MICROENCAPSULATION OF SHARK LIVER OIL POOL BY SPRAY DRYING

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***Abstract*– The aim of this paper is to study the spray-dried microencapsulation of shark liver oil using gum arabic and maltodextrin as encapsulating agents. A mix design, was developed where the main factor was the ratio between gum arabic and maltodextrin. Vitamin A content in microencapsulated and non-microencapsulated oil was determined by reversed-phase HPLC analysis, as well as the release of vitamin A from the dried product. The following parameters were also evaluated: encapsulation efficiency, loss on drying, surface morphology and particle size. The encapsulation efficiency of microencapsulated oil increased slightly as the concentration of gum increased. To reach higher encapsulation efficiency and lower moisture content of microencapsulated oil, the combination of gum arabic and maltodextrin should be maintained at 47% and 23%, respectively, according to established manufacturing conditions. The microencapsulation of oil by spray drying has no statistically significant effect on the vitamin A content response, or on its release rate.**

***Keywords* – gum arabic, maltodextrin, microencapsulation, shark liver oil pool, spray drying, vitamin A**

I. INTRODUCTION

Microencapsulation is a technology that helps protect core compounds, as well as reduce their reactivity with external factors, by reducing its interaction with the outside environment (e.g., heat, moisture, air, and light), decrease the transfer rate from the core to the outside, and control the release of the core compound, to avoid alterations in the physical characteristics of the original material, which can be modified more easily in order to facilitate handling, mask the core taste and dilute the core compound in the final product when it would be toxic in large quantities (Gharsallaoui *et al*., 2007; Nogueiro *et al*., 2013). In this manner, some heat-, temperature- or pH-sensitive compounds can be used more conveniently. This technology may also prevent the loss of some compounds, such as vitamins, proteins, enzymes and mineral salts, during certain industrial processes (Pothakamury and Barbosa-Cánovas, 1995). Water is the preferred solvent for most spraying processes, due to that the use of organic solvents produces toxicity and environmental problems (Kumar Das *et al*., 2011).

Typical wall materials for oil microencapsulation by spray-drying include proteins (sodium caseinate, whey proteins, soy proteins, gelatin) and hydrocolloids (modified starch and gum arabic). Hydrolyzed starches (glucose, lactose, corn syrup solids, and maltodextrin) are generally added as a secondary wall material to improve drying properties of the sprayed droplets (López *et al*., 2009).

Shark liver oil possesses multiple benefits, and is considered a raw material of natural origin with high potential of use in pharmaceutical formulations. This oil has a high content of vitamins and fatty acids, requiring appropriate storage conditions to ensure stability.

The aim of the present paper was the microencapsulation of shark liver oil pool through spray drying, in which the polymers gum arabic and maltodextrin were used as encapsulating agents. Its evaluation by FTIR and SEM, vitamin A content, active compound used as a chemical marker, as well as its release from the dried product were determined.

II. METHODS AND MATERIALS

Shark liver oil pool was obtained from the Fisheries Research Center (CIP, Cuba). Vitamin A acetate, as a chemical reference substance, was supplied by the Group of reference substances (CIDEM, Cuba). Arabic gum and maltodextrin were purchased from Panreac (USA). All other chemicals were of analytical grade.

A. Physical-chemical analysis of the 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, 2012; García *et al*., 2014a). Vitamin A content was determined through a validated high-resolution liquid chromatography method (García *et al*., 2008).

B. Spray drying of shark liver oil pool

Drying tests were performed in a B-191 BUCHI Minispray Dryer **(**Switzerland), with concurrent drying air flow and feed. The following parameters remained

constant: inlet temperature, 150°C; outlet temperature, 90°C; air flow rate, 600 L/h; and drying air flow rate, 60 m^3/h. The effects of gum arabic (GA) and maltodextrin (MD) were evaluated following a mix design at constant oil load (30% w/w). Table 1 shows the experimental matrix. Samples of microencapsulated oil pool were removed in order to determine loss on drying, encapsulation efficiency and yield.

C. Evaluation of the microcapsules

Loss on drying (LD)

Loss on drying was determined according to USP 35 (2012). Samples were weighed using a Sartorius R200D analytical balance (Germany), 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 the Eqs. 1-2, respectively (López *et al*., 2009; Bringas *et al.*, 2011; León *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.

Surface morphology and particles size

Photomicrographs were obtained in a JSM-6060 scanning electron microscope (Japan), with 3000X magnification, in order to more properly assess the morphological appearance of the dried product. The 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 sec.

Infrared Spectrometry (IR)

The following materials and equipment were used: FT/IR-4100 Jasco IR spectrophotometer (Japan); CLAIND CO_2 purifier (Japan); gas generator and a TGS detector. The study was conducted in the range of 650-3800 cm^{-1}.

HPLC vitamin A content determination

Vitamin A content in microparticles with the best characteristics was determined by a validated reversed-phase HPLC analysis, according to conditions described by García *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 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 microparticles in the dissolution medium, 20 mg of microencapsulated oil were taken in glass tubes containing 50 mL of artificial gastric juice (USP 35, 2012), at 37±0.5 ^{0}C for 60 minutes. The procedure was carried out in triplicate.

III. RESULTS AND DISCUSSION

A. Physical-chemical parameters of pool oil

The values obtained in the assessment of physical and chemical parameters demonstrated the quality of the shark liver oil pool, according to established specification limits (García *et al*., 2014a). The results obtained in the indexes of acidity, saponification, unsaponifiable matter and peroxide index show that the shark liver oil pool has adequate quality, with no evidence of possible degradation processes, such as the influence of the oil extraction process. The content of vitamin A and palmitic acid was in the established range.

B. Effect of encapsulating agents on the characteristics of microencapsulated oil

Microencapsulation is a technique where liquid droplets, solid particles or gas compounds, are entrapped in an encapsulating agent. The choice of an encapsulating agent is very important for encapsulation efficiency and microcapsule stability. Due to that one encapsulating agent alone may not have all the required characteristics, a combination of agents may be used (Nogueiro *et al*., 2013). Some carbohydrates (e.g. starch, maltodextrins, dextrose), gums (e.g. gum arabic, acacia gum, alginates, carrageenans), proteins (e.g. milk or whey proteins, gelatine) (Aghbashlo *et al*., 2012) and chitosan have been employed (Gouin, 2004; Krajewska, 2004; Gharsallaoui *et al*., 2007). Gum arabic is historically considered as one of the most important encapsulating agents (Nogueiro *et al*., 2013).

The correct selection of drying excipients is an important step to guarantee the stability and quality of the finished product. Thus, we evaluated the physical-chemical characteristics of microencapsulated oil by using two encapsulating agents, in order to analyze the adequacy of these excipients to generate a product with good properties. The effects of encapsulating agents on the properties of microparticles are presented in Table 1.

When only gum arabic was used to obtain microcapsules, this polymer by itself is capable of adsorbing at the oil/water interface, forming a viscoelastic film around the oil droplet which allows for microencapsulation, having a dual function as polymer and surfactant. On the contrary, maltodextrin alone did not allow for the microencapsulation of oil. However, the combination of both encapsulating agents favored the continuity of the protective film provided by gum arabic around the oil droplet. These results are in agreement with the reports of López *et al.* (2009) and Bringas *et al*. (2011).

Table 1. Experimental matrix and obtained responses

Run	GA (%)	MD (%)	Yield (%)	EE (%)	LD (%)
1	23	47	40.70 ± 1.84	45.65 ± 1.80	4.69 ± 0.13
2	47	23	42.70 ± 8.77	53.17 ± 4.11	3.85 ± 0.35
3	35	35	44.00 ± 5.66	54.95 ± 1.57	3.97 ± 0.27
4	70	0	33.50 ± 2.28	45.30 ± 2.30	4.45 ± 0.33
5	0	70	-	-	-

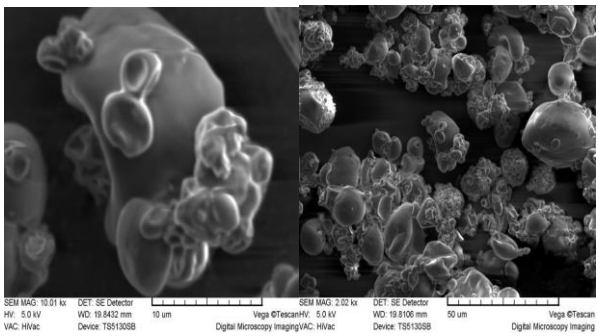

Figure 1. Scanning electron microscopy of microparticles.

Encapsulation efficiency of microencapsulated oil increased slightly as the concentration of gum increased. The optimum conditions for the preparation of microparticles were found to be the mixture of gum arabic at 47% and maltodextrin at 23%. As mentioned previously, a greater concentration of gum ensures a better encapsulation efficiency due to its dual role as polymer and surfactant. This may be explained by the fact that an increase in the gum solution viscosity and concentration prevents oil from leaving the droplet.

On the other hand, the effects of encapsulating agents did not affect the yield of the microencapsulated oil. Low yields were achieved, although those are similar in all variants. 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 other scales had been used.

Although the polymers used are hydrophilic, in all cases, the losses on drying values were below 5%, which ensures the integrity of the microcapsules and encapsulation efficiency in time. As shown (Table 1), when maltodextrin content in the mixture decreased, LD results were lower.

The calculated probability value (p) was lower than 0.05 (p = 0.0060). The factor (percent of maltodextrin and gum arabic) has a statistically significant effect on the encapsulation efficiency response. These results can be explained by the model equation: EE(%) = 44.181*GA + 1.33886*MD + 116.561*GA*MD, with an adequate r^2 value (r^2 = 97.0564). Consequently, the effect of encapsulating agents during the drying process significantly affects the encapsulation efficiency of resulting microparticles in a laboratory scale.

Therefore, to reach a higher encapsulation efficiency and lower moisture content of microencapsulated oil, the combination of gum arabic and maltodextrin should be maintained at 47% and 23%, respectively, according to established manufacturing conditions. This combination is ideal for obtaining microparticles with the smallest moisture content, which allows for a longer shelf life.

On the other hand, the microencapsulation process not only improves the oil's organoleptic characteristics when compared to non-microencapsulated oil, but it should also make it more stable, allowing for a longer shelf life (Yánez *et al.*, 2002; Goud and Park, 2005).

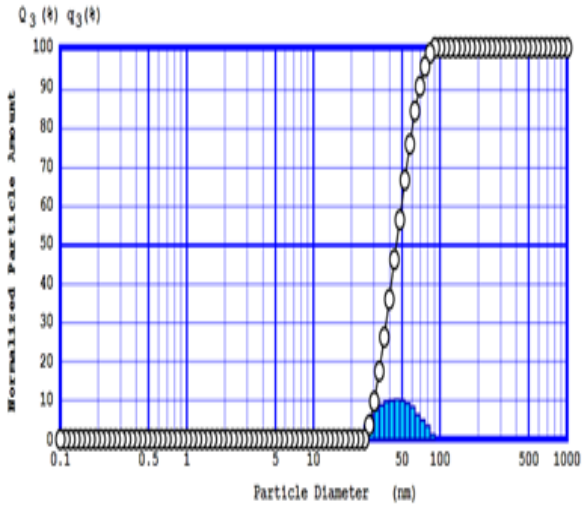

Figure 2. Particle size distribution of microparticles.

C. Surface morphology and particle size

Figure 1 presents typical SEM photomicrographs of the dried product. The 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 the oil content, which is lower than the polymer content (Snahidi and Han, 1993; Hoegger, 2002).

In addition, a smooth surface without the presence of pores is observed, which is essential for the microcapsules stability. The pores facilitate both the entry of oxygen and the exit of the 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 narrow size distribution (45.54 nm), without evidence of agglomeration (Fig. 2). The values are correct, since the maximum light intensity reached a value of 63.29, which is well between the acceptable 50-to-200 range.

D. Infrared Spectrometry

FTIR spectroscopy results are shown in Fig. 3. As observed in the microcapsules, the characteristic bands of fatty acids present in the oil appear minimized, between 2800 and 3000 cm^{-1}, 1600 and 1800 cm^{-1} and around 1400 cm^{-1}, which do not appear in the polymer spectrum. This indicates that the oil remains inside the microcapsule, though masked, and only superficial oil that is not encapsulated can be detected. Therefore, we were able to confirm the feasibility of using spray drying for encapsulation of the shark liver oil pool, by combining gum arabic and maltodextrin as encapsulating agents.

E. Content and vitamin A release

The dried product with the best characteristics was analyzed based on its vitamin A content (Table 2), and also its release rate (Fig. 4).

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

Vitamin A content (µg/g)	
Non-microencapsulated oil	Microencapsulated oil
200.32	199.83
200.51	200.27
200.42	200.34
200.84	200.16
200.62	200.83
200.53	200.74
X = 200.54	X = 200.36
SD = 0.1789	SD = 0.3727

t-Student calculated = 1.056
t tabulated (11; 0.05) = 2.20

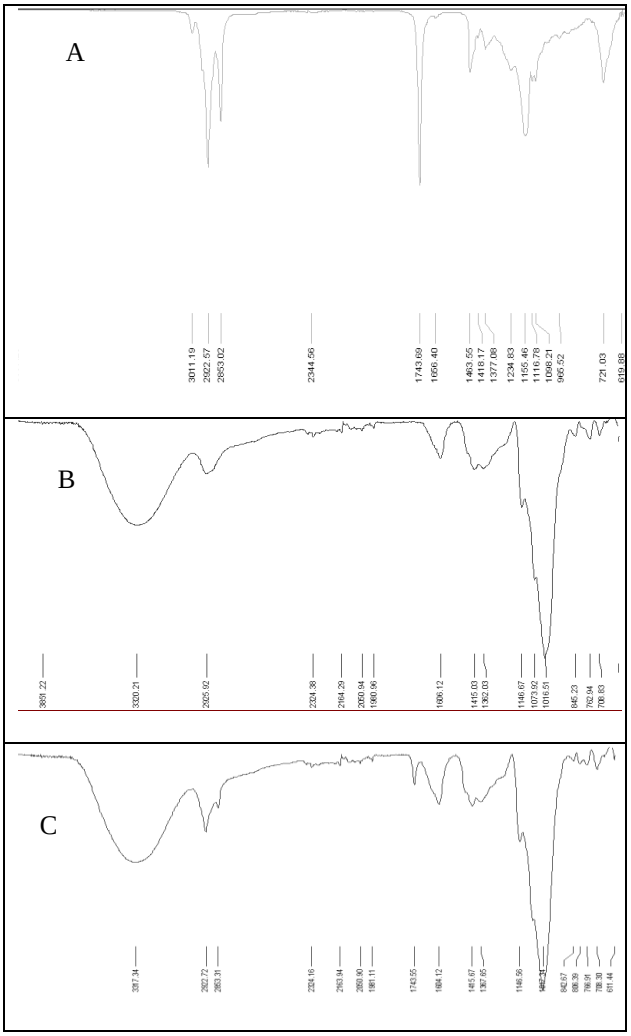

Figure 3. FTIR spectra of non-microencapsulated shark liver oil (A), encapsulating agents (B) and microencapsulated oil (C).

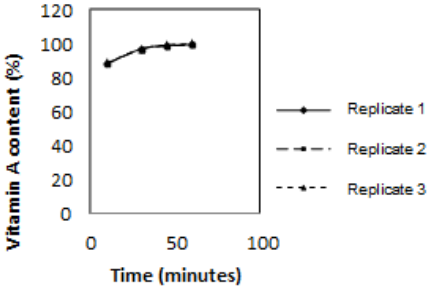

Figure 4. Vitamin A release from microencapsulated shark liver oil pool.

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. Further work would be needed to study the stability of microencapsulated shark liver oil.

Figure 4 shows the influence of encapsulating agents on the vitamin A release behavior from microencapsulated oil. The release rate of the encapsulated product was rapid, and more than 85% of its vitamin A content 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 used. Therefore, the release behavior for vitamin A was not affected by the viscosity of the gum solution.

IV. CONCLUSIONS

In order to microencapsulate shark liver oil with the best encapsulation efficiency and moisture content for its conservation, the combination of gum arabic and maltodextrin, as encapsulation agents, should be maintained at 47% and 23%, respectively, according to the established manufacturing conditions, allowing for a longer shelf life. Besides, this procedure solves the problem posed by the smell and taste of non-microencapsulated oil. Results indicate that the combination of encapsulation agents does not have a significant effect on vitamin A content or its release from the dried product.

This work showed that the shark liver oil pool was successfully encapsulated, opening new possibilities for its use as a natural active pharmaceutical ingredient.

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