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Improving *Xanthophyllomyces dendrorhous* astaxanthin stability by encapsulation using a fructan matrix

Mejoramiento de la estabilidad de astaxantina producida por *Xanthophyllomyces dendrorhous* mediante encapsulación utilizando una matriz de fructano

A. Torres-Haro[1], J.C. Mateos-Díaz[1], H. Espinosa-Andrews[2], G.A. Castillo-Herrera[2], M. Arellano-Plaza[1]*
[1]*Biotecnología Industrial,* [2]*Tecnología Alimentaria, Centre for Research and Assistance in Technology and Design of Jalisco State, A.C. Camino Arenero 1227, El Bajío del Arenal, Zapopan, Jalisco, Mexico, 45019.*
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Abstract
Astaxanthin is a pigment and powerful antioxidant that has gained a high interest as a bioactive food constituent, compared to other carotenoids. *Xanthophyllomyces dendrorhous* is one of the main producers of non-esterified (3R, 3´R)-astaxanthin, which is highly assimilable, however, its stability is low (half-life of 19 h at 30 °C). Microencapsulation has emerged as an interesting technique to increase astaxanthin stability, maintain its bioactivity and preserve interesting characteristics. In the present study, a bio-based food constituent fructan (inulin and/or agave fructans) containing microencapsulated non-esterified (3R, 3´R)-astaxanthin produced by *X. dendrorhous* and extracted using supercritical CO_2 was obtained and characterized. Using this technique, astaxanthin stability increased 26-fold compared to the free molecule. In addition, it was also demonstrated that microencapsulated astaxanthin using inulin as a matrix (91.3 ± 0.56 encapsulation efficiency), the capsules resulted well defined (size 2.66 ± 1.08 μm) and maintained their color tone (>70%) for at least 240 h under oxidation conditions. This work obtained a new bio-based stable fructan-astaxanthin microencapsulated food constituent with presumably increased solubility (82.00 ± 5.01 water solubility; size: 2.66 ± 1.08 μm) and bioavailability.
Keywords: Carotenoids, stability, prebiotic matrix, encapsulation, *Xanthophyllomyces dendrorhous*.

Resumen
La astaxantina natural se caracteriza por ser un pigmento y un poderoso antioxidante que sobre sale en comparación con otros carotenoides y, debido a estas propiedades, ha generado gran interés social como compuesto bioactivo aplicado a los alimentos y/o farmacéuticos. *Xanthophyllomyces dendrorhous* es uno de los principales productores de (3R, 3´R)-astaxantina (no esterificada), la cual es de alta asimilación, sin embargo, su estabilidad es baja y presenta una vida media de 19 h a 30 °C. La microencapsulación surge como una alternativa capaz de aumentar la estabilidad de la astaxantina, mantener su bioactividad y preservar sus características de interés social. En el presente estudio, se obtuvo un microencapsulado de (3R, 3´R)-astaxantina (extraída de *X. dendrorhous*) empleando matrices encapsulantes de base prebiótica (fructanos de agave y/o inulina). La astaxantina microencapsulada en inulina presentó una eficiencia de encapsulación de 91.3 ± 0.56, su estabilidad aumentó 26 veces en comparación con la molécula libre y mantuvo su tono de color por 240 h en condiciones de oxidación acelerada. Este trabajo obtuvo un nuevo componente alimentario microencapsulado de fructano-astaxantina estable de base biológica con presumiblemente mayor solubilidad (82.00 ± 5.01 de solubilidad en agua; tamaño de 2.66 ± 1.08 μm) y biodisponibilidad.
Palabras clave: Carotenoides, estabilidad, matriz prebiótica, encapsulación, *Xanthophyllomyces dendrorhous*.

* *Corresponding author. E-mail*: marellano@ciatej.mx;
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1 Introduction

It exists a great interest in carotenoids use in the pharmaceutical, cosmetic and food industries (Mata-Gómez *et al*. 2014; Avalos and Limón, 2015; Torres-Haro *et al*. 2022) because they possess attractive biological properties as powerful antioxidants, anti-inflammatories, radiation protectors, cell regeneration, among others. Elsewhere, different carotenoids such as β-carotene, phytoene, lycopene, zeaxanthin and astaxanthin have been applied in biotechnological processes (Bhosale, 2004; Mata-Gómez *et al*. 2014; Avalos and Limón, 2015; Torres-Haro *et al*. 2021a). Interestingly, astaxanthin (3,3´-dihydroxy-β, β´-carotene-4,4´-dione) is a xanthophyll that has stood out due to its higher biological activity compared to other carotenoids (antioxidant activity 10-fold higher compared to β-carotene) (Naguib, 2000; Mortensen *et al*. 2001). Moreover, astaxanthin has pro-vitamin A activity and it is considered as safe for human consumption.

It has been estimated that by 2025 above $2.7 billion USD will be generated due to astaxanthin sales (Torres-Haro *et al*. 2021a). It has been noted that astaxanthin derived from chemical synthesis abounds on the market (Bernhart, 1990; Mata-Gómez *et al*. 2014), however, natural astaxanthin presents higher bioabsorption and, subsequently, higher bioactivity compared to its chemical counterpart (Storebakken *et al*. 1987; Mata-Gómez *et al*. 2014; Torres-Haro *et al*. 2021a). Natural astaxanthin is produced in two main stereoisomers, (3R, 3'R and 3S, 3'S)-astaxanthin. Both free stereoisomers are highly assimilated and adsorbed by organisms; therefore, it is possible to obtain bioactive effects from both of them (Storebakken *et al*. 1987; Torres-Haro *et al*. 2021a). *Xanthophyllomyces dendrorhous* yeast and *Hematococcus pluvialis* microalgae are the main producers of (3R, 3´R)-astaxanthin and (3S, 3´S)-astaxanthin, respectively. However, *Hematococcus pluvialis* mainly synthesize esterified (3S, 3´S)-astaxanthin form, which is bound to fatty acids such as stearic, oleic or palmitic (Storebakken *et al*. 1987; Higuera-Ciapara *et al*. 2006; Domínguez-Bocanegra *et al*. 2007).

The astaxanthin esters bioabsorption may be limited or variable due to their molecular complexity, the organism ester hydrolysis capacity and, subsequently, the biological effects are limited (Storebakken *et al*. 1987; Bernhart, 1990; Torres-Haro, *et al*. 2021a). Interestingly, *Xanthophyllomyces dendrorhous* produces, mainly, non-esterified (3R, 3´R)-astaxanthin (Higuera-Ciapara *et al*. 2006; Amado and Vázquez, 2015; Torres-Haro *et al*. 2021b), a more attractive assimilable astaxanthin version with industrial production and market commercialization potential.

X. dendrorhous is a highly attractive astaxanthin producer at an industrial scale, due to its rapid cell growth, low-cost substrates assimilation and easy process scaling compared to *H. pluvialis* (Amado and Vázquez, 2015; Torres-Haro *et al*. 2021b). However, free astaxanthin (non-esterified form) is extremely unstable and susceptible to oxidation by several environmental factors (Zhou *et al*. 2018; Honda *et al*. 2021). This causes a loss in its bioactivity and limits its industrial application (Chen *et al*. 2019; Lin *et al*. 2016; Zhou *et al*. 2018; Honda *et al*. 2021). Thus, searching for novel strategies allowing the *X. dendrorhous* astaxanthin stabilization is most important to maintain biological properties.

The encapsulation process has emerged as a technique capable of maintaining and, even, increasing the bioactivity of different compounds susceptible to oxidation (Chen *et al*. 2019; Higuera-Ciapara *et al*. 2006; Lin *et al*. 2016; Martínez-Álvarez *et al*. 2020; Zapata-Luna *et al*. 2023). The astaxanthin microencapsulation consists of forming a layer around the biomolecule, decreasing its degradation when exposed to high temperatures, extreme pH conditions, high pressure, freeze-drying, oxygen presence, light, or even when exposed to digestive tract conditions (Zhou *et al*. 2018; Martínez-Álvarez *et al*. 2020). Generally, matrices such as chitosan, cyclodextrin, calcium alginate or liposomes have been used for bioactive compounds encapsulation, including astaxanthin (Lin *et al*. 2016; Zhou *et al*. 2018; Martínez-Álvarez *et al*. 2020; Zapata-Luna *et al*. 2023). Nevertheless, it could be possible to increase astaxanthin value using functional matrices to generate differentiated products with additional bioactive properties, promoting industrial applications of *X. dendrorhous* astaxanthin. Thereby, in the present study, the (3R, 3´R)-astaxanthin from *X. dendrorhous* 25-2 was for the first time encapsulated using prebiotic matrices based on chicory inulin and/or agave fructans generating an interesting stable product with additional prebiotic potential.

2 Materials and methods

2.1 Microorganism

X. dendrorhous 25-2 strain (astaxanthin overproducing non-specific mutant) was used for astaxanthin production in this study. The mutant strain was obtained from a wild-type ATCC 24202 strain by random mutagenesis using nitrosoguanidine and belongs to the Industrial Biotechnology Department microorganisms collection of the Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. (CIATEJ, AC).

2.2 Culture medium and biomass production

In the first instance, yeast was activated at a flask level of 250 mL, containing 50 mL of a chemically defined mineral culture medium at pH 6.0. The composition (g/L) is the following: 3.0, KH_2PO_4; 3.0, $(NH_4)_2SO_4$; 3.0, $Na_2HPO_4{\cdot}12H_2O$; 1.0, glutamic acid; 20.0, industrial glucose (from corn crops); 0.5, $MgCl_2{\cdot}6H_2O$; 0.0192, $ZnCl_2$; 0.0044, $MnCl_2{\cdot}4H_2O$; 0.0005, $CoCl_2{\cdot}6H_2O$; 0.0006, $CuCl_2{\cdot}2H_2O$; 0.0003, $(NH_4)_6Mo_7O_4{\cdot}4H_2O$; 0.0174, $CaCl_2$; 0.0116, $FeCl_2{\cdot}4H_2O$; 0.003, H_3BO_3; 0.005, pantothenic acid; 0.005, nicotinic acid; 0.125, inositol; 0.005, thiamine; 0.005, pyridoxine; 0.001, *p*-aminobenzoic acid; and 0.000012, biotin. Industrial glucose was obtained from ALMEX, Guadalajara, Jalisco, Mexico (Torres-Haro *et al*. 2021b). Flask-level cultures were incubated in a New Brunswick® Innova 44 rotary orbital (Hamburg, Germany) at 20 °C and 250 rpm (Tibayrenc *et al*. 2010; Torres-Haro *et al*. 2021b).

In the second instance, *X. dendrorhous* biomass production was obtained using a 110 L pilot level Applikon® bioreactor (Applikon Biotechnology, B.V., Delft, The Netherlands) with a feed-batch system, and it was inoculated with 10% volume of a previously activated strain (during 72 h) cultivated in a 15 L New Brunswick® bioreactor (Hamburg, Germany). The bioreactor culture conditions were pH 6.0, 20 °C (293 K) (Torres-Haro *et al*. 2021b).

2.3 Biomass recovery and drying

The biomass generated at 110 L pilot level bioreactor was concentrated to obtain 10% of biomass using a disk centrifuge GEA Westfalia (GEA Group, Jiutepec, Morelos, Mexico), and, subsequently, biomass was dried employing spray dryer Yamato DL410 (Yamato Scientific America Inc., Santa Clara, US) at 433 K (drying efficiency 95%) and stored until supercritical astaxanthin extraction (Torres-Haro *et al*. 2022).

2.4 Supercritical CO_2 astaxanthin extraction

A Thar Technologies-Waters®-SFE-500 supercritical fluid extractor (Waters Corporation, Milford, Massachusetts, US) was used for astaxanthin recovery (Torres-Haro *et al*. 2022). *X. dendrorhous* dried cells were placed into the extraction vessel (90 g biomass) and 15 g/min flow rate of carbon dioxide (medical grade, INFRA^{TM}, Guadalajara, Jalisco, Mexico) was fed to the extraction vessel by the bottom. The total extraction time was 4 h (Macías-Sánchez *et al*. 2008), temperature 313 K, pressure 32.5 MPa, and ethanol as co-solvent at 20% in the extraction process (Barajas-Álvarez *et al*. 2021; Torres-Haro *et al*. 2022).

2.5 Astaxanthin encapsulation using a prebiotic matrix

After supercritical extraction, the intracellular (3R, 3´R)-astaxanthin was collected for the encapsulation process using a prebiotic matrix. Chicory inulin (Sigma-Aldrich^{TM}, Saint Louis, Missuri, USA), agave fructans (Olifructine^{TM} Nutriagaves group, Guadalajara, Jalisco, Mexico) or a mixture of both (50-50% ratio) dissolved in distilled water were used and evaluated as encapsulating agents or prebiotic matrices. Astaxanthin is a non-polar compound, because of that it is necessary to form an aqueous emulsion and, subsequently, the microencapsulation. Thereby, an aqueous emulsion was formed using 1:1:2 (mass ratio) intracellular extract: tween 80 (polyoxyethylene sorbitano monooleate; Drogas la Paz S.A. de C.V., Guadalajara, Jalisco, Mexico): encapsulating agent. The compounds were dispersed by ultrasonication for 30 seconds at 80% amplitude using GEX 130 ultrasonicator equipment (Sigma-Aldrich^{TM}, Saint Louis, Missouri, USA). Astaxanthin dry capsules were obtained using a spray dryer Yamato DL410 equipment (Yamato Scientific America Inc., Santa Clara, US) maintaining the inlet temperature at 428 K, the outlet temperature at 340 K, atomization pressure 0.3 MPa and inlet flow for wet sample 0.22 L/h.

2.6 Astaxanthin capsules characterization

The generated astaxanthin capsules using different fructan matrices were characterized. Precipitated particles, after the encapsulation process using a spray dryer, were analyzed by scanning electron microscope (SEM) equipment (JEOL JSM-IT300), and the mean particle size was measured by Digimizer (version 6.1.1) image analysis software (Machado *et al*. 2014; Mezzomo *et al*. 2012). In addition, water solubility, water activity, moisture content (Gomez-Estaca *et al*. 2016; Martínez-Preciado *et al*. 2021), and encapsulation efficiency (Chen *et al*. 2019; Gomez-Estaca *et al*. 2016; Tirado *et al*. 2019) were evaluated for each astaxanthin capsule generated.

The moisture content of microcapsules (1 g) was determined using an oven at 75 °C during 48 h. The dried samples were cooled in a desiccator and weighed. The results were interpreted as relative moisture percent (Perez-Alonso *et al*., 2015).

The solubility of the capsules was determined by the gravimetric method (Gomez-Estaca *et al*., 2016; Martínez-Preciado *et al*., 2021). 0.5 g of capsules generated were added to 50 mL of distilled water and homogenized using an orbital shaker at 100 rpm for 30 min and 25 °C. After, the solution was centrifuged at 1400 g for 5 min using a centrifuge tube and, subsequently, 25 mL aliquot of the supernatant was transferred to another tube and maintained at 70 °C

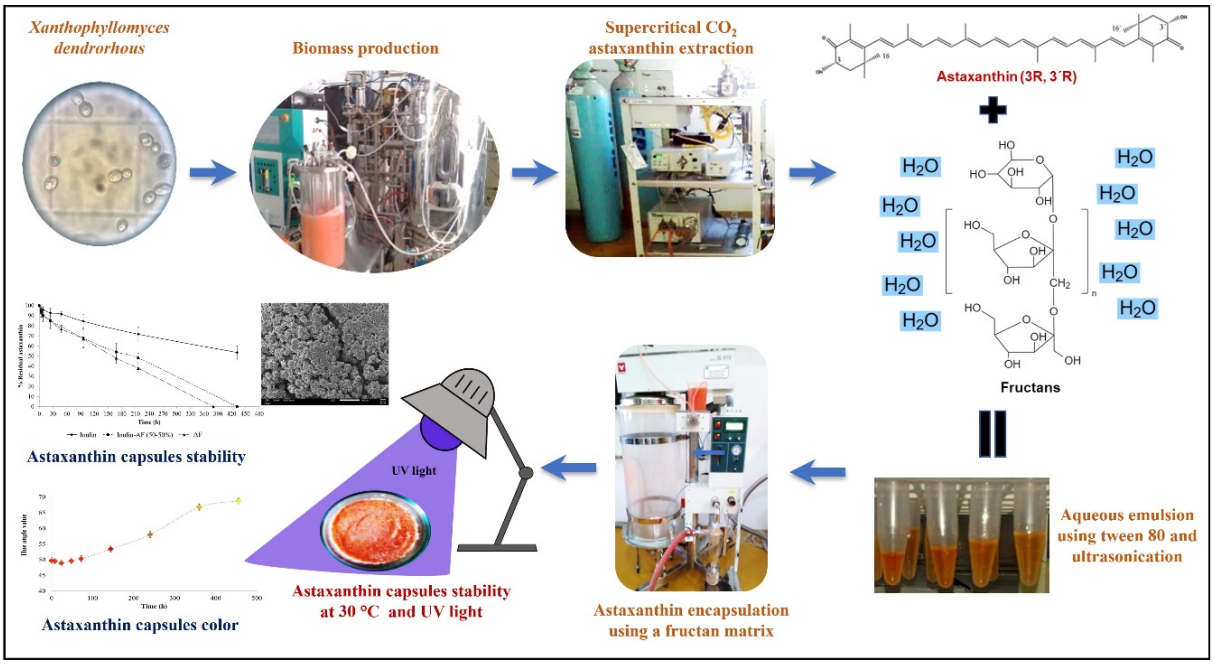

Figure 1. The methodological strategy used in this study.

until complete water evaporation. The solubility was calculated using Equation 1.

$$\text{Water solubility} = \frac{\text{Final weight} \times 2}{\text{Initial microcapsules weight}} \quad (1)$$

Encapsulation efficiency was obtained from the total astaxanthin quantification in the capsules generated and the surface astaxanthin quantification (non-encapsulated astaxanthin). The efficiency was calculated according to Equation 2 (Chen *et al*. 2019; Gomez-Estaca *et al*. 2016; Tirado *et al*. 2019).

$$\text{Encapsulation efficiency} = \frac{\text{Total astax} - \text{Surface astax}}{\text{Total astaxanthin}} \times 100 \quad (2)$$

2.7 *Astaxanthin capsules stability evaluation*

The astaxanthin stability was evaluated for 15 mg of sample (astaxanthin capsules) using a UV light chamber at a wavelength of 368 nm, 20 W, and 30 °C for 432 h. The residual astaxanthin amount was determined (Sedmak *et al*. 1990) and half-life time ($t_{1/2}$; Equation 4) was calculated based on kinetic reaction rate (Equation 3) (Perez-Alonso *et al*. 2015).

$$\ln\left(\frac{C_t}{C_0}\right) = -kt \quad (3)$$

$$t_{1/2} = \frac{\ln(2)}{k} \quad (4)$$

where C_0 is the initial astaxanthin content and C_t is the content at time t.

In turn, since the bioactive compound is a colorant, the loss of tone color was monitored using generated capsules (3 g) in a KONICA MINOLTA CM-5 spectrophotometer and data was expressed in color tone angle (*hue*; Equation 5) terms. Figure 1 depicts a diagram of the general methodology used to carry out the present study.

$$hue = \arctan\frac{b^*}{a^*} \quad (5)$$

where a^* and b^* are rectangular color coordinates.

3 Results and discussion

3.1 *Biomass production and astaxanthin extraction*

The final biomass production in a pilot scale bioreactor was 1.0 kg of dry biomass with an astaxanthin content of 215 μg/g biomass, quantified by solvent extraction due to it being an intracellular component (Sedmak *et al*. 1990). The total biomass yield was 0.448 g biomass/g substrate (using industrial glucose). The astaxanthin extraction from biomass was using only a supercritical extraction process and it was possible to obtain 54% astaxanthin recovery (Torres-Haro *et al*. 2022). Nevertheless, using combined extraction technologies, it has been possible to increase astaxanthin recovery. For example, Gio-Bin *et al*. (2002) obtained >75% astaxanthin recovery using supercritical extraction at 60°C and 50 MPa during 4 h.

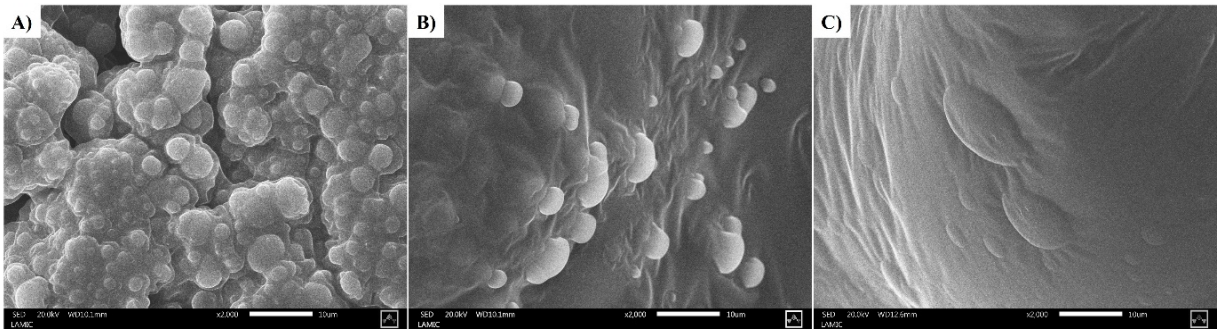

Figure 2. SEM analysis of precipitated particles after the encapsulation process using fructan matrices with a 2000 times magnification. A) (3R, 3´R)-astaxanthin capsules using an inulin matrix, B) (3R, 3´R)-astaxanthin capsules using an inulin-agave fructans matrix (50-50% ratio) and C) (3R, 3´R)-astaxanthin capsules using an agave fructans matrix.

Table 1. (3R, 3´R)-astaxanthin capsules characterization.

Attribute	AF	AF-Inulin	Inulin
Size (diameter; μm)	8.96 ± 3.97^a	5.79 ± 1.63^a	2.66 ± 1.08^b
% Water solubility	99.19 ± 0.77^a	98.97 ± 1.44^a	82.00 ± 5.01^b
Water activity	0.272 ± 0.017^a	0.411 ± 0.026^b	0.285 ± 0.008^a
% Moisture	2.06 ± 0.68^a	3.58 ± 1.00^a	2.57 ± 1.19^a
% Encapsulation efficiency	55.5 ± 7.77^a	78.5 ± 2.12^b	91.3 ± 0.56^c

Results are expressed as mean value ± standard deviation. AF: Agave fructans; AF-Inulin: Mixture agave fructans and inulin (50:50%). Different superscript letter on the same attribute indicates significant difference ($p<0.05$) between response variable of each row.

However, the mass astaxanthin recovered is attributed to the biomass pre-treatment process (cell breakage in a ball mill) before supercritical extraction.

3.2 Astaxanthin encapsulation using a prebiotic matrix

After supercritical extraction, the astaxanthin was collected for the encapsulation process using encapsulating agents such as chicory inulin, agave fructans or a mixture of both (50-50% ratio). Previously to encapsulation, an aqueous emulsion was formed using 1:1:2 (mass ratio) intracellular extract: tween 80: encapsulating agent.

Tween 80, resulted in being an attractive emulsification agent due to its amphiphilic properties, which decreases the surface tension of the mixture (prebiotic matrix, astaxanthin, and water), allowing the components to interact and generate a stable emulsion until the encapsulation process (spray drying process) was performed (Li *et al*., 2016; Tirado *et al*., 2019). In previous studies using tween 80 as a dispersing agent (oily phase-matrix 1:1 w/w), but different matrix agents, surface scanning electron micrographs showed astaxanthin homogenization from 20 to 95% in simulated gastric fluid (Lee *et al*., 2011) and 20 to 80% in aqueous systems (Hasan *et al*., 2007). Employing other dispersing agents, such as lipid or oil extracts, the dispersion in water is lower (from 10 to 28%) (Gomez-Estaca *et al*., 2016).

Subsequently, after obtaining astaxanthin capsules using agave fructans, inulin and/or a combination of both (50-50% ratio) as encapsulating agents, their characterization was performed, and results are shown in Table 1. The microcapsule sizes and moisture content depend on the encapsulating matrix. It has been reported that the diffusion coefficient of water is reduced when the moisture content is <7% (Martínez-Preciado *et al*., 2021; Pérez-Alonso *et al*., 2015). In this study, all astaxanthin microcapsules generated by spray dry technology showed a size lower than 10 μm and moisture content <5% (Table 1) and, probably, the shelf life could be extended.

3.3 Astaxanthin microcapsules morphologies

The astaxanthin microcapsules morphologies were analyzed (Figure 2). Inulin-agave fructans (50-50% ratio) and agave fructans microcapsules showed agglomerated particles (Figures 2b and 2c, respectively). This effect is probably due to agave fructans hydrophilicity (García-Curbelo *et al*. 2015).

In fact, agave fructans microcapsules presented higher water solubility compared to inulin (p-value<0.05; Table 1). Nevertheless, when using

only inulin as the encapsulating matrix (presenting hydrophobic characteristics), the capsules resulted well defined (Figure 2a; size 2.66 ± 1.08 μm) and, however, water solubility was 17% lower compared to agave fructans used as a matrix agent (p-value<0.05; Table 1). Probably, hydrophobic properties of astaxanthin and inulin allowed higher interaction to increase astaxanthin encapsulation efficiency (12.8 to 35.8% higher) compared to agave fructans use like encapsulating agent (p-value<0.05; Table 1).

It has been reported that the presence of larger particles increases the agglomeration process (Pérez-Alonso *et al*., 2015), and the same effect was observed in this study using agave fructans as encapsulating agent (size 5.79 to 8.96 μm; Figure 2). In addition, wall material and temperature during the drying process influence the morphological characteristics of the microcapsules (Martínez-Preciado *et al*., 2021; Pérez-Alonso *et al*., 2015). The microcapsule morphologies observed in the present study are typical using spray-drying process, as shown in Figure 2 (Martínez-Preciado *et al*., 2021).

The inulin microcapsules morphologies were semi-spherical, corrugated surfaces and, attractively, presence of fractures, cracks and other defects that could expose the astaxanthin were not observed. In the case of agave fructans and agave frcutans-inulin (50-50%) particles, they showed both semi-spherical morphologies and not well-defined. In addition, high agglomeration level and fractures were observed, which increased the astaxanthin degradation when it was exposed to oxidation conditions.

In the present study, the main objective was to obtain astaxanthin capsules to maintain the molecule integrity, but in addition, it was of interest to potentiate the final product biological activity by using a prebiotic matrix as an encapsulating agent (agave fructans and/or inulin use). This is also to promote a product which will be highly attractive in the market and/or society (Mata-Gómez *et al*., 2014; Avalos and Limón, 2015; Torres-Haro *et al*., 2022). Attractively, the best encapsulation efficiency was found using inulin as a protective material and tween 80 as an emulsifying agent, however, it is necessary to evaluate the stability of microencapsulated astaxanthin under oxidation conditions.

3.4 Microencapsulated astaxanthin stability

As previously mentioned, unesterified (3R, 3´R)-astaxanthin is extremely unstable and susceptible to environmental factors (Chen *et al*., 2019; Honda *et al*., 2021; Zhou *et al*., 2018). Figure 3 shows the (3R, 3´R)-astaxanthin degradation kinetic performed at 30 °C in the absence of oxygen and light. The calculated half-life ($t_{1/2}$; Equation 4) of unesterified astaxanthin was 19 h. Moreover, the astaxanthin microcapsules stability was evaluated and compared to the free astaxanthin (Table 2). Figure 4 shows the stabilities kinetics of the astaxanthin microcapsules and, subsequently, the half-life of astaxanthin degradation ($t_{1/2}$; 50% degradation) was calculated (Equation 4) for the capsules generated in this study under the described conditions (UV light and temperature).

Astaxanthin encapsulated using agave fructans and a combined treatment (inulin-fructans; 1:1) as matrices showed a $t_{1/2}$ of 165.03 and 210.04 h, respectively, while the active component encapsulated only with inulin showed the highest $t_{1/2}$ of 495.10 h.

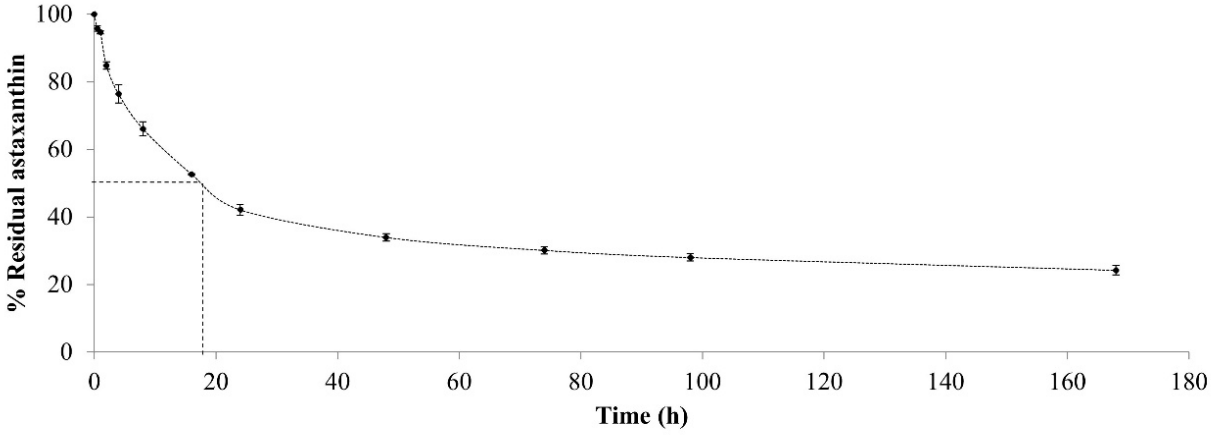

Figure 3. Unesterified (3R, 3´R)-astaxanthin degradation kinetic exposed to 30°C in the absence of light and oxygen. The results were expressed as residual astaxanthin and they are shown as mean with their standard deviation. $t_{1/2}$: half-life time.

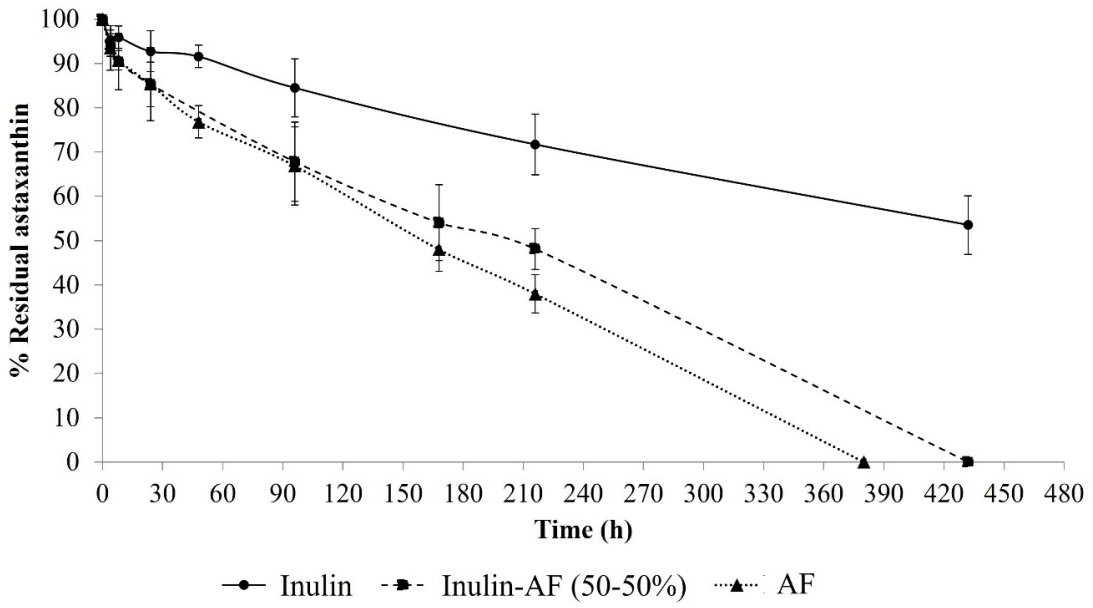

Figure 4. Astaxanthin capsules stability exposed to UV light (wavelength 368 nm; 20 W) and temperature (30 °C). The initial astaxanthin concentration in the capsules was 783 ppm. The results were expressed as residual astaxanthin and they are shown as mean with their standard deviation. AF: Agave fructans.

Table 2. Studies compilation of several astaxanthin encapsulation methods, using different matrices to increase molecule stability.

Astaxanthin Source	Matrix	Encapsulation method	Attractiveness	Reference
Extract from *X. dendrorhous*	Without matrix (control)	No encapsulated	No attractiveness (50% degradation at 19 h under oxidation conditions)	This study
Pure	Poly-vinyl alcohol-co-methoxy-cinnamate	Solvent displacement	Resistance at 70 °C (2 h)	Tachaprutinun *et al.* (2009)
Pure	Phosphatidyl choline	Thin-film method	Storage stability and antioxidant activity (>82% at 15 days)	Pan *et al.* (2018)
Pure	Ethyl cellulose	Supercritical emulsion extraction	Maintain antioxidant activity	Tirado *et al.* (2019)
Extract from shrimp	Starch (Hi-Cap 100)	Super critical fluid extraction of an emulsion	Color stability (>70% at 40 h)	Mezzomo *et al.* (2012)
Extract from shrimp	Gelatin and cashew gum	Coacervation	Color stability (between 40 and 60% at 43 days)	Gomez-Estaca *et al.* (2016)
Extract from *H. pluvialis*	Poly-hydroxybutyrate	Super critical fluid extraction of an emulsion	Stability to high pressure (between 80 and 100 bar)	Machado *et al.* (2014)
Extract from *H. pluvialis*	Chitosan	Coating	Resistance at 80°C (Stability >85% at 4 days)	Lee *et al.* (2011)
Extract from *X. dendrorhous*	Alginate	Ionic gelation	Maintain antioxidant activity (>70% at 6 days)	Kittikaiwan *et al.* (2007)
Extract from *X. dendrorhous*	Porous starch and gelatin	Loading astaxanthin	Astaxanthin retention rate at 55 °C (40% at 15 days)	Chen *et al.* (2019)
Extract from *X. dendrorhous*	Agave fructans	Spray dried	Prebiotic function and stability (>50% at 6 days under oxidation conditions)	This study
Extract from *X. dendrorhous*	Inulin-Fructans (1:1)	Spray dried	Prebiotic function and stability (>50% at 8 days under oxidation conditions)	This study
Extract from *X. dendrorhous*	Inulin	Spray dried	Prebiotic function and stability (>50% at 20 days under oxidation conditions)	This study

However, using the agave fructans or combined treatment (inulin-fructans; 1:1), the active component was completely degraded at 380 and 432 h, respectively. Probably, when agave fructans were used as encapsulating agents, a high permeability enhanced the presence of water, oxygen, and light, increasing astaxanthin degradation. Permeability effect or capsule rupture has been observed in the bioactive encapsulation process using other encapsulating agents such as maltodextrin, gum arabic, and aguamiel (agave sap) (Mezzomo *et al*., 2012; Pérez-Alonso *et al*., 2015).

Moreover, the hydrophilic nature of the agave fructans (capsules water solubility 99.19 ± 0.77%) decreased the hydrophobic astaxanthin interaction and limited its stability and encapsulation efficiency (Table 1; Figure 4). Nevertheless, it has been reported that using agave sap (containing fructans) as an encapsulating agent, it is possible to preserve color tone of the bioactive compound during and after the encapsulation process (Pérez-Alonso *et al*., 2015).

The higher stability (3R, 3´R)-astaxanthin encapsulated using an inulin-based matrix was attributed to the hydrophobic interaction of the inulin matrix with astaxanthin (water solubility 82.00 ± 5.01%; Table 1), leading to a more effective coating of the molecule (encapsulation efficiency 91.3 ± 0.56%; Table 1), resulting in a more stable product in the absence of light and oxygen (fractures absence; Figure 2A). In addition, inulin presents low permeability compared to agave fructans (Hasan *et al*., 2007; Pérez-Alonso *et al*., 2015) and, subsequently, it maintains the bioactive compound during a large time, similar to those reported in previous studies using different encapsulating agents such as poly-hydroxy-alkanoates, modified cellulose, gelatin, chitosan, alginate and other encapsulating agents (Table 2). Moreover, variable encapsulating agents and/or methods promoted different attractiveness in the final astaxanthin product, for example, different color stability, antioxidant activity, biosorption, and/or bioassimilation (Table 2).

It is important to observe that free astaxanthin stability was 26.06-fold lower than the (3R, 3´R)-astaxanthin encapsulated using the inulin prebiotic matrix (Figure 5). It is worth noting that in this kinetic experiment using free astaxanthin, there was no oxygen and/or light, thus the stability of encapsulated astaxanthin is by far higher because it was performed in the presence of oxygen, UV light, and temperature (Table 2).

3.5 Capsules color stability evaluation

The color maintenance study (attractiveness) of the generated capsules using an inulin matrix (as the best encapsulation treatment; efficiency 91.3 ± 0.56%; Figure 2A) was monitored under oxidation conditions (wavelength 368 nm; 20 W and 30 °C) and results are showed as tone color or *hue* angle function (Equation 5; Figure 5).

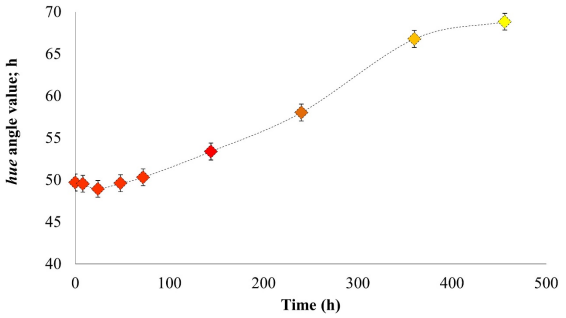

Figure 5. Astaxanthin capsules color determination using an inulin matrix and exposure to UV light (wavelength 368 nm; 20 W) and 30 °C. The results of color tone change were expressed as color tone or *hue* angle, and they are shown as mean with their standard deviation.

The *hue* angle was maintained without showing a significant (p-value>0.05), during the first 72 h of exposure, which shows that there was no color loss (red-orange color tone). This result shows a low loss of the red-orange color, related to the low astaxanthin degradation (degradation ≤8%; Figure 4). After 144 h of constant exposure, the *hue* angle changed to 53.38° (p-value<0.05) when a 20% astaxanthin degradation was reached (Figure 3), showing a slight color change. A color change (orange tone; *hue* angle = 57°) was observed after 240 h of exposure, associated with a 30% compound degradation. After 456 h of exposure, the color turned yellow (*hue* angle = 67.5°), and the degradation resulted >40% (Figure 4).

Similarly, astaxanthin encapsulation in other matrices has been reported to increase molecule stability, maintaining color and/or antioxidant activity (Table 2). Encapsulated astaxanthin stability is maintained between 6 and 364 days at 25°C and degradation is between 30 and 50% (Chen *et al*., 2019; Kittikaiwan *et al*., 2007; Lin *et al*., 2016; Zhou *et al*., 2018; Martínez-Álvarez *et al*., 2020). However, the astaxanthin degradation is variable depending on the encapsulating matrix (Table 2) and its encapsulation efficiency (Chen *et al*., 2019; Gomez-Estaca *et al*., 2016; Tirado *et al*., 2019). In the present study, oxidation conditions (presence of oxygen and UV light) were promoted to obtain results in a short time (10% degradation at 50 h of exposure and 50% degradation at 20 days; Table 2) and it was demonstrated inulin potential in astaxanthin encapsulation process to preserve its bioactivity, color and, attractively, to generate new food constituent.

Conclusions

Encapsulation of astaxanthin with agave fructans and a mixture of inulin-agave fructans (1:1) provided 8.40- and 10.69-fold, respectively, higher stability compared to free astaxanthin. Higher stability using agave fructans should be studied by testing other emulsifying agents and further matrix relations. The best encapsulation results were found using inulin as protective material and tween 80 as an emulsifying agent (91.3 ± 0.56% efficiency and 0.285 ± 0.008 water activity). The produced and supercritical CO_2 extracted unesterified (3R, 3´R)-astaxanthin form *X. dendrorhous* encapsulated on an inulin matrix was 26.06-fold more stable compared to free astaxanthin. Moreover, astaxanthin maintained >70% of its original tone color for at least 240 h under oxidation conditions, allowing the generation of a new well-defined (size 2.66 ± 1.08 μm) bio-based stable fructan-astaxanthin microencapsulated food constituent with presumably increased solubility (82.00 ± 5.01% water solubility; this study) and bioavailability.

References

Amado, I. R. and Vázquez, J. A. (2015). Mussel processing wastewater: A low-cost substrate for the production of astaxanthin by *Xanthophyllomyces dendrorhous*. *Microbial Cell Factories*. *BioMed Central 14*(1), 1-11. https://doi.org/10.1186/s12934-015-0375-5

Andrewes, A. G., Phaff, H. J. and Starr, M. P. (1976). Carotenoids of *Phaffia rhodozyma*, a red-pigmented fermenting yeast. *Phytochemistry 15*(6), 1003-1007. https://doi.org/10.1016/S0031-9422(00)84390-3

Avalos, J. and Carmen Limón, M. (2015). Biological roles of fungal carotenoids. *Current Genetics 61*(3), 309-324. https://doi.org/10.1007/s00294-014-0454-x

Barajas-Álvarez, P. Castillo-Herrera, G. A., Guatemala-Morales, G. M., Corona-González, R. I., Arriola-Guevara, E. and Espinosa-Andrews, H. (2021). Supercritical CO_2-ethanol extraction of oil from green coffee beans: optimization conditions and bioactive compound identification. *International Journal of Food Science and Technology 58*(12), 4514-4523. https://doi.org/10.1007/s13197-020-04933-1

Bernhart, K. (1990). Synthetic astaxanthin. The route of a carotenoid from research to commercialisation. *Carotenoids: Chemistry and Biology*, 337-363. https://doi.org/10.1007/978-1-4613-0849-2_24.

Bhosale, P. (2004). Environmental and cultural stimulants in the production of carotenoids from microorganisms. *Applied Microbiology and Biotechnology 63*(4), 351-361. https://doi.org/10.1007/s00253-003-1441-1

Boussiba, S. (2000.) Procedure for large-scale production of astaxanthin from *Haematococcus*. United States. Available at: https://patents.google.com/patent/US6022701A/en.

Domínguez-Bocanegra, A. R., Ponce-Noyola, T. and Torres-Muñoz, J. A. (2007). Astaxanthin production by *Phaffia rhodozyma* and *Haematococcus pluvialis*: a comparative study. *Applied Microbiology and Biotechnology*, 783-791. https://doi.org/10.1007/s00253-007-0889-9

Farías, L. J., Gschaedler, A., Sanchez, A., Cervantes, J., Arellano, M., Zamora, C., Amillastre, E. and Herrera, E.J. (2018). *Xanthophyllomyces dendrorhous* physiological stages determination using combined measurements from dielectric and Raman spectroscopies, a cell counter system and fluorescence flow cytometry. *Biochemical Engineering Journal 136*, 1-8. https://doi.org/10.1016/j.bej.2018.04.016

García-Curbelo, Y., Mercedes G., Bocourt, R., Collado, E., Nereyda, A. and Nuñez, O. (2015). Structural characterization of fructans from *Agave fourcroydes* (Lem.) with potential as prebiotic. *Cuban Journal of Agricultural Science 49*(1), 75-80. https://cjascience.com/index.php/CJAS/article/view/551

Gio-Bin, L., Sang-Yun, L., Eun-Kyu, L., Seung-Joo, H. and Woo-Sik, K. (2002). Separation of astaxanthin from red yeast *Phaffia Rhodozyma* by supercritical carbon dioxide extraction. *Biochemical Engineering Journal 11*(2-3), 181-187. https://doi.org/10.1016/S1369-703X(02)00023-2.

Gómez-Estaca, J., Comunian, T. A., Montero, P., Ferro-Furtado, R. and Favaro-Trindade, C. S. (2016). Encapsulation of an astaxanthin-containing lipid extract from shrimp waste by complex coacervation using a novel gelatin-cashew gum complex. *Food Hydrocolloids 61*, 155-162. https://doi.org/10.1016/j.foodhyd.2016.05.005

Hasan., A. S., Socha, M., Lamprecht, A., Ghazouani, F., Sapin, A., Hoffman, M., Maincent, P. and Ubrich, N. (2007). Effect of the microencapsulation of nanoparticles on the reduction of burst release. *International Journal of Pharmaceutics 344*(1-2), 53-61. https://doi.org/10.1016/j.ijpharm.2007.05.066

Higuera-Ciapara, I., Félix-Valenzuela, L. and Goycoolea, F. M. (2006). Astaxanthin: A review of its chemistry and applications. *Critical Reviews in Food Science and Nutrition 46*(2), 185-196. https://doi.org/10.1080/10408690590957188.

Honda, M., Kageyama, H., Hibino, T., Osawa, Y., Kawashima, Y., Hirasawa, K. and Kuroda, I. (2021). Evaluation and improvement of storage stability of astaxanthin isomers in oils and fats. *Food Chemistry 352*, 129371. https://doi.org/10.1016/j.foodchem.2021.129371

Kittikaiwan, P., Powthongsook, S., Pavasant, P. and Shotipruk, A. (2007). Encapsulation of *Haematococcus pluvialis* using chitosan for astaxanthin stability enhancement. *Carbohydrate Polymers 70*(4), 378-385. https://doi.org/10.1016/j.carbpol.2007.04.021

Lee, J. S., Park, S. A., Chung, D. and Lee, H. G. (2011). Encapsulation of astaxanthin-rich *Xanthophyllomyces dendrorhous* for antioxidant delivery. *International Journal of Biology and Macromolecules 49*(3), 268-273. https://doi.org/10.1016/j.ijbiomac.2011.04.021

Li, R., Chen, R., Liu, W., Qin, C. and Han, J. (2016). Preparation of enteric-coated microcapsules of astaxanthin oleoresin by complex coacervation. *Pharmaceutical Development and Technology 23*(7), 674-681. https://doi.org/10.1080/10837450.2016.1238483

Shen-Fu, L., Ying-Chen, C., Ray-Neng, C., Ling-Chun, C., Hsiu-O, H., Yu-Han, T., Ming-Thau, S. and Der-Zen, L. (2016). Improving the stability of astaxanthin by microencapsulation in calcium alginate beads. *PLoS ONE 11*(4), 1-10. https://doi.org/10.1371/journal.pone.0153685

Machado, F. R. S., Reis, D. F., Boschetto, D. L., Burkert, J. F. M., Ferreira, S. R. S. and Oliveira, J. V. (2014). Encapsulation of astaxanthin from *Haematococcus pluvialis* in PHBV by means of SEDS technique using supercritical CO_2. *Industrial Crops and Products 54*, 17-21. https://doi.org/10.1016/j.indcrop.2014.01.007

Machado, F. R. S., Trevisol, T. C., Boschetto, D. L., Burkert, J. F. M., Ferreira, S. R. S., Oliveira, J. V. and André, C. (2016). Technological process for cell disruption, extraction and encapsulation of astaxanthin from *Haematococcus pluvialis*. *Journal of Biotechnology 218*, 108-114. https://doi.org/10.1016/j.jbiotec.2015.12.004

Macías-Sánchez, M. D., Serrano, C. M., Rodríguez, M. R. and de la Ossa, E.M. (2008). Extraction of carotenoids and chlorophyll from microalgae with supercritical carbon dioxide and ethanol as cosolvent. *Journal of Separation Science 31*, 1352-1362. https://doi.org/10.1002/jssc.200700503

Martínez-Álvarez, Ó., Calvo, M. M. and Gómez-Estaca, J. (2020). Recent advances in astaxanthin micro/nanoencapsulation to improve its stability and functionality as a food ingredient. *Marine Drugs 18*(8), 1-25. https://doi.org/10.3390/md18080406

Martínez-Preciado, A. H., Silva-Jara, J. M., Flores-Nuño, B. A., Michel, C. R., Castellanos-Haro, M. E. and Macías-Rodríguez, M. E. (2021). Microencapsulation by spray-drying of Manilkara zapota pulp and probiotics (*Lactobacillus fermentum* A15): Assessment of shelf-life in a food matrix. *Revista Mexicana de Ingeniería Química 20*(2), 635-648. https://doi.org/10.24275/rmiq/Alim2166

Mata-Gómez, L., Montañez, J. C., Méndez-Zavala, A. and Aguilar, C. N. (2014). Biotechnological production of carotenoids by yeasts: an overview. *Microbial Cell Factories 13*(1), 12. https://doi.org/10.1186/1475-2859-13-12.

Mezzomo, N., Paz, E. D., Maraschin, M., Martín, Á., Cocero, M. J. and Ferreira, S. R. S. (2012). Supercritical anti-solvent precipitation of carotenoid fraction from pink shrimp residue: Effect of operational conditions on encapsulation efficiency. *Journal of Supercritical Fluids 66*, 342-349. https://doi.org/10.1016/j.supflu.2011.08.006

Mortensen, A., Skibsted, L. H. and Truscott T.G. (2001). The interaction of dietary carotenoids with radical species. *Archives of Biochemistry and Biophysics 385*(1), 13-19. https://doi.org/10.1006/abbi.2000.2172

Muñoz, J., Alfaro, M. D. C. and Zapata, I. (2007). Avances en la formulación de emulsiones.

Grasas y Aceites 58(1), 64-73. http://hdl.handle.net/11441/17634

Naguib, Y. M. A. (2000). Antioxidant activities of astaxanthin and related carotenoids. *Journal of Agricultural and Food Chemistry 48*(4), 1150-1154. https://doi.org/10.1021/jf991106k

Østerlie, M., Bjerkeng, B. and Liaaen-Jensen, S. (2000). Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration of astaxanthin. *Journal of Nutritional Biochemistry 11*(10), 482-490. https://doi.org/10.1016/S0955-2863(00)00104-2

Pan, L., Zhang, S., Gu, K. and Zhang, N. (2018). Preparation Of astaxanthin-loaded liposomes: Characterization, storage stability and antioxidant activity. *CyTA-Journal of Food 16*(1), 607-618. https://doi.org/10.1080/19476337.2018.1437080

Pérez-Alonso, C., Campos-Montiel, R. G., Morales-Luna, E., Reyes-Munguía, A., Aguirre-Álvarez, D. J. and Pimentel-González, D. J. (2015). Stabilization of phenolic compounds from *Opuntia oligacantha* Först by microencapsulation with agave sap (aguamiel). *Revista Mexicana de Ingeniería Química 14*(2), 579-588.

Sedmak, J. J., Weerasinghe, D. K. and Jolly, S. O. (1990). Extraction and quantitation of astaxanthin from *Phaffia rhodozyma*. *Biotechnology Techniques 4*(2), 107-112. https://doi.org/10.1007/BF00163282.

Storebakken, T., Foss, P., Schiedt, K., Austreng, E., Liaaen-Jensen, S. and Manz, U. (1987). Carotenoids in diets for salmonids. IV. Pigmentation of Atlantic salmon with astaxanthin, astaxanthin dipalmitate and canthaxanthin. *Aquaculture 65*(3-4), 279-292. https://doi.org/10.1016/0044-8486(87)90241-9.

Tachaprutinun, A., Udomsup, T., Luadthong, C. and Wanichwecharungruang, S. (2009). Preventing the thermal degradation of astaxanthin through nanoencapsulation. International *Journal of Pharmaceutics 374*(1-2), 119-124. https://doi.org/10.1016/j.ijpharm.2009.03.001

Tibayrenc, P., Preziosi-Belloy, L., Jean-Michel, R. and Ghommidh, C. (2010). Assessing yeast viability from cell size measurements. *Journal of Biotechnology 149*(1-2), 74-80. https://doi.org/10.1016/j.jbiotec.2010.06.019.

Tirado, D.F., Palazzo, I., Scognamiglio, M., Calvo, L., Della Porta G. and Reverchon, E. (2019). Astaxanthin encapsulation in ethyl cellulose carriers by continuous supercritical emulsions extraction: A study on particle size, encapsulation efficiency, release profile and antioxidant activity. *Journal of Supercritical Fluids 150*, 128-136. https://doi.org/10.1016/j.supflu.2019.04.017

Torres-Haro, A., Arellano-Plaza, M., Mateos-Díaz, J. C., Espinosa-Andrews, H. and Castillo-Herrera, G. A. (2022). Non-conventional high-pressure extraction process: A comparative study for astaxanthin recovery from *Xanthophyllomyces dendrorhous*. *International Journal of Food Science and Technology 57*, 1-10. https://doi.org/10.1111/ijfs.15466

Torres-Haro, A., Verdín, J., Kirchmayr, M. R. and Arellano-Plaza, M. (2021a). Metabolic engineering for high yield synthesis of astaxanthin in *Xanthophyllomyces dendrorhous*. *Microbial Cell Factories. BioMed Central 20*(1), 1-17. https://doi.org/10.1186/s12934-021-01664-6

Torres-Haro, A., Gschaedler, A., Mateos-Díaz, J. C., Herrera-López, E. J., Camacho-Ruíz, R. M. and Arellano-Plaza, M. (2021b). Improvement of a specific culture medium based on industrial glucose for carotenoid production by *Xanthophyllomyces dendrorhous*. *Processes 9*(3), 1-16. https://doi.org/10.3390/pr9030429

Wang, H. D. Chen, C. C., Huynh, P. and Chang, J. S. (2015). Exploring the potential of using algae in cosmetics. *Bioresource Technology 184*, 355-362. https://doi.org/10.1016/j.biortech.2014.12.001

Wang, L., Yang, B., Yan, B. and Yao, X. (2012). Supercritical fluid extraction of astaxanthin from *Haematococcus pluvialis* and its antioxidant potential in sunflower oil. *Innovative Food Science and Emerging Technologies 13*, 120-127. https://doi.org/10.1016/j.ifset.2011.09.004

Zapata-Luna, R. L., Davidov-Pardo, G., Pacheco, N., Ayora-Talavera, T., Espinosa-Andrews, H., García-Márquez, E. and Cuevas-Bernardino, J. C. (2023). Structural and physicochemical properties of bio-chemical chitosan and its performing in an active film with quercetin and

Phaseolus polyanthus starch. *Revista Mexicana de Ingeniería Química 22*(2). https://doi.org/10.24275/rmiq/Alim2315

Zhang, C., Chen, X. and Too, H. P. (2020). Microbial astaxanthin biosynthesis: recent achievements, challenges, and commercialization outlook. *Applied Microbiology and Biotechnology 104*(13), 5725-5737. https://doi.org/10.1007/s00253-020-10648-2

Zhou, T., Wang, X., Ju, Y., Shi, C. and Kan, G. (2018). Stability application and research of astaxanthin integrated into food. *Materials Science and Engineering 394*(2). https://doi.org/10.1088/1757-899X/394/2/022007