EFFECT OF NATURAL ANTIOXIDANTS ON THE PHYSICOCHEMICAL PROPERTIES AND STABILITY OF FREEZE-DRIED MICROENCAPSULATED CHIA SEED OIL

Running title: Freeze-dried microparticles with chia seed oil and natural antioxidants

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ABSTRACT

BACKGROUND: Chia oil possesses a very high content of polyunsaturated fatty acids, mainly α-linolenic acid. This characteristic makes this oil possess beneficial properties to health but gives it a high susceptibility to the oxidation process. The microencapsulation and the addition of natural antioxidants are alternatives to protect chia oil against oxidative deterioration. The aim of this study was to investigate the physicochemical characteristics and the oxidative stability of chia seed oil microencapsulated with different natural antioxidants (Guardian Chelox, which is a commercial blend of extracts from chamomile and rosemary, and essential oils from *Origanum vulgare*, *Origanum x majoricum,* and *Mentha* spicata) by freeze-drying using sodium caseinate and lactose as wall materials.

RESULTS: The main physicochemical properties of the microencapsulated chia oil were similar regardless the presence antioxidant. The moisture content was 38.1± 4.0 g kg^{-1}; the microencapsulation efficiency was higher than 85% in all cases.The freeze-drying microencapsulation significantly enhanced ($p \leq 0.05$) the oxidative stability of the chia oil. The addition of natural antioxidants conferred chia oil additional protection against lipid oxidation, depending on the type and concentration (500 or 1000 mg kg^{-1} of the emulsion previous to freeze-drying) of the antioxidant. Among them, Guardian Chelox (1000 mg kg^{-1}), presented the highest induction time obtained by the Rancimat accelerated oxidative stability test(t)and the lowest peroxide values after 90 d of storage (33%RH, 25±2°C). Overall, the microparticles with antioxidants presented a lower degree of yellowing during storage than the control system.

CONCLUSION: The use of different natural antioxidants confers freeze-dried microencapsulated chia seed oil additional protection against lipid oxidation. This

information is relevant for the application of this oil, which is a rich source of omega-3 fatty acids, in the food industry.

Keywords Chia oil; Omega-3 fatty acids; Microencapsulation; Natural antioxidants; Essential oils

INTRODUCTION

The oil obtained from chia seeds (*Salvia hispanica* L.) is rich in essential polyunsaturated fatty acids (PUFAs) (> 80%), mainly omega-3 (α-linolenic acid), which makes this oil possess beneficial properties to health. However, the high PUFAs content gives chia oil low stability to the oxidation process. The microencapsulation is a technique to protect and deliver different sensitive compounds, such as those present in chia oil, from the adverse influence of the environment. Recently, studies about chia seed oil microencapsulation have been carried out using different wall materials, microencapsulation processes and storage conditions [1-6].

The microencapsulation can be carried out by different methods, such as spray-drying and freeze-drying. The former is the most common process used in the industry because of its relatively low cost. However, the freeze-drying process is recognized as a method to produce high-quality dried food products as the drying is conducted under vacuum and at temperatures lower than ambient temperature[7]. This characteristic makes the freeze-drying process especially attractive for drying heat-sensitive and bioactive components, such as chia seed oil, minimizing the product damage caused by the high temperatures applied in the spray-drying process.

The selection of the wall materials is a determining point in developing microencapsulated oils since their properties affect the characteristics of the final product. As no single wall material possesses all the properties required of an ideal encapsulating agent, the use of a combination of proteins and carbohydrates has been found to be an

excellent alternative for microencapsulation [8]. Sodium caseinate rapidly confers low interfacial stress during emulsification previous to the drying process, and it is also a convenient wall material because of its good film-forming properties. On the other hand, lactose forms a continuous glass phase in which the protein chains are dispersed, thus improving the drying properties of the wall [9]. Different studies have shown high microencapsulation efficiency using these wall materials to encapsulate chia seed oil. In this sense, the mixture of a relatively low-cost carbohydrate such as lactose and sodium caseinate would be a cost-effective, functional, fat encapsulating alternative[2, 10, 11].

Another alternative to protect chia oil against oxidative deterioration is the use of antioxidants [12]. They are an extensive group of chemical compounds, synthetic or from natural sources, which can inhibit or retard oxidation processes. Although the food industry has been using synthetic antioxidants for a long time, their safety is questioned and exist doubts about the hazards on human health. This fact has led to a trend of the progressive substitution of synthetic antioxidants for the natural ones [13].

The antioxidants from plant sources, such as tocopherols, vitamin C, and flavonoids, are compounds of great value in the diet that can help to reduce the incidence of chronic diseases. Besides, there is a growing interest in the use of these natural antioxidants for extending the shelf-life of foods [14].

Spices and aromatic plants are mainly known for their antimicrobial activity with many applications in food preservation, pharmaceuticals, alternative medicine, and natural therapies. Besides, many essential oils from these plants are also known as natural antioxidants[15]. Many genera belonging to the Lamiaceae family, such as *Origanum*, *Rosemary*, and *Mentha*, posses biological and pharmacological activities. Different authors have reported that *Origanum vulgare* L. essential oil contains a high amount of polyphenols with antioxidant properties due to the presence of thymol and carvacrol, and a

synergistic effect among compounds containing oxygen [16, 17]. *Origanum x majoricum* Cambess, a hybrid between *O. majorana* L. and *O. vulgare* L., has a very high total phenolic content and its antioxidant activity against linoleic acid oxidation was comparable to that of BHA [18]. Concerning rosemary extract (*Rosmarinus officinalis* L.), it has potent antioxidant activity due to the presence of phenolic diterpenes, mainly carnosic acid and carnosol and other minor compounds such as rosmanol, epirosmanol and methoxyepirosmanol [19]. It is marketed in an oil-soluble or water-miscible form or as a dry powder[15]. Regarding *Mentha* genus, it showed a high radical scavenging capacity due to the presence of monoterpene ketones (menthone, isomenthone) [20]. The antioxidant action of *Mentha sp.* could also be due to the presence of aldehydes and substances with phenolic rings. In this sense, the phenolic compounds would act as donors of electrons or hydrogen atoms, stabilizing free radicals [21].

Moreover, species such as *Matricaria chamomilla* L (*Asteraceae*) has also presented antioxidant activity. Extracts and essential oil from this plant exhibited a high antioxidant and antimicrobial activity associated with a high total polyphenols content [22]. Besides, this species used in folk medicine for a long time, presents important anti-inflammatory, anti-neoplastic and immunoregulatory effects [23].

Few studies about microencapsulation of chia oil with natural antioxidants have been carried out [24], with no information published about the influence of different vegetable extracts or essential oils on the oxidative stability of chia seed oil microencapsulated with sodium caseinate and lactose by freeze-dried.

The objective of this study was to investigate the effect of the addition of natural antioxidants (commercial blend of chamomile and rosemary extracts, essential oils from *O. vulgare*, *O. x majoricum* and *Mentha spicata* L) on the physicochemical properties and

oxidative stability of the chia seed oil microencapsulated by freeze-drying using sodium caseinate and lactose as wall materials.

MATERIALS AND METHODS

Materials

Chia cold-pressed oil was supplied by Nutracéutica Sturla S.R.L (Burzaco, Buenos Aires, Argentina). Sodium caseinate (purity > 97%) was purchased from Sigma Chemical Company (St. Louis, MO, USA) and d-lactose monohydrate (purity >98%) from Anedra S.A. (San Fernando, Buenos Aires, Argentina). All reagents were analytical grade.

Guardian Chelox L, a commercial blend of chamomile and rosemary extracts with propylene glycol as carrier, was provided by Danisco USA Inc. (Madison, WI, USA). This blend contained 3.6 g kg^{-1} of total flavonoids and 2.8 g kg^{-1} of apigenin-7 glycosid according to the manufacturer.

Chia seed oil characterization

Fatty acid composition was analyzed by GC according to IUPAC 2.302 standard method [25]. Tocopherols were evaluated by normal phase HPLC with a Hewlett Packard system (HPLC Hewlett Packard 1050 Series, Waldbronn, Germany) equipped with a fluorescence detector Agilent 1100 Series (Agilent Technology, Palo Alto, CA, USA) [26].

Extraction and characterization of essential oils from *Origanum vulgare L.*, *Origanum x majoricum* Cambess, and *Mentha spicata L.*

Peppermint and both species of oregano, originally coming from Villa Dolores (Córdoba Province, Argentina), were established on weed-free plots in an experimental field situated in La Plata, Argentina (34° 52' S latitude and 57° 58´ W longitude, Typic Argiudoll soil). Crops were propagated vegetatively from plantings of the previous year, in spring (September) and autumn (May) using peppermint and oregano shoots, respectively. The harvesting was carried out during bloom (December-January), and the material collected

was dried in a forced-draft oven (200 cm x 160 cm x 80 cm) at 40 °C until to reach a constant weight. The volatile fraction was extracted by steam hydrodistillation with a Clevenger-type apparatus according to the *European Pharmacopoeia* [27]. Then, the volatile distillate was collected over anhydrous sodium sulfate and refrigerated until the chromatographic analysis was carried out.

The GC-MS analysis was performed using a gas chromatograph Finnigan TRACE GC (ThermoQuest, Milan, Italy) coupled to a mass spectrometer Finnigan TRACE MS (ThermoQuest, Manchester, GB) with a quadropole mass analyzer, electron impact ionization (70 eV), and autosampler A200S (CTC Analytics, Zwingen, CH). A Zebron ZB-5 MSi capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used. Helium was used as carrier gas at a flow rate of 1.0 mL/min. The inlet was operated in split mode (ratio 1:20) with an injection volume of 2.0 μL of the essential oil diluted in methyl-t-butyl ether (MTBE). The GC temperature program used was 50 ºC (5 min) and 10 ºC/min up to 280 ºC (12 min). Scan time and the mass range were 0.9 s and 15–650 *m*/*z*, respectively. The data analysis was made using a computer P3 800MHz under Windows NT 4.0 and ThermoQuest Software Xcalibur 1.2.

The identification of the essential oils compounds was carried out comparing their relative retention times with those of the authentic samples (Carl Roth GmbH, Karlsruhe, Germany). For the components whose authentic substances were not available, their identification was made by matching their EI mass spectra with NIST/NBS, Wiley libraries spectra, and with the literature data [28].

Preparation of oil-in-water (O/W) emulsions

Nine different O/W emulsions (total solid content=300 g kg-1 of the emulsion) containing chia seed oil (100 g kg -1 of the emulsion) and natural antioxidants were prepared (Table 1). To obtain the aqueous phases sodium caseinate (NaCas) (100 g kg-1 of the emulsion)

was dispersed in deionized water under constant stirring at 50°C for 3 h. After that, lactose (100 g kg-1 of the emulsion) was added to the NaCas dispersions while stirring at 25°C. These dispersions were stored overnight at 4°C to achieve the complete dissolution of these polymers. The essential oils from both species of Origanum and M. spicata were added into the oil phases, whereas the Guardian Chelox was incorporated to the aqueous phases, to achieve antioxidant concentrations of 500 or 1000 mg kg-1 of the emulsion in all cases.

The oily and the aqueous phases were blended using an Ultra-Turrax T25 high-shear probe mixer (Janke & Kunkel GmbH, Staufen, Germany) operated at 10,000 rpm for 60 s to give pre-emulsions. The resultant pre-emulsions were further homogenized at 600 bar with four recirculation cycles using a high-pressure laboratory valve homogenizer (Panda 2K, GEA Niro Soavi, Parma, Italy).

About 100 g of emulsion were placed in a tray (12.5 cm × 16 cm) with a thickness of 10 mm and frozen at -20±2 °C until further freeze-drying.

Preparation of microencapsulated chia seed oil by freeze-drying

Immediately 24 h previous to freeze-drying, samples were transferred at −80±2°C. A laboratory scale freeze-drying equipment with a capacity of ~500 g per batch was used to dry the frozen emulsions. The time needed for drying one batch was 48 h.

The dried samples were ground in a mortar and sifted using a plastic sieve (equivalent to ASTM No. 7) to obtain powders particles < 2.83 mm.

Characterization of the microparticles

Moisture content

The moisture content of the chia oil powders (2g) was measured by drying the samples for 24 h at 70 ºC and 29 in Hg in a vacuum oven (Instrumentación Científica S.A., Buenos Aires, Argentina). Moisture content was calculated from the weight difference [29].

Microencapsulation efficiency

To analyse the no encapsulated oil fraction (free oil), 4 g of powder was mixed with 200 mL of hexane and stirred for 15 min. The mixture was filtered (Whatman No. 4) and the solvent was evaporated at 40 ºC in a rotary vacuum evaporator (Büchi, Flawil, Switzerland) [30]. The total oil content of the microparticles was analyzed by the Rosse-Gottlieb method [2].

Microencapsulation efficiency (ME), expressed as percentage of total oil, was calculated from the **Eq. 1**:

$$\text{ME (\%)} = \left(\frac{\text{Total Oil-Free Oil}}{\text{Total Oil}}\right) \times 100 \quad \textbf{(1)}$$

Morphological analysis by Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was used to study the microparticles morphology and size. The microparticles were metalized with gold film (600 Å) in a sputter coater (Pelco 9100, Clovis, CA, USA). The samples were observed using a MA10 instrument (Carl Zeiss SMT Ltd., Cambridge, UK) under high vacuum mode operating at 5 kV.

Flowing properties

The aerated (ρ_A) and tapped (ρ_T) bulk densities were determined according to a modified method of Quispe-Condori, Saldaña and Temelli [31]. About 25 g of each sample (m_0) was poured through a funnel into a 100 mL glass graduated cylinder. Then, the cylinder was

slightly tapped to collect the powder sticking to the wall of the cylinder. The volume (V_0) was read directly from the cylinder and used to calculate ρ_A according to **Eq. 2**:

$$\rho_A = \frac{m_0}{V_0} \quad \textbf{(2)}$$

For ρ_T, the cylinder was tapped until a constant volume (V_T) was reached. ρ_T was calculated as follows:

$$\rho_T = \frac{m_0}{V_T} \quad \textbf{(3)}$$

Results of ρ_A *and* ρ_T *were expressed as kg* m^{-3}.

The powder flowability was evaluated using the Carr's Index or "percent compressibility" (*C*) and the Hausner Ratio (*H*), using **Eqs. 4** and **5**, respectively:

$$C = \frac{\rho_T - \rho_A}{\rho_T} \times 100 \quad \textbf{(4)}$$

$$H = \frac{\rho_T}{\rho_A} \quad \textbf{(5)}$$

Colour

A Minolta colorimeter (CR-400, Konica Minolta Sensing Inc., Japan) was used to record L* (lightness), a* (red-green component), and b* (yellow-blue component) values of samples. The samples were homogeneously distributed in a glass Petri dish (diameter 95 mm) with a thickness of 15 mm. For each sample, two color measurements with three replications each one was done in different regions of the Petri dish and averaged [32].

Whiteness index (WI) was calculated as described by Marcone and Kakuda [33], according to **Eq. 6**.

$$WI = L^* - 3b^* \quad \textbf{(6)}$$

The yellowness index (YI) was calculated as follows[34]:

$$YI = \frac{142.86 \times b^*}{L^*} \quad \textbf{(7)}$$

The change of color after 90 d of storage was followed by the ΔE index, which was obtained by **Eq. 8** [35]

$$\Delta E=\sqrt{\left[(\Delta L^{*})^{2}+(\Delta a^{*})^{2}+(\Delta b^{*})^{2}\right]} \quad \textbf{(8)}$$

Peroxide value (PV)

PV was determined spectrophotometrically according to Mancuso, McClements and Decker [36]. A sample of 0.5 g of powder was weighed into a test tube, suspended in 5 mL of distilled water and shaking until complete powder dissolution. An aliquot (300 µl) of this dispersion was shacked in a vortex apparatus for 10 s with 1.5 mL of an iso-octane/isopropanol solution (3:1 v:v) and then centrifuged for 2 min at 3400 g. A portion of the upper phase (10-100 µl) was mix with 2.8 mL of a methanol/butanol solution (2:1 v:v) and 30 µL of a thiocyanate/ferrous solution. This latter was prepared by mixing 0.144 M $FeSO_4$ and 0.132 M $BaCl_2$ (1:1 v/v), centrifuging 3 min and then, mixing the clear ferrous phase with 3.94 M NH_4SCN (1:1 v/v). After 20 min the absorbance was measured at 510 nm. Lipid hydroperoxide concentrations were determined using cumene hydroperoxide standard curve. The analyses were carried out in duplicate.

Accelerated oxidative stability by Rancimat

Accelerated oxidation tests for bulk and microencapsulated oil were performed using a Rancimat model 743 (Metrohm AG, Herisau, Switzerland). Particularly, 3 g of chia oil or 1.5 g of powder were heated under air flow rate of 20 L/h at 98°C. The induction times (t_i) expressed in hours were recorded to study the oxidative stability [37] .

Storage of microparticles

The storage of the powders and the chia bulk oil was carried out in glass Petri dishes (95 mm x 15 mm) or glass beaker (50 mL), respectively, at a relative humidity of 33%, 25±2ºC in the dark, for 90 days. Aliquots of the samples were taken periodically for color and PV analysis. The assay was carried out in duplicate.

Statistical analysis

Analyses were carried out in duplicate for all tests, and results presented as the average ± standard deviation (SD). Data were analysed using a one way analysis of variance (ANOVA) and Tukey test ($p \leq 0.05$). Relationships between variables were studied using correlation analysis by Pearson's test. The Statgraphics Centurion XV software (StatPoint, Inc., USA) was used for the statistical analysis.

RESULTS AND DISCUSSION

Characterization of chia oil

According to chia oil fatty acids (FA) composition results, the main FA was α-linolenic acid (C18:3*n*-3) (64.59±0.09%), followed by linoleic (C18:2*n*-6) (19.74±0.36%) and oleic (C18:1*n*-9) (6.49±0.01%) FA. Also, chia oil presented palmitic (C16:0) (7.30±0.02%) and stearic (C18:0) (2.21±0.01%) FA. The *n*−3/*n*−6 FA ratio of chia oil was 3.3, which is noticeably higher in comparison to those reported from other vegetable oils, e.g. canola oil (0.45), soybean oil (0.15), and olive oil (0.13) [38]. These results agree with findings reported in previous studies[39, 40] Chia oil also exhibited a total tocopherol content of 330.95 ± 6.61 mg kg^{-1} oil. Similar total tocopherol content (238–427 mg kg^{-1}) was informed by Ixtaina *et al.*[40]. In this sense, although chia oil presents a FA composition favorable from a nutritional point of view and possess natural antioxidants, the high amount of PUFAs results in a low oxidative stability.

Characterization of essential oils

The obtained yields of essential oils, expressed in dry matter, were 2.10±0.01 % (v/wt), 3.35±0.02 % (v/wt) and 2.50±0.01 % (v/wt) for *M. spicata*, *O. vulgare,* and *O.* x *majoricum,* respectively.

The major components of the three essential oils studied are listed in **Table 2**. As can be seen, *O.* x *majoricum* presented a higher number of different compounds than *O. vulgare*. Nine compounds (>1%) were identified in the essential oil of *O. vulgare,* being the monoterpene carvacrol the main component (72.00%). Other compounds were cymol (13.42%) and γ-terpinene (6.96%) and in minor concentrations β-myrcene (1.30%), α-terpinene (1.40%), and caryopyllene (1.00%) (**Table 2**). The chemical profile of the *O. x majoricum* exhibited twelve compounds with concentrations >1% including thymol

(26.20%), terpineol (23.10%), and terpinen-4-ol (11.94%). The GC-MS analysis also revealed the presence of carvacrol (9.08%), ɣ- (5.79%) and α-terpinene (3.66%) in this essential oil. In the case of *M. spicata*, their composition presented seven compounds (>1%). The main components of this essential oil were carvone (45.36%), d-limonene (36.37%), and menthone (5.05%) while smaller amounts of dihydrocarveol (3.08%), β-myrcene (2.15%), menthol (1.90%), and caryopyllene (1.20%) were also detected.

Characterization of the microparticles

Table 3 shows the properties of the control microparticles obtained by freeze-drying using sodium caseinate and lactose as the wall materials.

The moisture content, ME, bulk density, compressibility index, Hausner ratio, and morphology of all different microencapsulated chia oil were found to be similar ($p>0.05$) regardless the presence of the added antioxidant type. The moisture content of the chia oil microparticles was comprised within the recommended range for food powders (30-40 g kg^{-1} dry basis). The ME provides information about which fraction of the total oil was efficiently microencapsulated. As can be seen in **Table 3**, the ME of the freeze-dried chia oil microparticles (86.3±3.2%) was lower than those reported by Ixtaina *et al*.[2] (~95.0%) for microencapsulation of chia seed oil produced by spray-drying. The possible emulsion physical destabilization produced by the emulsifier dehydration during the freezing, previous to the freeze-drying process, could be the cause of the lower ME of freeze-dried microparticles in comparison with the spray-dried ones. Thus, the core material release can occur during the named stage [7]. Similar results were found by Copado *et al*. [10]. Regarding the bulk density, it varied between 310±10 and 410±20 kg m^{-3} for aerated and tapped density, respectively (**Table 3**). Bulk density is an important parameter to describe food powders. It depends on the particle size, the water content in the product and the

characteristics of the material. In general, a low bulk density of the powder is not desirable due to the requirement of a higher volume of the package [26]. Similar results of this parameter were obtained by Copado *et al*. [10], with values of ~301 and ~551 kg m^{-3} for aerated and tapped density, respectively. Also, Goyal *et al*. [41] found an equivalent behavior for microencapsulated flaxseed oil powder using milk proteins.

The compressibility in many powders is a measure of internal cohesion, flowability, and in some extent, deformability. A low compressibility indicates a less cohesive powder and a higher bulk density [42]. This fact is important for the homogeneous character and reproducibility of the microparticles to be subsequently included in food products. The compressibility index resulted in ~ 25%, which is between the limit of free-flowing and non-free flowing solid[43]. The *H* also indicated that the obtained powder presented intermediate flowing properties. According to Geldart *et al*.[44] , this parameter allows to distinguish between easy (*H*<1.25) and the difficult-to-fluidize (*H*>1.4) powders. In our case, the studied microparticles recorded *H* ~1.3 with properties in common with both types of powders. It should be noted that the *H* reflects the friction conditions in a moving mass of powder, not in a static situation[44].

The scanning electronic micrographs (SEM) of microparticles containing chia oil are shown in **Fig. 1**. In general, the particles exhibited a sheet form with an irregular geometry and a compact structure. This shape is different from the microcapsules obtained by spray dried, which are spherical and regular[2]. Besides, the external topography of freeze-dried powders showed walls with the presence of some protrusions and some little pores, which could be related to the generation of cavities of ice crystals or air bubbles retained during freezing (Fig. 1d).[32, 45]. Related to this, although the pores presence could not affect the microencapsulation efficiency, it would be a possible cause of lipid oxidation. In this sense, the literature suggests that particles highly porous could facilitate the diffusion of oxygen

from the air onto the particle surface, which gets into the inner part of the particle due to the amorphous state of the matrix. However, the freeze-drying microencapsulation process was able to protect the chia seed oil against lipid oxidation, as discussed below.

Effect of natural antioxidants on oxidative stability and colour of microencapsulated chia seed oil

Nine systems of microencapsulated chia oil with and without the addition of natural antioxidants were studied.

The oxidative stability of the chia oil was enhanced using the freeze-drying microencapsulation since all systems presented significantly ($p \leq 0.05$) higher values of t_i (2.97-21.74 h) than those corresponding to bulk chia oil (2.37 ± 0.07 h).

The accelerated oxidative test revealed that at the initial time (t=0 d) the addition of Guardian Chelox (GCH) significantly increased ($p \leq 0.05$) the t_i of the microencapsulated chia oil (t_i~20h), without significant differences ($p > 0.05$) between both concentrations tested (GCH500 and GCH1000) (**Fig. 2**). The t_i of samples with *O. vulgare* essential oils in both concentrations (Ov500 and Ov1000) did not show significant differences ($p > 0.05$) respecting the control system. Regarding t_i of the microparticles with essential oils of *O. x majoricum* (Om500 and Om1000) and *M.spicata* (M500 and M1000), they were significantly ($p \leq 0.05$) lower than that of the control microencapsulated oil. At the end of the storage (t=90 d) all the samples recorded lower t_i than that corresponding to t=0 d, which indicates the occurrence and evolution of the oxidative deterioration. However, GCH500, GCH1000, Ov500, Ov1000 and Om1000 systems showed higher values of t_i than the control (**Fig. 2**). Among them, GCH1000, a blend of extracts from rosemary and chamomile, presented the highest t_i thus showing to be the phenolic compounds from this antioxidant, the most effective to protect the microencapsulated chia oil from oxidation.

This natural antioxidants blend, rich in flavones and phenolic acids, such as apigenin-7-O-glucoside, carnosic acid, and carnosol, provides a two-step protection in the form of metal chelation and free radical scavenging. GCH was used in model food emulsions and real food systems, showing a synergistic interaction between the rosemary and chamomile extracts [46] .

The oxidative stability was also studied through the evolution of peroxide value (PV) during the microparticles storage in the dark at 25±2 °C and 33% RH **(Fig. 3)**. Initial PVs were low for all systems, suggesting that significant quantities of primary oxidation products were not detected immediately after the microencapsulation process. However, after 30 d of storage, there were some significant ($p \leq 0.05$) differences. At this time, the control system showed a PV higher than the other ones and the chia bulk oil. The oxidation process involves a free radical chain mechanism proceeding via three steps: initiation, propagation, and termination[47]. Thus, the microencapsulation by freeze-drying could have promoted the initiation step; the addition of natural antioxidants could have protected the chia oil during this process. Notably, at this time, all PV values were below the limit allowed for this type of oil (10 meq kg^{-1} oil[48]). After 60 d of storage, the control microencapsulated chia oil showed a PV significantly higher ($p \leq 0.05$) than the other systems. Besides, the bulk chia oil started to steeply increase its PV. At this time (t=60 d), both bulk and microencapsulated chia oil without antioxidants exceeded the upper limit established by the *Codex Alimentarius* [48] for the consumption of this type of oil. At the final of the storage period (t=90d), GCH1000 presented PV <10 meq/kg oil, showing that this blend of natural antioxidants was efficient to protect chia oil against lipid oxidation. Also, GCH500, Ov500 and Om1000 recorded PV~10 meq/kg, with no significant differences ($p > 0.05$) among them. The highest antioxidant efficiency of GCH could be related to the combination of

rosemary and chamomile extracts. Different studies have shown that while the former has free-radical scavenger activity, the latest shows strong metal chelation[47].

A study carried out by Danisco[46] has shown that the GCH (250-1000 mg kg^{-1}) used in mayonnaise, dressings, margarine, and spreads presented a similar antioxidant performance that EDTA (75-150 mg kg^{-1}).

Changes in color are associated with the oxidation of triacylglycerols and free fatty acid since this process can produce colored products. Thus, the color can be a sign of the oxidative deterioration degree of foods with a high-fat content [49].

At t=0, microencapsulated chia seed oil produced without antioxidants presented creamish color having mean values of L^* 90.72, a^* -2.63, and b^*16.29 (**Table 4**). In general, when antioxidants were incorporated, the powders were off-white, with higher whiteness indices (W) than the control. Converesly, the yellowness indices (Y) of powders with natural antioxidants were lower than the control system. This last index is related to the general product degradation caused by the processing conditions, light, temperature and chemical agents[50]. Therefore, the obtained results show that the addition of antioxidants could have protected microencapsulated chia seed oil during the microencapsulation process.

After 90 d of storage all the systems presented changes in the color parameters. These color changes were very distintct for the system without antioxidants and those with the addition of *M. spicata* essential oils, whereas the rest of the microcapsules presented distinct color changes, according to the differences in perceivable color classification proposed by Adekunte *et al*.[51] (very distinct $\Delta E>3$, distinct $1.5<\Delta E<3$ and small difference $1.5<\Delta E$). The highest color change was recorded by the control system, followed by that with 500 mg kg^{-1} of *M. spicata.* No significant ($p>0.05$) differences were found between ΔE of the rest of the powders. In general, at the end of the storage the powders were more yellowish as consequence of the colored products of the oxidation process. In this sense,

a positive correlation was found between the *Y* index at t= 90d and the PV at 30 d (r=0.8018, p=0.0000), 60 d (r=0.8676, p=0.0000) and 90 d (r=0.7567, p=0.0000). Also a negative correlation was detected between the *W* index and the PV at different storage times (*W* vs. PV_{30d} r=-0.7891 p=0.0000; *W* vs. PV_{60d}=-0.8621, p=0.0000; *W* vs. PV_{30d} r=-0.7515 p=0.0000). However, some differences were observed between the different systems. The highest b^* value, which takes positive values for yellowish colours, was recorded in the control microparticles. In addition, the control system recorded the lowest L^* and the highest a^* values of all the samples, wich indicates that this system suffered the highest deterioration during the storage. Also, microparticles with 500 mg kg^{-1} of *M. spicata* recorded higher values of b^* and *Y*, and lower *W* than the other ones.

CONCLUSIONS

This study provides an insight into the use of different natural antioxidants to give freeze-dried microencapsulated chia seed oil additional protection against lipid oxidation.

The addition of the natural antioxidants had no significant effect on the microencapsulation efficiency (>85%), moisture content (<40 g kg^{-1}), bulk density, compressibility index, Hausner ratio, shape, and surface condition of the microencapsulated chia seed oil. The microparticles were irregular with sheet-like shapes, a compact structure and with the presence of some protrusions.

Results of Rancimat analysis showed that the oxidative stability of the chia oil was improved by the freeze-drying microencapsulation since the initial t_i of the microencapsulated chia seed oil was five times higher than the bulk oil. The addition of natural antioxidants further increased the t_i of microencapsulated chia oil, depending on the type and concentration of the antioxidant. The best effects were recorded in microencapsulated chia seed oil with the addition of Guardian Chelox at both concentrations.

The evolution of hydroperoxide content, evidenced through the PV, showed that the systems with 1000 mg kg^{-1} of Guardian Chelox presented lower values than the upper limit allowed for this type of oil. Also, the addition of 500 mg kg^{-1} of Guardian Chelox and *O. vulgare* essential oil, and 1000 mg kg^{-1} of *O. x majoricum* protected the chia oil, although to a lesser extent, presenting PV~10 meq kg^{-1} after 90 d of storage at 25±2°C and RH 33%.

Regarding color changes during storage, the addition of natural antioxidants, except *M. spicata* extract, had significant effect on this parameter, achieving a decrease in the degree of yellowing during the storage.

Taking into account the results previously described, Guardian Chelox, an antioxidant based on the mixture of rosemary and chamomile extracts, was the most efficient of the natural antioxidants studied in conferring protection against oxidative deterioration to the microencapsulated chia oil.

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FIGURE LEGENDS

Figure 1. Micrographs obtained by Scanning Electronic Microscopy (SEM) of the outer morphology of freeze-dried chia oil microparticles **a)** 50x; **b)** 100x; **c)** 800x; **d)** 3000x; pores (po) and protrusions (pr) are indicated

Figure 2. Induction times (t_i) obtained from the Rancimat method (T= 98 °C, air flow 20 L/h) of freeze-dried microencapsulated chia seed oil (without and with the addition natural of antioxidants) at t=0 (immediately after microparticles production) and at t=90 d of microparticles stored at 25±2ºC and 33% RH. Values are the mean of the two independent batches (n=2) and bars indicate standard deviation

Figure 3. Peroxide value of chia oil and freeze-dried chia oil powders (without and with the addition of natural antioxidants) stored at 25±2 ºC and 33% RH during 90 days. Values are the mean ± standard deviation of two independent batches (n = 2)

Table 1. Composition of the different chia O/W emulsions previous to freeze-drying

Composition	Emulsion								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Chia seed oil (g kg^{-1}emulsion)	100	100	100	100	100	100	100	100	100
Water (g kg^{-1}emulsion)	700	700	700	700	700	700	700	700	700
Sodium caseinate (g kg^{-1}emulsion)	100	100	100	100	100	100	100	100	100
Lactose (g kg^{-1}emulsion)	100	100	100	100	100	100	100	100	100
Guardian Chelox (mg kg^{-1} emulsion)	-	500	1000	-	-	-	-	-	-
O. *vulgare* essential oil (mg kg^{-1} emulsion)	-	-	-	500	1000	-	-	-	-
O. *x majoricum* essential oil (mg kg^{-1} emulsion)	-	-	-	-	-	500	1000	-	-
Mentha spicata essential oil (mg kg^{-1} emulsion)	-	-	-	-	-	-	-	500	1000
Code	C	GCH500	GCH1000	Ov500	Ov1000	Om500	Om1000	M500	M1000

Table 2. Major chemical compounds (> 1%) in essential oils of *O. vulgare*, *O. x majoricum and M.spicata*

Peak	Compound	RT (min)	*O. vulgare*	*O. x majoricum*	*M. spicata*
			% relative		
1	β-phellandrene	9.18	ND	2.49	ND
2	β-myrcene	9.57	1.30	1.00	2.15
3	α- terpinene	10.08	1.40	3.66	ND
4	cymol	10.23	13.42	7.00	ND
5	d-limonene	10.32	ND	1.55	36.37
6	γ- terpinene	10.91	6.96	5.79	ND
7	terpineol	11.63	TR	23.10	ND
8	menthone	12.60	ND	ND	5.05
9	menthol	12.92	ND	ND	1.90
10	terpinen-4-ol	12.99	TR	11.94	ND
11	dihydrocarveol	13.28	ND	ND	3.08
12	carvone	14.01	ND	1.52	45.36
13	thymol	14.69	TR	26.20	ND
14	carvacrol	14.81	72.00	9.08	ND
15	caryopyllene	16.56	1.00	1.89	1.20
	others*		3.92	4.78	4.89

RT: retention time; ND: no detected; TR: traces (<1%)

* Sum of different compounds <1%

Tabla 3. Physicochemical properties of control microparticles containing chia seed oil obtained by freeze-drying.

Property	**Value**
Total oil (g kg^{-1})	325±2.0
Surface oil (g kg^{-1})	44.5±2.0
Microencapsulation efficiency (ME) (%)	86.3± 3.2
Moisture content (g kg^{-1})	38.1± 4.0
Bulk Density (kg m^{-3})	
Aerated	310±10
Tapped	410±20
Compressibility Index (%)	24.39±0.84
Hausner ratio (H)	1.32±0.01
Oxidative stability (t_i in h)	13.5± 1.6
Peroxide value (meq kg^{-1} oil)	0.8± 0.05

Mean values ± standard deviation (n=2).

Tabla 4. Color parameters of the powders containing chia seed oil without and with natural antioxidants during the storage at 25±2 ºC and 33% RH

Microencapsulated chia oil	t= 0 d					t= 90 d					ΔE
	L*	a*	b*	W	Y	L*	a*	b*	W	Y	
C	$90.72^{ab\ B}$	$-2.63^{a\ A}$	$16.29^{b\ A}$	$41.85^{a\ B}$	$25.65^{b\ A}$	$84.50^{a\ A}$	$1.095^{e\ B}$	$31.99^{c\ B}$	$-11.47^{a\ A}$	$54.13^{c\ B}$	17.41^{c}
GCH500	$91.33^{ab\ A}$	$-1.77^{b\ A}$	$14.64^{a\ A}$	$47.41^{b\ B}$	$22.91^{a\ A}$	$90.45^{bc\ A}$	$-1.78^{c\ A}$	$16.40^{a\ B}$	$41.25^{c\ A}$	$25.90^{a\ B}$	2.24^{a}
GCH1000	$89.72^{a\ A}$	$-1.29^{c\ A}$	$14.87^{ab\ A}$	$45.11^{ab\ B}$	$23.67^{ab\ A}$	$88.23^{b\ A}$	$-1.26^{d\ A}$	$15.85^{a\ B}$	$40.67^{c\ A}$	$25.67^{a\ B}$	1.84^{a}
Ov500	$92.25^{b\ A}$	$-2.11^{b\ B}$	$14.91^{ab\ A}$	$47.51^{b\ B}$	$23.10^{a\ A}$	$91.04^{c\ A}$	$-2.52^{a\ A}$	$17.54^{a\ B}$	$38.41^{c\ A}$	$27.54^{a\ B}$	2.98^{a}
Ov1000	$92.02^{b\ A}$	$-2.05^{b\ B}$	$14.62^{a\ A}$	$48.17^{b\ B}$	$22.72^{a\ A}$	$91.63^{c\ A}$	$-2.23^{b\ A}$	$16.14^{a\ B}$	$43.21^{c\ A}$	$25.18^{a\ B}$	1.97^{a}
Om500	$92.43^{b\ A}$	$-1.98^{b\ B}$	$14.45^{a\ A}$	$49.09^{b\ B}$	$22.33^{a\ A}$	$92.27^{c\ A}$	$-2.32^{ab\ A}$	$16.58^{a\ B}$	$42.53^{c\ A}$	$25.67^{a\ B}$	2.23^{a}
Om1000	$92.02^{b\ A}$	$-2.05^{b\ B}$	$14.31^{a\ A}$	$49.08^{b\ B}$	$22.22^{a\ A}$	$91.27^{c\ A}$	$-2.35^{ab\ A}$	$15.85^{a\ B}$	$43.72^{c\ A}$	$24.81^{a\ B}$	1.79^{a}
M500	$91.83^{b\ B}$	$-1.99^{b\ B}$	$15.10^{ab\ A}$	$46.51^{ab\ B}$	$23.50^{ab\ A}$	$90.07^{bc\ A}$	$-2.17^{b\ A}$	$22.74^{b\ B}$	$21.83^{b\ A}$	$36.09^{b\ B}$	7.89^{b}
M1000	$91.32^{ab\ A}$	$-1.95^{b\ B}$	$14.80^{a\ A}$	$46.91^{ab\ B}$	$23.16^{ab\ A}$	$90.74^{c\ A}$	$-2.43^{ab\ A}$	$17.66^{a\ B}$	$37.75^{c\ A}$	$27.81^{a\ B}$	3.04^{a}

Mean values (n=3). Different lowercase letters in each column indicate differences in each color parameter between systems with different antioxidants (Tukey test, p≤0.05). Different uppercase letters in each row indicate, for each system, differences in the color parameter between the initial (t=0) and final (t=90 d) storage time (Tukey test, p≤0.05).

Figure 1

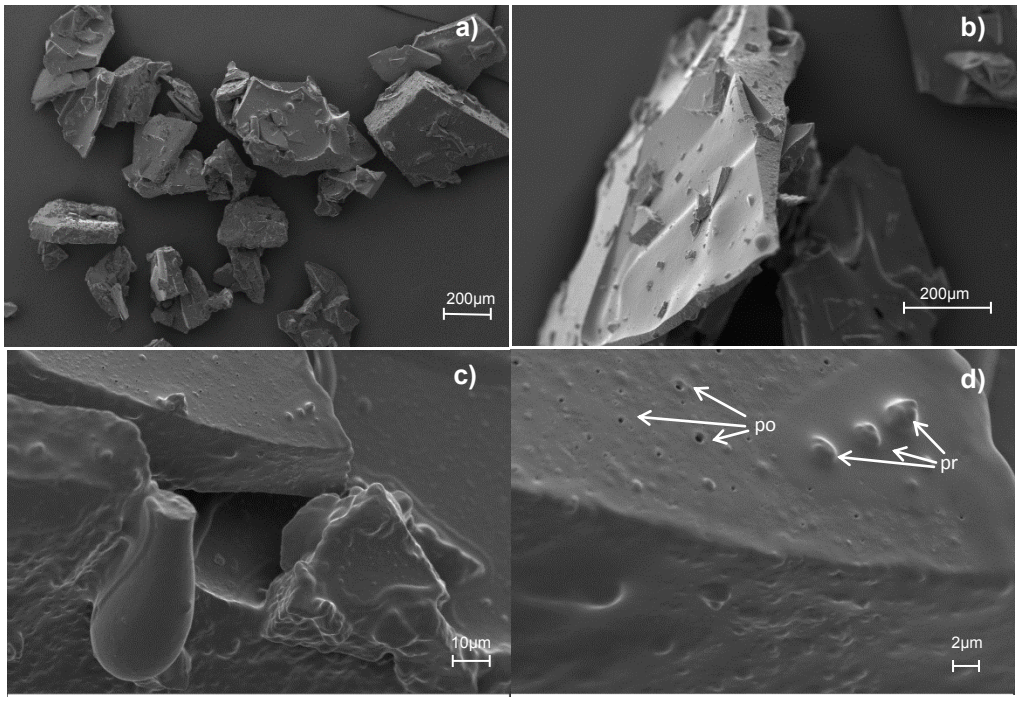

Figure 2

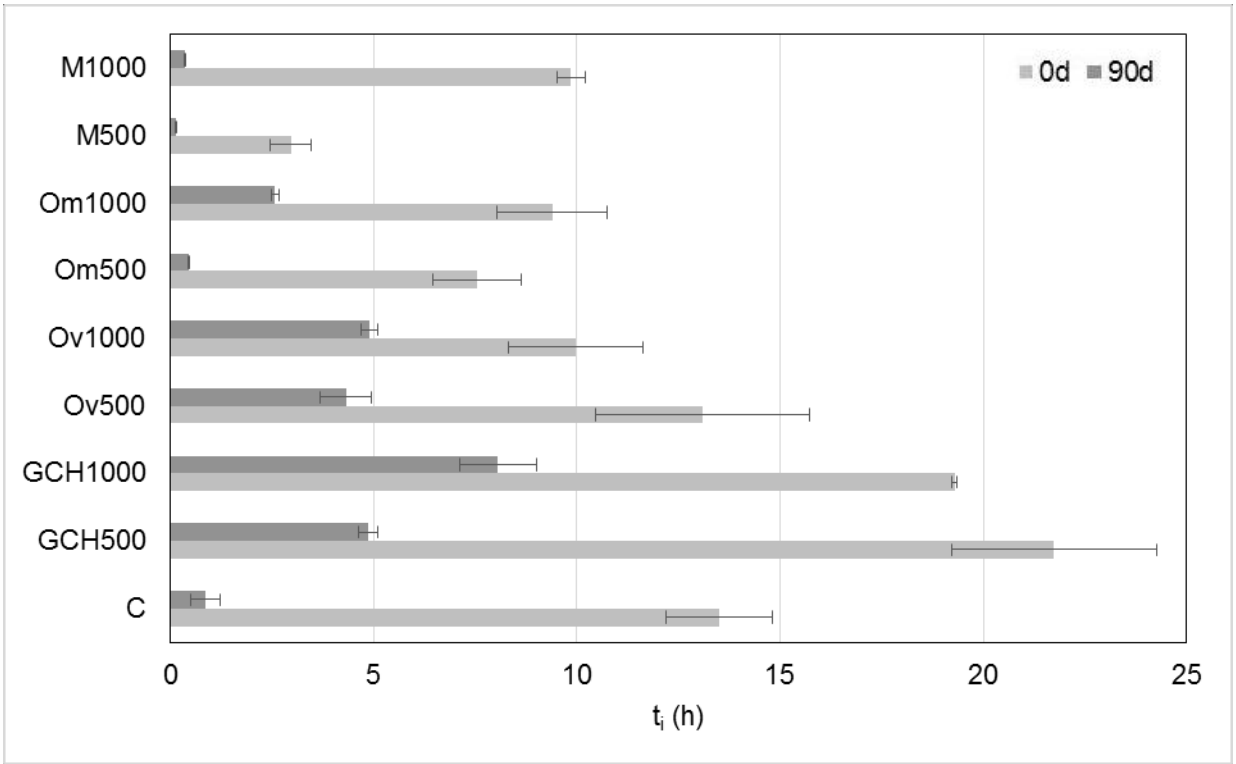

Figure 3