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Effects of Encapsulating Agents and Temperature in the Microencapsulation of Carotenogenic Extracts from *Sporidiobolus Salmonicolor* CBS 2636 and Storage Stability

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Abstract: The study aimed to encapsulate carotenogenic extracts produced by *Sporidiobolus salmonicolor* yeast using a spray dryer. The carotenogenic extract was characterized in relation to the total carotenoids and antioxidant activity. To evaluate the effects of the encapsulating agents (gum Arabic - AG and maltodextrin - MD) and temperature on the encapsulation efficiency (EE), an experimental design methodology was used. The microparticles were characterized in terms of product yield, EE, water activity, moisture content, color parameters (L*, a*, b*, and C *), particle microstructure, and storage stability in different conditions (amber and transparent glass). The extract showed 3852.75 µg/L carotenoid content and 1.11 mM of Trolox/g antioxidant activity. The greatest EE was 60% using 5% (v/v) extract, 2% (w/v) Tween 80, 90% (v/v) phosphate buffer (pH 7.0), drying air temperature of 130°C and 1:1 AG and MD (25g/L). The microcapsules showed 0.206 water activity, 1.1% moisture content, and color indexes of 72.57 L*, 22.60 chroma C*, and particle size of 3.48 µm (± 1.35). The carotenoids encapsulated presented stability of 63.50 and 50.57% stored for 40 days at 22 ± 2ºC in amber and transparent glass, respectively. Thus, encapsulation using a spray dryer employing the matrix composed of AG and MD is a promising alternative for preserving carotenogenic pigments and their application in food.

Keywords: carotenogenic extracts; gum arabic; maltodextrin; encapsulation efficiency.

1. Introduction

The industry has focused on dye application and maintaining their characteristics during processing and storage. Such compounds can be defined as substances incorporated in food in order to confer and intensify the color (making them more visually attractive), restore and/or standardize the original color (which was lost during processing), helping the consumer to identify the aroma and taste normally associated with the product. Generally, it can be classified as a natural or synthetic pigment [1–3]. A very important natural additive is carotenoid, a fat-soluble pigment responsible for colors that vary between yellow, orange, and red, which can be produced as a secondary metabolite of vegetables, algae, fungi, and some bacteria. These are the main pigments present in the human diet [4,5]. In 2017, approximately 1117 natural carotenoids from 683 sources were isolated and identified (8 arch; 170 bacteria and 505 eukaryotes) [6]. The global market for carotenoids has been growing, reaching US$ 1.5 billion

in 2017, and is expected to reach US$ 2.0 billion in 2022, at a compound annual growth rate (CAGR) of 5.7% in the period of 2017-2022 [7].

Obtaining pigments from natural sources by biotechnological processes has become attractive due to searching for natural products and replacing synthetic pigments [5,8]. Search for natural products to replace synthetic pigments to be an attractive niche of new biotechnological processes and applications. Biotechnological production of carotenoids presents the advantages of sustainability and cost-effectiveness and is considered natural and safe. Processing does not depend on climate change, soil composition, seasonality, or the time spent growing crops for harvesting and extraction. In addition, the process can be fully controlled and optimized to increase yields while reducing overall processing costs when using, for example, inexpensive substrates such as agro-industrial residues [5,9].

Carotenoids are used in food, nutraceutical, and pharmaceutical preparations due to their coloring, provitamin A, and antioxidant activity, which act to scavenge oxygen radicals and reduce oxidative stress [10–12]. However, the same characteristics that make carotenoids interesting for incorporation in food products create challenges regarding their conservation and stability, mainly due to susceptibility to isomerization and oxidation under the action of oxygen, light, and high temperatures, which may cause changes in color and loss of biological activity. In addition, have low water solubility, which makes them difficult their use in some food matrices [10,13].

One approach to bypass these processes is using the spray dryer microencapsulation technique to reduce the instability of these compounds and promote their gradual release in a controlled manner under specific conditions. Thus, it is possible to protect sensitive substances due to physical insulation due to the formation of membranes or walls that involve the particles of the encapsulated material, which proteins, lipids, or carbohydrates can constitute, as well as natural or synthetic polymers [14–19].

Polymeric carbohydrates with high activation energy prevent thermal and oxidative stress during storage. Among them, maltodextrin is widely used as a coating material due to its low cost, good solubility, non-toxicity, low viscosities even with high solids content, and easy availability [20–22]. However, has a low emulsification capacity, so it is preferable to use it in combination with other carrier agents, such as gum arabic, a dry exudate material obtained from *Acacia Senegal* (*L.*), constituted mainly by high molecular weight polysaccharides and their salts, which on hydrolysis produce arabinose, galactose, rhamnose, and glucuronic acid. At low levels (1 and 10%), the gum arabic acts as a film and moisture stabilizer [20,21,23].

Thus, considering that carotenoids are susceptible to loss of nutritional, biological, and technological properties when exposed to air, light, heat, and acids, the objective of this work was to evaluate the effects of the encapsulating agents (gum Arabic - AG and maltodextrin - MD). Temperature in the microencapsulation of carotenogenic extracts produced by *Sporidiobolus salmonicolor* CBS 2636 using a spray dryer. The storage stability of encapsulated carotenoids stored at 22ºC ± 2ºC in amber and transparent glass was also evaluated.

2. Materials and Methods

2.1. Microorganism and inoculum.

The *Sporidiobolus salmonicolor* CBS 2636 yeast (*Centraalbureau voor Schimmelcultures*, Holland) was used to produce carotenoids. The culture and inoculum preparation was performed according to Valduga *et al*. [24] and Colet *et al*. [25].

2.2. Production and recovery of carotenoids.

The bioproduction of carotenoids in a semi-continuous system was carried out in a bioreactor, according to Colet *et al*. [25]. Extraction and recovery of total carotenoids were performed according to Valduga *et al*. [24,26], with some modifications. After bioproduction, the fermented medium was centrifuged (model D-78532, Hettich Zentrifugen) at 4°C, 4534 xg for 10 min. Then, successive centrifugations (4°C, 4534 x g, 10 min) were performed with 10 mL distilled water, followed by 10 mL petroleum ether, to remove residual glycerol and subsequently with 10 mL of distilled water. Cell disruption was made with successive macerations using liquid nitrogen. Then, added dimethylsulfoxide (DMSO) was in a ratio 2:1 (DMSO:cell, v/v), heated (Unique UltraSonic Cleaner, model USC-1800A, Brazil) at 55°C for 30 min with periodic homogenization in a Digital Vortex Shaker (KASVI, model K40-10208, Brazil). Subsequently, was added 10 mL acetone:methanol mixture (7:3 v/v) and centrifuged at 4°C, 4534 x g, 10 min (MPW-351R refrigerated Laboratory Centrifuge). The supernatant was collected, and successive extractions were performed with the acetone:methanol mixture until the cells were colorless. The extract was solubilized in methanol to adjust the volume in a volumetric flask. The carotenogenic extract was characterized in terms of total carotenoids and antioxidant activity. In addition, this extract was used for encapsulation.

2.3. Characterization of the carotenogenic extract.

Total carotenoids (TC): The concentration of total carotenoids was estimated by measuring the absorbance value at 448 nm, described by Davies [27]. The extinction coefficient for β-carotene used was related to methanol $E^{1\%}_{1cm}$= 2550 [28]. The concentration of carotenoids was expressed in terms of total carotenoids (µg/L).

2.4. Antioxidant activity.

The ability to scavenge the 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+}$) cation radical was determined according to the methodology described by Rufino *et al*. [29] with adaptations. First, the carotenogenic solution was lyophilized at -40°C for 48 h to obtain the dry extract. Next, 0.1 g of dry extract was diluted in DMSO, and the volume was completed in a volumetric flask (5 mL) with ethyl alcohol (Quimex, Brazil). The solution was homogenized and transferred to an amber glass bottle. From the extract, 1250 to 20000 mg/L concentrations were prepared with standard Trolox solution and volume adjusted with ethyl alcohol. 20 µL of each extract dilution was transferred to test tubes with 2.0 mL of the ABTS radical and homogenized in a tube shaker. After 6 min of mixing, absorbances were recorded in a spectrophotometer at 734 nm. The ethyl alcohol was used as a blank. The results were expressed in µM Trolox /grams of carotenoid extract.

2.5. Carotenoid encapsulation.

The carotenogenic extract was concentrated in a rotary evaporator (IKA model RV 10 D, São Paulo, SP Brazil), protected with aluminum foil, at 65ºC, 165 rpm, and 600 mmHg. The wall materials used in the encapsulation were maltodextrin - MD (Globe ® A 1910 P.A. agglomerated maltodextrin, with dextrose equivalent of 20D (Ingredion ®)) and pure powdered Gum Arabic - AG (P.A. 85 % (Vetec ®, Brazil). Initially, the wall matrix was defined as maltodextrin: Gum Arabic in different proportions (0:1; 1:0; 1:1; 1:10 to 1, 5 and 10%) and Tween 80 (0, 2 and 4%) [30–32], extract (5, 10 and 15%) and drying temperature (100, 130 and 160ºC). For the tests, gum arabic and maltodextrin were dissolved in 45 mL phosphate buffer solution (pH 7.0) in an ultrasonic bath (Unique UltraSonic Cleaner, USC-1800A, Brazil), added 2 g of Tween 80 and dissolved in an ultrasonic bath at 50°C. Subsequently, 5 mL of carotenoid extract was added to the mixture, and the volume was completed with phosphate buffer. The drying by atomization was performed in a spray dryer (LabPlant, model SD-05, USA) operating in co-current, equipped with an injector nozzle (0.5 mm diameter), atomizing pressure from 0.08 to 0.12 bar and an average flow rate feed of 5.83 mL/min. The solution (concentrated extract of carotenoids/encapsulating materials/phosphate buffer solution pH 7.0/Tween 80) was kept under stirring in a magnetic stirrer (Fisatom, model 752A, Brazil) and pumped to the top of the atomizer, where it came into contact with the heated air in the drying chamber at the predefined temperature in the tests.

Subsequently, a rotatable central composite design (DCCR) 2^3 (quintuplicate of the central point - totalizing 19 experiments) was carried out evaluating the independent variables temperature (113 to 147ºC), AG (12.4 to 37.6 g/L), and MD (12.4 to 37.6 g/L). Each parameter of the independent variable was evaluated at three levels (low, central, and high), which were referred to as − 1, 0, and − 1, respectively. The fixed variables were the concentrated extract volume of carotenoids (5% v/v), phosphate buffer solution pH 7.0 (90% v/v), and concentration of Tween 80 (2% w/v). The dependent variables (responses) were encapsulation efficiency (EE), yield, L*, a*, b*, and chroma C* color indices, water activity (Wa), and moisture content. From the maximized condition in terms of EE, the morphology and average particle diameter were evaluated.

2.6. Characterization of the encapsulate.

2.6.1. Yield.

The yield (Y) of the powder encapsulation was determined using the total mass of solids before microencapsulation and the total mass of solids obtained after microencapsulation, expressed in percentage (%Y).

2.6.2. Encapsulation efficiency.

*T*he encapsulation efficiency (%EE) was based on the quantification of the superficial and internal carotenoids of the capsule since carotenoids are lipophilic and soluble in hexane, the matrix maltodextrin and gum arabic being soluble in phosphate buffer and insoluble in hexane [33]. The superficial carotenoids were quantified weighing 0.1 g of sample in centrifuge tubes, next added 5 mL of hexane, and homogenized in a vortex mixer (Kasvi, model K40-10208, Brazil) for10 s at 500 rpm, subsequently centrifuged at 10732 xg, 10 min at 25°C. The supernatant extract was separated to quantify the total superficial carotenoids (non-

encapsulated). To quantify the internal carotenoids, 5 mL phosphate buffer (pH 7.0) was added to the fraction that was left in the tube (a white precipitate) and vortexed for 3 min at 3000 rpm. Then, the tube was placed in an ultrasonic bath (Unique UltraSonic Cleaner, model: USC-1800A, Brazil) at 50°C for 4 min. Afterward, 10 mL of hexane was added, homogenized by vortexing, and centrifuged at 10732 x g, 25°C for 15 min. The supernatant extract was separated to quantify the total carotenoids of the internal fraction (encapsulated). The result was expressed as a percentage of encapsulation efficiency (%EE), as expressed in Equation 1 [34].

$$\%EE = \frac{\text{Total carotenoids} - \text{superficial carotenoids}}{\text{total carotenoids}} x\ 100 \quad (1)$$

where total carotenoids are the sum of the internal carotenoids (multiplied by the dilution) with the superficial carotenoids.

2.6.3. Morphological and mean particle diameter.

The morphological images were obtained by scanning electron microscopy (SEM) (Zeiss, model EVO LS25, Germany) at an operating voltage of 10kV. The capsule surface was previously covered with a gold layer with a metallizer (Sputter Coater SCD 050 - Balzers). The average particle diameter was measured by the Software Size Meter (version 1.1) using around 100 particles observed in the photomicrographs of the experiments.

2.7. Storage study.

The best condition from DCCR 2^3 in terms of encapsulation efficiency was evaluated regarding the stability of encapsulated carotenoids stored at 22ºC ± 2ºC in amber and clear glass. In the samples, the content of total carotenoids, L*, a*, b*, and chroma C*, moisture, and water activity were evaluated weekly. Results were expressed in terms of relative specific carotenoid concentration (%), relative L* color (%), and relative C* chroma (%).

2.8. Mathematical modeling of microcapsule stability.

The kinetic models (zero, one, and two order) [35] and Weibull [36] were adjusted for the concentration of specific carotenoids as a function of time, which are expressed in Equations 2, 3, 4, and 5, respectively.

$$[CE(t)] = [CE]_0 - kt \quad (2)$$

$$[CE(t)] = [CE]_0\ exp(k_0 + (-kt) \quad (3)$$

$$[CE(t)] = \frac{[CE]_0}{1+kt[CE]_0} \quad (4)$$

$$[CE(t)] = [CE]_0\ exp(-k(t^{\gamma})) \quad (5)$$

where [CE(t)] is the concentration of specific carotenoids at time t, $[CE]_0$ is the initial concentration of specific carotenoids, k is the reaction rate constant, γ is a shape parameter, and *exp* corresponds to the exponential function.

2.9. Analytical methodologies.

2.9.1. Total carotenoids.

The total carotenoids from the extracts were quantified by reading in a spectrophotometer (Pro-Analysis, model UV-1600, Santa Clara, CA) at 450 nm, using hexane as blank.

2.9.2. Specific carotenoids.

The determination of the encapsulated specific carotenoids was performed according to Equation 6 [27].

$$SC = \frac{ABS \times V \times 10^6}{E^{1\%}_{1cm} \times 100 \times m} \quad (6)$$

where SC is the concentration of specific carotenoids (µg/g), ABS is the mean absorbance read at 450 nm, V is the sample volume (mL), $E^{1\%}_{1cm}$ (2590) related to the extinction coefficient used (β-carotene) for hexane, and m is sample mass (g).

2.9.3. Water activity.

The water activity of the microparticles was determined with Water Activity Meter equipment (Novasina model AG - CH-8853, Lachen, Switzerland) after calibration and previous stabilization of the samples at 27°C.

2.9.4.Moisture.

Moisture was quantified using an infrared balance (Marconi, model ID200, Brazil). The automatic drying mode was programmed in which the material was kept at 105°C until it reached a constant mass [37].

2.9.5. Color index (L*, a*, b*, and chroma C*).

The values of L*(lightness), a*+ (a tendency to be redder) e b*+ (a tendency to be more yellow) were obtained in a colorimeter (Minolta Chroma Meter, model CR-400, Japan). The chroma index (C*) was calculated using the values obtained directly from the colorimeter. This parameter is related to saturation, which directly refers to the concentration of the coloring element and represents a quantitative attribute for intensity; that is, a high value is related to great color saturation [38].

2.10. Statistical analysis.

All the experiments were carried out in triplicate, as well as triplicate of the analysis. Results obtained in experimental design were performed considering a 95% confidence level ($p \leq 0.05$), using the software Statistica 5.0 (StatSoft Inc®, USA).

3. Results and Discussion

3.1. Concentration of carotenoids and antioxidant activity of the extract.

The concentration of total carotenoids was 3852.75 µg/L in a culture medium composed of 80 g/L of crude glycerol, 80 g/L of steep corn water, and 20 g/L of rice parboiling water. The carotenogenic extract showed antioxidant activity by eliminating the ABTS radical cation of 1.11 mM Trolox/g. The Trolox equivalent value (mM) was similar to that obtained by Miller *et al*. [39], 1.9 mM/g for β-carotene and 1.4 mM/g for Zeaxanthin. However, values of 2.57 mM/g [40] and 1.4 mM/g [41] were found for β-carotene, the latter being similar to the values obtained in the present study. This is justified because the major carotenoid produced by *S. salmonicolor* in a semi-continuous system is all-trans-β-carotene (85.2%) [25]. The ability of carotenoids to deactivate the ABTS radical increases with the extension of the conjugated double bond. However, the presence of cyclic end groups reduces the contribution of the double bond in the radical deactivation capability [39].

3.2. Carotenoid encapsulation.

3.2.1. Definition of matrix composition and drying conditions.

Table 1 presents the results of the encapsulation efficiency (%EE) of assays performed, aiming to define the levels of the RCCD 2^3 variables (Table 2), varying the concentration of extract, wall material (MD:AG) and Tween 80, as well as the drying temperature in the spray drier. In Run 1, it was not possible to evaluate the %EE, because, at 100°C, the material did not dry out. However, at 160ºC (Run 10), the lowest %EE (15.3%) was observed due to the degradation of carotenoids by the action of high temperature.

In Run 2, using 2% Tween 80, an increase in %EE is observed; this occurred due to the high percentage of surfactant added during the encapsulation process, which helped the interactions between the components through attraction, repulsion, and electrical charges, easing the formation of chemical bonds between the polymer in the wall [32]. The great amount of extract (Runs 5 and 6) did not result in a high %EE, and this is due to a deficiency of wall material, where part of the carotenoids remains on the matrix surface, which is degraded by the action of temperature, decreasing the EE (%). A low efficiency was observed in Runs 8 and 9 using isolated wall material.

Table 1. Encapsulation efficiency (%EE) as a function of spray drying temperature, extract concentration, Tween 80, and MD:AG ratio of wall material.

Run	T (ºC)	Carotenogenic extract (%)	Wall material (%) (MD:AG)	Tween 80 (%)	%EE*
1	100	5	1 (1:1)	2	-
2	130	5	5 (1:1)	2	$59.9^{a} \pm 2.04$
3	130	5	5 (1:1)	-	$24.5^{e} \pm 1.22$
4	130	5	5 (1:1)	4	$35.6^{c} \pm 1.78$
5	130	10	5 (1:1)	2	$29.5^{d} \pm 1.27$
6	130	15	5 (1:1)	2	$32.5^{cd} \pm 0.62$
7	130	5	10 (1:1)	2	$40.0^{b} \pm 1.78$
8	130	5	5 (0:1)	2	$40.4^{b} \pm 2.04$
9	130	5	5 (1:0)	2	$42.6^{b} \pm 1.04$
10	160	5	5 (1:1)	2	$15.3^{f} \pm 0.82$

Legend: T = temperature; MD = maltodextrin; AG = gum Arabic; %EE = encapsulation efficiency.
* means (n = 3) ± standard deviation followed by equal letters indicate no significant difference ($p<0.05$), Tukey's test.

Based on these results (Table 1), the variables temperature, temperature, concentration of AG, MD, Tween 80, and carotenoid extract to be used in the RCCD 2^3 matrix were defined (Table 2). The high yield (56.11%) was obtained using 130°C drying air temperature and a wall matrix composed of 37.6g/L AG, 25g/L MD, and 2% (w/v) Tween 80 and 5% (v/v) concentrated extract of carotenoids (Run 12). Thus, the high yields were related to high temperatures (130 and 140°C), which allowed fast drying, and great water loss inside the particles, reducing their adherence to the drying chamber walls and increasing the amount of powder recovered.

Equation 7 shows the second-order coded model, which describes the EE of carotenoids as a function of the independent variables within the ranges studied. The model was validated by variance analysis. The non-significant parameters were added to the lack of fit for the variance analysis (ANOVA). The correlation coefficient (R^2) obtained of 0.95, and the F calculated is 4.71 times higher than the F tabulated value (Fisher-Snedecor) allowed the construction of the contour curve presented in Figure 1.

$$\%EE = 60.3 - 2.42\ X_1 - 10.92.\ X_1^2 + 3.75.\ X_2 - 8.87.\ X_2^2 - 8.37.X_3^2 + 1.83.X_1.X_2 - 4.87.\ X_2.X_3 \quad (7)$$

where: X_1 = Temperature; X_2 = AG (g/L); X_3 = MD (g/L).

The region of maximum EE (~61%) is in the ranges from 128 to 132°C, 20 to 30g/L AG, and 19 to 29g/L MD (Figure 1). The results suggest that, although the increase in AG concentration was significant, the high concentrations did not result in high efficiency. The temperature has a negative effect on the EE, justified by the fact that carotenoids are sensitive to high temperatures. The AG (20 to 30 g/L) combined with MD (19 to 29 g/L) showed a positive effect on EE. This behavior is mainly due to the AG structure, a highly branched heteropolymer of sugars, glucuronic acid, and a portion of a protein covalently bonded to the carbohydrate chain. The AG is known for the property of forming films, trapping the core material, and acting as a good emulsifier in nonpolar substances allowing interaction with the hydrophilic and hydrophobic sections of the molecules. In the specific case of carotenoids, the interaction occurs in the hydrophobic region [42]. Consequently, the %EE is significantly affected by their presence. MD is a hydrolyzed short-chain starch that acts as a barrier against oxygen [43,44].

Similar results of 70% encapsulation efficiency were obtained in carotenoids from *Phaffia rhodozyma* NRRL Y-17268 microencapsulated by spraying at 130ºC using 1% xanthan gum, 0.15 g/L.h feed flow and 2% of Tween 80 [32].

3.3. Physicochemical and morphological characteristics of encapsulated.

The microcapsules were evaluated for water activity (Wa), moisture, color indices, and morphological characteristics.

Wa and moisture content are among the parameters influencing encapsulated bioactive compounds' stability. Thus, it is important to understand and have a good control of these parameters during the processing and storage of powders [45]. The Wa values ranged from 0.130 to 0.313, and the moisture from 0.5 to 1.8% (Table 1). The lowest Wa was obtained at the drying temperature of 130ºC, 37.6 g/L of MD, and 25 g/L of AG (Run 14).

Table 2. Design of the 2^3 matrix (coded and real values) and responses for RCCD of the yield (%Y), encapsulation efficiency (%EE), water activity (Wa), moisture (M) and color (L*, a*, b* and C*).

Run	Independent variables			Responses							
	T (ºC)	AG (g/L)	MD (g/L)	%Y	%EE	Wa	M (%)	L*	a*	b*	C*
1	-1 (120)	-1 (17.5)	-1 (17.5)	45.62	27.38	0.313	0.8	67.66	0.70	26.28	26.29
2	1 (140)	-1 (17.5)	-1 (17.5)	44.93	28.51	0.176	0.5	61.35	0.61	21.34	21.35
3	-1 (120)	1 (32.5)	-1 (17.5)	41.94	43.13	0.288	1.1	64.31	0.30	19.44	19.44
4	1 (140)	1 (32.5)	-1 (17.5)	45.06	35.56	0.219	0.5	66.07	0.32	17.44	17.44
5	-1 (120)	-1 (17.5)	1 (32.5)	49.23	42.44	0.250	0.8	72.81	0.28	18.32	18.32
6	1 (140)	-1 (17.5)	1 (32.5)	53.94	25.44	0.220	1.1	70.81	-0.21	18.32	18.32
7	-1 (120)	1 (32.5)	1 (32.5)	55.46	22.66	0.260	0.8	72.32	0.12	16.69	16.69
8	1 (140)	1 (32.5)	1 (32.5)	49.77	29.03	0.240	0.7	65.49	0.78	11.85	11.87
9	-1.68 (113)	0 (25)	0 (25)	49.28	34.12	0.134	1.1	73.59	-0.21	18.44	18.44
10	1.68 (147)	0 (25)	0 (25)	49.14	24.61	0.159	1.2	68.06	0.33	18.58	18.58
11	0 (130)	-1.68 (12.4)	0 (25)	51.36	21.89	0.160	1.2	72.43	0.27	16.55	16.55
12	0 (130)	1.68 (37.6)	0 (25)	56.11	48.39	0.178	1.4	72.72	-0.03	15.28	15.28
13	0 (130)	0 (25)	-1.68 (12.4)	48.17	32.30	0.154	1.4	69.63	0.39	19.77	19.77
14	0 (130)	0 (25)	1.68 (37.6)	54.72	40.79	0.130	1.8	78.08	0.11	18.04	18.04
15	0 (130)	0 (25)	0 (25)	50.11	59.91	0.182	1.0	69.02	0.14	24.22	24.22
16	0 (130)	0 (25)	0 (25)	49.83	61.73	0.216	1.1	71.36	0.05	24.70	24.70
17	0 (130)	0 (25)	0 (25)	50.33	58.06	0.189	1.2	69.98	0.68	20.79	20.80
18	0 (130)	0 (25)	0 (25)	54.61	59.60	0.215	1.0	70.12	0.29	23.24	23.24
19	0 (130)	0 (25)	0 (25)	51.22	60.81	0.229	1.3	82.36	0.14	20.08	20.08

Fixed independent variables: volume of concentrated extract of carotenoids (5% v/v), phosphate buffer solution pH 7.0 (90% v/v) and Tween 80 (2% w/v).

Legend: T (°C) = temperature; AG (g/L) = gum Arabic; MD (g/L) = maltodextrin.

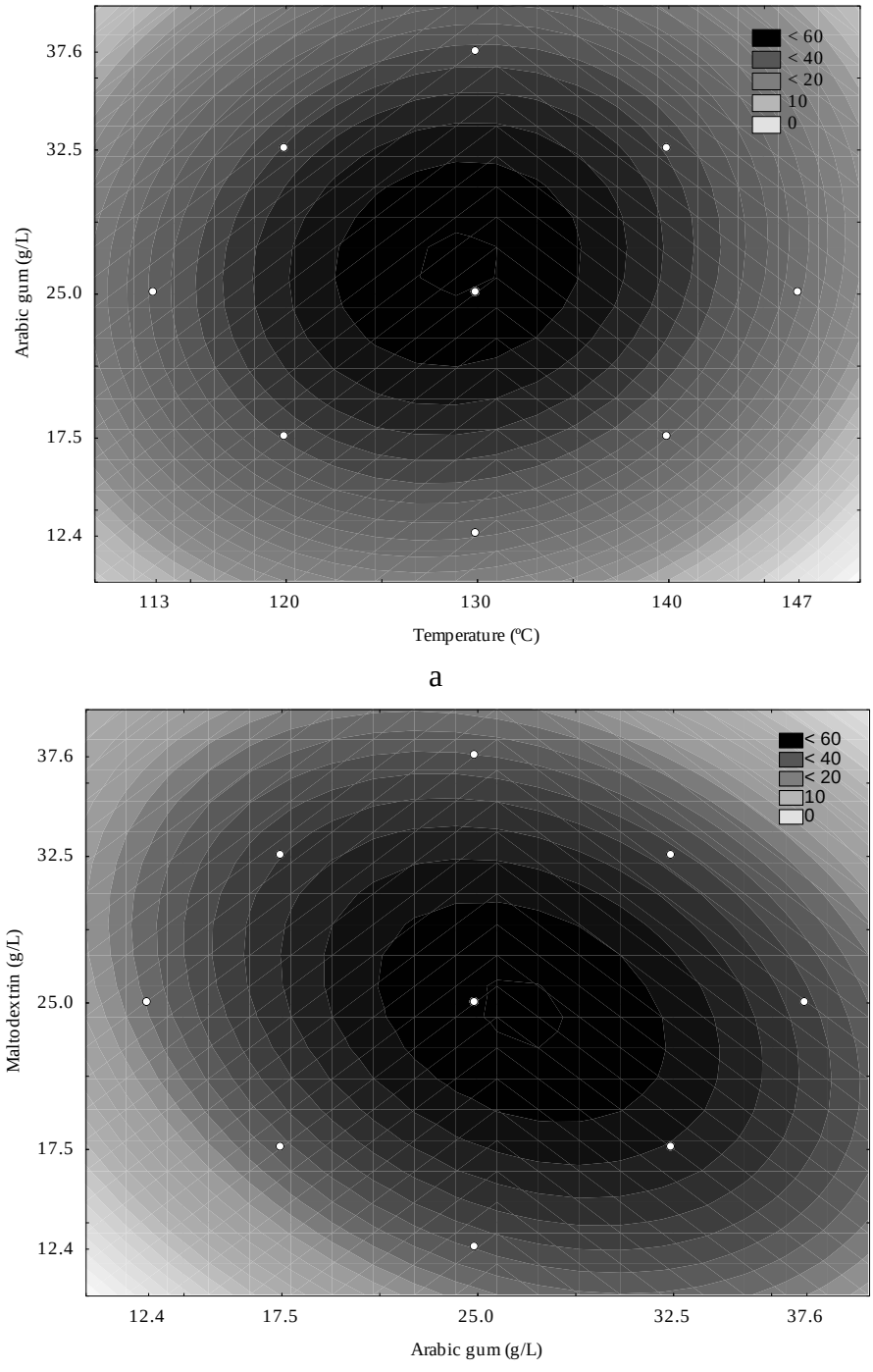
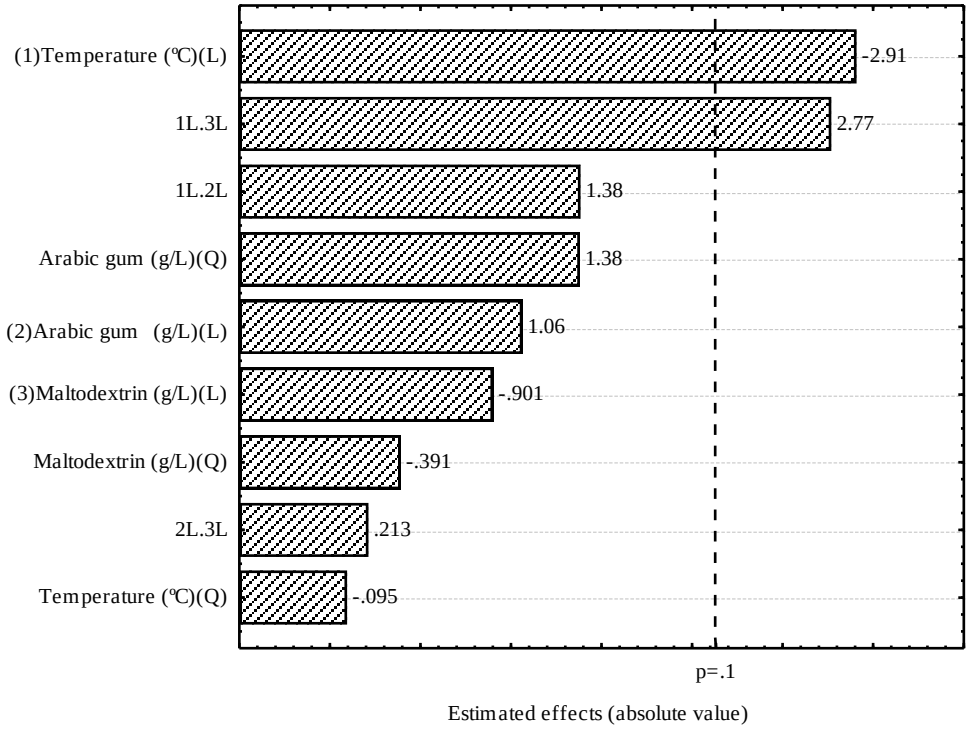


Figure 1. Contour curves for encapsulation efficiency (%EE) as a function of **(a)** temperature and AG**,** and **(b)** AG and MD**,** respectively.

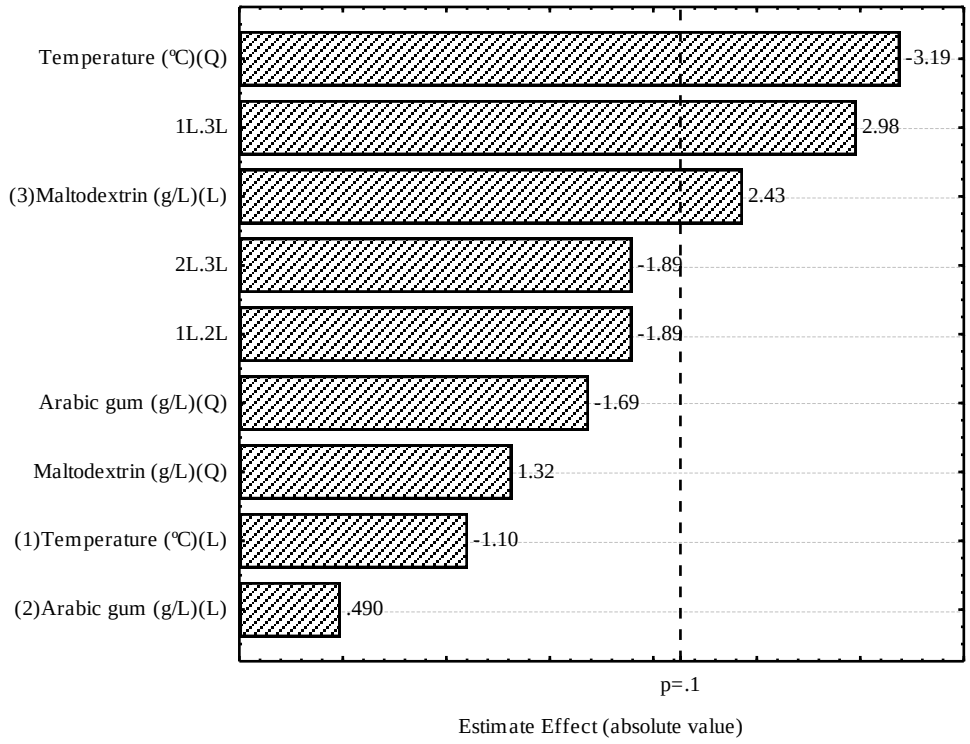
Regarding moisture, the tests with the lowest values were Run 2 (0.5%) at 140ºC of drying temperature and the same proportions of wall material 17.5 g/L (AG:MD), and Run 4 (0.5%) at 140ºC, but with high amount of AG (32.5 g/L) in relation to MD (17.5 g/L).

These results can be better visualized in the Pareto Chart (Figure 2), which describes the estimated effects of the variables studied in relation to the responses of Wa (Figure 2 a) at 90% confidence and moisture (Figure 2 b) at 95% confidence. The independent variable temperature and the correlation of temperature/MD were statistically significant ($p<0.05$) in carotenoids' encapsulation and drying process in relation to Wa and moisture. The temperature negatively affected both cases, indicating that a shift in the studied level from -1 to +1 would decrease both Wa and moisture. The water-holding capacity of a particle is closely related to

the powder porosity, its structure, and drying operation (differences between the inlet and outlet air temperatures and feed rate). Thus, high drying air temperature increases the heat transfer rate, which serves as a driving force for water evaporation [46], consequently generating powders with lower moisture content and Wa.



a



b

Figure 2. Pareto chart with the estimated effects (absolute value) of the independent variables evaluated in the 2^3 factorial design for encapsulation and drying of carotenoid extract in relation to **(a)** Wa and **(b)** moisture content**.**

On the other hand, the interaction between temperature and MD was significantly positive, indicating that with the increase in the correlation between these variables, there will possibly be a rise in the Wa and moisture of the capsules. In addition, the effect of MD was positive ($p<0.05$) also for moisture (Figure 2b). This indicates a tendency to increase the moisture with the amount of MD in the formulation, which would not be interesting since the lower the Wa and moisture, the more stable the microencapsulated ones.

The Wa values were less than 0.3 (except Run 1, Table 1), indicating that the microencapsulation is stable in relation to deterioration reactions, lipid oxidation, enzymatic browning, and microbial growth [47,48].

The moisture of the microencapsulates ranged from 0.5 to 1.8% (Table 1). These low values are due to the presence of MD (with dextrose equivalent of 20D) as wall material and inlet air temperature in the drying. MD, with low dextrose equivalent content [2], increases the glass transition temperature and reduces the viscosity of solids on the drying chamber wall, resulting in drier and more stable encapsulation.

In the literature, there are studies of microencapsulation with spray drying using MD and AG in different carotenogenic matrices (acerola pulp, tamarillo juice, peach palm residues, among others), also reporting that the operational parameters of drying (inlet air temperature and feed flow) and proportion of wall materials were the main factors that influenced the Wa and moisture [21,22,47,49].

The luminosity (L*) values (Table 1) ranged from 61.35 (Run 2) to 82.36 (Run 19). Table 1 shows that the L* values ranged from 61.35 (Run 2) to 82.36 (Run 19). As the encapsulation process was carried out using the encapsulating agents (AG and MD), both with light color, the increase in luminosity was already expected since these encapsulants dilute the dark orange color characteristic of the pure extract. The first-order model for the color parameter (L*) as a function of independent variables (temperature and MD) within the studied range is presented in Equation 8. The model was validated by variance analysis (ANOVA), and the R^2 obtained was 0.77. An R^2 value higher than 0.75 is acceptable, and the most suitable regression is the method suggested. The F calculated was 2.23 times higher than the F tabulated value (Fisher-Snedecor) allowed for constructing the contour curve presented in Figure 3 (a). The highest values of L* (Figure 3a) are found in the temperature ranges from 113 to 128°C and MD concentration above 35 g/L.

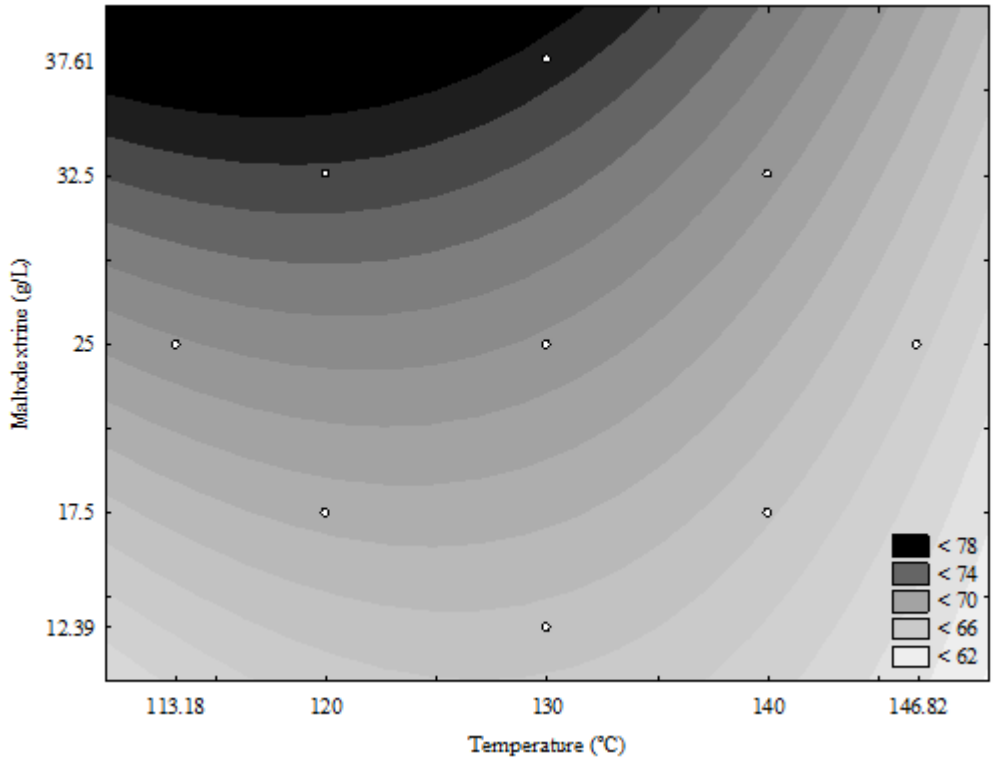
$$L^*=70.76 - 1.66 X_1 + 2.65 X_3 \quad (8)$$

where: L* = color parameter (luminosity) X_1 = Temperature (°C) and X_3 = MA (g/L).

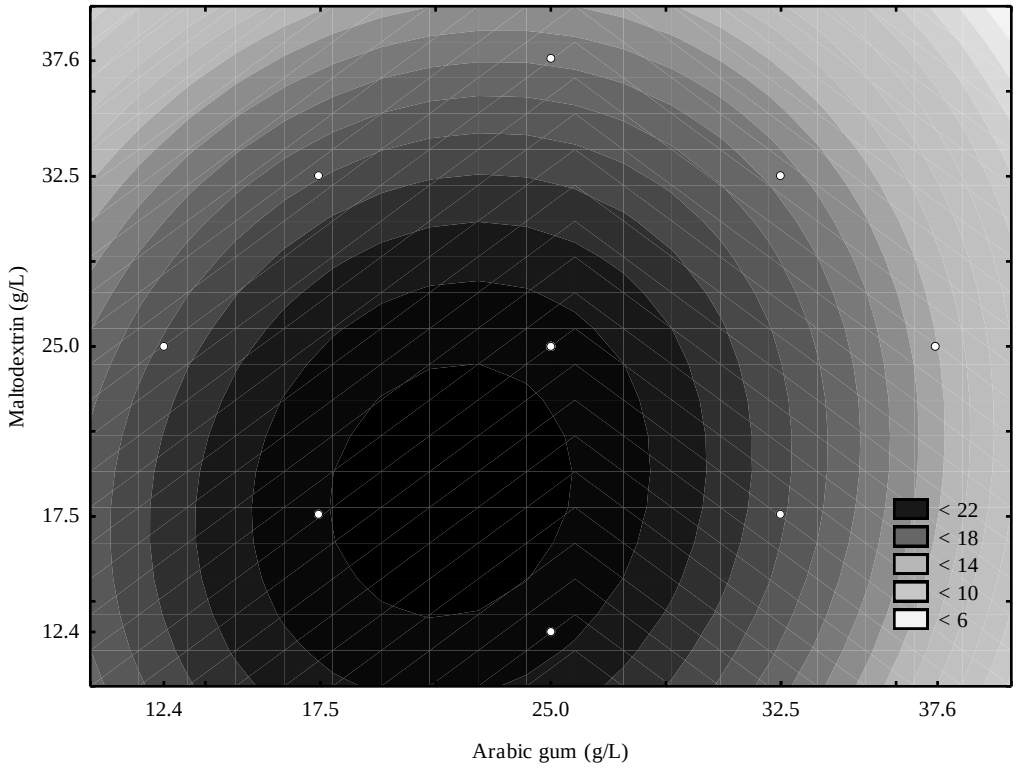
Regarding a* and b* index, the highest value was 0.78 and 26.28 (Run 8), respectively. Equation 9 presents the second-order coded model, which describes the C* color parameter as a function of the independent variables within the studied range. The model was validated by variance analysis with R^2 of 0.85 and F calculated 1.35 times greater than the tabulated value (3.63), which allowed the validation of the model and the construction of a contour curve (Figure 3).

$$C^*=22.56 - 2.11 X_2^2 - 1.63 X_3 \quad (9)$$

where: C* = chromaticity index (saturation), X_2 = AG (g/L) and X_3 = MD (g/L).



a



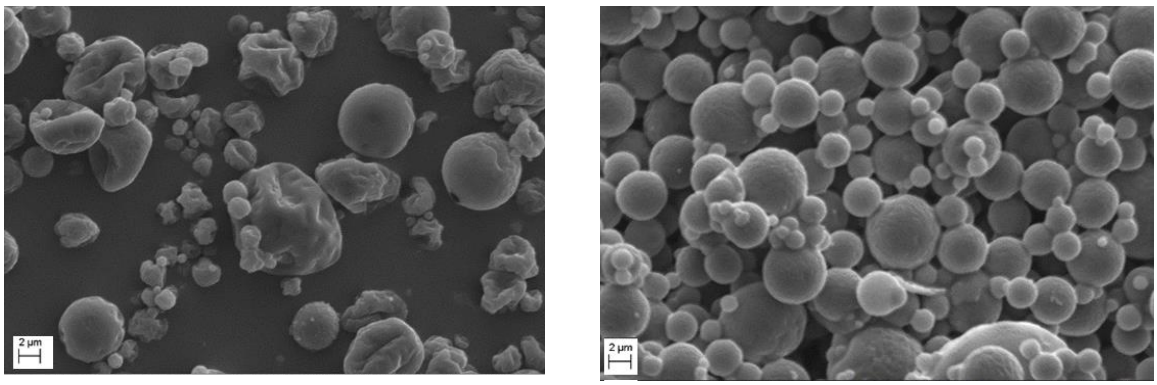
b

Figure 3. Contour curve for the **(a)** color parameter (L*) as a function of temperature and MD and **(b)** color C* as a function of AG and MD**.**

3.4. Microcapsule morphology.

The microcapsules presented a rounded shape and particles with similar sizes, without pores or cracks. Also, some concavities or flattening were observed on the surface, being visible in the wall material (Figure 4a). The microcapsules with the carotenoids (Figure 4b) are

more regular, presuming that the carotenoids are physically protected internally through the encapsulating materials (AG/MD/Tween 80).



(a) (b)

Figure 4. Electronic micrographs of **(a)** the microcapsules of the wall material and **(b)** the encapsulated carotenogenic extract**.**

Microcapsules with wall material have a different shape compared to encapsulated carotenoids, with some particles with concavities and flattening. The concavities or flattening on the capsule surfaces are typical of spray-dried products [43,50]. This usually results from the high drying temperatures or depends on the type of encapsulating material used in the process [51].

The average particle size of the AG/MD/Tween 80/carotenoid complex's microcapsules was 3.48 µm (±1.35). High concentrations of encapsulating agents in the solution promote an increase in particle size [52]. However, the microparticles obtained by the spray dryer showed a smaller particle size and spherical shape. This characteristic can facilitate their incorporation into foods due to the reduction of surface tension between the microcapsule and the food, reflecting great fluidity and flow [53].

3.5. Storage stability of microencapsulated carotenoids.

The reduction of the carotenoid's concentration (%) occurred predominantly with the photooxidation of external carotenoids.

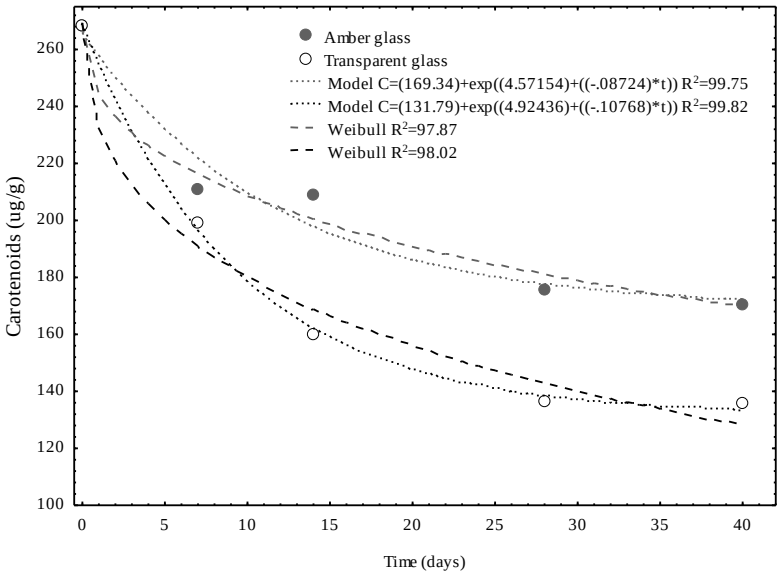


Figure 5. One-order and Weibull kinetic models of encapsulated carotenoid degradation as a function of time, stored at 22ºC in amber and transparent glass.

After 7 days of storage in amber glass, the value decreased by approximately 21%, remained practically stable until the 21st day, and the residual concentration was 63.50% on the 40th day, a degradation of approximately 36%. Already microencapsulated carotenoids stored in transparent glass packaging (Figure 5), the carotenoid concentration (%) decreased by 26% in the first 7 days and 49% after 40 days.

The degradation in the first 7 days can be explained by the rapid decrease of carotenoids that were not encapsulated, that is, and were on the capsules' surface, without the protection of the coating material. The degradation rate can be influenced by material properties, such as the thickness and internal porosity of the wall, which allowed the diffusion of oxygen through the matrix [47], storage conditions, and light and oxygen permeability of the packaging.

Troya [43] studied the stability of carotenoid capsules encapsulated in MD and AG stored at 40ºC and concluded that after 21 days, the carotenoids lost 22%.

Regarding Wa and moisture, few changes were observed during 40 days of storage at 22ºC. In the amber glass, the values ranged from 0.206 to 0.273 and 1.12 to 1.9%, and in the transparent glass, from 0.206 to 0.267 and 1.12 to 2.5%, respectively, indicating that the sample reached equilibrium. In addition, the Wa in both packages was less than 0.6, a value that is considered microbiologically safe [47].

The values of specific carotenoid concentration (µg/g) as a function of time of the encapsulated stored at 22ºC in amber and transparent glass were adjusted to the kinetic models of order one (Equation 3: $[CE(t)]= [CE]_0 \exp(k_0 +(-kt)$ and Weibull (Equation 5: $[CE(t)]= [CE]_0 \exp(-k(t^{\gamma}))$. Table 3 and Figure 5 show the k_0 parameter values, k (reaction rate constant), γ (shape parameter), and *exp* correspond to the exponential function of the models.

Table 3. k and γ parameters and statistical analysis of fit (R^2) of order one and Weibull kinetic models of encapsulated carotenoids degradation as a function of time, stored at 22ºC in amber and transparent glass.

Models/Samples	CE_0	k_0	k	γ	R^2
Order one					
Amber glass	169.34	4.57154	0.08724	-	99.75
Transparent glass	131.79	4.92436	0.10768	-	99.82
Weibull					
Amber glass	268.48	-	0.09394	0.430113	97.87
Transparent glass	269.29	-	0.14563	0.441357	98.02

All the degradation curves of encapsulated carotenoids show the same tendency (Figure 5), with the concavity facing up, proving that the shape parameter (γ) had already been indicated in Table 3, that is, $\gamma < 1$ for both models. The parameter k indicates that the higher this value, the faster the degradation of carotenoids with time. Therefore, for the encapsulated carotenoids stored at 22ºC in transparent glass, the k values were higher (Order one: k = 0.10768; Weibull: k = 0.14563) than those of amber glass (Table 3). The R^2 demonstrates a good fit of the experimental data and the predictive capacity of the models ($R^2 > 97.87$).

Thus, it is possible to verify that carotenoids encapsulated at 130ºC in AG/MD/Tween 80/extract matrix (25g/L/25g/L/2%/5%) stored at 22ºC ± 2ºC in amber glass maintained greater stability.

4. Conclusions

Maximum carotenoid encapsulation efficiency was 60% using 5% (v/v) carotenoid extract, 2% (v/v) Tween 80, 90% (v/v) phosphate buffer (pH 7.0), and 1:1 MD and AG (25 g/L) and drying air temperature of 130°C. The specific carotenoid concentration as a function of

time, of the encapsulated stored at 22ºC, in amber and transparent glass, were adjusted to the order one and Weibull kinetics non-linearized models, demonstrating the fit of the experimental data and the predictive capacity of the models. The microencapsulated carotenoid storage in amber glass was the one that was best preserved. Therefore, it has been proven that encapsulation by spray drying using AG and MD as wall materials is a promising alternative to increase the stability of carotenoids aiming their use as a potential food ingredient.

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Conflicts of Interest

The authors declare no conflict of interest.

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