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Spray drying of shark liver oil pool: Effects on physical-chemical properties and antioxidant capacity

[Secado por atomización de la mezcla de aceite de hígado de tiburón: Efectos sobre las propiedades fisicoquímicas y capacidad antioxidante]

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Abstract

Context: Spray-drying is a technique used to produce encapsulated products, thus improving the stability of components as well as their organoleptic characteristics.

Aims: To evaluate the effect of microencapsulation of shark liver oil pool by spray drying on its physical-chemical properties and antioxidant capacity.

Methods: A mix design was created with a constant load of oil, made possible by controlling the proportion between chitosan acetate and maltodextrin. Loss on drying, encapsulation efficiency and yield were determined for the microcapsules. Reversed-phase HPLC analysis was used in order to determine the vitamin A content in microencapsulated and non-microencapsulated oil, as well as its delivery from the dried product. Vitamin A was the active compound used as a chemical marker. The following parameters were also evaluated: organoleptic characteristics, moisture content, particle size, surface morphology and antioxidant capacity.

Results: The encapsulation efficiency of microencapsulated oil increased slightly as the concentration of chitosan acetate increased. In order to achieve a greater encapsulation efficiency and a lower moisture content in microencapsulated oil, the proportion between chitosan acetate and maltodextrin should be maintained at 35% and 35% each, according to established manufacturing conditions. Both polymers prevent oil from leaving the droplet. This is a very important factor in storage stability of vitamins and fatty acids, which are subject to oxidative deterioration.

Conclusions: Spray-drying microencapsulation of pool oil does not affect vitamin A content or delivery; fatty acid content and antioxidant capacity are also not affected.

Keywords: antioxidant capacity; chitosan acetate; maltodextrin; microencapsulation; shark liver oil pool; spray-drying.

Resumen

Contexto: El secado por atomización permite obtener productos encapsulados mejorando la estabilidad de los componentes, así como sus características organolépticas.

Objetivos: Evaluar el efecto de la microencapsulación de la mezcla de aceite de hígado de tiburón, por secado por atomización, sobre sus propiedades fisicoquímicas y capacidad antioxidante.

Métodos: Se desarrolló un diseño de mezcla, fijando la carga del aceite, cuyos factores fueron la proporción de acetato de quitosana y maltodextrina. A las microcápsulas le fueron determinadas las pérdidas por desecación, eficiencia de encapsulación y rendimiento. El contenido de vitamina A, compuesto activo utilizado como marcador químico, en el aceite microencapsulado y no microencapsulado se determinó por cromatografía, así como su liberación desde el producto seco. Además, se evaluaron los siguientes parámetros: características organolépticas, contenido de humedad, tamaño de partículas, morfología de la superficie y la capacidad antioxidante.

Resultados: La eficiencia de encapsulación del aceite microencapsulado aumentó ligeramente con el aumento de la concentración del acetato de quitosana. Para alcanzar una mayor eficiencia de encapsulación y menor contenido de humedad en el aceite microencapsulado, la combinación de acetato de quitosana y maltodextrina debe mantenerse al 35% y 35%, respectivamente. Ambos polímeros previenen la exudación del aceite, siendo importante para conservar la estabilidad de las vitaminas y ácidos grasos, sensibles a la degradación oxidativa.

Conclusiones: La microencapsulación del aceite, mediante secado por atomización, no afectó el contenido de vitamina A ni su liberación, así como tampoco el contenido de ácidos grasos ni su capacidad antioxidante.

Palabras Clave: acetato de quitosana; capacidad antioxidante; maltodextrina; mezcla de aceite de hígado de tiburón; microencapsulación; secado por atomización.

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INTRODUCTION

Free radicals are generated by exogenous chemicals or endogenous metabolic process in food systems or in the human body. Free radicals may cause oxidative damage to biomolecules, resulting in cell death and tissue injury. However, the ingestion of antioxidant supplements, or foods containing antioxidants, might reduce oxidative stress in the human body (Wang et al., 2006).

Investigations of fish oils have not only shown their importance as a dietary source of vitamins A, D and E, but also their richness in long-chained omega-3 fatty acids (Rabasco and González, 2000).

The unsaturation of fatty acids makes fish oil more vulnerable to spoilage than other oils. Fish oil spoils in two major ways, just as oils from animal and vegetable sources do: oxidative and hydrolytic spoilage (Cmolik and Pokorny, 2000). Oxidation is the most important cause for the deterioration of fish oil quality because of its high triglyceride concentration.

Unfortunately, the instability of fish oils during food or pharmaceutical processing, distribution or storage limits the activity and potential health benefits of fish oils. Fish oils are unstable because their high sensitivity to environmental factors, including physical and chemical conditions. Oxidative reactions in oils lead to the progressive appearance of a brown color and/or unwanted odors, with considerable loss in activity (Bae and Lee, 2008). Furthermore, many fish oils have an unpleasant taste, which must be masked before their incorporation into foodstuffs or oral medicines. Therefore, their delivery requires the formulation of a finished protecting product, which is able to maintain the structural integrity of vitamins and fatty acids until consumption, mask its taste, and increase its water solubility and bioavailability. Consequently, oil encapsulation may be useful to delay lipid peroxidation and increase the range of applications where oil could not otherwise be used.

Encapsulation is a process by which small particles of core products are packaged within a wall material to form microcapsules. One common technique to produce encapsulated products is spray-drying, which is a very rapid drying method due to the very large surface area created by the atomization of the liquid feed (Oliveira et al., 2006). The technology is relatively low-cost, fast, reproducible and easy to scale up when compared with other microencapsulation techniques, which justifies its preference for industrial purposes (Pu et al., 2011). One limitation of spray-drying technology is the type of encapsulating agent, which should be water soluble at an acceptable level (Desai and Park, 2005).

The choice of encapsulating agent is very important for encapsulation efficiency and microcapsule stability. Because one agent alone may not have all the required characteristics, a combination of encapsulating agents may be used (Nogueiro et al., 2013). Walls material for microencapsulation of oil by spray-drying must have emulsifying properties, high water solubility, low viscosity, and drying properties (Kagami et al., 2003; Bae and Lee, 2008). Typical wall materials include proteins (sodium caseinate, whey proteins, soy proteins, and gelatin) and hydrocolloids (modified starch and Arabic gum). Hydrolyzed starches (glucose, lactose, corn syrup solids, and maltodextrin) are generally added as a secondary wall material to improve drying properties of sprayed droplets (Kagami et al., 2003). Maltodextrin has been investigated as partial replacer for Arabic gum in spray-dried emulsions. Arabic gum is historically considered as one of the most important encapsulating agents (Bringas et al., 2011; Nogueiro et al., 2013). It has historically been used as a standard agent due to its excellent emulsification properties and low viscosity. Other authors have used similar polymers. For instance, Sánchez et al. (2002) used a combination of maltodextrin, Arabic gum, and gelatin to encapsulate shark liver oil.

The aim of this work was to determine the effect of microencapsulation of shark liver oil pool by spray drying in terms of its physical-chemical properties and antioxidant capacity.

MATERIAL AND METHODS

Chemicals

The shark liver oil pool was obtained from Cuba's Fisheries Research Center (CIP). A chemical reference substance of vitamin A acetate was supplied by CIDEM (Cuba). Chitosan acetate was prepared industrially in a Cuban company, in accord-

ance with the procedure reported previously (Cervera et al., 2011). Maltodextrin was purchased from Panreac (USA). Four fatty acids standards were obtained separately: palmitic (PA) C16:0 (BDH, Great Britain), stearic (SA) C18:0 (Merck, Germany), oleic (OA) C18:1 (Merck, Germany) and linoleic (LA) C18:2 (BDH, Great Britain). Boron trifluoride was procured from Panreac (USA) and n-hexane was purchased from Sigma-Aldrich (Germany). All other chemicals were of analytical grade.

Physical-chemical analysis of shark liver oil pool

Physical-chemical parameters such as organoleptic characteristics, specific gravity, refractive index, acidity, saponification, peroxide and unsaponifiable matter were determined according to the United States Pharmacopeia (USP 35, 2013). Vitamin A content was determined through a validated high-resolution liquid chromatography method (García et al., 2008). The values obtained (data is not shown) concerning physical and chemical parameters demonstrated the quality of shark liver oil pool, according to established specification limits (Garcia et al., 2014a).

Spray-drying of shark liver oil pool

Drying tests were performed in a model B-191 Büchi Minispray Dryer (Switzerland), with concurrent drying air flow and feed. The following parameters remained constant: inlet temperature at 150°C, outlet temperature at 90°C, air flow rate at 600 L/h and drying air flow rate at 60 m³/h. The effects of chitosan acetate (CA) and maltodextrin (MD) were evaluated based on a mix design, at constant load of oil (30% w/w). Table 1 shows the experimental matrix. Loss on drying, encapsulation efficiency and yield of microencapsulated oil were determined.

Evaluation of microcapsules

Loss on drying (LD)

Loss on drying was determined according to USP 35 (2013). Samples were weighed and dried to constant weight at 105°C (López et al., 2009; Bringas et al., 2011).

Encapsulation efficiency (EE) and yield

Encapsulation efficiency and yield were determined by eqs. 1-2, respectively (López et al., 2009; Bringas et al., 2011):

$$EE(\%) = \frac{\%Total - \%Free}{\%Total} \cdot 100 \quad (1)$$

$$Yield(\%) = \frac{A}{B} \cdot 100 \quad (2)$$

Where A is the amount of microparticles obtained, and B the amount of microparticles expected.

Table 1. Experimental matrix and obtained responses.

Run	Maltodextrin (%)	Chitosan acetate (%)	Yield (%)	Encapsulation efficiency (%)	Loss on drying (%)
1	23	47	29.70 ± 2.03	78.10 ± 1.38	8.61 ± 0.06
2	47	23	44.02 ± 1.84	78.90 ± 1.22	7.34 ± 0.05
3	35	35	46.30 ± 1.52	84.03 ± 0.14	4.14 ± 0.09
5	70	0	54.30 ± 1.72	-	4.39 ± 0.60
4	0	70	25.01 ± 1.03	52.33 ± 1.18	5.78 ± 0.09
Yield (%) = 30.71*CA + 45.0*MD + 36.81*CA*MD; p = 0.0116; r^2 = 96.12					
EE (%) =78.79*CA + 79.71*MD + 21.58*CA*MD; p = 0.0124; r^2 = 94.64					
LD (%) = 8.58*CA + 7.315*MD + 15.33*CA*MD; p = 0.0325; r^2 = 95.10					

EE: encapsulation efficiency; LD: loss on drying.

Surface morphology and particle size

Photomicrographs were obtained in a JSM-6060 scanning electron microscope (Japan), at 3000X magnification, in order to most properly assess the morphological appearance of the dried product. Particles were covered with gold and observed under high-vacuum conditions at an acceleration voltage of 10 kV.

Particle size was examined by means of a Shimadzu IG-1000 single-nanoparticle analyzer (Japan). The following conditions were established: frequency: 350 kHz; voltage: 30 Vpp, and time: 0.10 seconds.

Vitamin A content assay by HPLC

Vitamin A content in microparticles with the best characteristics was determined by a validated reversed-phase HPLC analysis, according to conditions described by Garcia et al. (2014b): Lichrosorb RP-18 column (5 µm) 250- 4 mm (Merck, Germany), mobile phase methanol - water (90:10) v/v, flow rate 1 mL/min and length wavelength of 325 nm. The procedure was performed using a high liquid resolution chromatograph (Merck, Germany).

Vitamin A release from microparticles

In order to suspend the microparticles in the dissolution medium, 20 mg of microencapsulated oil were added to glass tubes containing 50 mL of artificial gastric juice (USP 35, 2013), at 37 ± 0.5°C for 60 min. The procedure was carried out in triplicate (León et al., 2015).

Fatty acids content assay by GC

Fatty acids content in microparticles with the best characteristics was determined by a validated gas chromatographic analysis, according to conditions described by García et al. (2017): column HICAP-WAX (30 m x 0.53 mm ID x 1.0 µm) (Germany). The run was under temperature program as follows: initial column temperature of 180°C during 3 min, programmed to increase at a rate of 7°C/min up to 200°C. This temperature was maintained for 4 min. Injector and detector temperatures were maintained at 240°C. Hydrogen was used as the carrier gas, at a flow rate of 1.5 mL/mim with a split ratio of 30:1. To the determination of fatty acid methyl esters (FAME) by GC were employed four standard fatty acids: palmitic (PA) C16:0 (BDH, Great Britain), stearic (SA) C18:0 (Merck, Germany), oleic (OA) C18:1 (Merck, Germany) and linoleic (LA) C18:2 (BDH, Great Britain).

Antioxidant capacity by DPPH$^{\bullet}$ assay

The radical scavenging capacity of oil samples was measured using a method described by Brand-Williams et al. (1995), with slight modifications. The assay is based on the reduction of the free radical 2,2-diphenyl-1-picryl-hidrazyl (DPPH$^{\bullet}$) at 517 nm. An aliquot of isooctane solution (100 µL) containing different concentrations (1:2 serial dilutions from initial sample) of liver oil was added to 2.5 mL of DPPH solution (0.04 mM in isooctane) and vortexed. The optical density of DPPH solution was adjusted to 0.600 at 517 nm in a spectrophotometer UV (Pharmacia). Trolox (20 µM), a structural analogue of vitamin E, was used as standard. The experiment was carried out in darkness by duplicate in different days; samples were analyzed in triplicate after 30 min of reaction. Radical scavenging capacity was expressed as the percentage relative to DPPH$^{\bullet}$ absorbance diminishing.

***In vivo* antioxidant capacity of microencapsulated oil**

Sprague Dawley male rats, weighing 200-250 g were obtained from CENPALAB (Havana, Cuba) and were acclimatized to environmental conditions for seven days before tests. The animals (five animals/group) were housed under conventional conditions exposed to light-dark cycle of 12 h with free access to water and food. All studies followed Good Laboratory Practice standards in accordance with the ethical guidelines for investigations. Animal studies were performed with the approval of Pharmacy and Food Sciences College Institutional Animal Ethical Committee (CB162014). A minimum number of animals were used to obtain reliable results. The microparticles were suspended in 1 mL of CMC 0.5% and orally administered. Rats were supplemented with the microencapsulated oil (500 mg/kg/day) for 7 days. Control groups received 1 mL of non-encapsulated oil or CMC 0.5%, respectively, by oral cannulation. At the end of the experiment, blood samples were withdrawn from the tail

vein. These samples were immediately centrifuged at 2500 g, at 4°C for 10 min. The serum was collected and aliquots were stored at –80°C until analysis.

The serum antioxidant status was determined using a Randox TAS kit Cat No. 2332. In the assay ABTS (2,2′-azinodi-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and hydrogen peroxide (H_2O_2) to produce the radical cation $ABTS^{\bullet+}$. The relatively stable blue-green color, which is measured at 600 nm in a plate reader spectrophotometer (SUMA, Havana, Cuba). The results were expressed as mmol Trolox equivalent/L. In addition, the ferric reducing ability of plasma was determined by OxiSelect™ Ferric Reducing Antioxidant Power (FRAP) Assay Kit, which measures the ferric to ferrous iron reduction in the presence of antioxidants. Samples was analyzed by triplicated, and absorbance was measured at 590 nm in a plate reader spectrophotometer (SUMA). The results were expressed as µmol ascorbic acid equivalents.

Statistical analysis

For the statistical analysis, the program PASW Statistics (SPSS Inc. version 18) was used. The evaluations to the statistically-significant differences between the vitamin A and fatty acids content of non-microencapsulated oil and microencapsulated oil was calculated based in test t-Student. For antioxidant assays, the values are expressed as mean ± standard deviation of experimental values. Statistical analysis was performed with SPSS 12.0 software. For multiple comparisons, one-way ANOVA was used followed by Bonferroni post-hoc test. Values of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In order to choose the most appropriate wall component a mix design, at constant load of oil, was evaluated (Table 1). The selection was made according to microencapsulation yield, encapsulation efficiency and loss on drying. In general, the best results were obtained when CA and MD were used in equal proportion (35%:35%).

When only CA was used to obtain microcapsules, this polymer by itself was capable of adsorbing at the oil/water interface. It formed a viscoelastic film around the oil droplet that allows for microencapsulation, having a dual function as polymer and surfactant. In turn, MD alone did not allow for oil microencapsulation. However, the combination of both encapsulating agents favored the continuity of the protective film provided by chitosan acetate around the oil droplet. These results are consistent with those reported by López et al. (2009) and Nogueiro et al. (2013).

MD is used in combination with Arabic gum to contribute to structural continuity during the formation of the encapsulating film, thereby improving the efficiency of encapsulation of oils, with a lower molecular weight. By its nature, Chitosan presents a similar behavior to that of Arabic gum, so it adsorbed at the oil/water interface, forming a viscoelastic film and thus retaining the oil.

EE of microencapsulated oil became lower as the concentration of CA increased. High viscosity makes the spray-drying process difficult, as more microparticles will be formed. The droplets could be aggregated, which is why the yield was the lowest of all the experiments. It was found that optimum conditions for the preparation of microparticles were achieved by mixing CA at 35% and MD at 35%. The percentage of MD has a significant effect ($p<0.05$) on EE response. These results can be explained by the model equation (Table 1).

As mentioned before, CA concentration in the solution affected the yield of microencapsulated oil. In general, low yields were achieved. However, it must be pointed out that we have worked with small volumes, which explains the low performance, and that only laboratory equipment was used, without any device to facilitate product recovery, which could increase this parameter if working on a different scale.

Although polymers used are hydrophilic, in all cases LD values were below 10%, which ensures the microcapsule integrity and encapsulation efficiency over time. As shown (Table 1) when CA content in the mixture increased, LD results were higher. This is justified by the ability of the salt to absorb moisture (de la Paz et al., 2012). In three cases, the values of LD were over 5%. Meanwhile, when both polymers were used in equal percentages, values were below 5%, which corresponds to the equation obtained for this parameter (see Table 1). High moisture values

would adversely impact a powder's shelf life in the high-humidity conditions that are typical in Cuba.

The calculated probability values (p) for yield, EE and LD are summarized in Table 1. Values were lower than 0.05 (p=0.0116 for yield; p=0.0124 for EE and p=0.0325 for LD). In general, results showed that CA and MD effects did not exhibit the same pattern. MD percentage appeared as the statistically-significant main effect, having a positive influence in terms of yield and microencapsulation efficiency. The interaction between both factors (percentage of MD and CA) showed a significant negative effect on LD. These results can be explained by model equations and adequate r^2 values.

Consequently, the effect of encapsulating agents during the drying process significantly affects the EE of resultant microparticles on a laboratory scale. Therefore, in order to reach higher EE and lower moisture content of microencapsulated oil, the combination of CA and MD should be maintained at 35% and 35% each, according to established manufacturing conditions. This may be explained by the fact that both polymers prevent oil from leaving the droplet, which is a very important factor in providing storage stability for vitamins and fatty acids that are subject to oxidative deterioration. The formation of a semipermeable membrane around the emulsion droplet provides better retention of labile compounds.

On the other hand, the microencapsulation process not only improved the oil's organoleptic characteristics when compared to non-microencapsulated oil. It should also make it more stable, allowing for a longer shelf life (Goud and Park, 2005).

Fig. 1 presents typical SEM photomicrographs of the dried product. Photomicrographs show that the product is composed by spherical particles with some ripples, which is a characteristic of microcapsules with an encapsulated liquid in the core. This is also attributable to oil content, which is lower than polymer content (Chiappetta et al., 2006).

In addition, a smooth surface without pores is observed, which is essential for microcapsule stability. Pores facilitate both the entry of oxygen and the exit of encapsulated material, which results in a decrease in encapsulation efficiency and oxidation of compounds such as fatty acids (López et al., 2009; Bringas et al., 2011).

The dried product is composed by particles with a narrow size distribution (31.97 ± 0.099 nm), without evidence of agglomeration (Fig. 2). Values are correct, since the maximum intensity of light was 63.29, which is well within the acceptable 50-to-200 range.

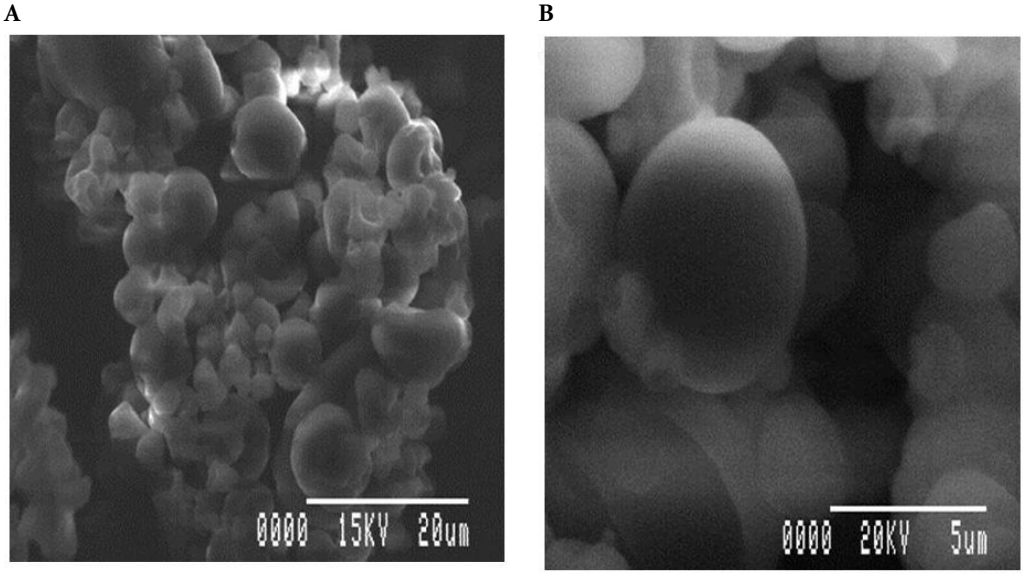

Figure 1. Scanning electron microscopy of microparticles.

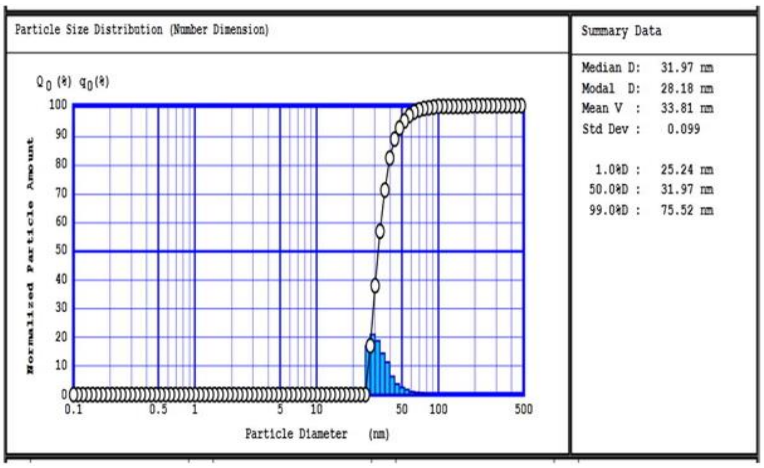

Figure 2. Particle size distribution of microparticles.

Results in Table 2 show a student-calculated t value that was lower than the tabulated t value, showing no statistically-significant differences between the vitamin A content of non-microencapsulated oil and that of microencapsulated oil. Consequently, this confirms the feasibility of microencapsulating shark liver oil by spray-drying, as a process that does not affect vitamin A content.

Table 2. Vitamin A content of non-microencapsulated oil and microencapsulated oil.

Non-microencapsulated oil (µg/g)	Microencapsulated oil (µg/g)
200.71	200.43
198.43	201.61
201.72	200.17
200.93	199.71
201.32	201.32
201.11	201.29
Mean ± SD = 200.70 ± 1.166	Mean ± SD = 200.76 ± 0.758
DSR (%) = 0.5810	DSR (%)= 0.3778
t-Student calculated = 1.056; t-tabulated (11; 0.05) = 2.20	

X: mean; DSR: relative standard deviation; n=6.

The time of exposition of microparticles to high temperatures is short (normally a few seconds) and temperature in the core of microparticles generally does not exceed 100°C, reducing the possibility of undesirable changes for thermo-sensitive compounds (de Azeredo, 2005). Further work would be needed to study the stability of microencapsulated shark liver oil.

Fig. 3 shows the influence of encapsulating agents on vitamin A release behavior from microencapsulated oil. The release rate of the encapsulated product was rapid, and more than 85% of vitamin A content was dissolved within 10 minutes. The results of the present study indicate that the release rate of vitamin A from microencapsulated oil was not influenced by the specific combination of encapsulating agents that was used. Therefore, the release behavior for vitamin A was not affected by the viscosity of chitosan solution.

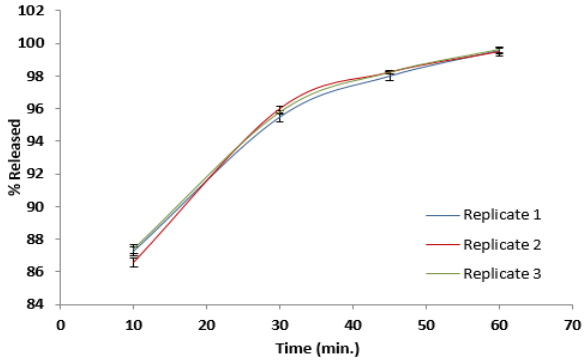

Figure 3. Vitamin A release from microencapsulated shark liver oil pool.

Although refined fish oils can provide nutrients of high biological value, crude fish oil has potential hazards. Highly unsaturated fatty acids are easily oxidized (Hossain, 2011). In addition, pollutants can accumulate in fish (Racine and Deckelbaum, 2007). Oxidation affects the color and taste of fish oil, and impairs its nutritional value. Oxidation can be increased by exposure to temperature, light, trace metals and certain enzymes (Calvo et al., 2010).

Table 3. Fatty acids content (μg/g of oil) of non-microencapsulated oil and microencapsulated oil.

Treatment	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
Non-microencapsulated oil	35.140	9.722	28.198	2.107
Microencapsulated oil	35.333	9.697	27.865	2.088
t-tab (11; 0.05) = 2.20	t calc = 1.08	t calc = 0.34	t calc = 1.64	t calc = 1.39

In order to demonstrate the applicability of the proposed microencapsulation process to protect the fatty acids in shark liver oil, we determined the selected fatty acids (palmitic, stearic, oleic and linoleic) in microencapsulated oil. Results in Table 3 show student-calculated t values that were lower than the tabulated t value, showing no statistically-significant differences between contents of selected fatty acids of non-microencapsulated oil and microencapsulated oil, at 95% confidence.

Consistent with García et al. (2017), the most abundant saturated fatty was palmitic acid, for both non-microencapsulated and microencapsulated samples. Consequently, this confirms the feasibility of microencapsulating shark liver oil by spray-drying as a process that does not affect fatty acid content. Further work is needed to study the stability of microencapsulated shark liver oil.

This proved the usefulness of the microencapsulation process by spray-drying as an alternative technology to protect vitamin A and palmitic acid in the oil. In addition, it was proven that microencapsulated oil with chitosan acetate is an alternative to the traditional use of Arabic gum.

Shark liver oils are known to exert antioxidants effects, which in turn have potential benefits in the prevention of human illnesses, such as cardiovascular diseases (Chan and Cho, 2009; Zzaman et al., 2014). Indeed, previous studies have demonstrated the correlation between fatty fish ingestion with antioxidant status in plasma and a decreased incidence of cardiovascular disease (Rajaram et al., 2009). Several methods have been developed for the characterization of antioxidant substances. Currently, there are methods that mimic the physiologically-formed reactive species (Halliwell, 2013). The objective of this work was not to characterize the oil antioxidant capacity, but demonstrate that technological process did not significantly affect its biological activity, in this case the antioxidant activity. DPPH assay is considered a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants, since the radical compound is stable and need not be generated (Brand-William et al., 1995). The DPPH radical scavenging capacity of both non-microencapsulated and microencapsulated oil was similar, with no statistical differences ($p>0.05$), as shown in Table 4. These results suggest that the microencapsulation process does not affect the antioxidant properties of shark liver oil samples. Once demonstrated that microencapsulation process did not affect the oil capacity to sequester the DPPH radicals, the antioxidant effect was verified *in vivo*. The results demonstrated that supplementation with the microencapsulated oil improve the serum antioxidant status.

Table 4. Free radical scavenging capacity determined by the DPPH· assay.

Treatment	DPPH· scavenging capacity (%)
Trolox	44.53 ± 0.47^{a}
Non-microencapsulated oil	29.63 ± 1.10^{b}
Microencapsulated oil (CA:MD)	28.31 ± 1.31^{b}
Maltodextrin	1.23 ± 0.05^{c}
Chitosan acetate	1.16 ± 0.07^{c}

The values are the mean ± standard deviation of the free radical scavenging capacity of shark liver oil. The antioxidant capacity of the oil pool was expressed as the percentage relative to DPPH· solution absorbance diminishing after 30 min of reaction. Trolox (20 μM) was used as antioxidant standard. Different letters represent statistical differences ($p<0.05$); n=3.

In order to evaluate the *in vivo* antioxidant capacity of the oil (Table 5), two different methods using different antioxidant mechanism were employed. TAS technique demonstrated that oil supplementation significantly incremented the free radical scavenger capacity of serum components from oil treated rats. In addition, the second method showed the reductive power of water soluble antioxidants present in animal´s serum treated with the shark liver oil. Thus, the microencapsulation process not only protects the oil from oxidation,

but also preserves one of its main biological properties, which is its antioxidant capacity. Shark liver oils are known to contain omega-3 fatty acids, which in turn have been used in the prevention of human illnesses, such as cardiovascular diseases (Chan and Cho, 2009; Zzaman et al., 2014). Indeed, previous studies have demonstrated the correlation between fatty fish ingestion with antioxidant status in plasma and a decreased incidence of cardiovascular disease (Rajaram et al., 2009).

Table 5. *In vivo* antioxidant effect of microencapsulated oil.

Treatment	TAS (mmol Trolox Eq)	FRAP (µmol ascorbic acid Eq)
Non microencapsulated oil	5.24 ± 0.32^{b}	336.51 ± 6.91^{b}
Microencapsulated oil	4.18 ± 0.46^{b}	290.25 ± 7.83^{c}
Control	1.89 ± 0.11^{a}	121.00 ± 4.59^{a}

The values are the mean ± standard deviation of serum total antioxidant status. Different letters represent statistical differences ($p<0.05$) between animal groups ($n=5$).

CONCLUSIONS

The combination of encapsulation agents (CA y MD) does not have a significant effect on vitamin A content or its release from the dried product. This work showed that shark liver oil pool was successfully encapsulated, opening new possibilities for its use as a natural antioxidant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contribution:

Contribution	García CM	Fernández M	López OD	Delgado-Roche L	Nogueira A	Castiñeira M	Medrano EA
Concepts or ideas	X	X	X			X	
Design	X	X	X	X			
Definition of intellectual content	X	X	X			X	
Literature search	X			X		X	X
Experimental studies	X			X	X		X
Data acquisition	X			X	X		X
Data analysis	X	X	X	X	X	X	X
Statistical analysis	X	X	X	X			
Manuscript preparation	X	X	X	X		X	
Manuscript editing	X	X					
Manuscript review		X					

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