Seabuckthorn fruit oleosomes as natural, microencapsulated oilbodies: separation, characterization, stability evaluation

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Introduction

The nutritional and medicinal value of sea buckthorn (SB) *(Hippophae rhamnoides L.*) berries is well known (Luetjohann, 1999; Kallio et al., 2002; Socaciu et al., 2002; Singh, 2006, 2007), due to their unique composition in bioactive phytochemicals including both lipophilic (PUFAs, carotenes, tocopherols) and hydrophilic (vitamin C, phenolic acids and flavonoids) antioxidants (Socaciu et al., 2002, 2003, 2006).
Ussually, the seeds of oleagineaous plants store oil in lipid bodies (of 1-5 μm) with physiological role in lipid trafficking surrounded by a layer of polar lipids embedded in supramolecular complexes (Deruere et al., 1994; Millichip et al., 1996; Frandsen et al., 2001; Murphy, 2004). The SB berries are able to store oils not only in seeds but also in the pulp, in small, discrete intracellular vesicles accumulated in chromoplasts. During ripening, chromoplasts accumulate such natural microencapsules (named oil bodies or oleosomes) which are distributed in the pectin network (up to 20 μm) (Beveridge et al, 2001; Socaciu et al, 2006). Several studies (Gomez et al, 1992; Lakshman et al, 1993; Socaciu et al, 2006, 2007a). Oleosomes accumulate and store mostly lipophilic molecules biosynthesized during photosynthesis and chloroplast to chromoplast transition. The single layer of such vesicles may include amphiphilic molecules (Murphy, 2004), mainly phospholipids, proteins as well carotenoid-lipoprotein complexes (CLPs). We stidied previously(Pintea et al, 2001, 2004; Socaciu et al, 2002, 2007a) the preparation and characterization of these complexes.
The SB oil is known for its rich composition in polyunsaturated fatty acids ($C_{16:1}$, $C_{18:1}$, $C_{18:2}$), sterols, tocopherols and carotenoids (up to 1800 ppm), strong antioxidants with UV-protective action and useful as ingredients for skin care products, dermocosmetics or food supplements (Heilscher et al, 1999; Socaciu et al., 2007b). New data are available about the composition and use of the isolated CLPs, replacing SB oil in cosmetic products (Pintea et al., 2001; Socaciu et al, 2002,2003,2007b) with good emulsifying properties, useful for food and non-food applications.
These studies continue our previous observations regarding the cLPs fractions separated from SB juice and aimed the morphological and physical-chemical characterization of oleosome vesicles which are present in these fractions. We investigated the size and behavior of oleosomes using optical microscopy and UV-Vis, FT-IR spectrometry, their stability in different conditions (dark/ light, 4ºC and 25ºC, different pH values, enzyme treatments) in order to be used as ingredient in cosmetic emulsions as innovative liposome-like ingredients.

Material and Methods

Two oleosome-rich fractions (low-density fraction F1 and high density F2) were obtained by centrifugation from the raw juice of SB berries, at two different temperatures (4ºC and 25ºC)(developed under EU project CRAFT- G5ST-CT-2002-50352). The fractions were separated and there were characterized by volume, lipid content (extraction in Soxhlet), carotenoid content (UV-Vis spectrometry). In order to check their stability, we used different conditions: dark and light (for 3 months), temperatures of 57ºC for 4 and 8 hrs, modifications of pHs from 2.8 to 3 and 5, with or without enzymatic treatment with a commercial pectinase Rohapect 10L (100, 200, 500 and 1000 ppm) in a sonication bath at 30% pulses for 5 minutes. FT-IR and UV-Vis spectrometry were used to evaluate the fingerprint and quality of each fraction and target molecules (carotenoids and lipids)

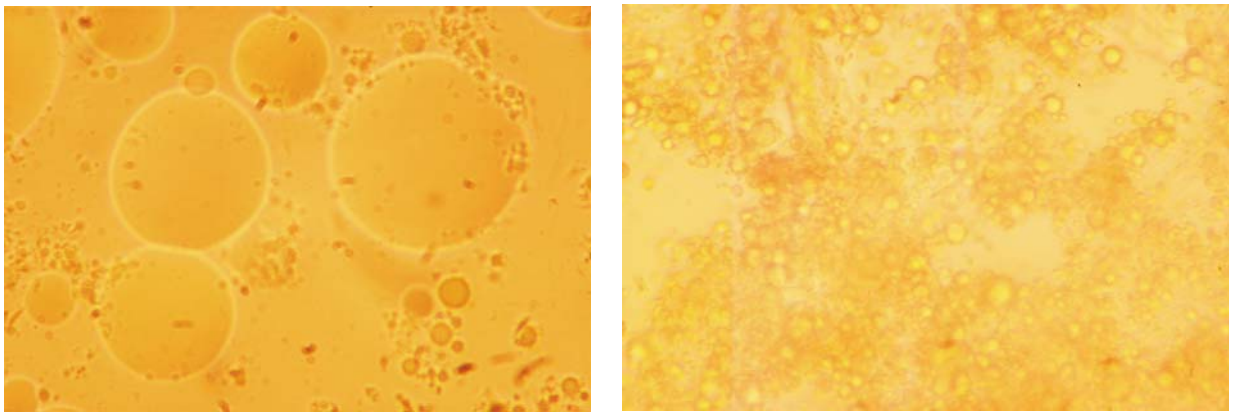
Results and discussion

One high-density (F2) and a low-density (F1) fraction were separated from the SB berry juice in a average ratio of 3:1, their appearance and composition being slightly different (Fig.1 and Table 1).

Temp.	Yield (% F1+F2 form juice)	Ratio F1/F2	Carotenoids (mg/kg d.m.)	Lipids (g/kg d.m.)	Ratio Lipids/carotenoids
4 ºC	14.5	1/3.2	F1: 1380 ±230 F2: 1250 ±120	F1: 900 ±60 F2: 550 ±80	F1:652 F2:440
25ºC	12	1/2.8	F1: 1380 ±230 F2: 1250 ±120	F1: 880 ±65 F2: 590 ±42	F1:637 F2:472

Table 1. Yields and comparative composition (total carotenoids and lipids) of fractions F1 and F2 separated from raw SB juice (d.m. – dry matter)

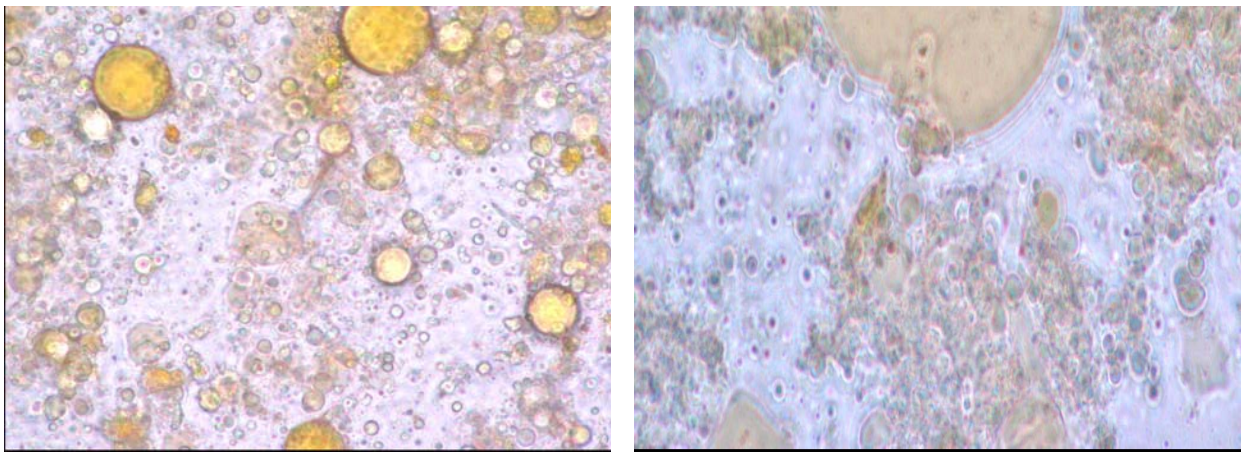
The optical microscopy shows oleosomes with different colors and dimensions (2-20 μm) as presented in Fig.1. The upper F1 fraction (A) is richer in carotenoids and lipids than the lower F2 fraction, which is richer in pectins, which insert better oleosomes in the network (B)



A. **B.**

Fig. 1. Images (400x magnitude)of the two oleosome fractions: A - upper low density F1 fraction , and B- high-density F2 fraction, separated from SB raw juice down

The stability of both F1 and F2 fractions kept in light and dark was different: in dark, a good stability was noticed for both fractions, but in light, F1 fraction was more labile (by FT-IR spectroscopy we identified peroxidation (data not shown).The HPLC fingerprint of carotenoids indicated similar compositions for both fractions and stability in dark and light(data not shown).
Same samples exposed at 57º C for 4 to 8 hours suffered modifications of the structure, especially F1 after 8 hrs (Fig.2 shows modifications of F1 fraction).
The pH of the fractions was initially 2,8. by addition of NaOH to raw juice it was reached the pH 3 and 5. It was noticed that an increase in pH did not influenced the yield of F1 fraction after centrifugation (Fig.3). By sonication and treatments with Rohapect 10L at pH 3, the increase of F1 fraction was significant (more than 3 times) comparing with the control.



A. B.

Fig.2. Modifications of the F1 fraction after heat treatments at 57C ºfor 4 (A) and 8 hrs (B).

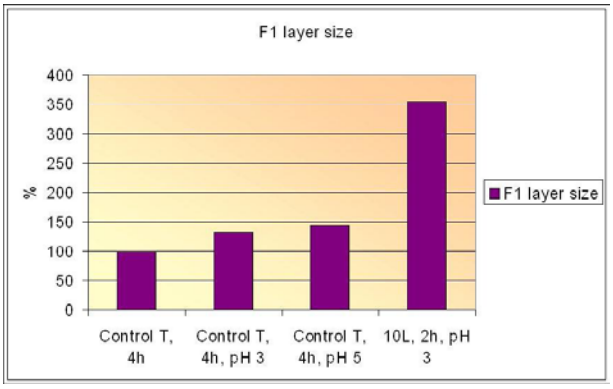


Fig.3. The yield (%)F1 fraction obtained from raw juice after incubation 4 hrs at pH= 3 and 5, and after 100 ppm Rohapect 10L treatment combined with sonication.

None of the samples showed damaged structures of the oleosomes after 200 ppm Rohapect tratement, combined with sonication. But when the enzyme quantity was higher than 500 ppm, and the temperature was high, the oil was released from oleosomes.

Conclusions

According to these experimental data, using fresh raw SB juice we obtained two oleosome-containing fractions, one of low density, more rich in lipids (F1) with higher size oleosomes and a high density fraction (F2), with smaller oleosomes inserted in the pectin network, their ratio F1/F2 being of aprox.1:3. The stability of first fraction was lower due to its higher content in lipids and sensitivity to oxidation under light and high temperatures. The F1 fraction can be obtained in higher concentrations by enzymatic treatment (Rohapect 200 ppm by sonication at 40ºC)and can be used as a good cosmetic cream ingredient or butter/margarine additive. The major, low density fraction F2 is more stable in watery environment and at higher temperatures, can be recommended as ingredient or additive for skin-care or food emulsions. These fractions represent an example of natural microencapsulated vesicles with interesting applications in food, cosmetic and biomedical areas.

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