchtop freeze-drier (Labconco, USA), until further analysis.

2.5. Water content

To estimate the water content, the beads (ca. 1 g) were filtered and accurately weighted before and after freeze-drying. The quantity of water was calculated according to Equation (1), where m_h is the mass of the hydrated beads and m_d is the mass of the dried beads. Measurements were performed in three independent batches.

$$Water\ (\%) = \frac{m_h - m_d}{m_h} \times 100 \quad (1)$$

2.6. Morphological characterization of the hydrogel beads

Bead morphology was studied by optical microscopy, taking images at 4× magnification using a digital microscopy system Olympus B×51 (Olympus Corporation, Japan). A digital camera head ProgRes CT3 (Jenoptik, Jena, Germany) and ProgRes CapturePro software (v 2.10.0.0) were used for image capturing. Size distributions were obtained from a minimum of 200 measurements, using the ImageJ (v. 1.52q) software.

2.7. Tannins content and antioxidant activity

The content of tannins in the microbeads was estimated using two different methods, summarised below: UV 280 nm assay and Folin-Ciocalteu (quantification of phenolic content) assay. Their antioxidant activity was also assessed through the ABTS assay. Both the feed solutions and the microbeads were analysed. A weight of 30 mg of dried beads was dissolved in 5 ml of sodium citrate (2% w/v) solution under magnetic stirring for 24 h, in order to dissolve the polysaccharides and release their contents.

UV 280 nm assay. The tannin content was directly determined by measuring the optical density at 280 nm using a 10-mm quartz cuvette (Piccardo and González-Neves, 2013), with a Cary 100 Bio UV–Vis spectrophotometer (Agilent, Ireland). Calibration was performed using aqueous solutions of the original tannin extracts (5–100 ppm) as reference standards.

Folin-Ciocalteu assay. The protocol of Moreno-Montoro et al. (2015) was slightly modified. Briefly, 60 μL of sodium carbonate (10%) were mixed with 15 μL of Folin-Ciocalteu reagent, 30 μL of sample and 195 μL of distilled water. The samples were incubated at 37 °C for 60 min, before measuring the absorbance at a wavelength of 595 nm. The amount of phenolic compounds was determined according to a calibration curve obtained with aqueous solutions of the original tannin extracts, CHE or QUE, (1–100 ppm) as reference standards.

ABTS assay. The antioxidant capacity was estimated in terms of radical scavenging activity, which was evaluated following the procedure by Re et al. (1999). Calibration was performed with aqueous solutions of the original tannin extracts, CHE or QUE, used as reference standard (1–50 ppm).

For ABTS and Folin-Ciocalteu assays, a Synergy HT microplate reader (Bio-Tek Instruments, USA) with temperature control (37 °C) was used to measure the absorbance at the aforementioned wavelengths on transparent 96-well polystyrene microplates (Biogen Científica, Spain).

All the measurements were performed in triplicate on independent duplicates and results were expressed as % mg tannins/mg microbeads. These three different techniques were used as direct or indirect methods to estimate the presence of tannins in the measured samples. All of them were used to calculate the microencapsulation efficiency (ME%) for both fresh and dried microbeads, and to study the effect of storage on the retention of tannins.

2.8. Microencapsulation efficiency (ME %)

The ME % was calculated according to the equation below:

$$ME(\%) = \frac{TTC_m}{TTC_i} \times 100 \quad (2)$$

where TTC_m was the total tannin content in the microbeads, and TTC_i was the total tannin content measured in the initial feed solutions used for microencapsulation, estimated as described in Section 2.7.

2.9. Release of tannins during storage

To study the effect of storage of fresh beads on the retention of tannins, the content of tannins within the samples was analysed at different times during storage. The first sampling was carried out after 90 min of curation in the gelling bath (T0). After that, 7 batches of 1.5 g of fresh beads were washed with distilled water and stored in 10 ml of distilled water, under refrigeration (at 4 °C). After selected time intervals (1 h, 3 h, 6 h, 24 h, 48 h, 7 d, 14 d) one batch of samples was filtered, stored at −80 °C and freeze-dried until further analysis. Tannin retention is expressed as percentage of the tannins initially encapsulated remaining in the beads after each selected time period.

$$Tannin\ release\ \% = \frac{TTC_{Tx}}{TTC_{T0}} \times 100 \quad (3)$$

where TTC_{Tx} was the total tannin content in the microbeads measured at each of the selected time intervals and TTC_{T0} was total tannin content in the microbeads measured at T0.

2.10. Statistical analysis

One-way ANOVA with Bonferroni post-hoc test was used to assess the statistical significance of the differences among samples. To compare two independent samples *t*-Student's test was performed. All the

statistical analyses were performed using the SPSS software (version 23, SPSS, Chicago, IL, United States).

3. Results and discussion

3.1. Rheological properties of the feed formulations

The viscosity of the different solutions prepared with alginate (AI) or pectin (PI), and adding different concentrations (10 and 20% w/w with respect to the mass of the different hydrocolloids) of quebracho tannin extract (AQ10I, AQ20I, PQ10I, PQ20I) or chestnut tannin extract (AC10I, AC20I, PC10I, PC20I) was measured. The rheological behaviour of biopolymer systems is the result of the interactions between macromolecules within them when subjected to mechanical stress, and therefore provides relevant insights about their molecular structure and interactions between components. The flow behaviour of the all the evaluated solutions was Newtonian, with a linear relationship between the shear stress and the shear rate. Thus, the viscosity was calculated from the slope of the shear stress *vs*. shear rate curves (Table 2) (Gunness et al., 2021).

The viscosity was statistically significant ($p < 0.05$) between the alginate- and the pectin-based feed formulations. This was expected given their different molecular structures. In particular, even though pectin was used in higher concentrations than alginate (2% w/v and 1.5% w/v respectively), its solutions resulted to be significantly less viscous (PI 0.104 ± 0.009 Pa s; AI 0.130 ± 0.014 Pa s). The addition of the tannin extracts, QUE and CHE, regardless of their different composition, did not result in significant viscosity changes. However, when tannins were added in the 20% w/w ratio to the alginate solutions (AQ20I, AC20I), a frequent obstruction of the nozzle was observed, which resulted in a greater difficulty to obtain microbeads by extrusion. For this reason, AQ20I and AC20I were discarded for the purposes of microbeads production.

3.2. Morphology

Fig. 1 shows optical micrographs of the obtained microbeads. In the case of alginate, both with and without the addition of tannins, spherical microbeads with no defects were obtained, as expected for the polysaccharide most widely used for microencapsulation purposes. On the contrary, the use of pectin alone (P) led to the formation of beads with a very irregular shape or a small vermiform appendix, and often the beads appeared broken or non-spherical (highlighted with arrows). These defects could have been partially due to the lower viscosity of pectin solutions compared to that of alginate. As several authors report, if the viscosity is too low, when the droplet impacts on the gelling bath, surface tension forces are too weak to counteract the effect of the impact an drag forces in the gelling bath, with a consequent deformation (Davarcı et al., 2017). Moreover, as discussed above, pectins and alginates have a different gelling mechanism, the former yielding weaker gels. It has to be taken into account that usually pectin is employed as excipient to improve the microstructure of other hydrogel network systems, in terms of size, compactness as well as their interconnectivity (Díaz-Rojas et al., 2004), rather than on its own.

The pectin used in this work was characterised by a certain level of amidation. This chemical modification of pectin is normally carried out to improve its gelling properties, without requiring the addition of other ingredients, such as sugars, in certain cases (Chan et al., 2017). As commented on above, despite the amidation, pectin alone did not result in good bead formation. However, the addition of tannins improved the shape of the microbeads considerably. Although PC10 and PQ10 beads still presented some imperfections, marked with arrows in Fig. 1, a further increase in tannin concentration allowed obtaining more spherical microbeads almost free of defects. In particular, PC20 had the most regular spherical shape among the pectin microbeads. The improved bead formation was therefore attributed to the presence of tannins, and their interactions with pectin.

It is understood that the presence of amide groups in proteins plays a role in their binding to tannins. Some authors also reported that tannins can react with non-protein organic N compounds similarly to their reaction with proteins, These interactions, that are influenced by the concentration and chemical structure, are facilitated by the ability of amide groups to form multiple hydrogen bonds with negatively charged carboxylate groups and their free electron pair (Adamczyk et al., 2011; Buchweitz et al., 2013). This mechanism by which amide-containing compounds form complexes with tannins would explain the improved hydrogel network formation in the pectin-based microbeads. To our knowledge, this is the first study that describes the successful production of pectin microbeads obtained without blending with other biopolymers, aided by the addition of tannin-rich extracts.

Table 2
Viscosity of the feed formulations. Results are expressed as Pa·s. Different letters indicate statistically significant differences by ANOVA and Bonferroni post-hoc tests ($p < 0.05$).

	Viscosity (Pa•s)	
AI	0.130^{A}	±0.014
AC10I	0.128^{A}	±0.008
AQ10I	0.128^{A}	±0.018
AC20I	0.129^{A}	±0.009
AQ20I	0.136^{A}	±0.006
PIF	0.104^{B}	±0.009
PC10I	0.109^{B}	±0.003
PQ10I	0.109^{B}	±0.003
PC20I	0.101^{B}	±0.005
PQ20I	0.101^{B}	±0.005

3.3. Size and water content of microbeads

Fig. 2 reports the size distribution and water content of the microbeads produced through the extrusion-external gelation method. Alginate microbeads were significantly smaller than pectin microbeads ($p < 0.001$). In general, when vibrating nozzle technologies are used for bead production, greater viscosities result in greater droplet sizes (Del Gaudio, Colombo, Colombo, Russo and Sonvico, 2005). Accordingly, given the higher viscosity of alginate-based feed formulations, bigger bead sizes could have been expected. However, other factors play a role in determining the bead size, such as the different gelling mechanisms of the polymers. The hydrogel networks that pectins form through the egg-box mechanism have been described to entail more defects than those of alginates (Fang et al., 2008), which explains the greater extent of shrinkage that the alginate droplets experienced upon crosslinking to form micro hydrogels. In fact, pectin beads without added tannins were the largest and most polydisperse among all (D = 273 ± 19 μm).

Interestingly, the addition of tannins resulted in a statistically significant reduction ($p < 0.05$) in bead size (PC10 D = 237 ± 12 μm; PQ10 D = 236 ± 13 μm; PC20 D = 254 ± 12 μm; PQ20 D = 241 ± 16 μm) compared to those obtained with pectin alone. The size of alginate beads also decreased slightly with the addition of tannins (A D = 222 ± 11 μm; AC10 D = 211 ± 10 μm; AQ10 D = 220 ± 9 μm), but to a lesser extent, which was not even significant in the case of quebracho tannins. Since no significant changes in viscosity were observed upon addition of the tannins, the decrease in size in the case of pectin was attributed to the interactions between the polysaccharide and the tannins discussed previously. These acted as cross-linkers, resulting in an improved hydrogel network formation and therefore less swelling, which was also confirmed by a lower water content in these beads. Previously, it has been described that tannic acid could act effectively as a cross-linking agent to harden the microbead structure, when obtained through complex coacervation (Zhang et al., 2011). Moreover, Mamet et al. (2017) reported that persimmon condensed tannins with a low degree of polymerization (DP 5), similar to those used in the present study,

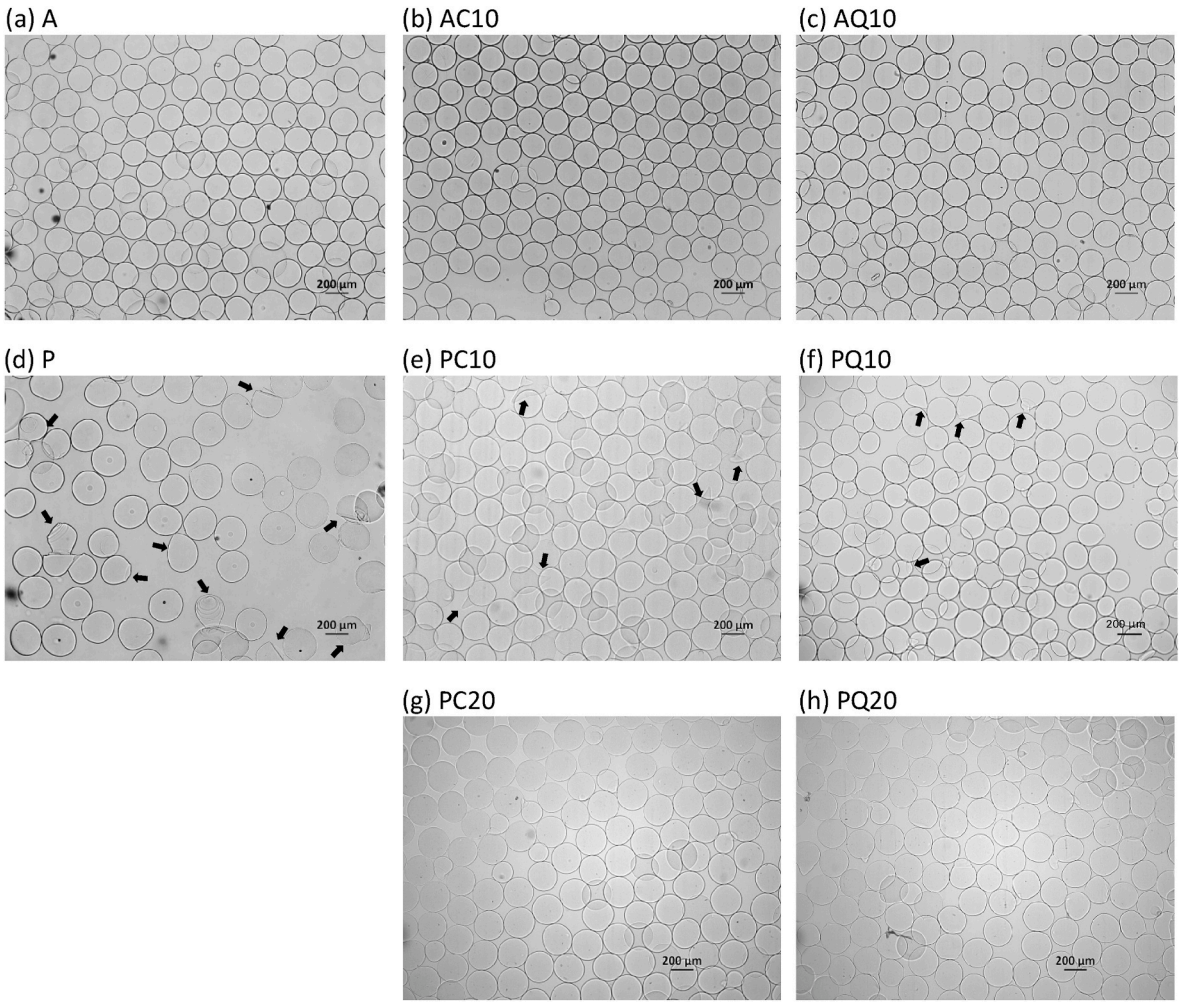

Fig. 1. Micrographs of the different systems of microbeads. Arrows in the images indicate bead defects. (a) A, (b) AC10, (c) AQ10, (d) P, (e) PC10, (f) PQ10, (g) PC20, (h) PQ20.

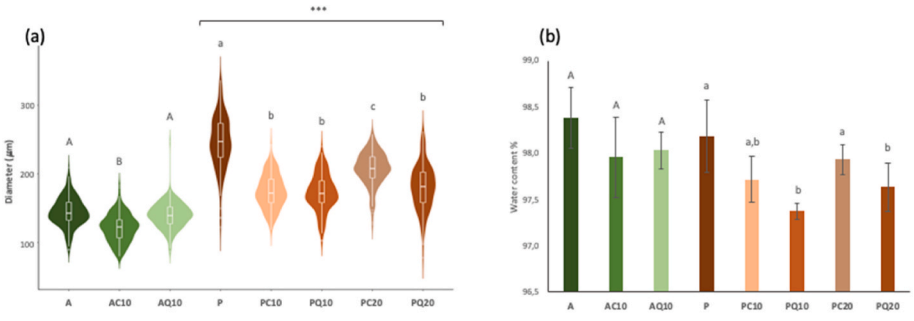

Fig. 2. Size distribution and water content of the microbeads. **(A)** Size distribution reported as diameter (μm) obtained from at least 200 microbeads per sample. *** Indicate statistically significant differences ($p < 0.001$) by Student's t-test between alginate- and pectin-based microbeads. Capital and small letters indicate statistically significant differences by ANOVA and Bonferroni post-hoc test ($p < 0.05$) among alginate- and pectin-based microbeads, respectively. **(B)** Water content of the microbeads. Capital and small letters indicate statistically significant differences by ANOVA and Bonferroni post-hoc test ($p < 0.05$) among alginate- and pectin-based microbeads, respectively. Three independent batches were analysed per sample.

improved pectin gelling properties (Mamet et al., 2017). In fact, these had a greater impact than those with higher degree of polymerization, as they could penetrate into the junction zones and stabilize them through hydrogen bonds, while the latter could not get into the junctions due to stearic hindrance. In view of these results, tannins can not only be used as bioactive compounds to be delivered with the microbeads, but also as cross-linkers to enhance pectin beads formation.

These findings are confirmed by the water content results. The presence of tannins resulted in a lower water content, thus the generation of smaller and denser microbeads. In the case of alginate the greatest changes are induced by the presence of chestnut extract, while when pectin was used, the smallest beads were obtained with the quebracho extract. This could be attributed to the different chemical structure of the tannins in both extracts, that seemed to play a role in the extent of interactions with the two biopolymers. As we reported in Section 2.1, the two tannin extracts are characterised by different chemical compositions, in particular CHE contains hydrolysable tannins while QUE is characterised by the presence of condensed tannins. This suggests that several factors, such as tannin molecular weight and degree of galloylation, may play a critical role on the affinity for pectin,

and resulted in one extract performing better than the other (Molino et al., 2019).

3.4. Microencapsulation efficiency

There is no universal method to quantify a phytocomplex such as those examined in this study. Therefore, the capacity of retention of tannins (CHE or QUE) by the different microencapsulation systems was tested using three different spectrophotometric techniques, i.e. UV 280 nm, ABTS and Folin-Ciocalteu assays, to provide an overview by evaluating different aspects: the presence of tannins in solution, as these compounds exhibit strong absorption at 280 nm, the scavenging capacity and the total polyphenol content, respectively. In order to reflect as precisely as possible the amount of tannins contained in the samples examined, reference calibration curves were generated with CHE or QUE for samples containing the respective extracts, and the obtained results were expressed as mg of tannins (CHE or QUE) per mg of encapsulation matrix (Table S1 of the Supplementary Material). Finally, the ME% was calculated as the percentage of the tannin content in the original feed solutions that was detected in the beads (Table 3). Although with slight variations in the absolute values, the results showed the same trends for the three techniques.

Pectin microbeads showed significantly better encapsulation efficiencies compared to alginate microbeads. Alginate is generally regarded as the polymer of choice for microencapsulation through extrusion-external gelation. Its success is due to the simplicity of the method to gel it, low cost and good biocompatibility. However, one of its main disadvantages is related to the high porosity of the beads, which is responsible for a final low encapsulation efficiency for small compounds. Indeed, the ME% for all the alginate-tannin systems studied in this work was very low (<16%). For this reason, alginate is mainly used as a delivery vehicle for large bioactive agents such as cells or probiotics, although in some cases this polymer has again proved to be scarcely efficient (Krasaekoopt and Bhandari, 2012). To mitigate these defects, the blending with other compounds or coating the microbeads is often necessary, especially when trying to entrap water-soluble and small bioactive compounds (Krasaekoopt and Bhandari, 2012; Mohammadi et al., 2018).

Pectin has received considerably less attention as a basic material for microencapsulation because of its more inefficient gelling mechanism which, as discussed in the previous section, results in beads with a greater extent of defects. The results in Table 3, however, show that the formation of interactions between pectin and tannins previously discussed not only results in microbeads with improved morphological characteristics, but also in a better encapsulation efficiency compared to alginate. The extent of interactions between the tannins and pectin through hydrogen bonds thus seemed to be greater compared to those with alginate, presumably enhanced by the presence of amide groups. It is worth mentioning that the pectin used for the study also contained a small amount of protein impurities (0.5%). The well-known ability of tannins to bind proteins, by establishing cross-links with the implication of a different nature of bonds, such as hydrophobic interactions and hydrogen bonds (Molino et al., 2019), might also have partially contributed to the improvement in ME%.

As expected, the tannins content of microbeads PC20 and PQ20 was the highest, since a greater amount of tannins was added to the feed formulations used to produce these samples. However, their encapsulation efficiencies were lower than those of samples PC10 and PQ10, which showed the best results. The microencapsulation efficiency generally decreases as the ratio matrix-to-encapsulated compound decreases (L. G. Gómez-Mascaraque and Lopez-Rubio, 2019), Indeed, by increasing the concentration of bioactive compound in PC20 and PQ20, the amount of pectin will be proportionally lower compared to PC10 and PQ10, leading to a lower retention by the matrix. In this particular case, the interactions in the pectin-tannin system seemed to reach a saturation point, beyond which further quantities of the bioactive compounds cannot be retained.

Table 3
Microencapsulation efficiency (ME %) measured with three different methods: UV 280 nm, ABTS and Folin-Ciocalteu assay and expressed as a percentage. Data are reported as means ± SD. CHE, chestnut tannin extract; QUE, quebracho tannin extract; Different capital letters indicate statistically significant differences within the same extract and method of analysis ($p < 0.05$) by ANOVA and Bonferroni post-hoc tests.

		UV 280 nm	Folin-Ciocalteu	ABTS
CHE	**AC10**	15.19 ± 0.794^{A}	6.217 ± 0.433^{A}	9.311 ± 0.544^{A}
	PC10	48.42 ± 12.20^{B}	33.20 ± 1.838^{B}	40.11 ± 4.593^{B}
	PC20	32.20 ± 1.318^{C}	27.53 ± 2.121^{C}	26.07 ± 1.593^{C}
QUE	**AQ10**	7.710 ± 0.785^{A}	4.889 ± 0.957^{A}	8.998 ± 0.571^{A}
	PQ10	38.37 ± 1.174^{B}	29.06 ± 4.451^{B}	53.73 ± 5.637^{B}
	PQ20	22.19 ± 1.177^{C}	28.11 ± 3.319^{B}	24.38 ± 3.709^{C}

3.5. Release of tannins during storage

The changes in the content of bioactive compounds during storage is an important factor to evaluate when working with products intended for functional food applications, to ensure that the activity of the compounds of interest is maintained during the shelf-life of the products. In the specific case of water soluble compounds encapsulated within hydrogel microbeads, it is of particular interest that the diffusion of these compounds out of the beads is as limited as possible. To assess the release of the tannins from the prepared beads during storage, the alginate and pectin systems containing the greatest tannins content (i.e alginate AC10 and AQ10 beads and pectin PC20 and PQ20 beads) were selected, and their content of tannins was monitored over a period of 14 days. Despite exhibiting a lower microencapsulation efficiency than PC10 and PQ10, PC20 and PQ20 were selected among the pectin-based systems for two reasons: their improved morphology and the greater amount of tannins they were able to carry compared to PC10 and PQ10. Fig. 3 illustrates the results obtained through three different methods (UV 280 nm, ABTS and Folin-Ciocalteu assays).

In general, a more sustained release was observed for pectin-based microbeads. In the case of alginate-based beads, a gradual and continuous release of the tannin extracts was observed over time, for both CHE and QUE. As reported by Goh et al. (2012) the release of water soluble compounds from alginate occurs by diffusion, and it mainly depends on the porosity of the beads, and in particular the pore size. Some approaches such as drying have been proposed to reduce the matrix porosity and thus limit the loss of bioactive compounds from the beads (Goh et al., 2012). While drying is a feasible approach to preserve ingredients, including microbeads, once incorporated within the final food products the diffusion towards the aqueous phase would resume, given that most foods have a high water content.

Pectin beads showed a reduced loss of tannin content, compared to alginate, especially through the Folin-Ciocalteu method, for which the loss of tannin content over the 14 days was AC10 = 77%, AQ10 = 81%, PC20 = 29%, PQ20 = 15%. These results again confirm the greater extent of interactions between tannins and pectin that allowed not only a greater encapsulation efficiency than for alginate-based systems, but also a slower release of the tannins and therefore a greater stability of the product over time.

The differences in release patterns were less obvious when monitored using the UV 280 nm method, and a similar trend was observed for all encapsulation systems through the ABTS assay. The differences between the results obtained by the three methods are due to the fact that they evaluate different parameters. Not all the phenolic compounds in a complex extract exhibit the same absorbance at 280 nm, nor have the same radical scavenging activity. On the contrary, not only is each individual phenolic compound unique in its chemical structure and functionality, but their antioxidant activity varies considerably depending on a number of factors, including the oxidation and

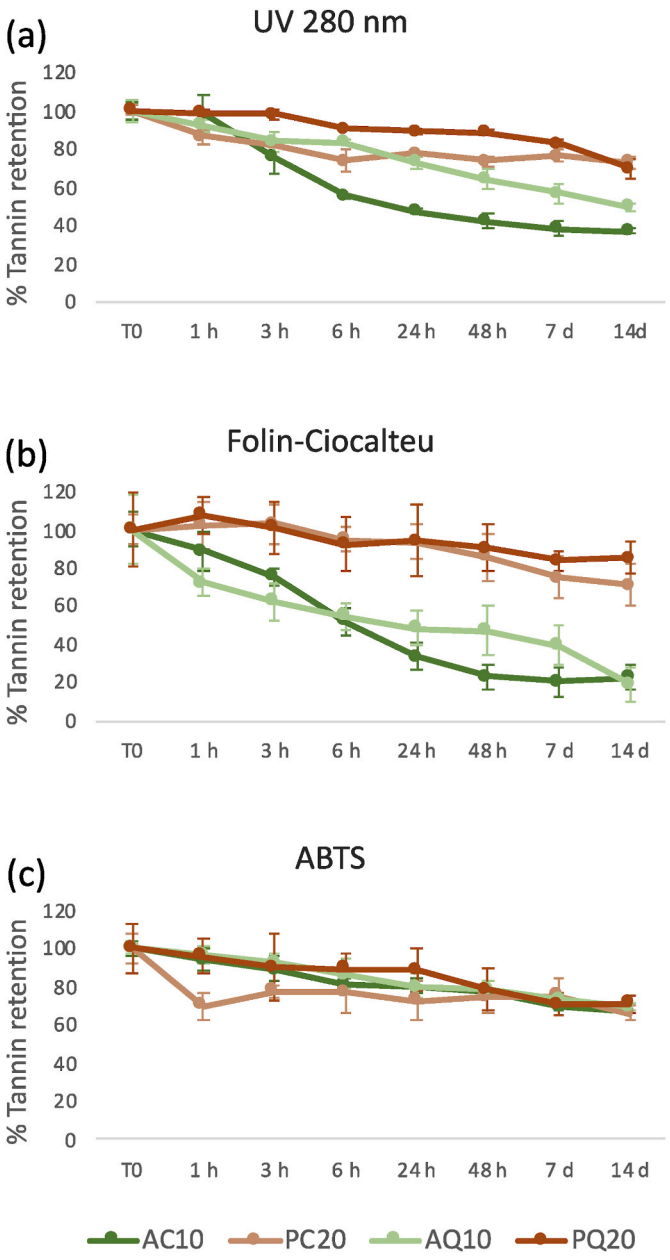

Fig. 3. Effect of long-term storage on ME% of fresh microbeads, evaluated with three different methods: (a) UV 280 nm, (b) Folin-Ciocalteu and (c) ABTS assays. Tannin retention is expressed as percentage of the tannins initially encapsulated (ME%) remaining in the beads after each selected time period. Measurements were performed in triplicate on independent duplicates.

oligomerization extent, and their interactions with other compounds (Falcó et al., 2018; Gómez-Mascaraque et al., 2016). As a result, although for pectin the decrease of tannins content is comparable to that of the their radical scavenging activity, for alginate the great loss in phenolics content over the 14 days of storage was not reflected in a similar loss in radical scavenging capacity. This suggests that the compounds that are still entrapped in the alginate network after day 14 are also the main contributors to antioxidant capacity of the extracts.

4. Conclusions

In this work, amidated pectin is proposed as an alternative encapsulation matrix to the most widely used biopolymer alginate, for the microencapsulation of tannin extracts through external gelation. In general, pectin-based beads were found to exhibit better performance than their alginate-based counterparts, which was attributed to the greater extent of interactions between the bioactive compounds and this amidated polysaccharide.

Most of the tannin content was lost during the encapsulation process for the alginate-based beads, as expected for water-soluble compounds with a relatively low molecular weight. However, the interactions between the tannins and pectin limited the loss of bioactive compounds from this matrix, resulting in a significant increase in encapsulation efficiency, from 2-fold to almost 6-fold (depending on the tannin extract and the method of analysis used). Being less viscous, pectin also allowed to incorporate a greater amount of tannins in the feed formulations that, combined with the better encapsulation efficiency of this matrix, also resulted in a greater tannin load in the final beads. Pectin microbeads loaded with 10% tannin extract (w/w with respect to pectin) yielded the best encapsulation efficiency, although the final tannin content could be further increased by adding a 20% (w/w) concentration of the extracts.

As the beads are intended for food use, the release of the tannins during storage was assessed over a period of two weeks. The results revealed that only a slight loss of tannins occurred during this period for the pectin-based beads, suggesting that the proposed encapsulation system would be a better alternative to alginate for incorporating phenolic compounds into fresh foods with a high water content.

Not only did the amidated pectin achieve greater microencapsulation efficiencies and loading capacities for both tannin extracts used in this work, and a more sustained release of the phenolic compounds, but incorporating tannins within the pectin matrices also proved to enhance the structural properties of the beads themselves, achieving improved morphologies, smaller bead sizes and reduced extent of swelling.

These promising results could be further improved by exploring strategies that have already been applied to alginate microbeads to enhance the encapsulation efficiency and delay the release of water-soluble bioactive compounds, such as the addition of fillers and/or external coatings. On the other hand, to validate their application in food systems, it is important that future work addresses the study of the structural changes that the proposed encapsulation system are subjected to during food processing and gastrointestinal digestion, and thus the subsequent release of the bioactive compounds.

Funding

This work was funded by Teagasc, the Irish Agricultural and Food Development Authority (Fermoy, Ireland), by the "Plan propio de Investigación y Transferencia" of the University of Granada under both programs "Intensificación de la Investigación, modalidad B" and by the European Research Commission (Research Executive Agency) under the research project Stance4Health (Grant Contract N 816303) granted to José A. Rufián-Henares.

CRediT authorship contribution statement

Silvia Molino: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **José Ángel Rufián Henares:** Validation, Supervision, Funding acquisition. **Laura G. Gómez-Mascaraque:** Conceptualization, Methodology, Validation, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This paper will form part of the doctoral thesis of S.M., conducted within the context of the "Nutrition and Food Sciences Program" at the University of Granada.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.01.014.

References

Adamczyk, B., Adamczyk, S., Smolander, A., Kitunen, V., 2011. Tannic acid and Norway spruce condensed tannins can precipitate various organic nitrogen compounds. Soil Biol. Biochem. 43 (3), 628–637. https://doi.org/10.1016/j.soilbio.2010.11.034.

Aguirre-Calvo, T.R., Molino, S., Perullini, M., Rufián-Henares, J.Á., Santagapita, P.R., 2020a. Effect of in vitro digestion-fermentation of Ca(II)-alginate beads containing sugar and biopolymers over global antioxidant response and short chain fatty acids production. Food Chem. 333 (August 2019), 127483. https://doi.org/10.1016/j.foodchem.2020.127483.

Aguirre-Calvo, T.R., Molino, S., Perullini, M., Rufián-Henares, J.Á., Santagapita, P.R., 2020b. Effects of: in vitro digestion-fermentation over global antioxidant response and short chain fatty acid production of beet waste extracts in Ca(ii)-alginate beads. Food Funct. 11 (12), 10645–10654. https://doi.org/10.1039/d0fo02347g.

Axelos, M.A.V., Thibault, J.-F., 1991. The chemistry of low-methoxyl pectin gelation. In: The Chemistry and Technology of Pectin. ACADEMIC PRESS, INC, pp. 109–118. https://doi.org/10.1016/b978-0-08-092644-5.50011-x.

Braithwaite, M.C., Tyagi, C., Tomar, L.K., Kumar, P., Choonara, Y.E., Pillay, V., 2014. Nutraceutical-based therapeutics and formulation strategies augmenting their efficiency to complement modern medicine: an overview. J. Funct.Foods 6 (1), 82–99. https://doi.org/10.1016/j.jff.2013.09.022.

Buchweitz, M., Speth, M., Kammerer, D.R., Carle, R., 2013. Impact of pectin type on the storage stability of black currant (Ribes nigrum L.) anthocyanins in pectic model solutions. Food Chem. 139 (1–4), 1168–1178. https://doi.org/10.1016/j.foodchem.2013.02.005.

Chan, S.Y., Choo, W.S., Young, D.J., Loh, X.J., 2017. Pectin as a rheology modifier: origin, structure, commercial production and rheology. Carbohydr. Polym. 161, 118–139. https://doi.org/10.1016/j.carbpol.2016.12.033.

Davarcı, F., Turan, D., Ozcelik, B., Poncelet, D., 2017. The influence of solution viscosities and surface tension on calcium-alginate microbead formation using dripping technique. Food Hydrocolloids 62, 119–127. https://doi.org/10.1016/j.foodhyd.2016.06.029.

Del Gaudio, P., Colombo, P., Colombo, G., Russo, P., Sonvico, F., 2005. Mechanisms of formation and disintegration of alginate beads obtained by prilling. Int. J. Pharm. 302 (1–2), 1–9. https://doi.org/10.1016/j.ijpharm.2005.05.041.

Díaz-Rojas, E.I., Pacheco-Aguilar, R., Lizardi, J., Argüelles-Monal, W., Valdez, M.A., Rinaudo, M., Goycoolea, F.M., 2004. Linseed pectin: gelling properties and performance as an encapsulation matrix for shark liver oil. Food Hydrocolloids 18 (2), 293–304. https://doi.org/10.1016/S0268-005X(03)00085-7.

Falcó, I., Randazzo, W., Gómez-Mascaraque, L.G., Aznar, R., López-Rubio, A., Sánchez, G., 2018. Fostering the antiviral activity of green tea extract for sanitizing purposes through controlled storage conditions. Food Control 84, 485–492. https://doi.org/10.1016/j.foodcont.2017.08.037.

Fang, Y., Al-Assaf, S., Phillips, G.O., Nishinari, K., Funami, T., Williams, P.A., 2008. Binding behavior of calcium to polyuronates: comparison of pectin with alginate. Carbohydr. Polym. 72 (2), 334–341. https://doi.org/10.1016/j.carbpol.2007.08.021.

Goh, C.H., Heng, P.W.S., Chan, L.W., 2012. Alginates as a useful natural polymer for microencapsulation and therapeutic applications. Carbohydr. Polym. 88 (1), 1–12. https://doi.org/10.1016/j.carbpol.2011.11.012.

Gómez-Mascaraque, L.G., Llavata-Cabrero, B., Martínez-Sanz, M., Fabra, M.J., López-Rubio, A., 2018. Self-assembled gelatin-ι-carrageenan encapsulation structures for intestinal-targeted release applications. J. Colloid Interface Sci. 517, 113–123. https://doi.org/10.1016/j.jcis.2018.01.101.

Gómez-Mascaraque, L.G., Lopez-Rubio, A., 2019. Encapsulation of plant-derived bioactive ingredients through electrospraying for nutraceuticals and functional foods applications. Curr. Med. Chem. 27 (17), 2872–2886. https://doi.org/10.2174/0929867326666191010115343.

Gómez-Mascaraque, L.G., Martínez-Sanz, M., Fabra, M.J., López-Rubio, A., 2019a. Development of gelatin-coated ι-carrageenan hydrogel beads by electric field-aided extrusion. Impact of phenolic compounds on their performance. Food Hydrocolloids 90, 523–533. https://doi.org/10.1016/j.foodhyd.2018.12.017. December 2018.

Gómez-Mascaraque, L.G., Martínez-Sanz, M., Hogan, S.A., López-Rubio, A., Brodkorb, A., 2019b. Nano- and microstructural evolution of alginate beads in simulated gastrointestinal fluids. Impact of M/G ratio, molecular weight and pH. Carbohydr. Polym. 223 (April), 115121. https://doi.org/10.1016/j.carbpol.2019.115121.

Gómez-Mascaraque, L.G., Sanchez, G., López-Rubio, A., 2016. Impact of molecular weight on the formation of electrosprayed chitosan microbeads as delivery vehicles for bioactive compounds. Carbohydr. Polym. 150, 121–130. https://doi.org/10.1016/j.carbpol.2016.05.012.

Gómez-Mascaraque, L., Lagarón, J.M., López-Rubio, A., 2015. Electrosprayed gelatin submicroparticles as edible carriers for the encapsulation of polyphenols of interest in functional foods. Food Hydrocolloids 49, 42–52. https://doi.org/10.1016/j.foodhyd.2015.03.006.

Gunness, P., Zhai, H., Williams, B.A., Zhang, D., Gidley, M.J., 2021. Pectin and mango pulp both reduce plasma cholesterol in pigs but have different effects on triglycerides and bile acids. Food Hydrocolloids 112 (September 2020), 106369. https://doi.org/10.1016/j.foodhyd.2020.106369.

Khanbabaee, K., van Ree, T., Ree, T. van, 2001. Tannins: classification and definition. Nat. Prod. Rep. 18 (6), 641–649. https://doi.org/10.1039/b101061l.

Khor, C.M., Ng, W.K., Kanaujia, P., Chan, K.P., Dong, Y., 2017. Hot-melt extrusion microencapsulation of quercetin for taste-masking. J. Microencapsul. 34 (1), 29–37. https://doi.org/10.1080/02652048.2017.1280095.

Krasaekoopt, W., Bhandari, B., 2012. Properties and applications of different probiotic delivery systems. In: Limited, W.P. (Ed.), Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals. Elsevier Masson SAS, pp. 541–594. https://doi.org/10.1533/9780857095909.4.541.

Mamet, T., Yao, F., Li, K. kai, Li, C. mei, 2017. Persimmon tannins enhance the gel properties of high and low methoxyl pectin. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 86 (October), 594–602. https://doi.org/10.1016/j.lwt.2017.08.050.

Mohammadi, N., Ehsani, M.R., Bakhoda, H., 2018. Development of caffeine-encapsulated alginate-based matrix combined with different natural biopolymers, and evaluation of release in simulated mouth conditions. Flavour Fragrance J. 33 (5), 357–366. https://doi.org/10.1002/ffj.3452.

Molino, S., Casanova, N.A., Rufián Henares, J.Á., Fernandez Miyakawa, M.E., 2019. Natural tannin wood extracts as a potential food ingredient in the food industry. J. Agric. Food Chem. 68 (10), 2836–2848. https://doi.org/10.1021/acs.jafc.9b00590.

Molino, S., Fernández-Miyakawa, M., Giovando, S., Rufián-Henares, J.Á., 2018. Study of antioxidant capacity and metabolization of quebracho and chestnut tannins through in vitro gastrointestinal digestion-fermentation. J. Funct.Foods 49 (May), 188–195.

Moreno-Montoro, M., Olalla-Herrera, M., Gimenez-Martinez, R., Navarro-Alarcon, M., Rufián-Henares, J.A., 2015. Phenolic compounds and antioxidant activity of Spanish commercial grape juices. J. Food Compos. Anal. 38, 19–26. https://doi.org/10.1016/J.JFCA.2014.10.001.

Paolino, D., Vero, A., Cosco, D., Pecora, T.M.G., Cianciolo, S., Fresta, M., Pignatello, R., 2016. Improvement of oral bioavailability of curcumin upon microencapsulation with methacrylic copolymers. Front. Pharmacol. 7 (December), 1–9. https://doi.org/10.3389/fphar.2016.00485.

Pasch, H., Pizzi, A., Rode, K., 2001. MALDI–TOF mass spectrometry of polyflavonoid tannins. Polymer 42 (18), 7531–7539.

Piccardo, D., González-Neves, G., 2013. Extracción de polifenoles y composición de vinos tintos Tannat elaborados por técnicas de maceración prefermentativa. Agrociencia Uruguay 17 (1), 36–44. https://doi.org/10.2477/vol17iss1pp36-44.

Qiao, P.Y., Li, F.F., Dong, L.M., Xu, T., Xie, Q.F., 2014. Delivering MC3T3-E1 cells into injectable calcium phosphate cement through alginate-chitosan microbeads for bone tissue engineering. J. Zhejiang Univ. - Sci. B 15 (4), 382–392. https://doi.org/10.1631/jzus.B1300132.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26 (9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

Redondo, L.M., Chacana, P.A., Dominguez, J.E., Fernandez Miyakawa, M.E., 2014. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Front. Microbiol. 5 (118), 1–7.

Santhanam, A.K., Lekshmi, M., Chouksey, M.K., Tripathi, G., Gudipati, V., 2015. Delivery of omega-3 fatty acids into cake through emulsification of fish oil-in-milk and encapsulation by spray drying with added polymers. Dry. Technol. 33 (1), 83–91. https://doi.org/10.1080/07373937.2014.934832.

Soares, S., Silva, M.S., García-Estevez, I., Großmann, P., Brás, N., Brandão, E., Meyerhof, W., 2018. Human bitter taste receptors are activated by different classes of polyphenols. J. Agric. Food Chem. 66 (33), 8814–8823.

Stokke, B.T., Draget, K.I., Smidsrød, O., Yuguchi, Y., Urakawa, H., Kajiwara, K., 2000. Small-angle X-ray scattering and rheological characterization of alginate gels. 1. Ca-alginate gels. Macromolecules 33 (5), 1853–1863. https://doi.org/10.1021/ma991559q.

Whelehan, M., Marison, I., 2011. Microencapsulation by Dripping and Jet Break-Up. BRG Newsletter e Bioencapsulation Innovations. https://doi.org/10.1201/b19038-16.

Zhang, Z.Q., Pan, C.H., Chung, D., 2011. Tannic acid cross-linked gelatin-gum Arabic coacervate microspheres for sustained release of allyl isothiocyanate: characterization and in vitro release study. Food Res. Int. 44 (4), 1000–1007. https://doi.org/10.1016/j.foodres.2011.02.044.