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DETERMINATION OF ENCAPSULATED SEA BUCKTHORN OIL OXIDATION USING FTIR-ATR SPECTROSCOPY

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Abstract: This work examines the oxidation process of Sea buckthorn oil encapsulated in ionotropically cross-linked alginate beads under oxidative conditions. The oxidation of oil was monitored by Fourier Transform Infrared Spectroscopy equipped with the universal ATR (FTIR-ATR) as an internal accessory. We considered as markers of oxidation processes in sea buckthorn oil. the following ratios between absorbances of some important bands: $A_{2853}/A_{3005-3007}$. A_{2853}/A_{1744}. $A_{2853}/A_{1159-1160}$. $A_{1744}/A_{3005-3007}$. $A_{1377}/A_{3005-3007}$. $A_{1159-1160}/A_{3005-3007}$. The values of all ratios indicated a first stage oxidation of encapsulated oil and a second or third stage oxidation of free oil. The encapsulated Sea bucktorn oil showed a good stability under oxidation conditions. Differential scanning calorimetric analysis was used to characterize the thermal behavior of free oil. the oil alginate beads and empty beads.

INTRODUCTION

Sea buckthorn oil (SBO) is well known for its high nutritional and biomedical value. due to the high content in antioxidants (PUFAs. carotenoids. tocopherols. phytosterols) with many applications as food supplement of additive to food or non-food products (dermocosmetics)[11].

This oil has a limited stability due to its lability to light. oxygen. irradiation. especially if incorporated in watery products like milk. yoghurt. cheese. juices. Microencapsulation can be the best alternative to improve the stability of SBO and its functionality. Fourier transform infrared (FTIR) spectra has been already found to be a versatile technique for evaluating the authenticity. stability and oxidation of edible oils in a simple. fast and accurate way [4. 5. 6].

The aim of this work was to monitor the stability of oil after encapsulation using FTIR-ATR. This technique became a powerful analytical tool in the study of oils and fats. It was applied also to identify the type of the phycocolloids by many seaweeds. The wide uses of these phycocolloids are based on their gelling. viscosifying and emulsifying properties [13].

Alginates are a family of polysaccharides occurring in brown seaweeds. They are composed of β-D-mannuronic acid and α-L-guluronic acid in varying proportions and sequential arrangements. Alginate is an anionic linear polysaccharide [10] and can form gels with multivalent cations such as calcium ions in aqueous media [1].

MATERIAL AND METHOD

Materials. Sodium alginate was purchased from Promova Biopolymer Norway. calcium chloride from Sigma Aldrich. sea buckthorn oil was extracted from the fruits of sea buckthorn. which were collected from Cluj county (Transilvania. North of Romania).

Beads preparation. Different concentration of sodium alginate (1.0% w/v. 1.5% w/v and 2.0% w/v) weredissolved in de-ionized water and were used to encapsulatethe sea buckthorn oil by ionotropically cross-linked gelation. The sea buckthorn-alginate emulsion obtained was dropped using a syringe with a needle (0.4 x 20 mm) into a hardening bath 2% (w/v) solution of $CaCl_2$ in water. After 30 minutes. the beads were separated from the hardening bath by filtration.

Light microscopy. Images of emulsions at magnification 20X were obtained using a light microscope Olympus BX40. Japan with a digital camera.

FTIR-ATR spectra. The FTIR spectra were obtained with a Fourier transform spectrometer Spectrum One (PerkinElmer). equipped with the universal ATR as an internal reflection accessory which have Composite Zinc Selenide (ZnSe) and Diamond crystals. Each spectrum was from 4000 to 650 cm^{-1}. Between measurements the crystal was cleaned with acetone. The oxidation process under UV light (254 μm) on time (after 1h. 4h and 6h) was monitored calculating the ratios between absorbances of some bands of the spectra of free oil and encapsulated oil in different alginate concentration

Differential Scanning Calorimetry analysis. Differential scanning calorimetric analysis was used to characterize the thermal behavior of the isolated substances. their physical mixture. and empty and loaded beads. Differential scanning calorimetry (DSC) thermograms were obtained using an automatic thermal analyzer system (DSC-60. Shimadzu. Japan).

Temperature calibration was performed using indium as a standard. Samples were crimped in a standard aluminum pan and holded for 1 minute at 20°C . than heated from 20 to 350°C at a heating rate of 350°C. than cooled from 350°C to 20°C at 10°C/min. under constant purging of dry nitrogen at 30 mL/min. An empty pan. sealed in the same way as the sample. was used as a reference. The characteristic endothermic peaks and specific heat of the melting endotherm were recorded.

RESULTS AND DISCUSSIONS

Confocal imaging. The light microscopy imaging of the sea buckthorn-alginate emulsion reveals the presence of polydisperse oil droplets whose size varies. All emulsion are instable after few minutes. but the most stable was the emulsion using 2% w/v alginate cc. 10 minutes. the emulsions must be drop into hardening bath immediately after their production.

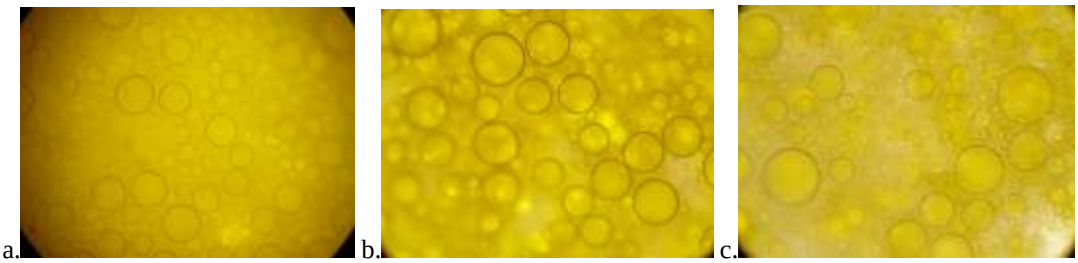


Fig. 1 Microscopic emulsion of sea buckthorn oil with sodium alginate: a. 2% w/v. b. 1.5% w/v. c. 1% w/v (light microscopy magnification 20X)

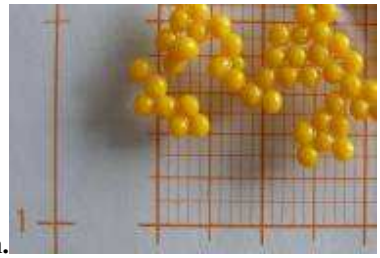
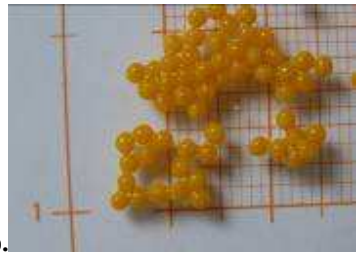
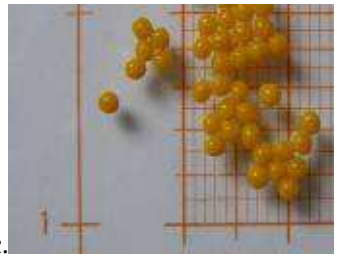
a.  b.  c. 

Fig. 2 Sodium alginate beads with sea buckthorn oil: a. 2% w/v. b. 1.5% w/v. c. 1% w/v

By dropping into the hardening bath ($CaCl_2$ 2% w/v) the emulsion of sodium alginate and se buckthorn oil orange beads with diameters of 2-3 mm and spherical shapes were obtained. The concentration of the sodium alginate influenced the diameter of the beads. Increasing concentrations of the sodium alginate determine harder beads.

FTIR-ATR spectra. FTIR spectra of sea buckthorn oil. sodium alginate. calcium alginate blank beads. and the calcium alginate beads containing sea buckthorn oil are shown in Fig 3.

The alginate FTIR spectrum showed the characteristic peaks at 3242 cm−1 (OH^- stretching). 1596 and 1407 cm^{-1} (COO^- asymmetric and symmetric stretching). 1081-1024 cm^{-1} (C-O-C antisymmetric stretching). and carboxyl and carboxylate at about 1000 to 1400 cm^{-1} [8].

In FTIR spectra of calcium alginate beads the asymmetrical band of carboxylate ion has shifted to lower frequencies from 1596 cm^{-1} to 1606 cm^{-1}. and the hydroxy band of sodium alginate has shifted from 3242 cm^{-1} to 3337 cm^{-1}. because of the interaction of sodium alginate and $CaCl_2$ [7]. The band at 1024 cm^{-1} is given by the guluronic units [9] in the all spectra.

In FTIR spectra of sea buckthorn some of the most significant bands are the following [2. 3. 4. 5. 6]: the band at 3485.77 $cm^{-1}$is assigned to the overtone of the glyceride ester carbonyl; band appearing at 3005.61 cm^{-1} in the spectrum to the CH stretching of =C-H bonding; the two intensive bands at 2922.86 cm^{-1} and 2853.64 cm^{-1} are assign to the aliphatic CH_2 asymmetric and symmetric stretching vibration. respectively; the band at 1744.38 cm^{-1} is assigned to the C=O stretching vibration of the ester carbonyl functional group of the triglycerides; at 1464.76 cm^{-1} is observed a band which is assigned to C=H scissors deformation vibration; the band near 1377 cm^{-1} is assign to the bending vibration of CH_2 groups; the bands at 1160.74 cm^{-1} and 1236.86 cm^{-1} are assign to the vibration of the C-O ester groups and CH_2 group; band near 1117 cm^{-1} is associated with the stretching vibration of the C-O ester group.

All spectra showed a band between 3200-3500 cm^{-1}. region that is assign to the hydroxyl or overtone. and we couldn't consider it as marker of oxidation. In the same case were some other bands at 1117 cm^{-1} and 1464 cm^{-1}. The band at 3005 cm^{-1} in the spectrum of sea buckthorn oil after oxidation is shifted to 3007 cm^{-1} in the spectrum of sea buckthorn oil calcium-alginate beads and the band at 1160 cm^{-1} in the spectrum of sea buckthorn oil calcium-alginate beads is shifted to 1159 cm^{-1}.

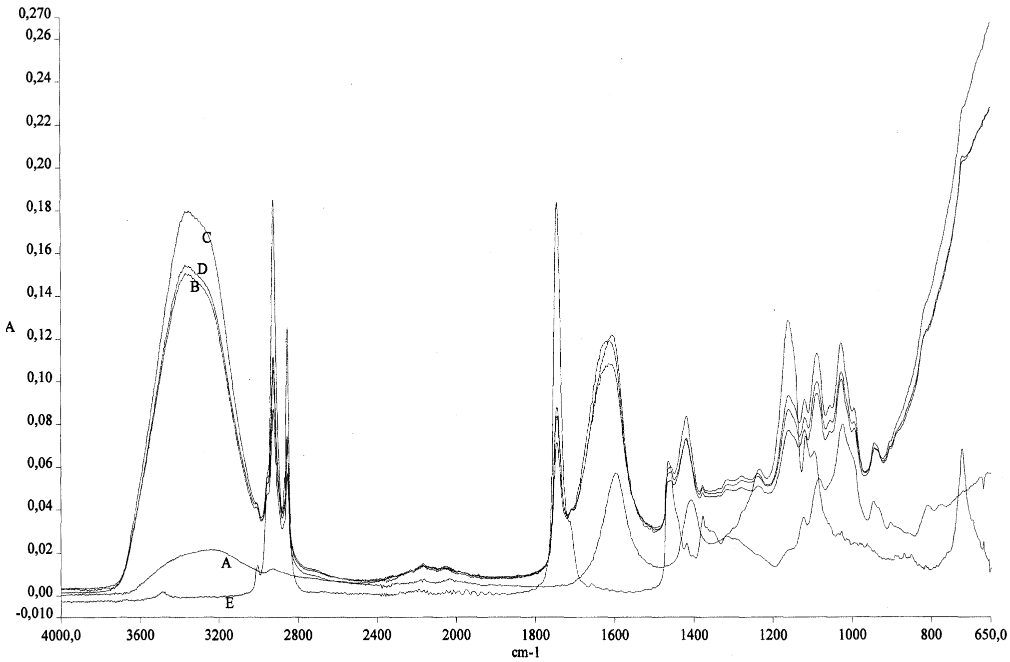


Fig. 3 FTIR-ATR spectra of: A-sodium alginate powder; B sea buckthorn oil in calcium alginate beads 1% w/v; C B sea buckthorn oil in calcium alginate beads 1.5% w/v; D B sea buckthorn oil in calcium alginate beads 2% w/v; E sea buckthorn oil free

We considered as markers of oxidation processes in sea buckthorn oil. the following ratios between absorbances of some important bands: $A_{2853}/A_{3005-3007}$. A_{2853}/A_{1744}. $A_{2853}/A_{1159-1160}$. $A_{1744}/A_{3005-3007}$. $A_{1377}/A_{3005-3007}$. $A_{1159-1160}/A_{3005-3007}$ [12].

Table 1

Changes in the band near 3005 cm^{-1}

Sample	After 1h UV				After 4h UV				After 6h UV			
Ratio between the absorbance	$A_{2853}/A_{3005-3007}$	$A_{1744}/A_{3005-3007}$	$A_{1377}/A_{3005-3007}$	$A_{1159-1160}/A_{3005-3007}$	$A_{2853}/A_{3005-3007}$	$A_{1744}/A_{3005-3007}$	$A_{1377}/A_{3005-3007}$	$A_{1159-1160}/A_{3005-3007}$	$A_{2853}/A_{3005-3007}$	$A_{1744}/A_{3005-3007}$	$A_{1377}/A_{3005-3007}$	$A_{1159-1160}/A_{3005-3007}$
oil	8.015	11.68	8.200	11.84	2.390	8.304	2.430	8.094	7.880	11.260	2.272	7.982
Oil beads (1%)	2.284	2.972	3.011	3.806	1.650	3.828	1.434	3.102	3.306	4.168	1.568	3.879
Oil beads (1.5%)	2.042	2.557	3.160	3.761	1.605	4.180	1.454	2.961	2.810	3.416	1.646	3.647
Oil beads (2%)	1.908	2.257	2.382	2.880	1.670	3.542	1.350	2.500	2.505	3.104	1.618	3.425

Table 2

Changes in the region between 1800 and 1000 cm^{-1}

Sample	After 1h UV		After 4h UV		After 6h UV	
Ratio between the absorbance	A_{2853}/A_{1744}	A_{2853}/A_{1160}	A_{2853}/A_{1744}	$A_{2853}/A_{1159\text{-}1160}$	A_{2853}/A_{1744}	$A_{2853}/A_{1159\text{-}1160}$
oil	0.769	0.736	0.692	0.987	0.700	0.987
Oil beads (1%)	0.686	0.990	0.791	0.786	0.793	0.852
Oil beads (1.5%)	0.799	0.688	0.840	0.756	0.822	0.770
Oil beads (2%)	0.845	0.766	0.827	0.673	0.804	0.729

According to the observations of Guillen et al. [4]. the values of the ratios: A_{2853}/A_{3007} are indicative parameters of the oxidation level. indicate a second or third stage oxidation of the pure oil. comparing with the first stage of oxidation of encapsulated oil; A_{2853}/A_{1160} indicate a second or third stage of pure and encapsulated oil. All data is possible to be influenced by the high absorption at 2853 cm^{-1}. Similar changes were observed in the ratios between absorbances of the other significants bands which are shown in the Tabels 1 and 2.

The encapsulated procedure protect the SBO against oxidation. proportional with concentration of alginate. best protected being the SBO oil beads 2%.

Differential Scanning Calorimetry analysis. The DSC thermograms of empty calcium alginate beads and sea buckthorn oil beads shows the following endothermic peaks: empty beads: 1% alginate 130.360°C ; 1.5% alginate 147°C and 2% alginate 138.033°C ; sea buckthorn oil beads: 1% alginate 126°C; 1.5% alginate 127.666°C and 2% alginate 143.670°C.

This confirms that the sea buckthorn oil and the concentration of the sodium alginate influence the endothermic peaks of the beads.

CONCLUSIONS

Sea buckthorn oil was successfully encapsulated by ionotropically cross-linked gelation using different concentrations of alginate and the stability of the encapsulated sea buckthorn oil in alginate beads was studied when irradiated with UV lights was monitored using FTIR

The values of IR absorption ratios indicated a second or third stage oxidation of free oil and a first stage oxidation of encapsulated oil. Encapsulation in sodium alginate improved sea buckthorn oil stability. FTIR has been found to be a very good technique to monitor the oxidation of the encapsulated sea buckthorn oil.

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