Olerosome-rich pectin network as a new, natural bioencapsulation matrix

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Introduction

The last decades, different categories of bioencapsulation matrices were tested, especially natural polymers (alginates, carragenans, dextrines, chitosans) as commercial products which selectively stabilize and assure controlled delivery of lipophilic or hydrophilic molecules (drugs, oils, vitamins, pigments, etc.).

Other hydrophilic matrices, as pectins, are also used and investigated as oral drug delivery systems with controlled-release applications because of their good compatibility. Pectin is a polysaccharide with a structural role in plant cell walls (Melia, 1991; Sande, 2005), obtained with low production costs, without any toxicity, used as stabilizer and gelling agent in food industry (drinks and lactic acid beverage, fruit jellies, yogurt). Pectin has also the ability to be a carrier in hydrophilic matrix controlled-release oral dosage forms (Sungthongjeen, 1999; Sriamornsak, 2007)

Fruit pulps and cell walls (such as olive) contain pectic polysaccharides which include arabinose linked to the C-4 of α-(1- 2)-linked L-rhamnose residues and interspersed in the α-(1- 4)-linked galacturonic acid (GalA) backbone which can be assembled by calcium bridges, playing an important role in cell–cell adhesion and cohesion (Ferreira, 2006). Similar structures were observed in Seabuckthorn fruits which contains 1.8% polysaccharides including pectins (0.5-1%), lignin, cellulose and other insoluble fibers, as well lipids associated with proteins (Singh, 2006)

Researches on pectin-based matrices and oral drug deliver systems are developing rapidly, most of the studies using pectin as a shield material. The basic poly-galacturonate structure of pectin shows close structural analogy to the poly-l-guluronate chain sequences of alginate (Liu, 2003).

The plant oils are encapsulated usually by adsorption on powders, such as maltodextrins and gums (arabic, xanthan), matrices which have emulsifying properties (at oil/matrix ratios inferior to 0.5) (Fuchs, 2006).

Our studies are focused on the characterization of natural biocomposites which are synthesized by plants and stored in fruits or seeds, and include combinations of natural polymers such as homo- and heteropolysaccharides (starch, cellulose, pectins), proteins, lipids (as oil-bodies or oleosomes). Such polymers make networks able to integrate, protect, and keep the functionality of different lipophilic or hydrophilic phytochemicals (Chedea, 2007; Trif, 2007;Socaciu 2007 a-d).

Such natural networks are interesting because, beside their natural and stable architecture, easy to obtain at cheap prices, they can be used as matrices for encapsulation, e.g. the inclusion of higher quantities of compatible synthetic or natural molecules (phytochemicals) because it provides biocompatibility, lack of toxicity and controlled release and complete biodegradability of the encapsulated structure.

We are presenting here the characterization of a supramolecular biocomposite structure found in seabuckthorn fruit pulp, a polysaccharide-oleosome matrix which naturally include both hydrophilic and lipophilic bioactive molecules. We propose the use of this matrix for encapsulation of pigments, oils, vitamins or drugs, with applications in biomedicine and cosmetics.

Morphologic characteristics of such structures, their spectroscopic fingerprint and stability, comparing with seabuckthorn oil are discussed.

Material and methods

Seabuckthorn (*Hippophae rhamnoides*) fruits were washed, grounded and filtered (through 1 mm sieves) to obtain a fine puree. The fruit pulp and the puree was examined microscopically and the pectin-cellulose biocomposite structure containing oleosomes was identified (Socaciu, 2006).
The puree, mixed with distilled water was then centrifuged at 5000 rpm and 3 fractions were obtained: a superior "low density fraction=LDF", an intermediate, turbid juice fraction and an inferior "high-density fraction=HDF". The LDF and HDF fractions were collected and microscopically examined (Socaciu, 2006). Both LDF and HDF were dissolved in ethanol an the UV-Vis spectra were registered (200-600 nm) and the pectin, protein, lipid, total carotenoids and water content were also determined (Socaciu, 2007a-d).
For encapsulation studies we used either the oil obtained by extraction of puree in petroleum ether (SBO) or the LDF or HDF. The SBO was encapsulated in alginate-carrageenan mixture (Socaciu, 2007a,c,d) and and the characterization of capsules (SBO-C) was made by UV-Vis, FTIR and NMR analysis (Trif, 2007). The LDF was mixed with lecithin, distilled water and soy oil to obtain a stable emulsion (LDF-E) and then encapsulated in alginate 2% (LDF-C) after hardening in a solution of CaCl2 0.5M, while HDF was directly homogenized with citrus peel pectins 10% (C1) or alginate 2% (C2) and jellified in the same hardening solution of Calcium Chloride 0.1M obtaining capsules HDF-C1 and HDF-C2. The holding time inside the CaCl2 solution was set at 4 h. The beads were recovered and rinsed with distilled water, then dried at room temperature by exposure to air (20°C; 70% RH)
These encapsulated products were tested for their macro- and microscopic properties, using physical and chemical analysis.

Results and Discussion

Bead size and morphology

Fig.1. shows the microscopic image of the polyscaharide-oleosome network in the Seabuckthorn fruit pulp (upper left), immediately after filtration: LDF (upper right) and HDF (down left) compared with SBO (down right). Different sizes of oleosomes are visible, from 5 to 20 μm, with bigger diameters in LDF. LDF was more rich in lipid components, stored in fewer, but larger oleosomes, while HDF contained many smaller oleosomes, dispersed in a pectin network, with better stability during storage (data not shown).
Fig.2. shows the capsules resulted from SBO (SBO-C) and LDF (upper photo) and from HDF-C1 and HDF-C2 (down), respectively. Their dimension is different due to their different compacted structures influenced by the matrix viscosity and gelling capacity in $CaCl_2$ solution. HDF-C1 are the most dense and structured capsules (average of 1 mm diameter) while HDF-C2 and SBO capsules have diameters of about 2 mm while LDF formed emulsified structures (micelles) of 0.8-1.5 mm. The stability of these 4 microcapsule types was different: at higher temperatures (45°C) HDF-C2 had the best stability while LDF lost stability and the lipophilic/hydrophilic phases were separated.

Physical-chemical analysis

Fig.3. shows the GC-FID chromatogram of Fatty acids found in SBO as fresh, free product (left amd the FTIR fingerprint (800-4500 cm^{-1}) of the SBO in free and encapsulated from (SBO-C) (right). According to our complementary studies regarding the stability of SBO in free and encapsulated form after UV irradiation (data not shown) the fatty acid composition was not significantly changed, an average of 5-7% decrease in unsaturated FA forms being noticed in free SBO and excellent stability of the encapsulated form.

The carotenoid, phenolic and chlorophyll fingerprint of HDF (C1 or C2) capsules is presented in Fig.4. Comparatively with the initial HDF, the encapsulated forms preserved 99-99.5% the pigment content and their antioxidant capacity (as determined by DPPH method, data not shown).

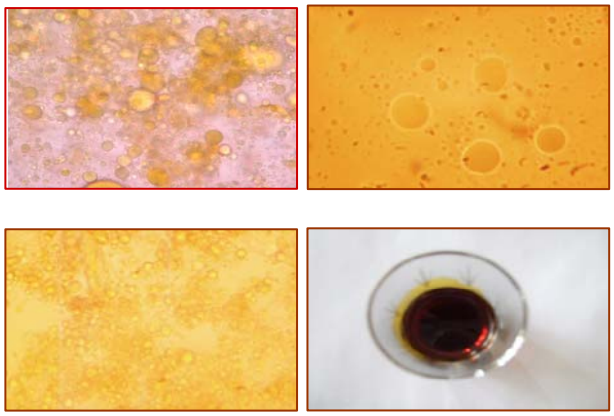

Fig. 1. Microscopic image (20x)of the polysaccha-ride-oleosome network in SB pulp (upper left),LDF (upper right), HDF and SBO (down left and right)

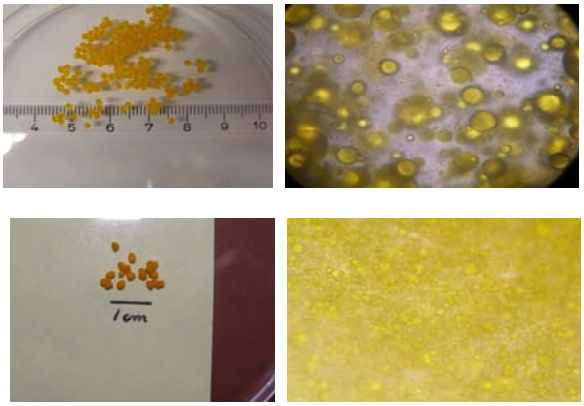

Fig.2.General aspect and dimensions of microcapsules SBO-C and LDF (up) and HDF-C1 and HDF-C2 (down)

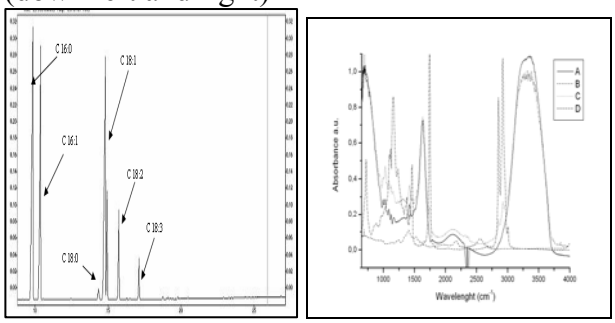

Fig.3. GC-FID and FTIR fingerprint of SBO , free (A) and encapsulated (SBO-C) in alginate. The fatty acid composition: 32.27%($C_{16:0}$), 25.46%($C_{16:1}$), 30.76%($C_{18:1}$), 6.97%($C_{18:2}$), 3.05% ($C_{18:3}$)

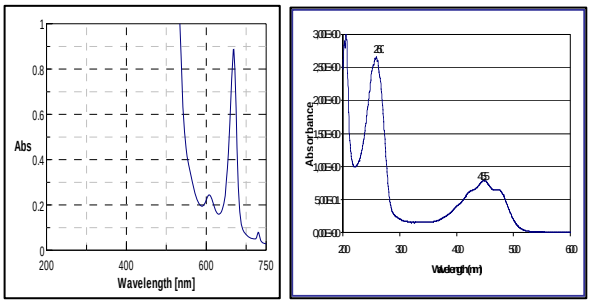

Fig. 4. UV-Vis spectra of HDF: phenolics (λ_{max}=280 nm), carotenoids (λ_{max}=450 nm and chlorophylls (λ_{max}=610 and 660 nm) identified.

Conclusion

Seabuckthorn fruits offer a complex biocomposite structure, made of heterogenic polysaccharides (pectins, lignins, hemicelluloses and cellulose) which inserts and stabilize the monolayered oleosome spheroids that store oils. This complex structure contains also pigment-lipoprotein complexes, lipophilic and hydrophilic vitamins distributed in pectin or oleosome fractions.

We were able to separate low- and high-density fractions (LDF and HDF) from the fruit pulp, the first one being more rich in oleosomes and the second, more rich in hydrophilic polyglucide network. The traditional SB oil-encapulated forms (SBO-C) have lower stability than LDF forms and are more expensive, considering the technological costs and safety problems of oil extraction into organic solvents.

The low-density fraction (LDF) is recommended to encapsulate oily drugs or biomolecules and/or to be encapsulated in hydrocolloids (alginates, carragenans), useful as ingredient for cosmetic formulations.

The second, high density fraction (HDF) can be used as such in cosmetic formulas or as nutraceutical/food supplement. By encapsulation in hydrocolloids or dextrins this fraction is better protected and can store different bioactive components (drugs, plant extracts) with targeted action. Further studies will evaluate the stability and release rate of different variants of encapsulated LDF or HDF with cosmetic or food applications.

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References

V. Chedea et al. (2007) *Stability studies of encapsulated carotenoid extract from orange waste using pullulan as microencapsulation matrix*. Int.Symp., Nanotech Insight, 10-17 March 2007, Luxor, Egipt, 156-157

M. Fuchs (2006) *Encapsulation of oil in powder using spray drying and fluidised bed agglomeration*, Journal of Food Engineering 75, 27–35

L.S.Liu (2003) *Pectin-based systems for colon-specific drug delivery via oral route*, Biomaterials 24 (2003) 3333–3343

C.D. Melia (1991) *Hydrophilic matrix sustained release systems based on polysaccharide carriers*. Crit. Rev. Ther. Drug Carrier Syst. 8 395–421.

S.A. Sande, (2005) *Pectin-based oral drug delivery to the colon*. Expert Opin. Drug Deliv. 2, 441–450.

C. Socaciu (2007c) *New technologies to extract and encapsulate natural food colorants- a review*. Proc. Int. Symp. USAMV Cluj-Napoca, 4-6 Oct. 2007, 63/64, 485-491

C. Socaciu et al. (2007b) *Seabuckthorn fruit oleosomes as natural, micro-encapsulated oilbodies: separation, characterization, stability evaluation.*15th Int. Symposium on Bioencapsulation, 6-8 Septembrie 2007, Wien, P3-19, 1-3

C. Socaciu et al. (2007d) *Quality and authenticity of seabuckthorn oils using succesive UV-Vis, FT-IR, NMR spectroscopy and HPLC-, GC- chromatography fingerprints*. Int. Seabuckthorn Association Conference, 12-16 august 2007, Quebec,Canada

C. Socaciu et. al. (2007a) Oleosome fractions separated from sea buckthorn berries: yield and stability studies, in: *Seabuckthorn (Hippophae L.): A Multipurpose Wonder Plant, Vol.III,*

V. Singh (ed.) (2006) *Seabuckthorn (Hippophae L.): A Multipurpose Wonder Plant, , Vol.II: Biochemistry and Pharmacology*. Daya Pub., Delhi, 605 pp.

C.Socaciu (2008) in *Food Colorants- Chemical and Functional Properties*. Socaciu C.(ed) CRC Press, Taylor & Francis, New York

J.A. Ferreira (2006) *Dimeric calcium complexes of arabinan-rich pectic polysaccharides from Olea europaea L. cell walls*, Carbohydrate Polymers 65, 535-543

P. Sriamornsak (2007) *Swelling and erosion of pectin matrix tablets and their impact on drug release behaviour*. Eur. J. of Pharmaceutics and Biopharmaceutics 67, 211–219

S. Sungthongjeen, et al. (1999) *Studies on pectins as potential hydrogel matrices for controlled release drug delivery*,. Drug Dev. Ind. Pharm. 25, 1271–1276.

M. Trif et al. (2007) *Application of FTIR spectroscopy to evaluate the oxidation of encapsulated seabuckthron oil*. 15th Int. Symp.on Bioencapsulation, Wien, Austria, P3-07, 1-3