

Original article

Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying

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Summary Pomegranate (*Punica granatum*) bioactive compounds (polyphenols and anthocyanins) of juice (PJ) and ethanolic extracts (PE) were encapsulated with maltodextrin (MD) or soybean protein isolates (SPI) by spray drying using a 2² statistical factorial design for each systems studied (PJ–MD, PJ–SPI, PE–MD and PE–SPI) considering the proportion of coating material and the inlet temperature as independent variables. The stability of the bioactive compounds microcapsules powders obtained under optimal conditions for each system was studied at 60 °C in oven for 56 days. The polyphenols encapsulating efficiency was significantly better in SPI matrix whereas for anthocyanins was in MD matrix. By the other hand, during the storage, the MD microcapsules provided a significant greater protective effect on the polyphenols and anthocyanins than SPI, as was shown by the lower degradation rate constants. When the microcapsules were added to yogurt the stability of the bioactive compounds followed a similar behaviour to those without encapsulation, except for PE–MD.

Keywords Anthocyanin, antioxidant activity, microcapsules, phenolic compounds, pomegranate.

Introduction

Pomegranate originated from the Middle East, extending throughout the Mediterranean area, eastward to China and India, and on to the American Southwest, California and México. The pomegranate tree thrives under arid and semiarid climatic conditions. The fruit is often deemed to be a large berry, generally harvested when fully ripe, and it possesses a waxy shiny surface of reddish yellow according to the varieties (Mars *et al.*, 1997). The fruit contain a large number of arils (seeds surrounded by a translucent juice sac) from which a valuable juice is obtained (Gil *et al.*, 1995a; Magerranov *et al.*, 2007). Pomegranate has been used for centuries in ancient cultures in folk medicine. All parts of the pomegranate plant (bark, stem, arils, whole fruit juice and leaves) have showed antioxidant activity. Studies about pomegranate polyphenols have shown prevention of cardiovascular disease, cancer and neurological damage in humans (Aviram *et al.*, 2002; Mertens-Talcott *et al.*, 2006; Seeram *et al.*, 2006; Lansky &

Newman, 2007). Flavonols and anthocyanins show anti-carcinogenic, antimicrobial (Opara *et al.*, 2009), anti-inflammatory and antioxidant activities (Lansky & Newman, 2007). The phenyl propanoids as chlorogenic, caffeic and coumaric acids may be responsible of the inhibition of tumour initiation and development in rats (Huang *et al.*, 2005).

The pomegranate juice (PJ) is a source of antocyanidins such as 3-glucosides and 3,5 diglucosides of delphinidin, cyanidin and pelargodinin. The phenolic compounds as gallagyl-type tannins (punicalagin); ellagic acid derivatives (ellagic acid glucoside and ellagic acid); hydrolysable tannins (galloyl glucose and other compounds) have been reported (Nawwar *et al.*, 1994; Gil *et al.*, 2000). Catechins, ellagitannins, gallitannins and quercetin glycosides have been reported in PJ too (Noda *et al.*, 2002). It also contains organic acids such as citric, ascorbic, malic and oxalic and small amounts of pectin. Different punicalagin content between commercial and experimental juices (only arils) have been reported, because the industrial process includes pomegranate rinds. Commercial PJ has one of the highest antioxidant activities compared to other fruit juices, red wine and green tea (Gil *et al.*, 2000).

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Pomegranate is an interesting source of polyphenols and anthocyanins which can be used as bioactive ingredients in foods. However, these molecules are unstable and the fresh juice has a short shelf life. In this context, the stabilisation of polyphenol and anthocyanin compounds for use in industrial purposes could be aided using microencapsulation technologies (Desai & Park, 2005). Microencapsulation is described as a technique wherein a bioactive compound is encapsulated by a biopolymer thereby protecting it from oxygen, water, light or other conditions in order to improve its stability and also to change liquid solutions to powders for easier handling (Gharsallaoui *et al.*, 2007). Encapsulation of quercetin has also been reported for increase its water solubility (Wu *et al.*, 2008).

The encapsulating agents used in this study were maltodextrin (MD) and soybean protein isolate (SPI). Maltodextrins of different dextrose equivalent (DE) are commonly used as wall material by its high water, solubility, low viscosity, low sugar content and their solutions are colourless. These properties make them useful ingredients in the food industry (Avaltroni *et al.*, 2004). Soybean protein is one of the most popular plant protein sources used as an ingredient in food formulation. The globulins glycinin and β -conglycinin are the major components of soybean isolates. These two globulins have different structures and functional properties (Arrese *et al.*, 1991; Pretuccelli & Añón, 1996). Soybean protein isolate has been used for their encapsulating and emulsifier characteristics in orange oil microcapsules, showing higher oil retention than whey protein isolate and arabic gum (Kim & Morr, 1996).

The objective of this research was to encapsulate PJ and ethanolic extract (PE) with MD or SPI by spray drying, to study the effect of the inlet temperature and the encapsulating agent content on the polyphenols and anthocyanins encapsulation (retention), and to analyse the matrix influence on the active compounds stability of the obtained powders.

Materials and methods

Materials

Pomegranate fruits (*P. granatum*) were obtained from a plantation located in the 'Las Cardas' Experimental Station that belongs to the University of Chile, Ovalle, Chile. Two pomegranate fruit genotypes (PG2 and PG3) were selected by their high bioactive compound content and a blend of arils of PG2:PG3 at a ratio of 1:2.5 (w/w) was used for juice and extract preparation. Maltodextrin from corn (MD) (DE = 12–20) (Inducorn, Santiago, Chile) and SPI (Prinal, Santiago, Chile) were used as wall materials. All other reagents were of analytical grade.

Juice and ethanolic extract preparation

Juice preparation

The arils from the pomegranate were manually obtained. The juice (4.07 L) from a 5.985 kg aril blend was obtained using a juice extractor (Moulinex T140-02).

Ethanolic extract preparation

The aril blend (600.41 g) was macerated with ethanol and water (1:1 w/w) (the addition of water included the arils water content) during a total of 12 h. Three extractions were made until the pulp was light red. Extracts were combined and concentrated in a Buchi RE120 evaporator at 40 °C until reaching a similar polyphenol content of the juice, obtaining 830 mL of PE. The juice and extract was frozen at –20 °C.

Preparation of the microcapsules

Encapsulation in MD or SPI were prepared as follows: PJ (20 g for MD or 6 g for SPI) or ethanol extract (24 g for MD or 7.5 g for SPI) was mixed with MD (10–40%) or SPI (3–12%) previously heated at 40 °C, with constant stirring. The SPI percentage used in the formulation before spray drying was lower than MD due to its high viscosity. Each preparation was homogenised with an Ultraturrax IKA T50 at 1400 × g for 5 min. The resultant solutions were fed to a mini spray dryer B191 (Büchi, Flawil, Switzerland). The spray dryer was operated at inlet temperature ranging from 140 to 160 ± 5 °C for MD and 100 to 140 ± 5 °C for SPI. The air flow, rate of feeding and atomisation pressure was 600 L h⁻¹, 10 mL min⁻¹ and 20 psi, respectively, for both encapsulating agents. The powders obtained were kept at –20 °C until analysed.

Pomegranate juice and ethanolic extract analysis

The soluble solids (°Brix), pH, and acidity were determined according to AOAC methods (AOAC, 1996). The total sugars were determined by the Antrona method (Osborne & Voogt, 1986) in an UNICAM UV/VIS spectrometer UV3.

The total phenolic content was determined according to the Folin Ciocalteu colorimetric method (Singleton & Rossi, 1965), and the results were expressed as milligram of gallic acid equivalents (GAE), according to a calibration curve (133.8–428.0 µg mL⁻¹; $R^2 = 0.9901$). The total anthocyanins were determined spectrophotometrically at 520 nm (Giusti & Wrolstad, 2001) and expressed as milligram of malvidin-3-glucoside equivalents per L. The identification was performed by HPLC using a Merck Hitachi L6200 pump, a Waters 996 photodiode-array detector, and a C18 column (5 µm × 4.6 mm i.d. × 25 cm, YMCTM Carotenoid S-5, Waters, Milford, USA). The mobile phases used

were solvent A [formic acid:water (10:90 v/v)] and solvent B (methanol) according to a program described by Prieto *et al.* (2005).

The antioxidant activity was evaluated in accordance with the radical scavenging DPPH method (Gil *et al.*, 2000). All the analyses were carried out in duplicate and averaged.

Microcapsule powder analysis

Total bioactive compounds

The coating material structure of the microcapsule was completely destructed by the following procedure: for MD, 200 mg of the microcapsules were accurately weighed and 2 mL of methanol:acetic acid:water (50:8:42 v/v/v) was added. This dispersion was agitated using a Vortex (1 min) and then an ultrasonicator twice for 20 min. The supernatant was centrifuged at 112 000 g for 5 min and then filtered. For SPI, 200 mg of the microcapsules were accurately weighed, and 1 mL of acetonitrile and 1 mL of methanol:acetic acid:water (50:8:42 v/v/v) were added, and then the same procedure described for MD was carried out. The amounts of phenolic and anthocyanin compounds were quantified as described above.

Surface bioactive compounds

For the determination of surface anthocyanin and phenolic compounds, 200 mg of microcapsules were treated with 2 mL of a mixture of ethanol and methanol (1:1). These dispersions were agitated in a Vortex at room temperature for 1 min and then filtered (0.45 µm Millipore filter). The amounts of phenolic and anthocyanin compounds were quantified as described above. The surface bioactive compound percentage (polyphenols or anthocyanins) and the microencapsulation efficiency (ME) of microencapsulated bioactive compounds were calculated according to eqns 1 and 2, respectively.

$$SB(\%) = \frac{\text{surface bioactive compounds}}{\text{theoretical total bioactive compounds}} \times 100 \quad (1)$$

$$ME(\%) = 100 - SB(\%) \quad (2)$$

Scanning electron microscopy

The outer structures of the microcapsules obtained under optimal conditions were studied by scanning electron microscopy (SEM). The samples were coated with gold/palladium using a Varian Vacuum Evaporator PS 10E and analysed using a LEO 1420VP (LEO Electron Microscopy Ltd, Cambridge, UK) operated at 20 kV. The scanned images were collected digitally

using EDS 7424 software (Oxford Instruments, Oxford, UK).

Accelerated storage stability test

Microcapsules of pomegranate (juice or ethanol extract) obtained under optimal conditions for each studied system (PJ-MD, PJ-SPI, PE-MD and PE-SPI) were stored at 60 °C in a forced-air oven (Memmert model BE 500, Schwabach, Germany) with controlled temperature and in absence of light for 56 days. Samples of 0.2 g of each powder were transferred to 100 × 150 mm clear glass vials. For determination of bioactive compounds (polyphenols and anthocyanins), duplicate vials were removed every 7 days. Pomegranate juice without encapsulation was used as a control.

Addition of microcapsules to a yogurt

Glass jars containing 50 g of natural yogurt Next® were added with 0.5 g of pomegranate encapsulated powders (obtained under optimal conditions) and storage at 5 ± 1 °C during 30 days in the absence of light. A control was prepared with concentrated PJ (60 °Brix). Samples were removed once a week for the determination of bioactive compounds according to Coisson *et al.* (2005). The yogurt assay was performed by triplicate.

Statistical design

The experiments were performed with a 2² central composed experimental design constituted by ten experiments for each encapsulating agent. The independent variables considered were the temperature of drying (140–160 and 100–140 °C for MD and SPI, respectively) and the coating material (10–40% and 3–12% for MD and SPI, respectively). The dependent variables were ME of microencapsulated anthocyanins and polyphenols. Response surface methodology was applied to optimise the ME of the bioactive compounds using Statgraphics software version 7.0 (Manugistics Inc., Statistical Graphics Corporation, Rockville, MA, USA).

Results and discussion

Juice and extract characteristics and bioactive compounds

Table 1 shows the physical and chemical analysis of the PJ and PE. The soluble solids and total sugar contents of PJ from arils were 15.97 °Brix and 17.72%, respectively, similar to those reported for thirteen varieties of PJ from Turkey (16–19 °Brix and 13.96–16.06%, respectively) (Poyrazoglu *et al.*, 2002). The soluble solids were also similar to those reported by Alighourchi *et al.* (2008) (12.1–18.33 °Brix), higher than Magerranov *et al.*

Table 1 Physical and chemical characteristics of PJ and PE from pomegranate

	PJ	PE
Total soluble solids (°Brix)	15.97 ± 0.06	13.80 ± 0.00
Total sugars (%)	17.72 ± 0.22	10.52 ± 0.31
pH	3.15 ± 0.07	3.03 ± 0.01
Acidity (% citric acid)	2.79 ± 0.13	1.02 ± 0.07
Total phenolics compounds (mg GAE L ⁻¹)	2128.6 ± 0.01	1717.9 ± 5.05
Total anthocyanins (mg malvidin 3-glucoside L ⁻¹)	882.3 ± 0.01	552.1 ± 6.12
Antioxidant activity DPPH (IC ₅₀ mg ⁻¹ mL ⁻¹)	2.12	1.73

PJ, pomegranate juice; PE, pomegranate ethanolic extract; GAE, gallic acid equivalent.

(2007) (11 °Brix) and lower than Gil *et al.* (1995a) (18.25 °Brix). The PE-soluble solids and total sugars contents were lower than those found for PJ due to a smaller extraction of those components during the extract preparation.

Several organic acids such as citric, malic, oxalic, acetic, fumaric, lactic and tartaric acids have been found in pomegranate fruit, being citric acid the main. The PJ acidity was higher than to those informed by other authors, who reported to be 0.045–0.414% (Alighourchi *et al.*, 2008) and 0.46–1.73% (Poyrazoglu *et al.*, 2002) expressed as citric acid. The pH was reported to be 3.04–4.07 (Alighourchi *et al.*, 2008) and 3.29–3.93 (Poyrazoglu *et al.*, 2002), similar to the value found in this study for PJ.

The PJ total phenolic content reached values of 2128.6 (mg GAE L⁻¹), higher than the value reported in PJ cv. Suruc of Turkey (1564 mg L⁻¹) (Vardin & Fenercioglu,

2003) and lower than in a sweet juice from Spanish pomegranate cv. Mollar (2750 mg GAE L⁻¹) (Pérez-Vicente *et al.*, 2004). Using other phenolic standard, Gil *et al.* (2000) reported a phenolic content of 2117 (mg L⁻¹ *p*-cumaric acid) from a fresh the juice from arils cv. Wonderful of California and Yunfeng *et al.* (2006) reported a value of 24.4 mg tannic acid g⁻¹ for the phenolic content of pomegranate pulp.

The PJ total anthocyanin content (882.3 mg malvidin-3-glucoside L⁻¹) was higher than that reported by Tzulker *et al.* (2007), which was between 100 and 300 mg of malvidin-3-glucoside L⁻¹ for juices prepared from the arils of twenty-nine Israeli pomegranate accessions. Other authors have reported that the anthocyanin content of pomegranates juices expressed as the sum of the individual anthocyanin contents, reached values of 6–120 mg cyanidin-3-glucoside L⁻¹ in juices from Tunisian pomegranates (Gil *et al.*, 1995b) and of 162–387 mg cyanidin-3-glucoside L⁻¹ in juices from fresh and frozen arils, and commercial juices from Californian pomegranates (Gil *et al.*, 2000).

The PE showed a lower total phenolic content (1717.9 mg of EAG g⁻¹) and total anthocyanins (552.1 mg of malvidin-3-glucoside L⁻¹) compared with the PJ, suggesting an incomplete extraction due to tissue complexity and the different polarity and/or solubility of the polyphenols in ethanol.

The anthocyanins identified in JP and PE were delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside, delphinidin-3-glucoside, pelargonidin-3,5-glucoside and cyanidin-3-glucoside as can be seen in Fig. 1A,B. These results showed the same anthocyanin profile as those reported by Miguel *et al.* (2004) for ‘Assaria’ pomegranate, by Gil *et al.* (2000) for ‘Wonderful’ pomegranate, and by Noda *et al.* (2002) who reported that delphinidin-3,5-diglucoside was a major anthocyanin

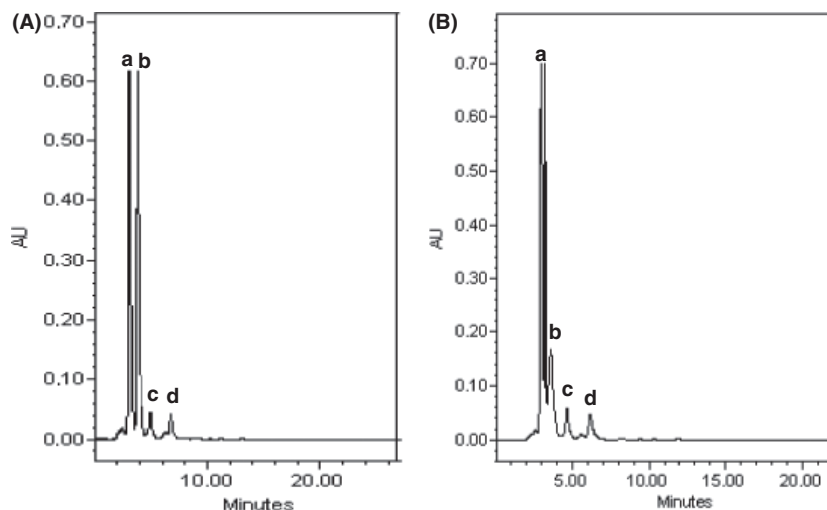


Figure 1 Chromatograms of pomegranate juice (A) and ethanolic extract (B), determined by HPLC. (a) Delphinidin-3,5-diglucoside; (b) cyanidin-3,5-diglucoside + delphinidin-3-glucoside; (c) pelargonidin-3,5-glucoside and (d) cyanidin-3-glucoside.

in juices from Californian pomegranates. In a recent paper, Alighourchi *et al.* (2008) in fifteen Iranian pomegranate varieties reported the presence of pelargonidin-3-glucoside too. A reduction in the cyanidin-3,5-diglucoside + delphinidin-3-glucoside peak was observed in PE according with the lower anthocyanins content observed in PE than PJ.

The antioxidant activity (diphenylpicryl-hydrazyl (DPPH)) of PJ and PE expressed as IC_{50} values were 2.12 and 1.73 mg mL⁻¹, respectively. Ricci *et al.* (2006) reported IC_{50} values for PJ (1.777 mg mL⁻¹) and aqueous extract (2.511 mg mL⁻¹, expressed as dry weight). The greater PE antioxidant activity could be attributed to other compounds extracted simultaneously (i.e. tocopherols) with ethanol and that would be playing an antioxidant function too.

Pomegranate bioactive compounds microencapsulation

The ME of polyphenols in PJ-SPI, PJ-MD, PE-SPI and PE-MD microcapsules were in the range of 36.6–62.8%, 51.4–82.8%, 52–81.5% and 52.9–82.8%, respectively. Similar polyphenols encapsulation efficiency has been reported by our group in previous studies about microencapsulated cactus pear juice with MD (10 DE) (39.41–74.78%) (Sáenz *et al.*, 2009). Wu *et al.* (2008) using other encapsulation method (nano-precipitation technique), reported an efficiency over 94% in microencapsulated quercetin using Eudragit[®] and polyvinyl alcohol as carriers. Other researchers reported for load ME values of 48.5–87.1% in microencapsulated polyphenols from *Illex paraguariensis*, with calcium alginate and calcium alginate-chitosan (Deladino *et al.*, 2008).

The encapsulation efficiency of anthocyanins in PJ-SPI, PJ-MD, PE-SPI and PE-MD microcapsules were in the range of 35.8–100%, 89.4–100%, 73–98.9% and

96.7–100%, respectively. In general, the encapsulation efficiency reached higher values for anthocyanin than polyphenol, showing the ability of MD and SPI to bind anthocyanins. Thus, the flavylum cation could be related with the better polymer-anthocyanin interaction. By the other hand, Ersus & Yurdagel (2007) studied the microencapsulation of anthocyanins pigments of black carrot by spray drying using MD with different DE and found that the greatest pigment retention was when used 20–23 DE.

The response surface methodology was applied to optimise the encapsulation efficiency of polyphenols and anthocyanins considering the linear, quadratic and cross-product forms for the independent variables studied (encapsulating agent and temperature) at $P \leq 0.05$ levels, for each system. In the case of polyphenols encapsulation, the encapsulating agent (A) linear form was significant in all the systems studied and their quadratic form (A²) had significant effect for PJ-SPI and PJ-MD. The temperature (B) linear form had a significant effect for PJ-MD and PE-SPI and their quadratic form (B²) for PJ-MD. The cross-product form (AB) was only significant for PJ-SPI.

For anthocyanins encapsulation, the linear form (A) was significant for PJ-SPI and the quadratic form (A²) for PJ-SPI, PE-SPI and PE-MD. The temperature linear form (B) was only significant for PJ-SPI and their quadratic form (B²) did not have effect as well as the crossproduct form (AB). Therefore, the encapsulating agent was the most important variable for the polyphenols encapsulation whereas for the anthocyanin encapsulation a smaller effect was observed according to its high ME.

Figure 2a–d shows the graphs obtained with the response surface methodology for the PJ-SPI, PJ-MD, PE-SPI and PE-MD designs, respectively. The effect of the temperature and the encapsulating agent on the

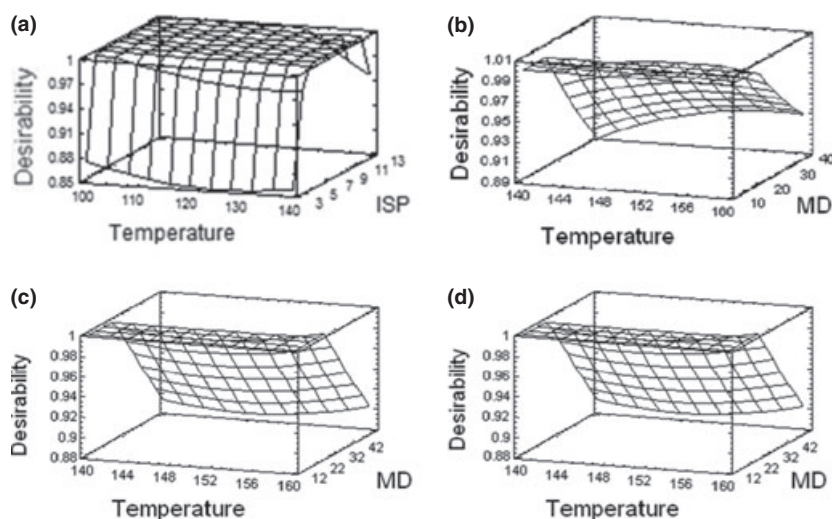


Figure 2 Graphs obtained by response surface methodology for PJ-SPI (a), PJ-MD (b), PE-SPI (c) and PE-MD (d).

encapsulation efficiency (expressed as desirability) is shown.

Microcapsule powder obtained under optimal conditions

Table 2 contains the concentration values of polyphenols and anthocyanins in the formulation and in the spray-dried powder obtained under optimal conditions from the encapsulated juice and ethanolic extract. The recovery percentages of bioactive compounds (polyphenols and anthocyanins) in all systems studied (PJ-SPI, PE-SPI, PJ-MD and PE-MD) were above 90%, showing that the drying temperature did not affect the recovery of the bioactive compound. The recovery of polyphenols over 100% could be a consequence of the hydrolysis of pomegranate polyphenols conjugated during the preparation of the samples or during the drying process (Turkmen *et al.*, 2005). In the β -carotene encapsulation with 25 DE MD, losses of 11% were obtained (Desobry *et al.*, 1997).

Table 3 shows the optimal conditions (percentage of encapsulating agent and inlet temperature) and the ME of the pomegranate bioactive compound microcapsules for PJ-SPI, PE-SPI, PJ-MD and PE-MD systems. The core material to coating material ratio was 1:1 for PJ-SPI, PJ-MD and PE-MD systems and 2:1 for PE-SPI system. These results suggest that the interaction between the bioactive compounds and the coating material could be more important in the PE-SPI system

allowing a greater bioactive compounds trapping than in PJ-SPI system. A higher core material to coating material ratio (3:1) for encapsulated cactus pear juice and ethanolic extract using MD (12–20 DE) was reported previously (Saénz *et al.*, 2009). In that research, the core/coating/water ratio and interaction between bioactive compounds and the coating material were discussed as parameters affecting encapsulation efficiency.

The polyphenols encapsulating efficiency was significantly better in SPI matrix whereas for anthocyanins was in MD matrix. This behaviour could be related with the bioactive compounds nature (i.e. charge: negative for polyphenols and positive for anthocyanins) and with polyelectrolyte structure (type and density charge), being SPI a poli(aminoacid) and MD a poli(glucosaccharide)), conditioning the bioactive-polymer interaction. Kim & Morr (1996) determined that SPI shows the better encapsulating efficiency of orange oil, respect to whey protein isolate, arabic gum and sodium caseinate as encapsulating agents.

Figure 3a–d presents SEM photographs of microcapsules for the PJ-SPI, PJ-MD, PE-SPI and PE-MD systems, respectively. The morphology of microcapsules with both encapsulating agents was irregularly spherical in shape with an extensively dented surface, attributed to the shrinkage of the particles during the drying process. Similar morphology was observed in microcapsules with different DE MDs (Díaz *et al.*, 2006; Cai & Corke, 2000).

Table 2 Bioactive compounds before and after the encapsulation of the juice and ethanolic extract of pomegranate in SPI or MD

System	Polyphenols			Anthocyanins		
	Theoretical mg GAE g ⁻¹	Experimental	% Recovery	Theoretical mg malvidin 3-glucoside g ⁻¹	Experimental	% Recovery
PJ-SPI	1.53	1.85 ± 0.023	121	0.64	0.59 ± 0.003	92
PJ-MD	1.84	1.76 ± 0.033	96	0.76	0.74 ± 0.023	97
PE-SPI	2.75	2.84 ± 0.182	103	0.83	0.84 ± 0.022	101
PE-MD	1.53	1.51 ± 0.014	99	0.46	0.47 ± 0.007	102

PJ, pomegranate juice; PE, pomegranate ethanolic extract; SPI, soybean protein isolates; MD, maltodextrin; GAE, gallic acid equivalent.

Table 3 Optimal conditions and ME of the pomegranate bioactive compounds microcapsules using SPI or MD

System	Temperature (°C)	EA (%)	Core/coating material	Polyphenols encapsulated (% ME)	Anthocyanins encapsulated (% ME)
PJ-SPI	120	7.5	(1:1)	76.2b	58.5a
PJ-MD	153	20.1	(1:1)	53.5a	86.6c
PE-SPI	100	3.75	(1:1)	82.9c	100d
PE-MD	153	24.2	(2:1)	71.0b	82.0b

PJ, pomegranate juice; PE, pomegranate ethanolic extract; SPI, soybean protein isolates; MD, maltodextrin; EA, encapsulating agent; ME, microencapsulation efficiency.

Different letters show significant differences between systems (by column) ($P < 0.05$).

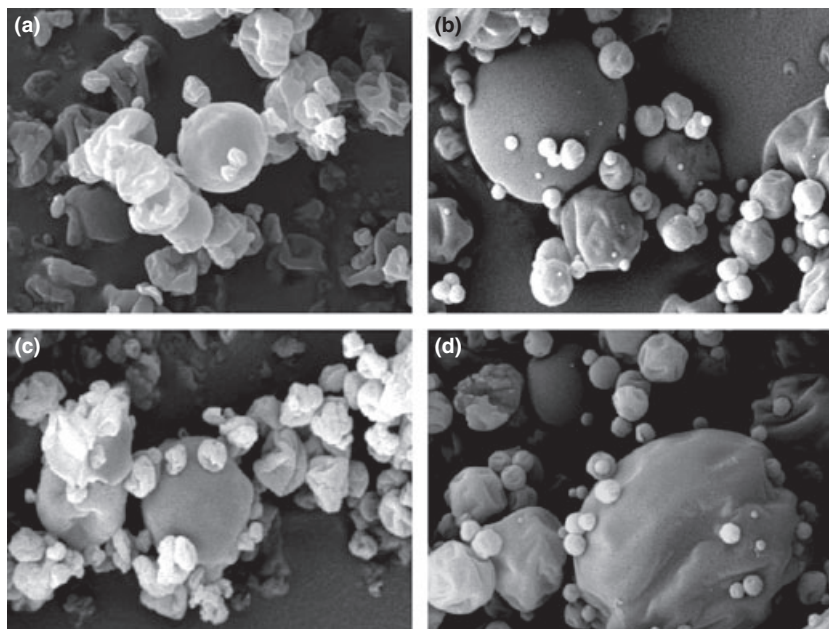


Figure 3 Scanning electron microscopic photographs of microcapsules for the PJ-SPI (a), PJ-MD (b), PE-SPI (c) and PE-MD (d) designs.

Storage stability evaluation

Figure 4 shows the evolution of polyphenols and anthocyanins retention percentages during the storage at 60 °C for PJ-SPI, PE-SPI and PE-MD systems. When SPI is used as a wall material both polyphenol and anthocyanin retention increase during the storage time (until 35 days), possibly due to the hydrolysis of the pomegranate conjugated polyphenols, and then decrease. Similar behaviour was also reported by other authors (Stewart *et al.*, 2000; Turkmen *et al.*, 2005). The polyphenol and anthocyanin retention diminish with storage time in MD microcapsules, except for PJ-MD system, where the polyphenol remains constant. Table 4 shows the first-order degradation rate constant for polyphenols and anthocyanin encapsulated, calculated between 35 and 56 days. PJ-MD system showed the lowest degradation rate for polyphenols and anthocyanins encapsulated. PJ-SPI, PE-MD and PE-SPI systems had higher polyphenols degradation rate however there was not significant differences between them. The anthocyanins show significant differences between all systems, being the highest degradation rate for the PE-SPI system, followed by PJ-SPI and PE-MD.

When compared the encapsulating agent effect on the polyphenols rate degradation constant, MD showed a significantly greater protective effect than SPI, for the juice systems. The same behaviour showed the anthocyanins degradation rate constant for the juice and also for the extract system. Contrary, Kim & Morr (1996) determined that SPI shows the lower lost limonene release rate on microencapsulated orange oil, respect to

whey, protein isolate, arabic gum and sodium caseinate as encapsulating agents. Dextrose equivalent of MDs has showed effect on the encapsulation yield but not effect was found during the storage in microencapsulated black carrot anthocyanins (Ersus & Yurdagel, 2007). The polyphenols and anthocyanins degradation in fresh juice (Fig. 4) was faster than that in microencapsulated juice, showing the importance of the encapsulating material in the degradation of bioactive compounds as has been reported for other functional compounds (Wagner & Warthesen, 1995).

The PJ system showed a significant lower degradation of polyphenols and anthocyanins than the PE system, suggesting that some components of the juice could aid the encapsulating process and therefore the stability of the bioactive compounds.

Table 4 shows the polyphenols degradation rate constant in yogurt for PJ-MD, PJ-SPI, PE-MD and PE-SPI systems. No statistical differences were observed between control ($7.58 \pm 0.73 \text{ day}^{-1}$) and PJ-MD, PJ-SPI and PE-SPI. The encapsulating agent (MD or SPI) showed significant effect on the polyphenols degradation rate constant for PE systems, having the MD the highest protective effect. When the microcapsules were added to yogurt the stability of the bioactive compounds followed a similar behaviour to those without encapsulation (juice), except for PE-MD. The microencapsulated anthocyanins disappear before 7 days, according to the observed by Coisson *et al.* (2005) during the addition of açai (*Euterpe oleracea*) juice to yogurt (10% w/w) where the anthocyanins were stable only 2 days at 4 °C. The anthocyanins degradation rate could be affected by the

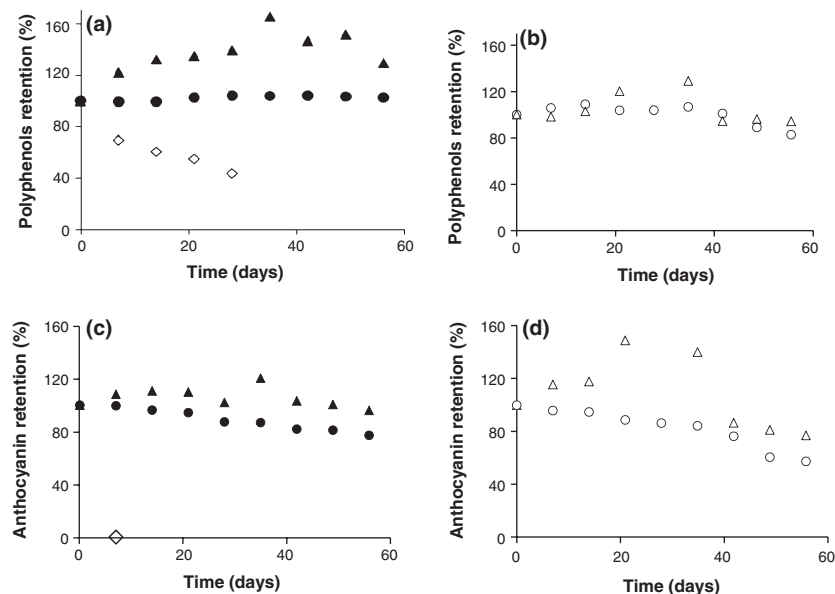


Figure 4 Evolution of the polyphenol and anthocyanins retention of microcapsules obtained under optimal conditions during storage at 60 °C. (a, c) Juice systems and (b, d) extract systems. PJ–MD (●), PJ–SPI (▲), control (◇), PE–MD (○) and PE–SPI (△).

Table 4 First-order degradation rate constant (k) of polyphenols and anthocyanins from pomegranate microcapsules obtained under optimal conditions stored at 60 °C and added to yogurt

System	Storage at 60 °C		Added to yogurt and storage at 4 °C
	Polyphenols $10^2 k_{\text{obs}} \pm 10^2 \text{SD} \text{ (days}^{-1}\text{)}$	Anthocyanins $10^2 k_{\text{obs}} \pm 10^2 \text{SD} \text{ (days}^{-1}\text{)}$	Polyphenols $10^3 k_{\text{obs}} \pm 10^3 \text{SD} \text{ (days}^{-1}\text{)}$
PJ–MD	$0.35 \pm 0.078\text{a}$	$0.49 \pm 0.046\text{a}$	$7.43 \pm 2.80\text{b}$
PJ–SPI	$1.36 \pm 0.354\text{b}$	$1.98 \pm 0.356\text{b}$	$6.22 \pm 0.98\text{ab}$
PE–MD	$1.27 \pm 0.125\text{b}$	$0.97 \pm 0.036\text{c}$	$5.34 \pm 0.55\text{a}$
PE–SPI	$1.57 \pm 0.040\text{b}$	$2.95 \pm 0.091\text{d}$	$7.48 \pm 0.65\text{b}$

PJ, pomegranate juice; PE, pomegranate ethanolic extract; SPI, soybean protein isolates; MD, maltodextrin GAE, gallic acid equivalent, SD, standard deviation. Different letters show significant differences ($P < 0.05$).

oxidation and consequently polymerisation of some phenolic compounds.

In conclusion, the bioactive compounds encapsulated obtained under optimal conditions showed a recovery over 90% after microencapsulation process. The encapsulation efficiency of bioactive compounds was higher when using SPI with respect to MD for PJ and PE system. However, the lowest bioactive degradation rate constant during storage at 60 °C was observed for MD systems. The bioactive compounds encapsulated added to yogurt showed a similar behaviour to those without encapsulation from juice, except for PE–MD. The pomegranate microcapsules studied could be used to design functional foods.

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