Application of FTIR Spectroscopy for determination of oxidation of encapsulated sea buckthorn oil

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Introduction

The application of sea buckthorn oil is to incorporate the oil into foodstuffs such as milk, yoghurt, cheese, butter, juice and snacks which represents new opportunities for food manufacturers, food supplements and nutraceuticals providing nutritional supports. The high content of polyunsaturated fatty acids and other oxygen sensitive lipid nutrients make the oil susceptible to oxidation, which limits the applications. Fourier transform infrared spectra has been already found to be a versatile technique for evaluating the oxidation of edible oils in a simple, fast and accurate way (Guillen, 2000).
The aim of this work was to monitor the stability of oil after encapsulation in sodium alginate using the Fourier transform infrared spectroscopy equipped with the universal ATR as an internal reflection accessory.

Materials and methods

Materials. Sodium alginate was purchased from Promova Biopolymer, 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) were used to encapsulate the sea buckthorn oil by ionotropicaly cross-linked gelation.

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 absorbance of some bands of the spectra of free oil and encapsulated oil in different alginate concentrations.

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 held at 20°C for 1 minute, then heated from 20 to 350°C at a heating rate of 350°C, then 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 Discussion

Confocal imaging. The light microscopy imaging of the sea buckthorn-alginate emulsion reveals the presence of polydisperse oil droplets whose sizes varies. All emulsions became instable after few minutes, most stable was the emulsion using 2% w/v alginate ~ 10 minutes, the emulsions were dropped into hardening bath immediately after were obtained. By dropping into the hardening bath ($CaCl_2$ 2% w/v) the emulsion of sodium alginate and sea buckthorn oil orange beads with a diameter between 2 and 3 mm and a spherical shape were obtained.

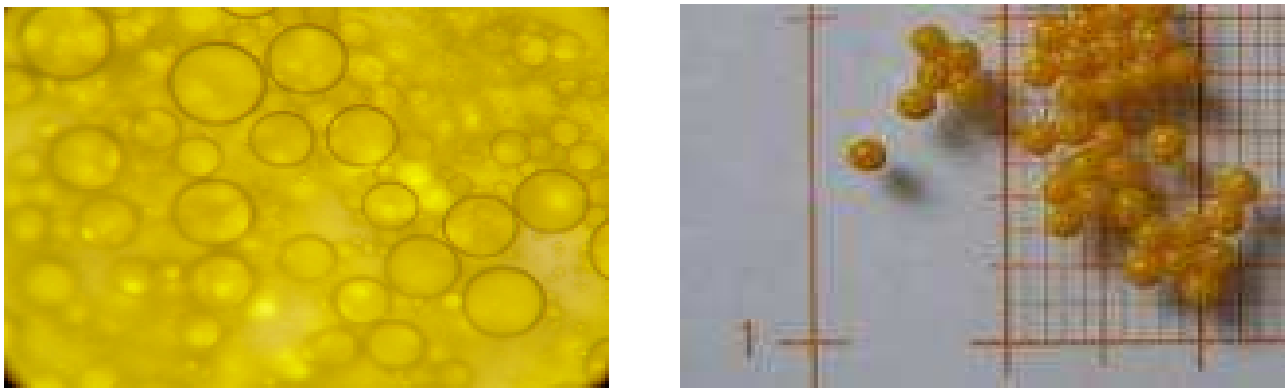

Fig. 1 From left to right: microscopic emulsion of sea buckthorn oil with sodium alginate 1,5% and sodium alginate beads 1,5% w/v with sea buckthorn oil 1,5%

FTIR-ATR spectra. FTIR spectra of sea buckthorn oil, sodium alginate, calcium alginate blank beads, and the calcium alginate beads with sea buckthorn oil are shown in Fig. 2. The alginate FTIR spectrum showed the characteristic peaks at 3242 cm−1 (OH^- stretching) (Lawrie, 2007), 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} (Mayur, 2005). In FTIR spectra of calcium alginate beads the asymmetric 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 $CaCl_2$ (Lawrie, 2007). The band at 1024 cm^{-1} is given by the guluronic units (Pereira, 2003) in all the spectra. In FTIR spectra of sea buckthorn some of the most significant bands are the following (Guillen, 1997, 1998, 1999, 2000): 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 3200-3500cm^{-1}, region that is assigned to the hydroxyl or overtone, and we couldn't consider it as marker of oxidation. In the same case were some other bands at 1117cm^{-1} and 1464cm^{-1}. The band at 3005cm^{-1} in the spectrum of sea buckthorn oil after oxidation is shifted to

3007cm^{-1} in the spectrum of sea buckthorn oil calcium-alginate beads and the band at 1160cm^{-1} in the spectrum of sea buckthorn oil calcium-alginate beads is shifted to 1159cm^{-1}.

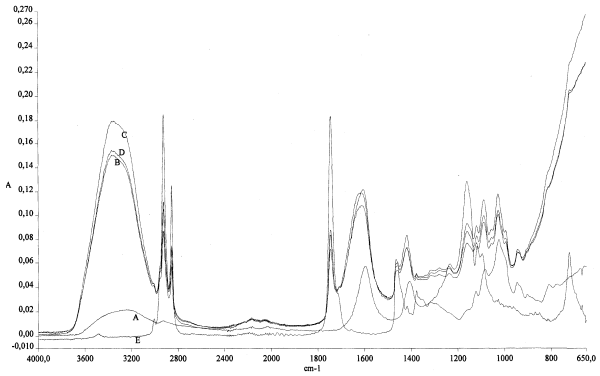

Fig. 2 FTIR-ATR spectra of: A sodium alginate powder; B sea buckthorn oil calcium alginate beads 1% w/v; C sea buckthorn oil calcium alginate beads 1,5% w/v; D sea buckthorn oil calcium alginate beads 2% w/v; E sea buckthorn oil

Sample	After 1h UV			
Ratio between the absorbance	$A_{2853}/A_{3005-3007}$	$A_{1744}/A_{3005-3007}$	$A_{1377}/A_{3005-3007}$	$A_{1159-1160}/A_{3005-3007}$
oil	8,015	11,681	8,200	11,844
Oil beads (1%)	2,284	2,972	3,011	3,806
Oil beads (1.5%)	2,042	2,557	3,160	3,761
Oil beads (2%)	1,908	2,257	2,382	2,880

Sample	After 4h UV			
Ratio between the absorbance	$A_{2853}/A_{3005-3007}$	$A_{1744}/A_{3005-3007}$	$A_{1377}/A_{3005-3007}$	$A_{1159-1160}/A_{3005-3007}$
oil	2,390	8,304	2,430	8,094
Oil beads (1%)	1,650	3,828	1,434	3,102
Oil bead (1.5%)	1,605	4,180	1,454	2,961
Oil beads (2%)	1,670	3,542	1,350	2,500

Table 1 Changes in the band near 3005 cm^{-1} after 1h and 4h UV

Sample	After 6h UV			
Ratio between the absorbance	$A_{2853}/A_{3005-3007}$	$A_{1744}/A_{3005-3007}$	$A_{1377}/A_{3005-3007}$	$A_{1159-1160}/A_{3005-3007}$
oil	7,880	11,260	2,272	7,982
Oil beads (1%)	3,306	4,168	1,568	3,879
Oil beads (1.5%)	2,810	3,416	1,646	3,647
Oil beads(2%)	2,505	3,104	1,618	3,425

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}	$A_{2853}/A_{1159-1160}$	A_{2853}/A_{1744}	$A_{2853}/A_{1159-1160}$
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

Table 2 From left to right: Changes in the band near 3005 cm^{-1} after 6h UV and Changes in the region between 1800 and 1000 cm^{-1} after 1h, 4h and 6h UV

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

According to the observations of Guillen et al. (2000), the values of the following ratios: $A_{2853}/A_{3005\text{-}3007}$ which could be an indicative parameter 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; $A_{2853}/A_{1159\text{-}1160}$ 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.

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 of sodium alginate and the stability of the encapsulated sea buckthorn oil was monitored using FTIR directly in the beads. The values of all 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 techique to monitorize the oxidation of the encapsulated sea buckthorn oil directly in the beads.

Acknowledgments

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