EFFECTIVENESS OF TILAPIA (*Oreochromis niloticus*) SCALE COLLAGEN IN ß-CAROTENE ENCAPSULATION

EFECTIVIDAD DEL COLÁGENO DE ESCAMAS DE TILAPIA (*Oreochromis niloticus*) EN LA ENCAPSULACIÓN DE ß-CAROTENO

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SUMMARY

The effectiveness of collagen extracted from Nile Tilapia (*Oreochromis niloticus*) fish scale on the encapsulation of β-carotene was evaluated. Two encapsulations were performed by lyophilization: encapsulation with soy protein (EP) and encapsulation with collagen and soy protein (ECP) in 1:4 ratio. The samples were physiochemically characterized, their fluidity properties were evaluated, and mathematical and kinetic models were used to predict the stability of the encapsulated β-carotene stored at 35°C for eight weeks. Collagen extraction yield was 13.85 %, collagen had the following proximal composition: protein 47 %, ash 40 %, fat 9 % and moisture 7.9 %. Initially, ECP showed a higher retention rate (TR = 83.4 %) than EP (TR = 53.3 %), while after storage at 35 °C for eight weeks, β-carotene retention in ECP was 0.84%, while in EP reached only 0.48 %. ECP encapsulation efficiency (66.7 %) was better than that of EP (42.6 %). High cohesiveness and intermediate fluidity were found in EP and intermediate cohesiveness and intermediate fluidity in ECP. According to results, collagen from this source proved to be a functional material for encapsulating β-carotene.

Index words: β-carotene encapsulated, collagen, kinetic model, tilapia scale.

RESUMEN

Se evaluó la efectividad del colágeno extraído de la escama del pez Tilapia del Nilo (*Oreochromis niloticus*) en la encapsulación de β-caroteno. Se realizaron dos encapsulaciones por liofilización: encapsulación con proteína de soya (EP) y encapsulación con colágeno y proteína de soya (EPC) en proporción 1:4. Las muestras se caracterizaron fisicoquímicamente, se evaluaron sus propiedades de fluidez y se utilizaron modelos matemáticos y cinéticos para predecir la estabilidad del β-caroteno encapsulado almacenado a 35 °C durante ocho semanas. Se obtuvo un rendimiento de extracción de colágeno del 13.85 %, el colágeno tuvo la siguiente composición proximal: proteína 47 %, ceniza 40 %, grasa 9 % y humedad 7.9 %. Inicialmente, EPC mostró una mayor tasa de retención (TR = 83.4 %) que EP (TR = 53.3 %), mientras que después del almacenamiento a 35 °C durante ocho semanas, la retención de β-caroteno en EPC fue de 0.84 %, mientras que en EP alcanzó solo 0.48 %. La eficiencia de encapsulación de EPC (66.7 %) fue mejor que la de EP (42.6 %). Se encontró alta cohesividad y fluidez intermedia en EP y cohesividad intermedia y fluidez intermedia en EPC. Según los resultados, el colágeno de esta fuente demostró ser un material funcional para encapsular el β-caroteno.

Palabras clave: β-caroteno encapsulado, colágeno, escama de tilapia, modelo cinético.

INTRODUCTION

β-carotene is a nutrient of great importance for human health, as it has antioxidant properties that help to reduce the risk of neurological or neurodegenerative diseases such as stroke or Alzheimer's disease, as well as stress disorders or cancer (Miękus *et al*., 2019); however, its low absorption capacity and its easy degradation from natural sources has led to the development of microencapsulation methods to improve its stability and bioavailability; thus, allowing to enrich foods (Pino and Aragüez, 2021). Likewise, its use as a nutraceutical ingredient or natural colorant is currently limited by its poor water solubility, high melting point, chemical instability, lipophilic character, and low bioavailability (Gutiérrez *et al*., 2013; Liang *et al*., 2013; Qian *et al*., 2012).

An alternative encapsulating material is collagen extracted from tilapia scales. These scales represent approximately 5 % of the fish live weight and are rich in collagen (41 to 84 %), this content depends on the species, the area in which they were caught, the region and their diet (El-Rashidy *et al*., 2015; Wang *et al*., 2013). The use of polymers in encapsulation is very common because of their good encapsulation properties; in this study collagen was used and soy protein isolate was blended to protect the β-carotene, as it was demonstrated that collagen and other polymer blends offer good protection to antioxidant compounds (Feng *et al*. 2024; Vargas-Muñoz *et al*., 2020). On the other hand, it is important to analyze the degradation kinetics of bioactive compounds in the encapsulates to compare the effect of the use of wall materials and determine the order and the kinetic rate constant of the degradation reaction. The reaction rate constant, in one or two-order reactions, may not be constant during a chemical reaction, so its proportionality will be associated with reaction rate and reactants concentration to predict product shelf-life (Bosch *et al*., 2013; Gaffney and Marley, 2018). The aim of

this study was to evaluate the effectiveness of collagen extracted from Nile tilapia (*Oreochromis niloticus*) scales in the encapsulation of β-carotene and to analyze its degradation kinetics in storage at 35 °C.

MATERIALS AND METHODS

Biological material

Tilapia (*Oreochromis niloticus*) scales were provided by an aquaculture hatchery located in Emiliano Zapata, Veracruz, Mexico. Scales were washed with purified water, dried at the sun for 5 h and stored in an airtight recipient until use. Soy protein isolate (90 % protein) was obtained from Zave Tecnolimentaria S.A de C.V, Mexico. Synthetic β-carotene > 93 % purity, EDTA, NaOH, NaCl and CH_3-COOH ($C_2H_4O_2$) were obtained from Sigma-Aldrich, Mexico.

Extraction of collagen from tilapia scales

Collagen extraction was carried out according to Quintero and Zapata (2017) with modifications. First, fish scales were washed with cold water. Non-collagenous proteins were removed by treating the samples with 0.1 M NaOH in a 1:10 (w/v) ratio under continuous stirring for 24 h, changing the alkaline solution at intervals of 12 h; the scale samples were then treated with 0.5 M EDTA (pH 7.5) at a 1:10 (w/v) ratio for 3 days, changing the solution every 24 h; then, the demineralized materials were washed with cold distilled water. Subsequently, the samples were treated with 0.5 M acetic acid for 3 days with continuous stirring. The supernatant was collected after centrifugation at 18,000 × *g* for 30 min. The supernatant was salted with NaCl to a final concentration of 2 M. Precipitates were obtained by centrifugation at 15,000 × *g* for 30 min. Finally, the precipitates were lyophilized at 0.05 mBar for 2 days (Labconco freeze dryer, Kansas City, MO, USA) and the moisture content was determined.

Collagen chemical composition

Moisture, ash, protein, and fat contents were determined according to the official methods established by the Association of Official Analytical Chemists (AOAC, 1980).

Encapsulation

Two suspensions of 13 % (w/v) and 0.25 % (w/w respect to wall material dry base) of β-carotene concentration were prepared. The first suspension was made with collagen and a soy protein isolate (1:4 ratio), while the second one was made only with the soy protein isolate as wall material; then, the mixtures were homogenized at 15,000 rpm (Oster blender) at 3-min intervals for 12 min, after freezing at -70

°C; finally, samples were freeze dried at -50 °C and 0.05 mBar for 2 days using a freeze dryer (Labconco Freezone, Kansas City, MO, USA). The above description provided two encapsulates, one with soy protein isolate (EP) and the other one with soy protein isolate and collagen (EPC).

Bulk density

Bulk density determination was performed as follows: 1 g of sample was weighed and placed in a 10 mL graduated cylinder, recording the volume displaced by the sample in mL (FEUM, 2014). Density was calculated with Equation 1.

$$P_g = m/v \qquad \text{(Eq. 1)}$$

Where ρ_g is the bulk density, m is the mass of the sample and v is the volume occupied by the sample.

Compacted density

Compacted density was carried out as established by FEUM (2014) with some modifications. One g of the sample was weighed and poured into a 10 mL graduated cylinder, followed by 'tapping', which consisted of raising the cylinder to a height of 10 cm and returning it to the surface, repeating this operation 250 times, recording the final volume occupied. Equation 2 was used for the calculations.

$$P_c = m/v_c \qquad \text{(Eq. 2)}$$

Where ρ_c is the compacted density, m the mass of the sample and v_c the compacted volume.

Particle density

Particle density is the mass of the solid fraction divided by the occupied volume, excluding intergranular spaces (Ambrose *et al.*, 2015). Particle density determination was performed as established by Jinapong *et al.* (2008). One g of sample was placed inside a 10 mL measuring cylinder, then 5 mL of petroleum ether were added and mixed with another 1 mL of ether, and the walls of the cylinder were washed. The particle density was determined using Equation 3.

$$\rho\ particle = \frac{Powder\ weigth\ (g)}{Volume\ ofether\ and\ suspended\ powder\ (mL)\ \text{-}6} \qquad \text{(Eq. 3)}$$

Hausner index

The Hausner index is a fluidity indicator that helps determine powders cohesiveness. HI values > 1.4 indicate high cohesiveness, values between 1.2 and 1.4 an intermediate cohesiveness, and values < 1.2 indicate low cohesiveness (Islam *et al.*, 2017). The Hausner index

determination was carried out using Equation 4.

$$HI = \rho_c / \rho_g \qquad \text{(Eq. 4)}$$

Where *HI* is the Hausner index, ρ_c the compacted density and ρ_g the bulk density.

Compressibility index

The compressibility index, or Car index (*CI*), is a fluidity indicator that determines the proponsity of a powder to compress (Ambrose *et al*., 2015). Compressibility values < 15, 15-20, 20-35, 35-45 and > 45 % represent excellent, good, intermediate, little, and very little fluidity (Islam *et al*., 2017). The Car index was calculated with Equation 5.

$$CI = \frac{\rho_c - \rho_g}{\rho_c} \times 100 \qquad \text{(Eq. 5)}$$

Porosity

The porosity results from the relationship between particle density and compacted density; this value represents potential permeability, as well as aeration through bulk solids (Ambrose *et al*., 2015). The porosity was calculated using Equation 6.

$$\varepsilon = \frac{\rho_p - \rho_c}{\rho_p} \times 100 \qquad \text{(Eq. 6)}$$

Where ε is the porosity, ρ_p the particle density and ρ_c the compacted density (Islam *et al*., 2017).

Assessment of degradation

Samples were incubated at 35°C for eight weeks. The quantification of β-carotene during storage was carried out every week according to Lan *et al*. (2021) with modifications. A β-carotene calibration curve in solution with acetone was performed, with a concentration range between 0 and 1.25 µg mL^{-1} (0.025, 0.5, 0.75, 1.00, 1.25 µg mL^{-1}), read in a spectrophotometer (Genesys 20, Thermo Scientific UV/Vis) at 475 nm. The samples (0.25 g) were diluted, mechanically agitated for 5 min, and filtered before evaluation of β-carotene degradation.

Kinetics of β-carotene degradation

Zero, first and second order equations (Equations 7, 8 and 9, respectively) were used to determine the reaction order followed by the samples and determined by linear regression. Parameters such as the reaction rate constant k, which was equal to the slope value, and the initial concentration $[A]_0$ were calculated.

$$[A] = -kt + [A]_0 \qquad \text{(Eq. 7)}$$

$$ln[A]_{-t} = -kt + ln[A]_0 \qquad \text{(Eq. 8)}$$

$$\frac{1}{[A]_t} = kt + \frac{1}{[A]_0} \qquad \text{(Eq. 9)}$$

Where *[A]* refers to the concentration, $[A]_0$ is the initial concentration, $[A]_t$ is the concentration at an arbitrary time, *t* is the time, and *k* is the reaction rate constant.

Half-life

The half-life of a reaction, $t_{1/2}$, is the time required for the concentration of a reactant to drop to half its original value. Equation 10 was used:

$$t_{1/2} = - ln_2 / k \qquad \text{(Eq. 10)}$$

Where k is the reaction rate constant.

ß-carotene retention

The percentage of ß-carotene retention (TR) in the emulsion was calculated using Equation 11 (Wang *et al*., 2013).

$$TR(\%) = C_a/C_b \times 100 \qquad \text{(Eq. 11)}$$

Where C_b refers to the carotene content in the emulsion and C_a refers to the carotene content in the samples after treatment (powder).

Encapsulation efficiency

Samples of the EP and EPC encapsulates were weighed (0.25 g) and diluted in acetone. The resulting solutions were read spectrophotometrically at 475 nm. Subsequently, the encapsulation efficiency (EE) was calculated from the ratio of the experimental and theoretical encapsulated mass, according to Equation 12.

$$EE (\%) = m_{exp}/m_{teo} \times 100 \qquad \text{(Eq. 12)}$$

Where m_{exp} is the experimentally encapsulated mass (g) and m_{teo} is the theoretically encapsulated mass

Image analysis

Pictures were taken every two weeks of the capsules stored at 35 °C for eight weeks with a camera (Xiaomi, Redmi note 8 pro, 64 MP, Beijing, China) with ultra-high

resolution. Once the storage time was over, image analysis was carried out using the Python 3.7.3 program, performing exponential regressions in Scipy 1.5.2, until obtaining the equation that describes the relationship of RGB (red, green, blue) colors with concentration. Images were converted to RGB colors by PIL 9.2.0.

Statical analysis

All determinations were made by triplicate and each value represents the mean of at least three measurements. Significant differences between means of the parameters studied from EP and EPC samples were determined by t-test analysis (SigmaStat 3.5). An alpha level of 0.05 (P ≤ 0.05) was used to determine significance. The values statistically different were indicated by different superscripts.

RESULTS AND DISCUSSION

Proximal composition of collagen extract

Results of proximal composition of the collagen extract are shown in Table 1. Collagen extraction yield was 13.8 % with a moisture content of 7.87 g/100 g d.s. (dried solids), the collagen ash content was 40 g/100 g d.s. and protein content was of 47 g/100 g d.s. Tilapia collagen extract showed a protein value of 47 %, this value can be close to the amount of pure collagen since collagen is the main

Table 1. Proximal composition of collagen extract.

Proximal composition	Results (g/100 g d.s.)
Moisture content	7.86 ± 0.038
Ash	40 ± 0.008
Fat	9 ± 0.036
Protein	47 ± 0.060

Data are expressed as means ± sd (standard deviation) (n = 3).

rotein extracted by the method used in this work, which correspond to 6.4 % (considering the extraction yield of 13.8 %). The extraction yield of collagen in the literature is reported with low concentration; for example, Pati *et al*. (2010) and Duan *et al*. (2009) achieved yields of 5 and 1.35 %, respectively; however, these results correspond to pure collagen. In this work collagen was not purified.

Flow properties

Table 2 shows the results of flow properties. In the case of bulk density for EP, EPC and collagen extract, EP had the lowest values, while collagen had the highest value of bulk density, indicating that EP has more roughness and voids than EPC, whereas collagen has a smoother surface and fewer cavities than EPC and EP according to Acosta-Domínguez *et al.* (2022) and Lüddecke *et al*. (2021), who demonstrated that the bulk density is proportional to the roughness and surface irregularities in the powders by AFM (Atomic Force Microscopy). The powder bulk density is a significant parameter for packaging and storage. In general, the bulk density of different food powders is in the range of 300 to 800 kg m^{-3}. Food products with lower bulk density are more susceptible to oxidation and subsequent poor storage stability due to increased air between their voids (Koç *et al*., 2017).

The incorporation of collagen to the EP samples increases the bulk density, which is desirable because it reduces storage, packaging and transport costs (Baysan *et al*., 2019). The compacted density was 0.26 g cm^{-3} for EP, 0.29 g cm^{-3} for EPC and 0.66 g cm^{-3} for the extracted collagen. Compaction compresses samples due to forces or pressures, causing a decrease in pore volume; therefore, its value is always higher than bulk density. In this case, collagen showed a higher compacted density than the encapsulated ones, which means the presence of smoother and less porous surfaces in the powders.

Table 2. Flow properties of EP, EPC, and collagen.

Property	EP	EPC	Collagen
Bulk density (kg m^{-3})	180[a] ± 0.002	220[b] ± 0.006	580[c] ± 0.0
Compacted density (kg m^{-3})	260[a] ± 0	290[b] ± 0.006	660[c] ± 0.0
Particle density (g cm^{-3})	1.66[a] ± 0.0	2.5[b] ± 0.0	5.0[c] ± 0.0
Hausner index	1.43[a] ± 0.018	1.34[b] ± 0.070	1.132[c] ± 0.0
Compresibility index (%)	30.26[a] ± 0.904	25.53[b] ± 3.91	11.68[c] ± 0.0
Porosity (%)	84.20[a] ± 0.00	88.05[b] ± 0.25	86.68[c] ± 0.0

Data are expressed as means ± sd (n = 3). Significant differences in each row are indicated by different superscripts (P ≤ 0.05).

The particle density presented values of 1.66, 2.5 and 5 g cm^{-3} for EP, EPC and collagen extract, respectively. This indicates that the mass of EP occupied a larger volume than that of EPC and collagen extract due to the presence of voids on the surface. Physical parameters such as bulk density, particle density and compacted density affect the powder flow properties and storage stability. Also, increasing bulk density reduces the amount of air trapped in the spaces between the particles, promoting a reduction of the risk of oxidation of powders during storage and increasing storage stability (Carneiro *et al.*, 2013). The results showed that the addition of collagen to the encapsulation caused a significant increase in bulk density, as it is known, powders with high bulk density require less storage space, as they reduce the volume of package compared to powders with low bulk density. In this work, collagen incorporation decreased the values of the Hausner index and compressibility index. Results of EP and EPC flow properties were similar to those of Elik *et al.* (2021), who encapsulated carotenoids by freeze drying and spray drying using maltodextrin, pectin and wax-like wall materials. They reported the following results of encapsulated carotenoids: bulk density 192.5 and 240.85 kg m^{-3}, Hausner ratio 1.32 and 1.68, Car index 24.51 and 40.62, for freeze drying and spray drying, respectively. According to Islam *et al.* (2017), compressibility values < 15, 15-20, 20-35, 35-45 and > 45 % represent excellent, good, intermediate, little, and very little fluidity, respectively. *HI* values > 1.4 indicate high cohesiveness, values between 1.2 and 1.4 indicate an intermediate cohesiveness, and values < 1.2 indicate low cohesiveness. Thus, using the classification proposed by Islam *et al.* (2017), in this work a flow with high cohesiveness and intermediate fluidity was obtained for EP, an intermediate flow and cohesiveness for EPC and excellent flow with low cohesiveness for collagen. In this case collagen decreased the cohesiveness of the encapsulation with soy protein isolate. Cohesiveness plays a fundamental role in determining the texture of food, directly influencing its ability to maintain shape and structure. In the case of food powders, cohesiveness has been linked to the water content, wetter powders are more cohesive and difficult to flow; however, the shape of the powder particle, size and interactions also affect cohesiveness and flowability (Düsenberg *et al.* 2023). Porosity of EP, EPC and collagen was 84.2, 88.1 and 86.7 %, respectively. Porosity represents the aeration capacity of bulk solids, resulting from the relationship between particle density and compacted density (Ambrose *et al.*, 2015). Low porosity may be caused by low oxygen permeability, which implies a lower risk of oxidation (Islam *et al.*, 2017). Based on the above, it is necessary to protect EP and EPC powders with a packaging material that protects them from the environmental factors that cause oxidation of β-carotene.

β-carotene degradation in storage

Table 3 shows β-carotene degradation, including storage time, β-carotene concentration, retention and images of encapsulating (a clear decrease in β-carotene content in EP and EPC can be observed). The β-carotene content was calculated using the equation: $y = 0.0013x − 2E-05$; $R^2 = 0.9861$, where y represents β-carotene concentration (mg/g d.s.).

After eight weeks, β-carotene content in EP was reduced from 1.06 to 0.009 mg/g d.s., while in EPC reduction was from 1.66 to 0.016 mg/g d.s. Retention of β-carotene in EP was significantly lower than in EPC prior to storage. The retention of β-carotene in EP and EPC was 53.3 and 83.4 %, respectively. After storage at 35 °C for eight weeks, β-carotene retention was 0.84 % in EPC and 0.48 % in EP. encapsulation efficiency of EPC (66.71 %) was better than that of EP (42.61 %), which may be caused because collagen has a higher affinity for carotenoids than soy protein. As can be seen, both samples showed a large loss in β-carotene content, mainly due to storage temperature; however, it is evident from these data that EPC presented a higher concentration of β-carotene than EP during storage conditions from start to finish.

Kinetics of the degradation of β-carotene in the storage

Figure 1 shows β-carotene concentrations in EPC and EP during storage at 35 °C. The data points were adjusted with kinetic models of 0, 1 and 2 orders.

According to Gaffney and Marley (2018), the reaction rate changes when reagents concentrations change. The way in which these concentrations change affects reaction speed. This is determined experimentally by measuring reaction rates as the concentrations of the reagents vary. The result is a constant of proportionality between the reaction rate and the reagents concentration. The order of a reaction in a kinetic model is a parameter that provides a mathematical description of time or concentration dependence. The best known models referring to shelf-life studies are zero reactions that represent a linear evolution (retention or degradation) of the parameter, while first and second order reactions represent an exponential evolution of the parameter (Bosch *et al.*, 2013).

In Figure 1 it is possible to see that the linearity of regression in the natural logarithmic scale showed that the degradation of β-carotene at 35 °C in the EP sample follows a first-order kinetics, where the value of the correlation coefficient indicated a good adjustment ($R^2 = 0.9945$). The kinetic constant was k = 0.6243, using this value to determine the half-life time, provided a value of

Tabla 3. Degradation of EPC and EP at 35 °C.

Time (weeks)	EPC			EP		
	Concentration (mg/g d.s.)	Retention (%)	Images	Concentration (mg/g d.s.)	Retention (%)	Images
0	1.66 ± 0.04	83.39		1.065 ± 0.047	53.267	
2	0.57 ± 0.11	28.76		0.34 ± 0.0	17.32	
4	0.14 ± 0.004	7.14		0.09 ± 0.0073	4.54	
6	0.029 ± 0.004	1.48		0.018 ± 0.006	0.9389	
8	0.016 ± 0.002	0.84		0.009 ± 0.001	0.48	

Data are expressed as means ± sd (n = 3).

7.77 days. Also, in Figure 1 it is possible to see that the linearity of the regression in the natural logarithmic scale showed that the degradation of β-carotene at 35 °C in EPC followed a first-order kinetics, where the correlation coefficient value indicated a good adjustment (R^2 = 0.9924) as well. The kinetic constant was k = 0.6131, using this value to determine the half-life time a value of 7.91 days was computed. Similar results were obtained by Kong *et al.* (2018), who encapsulated β-carotene in amylose/starch complexes with ascorbyl palmitate (ASCP), their results showed that degradation follows a first-order kinetic in all their treatments; in addition, they obtained the half-life time of 9.59 days for their β-carotene microcapsules stored at 30 °C for six weeks. This value is similar to that obtained in this investigation.

Image analysis

The results of the imagen analysis are showed in equations *yEP* and *yEPC*, both describe the relationship between color and β-carotene concentration of EP and EPC samples (0.25 g), respectively.

$$yEP = exp[654.08875069\, x_1 + (-454.74984032)\, x_0 + (-6769.05218523)\, x_0^2 x_1^2 + 9772.17853955\, x_0^4 x_1]$$

$$yEPC = exp[32.93805925\, x_0^2 + (-9.4069532)\, x_1^1 + (-5.96047463)\, x_2^2 + (-117.2297927)\, x_1^4]$$

Where: x_0 is red (r), x_1 is green (g) and x_2 is the blue of RGB (red, green and blue) scale of the photos taken, and y is the concentration of β-carotene.

Table 4 shows the scale of RGB variables determined by equations *yEP* and *yEPC*, and the concentration values obtained by imagen analysis. The error rate of equations *yEP* and *yEPC* was 7.2 and 1.5 %, respectively, which corroborates that the equations had a good fit determining the concentration of β-carotene in the EP and EPC samples during six weeks of storage.
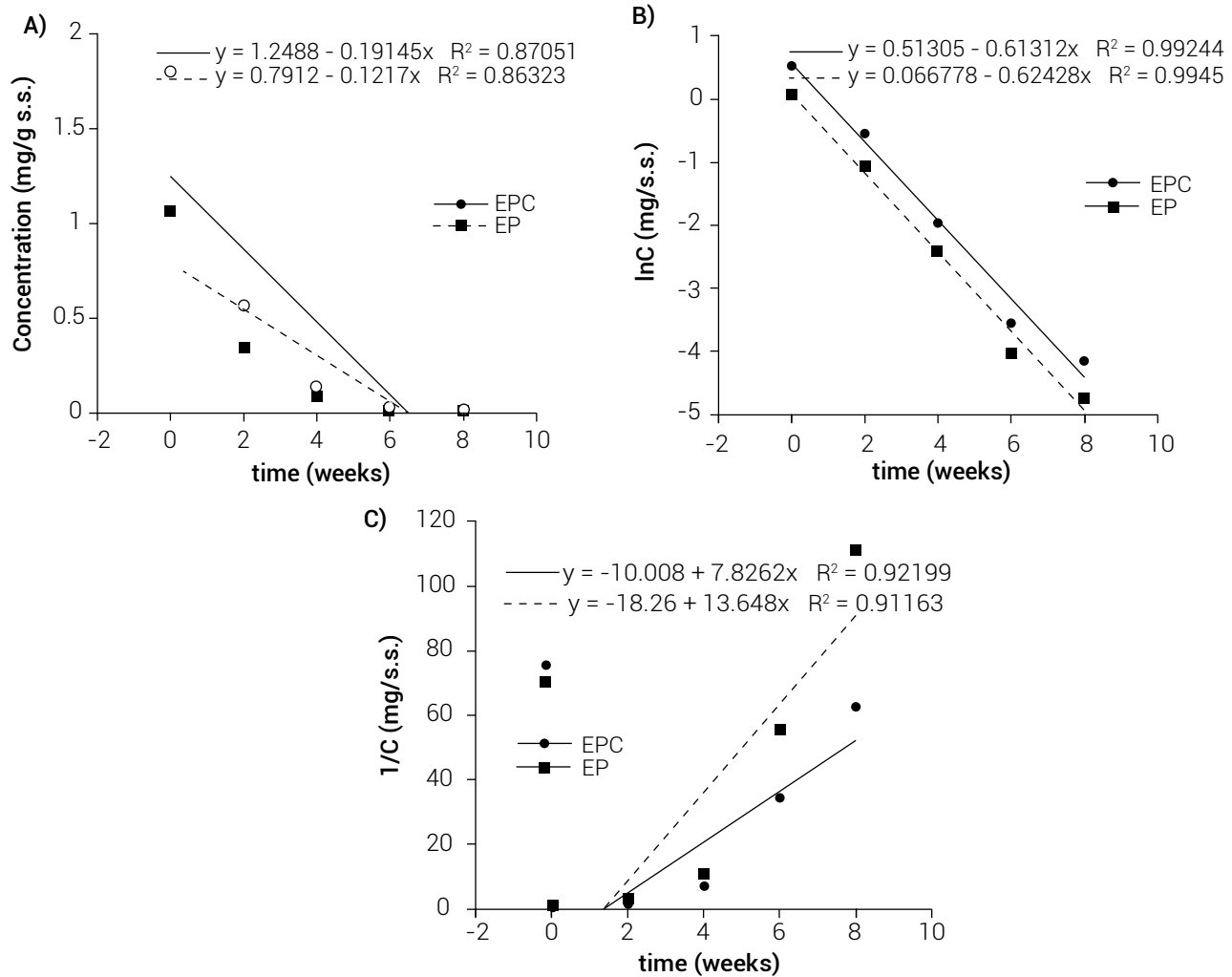
A)

y = 1.2488 - 0.19145x R^2 = 0.87051
y = 0.7912 - 0.1217x R^2 = 0.86323

EPC
EP

B)

y = 0.51305 - 0.61312x R^2 = 0.99244
y = 0.066778 - 0.62428x R^2 = 0.9945

EPC
EP

C)

y = -10.008 + 7.8262x R^2 = 0.92199
y = -18.26 + 13.648x R^2 = 0.91163

EPC
EP

Figure 1. Adjustment of kinetic models of β-carotene degradation during storage at 35°C A) zero, B) one, C) two orders.

Table 4. Variables determined by equation and of RGB scale.

Time (weeks)	EP				EPC				
	x_0	x_1	C (mg/0.25 g d.s)	C (mg/g d.s)	x_0	x_1	x_2	C (mg/0.25 g d.s)	C (mg/g d.s)
0	0.464	0.408	0.266	1.065	0.464	0.404	0.419	0.416	1.667
2	0.477	0.427	0.086	0.346	0.460	0.425	0.425	0.143	0.575
4	0.447	0.424	0.022	0.091	0.465	0.455	0.443	0.035	0.142
6	0.425	0.475	0.0006	0.0027	0.426	0.458	0.479	0.0076	0.0306

x_0, x_1, x_2 are the variables of RGB scale that represent the red, green, and blue color, respectively. EP: soy protein encapsulation, EPC: soy protein+collagen encapsulation. C: Concentration.

CONCLUSIONS

Collagen proved to be a functional material for encapsulating β-carotene, as EPC has a higher encapsulation efficiency and retention percentage than EP at 35 °C for the eight weeks span of this work. EP and EPC followed a first order kinetics and had a similar half-life as both presented similar kinetic constants. Also, EP was classified as a powder with high cohesiveness and intermediate fluidity, while EPC as a powder with intermediate cohesiveness and intermediate flow, so collagen decreased cohesiveness and increase bulk density of β-carotene encapsulate with soy protein isolate. On the other hand, image analysis provided an equation to predict the content of β-carotene encapsulated in the collagen and soy protein isolate matrix.

BIBLIOGRAPHY

Acosta-Domínguez L., Y. Cocotle-Ronzón, L. Alamilla-Beltrán and E. Hernández-Martínez (2021) Effect of a cryogenic treatment in the microstructure, functional and flow properties of soy protein isolate. *Food Hydrocolloids* 119:106871, https://doi.org/10.1016/j.foodhyd.2021.106871

Ambrose R. P. K., S. Jan and K. Siliveru (2015) A review on flow characterization methods for cereal grain-based powders. *Journal of the Science of Food and Agriculture* 96:359-364, https://doi.org/10.1002/jsfa.7305

AOAC, Association of Official Analytical Chemists (1980) Official Methods of Analysis. 13th edition. Association of Official Analytical Chemists. Washington, D. C., USA. 1038 p.

Baysan U., F. Elmas and M. Koç (2019) The effect of spray drying conditions on physicochemical properties of encapsulated propolis powder. *Journal of Food Process Engineering* 42:e13024, https://doi.org/10.1111/jfpe.13024

Bosch V., A. Cilla, G. García-Llatas, V. Gilabert, R. Boix and A. Alegría (2013) Kinetics of ascorbic acid degradation in fruit-based infant foods during storage. *Journal of Food Engineering* 116:298-303, https://doi.org/10.1016/j.jfoodeng.2012.12.003

Carneiro H. C. F., R. V. Tonon, C. R. F. Grosso and M. D. Hubinger (2013) Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering* 115:443-451, https://doi.org/10.1016/j.jfoodeng.2012.03.033

Duan R., J. Zhang, X. Du, X. Yao and K. Konno (2009) Properties of collagen from skin, scale and bone of carp (*Cyprinus carpio*). *Food Chemistry* 112:702-706, https://doi.org/10.1016/j.foodchem.2008.06.020

Düsenberg B., J. Schmidt, I. Sensoy and A. Bück (2023) Flowability of plant based food powders: Almond, chestnut, chickpea, coconut, hazelnut and rice. *Journal of Food Engineering* 357:111606, https://doi.org/10.1016/j.jfoodeng.2023.111606

Elik A., D. K. Yanık and and F. Göğüş (2021) A comparative study of encapsulation of carotenoid enriched-flaxseed oil and flaxseed oil by spray freeze-drying and spray drying techniques. *LWT - Food Science and Technology* 143:111153, https://doi.org/10.1016/j.lwt.2021.111153

El-Rashidy A. A., A. Gad, A. E. H. G. Abu-Hussein, S. I. Habib, N. A. Badr and A. A. Hashem (2015) Chemical and biological evaluation of Egyptian Nile Tilapia (*Oreochromis niloticas*) fish scale collagen. *International Journal of Biological Macromolecules* 79:618-626, https://doi.org/10.1016/j.ijbiomac.2015.05.019

Feng Y., L. Niu, D. Li, Z. Zeng, C. Sun and J. Xiao (2024) Effect of calcium alginate/collagen hydrolysates beads encapsulating high-content tea polyphenols on quality characteristics of set yogurt during cold storage. *LWT - Food Science and Technology* 191:115608, https://doi.org/10.1016/j.lwt.2023.115608

FEUM, Farmacopea de los Estados Unidos Mexicanos (2014) Farmacopea Herbolaria de los Estados Unidos Mexicanos. 12a edición. Secretaría de Salud. México.

Gaffney J. S. and N. A. Marley (2018) Kinetics and the rate of chemical reactions. *In*: General Chemistry for Engineers. J. S. Gaffney and N. A. Marley (eds.). Elsevier. Amsterdam, The Netherlands. pp:283-317, https://doi.org/10.1016/B978-0-12-810425-5.00009-6

Gutiérrez F. J., S. M. Albillos, E. Casas-Sanz, Z. Cruz, C. García-Estrada, A. García-Guerra, ... and M. L. Mussons (2013) Methods for the nanoencapsulation of β-carotene in the food sector. *Trends in Food Science & Technology* 32:73-83, https://doi.org/10.1016/j.tifs.2013.05.007

Islam M. Z., Y. Kitamura, M. Kokawa, K. Monalisa, F. H. Tsai and S. Miyamura (2017) Effects of micro wet milling and vacuum spray drying on the physicochemical and antioxidant properties of orange (*Citrus unshiu*) juice with pulp powder. *Food and Bioproducts Processing* 101:132-144, https://doi.org/10.1016/j.fbp.2016.11.002

Jinapong, N., M. Suphantharika and P. Jammong (2008) Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration. *Journal of Food Engineering* 84:194-205, https://doi.org/10.1016/j.jfoodeng.2007.04.032

Koç M., U. Baysan, E. Devseren, D. Okut, Z. Atak, H. Karataş and F. Kaymak-Ertekin (2017) Effects of different cooking methods on the chemical and physical properties of carrots and green peas. *Innovative Food Science and Emerging Technologies* 42:109-119, https://doi.org/10.1016/j.ifset.2017.06.010

Kong L., R. Bhosale and G. R. Ziegler (2018) Encapsulation and stabilization of β-carotene by amylose inclusion complexes. *Food Research International* 105:446-452, https://doi.org/10.1016/j.foodres.2017.11.058

Lan M., Y. Fu, H. Dai, L. Ma, Y. Yu, H. Zhu, ... and Y. Zhang (2021) Encapsulation of β-carotene by self-assembly of rapeseed meal-derived peptides: Factor optimization and structural characterization. *LWT - Food Science and Technology* 138:110456, https://doi.org/10.1016/j.lwt.2020.110456

Liang R., Q. Huang, J. Ma, C. F. Shoemaker and F. Zhong (2013) Effect of relative humidity on the store stability of spray-dried beta-carotene nanoemulsions. *Food Hydrocolloids* 33:225-233, https://doi.org/10.1016/j.foodhyd.2013.03.015

Lüddecke A., O. Pannitz, H. Zetzener, J. T. Sehrt and A. Kwade (2021) Powder properties and flowability measurements of tailored nanocomposites for powder bed fusion applications. *Materials and Design* 202:109536, https://doi.org/10.1016/j.matdes.2021.109536

Miękus N., A. Iqbal, K. Marszałek, C. Puchalski and A. Świergiel (2019) Green chemistry extractions of carotenoids from *Daucus carota* L. Supercritical carbon dioxide and enzyme-assisted methods. *Molecules* 24:4339, https://doi.org/10.3390/molecules24234339

Pati F., B. Adhikari and S. Dhara (2010) Isolation and characterization of fish scale collagen of higher thermal stability. *Bioresource Technology* 101:3737-3742, https://doi.org/10.1016/j.biortech.2009.12.133

Pino J. A. y Y. Aragüez (2021) Conocimientos actuales acerca de la encapsulación de aceites esenciales. *Revista CENIC Ciencias Químicas* 52:10-25.

Qian C., E. A. Decker, H. Xiao and D. J. McClements (2012) Physical and chemical stability of β-carotene-enriched nanoemulsions: influence of pH, ionic strength, temperature, and emulsifier type. *Food Chemistry* 132:1221-1229, https://doi.org/10.1016/j.foodchem.2011.11.091

Quintero J. y J. E. Zapata (2017) Optimización de la extracción del colágeno soluble en ácido de subproductos de tilapia roja (*Oreochromis* spp) mediante un diseño de superficie de respuesta. *Información Tecnológica* 28:109-120, https://doi.org/10.4067/S0718-07642017000100011

Vargas-Muñoz D. P., L. A. N. de Oliveira, L. C. da Silva, H. T. Godoy and L. E. Kurozawa (2020) Storage stability of 5-caffeoylquinic acid in powdered cocona pulp microencapsulated with hydrolyzed collagen and maltodextrin blend. *Food Research International*

137:109652, https://doi.org/10.1016/j.foodres.2020.109652

Wang B., L. Li, C. F. Chi, J. H. Ma, H. Y. Luo and Y. F. Xu (2013) Purification and characterization of a novel antioxidant peptide derived from blue mussel (*Mytilus edulis*) protein hydrolysate. *Food Chemistry* 138:1713-1719, https://doi.org/10.1016/j.foodchem.2012.12.002