Stability of Spray-Dried Encapsulated Carotenoid Pigments from Rosa Mosqueta (*Rosa rubiginosa*) Oleoresin

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ABSTRACT: Oleoresin of rosa mosqueta (*Rosa rubiginosa*) was encapsulated with starch or gelatin by spray-drying. Stability of the powders was studied at 25, 40, and 55°C in the dark. Degradation of *trans*-rubixanthin, *trans*-lycopene, and *trans*-β-carotene followed a pseudo-first-order kinetic model for both encapsulating agents. The gelatin matrix provided a greater protective effect over the main carotenoid pigments, as shown by the lower degradation rate constants and the longer half-life values at 21°C. In contrast, the carotenoid pigments showed the same degradation rate in starch, but *trans*-β-carotene was more stable in gelatin. The kinetic compensation effect obtained according to the calculated thermodynamic parameters suggests that the carotenoids are degraded by the same mechanism.

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KEY WORDS: Carotenoids pigments, encapsulated oleoresin, *Rosa rubiginosa*, stability.

Rosa mosqueta (*Rosa rubiginosa*), a member of the rose family, originated in the Mediterranean area and in Central Europe. In Chile, it grows wild in dry soils of low agricultural value. This plant fruit is characterized by its red color owing to the presence of carotenoid pigments. Lycopene and β-carotene have been determined as the main components of *R. canina* and *R. rugosa* species (1) and rubixanthin as the main component in *R. pomifera* (2).

Not only are carotenoids important as natural dyes and a source of provitamin A, but they also show biologic activities such as strengthening the immune system (3), decreasing the risk of degenerative illnesses such as cancer (4), preventing the risk of cardiovascular disease (5), preventing macular degeneration (6), and reducing the risk of cataracts (7). These beneficial effects on health have been associated with the antioxidant properties of carotenoids *via* deactivation of free radicals or singlet oxygen quenching (8).

For the purposes of making the application of carotenoids in food easier and more widespread and of increasing the shelf life of these pigments, they have been encapsulated in protective matrixes. Moreau and Rosenberg (9) pointed out that the simplest method for encapsulating is to emulsify the sensitive ingredient (usually an oil) in a solution containing a protective material, followed by drying such that the protective material then coats or entraps the oil matrix within it, providing a barrier to oxygen and water vapor.

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Encapsulation of carotenoids by spray-drying has been reported in the following works: paprika oleoresin in 15-dextrose equivalent (DE) maltodextrin, arabic gum, gelatin, and sodium caseinate (10); carrot carotenes in maltodextrin of different DE (11); and pure β-carotene in 25DE maltodextrin (12). In all of them, the encapsulating agents showed a protective function for the carotenoids against oxidative damage. The stability of the encapsulated product was influenced by the composition of the encapsulating agent (9).

Atomization, or spray-drying, is the most common method of encapsulation because of its short drying time and lower cost. Spray-drying has been used in the preparation of the dry powder of *Dunaliella salina* microalga (13) and tomato (14).

Rosa mosqueta shell could have industrial applications as a natural pigment source and as an important biological and nutritional agent owing to its high lycopene and β-carotene content, which is similar to tomato (15). The purpose of the present paper was to study the stability of the main carotenoids of rosa mosqueta-shell oleoresin (lycopene, rubixanthin, and β-carotene) encapsulated by spray-drying in starch or gelatin during storage.

EXPERIMENTAL PROCEDURES

Materials. Commercial dried shells of rosa mosqueta (*R. rubiginosa*) were provided by the Chilean company José Alaluf Ltd. (Santiago, Chile). Potato starch was obtained from Quimatic (Santiago, Chile), and Quavasavisco 1116 and gelatin (180° Bloom) were obtained from Prinal S.A. (Santiago, Chile).

Oleoresin. One kilogram of dried rosa mosqueta shell was put into a round-bottomed flask with 1 L of hexane for 3 h at room temperature. Three extractions were made until the pulp was light red. Extracts were combined and evaporated to 50 mL. The carotenoid pigments in hexane were added to a round-bottomed flask containing 70 g of refined sunflower seed oil. The solvent was evaporated first in a Büchi rotatory evaporator and then under a stream of nitrogen. The main carotenoid pigments present in the oleoresin were: rubixanthin, 1.80 mg/g; lycopene, 0.71 mg/g; and β-carotene, 0.60 mg/g. The oleoresin was held at –20°C under nitrogen until further experiments were carried out.

Encapsulation of rosa mosqueta oleoresin. Encapsulations in both starch and gelatin were prepared as follows: Starch

(0.48%) was combined with sucrose (18.4%), lecithin (0.18%), water (79.3%), and oleoresin (1.63%) with constant stirring. Gelatin (2.03%) was combined with sucrose (1.77%), lecithin (0.15%), water (94.28%), and oleoresin (1.77%) with constant stirring.

Each preparation was homogenized with a piston homogenizer. The resultant microemulsions were fed to a Niro spray-dryer (Niro Atomizers, Ltd.) equipped with a centrifugal wheel atomizer. The spray-dryer was operated at an inlet temperature of 150 ± 5°C for starch and 100 ± 5°C for gelatin. The outlet temperature was 70 ± 5 and 65 ± 5°C for starch and gelatin, respectively. The atomization pressure was 3 kg/cm^2 for starch and 5 kg/cm^2 for gelatin. The powders obtained were stored to exclude light and were kept at −20°C under nitrogen until analyzed.

Storage stability test. Rosa mosqueta powders encapsulated in spray-dried oleoresin, using starch or gelatin as the encapsulating agent, were stored at 55, 40, and 25°C in a forced-air oven with controlled temperature and in the absence of light. Samples of 0.1–0.2 g were transferred to 150 × 10 mm clear glass vials, which were covered with air and stoppered with Teflon caps. For determination of carotenoid levels by HPLC, duplicate vials were removed every 12 h from the 55 and 40°C storage conditions and every 24 h from the 25°C storage condition.

Extraction of carotenoids from the encapsulated product and rosa mosqueta shell. Each sample was reconstituted by adding 0.5 mL of water and vortexed; 2 ml of acetone was then added to the slurry and revortexed. Three extractions were made until no orange color remained. The combined acetone extracts were transferred into petroleum ether (15), then saponified with an equal volume of 10% potassium hydroxide in methanol, overnight at room temperature, with the addition of 1 mL of 0.1% BHT. The mixture was placed into a separatory funnel. The carotenoid solution was washed five times with water to remove the alkali and then filtered through anhydrous sodium sulfate. The solvent was evaporated to dryness under a stream of nitrogen. All extracts were redissolved in acetone and filtered through a 0.45-μm Millipore membrane (Millipore, Milford, MA) prior to analysis by HPLC. Initial *trans*-rubixanthin, *trans*-lycopene, and *trans*-β-carotene were determined prior to storage to calculate the retention percentages.

Standard solution. All-*trans*-β-carotene and all-*trans*-lycopene were obtained from carrots and tomatoes, respectively. All-*trans*-rubixanthin and all-*trans*-zeinoxanthin from rosa mosqueta were purified in-column as described by Rodriguez-Amaya (15). Concentrations of standards in hexane were determined by spectrophotometry using their respective $A^{1\%}_{1cm}$. Calibration curves were obtained for each carotenoid.

Chromatographic procedure. Carotenoid analysis was carried out by HPLC using a Waters symmetry column (C18, 5 μm particle size, 4.6 mm i.d. × 25 cm; Waters, Milford, MA). An isocratic mobile phase of methanol/acetonitrile/ethyl acetate (20:65:15 by vol) was used at a flow rate of 1 mL/min. A Merck-Hitachi L-6200A high-performance liquid chromatograph with a Merck-Hitachi D-2500 UV/vis detector at 450 nm was used. Carotenoid compounds were identified by comparing the peak retention times with standards.

Kinetic analysis. The data were best fit by a first-order kinetic model, $\ln C = \ln C_o - k(t)$. Degradation rate constants (k) were obtained from the slope of a plot of the natural log of the percentage retention of carotenoids vs. time.

The activation energy (E_a) and frequency factor (A) were determined from the Arrhenius model $k = Ae^{-(E_a/R)/T}$, where E_a/R is the slope and $\ln A$ is the intercept of the relationship between the natural log$k$ and ($1/T$) in degrees kelvin. For a first-order reaction, the half-life was determined at a specific temperature by the equation $t_{1/2} = \ln 2/k$.

The enthalpy of activation ($\Delta H^{\neq}$) was obtained by plotting $\ln(k/T)$ vs. ($1/T$), and the entropy of activation ($\Delta S^{\neq}$) was obtained from Equation 1 based on the transition state theory,

$$\ln(k/T) = \ln(k_B/h) + \Delta S^{\neq}/R - \Delta H^{\neq}/RT \quad [1]$$

where k_B is the Boltzmann constant and h is Planck's constant.

Statistical analysis. Experiments of storage were duplicated for each temperature. The linear regression (95% confidence limit) was used to determine the reaction order, rate constants, and activation energies. To determine the statistical differences among rate constants for matrixes and carotenoids, a multivariate ANOVA was performed. All the statistical analyses were calculated by using Statgraphics, version 7.0 (Manugistics Inc., Statistical Graphics Corporation, Rockville, MA).

RESULTS AND DISCUSSION

Two carotenes, *trans*-lycopene (126.2 μg/g) and *trans*-β-carotene (47.5 μg/g), and two xanthophylls, *trans*-rubixanthin (113.0 μg/g) and *trans*-zeinoxanthin (5.9 μg/g), were identified as the main carotenoid pigments in the commercial shell of rosa mosqueta, with lycopene being the predominant one (Fig. 1).

The high content of carotenoid pigments found in the commercial shell of rosa mosqueta confirms that this industrial by-product is not only an important and powerful natural dye for foods but also a good source of bioactive compounds.

The distribution percentages of *trans*-rubixanthin, *trans*-lycopene, and *trans*-β-carotene (58, 23, and 19%) obtained from the oleoresin of rosa mosqueta changed compared with the original distribution obtained from the commercial shell (39, 44, and 17%). Losses of lycopene occurred during oleoresin preparation, a process that involves extraction, filtration, and elimination of the solvent.

Table 1 contains the concentration values of carotenoid pigments in the formulation and in the spray-dried powder obtained from the encapsulated oleoresin. The recovery porcentages of carotenoids pigments were calculated from dried matter. The recovery of *trans*-rubixanthin in the starch or gelatin matrix was 60%. The recovery of *trans*-lycopene and

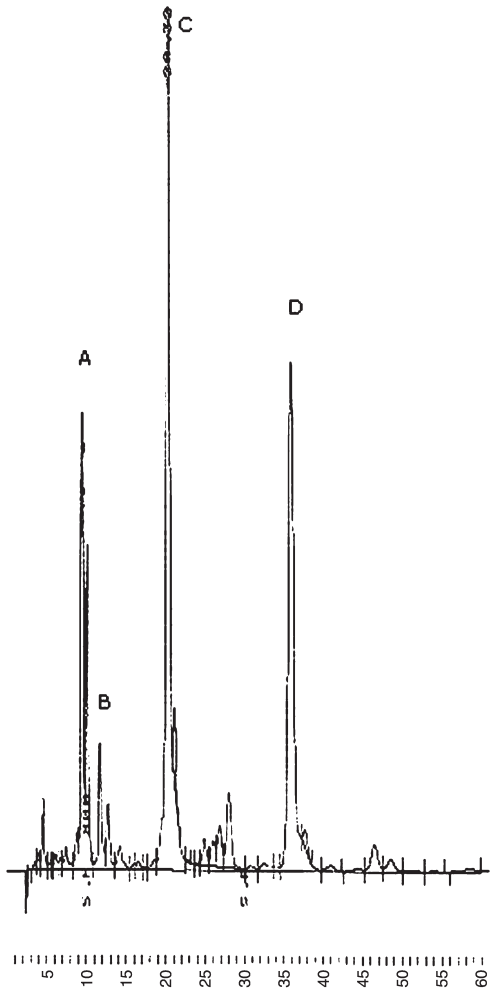

FIG. 1. Chromatographic separation of the major carotenoid pigment in rosa mosqueta shell. (A) *Trans*-rubixanthin; (B) *trans*-zeinoxanthin; (C) *trans*-lycopene; (D) *trans*-β-carotene.

trans-β-carotene in gelatin reached 72 and 99%, respectively, whereas in the starch matrix the recoveries were lower, at 54 and 71%, respectively.

A recovery of over 89% of β-carotene in spray-dried microalga *D. salina* has been reported (13). Another study showed 11% losses of β-carotene in pure β-carotene encapsulated with 25DE maltodextrin (12).

Inlet drying temperatures in the gelatin matrix (100°C), which were lower than those in the starch matrix (150°C), allowed a higher recovery of the pigments in gelatin, whereas higher drying temperatures in the starch matrix produced losses due to carotenoid decomposition. The moisture content of the powders obtained when gelatin and starch were used as encapsulating agents reached values of 4.4 and 2.8%, respectively.

Storage stability evaluation. The kinetics of degradation of *trans*-rubixanthin, *trans*-lycopene, and *trans*-β-carotene were monitored by storing oleoresin powders encapsulated in starch or gelatin at 55, 45, and 25°C, respectively. Reaction orders, rate constants, and half-life values were determined.

Figure 2 shows the natural logarithm of the percentage retention vs. time (h) for *trans*-rubixanthin (A), *trans*-lycopene (B), and *trans*-β-carotene (C) encapsulated in starch at 55, 40, and 25°C. Figure 3 (panels A, B, and C) corresponds to those encapsulated in gelatin. The degradation of these carotenoids followed pseudo-first-order behavior for both of the encapsulated products for all the temperatures studied. The correlation coefficient was used as a parameter to determine the reaction order. Isomerization of carotenoid pigments during the storage of powders was not observed; similar results were found by Wagner and Warthesen (11).

Previous researchers have shown that the degradation of α- and β-carotenes follows first-order kinetics in stored, spray-dried carotenoid pigments from carrot encapsulated in maltodextrin with different DE (11). Similar conclusions for the degradation of spray-dried β-carotene in *D. salina* microalga have been reported (13). Other studies have reported second-order kinetics during the storage of encapsulated paprika oleoresin (10) and a sigmoidal degradation for model systems of synthetic all-*trans*-β-carotene with microcrystalline cellulose (16).

Table 2 shows pseudo-first-order deterioration rate constants (k), obtained from the slopes of logarithmic plots of the percentage retention vs. time (h). An increase in storage temperature led to an increase in the rate constant (k) for *trans*-rubixanthin, *trans*-lycopene, and *trans*-β-carotene in starch- and gelatin-encapsulated products.

When carotenoids were not encapsulated, higher values of k for β-carotene were observed (1.2×10^{-2} h^{-1}) in spray-dried carrots (11) and in the microalga *D. salina* (13) stored at 21 and 28°C, respectively.

TABLE 1
Composition of the Major Carotenoid Pigments Before and After Encapsulation of Rosa Mosqueta Oleoresin in Starch or Gelatin

	Starch			Gelatin		
Carotenoid pigments	Before spray-drying[a] (μg/g)	After spray-drying[b] (μg/g)	Recovery (%)	Before spray-drying[a] (μg/g)	After spray-drying[b] (μg/g)	Recovery (%)
trans-β-Carotene	47.3	33.8 ± 0.2	71	185.3	183.9 ± 8.8	99
trans-Lycopene	56.0	30.2 ± 0.8	54	220.3	159.0 ± 1.5	72
trans-Rubixanthin	141.5	81.3 ± 2.4	57	556.9	334.5 ± 2.4	60

[a]Values were calculated according to the formulation and expressed as total solids.
[b]Values are expressed as $x \pm$ SEM ($n = 2$).

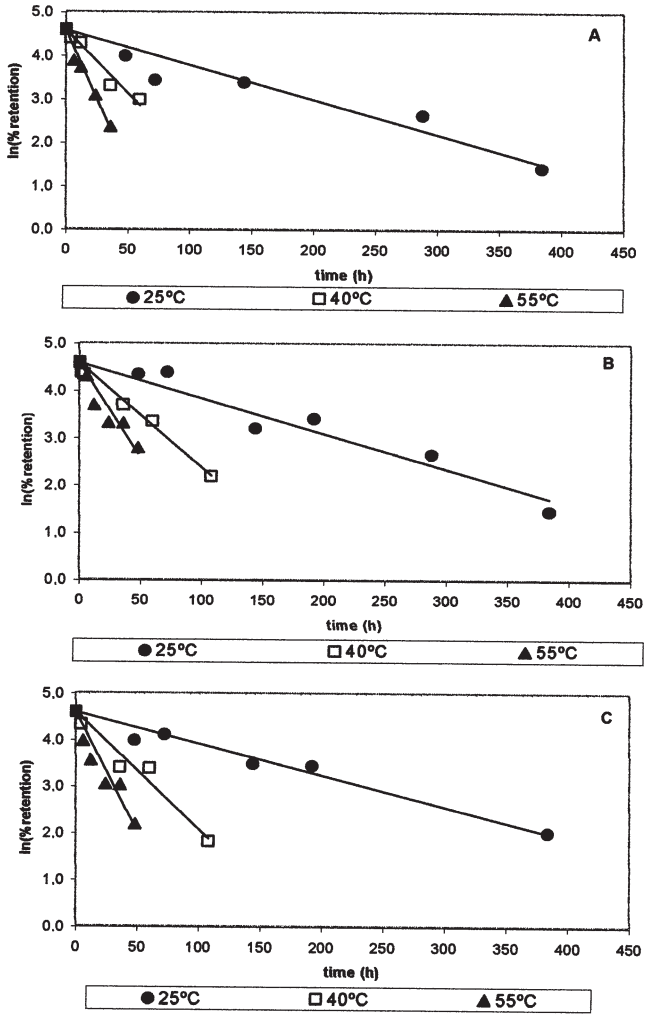

FIG. 2. First-order degradation plots for *trans*-rubixanthin (A), *trans*-lycopene (B), and *trans*-β-carotene (C) in spray-dried rosa mosqueta oleoresin encapsulated in starch. Each point represents an average of duplicate experiments at each temperature.

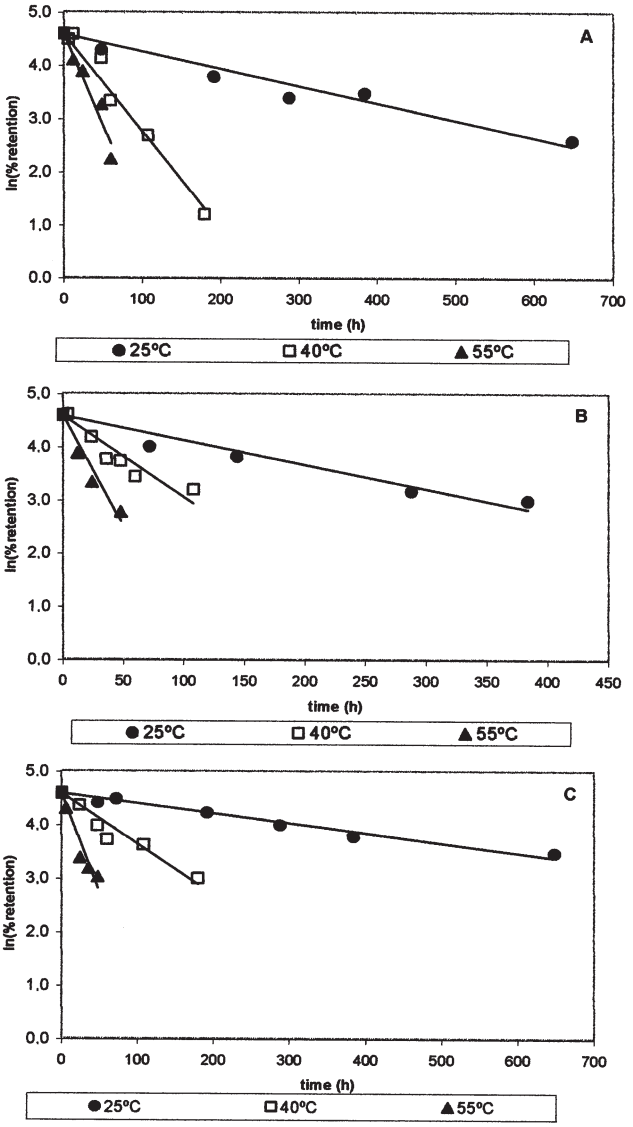

FIG. 3. First-order degradation plots for *trans*-rubixanthin (A), *trans*-lycopene (B), and *trans*-β-carotene (C) in spray-dried rosa mosqueta oleoresin encapsulated in gelatin. Each point represents an average of duplicate experiments at each temperature.

The degradation rate of the main carotenoid pigments of rosa mosqueta-shell encapsulated oleoresin powders was significatively higher ($P < 0.05$) in starch than gelatin, showing the importance of the encapsulating material in the degradation of carotenoid pigments. The matrix effect also has been observed in encapsulated oleoresin paprika, where a better protection of 15DE maltodextrin with respect to arabic gum, gelatin, and sodium caseinate was reported (10). The encapsulation of carrot with maltodextrins of different DE has been studied as well (11).

Degradation of *trans*-rubixanthin, *trans*-lycopene, and *trans*-β-carotene occurred at the same rate ($P > 0.05$) in starch. Nevertheless, in the gelatin-encapsulated product, the *trans*-β-carotene showed a degradation rate lower than that for the other carotenoid pigments. Similar results in starch have been reported in the degradation of α- and β-carotenes in a vegetable juice system (17) and in carrot encapsulated in starches of different DE (11). On the other hand, in lipid oxidation, differences in the degradation rates of carotenoids have been reported, which have been associated with their structure. Henry *et al.* (18) reported the following degradation rate for safflower seed oil: lycopene > all-*trans*-β-carotene, 9-*cis*-β-carotene > lutein, whereas Anguelova and Warthesen (19) reported the degradation rate: lycopene > β-carotene > α-carotene in methyl linoleate.

Table 3 shows the Arrhenius parameters, half-lives, and thermodynamic parameters for the main carotenoids of rosa mosqueta oleoresin encapsulated in starch and gelatin. The activation energies were higher for the carotenoids encapsulated in gelatin. The energy of activation for β-carotene encapsulated in starch was 13.1 kcal/mol, a value similar to that reported for β-carotene encapsulated in maltodextrin (14.2 kcal/mol) (12). The half-life values estimated at 21°C were lower in the carotenoid pigments encapsulated in starch compared with

TABLE 2
Degradation Rate Constants (*k*) of Spray-Dried Rosa Mosqueta Oleoresin Encapsulated with Starch or Gelatin

	T (ºC)	*trans*-β-Carotene (h^{-1})	*trans*-Lycopene (h^{-1})	*trans*-Rubixanthin (h^{-1})
Starch	25	$6.7 \times 10^{-3} \pm 5 \times 10^{-4}$	$7.5 \times 10^{-3} \pm 8 \times 10^{-4}$	$8.0 \times 10^{-3} \pm 9 \times 10^{-4}$
	40	$2.5 \times 10^{-2} \pm 3 \times 10^{-3}$	$2.2 \times 10^{-2} \pm 3 \times 10^{-3}$	$2.9 \times 10^{-2} \pm 4 \times 10^{-3}$
	55	$5.1 \times 10^{-2} \pm 5 \times 10^{-3}$	$4.0 \times 10^{-2} \pm 6 \times 10^{-3}$	$6.4 \times 10^{-2} \pm 1 \times 10^{-2}$
Gelatin	25	$1.9 \times 10^{-3} \pm 1 \times 10^{-4}$	$4.6 \times 10^{-3} \pm 6 \times 10^{-4}$	$3.2 \times 10^{-3} \pm 3 \times 10^{-4}$
	40	$9.5 \times 10^{-3} \pm 1 \times 10^{-3}$	$1.5 \times 10^{-2} \pm 1 \times 10^{-3}$	$1.8 \times 10^{-2} \pm 3 \times 10^{-3}$
	55	$3.7 \times 10^{-2} \pm 4 \times 10^{-3}$	$4.1 \times 10^{-2} \pm 5 \times 10^{-3}$	$3.4 \times 10^{-2} \pm 5 \times 10^{-3}$

[a]Values are reported as mean ± SEM; 95% confidence interval. Values were obtained from plots of the slopes of ln(% retention) vs. time.

TABLE 3
Arrhenius Parameters, Half-Life Values, and Thermodynamic Parameters for Major Carotenoid Pigments of Rosa Mosqueta Oleoresin Encapsulated in Starch or Gelatin by Spray-Drying

Carotenoid	Activation energy[a] (kcal/mol)	Intercept (ln*A*)	r^2 (Arrhenius plot)	Calculated half-life at 21°C (d)	Enthalpy of activation[b] (kcal/mol)	Entropy of activation[b] (cal/mol K)	r^2
Starch							
trans-β-Carotene	13.1 ± 1.5	17.2 ± 2.5	0.979	5	12.6 ± 0.05	–42	0.98
trans-Lycopene	10.9 ± 1.1	13.5 ± 1.8	0.982	5	10.2 ± 1.2	–50	0.98
trans-Rubixanthin	13.4 ± 1.1	17.9 ± 1.8	0.988	5	12.9 ± 1.1	–41	0.98
Gelatin							
trans-β-carotene	19.2 ± 0.1	26.2 ± 0.2	0.999	23	18.6 ± 0.1	–25	0.99
trans-Lycopene	14.2 ± 0.2	18.7 ± 0.3	0.999	9	13.5 ± 0.05	–40	0.99
trans-Rubixanthin	15.4 ± 3.5	20.4 ± 5.7	0.947	11	14.8 ± 3.2	–36	0.94

[a]Values were obtained from slopes of Arrhenius plots.
[b]Values were obtained from transition state theory equations. *A*, frequency factor.

those encapsulated in gelatin. The presence of a large proportion of long-chain saccharides has been reported to cause the barrier to be inflexible and more permeable to oxygen. This means a decrease of the protective effect toward carotenoid pigments (11). Longer half-life values for β-carotene (between 145 and 431 d) at 21°C have been reported for carrot pigments encapsulated in maltodextrin (11). In other studies, the half-life at 25°C for commercial β-carotene encapsulated in 25DE maltodextrin was 50 d (12).

The presence of lipid in the encapsulated product would have an important influence on the pigments' half-life values, promoting their degradation because they act as antioxidants through free radical scavengers. This explains the difference in half-life values observed for encapsulated oleoresin and oleoresin without lipid in the matrixes.

The enthalpy of activation ($\Delta H^{\neq}$) and entropy of activation ($\Delta S^{\neq}$) were obtained as the slope and intercept, respectively, from a plot of ln(k/T) vs. 1/T. The enthalpies of activation were greater for those encapsulated in gelatin, together with a less negative entropy of activation. A linear relationship was obtained for a plot of the enthalpy of activation vs. the entropy of activation (r^2 = 0.998 and 0.999 for starch and gelatin, respectively). The compensation effect suggests that each carotenoid is degraded by a similar mechanism (20); the same effect was reported by Henry *et al.* (18) in the oxidative degradation kinetics of lycopene, lutein, and 9-*cis*- and all-*trans*-β-carotene. The similarity among the values of degradation rate constants, half-life values, and activation energies in each matrix does not eliminate the possibility that the process is controlled by the permeability of oxygen into the matrix.

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