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α- and β-Carotene Stability During Storage of Microspheres Obtained from Spray-Dried Microencapsulation Technology

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This study was aimed at comparing the stability of carotenes (α- and β-carotene) in oil solutions with their stability when spray-dried encapsulation is applied. The carotenes were isolated from carrot. A storage test was subsequently performed. The stability of carotenes in oil solutions was determined with the HPLC method. The color of the samples was also analyzed. The oil solutions of carotenes were microencapsulated with the spray--drying method. A mixture of gum Arabic and maltodextrin was used as a matrix.

Degradation of carotenes during storage of the oil solutions followed first-order kinetics. The energies of activation were 58.7 and 33.6 kJ/mol for α- and β-carotene, respectively. Among the studied factors (time, daylight, temperature), it was the time and the temperature that influenced carotenes degradation the most. Spray-drying encapsulation caused a significant decrease in the content of carotenes. However, retention of pigments stored in microspheres was longer than retention of pigments stored as oil solutions.

INTRODUCTION

In recent years, the use of natural pigments has been steadily increasing, primarily because of changes in consumer preference towards more natural products known to exhibit specific functional properties [Ramoneda *et al*., 2011].

Carrot is widely known as an efficient source of carotenoids, especially of α- and β-carotene [Marx *et al*., 2003; Lemmens *et al*., 2010]. Not only is β-carotene known for its dyeing properties, but also for its health effects as a vitamin A precursor [Liu *et al*., 2010; Ramoneda *et al*., 2011]. It is transformed into vitamin A in 100% while α-carotene is only in 50%. Moreover β-carotene is an effective antioxidant [Desobry *et al*., 1997; Przybysz *et al*., 2016], especially regarding its ability to quench a singlet oxygen and interact with free radicals. However, other mechanisms of antioxidation have been reported as well: modulation of carcinogen metabolism, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of cell-to-cell communication, and filtering of blue light [Rodriquez-Amaya & Kimura, 2004].

The concern about carotenoids has recently increased also because of their contribution to prevent lifestyle diseases, *e.g.* heart diseases, cancer or macular degeneration [Meléndez--Martínez *et al*., 2007; Aparicio-Ruiz *et al*., 2011]. On the other hand, β-carotene and α-carotene as unsaturated compounds are highly sensitive to oxygen, light, and heat [Elizalde *et al*., 2002; Ramoneda *et al*., 2011], and their oxidation products show very low level of pigmentation or even do not show it at all [Wagner & Warthesen, 1995].

Thus microencapsulation, as a part of food production process, is highly recommended with the aim to improve stability period of sensitive and functional food ingredients such as β-carotene [Ramoneda *et al*., 2011; Thirundas *et al*., 2014]. The method enables transforming liquids into stable and free-flowing powders which are easy to handle and incorporate into a dry food system [Wagner & Warthesen, 1995; Wang *et al*., 2014]. The stability and retention time of a pigment during microencapsulation and later on during storage strongly depends on the type of wall material employed to form the particles. The physical structure of the matrix changes during storage. This may lead to increased permeability and gas diffusivity, which can influence the degradation reaction rate and decrease the stability of the microencapsulated substance (the active ingredient) [Elizalde *et al*., 2002]. Therefore, the feasibility of introducing some microencapsulated particles into the final product depends on the selection of a matrix. Both maltodextrin and gum Arabic may be applied as the coating material. Gum Arabic is an effective carrier because of its high solubility in water, low solution viscosity and ability to form a protective film around the particles of the dispersed phase of the emulsion [Dickinson *et al*., 1989; Chanamai & McClements, 2002]. Maltodextrin is less expensive than gum

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Arabic and constitutes an effective anti-oxygen barrier, but on the other hand it has poor emulsifying ability [Anwar *et al*., 2010]. Thus gum Arabic combined with maltodextrin may be a perfect matrix to protect β-carotene during spray--dried microencapsulation process.

The aim of this study was to obtain carotene pigments (α- and β-carotene) from carrot taproot and to assess the influence of their oil solutions storage in different conditions: without access to daylight at 20±2 °C, with access to daylight at 20±2 °C, and without access to daylight at 4±2 °C on their stability. Moreover, the study compares trends in the stability of the carotenes stored in oil solutions and microspheres obtained by spray drying.

MATERIALS AND METHODS

Isolation of α- and β-carotene from carrot

Carotenes were separated from *Daucus carota* subsp. *Sativus*, originating from experimental fields of the Warsaw University of Life Sciences (Poland). Carrot was crumbled and juice was subsequently squeezed with a Bauknecht robot. Protein present in carrot juice was coagulated with 25% HCl. The juice was then neutralized to pH 4.7 and heated to 89±1°C. The coagulated protein-carotene precipitate was sedimented in cooling conditions and stored for about 20 h. Then, the precipitate was centrifuged for 10 min at 25,000×*g* in a Sigma Centrifuge MPW-340. Next, the centrifuged coagulate was frozen, dehydrated and lyophilized with the use of a Christ Alpha 1–4 LSC Freeze Dryer. Three--stage *n*-hexane extraction of carotenes from the lyophilizate was performed. The extract was recovered by vacuum filtration throughout a Büchner funnel with the use of a vacuum pump and freed from *n*-hexane by evaporation in a Buchi Rotavapor R–210. The lyophilized powder was subsequently dissolved in refined rapeseed oil (Uniwersalny, Aro, Poland) [Przybysz *et al*., 2012]. Finally, 0.073% α- and β-carotene oil solution was obtained. The oil had been chosen due to its oxidation stability.

HPLC qualitative and quantitative analysis of carotene oil solutions

A modified Chen *et al*. [1995] method was applied to prepare HPLC samples. To this end, 100±0.0001 mg of the oil solution of carotenes was weighed on an analytic scale. Afterwards, 3 mL of hexane, 1 mL of 0.5 mol/L KOH methanol solution and 20 μL of a hexane solution of BHT antioxidant was added in order to inhibit carotene oxidation in the analyzed samples. The solution was thoroughly stirred. The samples were incubated in a CWE-4A incubator (Elkon, Łódź, Poland) for 6 h at 30±2°C. Afterwards, 3 mL of distilled water were added and the solution was stirred again. Next, 1.5 mL of hexane layer was collected, transferred to bottles containing anhydrous sodium sulfate (VI) and stirred again. After 5 min, 1.3 mL of the supernatant was collected and evaporated under a stream of nitrogen. Then, 1.5 mL of acetonitrile was added and stirred. The mixture was filtered until it had become clear. Finally, 1.5 mL of the solution was pipetted to 2 mL vials. Extraction was performed in 3 replications.

HPLC qualitative and quantitative analysis

High performance liquid Shimadzu chromatograph with spectrophotometric detection (UV-VIS (DAD)) was used to determine the content of carotenes (α- and β-carotene). Chromatographic separation was performed in the Applied Biosystems Spheri-5 RP C-18 column (10 mm length of column, 1.0 mm diameter of column, diameter of grains 5 μm). Acetonitrile was used as a mobile phase at the flow rate of 0.12 mL/min. Thermostat temperature of the column was 22±0.1°C [Szterk *et al*., 2008].

Furthermore, identification of α- and β-carotene forms was based on mass spectrum results obtained with liquid chromatography–mass spectrometry detection with electrospray ionization. In the assay, we used Shimadzu liquid chromatograph LCMS-220V [Szterk *et al*., 2008].

Color measurements of carotene pigments

Color of oil carotene solutions was measured according to the CIE L*a*b* system with the use of a Konica Minolta CM-3600d colorimeter. The transmission assay was performed in 2 cm thick cuvettes with the use of 10° observer and D_{65} illuminant. In the L*a*b* color space, the parameter L* describes brightness (ranging from 0 to 100). Parameter a* describes green-red ratio (ranging from -150 to 100), green tones take negative values and red tones take positive values. Parameter b* defines the blue-yellow ratio (ranging from -100 to 150), blue tones take negative values, and yellow tones take positive values. Values of parameters a* and b* enable calculating chromaticity C* ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue h ($h = \text{arctg}(b^*/a^*)$): h=0° represents red, 90° – yellow, 180° – green, 270° – blue. All parameters were measured in three parallel repetitions and results are given as their arithmetical averages [Sánchez-Moreno *et al*., 2003; Liu *et al*., 2010].

Determination of carotene oil solutions stability

The oil solution of carotenes was placed in colorless vials (vial capacity 25 mL) and subsequently subjected to a 12-week storage test. The following storage conditions were applied: without access to daylight at 20±2°C, with access to daylight at 20±2°C and without access to daylight at 4±2°C. The samples stored without the access to daylight were protected from light with aluminum foil coating. Tests covered qualitative and quantitative determination of carotene content and color measurements of oil solutions. The first assays were performed on the day when the samples had been prepared and repeated every 2 weeks for 3 months.

Spray-dried microencapsulation

Microspheres were obtained by spray-dried microencapsulation. The core of microspheres consisted of carotene oil solution, while the matrix was made of gum Arabic VALCOAT WM 960 (Valmar, Sudan) and maltodextrin GLUCIDEX IT 12 (DE 12 Jar-Jaskulski Aromaty, Poland). The wall material ingredients were used in the 1:1 ratio. The composition of the final spray-dried emulsion was 5% of core, 30% of matrix and 65% of distilled water, and these proportions had been determined experimentally in order to achieve the highest possible microencapsulation efficiency.

Preparation of oil in water emulsion (O/W)

A carrier solution was prepared by two-stage dispersion of the matrix made of gum Arabic and maltodextrin in distilled water. At first, gum Arabic was added to distilled water at 40°C, and stirred with a laboratory stirrer RW 20 DZM (Janke & Kunkel, Germany) at 380×*g*. After 30 min, maltodextrin was added while stirring at the same parameters. The continuous phase was left at room temperature (20±2 °C) for about 24 h in order to fully hydrate the carrier. On the following day, the carotene oil solution of the core was added to the wall material solution and the mixture was stirred together for 10 min with a laboratory stirrer at 380×*g*. Emulsions were prepared in a two-stage homogenization in a high-pressure homogenizer APV-1000 (APV) (number of passes in APV – one). At the first stage, homogenization pressure of 55 MPa was applied and at the second stage the pressure was 18 MPa [Przybysz *et al.,* 2016].

Drying

The final emulsion was dried in a laboratory spray drier (A/S Niro Atomizer, Denmark; spraying mechanism – atomizing disc). The emulsion was fed to the atomizing disc using a peristaltic pump Elpan 372.1 at 4×*g*, at atomization pressure of 2 kg cm^{-2}. The emulsion was heated to 40±2°C prior to its introduction into the dryer in order to reduce its viscosity. This facilitated atomization of the emulsion and resulted in more efficient microencapsulation. Counter flow drying was applied at the inlet air temperature of 190±5°C and at the outlet air temperature of 80±5°C. The microspheres were obtained in six parallel repetitions [Przybysz *et al.,* 2016].

The qualitative and quantitative analysis of α- and β-carotene in microspheres with HPLC

The microspheres (1 g) were weighed on an analytical scale to Falcon centrifuge tubes, then 500 mg of L(+) ascorbic acid, 8 mL of EtOH, and 8 mL of 60% KOH in H_2O were added. The tube content was homogenized, capped and transferred to an incubator. Incubation parameters were as follows: time 15 min, temp. 70°C, speed 250×g. After incubation, 8 mL of distilled water and 8 mL of petroleum ether were added to the tubes. Contents of the tubes were mixed and centrifuged at 5000×*g* for 5 min at 0°C. After centrifugation, a layer of β-carotene was taken into a round bottom flask of 100 mL; wherein the extraction was carried out in three steps. Hexane was evaporated and the sample was dissolved in *n*-hexane for HPLC (developed by authors based on Rodriquez-Amaya [2001] and Rodriquez-Amaya & Kimura [2004]).

The qualitative and quantitative analysis of α- and β-carotene on the surface of the microspheres with HPLC

The microspheres (1 g) were weighed on an analytical scale into a conical flask, then 25 mL of the extraction mixture (acetone:hexane, 1:1) were added. The solution was stirred for 1.5 min using a magnetic stirrer. After settling of the microspheres at the bottom of the conical flask, the clear solution was pipetted to the round 50 mL bottom flask. Hexane was evaporated and the sample was dissolved in *n*-hexane for HPLC (developed by authors based on Rodriquez-Amaya [2001] and Rodriquez-Amaya & Kimura [2004]).

HPLC analysis

The HPLC analysis was carried out the same as for the determination of the content of pigments in the oily solution.

On the basis of the determined total content of carotenes inside the microspheres and on their surface, we calculated the microencapsulation efficiency (ME) according to the equation proposed by McNamee *et al*. [2001]:

$$ME = (c_b - p_b)/c_b \times 100 \quad (1)$$

where ME – microencapsulation efficiency (%), c_b – total pigment content in microspheres (mg/100 g), p_b – total pigment content on the surface of the microspheres (mg/100 g).

The microencapsulated carotenes were stored for 3 months at 20±2°C without access to daylight. Contents of α- and β-carotene inside the microspheres and on their surface were determined on the day when the samples had been prepared and then after 1, 2 and 3 months of storage.

Scanning electron microscopy (SEM)

The analysis of microencapsulated carotenes morphology was based on images taken with a scanning electron microscope Hitachi TM 3000 Tabletop Microscope (Tokyo, Japan) and Multi Scan Base v. 18.03 program (Computer Scanning System, Warsaw, Poland). The microspheres were immobilized on the SEM table with double-sided adhesive tape. Prior to the observation, the samples had not been sputtered with any kind of coating material. This study presents only selected images of microspheres.

Kinetic parameters

Changes in pigment concentrations (mg/100 g) were used to calculate kinetic parameters of the degradation reaction. A graphic method was applied to determine kinetic equation parameters and to estimate the kinetic constants (the degradation rate constant k and the activation energy E_a). Based on the integral calculations, it was found that the reaction of degradation of both α- and β-carotene form was the first order reaction. The degradation of α- and β-carotene rate constants were determined on the basis of the slope of linear regression coefficient (a linear function is of the form $y = ax$) for the first order reactions. The dependence of the degradation reaction rate constant on temperature is presented below according to the Arrhenius equation:

$$\ln(k) = B - E_a/RT \quad (2)$$

where E_a – activation energy of the reaction a given in J/mol, R – the gas constant in J/molK. The activation energies of α- and β-carotene were determined according to a slope coefficient of the line showing dependence between natural logarithms of degradation rate constants and inverse temperatures in kelvins. The slope coefficient can be calculated from the expression – E_a / RT from the equation 2.

According to the active complex theory, enthalpies ($\Delta H^{\#}$) and activation entropies ($\Delta S^{\#}$) can be calculated from the Eyring equation:

$$\ln(k/T) = -\Delta H^{\#}/RT + \Delta S^{\#}/R + \ln(k_b/h) \quad (3)$$

where K – rate constant at temperature T, k_B – Boltzmann constant, R – universal gas constant, h – Planck's constant [Aparicio-Ruiz *et al*., 2011].

Statistical analysis

All samples were obtained at least in triplicate and their measurements were performed in at least two parallel repetitions. Statistical analysis of results was performed using Statgraphics Plus 5.0 statistical package. In order to test the hypothesis of the existence of a significant effect of daylight and temperature and storage time on the obtained results, the ANOVA was performed on two factors: the combination of daylight and temperature (3 levels: with access to daylight at 20±2°C, without access to daylight at 20±2°C, without access to daylight at 4±2°C) and storage time (7 levels: 0, 2, 4, 6, 8, 10, 12). The significance of differences between mean values was verified with Tukey's test at the significance level of p = 0.05 and homogeneous groups average were marked using alphabetic classification.

RESULTS AND DISCUSSION

Stability of carotenes oil solution

In the examined samples, α-carotene to β-carotene ratio was 1:2 according to the applied chromatographic analysis. This proportion is typical of the carrot [Rodriquez-Amaya, 2001; Rodriquez-Amaya & Kimura, 2004]. Szterk *et al*. [2008] managed to obtain a mixture of α-carotene and β-carotene at 1:1.7 ratio. The proportions between the content of α and β form depend on carrot cultivar and multiple environmental factors. However, the β form always dominates [Rodriguez--Amaya, 2001; Szterk *et al*., 2008]. Chromatographic separation of α- and β-carotene is shown in Figure 1.

The α- and β-carotene spectra were obtained in visible light. The α form wavelength of the maximum of absorbance was at 446 nm, whereas β form maximum was at 453 nm. Similar results were found by other researchers. Szterk *et al*. [2008] reported the maximum absorbance wavelength of α-carotene at 445 nm and of β-carotene at 450 nm using HPLC-MS.

The total concentration of α- and β-carotene in the investigated samples for different storage conditions are presented as a function of time and marked as single data points (Figure 2). On the day when the samples had been prepared, the total content of α and β form in oil solution was 36.6 mg/100 g and 73.0 mg/100 g, respectively. With time the content of α- and β-carotene decreased regardless of the storage conditions applied. We have observed a linear, negative and strong correlation between time and concentration of both forms of carotenes in the oil solution stored in different conditions based on regression analysis of the values of determination coefficients ranging from 88.4% to 93.3% (α form) and from 85.5% to 91.4% (β form). After 12 weeks of the storage test, the greatest decrease in β-carotene content was observed in the oil solutions stored at 20±2°C without access to daylight (78.8% of the initial content of β-carotene had degraded). The smallest decrease was noted in the samples stored at 4±2°C without the access to daylight (47.1% of the initial content of β-carotene had degraded). Similar results were noted for the form α. By the end of the experiment, α-carotene had degraded in 75.4% in samples stored at 20±2°C without access to daylight, and in 27.3% at 4±2°C without access to daylight. Based on those results we found that low temperature significantly slowed down the oxidation of both α and β form of carotene. Similar results were found by other researchers. Many studies [Marx *et al*., 2003; Lem-

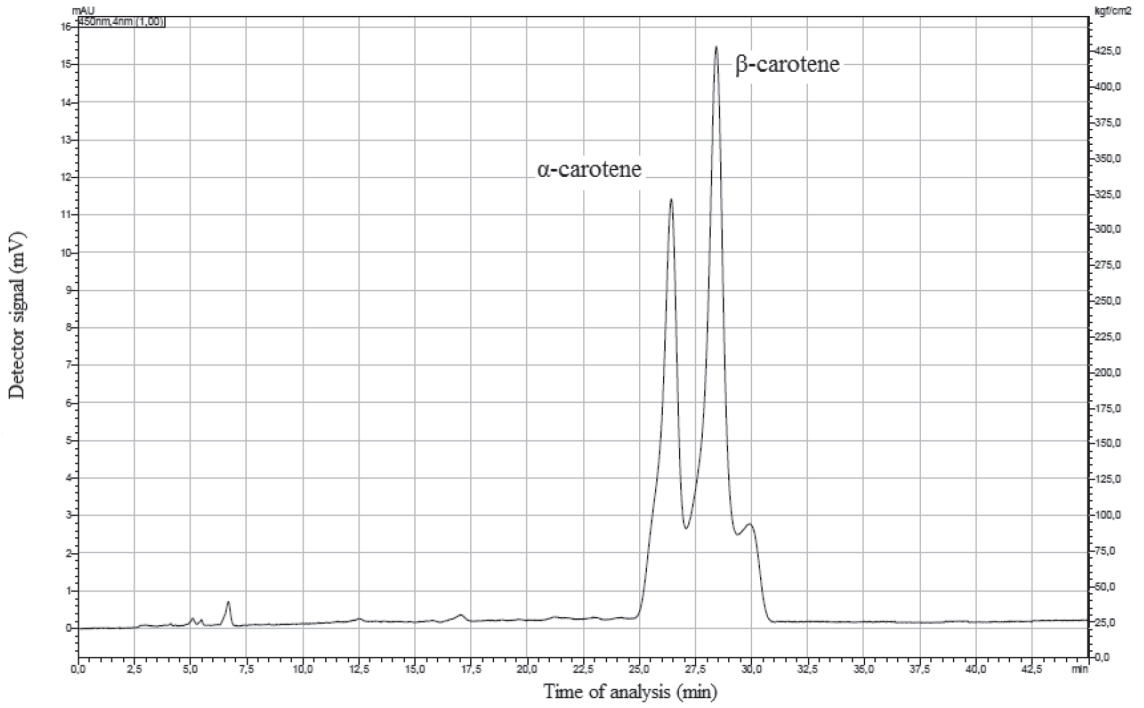

FIGURE 1. Chromatographic separation of α- and β-carotene, detection at λ=450 nm.

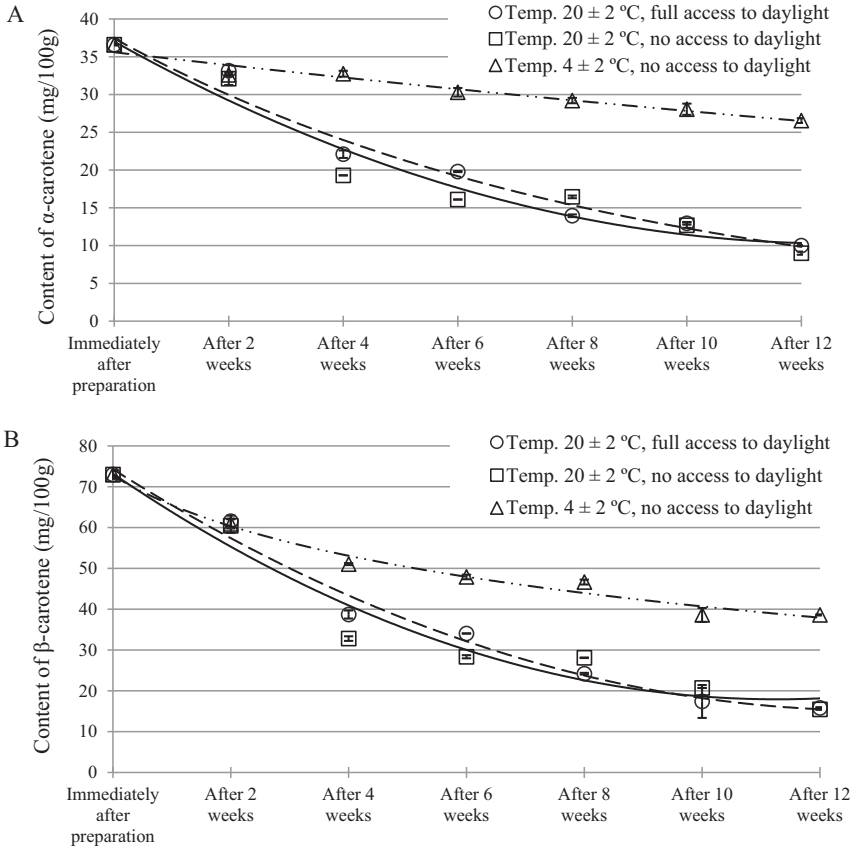

FIGURE 2. The effect of time and storage conditions on the content of α-carotene (A) and β-carotene (B) in oil solutions.

mens *et al*., 2010; Knockaert *et al*., 2012; Demiray *et al*., 2013] have proven that the storage temperature is the main factor which determines carotene stability. The process of β-carotene degradation was rapid in the first few weeks of the storage test (2–6 weeks) regardless of the conditions applied. Less violent β-carotene degradation was observed in the final period of the experiment. Similarly to β form degradation, degradation of the α form in oil solutions stored at 20±2°C both with and without access to daylight was observed at the beginning of the storage test. However, this trend has not been observed in oil solutions stored at 4±2°C without access to daylight. According to other researchers' studies [Rodríguez-Huezo *et al*., 2004], the process of carotene degradation is the fastest during the first weeks of the storage test.

Kinetic analysis

Kinetics of carotene degradation is important in terms of predicting changes during food processing in order to produce safe food of high nutritional quality [Knockaert *et al*., 2012]. Both α- and β-carotene degradation in oil solutions follows the first order kinetics. Degradation reaction rate constants were determined on the basis of presenting the natural logarithm (C_S^0/C_S) as a function of time for the applied reaction conditions. C_S^0 is the initial concentration of pigment (mg/100 g) and C_S is the concentration after 2, 4, 6, 8, 10 and 12 weeks of storage. The coefficients of determination (R^2) ranging from 0.9355 to 0.9776 (α form) and from 0.9057 to 0.9823 (β form) confirm that the reaction is first order, which is consistent with the results presented by Demiray *et al*. [2013]. The degradation rate constants (k) and other degradation kinetic parameters of the analyzed carotenes in oil solutions stored in various conditions are shown in Table 1. Degradation rate constants ranging from 0.0277 to 0.1141 (α form) and from 0.0602 to 0.1346 (β form) ($weeks^{-1}$), indicate that β-carotene is more susceptible to degradation than α form. Degradation rate constants for the tested pigments were strongly dependent on their storage temperature. As the storage temperature increases the carotenes degradation rate constants goes up. This can be interpreted in terms of the collision theory as an increase in the frequency of effective collisions between molecules. The influence of temperature on the degradation rate constant has been characterized by the activation energy and estimated at 58.7 kJ/mol and 33.6 kJ/mol for α- and β-carotene, respectively. Thus, in the case of α-carotene degradation, the transition of substrates into products required to overcome greater barrier energy than in the case of β-carotene degradation. This corroborates the fact that there is a stronger dependence between α form degradation rate and the storage temperature than in the case of β form degradation. The obtained results are similar to other researchers' findings [Achir *et al*., 2010, 2011; Pénicaud *et al*., 2011; Aparicio-Ruiz *et al*., 2011; Demiray *et al*., 2013] available in the literature on kinetics of degradation of β-carotene during heat treatment. Activation energies reported in the literature are in the wide range from 20 to 171 kJ/mol, depending on the source of β-carotene, reaction

TABLE 1. Kinetic parameters of degradation α- and β-carotene.

Kinetic parameters	α-Carotene	R^2	β-Carotene	R^2
$k_{20\pm2^oC \text{ with access to daylight}}$ (weeks^{-1})	0.1085	0.9776	0.1346	0.9823
$k_{20\pm2^oC \text{ without access to daylight}}$ (weeks^{-1})	0.1141	0.9431	0.1321	0.9356
$k_{4\pm2^oC \text{ without access to daylight}}$ (weeks^{-1})	0.0277	0.9355	0.0602	0.9057
E_a Activation energy (J/mol)	58702.66		33574.43	
Ln Z	21.89		11.76	
Z-Pre-exponential	$3.2x10^9$		$1.2x10^5$	
$\Delta H^{\#}$ (kJ/mol)	56.3		31.2	
$\Delta H^{\#}$ (J/molK)	-71.0		-155.1	

medium and the processing conditions. Activation energies of the complete degradation of β-carotene (the standard all-*trans*-β-carotene dissolved in hexane) obtained in this study are similar to those obtained by Chen & Huang [1998]. In turn, Knockaert *et al*. [2012] and Demiray *et al*. [2013] determined activation energies of the complete degradation of β-carotene at 45±8.6 and 40.17 kJ/mol, respectively.

Color changes during storage

Both, α- and β-carotene belong to the same group of carotenoids called carotenes, but they have different color. The difference in color of the analyzed compounds is caused by differences in the chemical structure related to the number of conjugated double bonds in their molecules. α-carotene has 10 conjugated double bonds in the polyene chain and is yellow, while β-carotene has 11 conjugated double bonds and is orange [Britton, 1995; Rodriguez-Amaya, 2001; Meléndez-Martínez *et al*., 2007]. Thus, the color of oil solutions of the analyzed carotenes results from individual color properties of: α-carotene, β-carotene, and oil itself. Immediately after preparation of the carotene oil solutions, parameter L* value was 59.87 and both chromatic color parameters took positive values: a* (indicating the predominant red color) was 57.58 and b* (demonstrating the dominant yellow) was 103.02 (Figure 3). With time, color brightening was observed in all carotene oil solutions (gradual increase in parameter L* value), a decrease of a red-green ratio and an increase of a yellow-blue ratio regardless of the storage conditions. However, the percentage change of those parameters depended significantly on storage conditions. There was a statistically significant correlation between temperature, daylight, storage time and the value of brightness (L*) (Table 2). The carotene oil solutions stored for 12 weeks at 20±2°C (both with and without access to daylight) were characterized by higher values of brightness in comparison with the solution stored at 4±2°C without access to daylight. The observed color brightening resulted from pigment isomerization [Orset *et al*., 1999]. Isomerization being the first stage of carotene degradation occurs easily at higher temperature, in daylight, in acidic environment and in contact with catalysts. It results in the transition

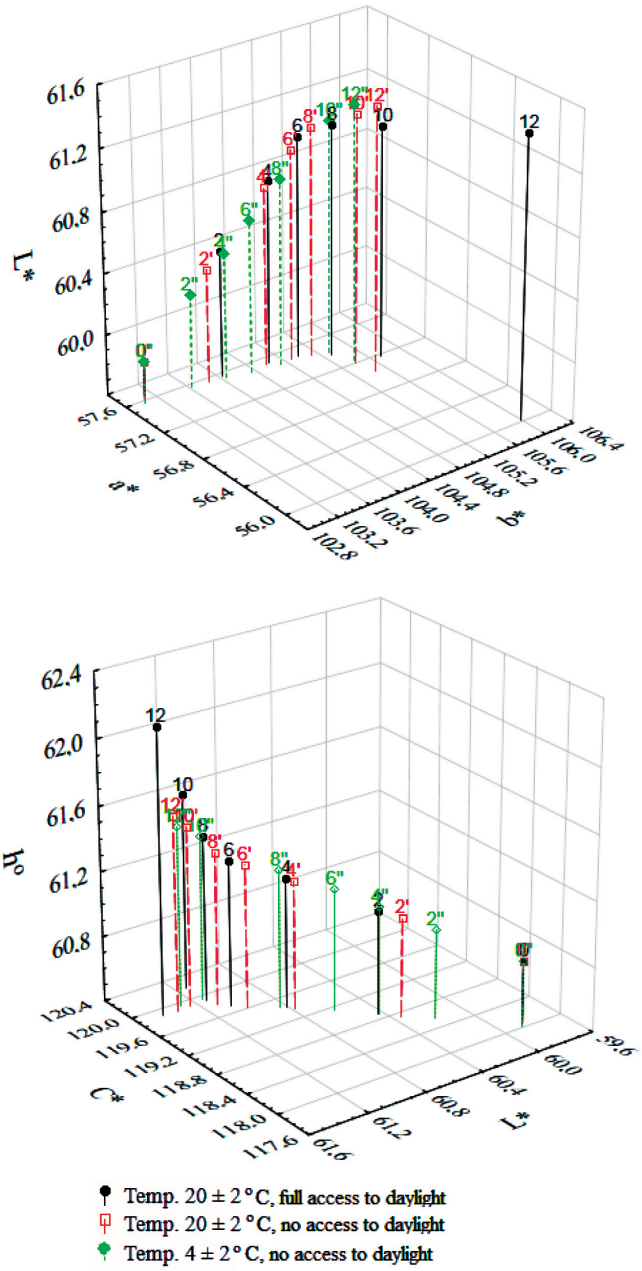

FIGURE 3. Effect of temperature, daylight access and storage time on color parameters of carotene oil solutions.

of the standard carotene from *trans* isomers into *cis* isomers [Rodriguez-Amaya, 2001], which color is considerably less intense than in the case of the *trans* form [Chen *et al*., 1995]. The largest decrease in the red-green ratio and the largest increase in the yellow-blue ratio caused by carotene degradation was observed in the solutions exposed to daylight at 20±2°C ($\Delta a^*=1.56$; $\Delta b^*=2.98$). Daylight limitation while maintaining the same storage temperature resulted in 2.9 times smaller decrease in the red-green ratio and 1.2 times smaller increase in the yellow-blue ratio. The smallest decrease in both red-green and yellow-blue ratio were found in the samples stored at 4±2°C without access to daylight ($\Delta a^*=0.35$; $\Delta b^*=2.39$). This proves the greatest carotene retention time in oil solutions and was confirmed by the smallest loss of the initial content

TABLE 2. Carotene content and color parameters of the analyzed pigments in oil solutions as affected by temperature, light and storage time.

Main effects	Content of carotenes (mg/100 g)		Color parameters				
	α-carotene ($\overline{x}$ ±SD)	β-carotene ($\overline{x}$ ±SD)	brightness L* ($\overline{x}$ ±SD)	parameter a* ($\overline{x}$ ±SD)	parameter b* ($\overline{x}$ ±SD)	chroma C* ($\overline{x}$ ±SD)	hue h ($\overline{x}$ ±SD)
Access to daylight and temperature	#	#	#	#	#	#	n.i.
Access to daylight, temp. 20±2°C	21.22±9.85^{b}	37.81±21.28^{b}	60.86±0.53^{c}	57.24±0.54^{a}	104.81±0.99^{c}	119.43±0.72^{c}	61.29±0.35^{a}
No access to daylight, temp. 20±2°C	20.32±9.81^{a}	36.98±20.59^{a}	60.81±0.51^{b}	57.40±0.20^{b}	104.61±0.86^{b}	119.33±0.68^{b}	61.24±0.27^{a}
No access to daylight, temp. 4±2°C	30.86±3.32^{c}	50.96±11.96^{c}	60.63±0.49^{a}	57.47±0.12^{c}	104.35±0.84^{a}	119.13±0.68^{a}	61.16±0.24^{a}
Storage time (weeks)	#	#	#	#	#	#	#
0	36.60±0.11^{g}	73.00±1.53^{g}	59.87±0.01^{a}	57.58±0.01^{g}	103.02±0.01^{a}	118.02±0.01^{a}	60.80±0.01^{a}
2	32.62±1.11^{f}	60.97±2.28^{f}	60.33±0.10^{b}	57.56±0.01^{f}	103.83±0.18^{b}	118.72±0.15^{b}	61.00±0.04^{b}
4	24.72±6.37^{e}	40.85±8.39^{e}	60.67±0.19^{c}	57.53±0.01^{e}	104.42±0.30^{c}	119.22±0.26^{c}	61.15±0.07^{c}
6	22.07±6.61^{d}	36.77±9.01^{d}	60.89±0.22^{d}	57.49±0.01^{d}	104.74±0.31^{d}	119.48±0.27^{d}	61.24±0.07^{d}
8	19.87±7.33^{c}	32.98±10.74^{c}	61.03±0.14^{e}	57.43±0.05^{c}	105.04±0.27^{e}	119.71±0.22^{e}	61.34±0.08^{e}
10	17.90±7.90^{b}	25.57±10.93^{b}	61.23±0.08^{f}	57.26±0.12^{b}	105.47±0.20^{f}	120.01±0.14^{g}	61.51±0.09^{f}
12	15.18±8.82^{a}	23.28±11.87^{a}	61.36±0.07^{g}	56.76±0.58^{a}	105.62±0.30^{g}	119.91±0.03^{f}	61.58±0.33^{g}

Explanatory notes: – # statistically significant at <0.05; -n.i. – insignificant; – mean values for each level of factor in columns and designated by the same letter do not differ statistically significantly; $\overline{x}$ – mean value; SD – standard deviation; all measurements were done in triplicate

of α- and β-carotene (27.3 and 47.1%, respectively). Carotene degradation occurs as a result of oxidation and is initiated by isomerization. *Cis* isomers are more reactive and their oxidation is easier. As a result of their oxidation, carotyl peroxides are formed. In epoxidation reactions with radicals, carotyl peroxides give epoxy carotenoids or dioxetanes. Dioxetanes can degrade into aldehydes and ketones [Mordi *et al*., 1993]. A decrease of the red-green ratio and an increase of brightness during storage of β-carotene encapsulated in maltodextrin matrixs have also been observed by Desobry *et al*. [1997]. Elizalde *et al*. [2002] made a similar observation while studying changes in color during β-carotene stored on the surface of an amorphous matrix made of gelatin and trehalose. Chroma of carotene oil solutions immediately after they had been prepared was 118.02. With time, we observed slight increase in color saturation in all of the oil solutions which means that their color was perceived as more vivid. After 12 weeks of solution storage, the highest chroma value was observed at 4±2°C without access to daylight (119.94). In contrast, the solutions stored at higher temperature were characterized by lower color chroma and daylight did not affect the change of color saturation. The value of the h parameter representing solution hue immediately after its preparation was 60.80°. With time of storage, we observed its gradual increase in all samples in comparison to the baseline. After 12 weeks of storage, the h parameter was on average ranging from 61.51° in the solution stored at 4±2°C to 62.15° for the solution stored at 20±2°C. There was no significant correlation between the hue angle and temperature and daylight. This parameter depended significantly only on the time of storage (Table 2). While analyzing the color of acetone solutions of α- and β-carotene isolated from natural sources (palm oil) Meléndez-Martínez *et al*. [2007] obtained chroma values of C* 42.90 and 45.00 for α- and β-carotene solution, respectively. The hue angle h was 98.96° (α form) and 93.96° (β form). However, noteworthy is that the type of organic solvent, in which carotenes were dissolved, affects carotenoid spectra obtained and thus influences their light absorption [Britton, 1995].

Correlation between color parameters and content of the analyzed pigments

On the basis of the described color parameters L*, a*, b*, we searched for a correlation between those parameters and carotene content in the solutions examined. For all of the stored samples there was a very strong correlation between all the color parameters L* (brightness), b* (yellow-blue ratio) and the content of α- and β-carotene. Coefficients of Pearson correlation between concentrations of β-carotene and values of parameters L* and b* ranged from -0.96 to -0.99 (L*) and from -0.97 to -0.98 (b*). They depended on storage conditions. For α-carotene, values of these coefficients ranged from -0.96 to -0.98. On the contrary, a correlation between the parameter a* (red-green ratio) and the content of α- and β-carotene was found moderately strong. Coefficients of Pearson correlation between the level of α-carotene and value of parameter a* ranged from 0.65 to 0.77; and for β-carotene, these coefficients ranged from 0.61 to 0.79 depending on the storage conditions. According to Desobry *et al*. [1997] and Elizalde *et al*. [2002], the chromatic coordinate b* was not a good indicator of β-carotene retention because it characterizes yellowness to blueness and these colors were not dominant in the case of the aforementioned

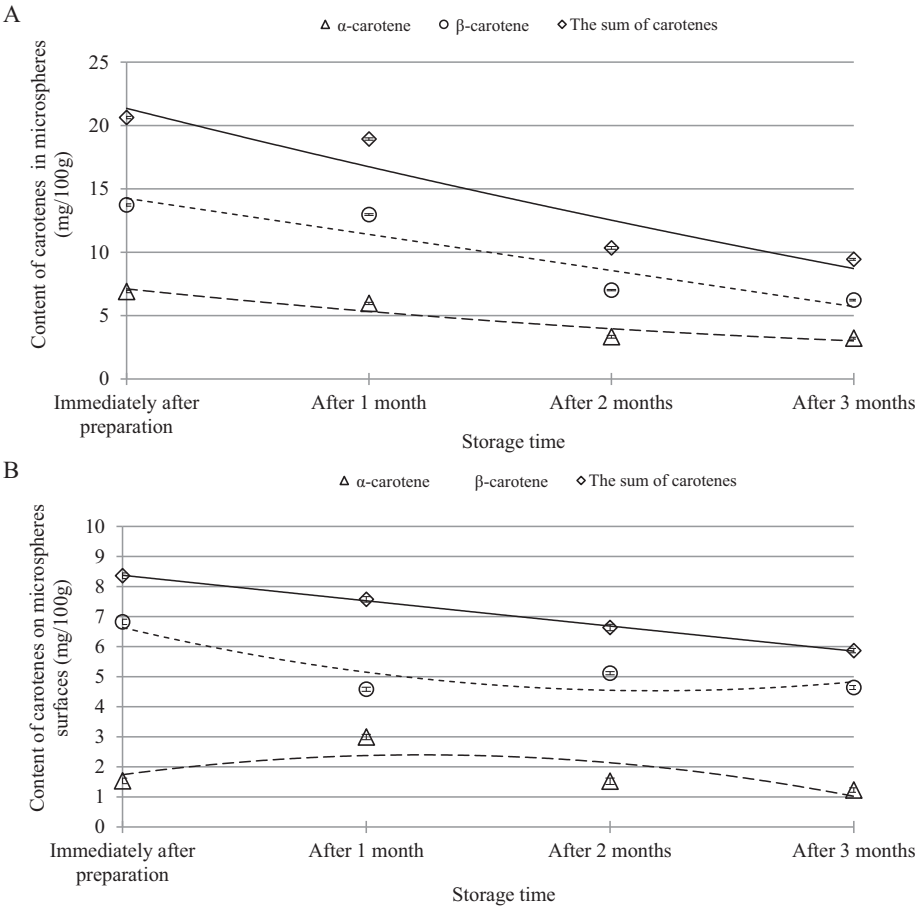

FIGURE 4. Effect of storage time on the total content of carotenes (A) and the content of carotenes on microsphere surfaces (B).

pigment. Chromatic coordinate a* is much more sensitive to changes in color of a β-carotene solution. Based on the above analysis, it can be concluded that parameters L* and b* are most useful for determination of the content of α- and β-carotene in their oil solutions. The described high correlation makes it possible to use them to measure changes in the content of α- and β-carotene during storage of oil solutions of the analyzed pigments.

Stability of microencapsulated carotenes

Figure 4 shows the carotene content on microsphere surface and the total carotene content in microspheres. Immediately after carotene microencapsulation, the total carotene content was 20.6 mg/100 g. With storage time we observed decline in the total carotene content (confirmed by regression analysis – the coefficient of determination was 89%). The greatest loss of total pigment content was observed between the first and the second month of storage (-8.6 mg/100 g). The total content of carotenes in the second month was very similar to the total content in the third month of storage. Results obtained confirmed findings from earlier other studies, which prove that the initial period of carotene degradation is more violent at the early stages of microsphere storage and the later on the process tends to be slowing down [Rodríguez-Huezo *et al*., 2004].

The carotene content on microsphere surface immediately after microencapsulation was relatively high (8.4 mg/100 g), which was 40.6% of the total microencapsulated pigments). In theory, the pigment placed on microsphere surface should degrade as first due to the lack of protection form environmental factors by the matrix. However, the surface pigment decrease was only 29.8% during the entire storage time, which might indicate a slower rate of its degradation on microsphere surface than predicted. However, the relatively large amount of the pigments on the surface may be associated with changes in the total carotene content in the microspheres and may suggest matrix cracking during their storage, which might had caused pigments dislocation from the interior of a microcapsule through gaps to its matrix surface.

The microencapsulation efficiency (ME) reflects the real amount of the pigments enclosed within a matrix [Anwar & Kunz, 2011]. Thus, the more carotenes are located on microcapsule surface, the smaller is the microencapsulation process efficiency. In the case of the applied maltodextrin – Arabic gum matrix, the microencapsulation efficiency was 59.4%. Results obtained in this study confirm that this type of matrix ensures fairly good protection of carotene oil solution droplets (after 3 months of storage, the total degradation of pigments was 54.2 %). Wagner & Warthesen [1995] found that after 8 weeks the total β-carotene degradation was 90% regardless of storage conditions. They also observed that the retention time of β-carotene was the same in microspheres exposed to fluorescent light as well as in those stored without access to daylight.

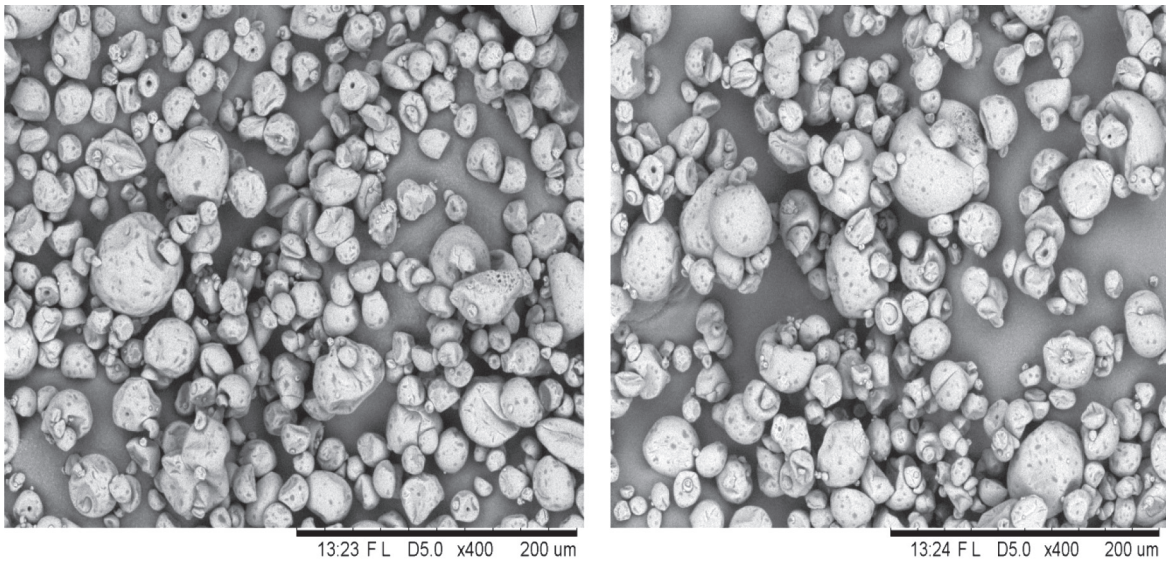

FIGURE 5. Morphology of the microspheres (magnification at 400x).

Microstructure

The scanning electron microscope analysis (SEM) (Figure 5) showed that the obtained particles had the smallest possible vacuole volume. Their shape was irregular and they varied in size. Numerous indentations as well as characteristic holes were observed on their surface. The shape and the structure of the obtained particles reflect their shrinkage during the drying process. Shrinkage of microspheres can be explained by the transport of moisture from the interior of the particle out with decreasing drying speed [Walton, 2000]. At high temperature, moisture evaporates more rapidly from the emulsion droplets and a hard layer on microspheres surface is produced more violently. Rapid evaporation of water results in simultaneous microsphere shrinkage and collapse of its structure.

Few particles had surface defects such as torn away parts exposing pores in the matrix that allowed pigments to migrate from the interior of a microspheres to the surface. Solval *et al*. [2012] noticed that if the inlet air temperature was at least 190ºC, particles cracked and this often resulted in surface defects characteristic of high temperature drying. Furthermore, the pigments closed inside microspheres could have escaped to the surface through characteristic holes in microsphere matrix. These phenomena support the earlier hypothesis, explain and interpret relatively high pigment content on the surface of microsphere matrix in relation to the total carotene content inside microspheres. This is true not only for microspheres at the end of the storage test, but for all microspheres during the entire storage time. Darker spots which can be seen on the surface of microspheres result from irregularities of their surface and the fact that prior to analysis the material had not been coated with gold, which would have dissipated the charge.

The comparison of stability of carotenes stored in oil solutions and in the microspheres

The process of preparing the emulsion and encapsulation itself had significantly affected the decrease in carotene content. Stability of carotenes in oil solutions and microspheres was compared in the same storage conditions (20±2ºC without access to daylight). After microencapsulation, carotene content was 5.3 times lower than in oil solutions immediately after preparation. However, the retention time of the microencapsulated pigments was greater during the entire test (54.2%) than the retention time of carotenes in oil solutions (22.4%). The significant decrease in carotene content after the drying process can be strongly related to oil oxidation during microsphere preparation process. This is because β-carotene may behave as an effective antioxidant to prevent oxidation of the oil, which is particularly susceptible to it during preparation of the emulsion (stirring, homogenization) and the drying process.

In addition, extended drying time also increases the rate of carotene degradation. Based on the results obtained we may conclude that microencapsulation process improves pigment retention time during storage and that gum Arabic and maltodextrin martix ensure good protection of carotenes against environmental factors.

CONCLUSIONS

HPLC analysis showed that α-carotene and β-carotene ratio in carrot was 1:2.

Storage of carotene oil solution at 4±2°C significantly slowed the process of pigment degradation, in comparison to its storage at 20±2°C.

Both α- and β-carotene degradation followed the first order kinetics mechanism. Activation energies were 58.7 and 33.6 kJ/mol for α- and β-carotene, respectively.

The brightening of color of oil solutions was the most violent during the first six weeks of the storage test, regardless of the storage conditions applied. Carotene oil solutions stored at 20±2°C with access to daylight had the highest brightness, the smallest intensity of red color and the greatest intensity of the yellow color.

Carotene retention time during storage as microspheres was greater than the retention time of pigments stored in the oil

solutions under the same conditions. The matrix composed of a mixture of gum Arabic and maltodextrin ensured good protection of carotenes against environmental factors.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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