Developing of Nutraceuticals Systems Containing Bioactives from Salvia Hispânica Seed

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Research Article

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Abstract

This paper describes the research on the encapsulation of chia bioactives, which was derived from the extraction of Salvia hispânica seed using ethanol, employing percolation method and by Soxhlet. The obtained materials were characterized solution by nuclear magnetic resonance analysing the carbon-13 and hydrogen nuclei. The extracts were encapsulated with polycaprolactone (PCL) using nanopreciptation method. The nanoencapsulated materials were also analyses as their molecular homogeneity through low-field nuclear magnetic resonance in the time domain (NMR-DT). For that the determination of proton spin-lattice relaxation time was used to confirm the nanoencapulation. The measurements of size and zeta potential were also done employing DLS. From the results was conclude that the bioactives extracted from the seeds are associated to triacylgliceride major composed by α-linolenic acid (C18:3) and by linoleic acid (C18:2). It was also observed that the nanoencapsulation showed to be effective to produce particles with size between 203 a 430 nm, this size is appropriated to nutraceuticals formulations and/or to be added to food preparations.

Keywords: Encapsulation; Bioactives compounds; Polymeric encapsulation; PCL.

Introduction

The chia is a small oval shaped seed about 2 mm in brown colour and it belongs to Labiatae, being native to southern Mexico and northern Guatemala, their benefices have been detected a long time ago. For several centuries, these seeds were used as a main food for the Indians of southwest America (8).

The seed chia is rich in fatty acids, main bioactives, among them docosahexaenoic acid precursor α-linolenic acid (DHA) and eicosapentaenoic acid (EPA). Proportionally, this seed presents significant quantity of lipids, around 40% of total mass of seed, being almost 60% of omega 3 and 6, more 30% of total weight from dietic fiber, 19% of protein, as well as minerals, vitamins and natural antioxidants (3, 4).

The benefits of a high fatty acid diet and specific supplementation are numerous, among them it is worth mentioning the benefits associated with cardiovascular disease, reduced lipid levels, induced insulin resistance, and reduced visceral adiposity. Produces microcirculation benefits for better tissue oxygenation (6), helps treat and prevent Alzheimer's disease (5), important function in the formation, development and functioning of the brain and retina in the maternal gestational period (5).

Objectives

The main objective of this work was to extract the bioactives of chia seeds and their nanoencapsulation in polycaprolactone through nanoprecipitation for nutraceuticals generation.

Materials and Methods

Materials

The materials used there was no treatment and they were purched: Seed chia (Salvia hispanica); Absolute ethyl alcohol P.A.; Polycaprolactone (PCL) (Mn = 10000) – Aldrich Chemistry; Polyethileneglicol-b-poly(propileneglicol)-b-poly(ethylenoglicol) - Pluronic® F-68 – Aldrich Chemistry; Acetone P.A. – Isofar.

Chia seed bioactive extraction

The chia seed bioactive was extracted by percolation with ethanol for 3 days, the samples went through a vacuum filtration process to separate the extract and the shells (Figure 1). Subsequently, extraction was carried out through Soxhlet, with ground chia seeds in ethanol, after the solvent was evaporated and the extract was kept at desiccator (Figure 2).

Figure 1: (a) Grinded chia seed and (b) Soxhlet extraction.

Figure 2: Oil (product) extracted from chia seed.

Preparation of nanoparticles of PCL

The nanoprecipitation reaction was carried out to produce polymeric nanoparticles. A standard solution was prepared without the addition of the extracted bioactive, with 0.2g of PCL in 50mL of acetone, under heating and magnetic stirring until the polymer dissolves. Then a 0.2g solution of Pluronic® F-68 and 100mL of distilled water was prepared. Solution 1 was prepared by mixing 0.4 g of PCL in 100 ml of acetone, after was added 0.002mL of chia oil, under heating and magnetic stirring until the polymer dissolves. Then a 0.4g solution of Pluronic ® F-68 e 200mL of distilled water under stirring. Nanoprecipitation solution 2 was carried out under the same conditions, however 0.04g of chia oil was added. The organic solution was poured with constant flow into the aqueous solution with constant magnetic stirring for 3 days until the solvent evaporated.

Characterization

NMR measurements

Acquisition conditions of H-1 nuclei: Frequency of H-1 300.06 MHz; Spectral window (SW): 4800 Hz; Acquisition time AT: 2.5s; Interval between of 90º pulses D1: 20s; Number of transients NT: 16; Temperature of Analysis: 40°C.

Acquisition conditions of C-13 nuclei: Frequency of ^{13}C: 75.4 Hz; Spectral window (SW): 20.700 Hz; Acquisition time: AT: 1; Interval between of 90º pulses: D1: 2s; Number of transients: NT: 5200; Temperature of Analysis: 40°C. The attached proton test (APT) technique was also acquired to confirm the carbon-13 assignments. The conditions used was the same for C-13 pulse sequence, using the standard measurements, that comes in the equipment. From this technique the carbon -13 linked to an even number hydrogens and carbonyl groups are located in phase in the spectrum and for the carbons-13 linked to an odd number of hydrogens are located in anti-phase in the spectrum.

Low-field NMR: MARAN Ultra 0,54 T (23,4 MHz para o^{1}H), Oxford Instruments, 18mm probe; Temperature: 30±2° C; Pulse sequence 1: inversion-recovery; delay (ms): 0,1-5000.

CPMG: Carr-Purcell-Meiboom-Gill [p90x – (t – p180y - t) n] echo time (2t, us): 1200.

Particles size

The samples were analyzed in Nanosizer Zeta – Model: Nicomp ZLS Z3000 - DLS / PALS, to obtain the distribution size and potential zeta of the nutraceuticals.

Thermal analysis (TGA)

The measurements were carried out in TGA, Model TGA Q500 V6.7 Build 203 – Universal V4.5A TA Instruments, using 10 ºC/min in nitrogen atmosphere, varying temperature from 30 to 700 ºC.

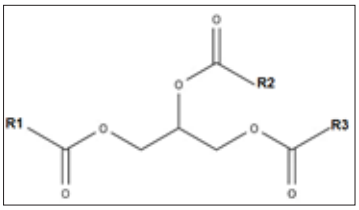
Infrared analysis (FT-IR)

The FT-IR analyses were carried in a Model Frontier *FT-IR/FIR,* with Number of scans: 4 and Resolution: 4

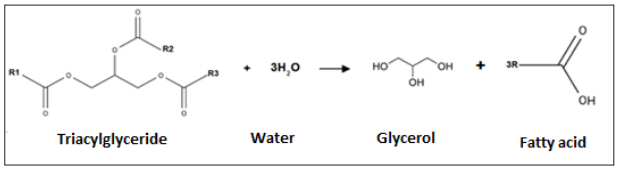
Results and Discussion

C-13 NMR solution results and analysis

The analysis of the spectra showed that the extracted material refers to a triacylglyceride (Figure 3) as a major component, indicating the main compounds found in the chia seed, which is rich in fatty acid, according to the literature (BUSHWAY, 1981), specifically α-linolenic acid (C18:3 – structure containing 18 carbons and 3 unsaturation in the carbon chain) and linoleic acid (C18:2 – structure containing 18 carbons and 2 unsaturation in the carbon chain).



(a)



(b)

Figure 3: (a) Triacylglyceride chemical structure and (b) Triacylglyceride Formation.

In the C-13 solution NMR spectrum four regions was detected for the product extracted. The region 1 was delimited between 10 to 40 ppm, which refers to the aliphatic part of the carbon chain, containing CH_3 (methyl) e CH_2 (methylene) groups from

long aliphatic chains; region 2, related to the range from 60 to 80 ppm, is the regions of carbons linked to CH-O e CH_2-O; the third region was delimited from 110 to 135 ppm that was related to the unsaturated carbons, -C=C-, present in the fat acid and the last region belongs to the carbonyl group (C=O) located from 170 to 180 ppm. Figure 4 shows the C-13 NMR solution spectrum with the assignment of the regions.

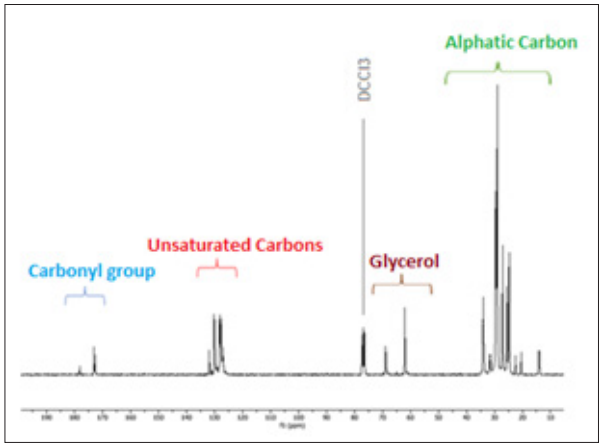
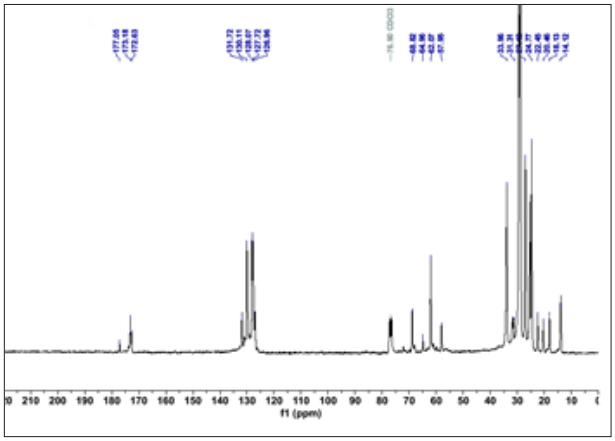


Figure 4: ^{13}C NMR spectrum in triacylglyceride-containing chia seed extract solution.

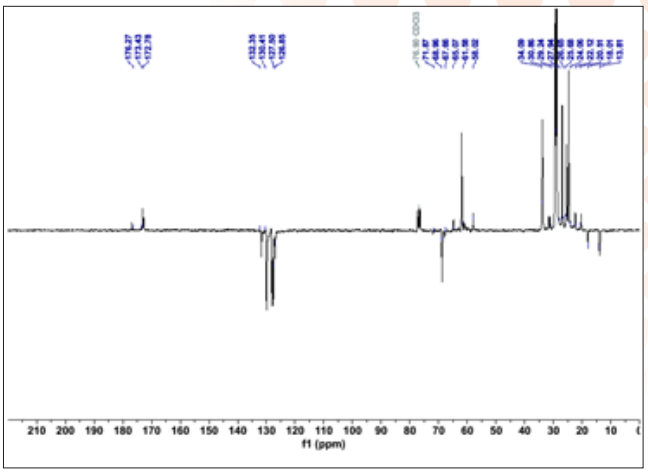
Table 1 exhibits the assignments of C-13 types and Figures 5 and 6 shows the C-13 NMR solution spectrum of the chia extract obtained from Soxhlet (a) using ethanol and the C-13 APT solution spectrum (b), which was acquired to confirm the assignments.

δ (ppm)	Carbon Types
14 – 34	CH_3 e CH_2
58 – 69	CH_2-O e CH-O
127 -133	C=C
173 – 177	C=O

Table 1: Assignments of C-13 NMR solution spectrum of chia extract from ethanol.

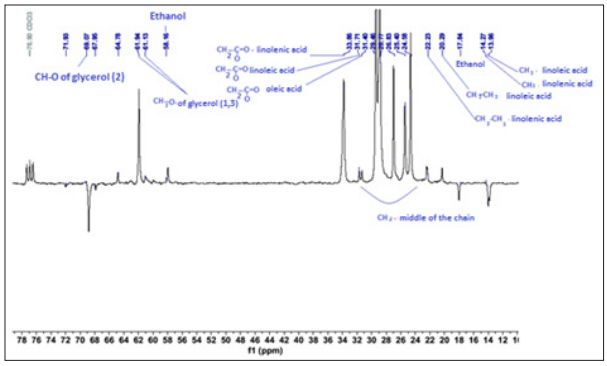


(a)

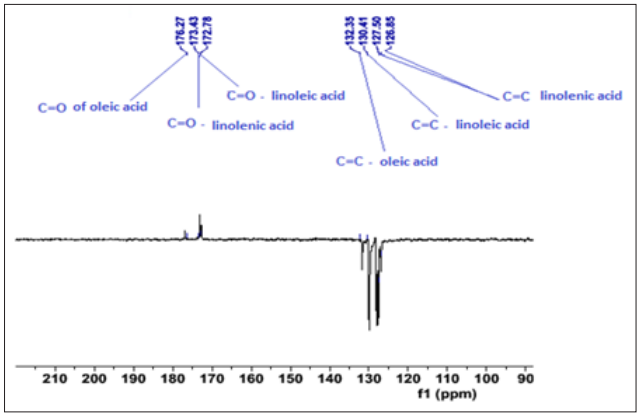


(b)

Figure 5: a) C-13 NMR solution spectrum of chia seed extract in ethanol, through Soxhlet.
b) C-13 NMR solution spectrum using APT technique for the same extract.



(a)



(b)

Figure 6: (a) APT ^{13}C spectrum and an expansion of the signals in the range from 10 to 78 ppm,
(b) APT spectrum with expansion in the range from 90 to 180 ppm.

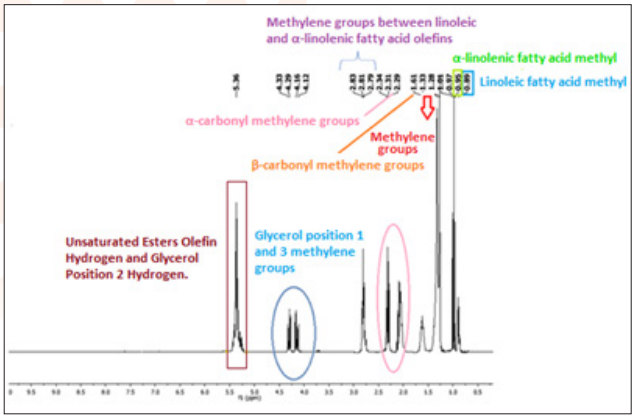
Analyzing the spectra, it was observed that from this system, it was able to extract the triacylglyceride composed of α-linolenic acid and linoleic acid, which are the major compounds and the object of study, however it was possible to observe that

this system was still able to extract a free fatty acid with displacement closer to 180 ppm that the literature refers to such a compound (1).

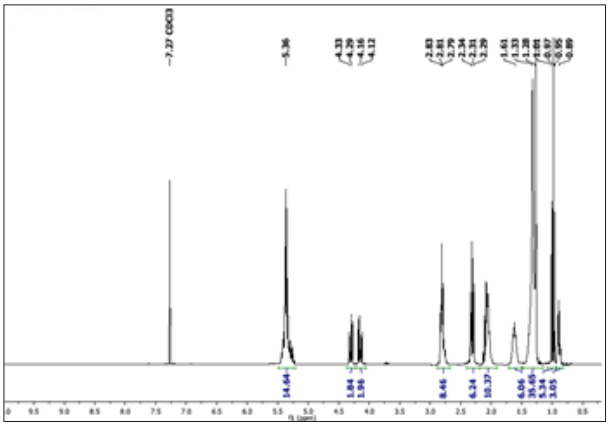
In summary, the 13C MNR spectra of chia extract, using ethanol, corroborates the data in the literature, were the signals located from 173 to 173 ppm corresponds to α-linolenic fatty acid correlating its content found in chia seed (Table 2) and in the 1 and 3 position the glycerol, signal at 172 corresponds to linoleic fatty acid in position 2 (2).

^{1}H NMR solution results and analysis

Hydrogen nucleus NMR analysis was performed to better detail the results obtained. Comparing the obtained spectrum showed in Figure 7, with the one from the literature (7) (Figure 8), we can see signals in the same area, suggesting that the compounds found from the extraction was α-linolenic acid and linoleic acid, with a higher content of α-linolenic acid.



(a)



(b)

Figure 7 & 8: (a) ^{1}H NMR solution spectrum divided into regions of the chia seed extract obtained from ethanol Soxhlet extraction and
(b) ^{1}H NMR solution spectrum of the chia seed extracted obtained from ethanol Soxhlet extraction

In the ^{1}H NMR solution spectrum, one can observe that the CH3 characteristic triplet of linoleic acid ester is centered in 0.9 ppm and the typical triplet α-linolenic acid methyl is located at 1 ppm; it is also observed that the chemical shift fat around 1.3 ppm corresponds to the methylene groups (CH_2) except the ones that belongs to α-carbonyls and β-carbonyls. The chemical shift centered at 1.6 ppm corresponds to β-carbonyl methyl groups. The signals from located from 2.3 to 2.8 ppm are derived from aliphatic groups of the aliphatic chains. The ^{1}H signals located between 4.1 to 4.3 ppm are derived from the methylene hydrogens of 1 and 3 position of glycerol and the signals at 5.3 ppm are derived from the olefinic hydrogens (H-C=C-H) and the hydrogens of position 2 of glycerol. Thus, after analyzing this spectrum, it corroborates the C-13 NMR solution data and, confirms the extraction of a triacylglyceride constituted by the α-linolenium fatty acids, mainly, the linoleic acid.

Dynamic light scattering (*DLS*)

The dynamic light scattering technique (DLS), was employed to measure the size of the particles formed after nanopreciptation method. It was also evaluated the dimensional stability of the particles. Two samples and a standard were analyzed, and the compositions of them are exhibited in Table 2.

NANOPRECIPITATION			
SAMPLES	PCL (g)	PLURONIC F 68 (g)	CHIA OIL (mL)
Standard sample	0.2	0.2	---
Sample 1	0.4	0.4	0.002
Sample 2	0.4	0.4	0.4

Table 2: Composition of samples analyzed by DLS.

In the standard sample, without adding the bioactive studied, particles with diameters with an average value of 212.8 nm was observed with the distribution size between 175.4 to 385.6 nm (Table 3); the graphs in Figure 9 and 10 show the behavior of the results found.

Distribution (%)	Size (nm) ± 1
25	175
50	213
75	251
90	294
99	386
80	262

Table 3: Particle size distribution of the standard solution.

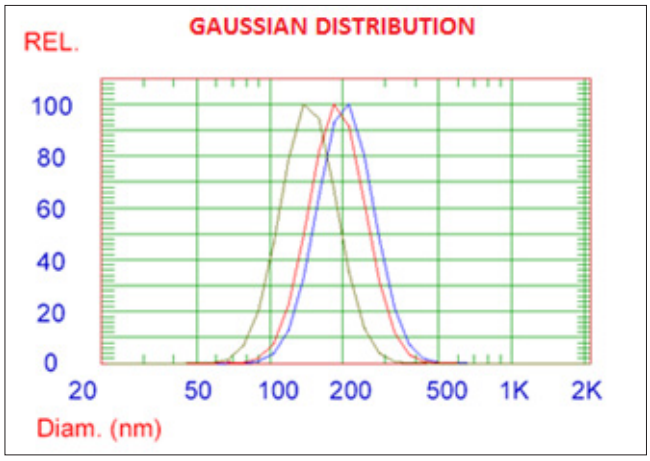


Figure 9: Gaussian Distribution of standard solution.

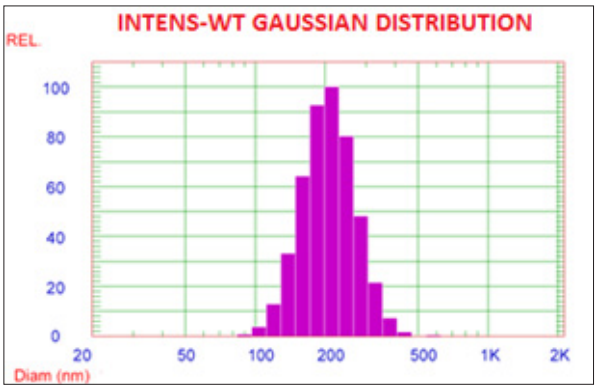


Figure 10: Weighted Intensity – Gaussian Distribution of standard solution.

In sample 1, particles with sizes ranging from 203.4 to 351.5 nm were obtained, with an average value of 231.9 nm, which can be seen in Figure 11 and 12 and the size distribution is shown in Table 4.

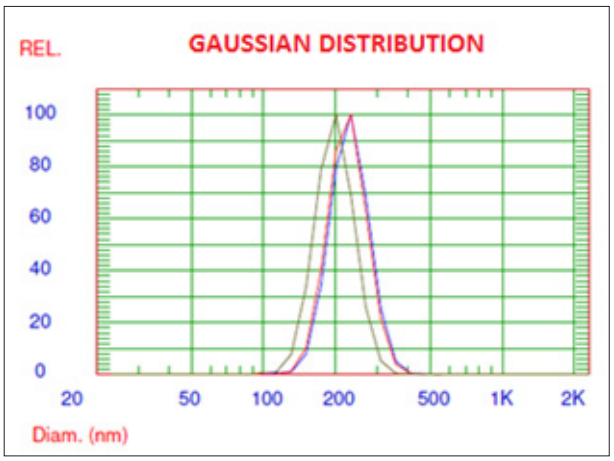


Figure 11: Gaussian Distribution of sample 1, determined in the DLS measurements.

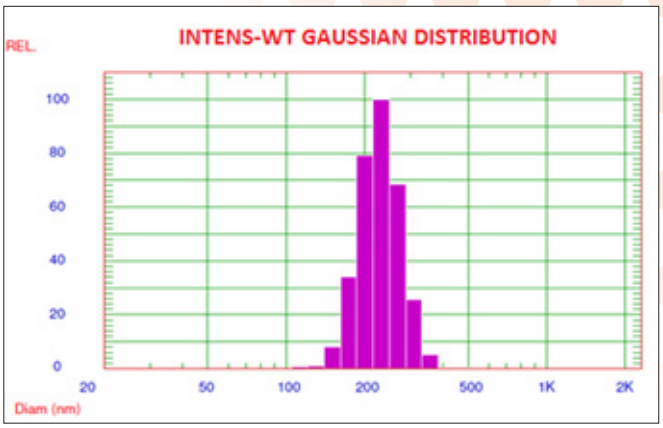


Figure 12: Weighted Intensity – Gaussian Distribution of sample 1, determined in the DLS measurements.

Distribution (%)	Size (nm) ± 1
25	203
50	232
75	261
90	291
99	351
80	269

Table 4: Distribution of particles size from sample 1, determined in the DLS measurements.

Sample 2 was also analyzed by DLS, where particle sizes were analyzed, when compared to sample 1, a small increase in particle size was observed, as shown in Figures 13 and 14.

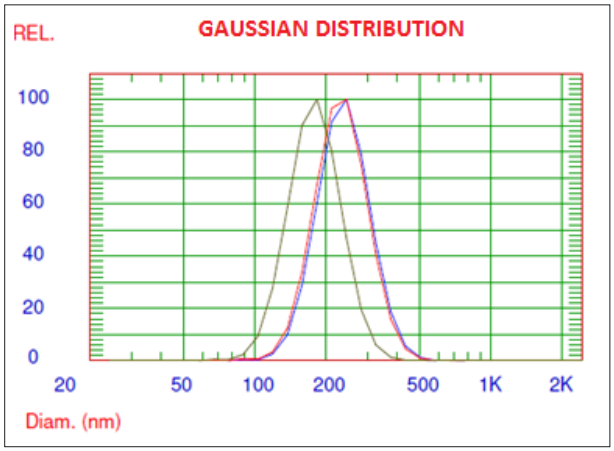


Figure 13: Gaussian Distribution of sample 2, determined in the DLS measurements.

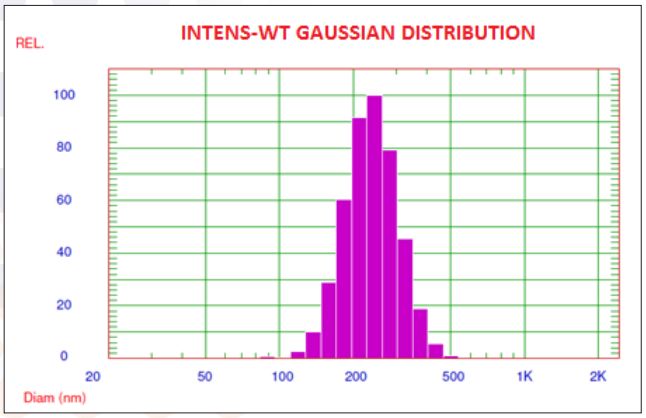


Figure 14: Weighted Intensity – Gaussian Distribution sample 2, determined in the DLS measurements.

According to Table 5, the particles of sample 2 varied from 203.2 to 429.9 nm, with an average value of 244.1 nm, a size also appropriate for this type of bioactive encapsulation system.

Distribution (%)	Size (nm) ± 1
25	203
50	244
75	286
90	331
99	429
80	298

Table 5: Distribution size of the particles of sample 2, determined in the DLS measurements.

The analyzes show that the nanoparticles were formed with appropriate sizes for the bioactive encapsulation system, it was noticed a production of larger nanoparticles in sample 2 when compared with the standard sample, characterizing the encapsulation of the bioactive in study.

Nuclear magnetic resonance in the time domain (NMR-DT) of nanoprecipitation

The products from nanoprecipitations were also analyzed by relaxometry, to verify the encapsulation, the spin-spin relaxation time was determined because it is better representative for materials in solution, the corresponding T_2H values are shown in Table 6.

SAMPLE	$T_2H \pm 2\%$
Standard solution	2.6 s
Nano 1	2.7 s
Nano 2	2.5 s

Table 6 : T_2H values of the nanoprecipitation solutions determined by the CPMG technique (median time).

Analyzing the nanoencapsulation systems, it is observed that the T2H relaxation time value is in the order of seconds depending on the water and the domain curves. Figure 15 shows a very homogeneous system, indicating that the encapsulation was more effective in the Nano 2 solution, as it presents a narrower curve and with greater intensity, showing a more homogeneous system and with better bioactive-polymer interaction.

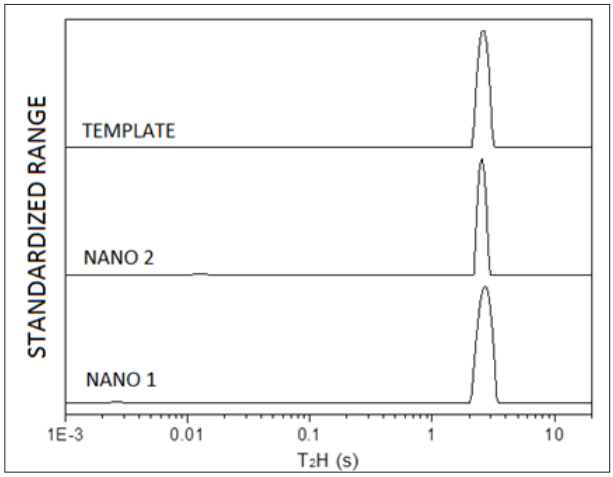


Figure 15: Distribution curves of domains from transversal relaxation obtained from CPMG.

Conclusion

Through this work, it is possible to study the extraction of bioactive chia seeds, given their innumerable health benefits, where it was characterized by several analyzes, confirming its chemical composition rich in fatty acids.

Through extraction, and different techniques, it was possible to extract from the chia seed, the triacylglycerides formed by α-linolenic fatty acids, mostly according to the composition of the seed, and the linoleic. The NMR characterization of 13C and 1H, with the aid of the APT technique, was the most effective technique for characterizing the bioactive. The nanoprecipitation technique employed for the encapsulation of bioactive substances was effective, being proven by the NMR-DT technique and the size of the nanoparticles was determined by the DLS, presenting an ideal size for the desired purpose.

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