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Encapsulation of grape seed phenolics from winemaking byproducts in hydrogel microbeads – Impact of food matrix and processing on the inhibitory activity towards α-glucosidase

Davide Pedrali, Sara Barbarito, Vera Lavelli *

DeFENS, Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, via Celoria 2, 20133, Milano, Italy

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ABSTRACT

Hydrogel-based encapsulation systems were developed for grape seed phenolics recovered from winemaking byproducts. Proanthocyanidins, monomeric flavanols, flavonols and anthocyanins were extracted from grape seeds with 60% ethanol. Encapsulation of solubilized grape seed phenolics in microbeads with alginate-Ca^{++} or alginate-chitosan-Ca^{++} achieved efficiency of 78 and 92%, respectively, higher than those of pure catechin and proanthocyanidins.

Encapsulated phenolics showed inhibitory activity towards the brush border enzyme α-glucosidase, which was chosen as a model bioactivity assay. Grape seed phenolics were retained in the hydrogel structure after mixing of the microbeads with bovine milk or rice drink, pasteurization of these beverages to achieve 7 decimal reductions of *Mycobacterium avium* subsp. *paratubercolosis* (equivalent to 72 °C for 91 s) as well as after intense heat treatment of the rice beverage to achieve 7 decimal reductions of *Bacillus cereus* (95 °C for 20 min).

1. Introduction

Grape seeds are by-products of winemaking that can be extracted, dried and purified to produce a phenolic-rich extract that has Generally Recognized as Safe (GRAS) status approved by Food and Drug Administration (FDA). Proanthocyanidins are the major components among grape seed phenolics. Monomeric flavanols, flavonols and anthocyanins are also found in grape seed extract (Lavelli, Kerr, García-Lomillo, & González-SanJosé, 2017; Maier, Schieber, Kammerer, & Carle, 2009). Grape seed phenolic extract has received considerable attention as a nutraceutical product since its antioxidant potential is twenty and fifty fold greater than those of vitamins E and C, respectively (Shi, Yu, Pohorly, & Kakuda, 2003).

The bioactive properties of grape seed phenolics have been investigated through human intervention studies involving both healthy subjects and subjects affected by relevant diseases. In type-2 diabetic subjects, the supplementation of grape seed phenolics improved markers of vascular risk such as endothelial dysfunction, oxidative stress, inflammation and insulin resistance (Kar, Laight, Rooprai, Shaw, & Cummings, 2009). Considering subjects with pre- and stage I hypertension, the intake of grape seed phenolics was found to significantly lower systolic blood pressure (Ras et al., 2013). In hyperlipidemic patients, intervention with grape seed phenolics improved lipid profiles (Razavi et al., 2013). A meta-analysis of the randomized controlled trials performed with grape seed phenolics supported its health effects, although it was evidenced that there is a need for larger trials that evaluate the effects of different dosages of grape seed phenolics and proceed for longer follow-up durations (Feringa, Laskey, Dickson, & Coleman, 2011). Review studies on this topic also pointed to numerous health effects of grape seed phenolics, while it was concluded that better designed studies are required before any recommendations of intake quantity and period (Rasines-Perea & Teissedre, 2017; Sri Harsha & Lavelli, 2019).

In all the above-mentioned studies, grape seed phenolics were formulated in capsules and administered as for drugs under fasting conditions. Hence, a phenolic-rich fraction was extracted, dried and

* Corresponding author.
E-mail address: vera.lavelli@unimi.it (V. Lavelli).

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formulated as for drugs. Another approach could be to deliver proanthocyanidins with foods. To this aim, encapsulation technologies have been proposed. One criterion to design the encapsulation system was to provide thermal stability to proanthocyanidins, in order to prevent their degradation that may occur during food processing and storage. By using differential scanning calorimetry, increased thermal stability was demonstrated for proanthocyanidins encapsulated in zein (Wang et al., 2016) or alginate and pectin mixtures (Chen and Zhang, 2019) due to the formation of hydrogen bonds with the protein- and the polysaccharide-based carriers.The encapsulation of proanthocyanidins was also designed to allow their pH controlled release. To this aim, proanthocyanidins were encapsulated in alginate, which is well-known to enable a pH dependent release of encapsulated compounds (Xu, Zhan, Fan, Wang, & Zheng, 2007). The combination of alginate and pectin allow to modulate the release rate, since the release increases gradually as the ratio of pectin increases (Chen and Zhang, 2019). Alternatively, grape seed phenolics were encapsulated in liposomes consisting of soy phospholipid bilayers coated with layers of chitosan and pectin and it was demonstrated that the release kinetics can be controlled by modulating the coating layers (Gibis, Ruedt, & Weiss, 2016).

Moreover, the encapsulation of proanthocyanidins was designed to improve their cell permeation. The use of chitosan and lauryl succinyl chitosan as carriers for proanthocyanidins increased their cell permeability *in vitro* through Caco-2 cells (Munoz, Kappes, Roeckel, Vera, & Fernandez, 2016). Proanthocyanidins encapsulated in chitosan microcapsules were also found to exert a better pharmacological effect *in vitro* towards the human lung and colorectal carcinoma cells A549 and Caco-2 cells with respect to the free proanthocyanidins (Iannone et al., 2017).

Previous studies have considered the application of alginate and chitosan in food systems (Spagna et al., 1996; Caleja et al., 2016; Arab et al., 2019; de Moura et al., 2019).

A relevant challenge to consider to design the addition of grape seed extract into foods is that its main components, i.e., proanthocyanidins, interact with food proteins, resulting in a decreased bioactivity (Joshi, Howell, & D'Souza, 2017). In fact, polyphenols and proteins form intermolecular complexes through hydrogen bonding or hydrophobic interactions, with binding constants that are negligible for catechin and epicatechin but especially high for proanthocyanidins. Moreover, the binding of grape seed proanthocyanidins to proteins was shown to be a cooperative process (Poncet-Legrand, Gautier, Cheynier, & Imberty, 2007). Despite various encapsulated nano- and microstructures have been developed to deliver proanthocyanidins, the behavior of encapsulated proanthocyanidins in model food matrices has not been thoroughly investigated so far.

The aim of the present study was to investigate model food matrices for the delivery of proanthocyanidin-rich grape seeds phenolics encapsulated in hydrogel. Hence, a raw grape seed extract was encapsulated in alginate-Ca^{++} or alginate-Ca^{++}-chitosan and the encapsulation efficiency was studied in comparison with those of its purified monomeric and oligomeric flavanol components, i.e., catechin and proanthocyanindins, Then, the alginate-Ca^{++} and the alginate-Ca^{++}-chitosan microbeads loaded with the grape seed extract were added to bovine milk and rice drink. The functionality of grape seed extract after encapsulation in the microbeads and after addition to the beverages (before and after heat treatment) was investigated by measuring their inhibitory activity towards the brush border enzyme α-glucosidase, chosen as a model bioactivity assay.

2. Materials and methods

2.1. Chemicals and materials

Low molecular weight sodium alginate from brown algae, low molecular weight chitosan (80% deacetylated chitin from shrimp shell), analysis grade calcium chloride 2-hydrate, HPLC-grade solvents, rat intestinal acetone powder, *p*-nitrophenyl α-D-glucoside (*p*-NPG) and acarbose were obtained from Sigma–Aldrich Italia (Milan, Italy). Standards of catechin, epicatechin, procyanidin B1, procyanidin B2, delphinidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, quercetin 3-*O*-glucuronide, quercetin 3-*O*-glucoside, quercetin and kaempferol were purchased from Extrasynthese (Lyon, France). Proanthocyanidins capsules from grape seeds were purchased by Schiha Vital GmbH (Burgenland, Germany). Grape pomace samples (Barbera variety) were kindly provided by a winery located in Northern Italy.

Skimmed cow milk was purchased from Latteria Soresina (Soresina, CR, Italy) and contained per 100 mL: fat 1.6 g, carbohydrate 5 g, protein 3.2 g and salt 0.1 g, providing 47 kcal. Rice beverage was purchased from Riso Scotti (Milan, Italy) and contained per 100 mL: fat 0.9 g, carbohydrate 13.1 g, protein 0.2 g and salt 0.1 g, providing 61.3 kcal.

2.2. Phenolic extraction from grape seeds

At the winery, grape pomace was sieved (with a 5 mm sieve) to separate the skins from the seeds and these components were frozen and transported to the lab. The seeds were dried at 50 °C for about 8 h to a final moisture content of 3 g/100 g. Dried grape seeds were milled by a grinder (Sunbeam Osterizer blender, Boca Raton, FL, USA). The powders obtained were sieved to standardize the particle size by using the Octagon Digital sieve shaker (Endecotts Ltd., United Kingdom), with certified sieves (openings: 250 and 125 μm) for 10 min at amplitude 8. The grape seed fraction with particle sizes in the range 250–500 μm was used for phenolic extraction. Phenolic extract was obtained according to the method reported by Lavelli, Sri Harsha, and Spigno (2016). Briefly, 25 g of grape seed fraction was extracted with 200 mL of 60% aqueous ethanol with continuous stirring for 2 h at 60 °C. Ethanol was chosen because it is a food grade solvent, even if the recovery of phenolics (evaluated as described under the paragraph 2.5) was 91% of the amount recovered with 70% acetone as proposed previously for the recovery of proanthocyanidin-rich fractions (Lavelli & Vantaggi, 2009; Hanlin, Kelm, Wilkinson, & O. Downey, 2011). The mixture was then centrifuged at 2000*g* for 10 min and the extract recovered.

2.3. Hydrogel formation

Microbeads were obtained by a vibrating nozzle method using a Buchi B-390 encapsulator (Buchi Italia, Milan) as described previously to encapsulate grape skin phenolics (Lavelli & Sri Harsha, 2019). The inlet solution was prepared by dissolving sodium alginate at a final concentration of 1.5%, in 400 mL of water, mixing carefully by a T 25 Ultraturrax (IKA Werke, Staufen, Germany) and then adding 100 mL of the grape seed extract (containing 4.9 g/L of total phenolics (determined as indicated under the paragraph 2.5) and 2.6 g/L of proanthocyanidins (determined as indicated under the paragraph 2.6) or 100 mL of a 2.6 g/L solution of catechin in 60% ethanol, or 100 mL of a 3.6 g/L solution of proanthocyanidin capsules (to have a final proanthocyanindin concentration of 2.6 g/L). Operating conditions were: 300 μm nozzle, nitrogen pressure to deliver the inlet solution to the nozzle: 0.5 bar; vibration frequency used to break up the laminar liquid: 1000 Hz; voltage used to create an electrostatic field between the nozzle and the hardening solution in order to prevent the coalescence of the microdrops:1400 V; distance between the nozzle and the hardening solution: 20 cm.

As hardening solutions, 0.2 mol/L calcium chloride or 0.2 mol/L calcium chloride added with 0.5% of chitosan were used. For complete hardening, microcapsules were allowed to stir in the hardening solution for 10 min. After that, the beads were filtered with a Whatman n. 4 paper filter and the filtrate was recovered. The beads were then washed with water. A total of three replicates were run in each conditions. For every replicate, the inlet solution, hardening solution and washing solution volumes were 50 mL, 150 mL and 100 mL, respectively and 42 ± 2 g of

microbeads were obtained.

2.4. Moisture content

Moisture content of the microbeads was determined by drying in a vacuum oven at 70 °C and 50 Torr for 18 h.

2.5. Total phenolics

The Folin–Ciocalteu assay was performed in duplicate on the ethanolic extract of grape seeds and the hardening solutions. The reaction mixture contained 0.5 mL of the extract diluted with 60% ethanol or 0.5 mL of the hardening solution, 6.0 mL of distilled water, 0.5 mL of Folin–Ciocalteu reagent and 3 mL of 10% Na_2CO_3. The mixtures were incubated for 90 min at room temperature and the absorbance was recorded at 760 nm against a blank with no extract addition. A calibration curve was built using gallic acid. Total phenolics were expressed as milligram of gallic acid equivalents (GAE) per kilogram of dry weight (d.w.) for the grape seeds and milligram GAE per liter for the grape seed extract and the hardening solutions.

2.6. Proanthocyanidins

Proanthocyanidin content was analysed in duplicate as described previously on the ethanolic extract of grape seeds and on the commercial proanthocyanidin capsules (Lavelli et al., 2016). Briefly, 1 mL of the extract diluted with 60% ethanol or 1 mL of commercial proanthocyanidin capsules dissolved in 60% ethanol was added to 6 mL of *n*-butanol: HCl (95:5, v/v) and 0.2 mL of 2% $NH_4Fe(SO_4)_2 \times 12\ H_2O$ in 2 M HCl. Hydrolysis was carried out at 95 °C for 40 min. The reaction mixtures were cooled and the absorbance was measured at 550 nm against a blank made as for the sample, but incubated at room temperature. Proanthocyanidin amount was determined using 0.1736 mg/mL as conversion factor and expressed as milligram of per kilogram of d.w. of grape seeds or milligram of per kilogram d.w. of proanthocyanidin capsules or milligram per liter for the grape seed extract.

2.7. Phenolic analysis by HPLC

The phenolic contents of the grape seed extract and of the commercial proanthocyanidin capsules were analysed in duplicate as described previously (Lavelli et al., 2016) using a model Shimadzu LC-20 AD pump coupled to a model Shimadzu SPD-M20A photodiode array detector and an RF-20 AXS operated by Labsolution Software Shimadzu, Kyoto, Japan). A 2.6 μm Kinetex C18 column (150 × 4.6 mm; Phenomenex, Bologna, Italy) was used for the separation, at a flow-rate of 1.5 mL/min. The column was maintained at 40 °C. The separation was performed by means of a linear gradient elution. Eluents were: (A) 0.1% H_3PO_4; (B) acetonitrile. The gradient was as follows: from 6% B to 20% B in 18 min; from 20% B to 60% B in 7 min; from 60% B to 90% B in 19 min; 90% B for 10 min and then 6% B for 5 min. DAD analysis was carried out in the range of 200–600 nm. Anthocyanins and flavonols were quantified by calibration curves built with external standards with the diode array detector set at 520 and 354 nm, respectively. Flavanols, were quantified by calibration curves built with external standards with the fluorimetric detector set at λ_{ex} 230 and λ_{em} 320. Results were expressed as milligram per kilogram d.w. grape seeds.

2.8. In vitro α-glucosidase inhibition assay

In vitro α-glucosidase inhibition assay was performed as described previously (Lavelli, Sri Harsha, Laureati, & Pagliarini, 2017). In brief, a crude α-glucosidase solution was prepared by dissolving 200 mg of rat intestinal acetone powder in 4 mL of 50 mmol/L ice-cold phosphate buffer (pH 6.8) and sonicating for 15 min at 4 °C. The suspension was vortexed for 20 min and then centrifuged at 10 000 g at 4 °C for 30 min. The resulting supernatant was used for the assay. Before the enzymatic reaction, 1.600 mL of 0.1 mol/L phosphate buffer, pH 6.8; 0.2 mL of the enzyme solution and 600 mg of microbeads or 0.1 mL of the grape seed extract were added in tubes and preincubated for 10 min at 37 °C. Then, 0.200 mL of 1 mmol/L *p*-NPG was added as substrate, and the mixture was further incubated at 37 °C for 25 min. The assay mixture was centrifuged at 10000 *g* for 3 min, and the absorbance of the clear supernatant was recorded at 405 nm. The control was run by addition of the phosphate buffer replacing the sample. A sample blank and a control blank were run without addition of substrate and without addition of both substrate and sample, respectively. Acarbose was used as a positive control.

Percentage of inhibition was calculated using the formula:

$$\%\ \text{Inhibition} = [(A_c - A_s)/A_c] \times 100$$

where A_c and A_s are the values of absorbance of the control and sample, respectively.

The inhibitory activity of the microbeads is reported as microgram acarbose equivalents per gram of microbeads and that of the grape seed extract is reported as microgram acarbose equivalent per liter of grape seed extract.

2.9. Encapsulation efficiency

The mass of phenolics (TP, total grape seed phenolics or catechin or proanthocyanidins depending on the system considered) encapsulated in the microbeads was calculated by subtracting the mass of phenolics in the hardening solution (h) from the mass of phenolics in the inlet solution (i), as mentioned in previous studies (Guo, Giusti, & Kaletunc, 2018).

For the grape seed extract, TP were evaluated as gallic acid equivalents as described in 2.5. For commercial proanthocyanidins and catechin, TP were evaluated by measuring the absorbance at 280 nm.

The percent encapsulation efficiency (EE) was hence calculated according to Eq. (1):

$$\%EE = 100 \times (\text{g TP/L}_i \times 0.050\ L_i - \text{g TP/L}_h \times 0.15\ L_h)/(\text{g TP/L}_i \times 0.050\ L_i) \quad (1)$$

where 0.050 L and 0.150 L are the volumes of inlet and hardening solutions used for each trial.

The percent recovery of Acarbose eq. (R Ac eq.) was calculated as:

$$\%R\ Ac\ eq = 100 \times (\mu g\ Ac\ eq/g_{microbeads} * 42\ g_{microbeads})/(\mu g\ Ac\ eq/L_{grape\ seed\ extract} * 0.01\ L) \quad (2)$$

where 0.01 L is the volume of grape seed extract used for each trial (0.05 L of inlet solution contained 0.01 L of grape seed extract) and 42 g is the mass of microbeads obtained for each trial.

2.10. Processing of milk and rice beverage added with the grape seed phenolics encapsulated in the microbeads

An amount of 0.6 g of the microbeads loaded with the grape seed extract was added to 2 mL of milk or to 2 mL of rice drink into different glass tubes (10 mL capacity). A set of the tubes was then heated to 75 °C in thermostat and then cooled in ice. During heating, the temperature of milk was monitored continuously by using a thermocouple set in one of the tubes.

For bovine milk, the pasteurization effectiveness was calculated by considering *Mycobacterium avium* subsp. *paratuberculosis* as the reference target microorganism, using a decimal reduction value, D, at 72 °C of 13 s and a z value of 10 °C (Mullan, 2019). The D values for the target microorganism was calculated as a function of temperature using the Bigelow's model, as reported below:

$$D = D_{ref} * 10^{(Tref - T)/z}$$

$T_{ref} = 72$ °C.

The rice drink was heated as described for bovine milk and also at 95 °C for 20 min. *Bacillus cereus* was considered as a target microorganism for the rice drink, using D value of 3.1 min at 95 °C (Kim et al., 2012).

The 1/D values were then plotted as a function of time and the resulting curves were then integrated to evaluate the total decimal reductions (Earle, 2013). Another set of tubes was not heated.

The unheated and heated tubes were then centrifuged at 10000 *g* for 3 min to separate the microbeads from the liquid. The supernatant was removed and the beads were washed with 0.1 M ice-cold phosphate buffer (pH 6.8) before centrifugation at 10000*g* for 3 min. After the washing step, no opacity due to residual beverage solution was present on the microbeads. The inhibitory activity of the recovered microbeads towards α-glucosidase was then evaluated as described in 2.8.

2.11. Statistical analysis of data

Experimental data were analysed by one-way ANOVA using the Tukey test as a multiple range test using Statgraphics 5.1 (STCC Inc.; Rockville, MD). Results are reported as average ± standard deviation (SD).

3. Results and discussion

3.1. Encapsulation of grape seed phenolics in hydrogel

Phenolic profile of grape seeds recovered after winemaking was mainly characterized by the presence of proanthocyanidins, with minor amounts of monomeric flavanols, flavonols and anthocyanins. In the commercial proanthocyanidins capsules, only oligomeric and monomeric flavanols were present (Table 1). The total amount of phenolics in grape seeds was 39.2 g/kg d.w. and the concentration of phenolics in the grape seed extract used for encapsulation was 4.9 g/L.

In general, grape seed phenolics are extracted with alcoholic solvents (Pinelo, Rubilar, Jerez, Sineiro, & Núñez, 2005) and then dried to be used as food ingredients or additives (Lavelli et al., 2016). In the current study, phenolics were recovered from the grape seed extract through direct encapsulation in alginate microbeads in the presence of Ca^{++} ions alone or with Ca^{++} ions and the copolymer chitosan. In parallel, catechin and proanthocyanidins microbeads were obtained with the same carrier polymers. The presence of chitosan resulted in higher solids content than in the presence of Ca^{++} ions alone in all the types of microbeads, indicating a more compact hydrogel structure (Table 2).

Table 1
Phenolic compounds (mg/kg d.w.) in grape seeds recovered from winemaking by-products and commercial proanthocyanidins capsules.

	Grape seed from winemaking byproducts	Commercial proanthocyanidins capsules
Anthocyanins		
Delphinidin-3-O-glucoside	9.1 ± 0.6	
Cyanidin-3-O-glucoside	6.4 ± 0.5	
Petunidin-3-O-glucoside	11.5 ± 0.7	
Peonidin-3-O-glucoside	12.6 ± 0.2	
Malvidin-3-O-glucoside	52.6 ± 1.6	
Flavonols		
Quercetin 3-O-glucoside	17.0 ± 0.1	
Quercetin 3-O-glucuronide	24.7 ± 0.9	
Quercetin	75.5 ± 2.2	
Kaempferol	9.3 ± 0.5	
Flavanols		
Procyanidin B1	62.5 ± 2.2	10600 ± 100
Catechin	589 ± 10	52000 ± 100
Procyanidin B2	159 ± 2	14000 ± 100
Epicatechin	326 ± 1	48412 ± 100
Procyanidin A2		3700 ± 100
Oligomeric proanthocyanidins	21000 ± 1600	736000 ± 6000
Total phenolics	39200 ± 1600	

Encapsulation efficiency for catechin was ~40%. Similarly, Aizpurua-Olaizola et al. (2016) found a low encapsulation efficiency for catechin, equal to 30%. Indeed, alginate hydrogel is a very porous structure, which limits its performance as encapsulating system for the small molecules, unless fillers, such as cocoa and carob powder are added to the structure (Bušić et al., 2018). Addition of chitosan did not result in increased encapsulation efficacy for catechin. It may be assumed that catechin, being a small molecule, can be easily released from the alginate-Ca^{++} hydrogel, both in the presence and in the absence of chitosan. Similarly, Kim, Lee, and Lee (2016) found that catechin can be released easily from alginate hydrogel. Encapsulation efficiency for proanthocyanidins was double than that of catechin, probably due to their high molecular weights that limit their diffusion in the hydrogel. However, the encapsulation efficiency of proanthocyanidins was not significantly affected by the presence of chitosan. The encapsulation efficiency for grape seed phenolic extract in alginate-Ca^{++} was 77.4%, the same as that of proanthocyanidins, which are the prevalent phenolics in grape seed extract. In the presence of chitosan, the encapsulation efficiency for grape seed phenolic extract was significantly higher, equal to 92.3%, indicating that some compounds present in grape seed extract could favor the formation of the alginate/chitosan hydrogel. Compounds that potentially strengthen the alginate/chitosan hydrogel could be the di- or tri-carboxylic acids that can form linkages with the amino-groups of chitosan. In fact, tartaric, malic, and citric acids are present in grapes (Ali, Maltese, Choi, & Verpoorte, 2010). In particular, grape seeds are rich in tartaric acid, which is recovered from this source after winemaking for further industrial uses (Palma & Barroso, 2002).

3.2. Use of grape seed extract encapsulated in hydrogel microbeads in milk and rice drink to deliver inhibitors towards α-glucosidase

The inhibitory activity towards the brush border enzyme α-glucosidase was chosen as a model bioactivity assay for the grape seed extract encapsulated in the microbeads The α-glucosidase inhibitory activity was 330 and 352 μg acarbose equivalents/g microbeads for the alginate-Ca^{++} and alginate-Ca^{++}-chitosan microbeads, respectively (Table 3). Considering the α-glucosidase inhibitory activity of the free grape seed extract, (2.45 g acarbose equivalents/L), the recovery yields for acarbose equivalents (calculated as indicated under the paragraph 2.9) were 60 and 57% for the alginate-Ca^{++} and alginate-Ca^{++}-chitosan microbeads, respectively. The relatively low recovery of the inhibitory activity can be due to interactions occurring between phenolics and the

Table 2
Solids content (g/100 g) and percent encapsulation efficiency (g of phenolics encapsulated/100 g phenolic in the inlet solution) of the alginate-Ca^{++} and alginate-Ca^{++}-chitosan microbeads loaded with catechin, commercial proanthocyanidins and grape seed extract.

Polymer	Phenolics	Solids content	Encapsulation efficiency
Alginate	catechin	$4.91^{a} \pm 0.56$	$37.7^{d} \pm 5.1$
Alginate/Chitosan	catechin	$6.17^{bc} \pm 0.48$	$41.6^{d} \pm 0.1$
Alginate	proanthocyanidin	$5.47^{ab} \pm 0.77$	$78.5^{bc} \pm 0.4$
Alginate/Chitosan	proanthocyanidin	$7.29^{c} \pm 0.54$	$84.4^{b} \pm 0.7$
Alginate	seed extract	$4.46^{a} \pm 0.17$	$77.4^{c} \pm 0.9$
Alginate/Chitosan	seed extract	$7.06^{c} \pm 0.89$	$92.3^{a} \pm 0.8$

Results are average ± SD for three replicates, each of them analysed in duplicate. Different letters in the same column indicate significant differences among samples (Tukey test, $p < 0.05$).

Table 3
Inhibitory activity towards α-glucosidase (μg acarbose equivalents/g microbeads) of grape seed extract encapsulated in microbeads of alginate-Ca^{++} or alginate-Ca^{++}-chitosan after gelation, mixing with bovine milk or rice drink and heat treatment of the beverages.

Polymer	Food	Processing step	Acarbose eq
Alginate		gelation	$352^{ab} \pm 34$
Alginate/ Chitosan		gelation	$330^{a} \pm 38$
Alginate	bovine milk	mixing	$326^{ab} \pm 23$
Alginate	bovine milk	heating equivalent to 72 °C for 91 s	$282^{a} \pm 24$
Alginate/ Chitosan	bovine milk	mixing	$288^{a} \pm 16$
Alginate/ Chitosan	bovine milk	heating equivalent to 72 °C for 91 s	$302^{a} \pm 46$
Alginate	rice drink	mixing	$346^{ab} \pm 18$
Alginate	rice drink	heating equivalent to 72 °C for 91 s	$337^{ab} \pm 18$
Alginate	rice drink	heating at 95 °C for 20 min	$471^{c} \pm 5$
Alginate/ Chitosan	rice drink	mixing	$354^{ab} \pm 66$
Alginate/ Chitosan	rice drink	heating equivalent to 72 °C for 91 s	$314^{a} \pm 16$
Alginate/ Chitosan	rice drink	heating at 95 °C for 20 min	$422^{bc} \pm 9$

Results are average ± SD for three replicates, each of them analysed in duplicate. Different letters in the same column indicate significant differences among samples (Tukey test, $p < 0.05$).

carrier polymers. In fact, alginate can interact with phenols mainly through the formation of hydrogen bonds involving the hydroxyl groups of phenols and both the hydroxyl and the carboxyl groups of alginate (Plazinski & Plazinska, 2011). Moreover, additional interactions occur between phenolic compounds and chitosan, consistently with previous studies (Gibis et al., 2016; Spagna et al., 1996). In fact, chitosan can absorb proanthocyanidins and other phenolics from white wine, most likely due to its functional groups that interact with phenolic compounds by weak interactions such as hydrogen bonding and van der Waals forces (Spagna et al., 1996). Accordingly, in a previous study by Fabra, Falco, Randazzo, Sanchez, & Lopez-Rubio (2018), lower antioxidant and antiviral activity was observed for grape seed phenolics encapsulated in alginate than for free grape seed phenolics.

To investigate the effect of food processing on the encapsulated grape seed phenolics, bovine milk and rice drink were used as model foods. Firstly, the effect of mixing on microbeads' bioactivity was investigated to assess possible release of grape phenolics from the microbeads. The inhibitory activity towards α-glucosidase for the microbeads recovered from the beverages after mixing was not significantly different from that observed for the microbeads after gelation (Table 3). This result indicates that the bioactive compounds were not lost from the hydrogel. Moreover, the complete recovery of the inhibitory activity of the microbeads after mixing suggests that encapsulation prevented grape seed proanthocyanidins from forming complexes with proteins. This effect is particularly relevant for bovine milk. Indeed, in a previous study addition of free proanthocyanidins to bovine milk resulted in a no significant bioactivity due to proanthocyanidin interactions with proteins (Joshi et al., 2017).

The effect of pasteurization was then studied. Regarding bovine milk, to calculate the effectiveness of heat treatment, the kinetic parameters for the thermal destruction of *M. avium* subsp. *paratubercolosis* were used. In fact, while *Listeria monocytogenes* is generally considered as target for milk pasteurization (Artíguez, Arboleya, & de Marañón, 2012; Bermúdez-Aguirre, Corradini, Mawson, & Barbosa-Cánovas, 2009). However, concern was raised for the possible contamination of milk by the pathogenic microorganism *M. avium* subsp. *paratubercolosis*, which is more thermal resistant than *L. monocytogenes*. Indeed, pasteurization conditions to achieve >5 log reductions of *M. avium* subsp. *paratubercolosis* are being used in the industry (Mullan, 2019). The heat treatment applied in the current study achieved 7 D of the target microorganism and hence it was intense enough to ensure safety of the product; moreover, it did not affect the inhibitory activity towards α-glucosidase.

There is not a reference treatment to ensure safety of rice-based drinks. In some studies, *B. cereus* has been considered the most heat resistance pathogen that can be present in rice-based foods. In this study, rice drink was treated under the same conditions as bovine milk and also more intensively, i.e., at 95 °C for 20 min to achieve around 7 D of *B. cereus* (Kim at al., 2012). The less intensive treatment did not affect microbeads ability to inhibit α-glucosidase. The inhibitory activity towards α-glucosidase for the intensively heat treated microbeads was found to be 471 and 422 μg acarbose equivalents/g microbeads for the alginate-Ca^{++} and alginate-Ca^{++}-chitosan microbeads, respectively (Table 3). Hence, the inhibitory activity increased with respect to that of the unheated microbeads.

Interestingly in none of the model foods there was a decrease in the inhibitory activity towards α-glucosidase upon heat treatment. On the other hand, the presence of proteins and carbohydrates in these model foods especially milk, promotes heat induced reactions, which should decrease proanthocyanidin content (Oracz, Nebesny, & Żyżelewicz, 2019; Saito, Nakajima, Matsuura, Tanaka, & Ubukata, 2004). It could be hypothesized that encapsulation in alginate and chitosan hydrogel prevented proanthocyanidin degradation. Similarly, grape proanthocyanidins have been found to be stable upon heat treatment in gelled foods (Cappa, Lavelli, & Mariotti, 2015) and in encapsulated structures (Chen and Zhang, 2019; Wang et al., 2016). On the other hand, heating affects alginate gel structure by promoting an interchange between the hydrogen bonds. In particular, Hou and Wu (2019) found that during heating hydrogen bonds between water molecules and the polar groups along the alginate chains are broken, which consequently results in the formation of inter/intra-molecular hydrogen bonds within alginate chains. Hence, the observed increase in enzyme inhibition efficacy by the microbeads after intense heat treatment suggests that grape proanthocyanidins were retained in the hydrogel structure, although there was a decrease in the interactions between phenolics and the carrier polymers.

4. Conclusions

The results of this study lead to the following conclusions: a) as an alternative process to drying, phenolics extracted form grape seeds can be encapsulated in alginate-Ca^{++} or alginate-Ca^{++}-chitosan hydrogels with encapsulation efficiency up to 92%,; b) encapsulation in hydrogel prevented interactions between grape seed proanthocyanidins and the food matrices such as bovine milk and rice drink, since their activity was not affected by mixing; c) in both the food matrices considered, grape seed phenolics were retained in the hydrogel structure during food processing. As alginate and chitosan encapsulation are already being used for pH control release of drugs and are applied in food formulation, it can be assumed that their use for the recovery of healthy compounds from the residues of food processing can also open up safe and efficient approaches to minimize food waste.

Industrial relevance

Phenolic compounds of grape seeds mainly consisting of proanthocyanidins, possess outstanding antioxidant properties and numerous health benefits. Hence, these compounds are extracted from wine-making byproducts in diluted solutions and then dried to produce a powder for nutraceutical or food applications. This study demonstrated that solubilized grape proanthocyanidins, most likely due to their high molecular weights, could be recovered efficiently in porous hydrogel microbeads made of alginate or alginate/chitosan, thus avoiding the

energy-consuming drying phase. Encapsulation in hydrogel also prevented interactions between proanthocyanidins and model food matrices such as bovine milk and rice drink. Moreover, grape seed proanthocyanidins were retained in the hydrogel during beverage processing such as pasteurization (72 °C per 91 s) and intense heat treatment (95 °C for 20 min). As alginate and chitosan encapsulation are already being used for pH control release of drugs, use of this technology can open up safe and efficient approaches for the recovery of healthy compounds from food byproducts and their reuse into the food chain.

CRediT authorship contribution statement

Davide Pedrali: Investigation. **Sara Barbarito:** Investigation. **Vera Lavelli:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

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