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FATTY ACIDS PROFILE OF CHIA OIL-LOADED LIPID MICROPARTICLES

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ABSTRACT - Encapsulation of polyunsaturated fatty acid (PUFA)is an alternative to increase its stability during processing and storage. Chia (*Salvia hispanica* L.) oil is a reliable source of both omega-3 and omega-6 and its encapsulation must be better evaluated as an effort to increase the number of foodstuffs containing PUFAs to consumers. In this work chia oil was extracted and encapsulated in stearic acid microparticles by the hot homogenization technique. UV-Vis spectroscopy coupled with Multivariate Curve Resolution with Alternating Least-Squares methodology demonstrated that no oil degradation or tocopherol loss occurred during heating. After lyophilization, the fatty acids profile of the oil-loaded microparticles was determined by gas chromatography and compared to in natura oil. Both omega-3 and omega-6 were effectively encapsulated, keeping the same omega-3:omega-6 ratio presented in the in natura oil. Calorimetric analysis confirmed that encapsulation improved the thermal stability of the chia oil.

Keywords: encapsulation, polyunsaturated fatty acid, solid lipid microparticles, *Salvia hispanica*, MCR-ALS.

INTRODUCTION

The increasing demand for functional foods has directed the market towards offering omega3-enriched foodstuff. It is a modern lifestyle challenge to meet the required amount of polyunsaturated fatty acids (PUFAs) in order to minimize the risk of chronic disease (Garg et al., 2006). Chia (*Salvia hispanica* L.) oil have high nutritional value since most of its constituents are triglycerides with PUFA acids present in larger proportions and omega-3 content between 60 and 68% (Capitani et al., 2012). PUFAs are known to help reduce triglycerides and cholesterol levels and other beneficial effects have been observed regarding coronary heart disease, hypertension and inflammatory disorders(Borneo et al., 2007; Harris et al., 2008). Although fish oil is a less expensive source of omega-3, its use in food formulations has been questioned due to unpleasant sensory properties even after encapsulation (Martínez et al., 2012; Muchow et al., 2009; Rodea-González et al., 2012).

PUFAs are susceptible to oxidation(Ixtaina et al., 2011), which can lead to a decrease in nutritional and sensory quality. Encapsulation techniques are a promising approach in order to protect substances from harm and to meet shelf-life requirements (Gökmen et al., 2011; Gouin, 2004). There are a number of encapsulation techniques to encapsulate liquid lipids (TAMJIDI et al., 2013a) such as spray drying (Carneiro et al., 2013; Jimenez et al., 2006; Rodea-González et al., 2012), cyclodextrin complexation (XU et al., 2013), complex coacervation (Tamujidi et al., 2012) and ultrasonic atomization (Klaypradit; Huang,

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2008). Borneo et al. (2007) obtained cream-filled sandwich cookies containing encapsulated omega-3, demonstrating the possibility of producing shelf-stable foods with high levels of omega-3. Gökmen et al. (2011) observed a reduction in the thermal oxidation of omega-3 from flaxseed oil after its encapsulation. Rodea-González et al. (2012) produced microparticles containing chia oil by spray drying using whey protein concentrate-polysaccharide matrices.

Hot homogenization is also an interesting technique due to the natural compatibility between PUFAs and the solid lipid matrix (Lacatusu et al., 2013; Muchow et al., 2009; Salminen et al., 2013). Encapsulation by hot homogenization is also favored by the fact that the liquid oil hinders the solid lipid crystallization, generating a less ordered microstructure or even an amorphous phase (Tamjidi et al., 2013b). Although no solvent is required, relatively high temperatures are needed for the proper mixing of encapsulant and the encapsulated compound. For this reason, attention must be paid to the possibility of thermal degradation when unsaturated lipids are to be encapsulated. Oil degradation may be detected by UVVis spectroscopy, but the complexity of the obtained spectra requires further signal treatment. Multivariate Curve Resolution (MCR) is a suitable chemometric technique since it can identify mixed signals and recover the relative concentration of the substances and their respective spectra (Março et al., 2014).In the case of unsaturated fatty acids, thermal degradation can be evaluated by the formation of degradation products such as conjugated trienes and dienes and hydrolysis products (Gonçalves et al., 2014).

Moreover, some key points are still to be investigated in the encapsulation of chia oil by the hot homogenization procedure. First, the encapsulation efficiency of each PUFA must be determined since it can be affected by the interactions between them and the encapsulant. Second, the possibility of thermal degradation must be checked since the system must be heated during the particle production step. The objective of this work was to extract the oil from chia seeds and encapsulate it in stearic acid microparticles by the hot homogenization technique. Multivariate Curve Resolution (MCR) was applied to determine if damage to the oil occurred due to heating. Encapsulation efficiency was determined for both omega-3 and omega-6 using gas chromatography.

EXPERIMENTAL SECTION

Materials

Chia seeds were acquired from the local market. Stearic acid (Sigma-Aldrich, 99.5%) and Tween 80 (Dinâmica, 97%) were used as encapsulant and surfactant, respectively. Distilled water was used as continuous phase. Methanol (Isofar, 99.8%), chloroform (Vetec, 99.5%), ammonium chloride (Vetec, 99.5%) and sulphuric acid (Vetec, 95%) were used in the transesterification reaction. KBr (Sigma-Aldrich, spectrophotometric standard) was used in the spectrophotometric analyses.

Chia oil extraction

Total moisture of the chia seeds was determined and then adjusted to 80% by adding distilled water. Then, the extraction was performed according to the methodology described by Bligh and Dyer (1959). Briefly, triturated chia seeds (15g) were added to methanol (30 mL) and chloroform (15 mL) under mild stirring. Then, 15 mL distilled water was added and stirred for another 5 minutes. The resulting solution was filtered and 20 mL more chloroform was added. After 5 minutes stirring, the solution was filtered again. Solvent was removed under vacuum (-400 mmHg e 35ºC) and the chia oil was stored at 10°C protected from light.

Quantification of the degradation products by MCR-ALS

Quantification of the oil degradation products was carried out to determine if chia oil was prone to degrade at the temperature used in the encapsulation procedure (75°C). A sample of in natura chia oil (before encapsulation) was heated and aliquots were collected at 28, 30, 40, 50, 60, 70 e 75ºC. The sample was then kept at 75ºC and additional aliquots were collected after regular time intervals for 120 minutes. UV-Visible spectra (Ocean Optics, Red Tide USB650, 1 nm resolution) were obtained and the formation of degradation products was evaluated by Multivariate Curve Resolution Alternating Least-Squares method (MCR-ALS) as described by Gonçalves et al.(2014). Spectra recovered by MCR-ALS were attributed to their respective compounds according to Valderrama et al. (2011).

Microparticles production

Chia oil-loaded microparticles were obtained by the hot homogenization technique(Gonzalez-Mira et al., 2010) such as non-steroidal anti-inflammatory drugs (NSAIDs. Aqueous phase was prepared dissolving Tween 80 (0.300 g) in distilled water (25 g) and heating to 75°C under gentle stirring. Separately, stearic acid (0.625g) was melted at 75°C in a borosilicate double walled vessel. Chia oil was then added to the molten lipid and mixed for 1 minute. Then, the aqueous phase was added to the vessel and stirred for 3 minutes, resulting in an oil-in-water macroemulsion. Sonication (Fisher-Scientific – Ultrasonic Dismembrator 120 W, 1/8” tip) was carried out for 3 minutes in a pulse regime (30 seconds on and 10 seconds off). The sonicated mixture was cooled in an ice bath, resulting in the

formation of solid lipid particles dispersed in water. They were freeze dried before analysis. The same procedure was also carried out without the addition of chia oil to obtain blank microparticles.

Transesterification and Gas Chromatography (GC)

Fatty acids quantification was performed by CG using methyl tricosanoate (23:0, Sigma-Aldrich) as internal standard according to Hartman and Lago methodology (Milinsk et al., 2008).Fatty acid methyl esters (FAMEs) were separated and identified using chromatograph standards (Sigma-Aldrich, F.A.M.E. Mix C14-C22). The equipment setup was as follows: gas chromatograph (Shimadzu, GC-2010 Plus AF) equipped with Split/ Splitless capillary injector, flame ionization detector (FID), flow and pressure automatic controllers and a 100% dimethylpolysiloxane capillary column (Rtx-1, 30m x 0.25mm x 0.25μm). Transesterifications were performed in triplicate. Equation 1 was used in the FAMEs quantification(Joseph and Ackman, 1992).

$$M_x = \frac{A_x M_{23:0} F_{CT}}{A_{23:0} M_s F_{MEA}} \quad (1)$$

where:
Mx= Fatty acid concentration in the sample (mg.g-1oil);
M23:0 = internal standard mass (mg);
Ms = sample mass (g);
AX = peak area for each fatty acid;
A23:0 = peak area of the internal standard;
FCT = theoretical correction factor;
FMEA = conversion factor.

Microparticles characterization

Fourier transform infrared spectroscopy (FTIR) was used to qualitatively evaluate chia oil encapsulation. Lyophilized samples or in natura oil were mixed with dry KBr and spectra were acquired in a Shimadzu spectrometer (IR Affinity-1, 32 cumulative scans) from 4000 to 400 cm-1.This procedure was carried out in triplicate. Images from the microparticles were taken using an optical microscope (BIOVAL, L2000A) coupled to a digital camera. Differential Scanning Calorimetry (DSC, Perkin Elmer 4000) was used to investigate the thermal behavior of in natura chia oil, oil-loaded microcapsules and blank microcapsules. In the first set of experiments, approximately 5 mg of samples were placed on open aluminum lids and heated from 0°C to 440°C at 20°C/min under air atmosphere (100 mL/min) in order to investigate if encapsulation influenced the thermal stability of the chia oil. In a second set of experiments, approximately 5 mg of samples were placed on closed aluminum lids and heated from 0°C to 250°C at 20°C/min under a nitrogen atmosphere (10 mL/min) to determine the enthalpy of fusion and melting temperature of the encapsulant.

To determine encapsulation efficiency (EE%, Equation 2), an aliquot of the microparticles dispersion was filtered in Amicon filters (100 kDa, Millipore) using an ultracentrifuge at 14,500 rpm for 15 min. The liquid phase containing the non-encapsulated fatty acids ([FA] non-encapsulated) was transesterified as described above. Also, the total concentration of fatty acids ([FA]total, encapsulated plus non-encapsulated) was determined for the lyophilized microparticles.

$$EE(\%) = 100\frac{\left[FA\right]_{total} - \left[FA\right]_{non-encapsulated}}{\left[FA\right]_{total}} \quad (2)$$

RESULTS AND DISCUSSION

Chia oil composition

Chia seeds presented 9.5% humidity, which is in accordance with values found in the literature (Ixtaina et al., 2011). Total lipid content was 19.80%, while values from 20.30 to 33.60% were reported when hot hexane or pressing were used for extraction (Ixtaina et al., 2011). It is worth noting that Bligh-Dyer (Bligh and Dyer, 1959) is a cold method thus minimizing lipid oxidation during extraction.

Thermal degradation of in natura chia oil by MCR-ALS

Figure1 presents the relative concentration of tocopherol and degradation products (conjugated dienes/ trienes and hydrolysis products) for the in natura chia oil (before its encapsulation) during heating to 75°C then keeping at this temperature for 2 hours. In Figure 2, UV-Vis spectra recovered by MCR-ALS of tocopherol and degradation products are presented.

It is worth noting that the concern about thermal degradation arises from the fact that the encapsulation procedure demands heating to melt the lipid encapsulant, which could damage the unsaturated fatty acids present in chia oil. UV-Vis spectra detected the presence of tocopherol (Ixtaina et al., 2011) and that its concentration started decreasing only after approximately 2 hours at 75°C. Hydrolysis products were also formed after this heating time. Gonçalves et al. (2014) have demonstrated that temperature and oil composition are key factors for tocopherol concentration decrease and oil degradation for

a number of different edible oils. During the encapsulation procedure adopted in this work, oil is heated at 75°C for only 7 minutes, meaning that one may not expect chia oil degradation caused by the encapsulation conditions (time and temperature).

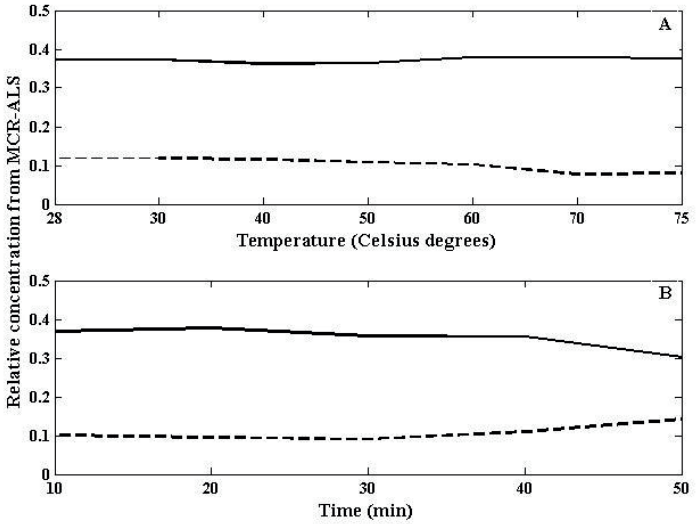

Figure 1. Relative concentration profiles of tocopherol (—) and conjugated dienes/trienes and hydrolysis products (- - - -) for in natura chia oil (before encapsulation) (A) in different temperatures and (B) at 75°C for 2 hours.

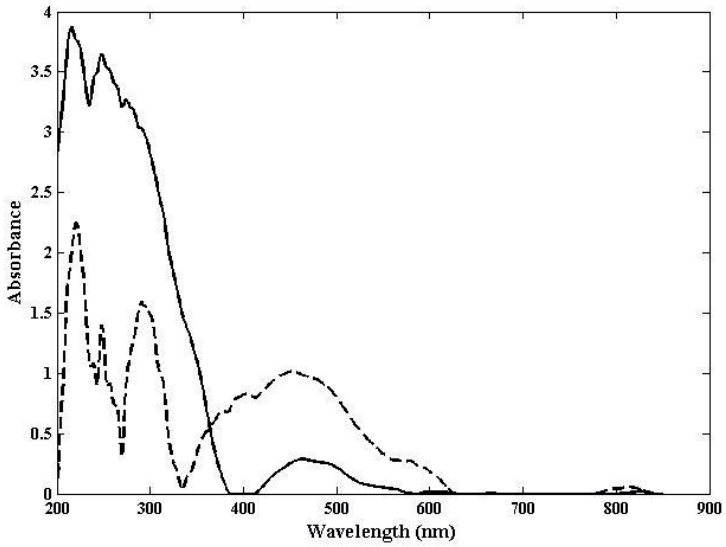

Figure 2. UV-Vis spectra recovered by MCR-ALS of (- - - -) tocopherol and (——) conjugated dienes/trienes and hydrolysis products.

Fatty acids identification and quantification

Figure 3 presents the separation of the fatty acids methyl esters of the in natura chia oil before its encapsulation, while the concentration (mg.g-1) of each compound is presented in Table 1.

The most concentrated fatty acids found were palmitic (C16:0, 67.88 mg.g-1), oleic (C18:1n-9, 54.09 mg.g-1), linoleic (LA, 18:2n-6, omega 6, 181.94 mg.g-1) and alpha-linolenic (LNA, 18:3n-3, omega-3, 565.52 mg.g-1). Linoleic and alpha-linolenic essential fatty acids (LA e LNA) were identified at 12 and 14 minutes, respectively and corresponded to 20.21% and 62.80% of the total lipid in chia oil. These results are in accordance with those presented by Capitani et al. (2012)and Martínez et al. (2012).

Edible oils presenting a ΣPUFA:ΣSFA ratio above 0.45 and n-6:n-3 ratio from 1:1 to 2:1 are recognized as ideal to human dietary intake (Wood et al., 2004), meaning that the oil presented high nutritional quality.

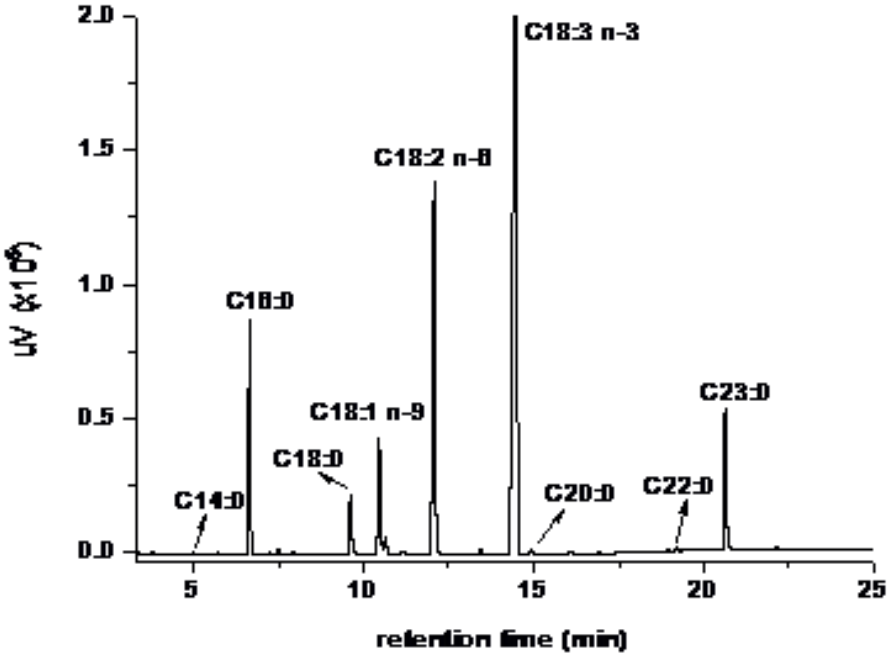

Figure 3. In natura chia oil chromatogram (before encapsulation).

Table 1. Fatty acids concentration in in natura chia oil (before encapsulation).

Fatty acid	Concentration (mg.goil-1)
C14:0 (myristic acid)	0.32 ± 0.01
C16:0 (palmitic acid)	67.88 ± 1.75
C18:0 (stearic acid)	27.91 ± 0.73
C18:1 n-9 (oleic acid)	54.09 ± 1.36
C18:2 n-6 (linoleic acid)	181.94 ± 5.75
C18:3 n-3 (α linolenic acid)	565.52 ± 24.4
C20:0 (arachidic acid)	2.35 ± 0.04
C22:0 (behenic acid)	0.78 ± 0.03
Ratios and sums*	
ΣSFA	99.24
ΣMUFA	54.09
ΣPUFA	747.46
ΣPUFA:ΣSFA	7.53
n-6:n-3	0.32

*ΣSFA = saturated fatty acids; ΣMIFA = monounsaturated fatty acids; ΣPIFA = polyunsaturated fatty acids.

Chia oil-loaded particles were lyophilized and the powder was transesterified and compared to in natura oil (Figure 4). Also, non-encapsulated oil was separated from the particles by filtration and subjected to transesterification (Figure 5). Encapsulation efficiency (EE%) of omega-3 and omega-6 are presented in Table 2.

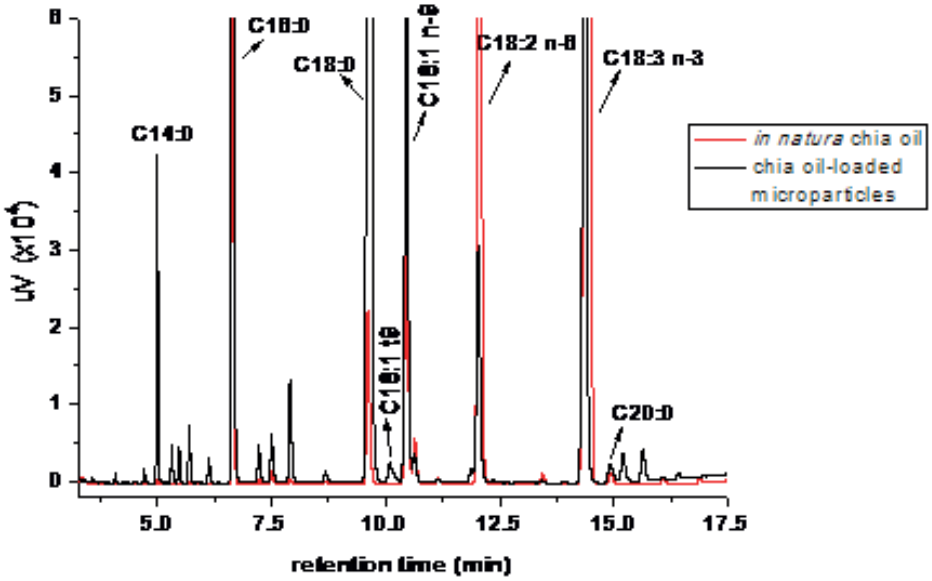

Figure 4. Chromatograms of in natura chia oil (red) and oil-loaded particle (black).

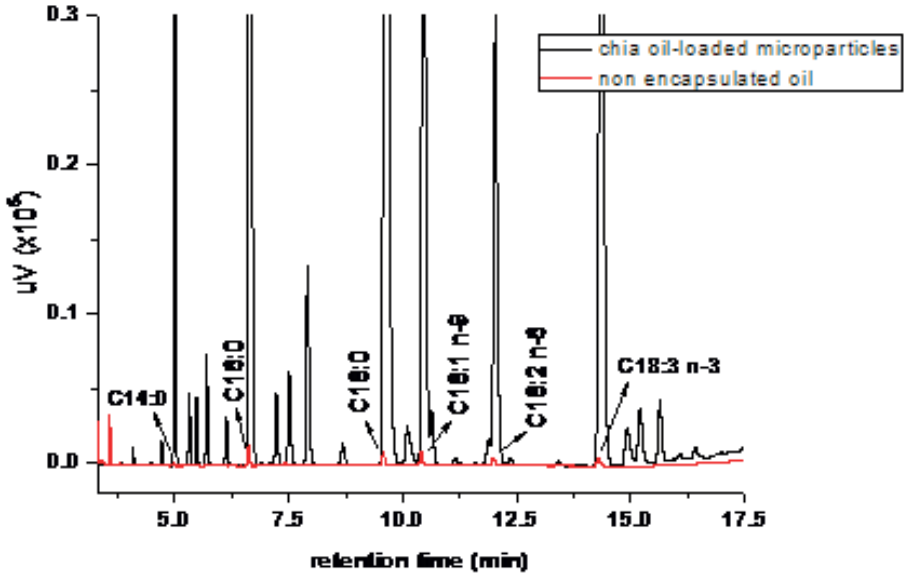

Figure 5. Chromatograms of non-encapsulated chia oil (red) and oil–loaded particles (black).

Table 2. Fatty acids concentration of the chia oil-loaded microcapsules and the non-encapsulated oil.

	Concentration (mgFA/gparticles)	
Fatty acid	Oil-loaded microcapsules	Non encapsulated oil
C14:0 (myristic acid)	13.24 ± 0.15	0.84 ± 0.15
C16:0 (palmitic acid)	165.07 ± 64.91	23.67 ± 1.96
C18:0 (stearic acid)	682.17 ± 213.04	24.35 ± 7.35
C18:1 n-9 (oleic acid)	90.67 ± 0.80	21.51 ± 5.09
C18:2 n-6 (linoleic acid)	124.01 ± 0.19	9.57 ± 2.37
C18:3 n-3 (α linolenic acid)	388.71 ± 17.04	18.11 ± 3.29
C20:0 (arachidic acid)	7.35 ± 0.28	-
C22:0 (behenic acid)	1.34 ± 0.72	-
	Ratios and sums*	
ΣSFA	869.17	48.86
ΣMUFA	90.67	21.51
ΣPUFA	512. 72	27.68
ΣPUFA:ΣSFA	0.60	0.57
n-6:n-3	0.32	0.53

*ΣSFA = saturated fatty acids; ΣMIFA = monounsaturated fatty acids; ΣPIFA = polyunsaturated fatty acids.

Table 3. Encapsulation efficiency (EE%) of omega-3 and omega-6.

Fatty acid	Encapsulation efficiency (EE%)
C18:3 n-3 (omega-3)	95.4 ± 0.6
C18:2 n-6 (omega-6)	92.3 ± 1.9

Omega-3 and -6 peaks can be found in the microparticles but in less intensity when compared to in natura oil because particles are composed of the encapsulant (stearic acid) and oil in 1:1 (m:m) proportion. Unidentified peaks can also be observed, probably related to impurities in the stearic acid. No significant difference between encapsulation of omega-3 and omega-6 could be found ($p>0.05$), which means that the omega-3:omega-6 ratio in the particles and in in natura oil is statistically the same (3.14 ± 0.14 and 3.11 ± 0.05, respectively). This is important since an imbalance of the omega6:omega3 ratio as presented by modern Western food intake is related to a number of chronic diseases and metabolic disorders (Simopoulos, 2008). These results demonstrate that the encapsulated oil presents the same proportion of in natura chia oil, meaning that the microcapsules may be used to protect the oil and to formulate products with high nutritional value.

The literature reports typical encapsulation efficiency values for omega-3 and -6 from 70 to 80% (Rodea-González et al., 2012), 57.2 to 89.6% (Jimenez et al., 2006), 62.3 to95.7% (Carneiro et al., 2013) and 45.8% to 58% (Xu et al., 2013), depending on the encapsulant and the encapsulation technique. Solid lipid nano or microparticle systems are often suitable for oil encapsulation due to the compatibility between the oil and the encapsulant matrix. Lacatusu et al. (2013) encapsulated fish oil in nanostructured lipid carriers with 88.5% efficiency. Unfortunately some works in the literature did not present information on efficiency values, possibly assuming total encapsulation (Muchow et al., 2009; Salminen et al., 2013).

Particles characterization

Optical microscopy images of chia oil-loaded particles and blank particles (no oil added) are presented in Figures 6 and 7, respectively. Figure 8 presents infrared spectra of in natura chia oil, chia oil-loaded particles and blank particles. All spectra were normalized in order to allow comparison. Figure 9 (a) presents the DSC thermograms of the chia oil-loaded microparticles and blank microparticles (no oil added) under nitrogen atmosphere. In Figure 9 (b) thermograms of chia oil and chia oil-loaded microparticles under air atmosphere are presented.

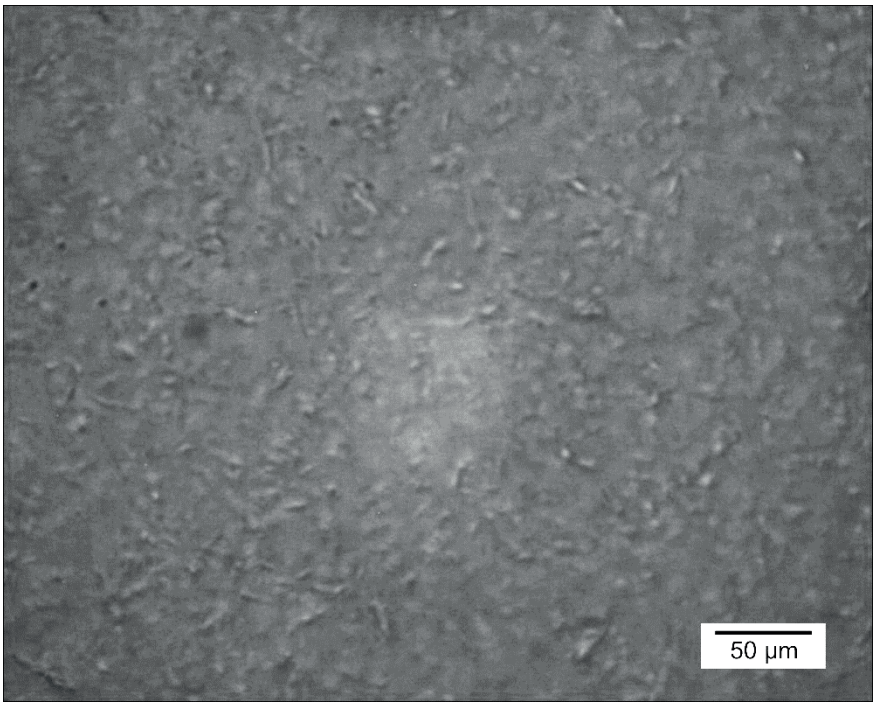

Figure 6. Optical microscopy image of blank microparticles (no chia oil added).

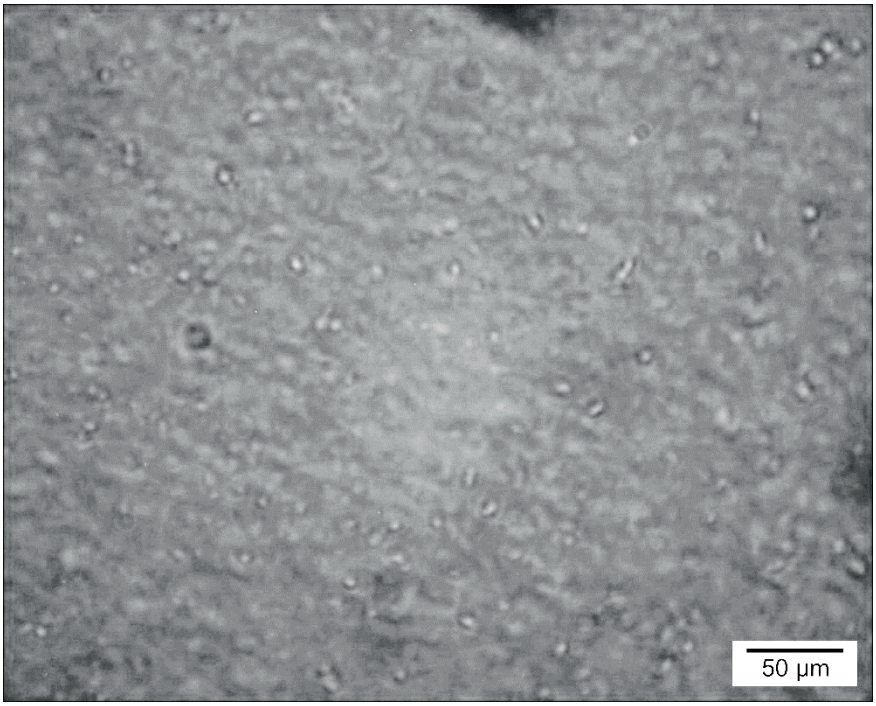

Figure 7. Optical microscopy image of chia oil-loaded microparticles

Absorption band at 3010 cm-1 associated to =C-H groups was found at the chia oil spectrum as expected (Vidal et al., 2013). Blank particles did not show this band since stearic acid did not present any unsaturation on its chemical structure. This band was also present at the oil-loaded particles but in a much lower intensity. The decrease in intensity could be an indication of efficient entrapment of encapsulated compounds, also corroborating the previously results found (optical microscopy and gas chromatography).

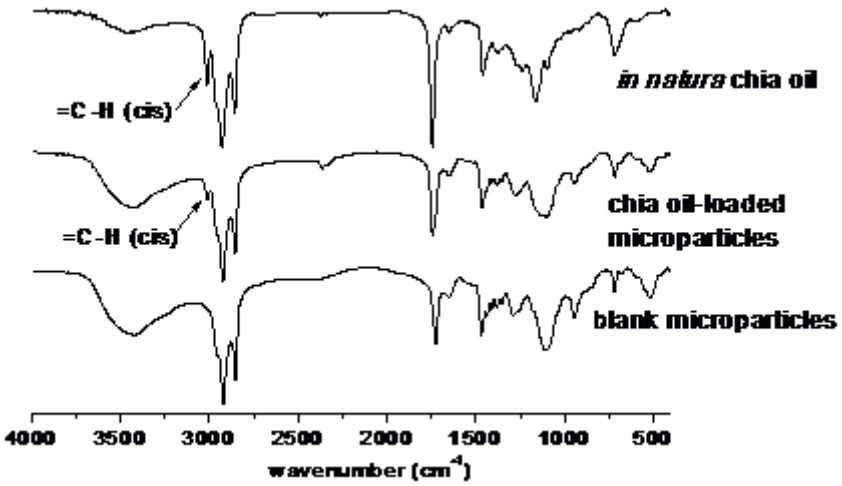

Figure 8. FTIR spectra of in natura chia oil, chia oil-loaded particles and blank microparticles (no oil added).

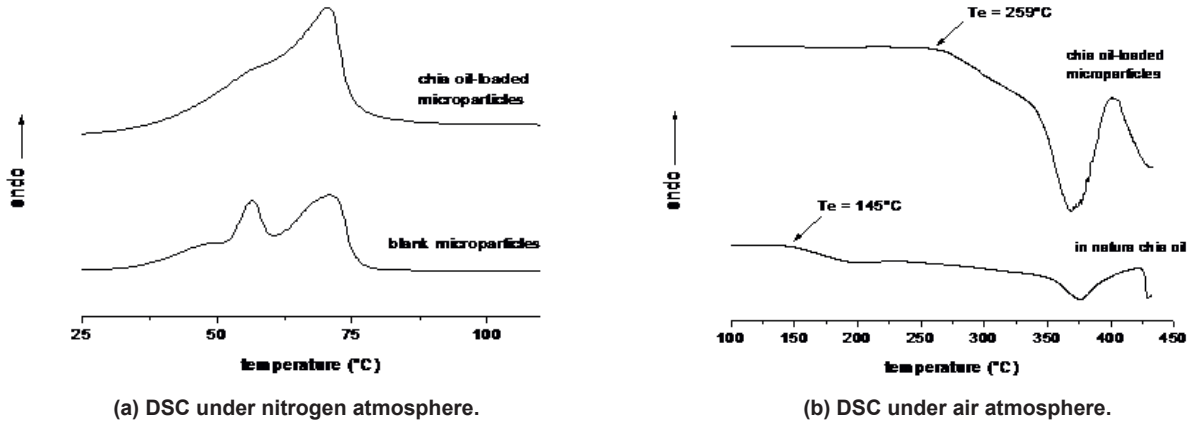

Figure 9. DSC thermograms of in natura chia oil, chia oil-loaded particles and blank microparticles (no oil added).

In natura (before encapsulation) chia oil begins oxidation at approximately 145°C. Grampone et al. (2013) reported an oxidation temperature of 176°C using pure oxygen as oxidizing atmosphere at a higher rate.This could explain the difference, along with discrepancies in oil composition, as the omega-6 concentration is not reported by the authors. The same may be concluded when comparing to the results from Ixtaina et al. (2012), who reported an oxidation temperature of (168.2 ± 2.8)°C. DSC thermograms demonstrated that the encapsulation of chia oil increased its oxidative stability since oxidation began at approximately 259°C. This behavior was also described in the encapsulation of oregano oil in chitosan nanoparticles (Hosseini et al., 2013), eugenol (Woranuch and Yoksan, 2013) and also carvacrol (Keawchaoon and Yoksan, 2011) using thermogravimetric analysis.

CONCLUSION

Chia oil was extracted by using the Bligh-Dyer method to minimize oil degradation during extraction. UV-Vis spectroscopy coupled to multivariate analysis (MCR-ALS) demonstrated that no oil degradation or tocopherol loss were expected to occur under the experimental conditions (heating time and temperature) applied in the hot homogenization procedure used in this work. Chia oil waseefficiently encapsulated in micrometric stearic acid particles as demonstrated bygas chromatography, UV-Vis, DSC and FTIR spectroscopy. Encapsulation efficiencies for omega3 and omega6 were similar, meaning that the n-6:n-3 ratio of the particles is very close tothe one presented byinnatura oil.Differential Scanning Calorimetry showed

an increase in the oxidative stability of the encapsulated oil, which may indicate that such microparticles are suitable to formulate food products where long shelf life is needed or when heating is applied during production such as in baked products.

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