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Spray freezing drying microencapsulation of krill oil enhances digestion and storage stability

La microencapsulación de aceite de krill mediante secado por aspersión en frío mejora su digestión y estabilidad de almacenamiento

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Abstract

This study explores microencapsulation of krill oil (KO) by Spray-Freezing-Drying (SFD) as an effective process to protect KO from degradation due to environmental factors. Gum arabic (GA) and whey protein concentrate (WPC) were employed as wall materials and the resulting KO microcapsules were characterized in terms of color, astaxanthin content, water activity, antioxidant activity, microencapsulation efficiency and morphology. The bioaccessibility of both types of KO microcapsules was evaluated (by *in vitro* digestion), thermodynamic parameters were calculated through a moisture adsorption study for the prediction of the maximum stability of the microcapsules, and the kinetic stability of the astaxanthin content during the storage of the microcapsules was analyzed. The encapsulation efficiency of KO was around 96% in both treatments; however, a less porous structure and a slightly higher astaxanthin content were found in the microcapsules made with WPC. A significant increase in the bioaccessibility values of the microencapsulated KO compared to the free KO was found, however, no significant differences were found between the bioaccessibility, and antioxidant activity values obtained for both types of KO microcapsules. The moisture content of the monolayer was relatively low for both samples. It was found that the maximum stability occurred in the zone of minimum integral entropy with water activity values of 0.26 and 0.14 for KO microencapsulated with WPC and GA, respectively; this is confirmed by studying the effect of water activity on the degradation of astaxanthin content in both types of microcapsules stored at 35 °C.

Keywords: Microencapsulation, Spray-freeze-drying, Thermodynamic properties, Storage stability, Bioaccessibility.

Resumen

Este estudio explora la microencapsulación de aceite de krill (KO) mediante secado por aspersión en frío (SFD) como un proceso eficaz para proteger el KO de la degradación debida a factores ambientales. Se emplearon goma arábiga (GA) y concentrado de proteína de suero de leche (WPC) como materiales de pared y las microcápsulas de KO resultantes se caracterizaron en términos de color, contenido de astaxantina, actividad de agua, actividad antioxidante, eficiencia de microencapsulación y morfología. Se evaluó la bioaccesibilidad de ambos tipos de microcápsulas de KO (mediante digestión *in vitro*), se calcularon parámetros termodinámicos mediante un estudio de adsorción de humedad para la predicción de la máxima estabilidad de las microcápsulas, y se analizó la estabilidad cinética del contenido de astaxantina durante el almacenamiento de las microcápsulas. La eficiencia de encapsulación de KO resultó alrededor del 96% en ambos tratamientos; sin embargo, se encontró una estructura menos porosa y un contenido de astaxantina ligeramente mayor en las microcápsulas elaboradas con WPC. Se encontró un aumento significativo en los valores de bioaccesibilidad del KO microencapsulado en comparación con el KO libre, sin embargo, no se encontraron diferencias significativas entre los valores de bioaccesibilidad y actividad antioxidante obtenidos para ambos tipos de microcápsulas de KO. El contenido de humedad de la monocapa fue relativamente bajo para ambas muestras. Se estableció que la máxima estabilidad se presentó en la zona de mínima entropía integral con valores de actividad de agua de 0.26 y 0.14 para KO microencapsulado con WPC y GA, respectivamente; esto se confirmó mediante el estudio del efecto de la actividad del agua sobre la degradación del contenido de astaxantina en ambos tipos de microcápsulas almacenadas a 35 °C.

Palabras clave: Microencapsulación, Secado por aspersión en frío, Propiedades termodinámicas, Estabilidad en almacenamiento, Bioaccesibilidad.

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1 Introduction

In the last decade, krill oil (KO) has been investigated due to its important fatty acid composition and antioxidant power, which help improve human health when consumed. Astaxanthin (a well-documented powerful antioxidant) is the main biomolecule found in KO and it is the responsible for its intense red color, besides that an important amount of essential fatty acids (like eicosapentaenoic and docosahexaenoic acids) can also be found in its composition (Ahn *et al*., 2018; Köhler *et al*., 2015; Kwantes and Grundmann, 2015; Xie *et al*., 2019).

Due to the highly sensitive antioxidants and lipids present in KO, several detrimental reactions take place once it is exposed to environmental conditions; its nature (as a pure fat food product) makes it difficult for the human body to absorb. Since the bioaccessibility of molecules is related to the amount of them that can be solubilized in the gastrointestinal environment and accessible for their absorption in the small intestine, and it depends on the nature of compounds, fat-soluble components must be incorporated into mixed micelles before absorption, so lipophilic molecules are less available to be absorbed due to its nature (Granado-Lorencio *et al*., 2007).

One solution to those problems is using methods and techniques that help extend its properties along with improve solubilization in water-rich environments so that people can profit from its nutritional advantages. Microencapsulation can protect functional compounds, such as krill oil, by covering them with a layer (wall material) that makes it difficult for certain compounds (e.g., oxygen) to interact with the bioactive ingredient (core material). Thus, functional compounds are protected during storage and could be easily incorporated into micelles in the gastrointestinal system. In fact, it has been reported that microencapsulation systems containing w3 fatty acids can be used as suitable technology to produce functional foods (Bakry *et al*., 2016).

Microencapsulation can be done by different techniques, such as emulsion, inclusion encapsulation, extrusion, spray-drying (SD), freeze-drying (FD), etc., of which SD and FD are the most used techniques to produce microencapsulated products. However, the high temperatures during SD cause a loss of bioactive compounds that can be considerable if the process parameters are not adequately controlled. On the other hand, FD does not imply high temperatures but high operating costs due to the low temperatures, high vacuum, and long residence times required. It has been reported that the residence time during FD can be significantly reduced by improving the diffusion of water through the solid by decreasing the particle size, thus accelerating the process, and reducing costs (Dolly *et al*., 2011). A novel microencapsulation method that allows such particle size reduction is spray-freeze-drying (SFD); in the first phase of the process (called spray freezing into cryogenic liquid), a solution or emulsion is atomized into droplets, and their solidification takes place immediately due the contact with a freezing liquid (e.g., liquid nitrogen). After that, solid particles are freeze-dried until a dry powder is obtained. The main advantages of the SFD are that the process does not need elevated temperatures, so thermosensitive molecules can be well protected (Flores-Andrade *et al*., 2019), and residence times are shorter than FD. It has been reported in different studies that the use of SFD has many advantages with a substantial impact on the final quality of the products (Dutta *et al*., 2018).

Considering the increase in demand for functional foods, it has been proposed that future studies on microencapsulation of functional lipids focus on improving their stability during storage and in the human body by carrying out *in vitro* and in vivo studies (Delshadi *et al*., 2020); for that reason, it is necessary to study the impact of SFD microencapsulation on the digestibility and storage stability of KO. The study of the digestibility of functional oils is generally achieved using standardized *in vitro* digestion models (Venugopalan *et al*., 2021). Besides to evaluate storage stability, it is necessary to study microcapsules behavior when they are exposed to different atmospheres since water plays a vital role during storage. The construction of moisture adsorption isotherms (WSI) and the thermodynamic analysis of the data provide consistent scientific criteria that help to predict storage conditions that impact the shelf life of dry powdered foods. A WSI thermodynamic analysis involves differential and integral enthalpy (ΔH) and entropy (ΔS) analysis along with Gibbs free energy (ΔG); ΔG is a quantitative measure of the sorbent affinity and indicates the degree of spontaneity during the adsorption process (Arslan-Tontul, 2021), which means the degree of hygroscopicity of the food sample. The differential enthalpy or isosteric heat of sorption indicates the strength of water molecules adsorbed by solid particles (Azhar *et al*., 2021), as it reflects the energies of the water molecules bound at a certain hydration point. Several authors have proved that the point where integral entropy finds its minimum ($\Delta S_{\min}$) could be assumed as the best water activity (a_w) for preserving food, and that occurs because the water-food bond is the strongest one (Acosta-Domínguez *et al*., 2021; Cano-Higuita *et al*., 2015; Escalona-García *et al*., 2016; Osorio-Tellez *et al*., 2021; Velázquez-Gutiérrez *et al*., 2021).

Novelty impact: The need for new products rich in omega-3 oils and antioxidant compounds has increased due to the increasing incidence

of cardiovascular diseases. However, lipids and antioxidants are prone to oxidation by environmental conditions; therefore, microencapsulation has been employed for decades to produce more chemically stable oils and antioxidants. However, conventional microencapsulation techniques have disadvantages such as high temperatures or high energy expenditure. Therefore, it is necessary to study new and alternative microencapsulation techniques with low-temperature processes, such as Spray-Freeze-Drying, to protect bioactive compounds from oxidation. SFD is a novel microencapsulation technique, and its potential is still being explored in the pharmaceutical and food industries. Little published information exists about the digestibility and stability of microcapsules obtained by SFD, and it is possible that this technique can improve the bioaccessibility and stability of Krill oil. In this sense, this study aims to explore using the spray freeze-drying microencapsulation technique to protect KO and evaluate its effect on the storage stability and bioaccessibility of microcapsules.

2 Materials and methods

2.1 *Materials and chemical reagents*

Krill oil was purchased from Natura Extracta (Guadalajara, Jalisco, México), gum arabic (GA) and whey protein concentrate (WPC) were acquired from Droguería Cosmopólita (México City, México), ethyl acetate, HCl, petroleum ether, LiCl, $MgCl_2$, $Mg(NO_3)_2$, NaCl, KCl and BHT were of analytical grade. Pepsin, pancreatine and porcine bile extract were from Sigma Chemical Co. (St. Louis, USA).

2.2 *Preparation of emulsions and spray freezing drying*

For emulsions preparation aqueous solutions of GA and WPC were made with the help of a homogenizer working at 5000 rpm for 10 min (both solutions had a concentration of 3 g solids per 10 mL distilled water); once complete solute dissolution was achieved, and in order to prepare oil-in-water emulsions, KO was added (at a ratio of 1:4 KO:GA or WPC) dropwise and homogenized at the conditions stated above. A Büchi Mini Spray Dryer 290 (B290, Flawil, Switzerland) was employed to produce KO microcapsules. In the equipment, the glass equipment was removed, and heating was switched off, then a container with 1.5 L of liquid nitrogen was placed at the height of 45 cm from the nozzle (4 x 10-4 m) and the feed rate of the equipment was set at 10 mL/min with an atomization pressure of the air gas of 543.80 kg/m2. The frozen microcapsules produced were rapidly recovered and freeze-dried at a temperature of -84°C with a vacuum pressure of 0.034 mbar for 72 h (Labconco freeze dryer 4.5 Plus, Kansas, USA). Finally, dried microcapsules were recovered from the particle vessels and placed in dark bottles until employed for further analysis.

2.3 *Microencapsulation efficiency (ME)*

To assess microencapsulation efficiency (ME), 1.5 g of KO microcapsules were mixed with 27 mL of petroleum ether, a handshake for 10 min, and then filtered; the filtrate was recovered and evaporated to dryness in a rotavapor at 60 °C. The oil at the microcapsule's surface (w_f) was calculated by gravimetry. Total oil (w_t) present in KO microcapsules was determined using the procedure described above right after the disintegration of microparticles, for which a 1:10 microcapsules: distilled water ratio was employed. wt was obtained gravimetrically, and all the values were substituted in Eq. (1) for ME calculation (Shi *et al*., 2018).

$$ME(g/100g) = \frac{w_t - w_f}{w_t} \times 100 \tag{1}$$

2.4 *Astaxanthin content*

The astaxanthin concentration was determined by the spectrophotometric method (Carvalho Lago and Noreña, 2015; Vakarelova *et al*., 2017) for which aliquots of the emulsion (2 g) and of the KO microcapsules (1 g) were added separately to 10 mL of ethyl acetate and then handshake during 10 min, mixture was subjected to vortex at 5000 rpm for 10 min for solids separation. The supernatant was recovered and placed in the quartz cell, which was introduced into the spectrophotometer (Thermo Scientific) and read its absorbance at 480 nm (A). Astaxanthin concentration was determined through the Eq. (2):

$$\text{Astaxanthin concentration}\left(\frac{\mu g)}{g}\right) = \frac{A * V_t(mL) * 10^4}{A_{(1\%,1cm)} * P(g)} \tag{2}$$

Where V_t = Total extract volume; P = sample weight; $A_{(1\%,1cm)}$ =2150 (astaxanthin specific absorbance in ethyl acetate).

2.5 *Antioxidant activity*

The antioxidant activity (AA) was determined using $ABTS^+$ assay. First, a 7 mM ABTS solution was prepared from ABTS (Sigma-Aldrich), and a 2.45 mM potassium persulfate solution. Subsequently, these solutions were mixed in a ratio of 0.5:1 (persulfate solution: ABTS solution), allowing them to stand for 16 hours in the dark. The $ABTS^+$ stock solution was found to be diluted with ethanol to an absorbance

of 0.70 (± 0.02) at 734 nm. A calibration curve was made with different concentrations of Trolox, starting from a concentration of 1.5 mM. 30 μL of Trolox were taken, and 3 mL of the $ABTS^+$ solution was added. After standing for 6 minutes, absorbance readings were taken on a spectrophotometer (Thermo Scientific) against an ethanol blank at 734 nm. To measure the antioxidant activity of the samples, one gram of these was dissolved in 10 mL of ethanol; the resulting mixtures were centrifuged at 3000 rpm for 10 min. 30 μL of supernatant were added to 3 mL of the $ABTS^+$ solution was left to settle for 6 minutes at room temperature, and different absorbance readings were carried out against an ethanol blank at 734 nm. Absorbance values were compared to the reference curve constructed with Trolox, and the results are reported as mMTE/g (mM Trolox equivalents/gram of sample).

2.6 Color

Chroma (Ch) and hue angle (h) were calculated with equations (3) and (4) using CIELab values obtained from a colorimeter (Konica Minolta, INC B8210364, Japan). Brightness of the sample is represented by L, a* represents colors in range of red (+) and green (-) and b* values in the range of yellow (+) and blue (-) (Jimenez-Fernandez *et al*., 2021).

$$Ch = \sqrt{a^{*2} + b^{*2}} \tag{3}$$

$$h = \tan^{-1}\frac{b^*}{a^*} \tag{4}$$

2.7 Particle size and morphology

The particle size of the samples was determined randomly using optical microscopy (Cole-Parmer 48923-40) and the Motic Image Plus 2.0 software. Three hundred fields were taken, and the particle size of the samples was determined randomly. Scanning electron microscopy (SEM microscope Jeol model JSM-5600lv) was employed for microcapsule structure visualization. First, KO microcapsules were coated with gold-palladium and then examined at 15 kV (Jimenez-Fernandez *et al*., 2021).

2.8 In vitro bioaccessibility

In vitro bioaccessibility of krill oil and KO microcapsules was evaluated under simulated gastrointestinal conditions; first, one gram of the sample was mixed for 10 min with a basal saline solution made of 5 mM KCl, 150 mM BHT, and 140 mM NaCl, then the mixture was acidified till pH 2 with 1.0 M HCl; once an acid medium was achieved, a simulated gastric fluid made of 3.2 g/L pepsin in 1 M HCI was added and set to incubation at 37 °C during one hour; at the end of that process pH of the mixture was adjusted to 7.5 using 1.0 M NaOH and, simulated intestinal fluid made of 4.76 mg/mL pancreatin and 5.16 mg/mL porcine bile extract in phosphate buffer solution (pH 7.5) was added. During two h the liters (V) of NaOH 0.1 M needed for maintaining the pH of the mixture at 7.5 was recorded and employed for bioaccessibility calculation using equation five (El-Messery *et al*., 2020).

$$\text{Bioaccessibility } (\%) = \frac{VM_w}{W_{lipid}} \times 10 \tag{5}$$

M_w represents the molecular weight of the lipid and W_{lipid} is the total lipid content in the sample.

2.9 Water vapor sorption isotherm

One gram of each sample was placed in triplicate vacuum desiccators (13 kPa) containing P_2O_5 for approximately 15 days. After that time, the samples were placed in microclimates prepared at 35 °C with saturated saline solutions (a_w from 0.11 to 0.85).

When the difference between two consecutive weightings was less than 1 mg/g of solids samples, equilibrium was assumed. The Guggenheim-Anderson-de Boer (GAB) equation (Equation 6) was employed for data modeling (Weisser, 1985).

$$M = \frac{M_0 C k a_w}{(1 - k a_w)(1 - k a_w + C k a_w)} \tag{6}$$

Where M, a_w and M_0 represent the moisture content of the sample, water activity and the monolayer moisture content respectively; C and k are constants related to the properties of multilayer molecules and bulk liquid.

The mean relative deviation (SD) modulus (Equation 7) was used to determine a good fit of the equation to the experimental data.

$$E\% = \frac{100}{n}\sum_{i=1}^{N}\frac{|M_i - M_p i|}{M_i} \tag{7}$$

M_i is the observed moisture content at a certain point and, M_{pi} stands for the predicted moisture content at the same level, and n is the quantity of observations taken. If E<10 % it is generally assumed a very good fit.

2.10 Determination of thermodynamic parameters differential and integral thermodynamic properties

ΔG was calculated employing Gibbs' formula (equation 8) (Iglesias *et al*., 2022)

$$\Delta G = RT \ln a_w \tag{8}$$

where R (J/mol K) is the universal gas constant; T is the absolute sorption isotherm temperature, and water activity is represented by a_w.

2.10.1 *Differential properties*

The changes of the differential enthalpy during the adsorption process was calculated with Othmer´s equation (9).

$$\frac{d\ln P_v}{d\ln P_v^0} = \frac{(H_v)_T}{(H_v^0)_T} \tag{9}$$

Where P_v (Pa) is the vapor pressure of water over the wall material; P_v^0 (Pa) is the vapor pressure of pure water at the temperature of sorption; $(H_v)_T$ (J/mol) is the differential molar enthalpy of sorption, and $(H_v^0)_T$ (J/mol) is the enthalpy of condensation of pure water. If equation 9 is integrated at a constant moisture (M, g water/100 g of dry solids) equation 10 is obtained and CI (adsorption constant) is added:

$$\ln P_v = \left(\frac{H_v(T)}{H_v^0(T)}\right)_M \ln P_v^0 + CI \tag{10}$$

Equation 11 helps to calculate the net molar differential enthalpy:

$$(\Delta H_{dif})_T = \left(\frac{H_v(T)}{H_v^0(T)} - 1\right)_M H_v^0(T) \tag{11}$$

$(\Delta H_{dif})_T$ was estimated at different temperatures employing steam tables by calculating $H_v(T)/H_v^0(T)$ with Equation 10 and its subsequent substitution in Equation 11. And the molar differential entropy was estimated with the values obtained for enthalpy changes using Eq. 12.

$$(\Delta S_{dif})_T = \frac{-(\Delta H_{dif})_T - RT\ln a_w}{T} \tag{12}$$

2.10.2 *Integral properties*

The net molar integral enthalpy of krill oil microcapsules $(\Delta H_{int})_T$ was calculated using an analogous equation to differential enthalpy (Eq. 13), at a constant diffusion pressure (φ):

$$(\Delta H_{int})_T = \left(\frac{H_{vi}(T)}{H_v^0(T)} - 1\right)_M H_v^0(T) \tag{13}$$

The molar integral enthalpy of water adsorbed in food and the molar integral enthalpy of condensation of pure water are represented by $H_{vi}(T)$ and $H_v^0(T)$ respectively (both enthalpies have the same units of J/mol). The diffusion pressure (φ) can be calculated using Eq. 14:

$$\varphi = RT\frac{W_{ap}}{W_v}\int_0^{a_w} M d\ln a_w \tag{14}$$

Where W_{ap} and W_v are the molecular weight of the adsorbent (g/mol) and molecular weight of water (g/mol), respectively. Once the values for $(\Delta H_{int})_T$ are obtained, molar integral entropy changes can be calculated using Eq. (15) (Nunes and Rotstein, 1991).

$$(\Delta S_{int})_T = \frac{-(\Delta H_{int})_T - RT\ln a_w}{T} \tag{15}$$

2.11 *Storage stability*

To evaluate the effect of different water activities (0.108, 0.318, 0.515, and 0.743) on KO microcapsules, saturated solutions of LiCl, $MgCl_2$, $Mg(NO_3)_2$, and NaCl were prepared and then placed in desiccators. The temperature was set at 35 °C, and once the equilibrium was reached, fresh KO microcapsules (zero-time samples) were introduced. Three samples of each a_w were withdrawn every 5 days over 35 days for astaxanthin concentration determination.

2.12 *Degradation reaction rate calculation*

For each sample of KO microcapsules and all water activities, the degradation rate or the change in the amount of astaxanthin (k_v) was related to treatment time in days (t) and expressed as a percentage. According to the literature, the system tested followed zero and first-order kinetics; therefore, the best correlation coefficient (R) result was selected.

2.13 *Statistical analysis*

Analysis of variance (one-way ANOVA) was employed, and differences between pairs of means were assessed based on confidence intervals using the Tukey test with a significance level of 0.05.

3 Results

3.1 *Characterization of SFD microcapsules*

3.1.1 *Particle size and morphology*

During the SFD microencapsulation process, droplets of both krill oil emulsions (WPC and GA) are atomized and immediately frozen by immersion in liquid nitrogen; this process retains the size and shape of the particles. The different krill oil microcapsules exhibited monomodal size distributions and a similar particle size, 126.96 μm and 122.70 μm for the microcapsules made with WPC and GA, respectively. Similar particle sizes have been reported by other authors during microencapsulation by SFD, Parthasarathi and Anandharamakrishna (2016) reported 145.3 μm for vitamin E microencapsulated in whey protein, Rajam and Anandharamakrishnan (2015) reported 105.07 μm for L. plantarum encapsulated in denatured whey protein isolate and Hundre *et al.* (2015) reported 120.1 μm for vanillin encapsulated in β-cyclodextrin; however, in the same studies, the authors reported smaller particle sizes when using other wall material combinations.

Table 1. Effect of wall material on microencapsulation efficiency, astaxanthin content, antioxidant activity, a_w and color in Krill oil microcapsules.

Wall material	ME (%)	AST (μg/g)	AA (mMTE/g)	a_w	Color		
					L	C	h (°)
WPC	96.907 ± 2.440^a	55.004 ± 0.048^a	0.432 ± 0.001^a	0.10	91.146 ± 0.041^a	13.238 ± 0.114^a	45.815 ± 0.150^a
GA	96.772 ± 2.819^a	54.175 ± 0.069^b	0.432 ± 0.001^a	0.13	87.053 ± 0.015^b	22.282 ± 0.031^b	56.298 ± 0.116^b

Values are expressed as mean ± standard error (n = 3). Different letters (a-d) in the same column indicate significant difference between values in the same column p <0.05. ME, AST and AA refer to microencapsulation efficiency, astaxanthin concentration and antioxidant activity respectively.

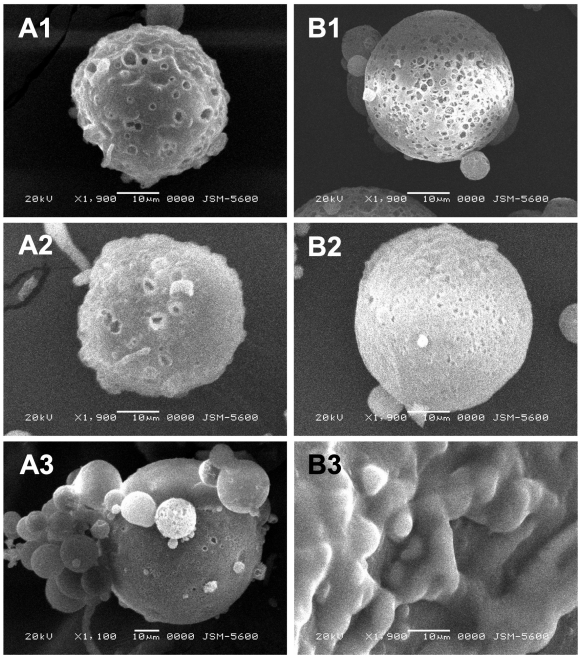

Figure 1. SEM images of spray-freeze-dried microcapsules containing krill oil produced with (A) GA and (B) WPC and stored at 35 °C and 1) a_w = 0.108, 2) a_w = 0.318 and 3) a_w = 0.743.

According to the authors, the particle size of the microcapsules could be affected by the type of wall material used due to the changes in the viscosity of the emulsions since the feeding solutions with higher viscosity form larger droplets during the stage of atomization.

On the other hand, the external structure of the SFD microcapsules made with WPC and GA are shown in Figure 1 (A1 and B1, respectively). Both types of microcapsules showed spherical shapes; however, the different wall materials used in their formulation produced differences in the surface of the spheres. The morphological differences in the surface of SFD microcapsules could be related to the differences in the film-forming properties of the wall materials used for encapsulation (Rajam and Anandharamakrishnan, 2015). The microcapsules made with GA showed a rougher surface with a more significant number of tiny micropores due to the sublimation of ice crystals during secondary drying. This numerous fine pores on the surface of the spherical particles produced by SFD could enhance the rehydration properties of the microcapsules (Karthik and Anandharamakrishnan, 2013), Meanwhile, microcapsules made of WPC have fewer micropores and a smoother outer surface due to the typical film-forming property of whey protein. Similar structures have been reported in several studies in which whey protein isolate (WPI) was used as the wall material during SFD encapsulation (Ishwarya *et al*., 2015). In addition, this less porous structure of microcapsules made with WPC could be beneficial since it has been reported that this type of structure improves the mechanical resistance of the particles during transport and reduces the risk of oxidation of the particles (Elik *et al*., 2021).

3.1.2 Encapsulation efficiency

The effect of the wall material on krill oil microcapsules properties is summarized in Table 1; as can be seen, SFD leads to microencapsulation efficiencies of around 96 % in both treatments; those values reflect the amount of KO that is protected by the wall material and less exposed to the environmental conditions and consequently influence the storage stability of the microcapsules. The high encapsulation efficiency achieved in this study may be because, during the formation of the different emulsions, the proteins present in the wall materials are adsorbed at the interface, where they most likely interact with the phospholipids of the KO, forming a continuous and homogeneous film around the KO droplets due to their properties helping to stabilize the micelles. Therefore, the KO is easily trapped by the wall materials during the formation of the microcapsules in the first stage of the SFD, which leads to a high ME value. Similar ME values have been reported in studies using protein-rich wall materials and SFD microencapsulation technique; for example ME values of 87-95 % have been reported for *L. plantarum* microcapsules using whey protein isolate (WPI) as wall material (Rajam and Anandharamakrishnan, 2015), a ME value of 89.3 % for vitamin E encapsulated in whey protein (Parthasarathi and Anandharamakrishnan, 2016), and a ME value of 90.8% for fish oil encapsulated with GA (Pang *et al*., 2017).

On the other hand, lower values of ME have been reported with other microencapsulations techniques; values of 59-85.67% have been founded in fish oil

microencapsulated by FD and SD techniques using WPC and WPI as wall materials (Aghbashlo *et al*., 2013). Besides, an ME value of 59.63% was reported for flaxseed oil microencapsulated by FD using GA as wall material (Tonon *et al*., 2012). ME values of 70.73% to 90.37% were found for sesame oil microencapsulation by SD using mixtures of mesquite gum and maltodextrin as wall material (Fuentes-Ortega *et al*., 2017). Additionally, Hinnenkamp *et al*. (2021) reported a ME of 94.2 % during the SD process of fish oil when a mixture of WPC and maltodextrin was employed as wall material, Bakry *et al*. (2019) employed complex coacervation for microencapsulation of tuna oil using myofibrillar protein extracted from *Ctenopharyngodon idellus* as wall material and found a ME of 91.1 %. Meanwhile, Plazola-Jacinto *et al*. (2019) reported ME values from 48.7 to 55.42 % for avocado leaves oily extracts microencapsulated by a spray dried using a mix of GA and maltodextrin as wall material, also an ME value of 83 % was obtained for a microencapsulation of AST by SD utilizing a mixture of carrageenan, chitosan and lupin protein isolate as wall material (Morales *et al*., 2021). It is clear that ME is affected by the type of wall material and the microencapsulation method used; when comparing the ME values obtained in this study with those published for functional oils microencapsulation, it is noted that the SFD process combined with the use of protein-rich wall materials allows high oil encapsulation efficiencies. It has been reported that the food industry aims to produce microcapsules with surface oil less than 2% (w/w) and encapsulation efficiency greater than 98% (w/w) (Gordon *et al*., 2018); therefore, the development of KO microcapsules in this study could be suitable for the food industry.

3.1.3 Water activity (a_w)

In general terms, a_w is one of the most critical parameters in dried food products; indicate the amount of water that is not bound to molecules, generating quality deterioration and changes in the physical properties of microcapsules (Borrás-Enríquez *et al*., 2023), a_w of microcapsules depends upon wall materials and drying conditions, and values under 0.4 guarantee stability against browning, hydrolitical reactions, lipid oxidation, auto-oxidation, and enzymatic activity (Caliskan and Nur Dirim, 2013). In the present study, low values of a_w are founded (Table 1); similar values have been reported by Bssijeh *et al*. (2020) in freeze-dried astaxanthin microcapsules prepared with WPI, by Zhu *et al*. (2021) in freeze-dried carotenoids and fish oil co-microcapsules prepared with WPI, and by Koc *et al*. (2022) in ultrasonic SFD transglutaminase microcapsules prepared with inulin and gum arabic.

Due to low a_w values found in both KO microcapsules they can be accepted as microbiologically and oxidatively stable. However, the shelf life of microcapsules depends on their moisture sorption characteristics and storage conditions, which are discussed in section 3.2.

3.1.4 Antioxidant activity and astaxanthin content

On the other hand, antioxidants are molecules that can inhibit the oxidation of others, they can scavenge free radicals protecting the human organism, so they may help to retard the progress of many chronic diseases. The antioxidant activity of KO microcapsules is mainly due to their astaxanthin content and, to a lesser extent, their omega-3 fatty acid content. As can be seen in table 1, TEAC values and astaxanthin concentration for both KO microcapsules were the same, which was expected due to the high encapsulation efficiency achieved. The slight astaxanthin concentration variations could be attributed to the minor differences associated with the nature of pure KO.

Similar values of antioxidant activity have been reported by Foo *et al*. (2020) in freeze-dried fucoxanthin microcapsules prepared with maltodextrin/GA as wall material (45 mMTE/g) and Xu *et al*. (2021), who prepared KO emulsions by ultrasound using a mix of chickpea protein/Ginseng saponin powders as wall material (0.3 mMTE/L). Astaxanthin values of both kinds of KO microcapsules are higher than those reported by Haq and Chun (2018), who encapsulated astaxanthin-rich salmon oil using particles from gas saturated solutions process (40 μg/g) and Montero *et al*. (2016) in AST extracted from shrimp waste and encapsulated by spray drying using GA as wall material (3 μg/g).

3.1.5 In-vitro bioaccessibility of KO microcapsules

Microencapsulation can help deliver a bioactive compound to specific parts of the gastrointestinal tract, so it is essential to estimate the amount of KO released at the end of human digestion by analyzing its bioaccessibility. This term represents the proportion of digest that is available for uptake by the intestinal absorptive cells, and it could be employed as a predictor of bioavailability (Grace *et al*., 2022). KO absorption involves hydrolysis of the triacylglycerides and phospholipids in the lumen of the intestine, followed by absorption of the hydrolyzed products by enterocytes. However, the amount of fatty acids that can be solubilized in the gastrointestinal environment and be accessible for absorption in the small intestine is limited by the lipophilic nature of these compounds since they must be incorporated into mixed micelles before absorption (Granado-Lorencio *et al*., 2007). Figure 2 shows the intestinal digestion kinetics of KO microcapsules and KO not microencapsulated.

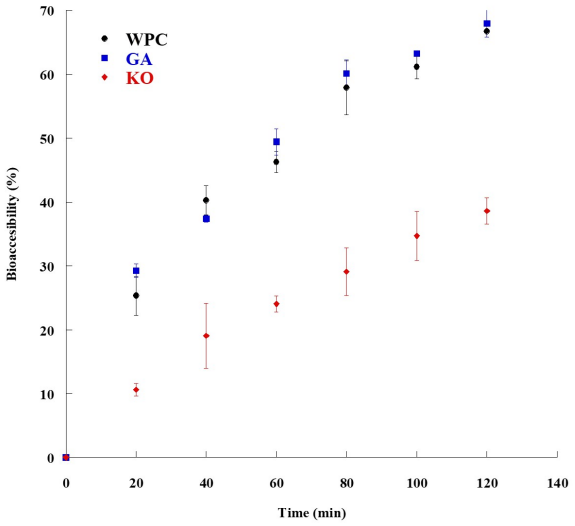

Figure 2. *In vitro* bioaccessibility of krill oil microcapsules.

It can be seen that SFD microencapsulation allows a considerable increase in the bioaccessibility of KO fatty acids compared to the digestion of KO that is not microencapsulated. The improvement in bioaccessibility shown by both microcapsules may be due to the emulsification properties of wall materials that increase the incorporation of fatty acids into pancreatic lipases during digestion since it has been reported that the rate and extent of catalytic activity of pancreatic lipase are controlled by the composition and relative surface area of the substrate interface (Joyce *et al*., 2018). During interaction with an interface, the lipase undergoes a conformational change, exposing its catalytic domains to the substrate and increasing the amount of fatty acids incorporated into micelles. So, KO emulsification caused by wall materials facilitates higher enzymatic activity due to a larger surface area of the lipid-in-water interface.

Additionally, at the end of the 120 min of *in vitro* small intestine digestion, the bioaccessibility of both KO microcapsules was the same ($p<0.05$). Similar bioaccessibility values (66.6 - 69.6 %) were reported by Fu *et al*. (2021) for KO microcapsules produced by FD using yeast cell microcarriers as wall material. They attribute the results to the influence of the digested particles on the solubilization capacity of the mixed micellar phase. Also, they argue that the phospholipids from KO and the products released after the microcapsule's digestion can intensify the solubilization capacity during the micellar phase by multiplying the number of nonpolar sites available to attract hydrophobic molecules. Moreover, Grace *et al*. (2022) found that wall material influences the bioaccessibility of carotenoids microencapsulated with different methods (Spray-Drying vs Freeze-Drying). They reported that WPI showed higher values (56.6%) than soy protein isolated (53.1%); according to their theory, animal proteins are easily digestible because they have smaller molecular size, more flexible structures, and the ability to dissociate in an aqueous phase when comparing to vegetable proteins which have larger molecular sizes, inflexible geometries, and hydrophobic amino acids on the surface, so there is a decrease in solubility, a rise in viscosity, and a tendency for aggregation and precipitation. Furthermore, Wang *et al*. (2022) reported values of 45 % for astaxanthin bioaccessibility for KO emulsions prepared with WPI; according to them, during the digestion process, astaxanthin might be trapped into the micellar phase and transported to cells, so the barrier material has a certain degree of influence on its bioaccessibility. In this sense, Rahmani-Manglano *et al*. (2022) studied the effect of the emulsified type (whey protein concentrate hydrolysate and Tween 20) on the *in vitro* digestion of fish oil microcapsules obtained by Spray-Drying, they found that all different microcapsules showed the same digestion profile, but only those prepared with whey protein concentrate hydrolysate (WPCH) showed the highest amount of free fatty acids released, they argue that during the intestinal process bile salts moved proteins in the wall material from the oil in water boundary leading to a better re-emulsification compared to Tween 20 microcapsules.

Based on the results obtained in this study, it can be assumed that the GA and WPC microcapsules produced by SFD could act as an efficient barrier to promote a controlled release of KO during digestion and allow good re-emulsification of the KO, facilitating higher enzymatic activity, thereby improving its bioaccessibility.

3.2 Prediction of suitable storage conditions for SFD microcapsules

Microencapsulation protects a bioactive compound from adverse factors during storage, thus prolonging its shelf life. To determine the ideal storage conditions for the microcapsules, a thermodynamic study of moisture adsorption was carried out.

As expected for an adsorption process in dry foods, the equilibrium moisture content of KO microcapsules increased as it did. It is well known that the adsorption process is exothermic. Therefore, the moisture gained was higher at lower temperatures. A crossover at values above 0.7 was observed in all isotherms from both kinds of KO microcapsules (Figures 3 and 4), which is essentially due to a rise in the solubility of sugars and solute dissolution; besides that, at high values, the soluble components in the food material tend to gain more water, which is further accentuated by the temperature (Paul and Das, 2019).

GAB's equation constants (M_0, C, and K) obtained by the mathematical modeling of experimental points are presented in Table 2, along with regression

Table 2. Estimated values of the GAB equation parameters for KO microcapsules.

T (°C)	M_0 (g water/ 100 g dry solids)	C	K	R^2	E (%)
WPC					
25	5.888	14.832	0.858	0.999	3.173
35	4.485	20.134	0.989	0.997	5.811
45	4.009	11.879	1.027	0.997	6.16
GA					
25	7.486	14.521	0.855	0.999	3.173
35	5.954	18.451	0.953	0.998	5.811
45	5.659	8.086	1.017	0.995	6.16

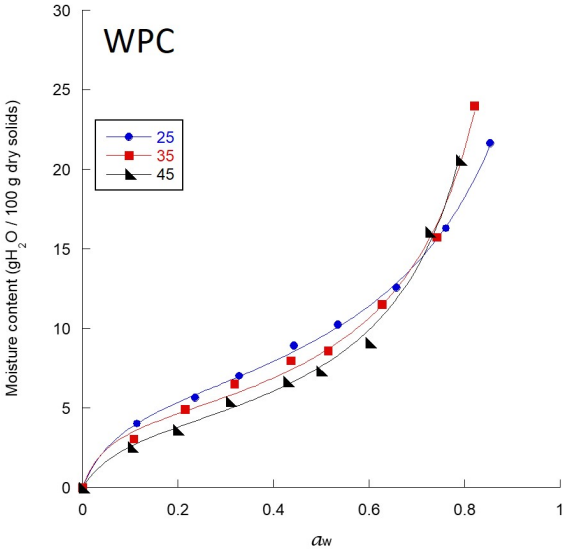

Figure 3. Moisture adsorption isotherms of microencapsulated krill oil in WPC.

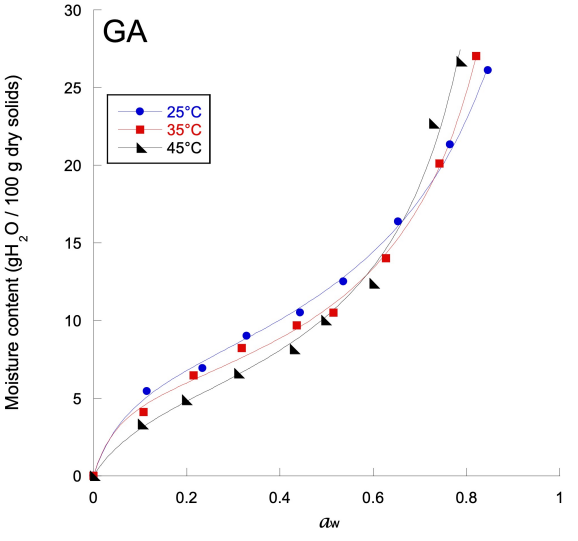

Figure 4. Moisture adsorption isotherms of microencapsulated krill oil in GA.

coefficient (R^2) and the relative mean deviation (E) values, which indicate that the model employed was appropriate.

As can be seen, as temperature increases, all monolayer values tend to be lower, which is associated with the energy required for the water molecules to disengage from their adsorption sites. Also, a decrease in M_0 as temperature rises represents a reduction in the amount of water binding sites. According to the GAB fit, the corresponding value of the monolayer for the KO microcapsules at 35 °C was 0.18 and 0.19 a_w for the microcapsules obtained with WPC and GA, respectively. The monolayer value represents the amount of water adsorbed by the binding sites in the food material, forming a single layer of water molecules; below that value, most degradation processes slow down, making the product more stable (Arslan-Tontul, 2021).

C values are associated with thermal effects, and due to the nature of the exothermic process, it can be assumed that the strong interaction between water molecules and KO microcapsules surface produces temperature lowering and increases C. The estimated values for K in the GAB model are close to the unity. The physical meaning of that constant is related to the heat of adsorption of the multilayer and coupled with higher C values, may indicate that the heat of adsorption of the first layer of water molecules is higher than that of the subsequent layers (Yogendrarajah *et al*., 2015).

Similar GAB values were reported by Koc *et al*. (2022) for transglutaminase microencapsulated by ultrasonic SFD using GA/inulin as wall material (M_0=8.00, C=16.2, k=1.09), Mosquera *et al*. (2012) for freeze dried strawberry pulp added with GA (M_0=6.5, C=13.94 and k=1.03), Tavares and Noreña (2021) for garlic extract encapsulated by Freeze-Drying using WPI and GA as wall materials (M_0=9.3. C=7.9, k=0.922 and M_0=8.5, C=5.4, k=0.917 respectively), and by Esquerdo *et al*. (2019), for Freeze-dried nanocapsules of unsaturated fatty acids prepared with chitosan (M_0=8.24, C=6.13, k=0.93).

3.2.1 Gibbs free energy

In thermodynamics, at constant pressure and temperature, Gibbs free energy describes the maximum amount of energy that exists for being used in a process (Arslan-Tontul, 2021). Also, it serves for

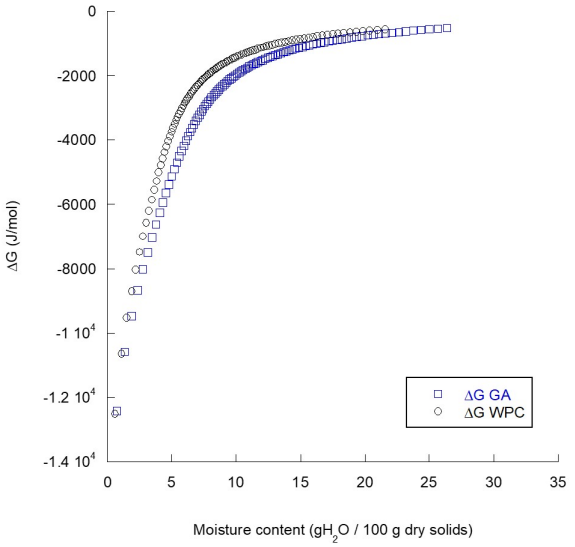

Figure 5. Changes in Gibbs free energy as a function of moisture content at 35 °C for krill oil microcapsules.

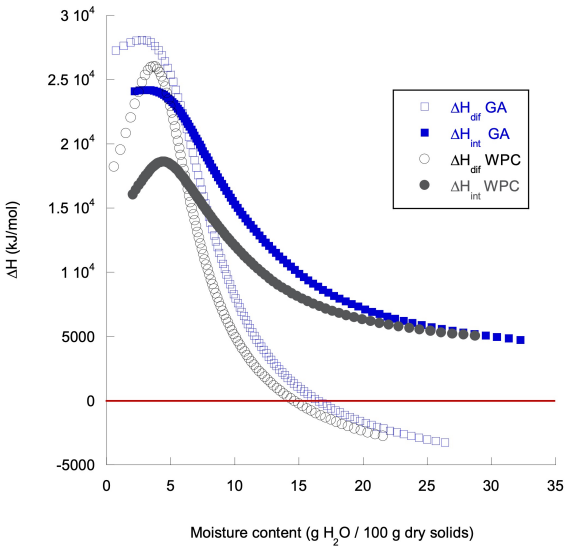

Figure 6. Differential and integral adsorption enthalpy as a function of moisture content at 35 °C for krill oil microcapsules.

quantifying the dry food-water affinity and it helps to represent the energy needed to move a molecule of water from the environment to the adsorbed state (Carvalho Lago and Noreña, 2015).

As can be seen in Figure 5, for both KO microcapsules, all ΔG values were negative and approached zero as moisture content increased, which could be related to the few water binding sites at the surface of the KO microcapsules due to a higher water content (Turker and Isleroglu, 2021). In general, if ΔG values are equal or below zero the adsorption process is spontaneous. Similar findings were reported by Hoyos-Leyva *et al*. (2018), Sapada *et al*. (2012), Turker and Isleroglu (2021), and Viganó *et al*. (2012) in L-ascorbic acid microcapsules obtained by spray drying, microcapsules with hydrolyzed pinhão, lyophilized mahaleb powder and freeze-dried pineapple pulp respectively.

GA microcapsules presented more negative ΔG values, which denotes a higher degree of hygroscopicity; this could be attributed to both the wall material and the SFD technique since it has been reported that SFD generates a large number of micropores that came after the sublimation of fine ice crystals at the end of the lyophilization process, so it exists to a certain degree an extremely porous and highly hygroscopic sponge-like surface (Flores-Andrade *et al*., 2019; Isleroglu *et al*., 2019; Pascual-Pineda *et al*., 2019) Also, wall material influences the amount and size of the micropores; protein-based SFD microcapsules have been reported to exhibit a structure with smooth outer surfaces with small fine pores (Dolly *et al*., 2011; Hundre *et al*., 2015; Rajam and Anandharamakrishnan, 2015), whereas polysaccharide-based SFD microcapsules exhibit a nanoporous surface (Pascual-Pineda *et al*., 2019).

3.2.2 Differential and integral enthalpy

The differential enthalpy (ΔH_{dif}) or isosteric heat is related to the energetic changes that take place in foods at a certain level of hydration, and it is the result of differential changes in the moisture content of a dry product (Viganó *et al*., 2012).

Water adsorption occurs at the most active sites which involves higher interaction energy; as active sites became saturated moisture binding starts at few active sites leading to less heat of sorption (Iglesias *et al*., 2022).

Figure 6 shows the (ΔH_{dif} changes for all KO microcapsules as a function of moisture content. At the beginning, as moisture content increase there is a constant rise of differential enthalpy until it reaches maximum values of 25,916.6 J/mol (a_w= 0.122) and 28,055.5 J/mol (a_w= 0.05) for KO microcapsules prepared with WPC and GA respectively. A higher value of ΔH_{dif} means that the binding degree is stronger, so the adsorbate becomes firmly attached to the active spots on the surface of the KO microcapsules at a low amount of moisture (Paul and Das, 2019). It can be notice that for both kind of microcapsules at a moisture content around 15 g water / 100 g of dry solids solutes with a low molecular weight exhibit incipient solubilization (a cross over with zero in Y axis), that moisture content corresponds to 0.7 of a_w.

On the other hand, integral enthalpy ((ΔH_{int}) can be understood as transitional enthalpy that brings information about the energy changes needed for de adsorption phenomenon (Viganó *et al*., 2012). In Figure 6, also it can be observed that ΔH_{int} increased until it reaches a maximum of 18,625 J/mol (0.18

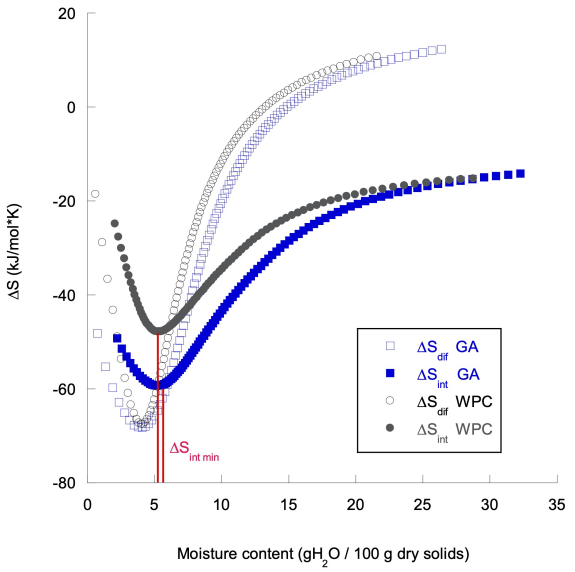

Figure 7. Differential and integral adsorption entropy as a function of moisture content at 35 °C for krill oil microcapsules.

a_w) and 24,166 J/mol (0.06 a_w) for WPC and GA microcapsules respectively. Water molecules are well adsorbed on the most available sites on the surface of the food material, as moisture content increases the binding sites are filled up and the ΔH_{int} gradually declines till there is a water multilayer formation.

According to the shape of ΔH_{int} curve from 0 to 10 g water / 100 g of dry solids (which corresponds to 0 to 0.5 a_w) it could be infer that KO microcapsules prepared with WPC have a more homogeneous sorption surface in terms of energy and they could be more stable to a_w variations. Based on the differential and integral enthalpy results obtained (greater than the enthalpy of vaporization of pure water at low moisture contents), it can be supposed that the interaction adsorbate - adsorbent was higher than the energy that keeps bounded the molecules of water in the liquid state.

Due to a crossover between the differential and integral enthalpy curves, which occurred above the enthalpy of vaporization, it is possible to infer that the water (in vapor state) interacted with high energy, and at this point, the minimum integral entropy was obtained. Similar results have been published for pure and blended carbohydrate polymers (Pérez-Alonso *et al*., 2006), canola oil microcapsules produced by spray drying (Bonilla *et al*., 2010) and spray-dried microcapsules of extra virgin olive oil (Zungur Bastıoğlu *et al*., 2017).

3.2.3 Differential and integral entropy

At a particular hydration level, the sum of the integral entropy plus the change of order/disorder when a new water molecule is adsorbed into the system is represented by the differential entropy. Figure 7 shows the behavior of the differential and integral entropies when moisture content changes in the KO microcapsules.

Differential entropy values showed a decrease to a minimum and then increased in magnitude as moisture content raised; for KO microcapsules prepared with WPC and GA the minimum was at -67.77 and 3.98 g water / 100 g dry solids (approximately) which corresponds to a 0.13 and 0.08 a_w respectively. Changes in differential entropy can be associated with different amounts of the available adsorption sites of the dry material. When there is a low moisture content, the water molecules are positioned in high energy binding sites, which promotes less rotational freedom and randomness degree, which causes lower entropy values. High energy sites in dry foods begin to be saturated when moisture content increases, so there is an increase in the randomness and the system entropy due the motion of adsorbed water molecules (Lara *et al*., 2020).

On the other hand, the integral entropy values for KO microcapsules decrease to a minimum that is related to the better accommodation of the water molecules on the surface of the microcapsules and their greater stability (Cano-Higuita *et al*., 2015; Paul and Das, 2019). According to Luna-Flores *et al*., (2023), as available sites become saturated, the rotational movement of water molecules decreases. Therefore, an increase in moisture content decreases entropy and, thus, results in an ordered system. After this minimum, there is an increase in the integral entropy related to more freedom of the held water molecules and the creation of multilayers. When the moisture content is high, the entropy will be close to free liquid water (Guadarrama-Lezama *et al*., 2014).

The point of minimum integral entropy was calculated at - 45.8 and -59.3 J/molK for WPC and GA microcapsules, respectively; those values correspond to moisture contents of 5.63 g water / 100 d.s. (0.26 a_w) and 5.15 g water / 100 d.s. (0.14 a_w) respectively. The existence of adsorption and/or changes in the adsorbent's structure is demonstrated by the negative values obtained for the integral entropy (Bonilla *et al*., 2010; Carvalho Lago and Noreña, 2015).

Guadarrama-Lezama *et al*. (2014) found a similar behavior for integral entropy in carotenoids microcapsules produced by spray drying using GA and maltodextrin as barrier materials, they found that the best storage conditions (based on the minimum integral entropy) were at 25 °C and a_w of 0.2. Further, Pavón-García *et al*. (2015) reported that the point of minimum integral entropy (0.19 a_w) was related to the most suitable conditions for storing a nutraceutical system microencapsulated using spray drying and a mixture of different wall materials.

Additionally, in both treatments, it was possible to observe a small area where the minimum

integral entropy did not change significantly. In the microcapsules made with WPC, this area ranged from a moisture content of 4.86 to 6.31 g water / 100 d.s. (0.219 - 0.355 a_w) and in the microcapsules made with GA from 4.86 to 5.73 g water / 100 d.s. (0.125 - 0.186 a_w). Pascual-Pineda *et al*. (2019), reported a zone of minimum entropy for red onion microcapsules produced by SFD in the range of 0.402-0.544 a_w and they found that it was correlated to the highest anthocyanin stability. Additionally, Escalona-García *et al*. (2016) reported a zone around the minimum integral entropy in a range of water activities from 0.617 to 0.668, which was related to the best storage conditions of microencapsulated chia oil produced by spray drying using a mixture of WPC and mesquite gum as wall material.

According to the results obtained, the KO microencapsulated through SFD presents its maximum storage stability at low relative humidity values, being the microcapsules made with GA more susceptible to degradation than those made with WPC.

4 Storage stability

Astaxanthin stability in both KO microcapsules was measured for 35 days, samples were placed in microclimates with four different water activities to simulate the most common atmospheres; results were fitted to different degradation models, the best being the one corresponding to a first-order reaction due to the highest regression coefficient, which is also in agreement with other authors who have studied the degradation of carotenoids in different food matrices (Liu *et al*., 2019; Ordóñez-Santos and Martínez-Girón, 2020; Song, Meng, *et al*., 2018; Song, Wei, *et al*., 2018; Valerio *et al*., 2021; Xiao *et al*., 2018).

According to Song *et al*. (2018), non-enzymatic oxidative degradation is the major cause of carotenoid deterioration when molecular oxygen is present. As can be seen in Table 3, KO microcapsules with WPC and GA stored in microclimates with low a_w produced the lowest degradation rates (k_v), and this increases with the increase in humidity in the atmosphere of the microclimates, presenting the greatest degradation of astaxanthins in the samples stored at a_w of 0.743, especially in those made with GA, which may be due to the loss of its structural integrity (Figure 1-A3) since increasing the storage a_w, water may act as a plasticizer over the time (Calderón-Castro *et al*., 2022).

Due to a high unsaturated structures carotenoids are more vulnerable to oxidation, isomerization and other chemical reactions during processing and storage (Ordóñez-Santos and Martínez-Girón, 2020). So, the degradation of astaxanthins in the stored KO microcapsules could be related to interactions between water molecules in the surrounding atmosphere and the wall material, which promotes the oxidative effects resulting from its dissolution and swelling, which makes the catalytic sites more exposed.

The thermodynamic study of moisture adsorption predicted that the greatest stability in the microcapsules with WPC and GA would be found at water activities of 0.26 and 0.14, respectively, which agrees with the results obtained from storage (Table 2). High rates of astaxanthin degradation can be observed in the samples stored in microclimates with water activities further away from the a_w where the minimum integral entropy was found.

Furthermore, when comparing the c values of the different microcapsules stored at the same a_w, it can be seen that the k_v values of the microcapsules made with GA as wall material are higher than the k_v values of the microcapsules containing WPC, indicating faster degradation. And since both wall materials demonstrated high oil encapsulation efficiency, this difference in degradation rates is not related to surface oil content in the samples, but to the physicochemical characteristics of each wall material. The GA seems to allow a greater diffusion of oxygen molecules through the walls of the microcapsules.

Santos *et al*. (2021) studied the storage stability at 25 °C and 32.8 % relative humidity of spray dried microparticles containing carotenoid-rich tucuma oil and GA, they found that after 125 days the initial concentration was reduced by 25 %. They argue that microparticles have surface cracks, which may let bioactive compounds exposed to the ambient due to the larger contact surface.

Some studies have shown that other wall materials, such as maltodextrins, starches, and modified starches, have high rates of degradation of bioactive compounds in microcapsules. In this sense, Li *et al*. (2019) reported a reduction in β-carotene concentration around 60 % in microcapsules produced by SFD using a mixture of maltodextrin and modified starch as wall material, and Spada *et al*. (2012) reported a high k_v value in β-carotene microencapsulated with pinhao starch and stored at 25 °C.

Moreover, Sun *et al*. (2018) studied the behavior of fucoxanthin microcapsules obtained by spray drying using whey protein isolate as wall material during storage at 37 °C, their results were adjusted to a first-order model, obtaining a k_v value of -13.51×10^{-3}, which is greater than all those obtained in the present study for microcapsules with WPC. This shows that the encapsulation method also has a noticeable effect on the stability of the KO microcapsules. In this sense, Zhu *et al*. (2021), conducted experiments with β-carotene microcapsules produced by spray drying and freeze drying and stored at 55°C, they found that after 4 weeks spray dried microcapsules were more unstable than those produced by freeze drying.

Table 3. Kinetic constants for the degradation of astaxanthin in Krill oil microcapsules stored at 35 °C in four different water activities.

Wall material	a_w	Slope ($-k_v \times 10^{-3}$)	Origin ($\ln C/C_0$)	R
WPC	0.108	3.579±0.001	-0.058±0.005	0.713
	0.318	3.737±0.005	-0.025±0.010	0.932
	0.515	5.470±0.001	-0.029±0.004	0.986
	0.743	7.171±0.000	-0.148±0.020	0.733
GA	0.108	14.544±0.003	-0.026±0.003	0.943
	0.318	14.669±0.006	-0.171±0.001	0.854
	0.515	27.508 ±0.002	-0.290±0.001	0.88
	0.743	38.384±0.001	-0.018±0.003	0.956

$C = C_0 \exp(Kt)$. C is the concentration of astaxanthin in μg/g; t is the time in days

They argue that the possible reason could be due to the fact that spray drying produces small particles with a large surface area, which leads to degradation/oxidation of the active principle along with undesirable chemical reactions with other species; in the same paper, they stated that spray-dried products with smaller particle distribution and higher surface areas have a fast degradation rate because they have higher dissolution rates of the enclosed active compounds in exposed areas.

Conclusion

The results showed that the SFD technique could be successfully used for KO microencapsulation using WPC or GA as wall material since both wall materials exhibited microcapsules with high percentages of encapsulation efficiency and bioaccessibility, as well as significant astaxanthin content and antioxidant activity. However, the wall material significantly affected the stability of the KO microcapsules during storage. The thermodynamic analysis showed that the suitable conditions for storing WPC microcapsules are in a higher range of water activities than GA microcapsules. These suitable conditions were corroborated through the study of the degradation of the astaxanthin content during the storage of the microcapsules, which showed that the values of the degradation rate in the GA microcapsules are significantly higher than the values obtained in the WPC microcapsules in all water activities tested.

Considering the increase in demand for functional foods, the results obtained in KO microencapsulation are promising; however, future studies using in vivo models are necessary to establish the effective dose and confirm the beneficial effects of consuming KO microcapsules. If their beneficial effects are confirmed, KO microcapsules can be used in water-based food products since, due to the properties of their wall materials, they could interact with hydrophilic molecules and allow a controlled release of both the w3 fatty acids and astaxanthin, which can provide various benefits to the consumer.

Additionally, it could be concluded that with a good selection of the wall material, the SFD process could find applications for high-value bioactive compounds, especially compared to traditional drying techniques.

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