Co-crystallization of paprika oleoresin and storage stability study

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Abstract

Paprika oleoresin was co-crystallized to formulate a natural and hydrophilic dye for use in food applications. The color stabilities of the co-crystallized and free oleoresins were evaluated at different temperatures (25 and 35 °C, 70% RH) and light exposures (light 70 watts and dark, 25 °C, 70% RH). Co-crystallization from supersaturated sucrose syrup (S = 1.32) was found to be optimum for the formation of sugar agglomerates in a few minutes, with the paprika oleoresin entrapped inside. With time, decreases in the values of the a* parameter and the β-carotene content were observed with increasing temperature and with the incidence of light. The color degradation kinetics were dependent on both factors. The loss of color was greater for the free oleoresin as compared to the co-crystallized, suggesting that the co-crystallization process improves oleoresin retention in the sugar matrix, making it less susceptible to losses due to color degradation.

Keywords: co-crystallization; paprika oleoresin; β-carotene; stability; kinetics; color.

Practical Applications: Co-crystallization process was used to encapsulate paprika oleoresin in sucrose matrix having natural color as the main technological feature of the product and developing an ingredient with good properties for application in sugar products. This approach created an ingredient with a hydrophobic compound incorporated in a hydrophilic matrix. It is an alternative process to handle and entrap a range of ingredients, aiming to improve the stability of the encapsulated material. β-carotene had good storage stability and degradation kinetics provided information to determine best storage conditions.

1 Introduction

The restriction or rejection of ingredients considered by consumers to have some potential risks has been reflected on the global market, with a trend towards natural products, which in many cases is strongly linked to the substitution of artificial additives, such as colors and flavors (Queiroz & Nabeshima, 2014). According to Munawar & Jamil (2014), consumers prefer natural sources to provide color in foods rather than synthetic ones, which are responsible for a variety of allergic reactions and attention deficit hyperactivity disorder (ADHD) in children.

Despite of synthetic dyes have lower production costs, higher stability and good dyeing capacity, their use have been declining every year in developed countries and several researches have been directed with the objective to produce dyes from natural sources (Hassani & Sharifi, 2012; Lopes et al., 2007).

Dried ground paprika, or the same product in the oleoresin form, is widely used as natural color in the food industry, having the red color due to the high concentration of fat-soluble carotenoid type pigment (Koncsek et al., 2016). Because of hydrophobic character, its application as oleoresin can be difficult in aqueous systems and hence its incorporation into a sugar matrix via co-crystallization can create an alternative means of using this extract in sugared products. Additionally, carotenoids are susceptible to isomerization and oxidation, which results in the loss of their properties and encapsulation technologies, in general, become a way to preserve and/or protect them from

adverse environmental conditions (oxygen, water, heat, light and metals) (Mahfoudhi & Hamdi, 2015).

The encapsulation of active compounds by co-crystallization technology, is a relatively simple method to be used commercially, which offers an economic and flexible alternative for preserving and handling active components used in the food industry. Furthermore, co-crystallization improves the stability of the encapsulated material (Sardar & Singhal, 2013; López-Córdoba et al., 2014). Desai & Park (2005) mention other advantages regarding the conversion of liquid materials into dry powdered form without additional drying, and direct tableting characteristics of the co-crystallized products due to their agglomerated structure, finding wide application in candy and pharmaceutical industries. Oriani et al. (2016) proposed other technique through spray chilling to deliver ginger oleoresin in solid microparticles.

Munin & Edwards-Lévy (2011) and Fang & Bhandari (2010) published reviews focused on different techniques to encapsulation of polyphenols and commented that the main advantages of the co-crystallization technique are that the granular product obtained possesses a very low hygroscopicity, good fluidity, and better stability. The encapsulation of a yerba mate extract containing flavonoids was successfully performed by co-crystallization in a saturated sucrose solution. The process changed it from a cohesive material to a non-cohesive product, and notably reduced its hygroscopic characteristics without affecting its high solubility

(Deladino et al., 2007). Sardar et al. (2013) studied the thermo-oxidative stability of the active components of co-crystallized cardamom oleoresin, and obtained good retention of α-terpinyl acetate, one of the active components of cardamom.

Thus, co-crystallization could be an alternative for the development of specific ingredients with technological and functional properties for application in many products, since the process has been shown to be efficient in improving properties such as solubility, wettability, homogeneity, dispersibility, hydrating, anti-caking and stability of the encapsulated material (Deladino et al., 2007; López-Córdoba et al., 2014).

There have been very few reports of the application of the co-crystallization process and in the present work, it is established the procedure for the production of a sucrose co-crystallized with paprika oleoresin entrapped in the porous matrix, having natural color as the main technological feature of the product. The co-crystallization process was used as a way to carry a hydrophobic compound into a hydrophilic matrix for applications in sugar systems, as well as evaluating its color stability during storage at different temperatures (25 and 35 °C, 70% RH) and light exposures (70 watts and dark, 25 °C, 70% RH), calculating the kinetic parameters also.

2 Materials and methods

2.1 Materials

Commercial refined sugar (Açucareira Boa Vista, Limeira, Brazil); Paprika oleoresin (New Max Company, Americana, Brazil); Refined soybean oil (COAMO, Campo Mourão, Brazil); Thermal oil (XIAMETER® PMX-200 Silicone Fluid, 5-20 cSt, Dow Corning Corporation, Midland, USA).

2.2 Reagents

Formamide (EMSURE® ACS, Merck S.A); Karl Fischer (CombiTitrant 5, Merck S.A, Darmstadt, Germany); Methyl alcohol (EMSURE® ACS, ISO, Reag. PhEur, Merck S.A, Darmstadt, Germany); absolute ethyl alcohol (Sinth, Diadema, Brazil).

2.3 Production of the co-crystallized paprika oleoresin

Co-crystallized particles were prepared as described by Sardar & Singhal (2013), with modifications. Briefly, commercial refined sugar (800 g) and distilled water (240 g) were preheated in a stainless steel pan (atmospheric conditions) to 119 °C, and then transferred to a thermostatic bath containing thermal oil to control the temperature accurately (Figure 1) (Silva et al., 2016). The syrup reached the final temperature of 124 °C, ranging from 0.5-1.0 °C/min, corresponding to a supersaturation ratio (S) of 1.32, where $S = C/C_0$, C being the concentration of sucrose in the solution (g sucrose/100 g solution) and C_0 the saturation concentration of the sugar at approximately the same temperature (Hartel, 2001). A slight turbidity of the sucrose syrup indicated the beginning of the crystallization process when the thermal level was kept constant, and the active component (16 g of a blend of paprika oleoresin and soy oil, 10% w/w, representing 4% of the blend in the co-crystallized product) was added with mild stirring (50 rpm, using a vertical stirrer: TECNAL –

model TEC039/1, Piracicaba, Brazil) for a few seconds. Stirring was then increased to 450 rpm until complete crystallization (approx. 3 minutes). The co-crystallized product was screened (3.36 mm sieve) and cooled to 25 °C (room temperature). The latent heat of crystallization was sufficient to evaporate the moisture, avoiding an additional drying step.

2.4 Characterization of the co-crystallized product

Granulometric analysis

The percentage of material retained on each sieve with predefined openings of 2.38 mm and 1.70 mm was determined using a sieve shaker (Produtest, São Paulo, Brazil) with the rheostat activated at position 8 for 10 minutes. The agglomerates smaller than 1.70 mm were used in the characterization and stability study.

Moisture content

This was determined by volumetric Karl Fischer titration (Titrando 901, Methrom Pensalab, São Paulo Brazil) with a methanol:formamide solution (2:1, v/v) as the solvent.

Water activity (a_w)

This was determined at 25 °C using an electronic water activity meter equipped with a temperature-controlled system (Aqua Lab series 4 model TEV, Decagon Devices Inc., Pullman, USA).
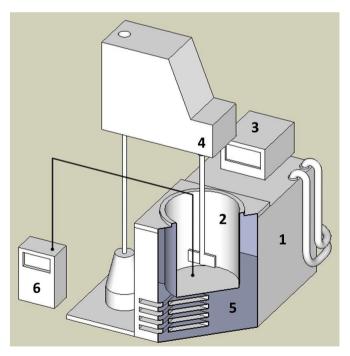

Figure 1. Schematic diagram of the co-crystallization system used. 1. Thermal bath (Ecoline Staredition RE212, Lauda, Lauda-Königshofen Germany); 2. Vat for concentration; 3. Control unit for programming the temperature of the thermal oil; 4. Stirring/beating system TE039/1, Tecnal, Piracicaba, Brazil); 5. Thermal oil; 6. Temperature recorder (Almemo® 2390-5, Ahlborn GmbH's, Germany). Source: Silva et al. (2016).

Quantification of total paprika oleoresin entrapped in the co-crystallized (TPE)

The quantification of paprika carried in the co-crystallized was based on assumption proposed by Sartori et al. (2015) and methodology described by Alvim et al. (2016), with modifications. Sartori et al. (2015) proposed quantify the encapsulation of ascorbic acid and defined as total encapsulation efficiency the ratio between the total amount of ascorbic acid present in the sample and the initial amount of acid ascorbic added before encapsulate production. Similarly, it was proposed quantify the Total Paprika Entrapped (TPE) considering the ratio between the total β-carotene present in the sample (β-car$_T$) and the β-carotene amount added in the co-crystallization process (β-car$_0$), as a percentage, according Equation 1.

$$TPE\ (\%) = \ (\beta - car_T\ /\ \beta - car_0\ ).100 \tag{1}$$

Although capxantin and capsorubin are the main oleoresins, representing 80% of the extractable pigments from paprika, β-carotene plays an important role in the color of the product and is more susceptible to oxidation. Thus, it was defined as the active compound to be quantified in order to evaluate the paprika entrapped.

β-caroteno was quantified by spectroscopy (UV-VIS 310 Spectrophotometer, Allcrom, Shanghai, China), measuring the absorbance at 452 nm of the co-crystallized agglomerates dissolved in water and ethanol. According to Alvim & Grosso (2010), the spectrum of ground paprika (ranging between 290 and 700 nm), indicated maximum absorbance at 452 nm, the absorbance ranging between 410 and 510 nm, associated with the carotenoids found in paprika and its oleoresins, depending on the type of solvent used. Sample preparation consisted of dissolving 2 g of the co-crystallized product in 3 mL of distilled water using a 100 mL volumetric flask and stirring for 2 minutes. Absolute ethanol was then added to complete the volume and stirred for a further 2 minutes until total homogenization. A standard curve prepared from successive dilutions of a 10% solution of oleoresin of paprika was used for quantification. The value reported was the mean of six replicates.

Dissolution time (Solubility)

This was determined by delivering a known mass of the co-crystallized product (10 g) into 100 mL of distilled water at 22 °C with manual stirring. Aliquots were removed at different times and the dissolved sucrose mass in the solution was determinated using an Abbe digital Refractometer Q767BD (Quimis, São Paulo, Brazil), until solution reached equilibrium (Sardar & Singhal 2013; López-Córdoba et al., 2014).

Bulk density (g/cm³)

It was evaluated by measuring the volume of 30 g of co-crystallized product deposited freely by gravity into a 250 mL graduated cylinder. The cylinder was slightly beat against a flat surface until the volume occupied by the powder reached a constant value, and the density was calculated dividing the mass by the bulk volume (Astolfi-Filho, 2003; López-Córdoba et al., 2014).

Particle size distribution

The size distribution and mean diameter were determined by laser scattering using a LA 950-V2 equipment (Horiba, Kyoto, Japan). The particle was dispersed in absolute ethyl alcohol and result was determined for an average fraction of 0.1000 g sample, making 5 readings for each measurement. The mean particle size was expressed as the average diameter of a sphere of the same volume (De Brouckere diameter – D$_{[4,3]}$), and the polydispersity was given by the span index, which was calculated according to Equation 2 (Sartori et al., 2015; Alvim et al., 2016; Oriani et al., 2016).

$$SPAN\ = \ (D_{90}\%\ -\%\ D_{10})\ /\ D_{50}\% \tag{2}$$

The diameters of the cumulative distributions D$_{0.1}$, D$_{0.5}$ and D$_{0.9}$ (corresponding to 10, 50 and 90% of accumulated size distribution).

Morphology

The co-crystallized morphology was observed under optical microscopy, using a Microscope BX41 (Olympus, Japan) supported by polarized light filter and external halogen light source (LGPS, Olympus, Japan) at 40 and 100 magnification and captured using a digital camera (Q-color, Olympus America Inc., USA).

2.5 Storage study

Co-crystallized paprika oleoresin was submitted to two treatments in order to investigate its color stability. One treatment aimed to evaluate the effect of temperature on color degradation and the other to evaluate the effect of light. Raw sucrose and paprika oleoresin were blended and used for control purposes. This sample will be referred to as "free oleoresin".

For the effect of temperature, the samples were kept in two chambers at 25 °C and 35 °C and 70% of relative humidity (RH) for 4 weeks (BOD Eletrolab 131 with mechanical humidity controller, São Paulo, Brazil). During storage the temperature and RH were monitored with support of a thermohygrometer (Testo 608-H1, Lenzkirch, Germany) placed inside the chambers.

For the effect of light, the samples were exposed to constant illumination (70 Watts lump) and kept in a dark chamber, both at 25 °C/70% RH for 4 weeks.

In both studies the samples were produced in triplicate and the analyzes performed in triplicate per sample, totalizing nine measures expressed as means in the final results.

After pre-selected times, samples were removed and analyzed for β-carotene retention and color.

The oleoresin concentration or β-carotene retention were evaluated as previously described in the section on quantification of total paprika oleoresin entrapped in the co-crystallized.

The color was determined instrumentally using a CR-410 spectrophotometer (Konica Minolta Inc., Osaka, Japan), with the chromatic characteristics expressed by the *CIE L*a*b** system, with ten triplicates.

2.6 Color degradation kinetics

Color degradation was calculated using a first-order kinetic model (Equation 3), where ´C_o` and ´$C_t$` are the quality parameters at zero and t times (days), respectively, and ´k` is the first-order kinetic constant. A first-order model was used to fit the thermal degradation of β-carotene and color changes with time, as used by other authors (Spada et al., 2012; Chen et al., 2009; Mahfoudhi & Hamdi, 2015). Despite the fact that β-carotene degrades into various oxidized and cleavage molecules, the majority of the kinetic models used to describe β-carotene degradation are single-response kinetic models (Colle et al., 2015).

Although the main changes suffered by carotenoids are degradation and isomerization, oxidation is by far the major cause of its degradation and exposure to oxygen, water, heat, light and metals in the environment may accelerate this process (Mahfoudhi & Hamdi, 2015).

The half-time ($t_{1/2}$) was also calculated (Equation 4) (Spada et al., 2012).

$$\ln(\frac{C_t}{C_0}) = \pm kt \tag{3}$$

$$t_{1/2} = \frac{0.693}{k} \tag{4}$$

The temperature dependency of the reaction rates for degradation is usually quantified by Arrhenius model (Equation 5), where E_a is the activation energy of the (k) reaction (kJ/mol), R is the universal gas constant (8.3145 J/mol.K) and T is the absolute temperature (K).

$$\ln \frac{k_2}{k_1} = \frac{E_a}{R} \left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{5}$$

A first-order model was also used to fit the β-carotene degradation and color changes with time for the effect of light.

2.7 Data analysis

All experiments and/or measurements were carried out at least in triplicate. The mean ± standard deviation (SD) was reported, unless otherwise specified.

The experimental data were fitted to a first-order kinetic model calculated using the Statistica 12 software for Windows (Statsoft Inc., Tulsa, OK, USA).

3 Results and discussion

Co-crystallization from a supersaturated sucrose syrup (S = 1.32) was found to be optimum for the formation of sugar agglomerates with paprika oleoresin entrapped inside, taking only a few minutes. According to Hartel (2001), this level of supersaturation provides sufficient driving force for the fast crystallization of sucrose. It was observed that the addition of paprika oleoresin to the system did not delay the phase transition, which occurred after approximately 3 minutes of agitation. Furthermore, the latent heat of crystallization was sufficient to evaporate the moisture without additional drying.

3.1 Characterization of the co-crystallized product

Granulometric analysis

Separation of the co-crystallized product using sieves resulted in 68.5% of agglomerates smaller than 1.70 mm (fraction used in the characterization and stability study) and 31.5% which were retained on the 2.38 mm sieve.

Total Paprika Entrapped (TPE%), calculated from the standard curve for paprika oleoresin (y = 0.0960 x + 0.043, R^2 = 0.989) was found to be 95.02 ± 2.09%, indicating that co-crystallization was able to retain most of the paprika oleoresin added in the agglomerated matrix.

The properties of the co-crystallized product were: moisture content 2.67 ± 0.23 (%, w/w), a_w 0.590 ± 0.006, dissolution time 110 ± 5 s and bulk density 0.789 ± 0.006 g/cm³.

Both the moisture content and water activity of the co-crystallized product presented low values, so it can be considered safe with respect to microbiological stability (Barbosa-Cánovas et al., 2007). It is important to note that the process used was able to eliminate sufficient moisture during phase transition, avoiding further drying, as is sometimes required for co-crystallized products (Sardar & Singhal, 2013; López-Córdoba et al., 2016). The co-crystallized was characterized as a granular, dry and free-flowing particle.

The dissolution time was similar to those found for other co-crystallized products (112, 128 and 135 s for different co-crystallized extracts of yerba mate/Deladino et al., 2007 and 100-108 s for cardamon oleoresin encapsulated in a co-crystallized system/Sardar & Singhal, 2013). On the other hand, Astolfi-Filho (2003), analyzing co-crystallized products made with passion fruit juice, obtained fast dissolution of 36.7-60.0 s. It was observed that longer dissolution times were related to hydrophobic active components, when compared to pure sucrose (97 s/Deladino et al., 2007). This time was defined when no changes in the soluble solids content determined by the retractometer were noticed.

The bulk density was similar to that found by Vázquez & Beristain (1998) studying a co-crystallized cucumber concentrate, 0.762-0.862 g/cm³, and greater than that of the pure sucrose agglomerate, as indicated by Deladino et al. (2007) (0.651 ± 0.079 g/cm³).

Particle size distribution and morphology

The particles smaller than 1.70 mm presented a mean diameter expressed as $D_{[4,3]}$ = 756.7 ± 48.6μm.

$D_{(0.1)}$=177.4 ± 14.9 μm; $D_{(0.5)}$=749.6 ± 37.1; $D_{(0.9)}$=1,318.7 ± 103.5 μm which represent the diameter of accumulated distribution of 10%, 50% and 90% of the total particles, showed a behavior of a monomodal size distribution with high polydispersity (Span = 1.5 ± 0.1). Particles showed a typical polygonal shape, irregular aspect corresponding to cluster-like agglomerates, characteristic of co-crystallized products and orange color of paprika oleoresin in the void spaces as it can observed in the Figure 2.

3.2 Color changes during storage

The results for the determination of the chromatic characteristics during storage showed that a* (redness) decreased and L* (lightness) increased. According Spada et al. (2012), a* decreased and L* increased in study of β-carotene microencapsulated in starch suggest that β-carotene oxidation was responsible for reducing the intensity of the red color and expose the white color of starch. The chromatic coordinate b* was not a good indicator of β-carotene retention, since this parameter characterizes the extent of blueness to yellowness, colors not dominant in the paprika oleoresin from red pepper. Similar stability results were reported by Mahfoudhi & Hamdi (2015) for β-carotene encapsulated by freeze-drying.

Tables 1 and 2 present the results obtained for the chromatic coordinate a* and for the concentration of colored compounds as calculated from the standard curve for oleoresin, obtained in the study of degradation with time against temperature variation and with the presence/absence of light, respectively.

A decrease in the a* values was observed with an increase in temperature and with the incidence of light, suggesting that color degradation was dependent on both factors. However, it can be seen that the loss of color was more pronounced for the free oleoresin sample than for the co-crystallized sample, indicating that co-crystallization promoted greater protection of the natural paprika color when exposed to critical environmental conditions.

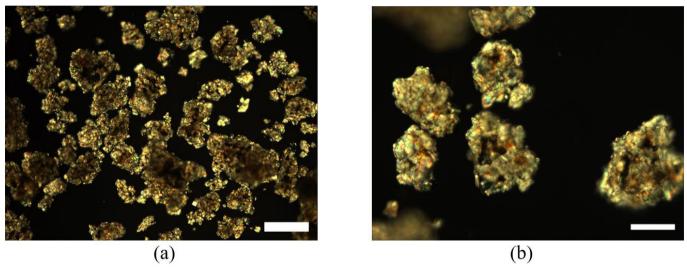Co-crystallization also promoted greater β-carotene stability than in the free paprika oleoresin. In this process, the

Figure 2. Images obtained by optical microscopy of the Paprika co-crystallized. (a) correspond to 40x/ scale 500μm (b) correspond to 100x/scale 200μm.

Table 1. Results for a* and for the concentration of colored compounds as a function of temperature for the co-crystallized and free oleoresin samples.

| Time (days) | Free oleoresin sample | | | |
| | 25 °C | | 35 °C | |
	a*	Concentration (mg/mL)	a*	Concentration (mg/mL)
0	30.67 ± 0.25	0.509 ± 0.002	29.36 ± 0.57	0.509 ± 0.002
7	25.59 ± 0.55	0.318 ± 0.011	20.71 ± 0.40	0.192 ± 0.001
15	23.30 ± 0.55	0.203 ± 0.022	16.40 ± 0.36	0.142 ± 0.011
18	21.00 ± 0.44	0.132 ± 0.020	13.50 ± 0.06	0.102 ± 0.004
21	19.60 ± 0.35	0.122 ± 0.007	10.70 ± 0.06	0.042 ± 0.006
28	16.60 ± 0.23	0.070 ± 0.002	10.50 ± 0.25	0.031 ± 0.001
Time (days)	Co-crystallized sample			
	25 °C		35 °C	
	a*	Concentration (mg/mL)	a*	Concentration (mg/mL)
0	27.64 ± 0.47	0.376 ± 0.014	27.91 ± 0.80	0.376 ± 0.014
7	27.08 ± 0.26	0.335 ± 0.003	25.37 ± 0.89	0.324 ± 0.002
15	24.68 ± 0.46	0.332 ± 0.005	22.53 ± 0.12	0.304 ± 0.004
18	22.96 ± 0.16	0.321 ± 0.009	21.36 ± 0.61	0.293 ± 0.008
21	23.74 ± 0.50	0.318 ± 0.010	21.77 ± 0.92	0.292 ± 0.013
28	20.32 ± 0.55	0.314 ± 0.002	18.48 ± 0.31	0.243 ± 0.013

crystalline structure of sucrose is modified from perfect to irregular agglomerated crystals to provide a porous matrix in which the oleoresin can be incorporated, improving its stability. According to Bhandari et al. (1998), when sensitive compounds are locked inside the porous structure of agglomerates, they are protected against oxidation, for example. Other authors have also reported that co-crystallization improved the stability of the active component entrapped in the sugar matrix (Deladino et al., 2007; Sardar et al., 2013).

Figure 3 shows the retention of β-carotene in the free oleoresin and co-crystallized samples stored at 25 and 35 °C (a) and in the presence and absence of light (b) with time.

The retention of β-carotene was influenced by temperature and notably by the co-crystallization process. After 28 days of storage, the co-crystallized sample still retained 84% and 65% of the β-carotene at 25 and 35 °C, respectively, whereas the free oleoresin only retained 14% and 6% at the same temperatures.

The effect of light on the loss of β-carotene was pronounced for both free and co-crystallized oleoresin, although retention was higher in the co-crystallized sample. Retentions of 68% and 73% were observed in the free oleoresin and co-crystallized samples, respectively, in the absence of light. For the samples exposed to light the retention was 2% (free oleoresin) and

Table 2. Results for a* and for the concentration of colored compounds as a function of light for the co-crystallized and free oleoresin samples.

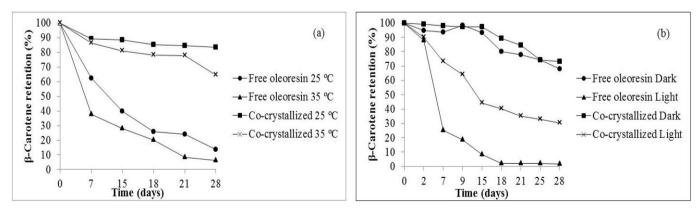| Time (days) | Free oleoresin sample | | | |
| | Dark | | Light | |
	a*	Concentration (mg/mL)	a*	Concentration (mg/mL)
0	30.89 ± 0.61	0.352 ± 0.006	31.11 ± 0.50	0.350 ± 0.021
2	28.49 ± 0.36	0.334 ± 0.003	26.65 ± 0.72	0.307 ± 0.019
7	27.53 ± 0.35	0.330 ± 0.024	6.24 ± 0.25	0.088 ± 0.001
9	25.12 ± 0.72	0.346 ± 0.032	2.20 ± 0.06	0.065 ± 0.015
15	23.43 ± 0.40	0.329 ± 0.004	0.65 ± 0.06	0.029 ± 0.007
18	23.38 ± 0.64	0.282 ± 0.020	0.61 ± 0.00	0.007 ± 0.000
21	22.39 ± 0.35	0.274 ± 0.008	0.50 ± 0.00	0.007 ± 0.016
25	16.59 ± 0.32	0.261 ± 0.005	0.31 ± 0.00	0.007 ± 0.017
28	11.57 ± 0.15	0.239 ± 0.003	0.30 ± 0.00	0.006 ± 0.001
	Co-crystallized sample			
Time (days)	Dark		Light	
	a*	Concentration (mg/mL)	a*	Concentration (mg/mL)
0	31.18 ± 0.25	0.401 ± 0.008	31.01 ± 0.38	0.421 ± 0.009
2	28.22 ± 0.44	0.397 ± 0.008	25.57 ± 0.78	0.379 ± 0.033
7	27.73 ± 0.32	0.393 ± 0.001	23.09 ± 0.69	0.308 ± 0.004
9	27.22 ± 0.56	0.391 ± 0.001	20.92 ± 0.64	0.269 ± 0.001
15	24.62 ± 0.78	0.390 ± 0.005	17.00 ± 0.82	0.187 ± 0.002
18	23.86 ± 0.50	0.358 ± 0.005	17.19 ± 0.38	0.169 ± 0.004
21	22.03 ± 0.59	0.339 ± 0.030	16.80 ± 0.14	0.148 ± 0.003
25	18.52 ± 0.40	0.298 ± 0.003	12.47 ± 0.80	0.139 ± 0.010
28	15.92 ± 0.64	0.294 ± 0.002	10.65 ± 0.35	0.128 ± 0.017

Figure 3. The retention of β-carotene in the free oleoresin and co-crystallized samples as a function of time and storage conditions (a) 25 and 35 °C, (b) presence and absence of light.

30% (co-crystallized) after 28 days of storage. It is important to mention that the free oleoresin sample stored under light presented an intense loss of β-carotene, which occurred rapidly in the first days of storage.

3.3 The kinetics of color degradation

The results showed that the values calculated assuming a first-order kinetic model fitted the experimental data with correlation coefficients (R^2) ranging from 0.80 to 0.99. Tables 3 and 4 show the kinetic parameters for color degradation of the free oleoresin and co-crystallized samples stored under different conditions.

The first-order kinetic model constant (k) showed an increase in color degradation with increasing temperature for both samples, but in general way, it were observed higher k values for free oleoresin samples when compared to the co-crystallized, suggesting that the co-crystallization process improve the color stability.

The estimated half-life time for the co-crystallized oleoresin was higher, 115 and 50 days for β-carotene at 25 and 35 °C, respectively, as against 10 and 7 days for the free oleoresin at the same temperatures (Table 3).

Spada et al. (2012) reported a half-life of 10 days for free β-carotene stored at 25 °C in the dark and a good fit for the first-order kinetic model in a study of β-carotene microencapsulation with *pinhão* starch. Similar results were observed for β-carotene encapsulated using freeze-drying with almond and arabic gum, as shown by Mahfoudhi & Hamdi (2015).

Several authors have reported the protective effect of different encapsulation processes against β-carotene oxidation, and the present study suggested that co-crystallization in a sucrose matrix could also be effective in delaying its loss with time.

Table 3 shows an adverse effect for activation energy between the samples. Although in both cases there was an increase of the reaction speed for the two parameters (a*color parameter and β-carotene concentration), it observed that they did not follow a same trend in the temperature dependence. For free oleoresin the decrease of a* presented the activation energy of 11.13 kcal/mol compared to the loss of β-carotene with Ea=6.13 kcal/mol. On the other hand, for the co-crystallized the activation energy for the loss of β-carotene (15.34 kcal/mol) was 3 times higher when compared to the reduction of the color given by the parameter a* (4.9 kcal/mol). According Elizalde et al. (2002) results for retention of β-carotene encapsulated in a trehalose-based matrix showed that the surface color change was not a sensitive indication of carotene retention, since the active compound was inside of the particle, suggesting that a high percentage of trapped β-carotene need to be lost in order to detect a sensitive change of a* color parameter. In the co-crystallization process the oleoresin was entrapped more in the pores of the crystals and less exposed on the surface compare to the free oleoresin and possibly the change in particle characteristic may have been the cause of this adverse effect found.

The results obtained in the stability study when exposed to light showed an increase in the reaction rate for both free and co-crystallized oleoresin exposed to constant illumination, but it can be seen that the values are even higher for the free oleoresin when compared with the co-crystallized sample. Assuming a fit for the first-order kinetic model, the estimated times required for β-carotene to degrade to 50% of its original value would be 4 and 55 days for free oleoresin in the light and dark, respectively, and 15 and 61 days for the co-crystallized sample, as can be seen in Table 4.

Although the effect of light was more evident in the degradation of β-carotene, greater retention was observed when it was trapped in the crystallized matrix.

Table 3. Kinetic parameters for color degradation of the free oleoresin and co-crystallized samples stored at 25 and 35 °C.

	Free oleoresin sample				Co-crystallized sample			
	a* color parameter		Concentration (mg/mL)		a* color parameter		Concentration (mg/mL)	
Temperature (°C)	25	35	25	35	25	35	25	35
k	0.0212	0.0390	0.0709	0.0992	0.0108	0.0141	0.006	0.0139
r^2	0.98	0.95	0.99	0.94	0.91	0.97	0.84	0.94
$t_{1/2}$ (days)	33	18	10	7	64	49	115	50
Ea (Kcal/mol)	11.13		6.13		4.9		15.34	

Table 4. Kinetic parameters for color degradation of the free oleoresin and co-crystallized samples stored in the absence and presence of light.

	Free oleoresin sample				Co-crystallized sample			
Storage	a* color parameter		Concentration (mg/ mL)		a* color parameter		Concentration (mg/mL)	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
k	0.1765	0.0273	0.1592	0.0125	0.0331	0.0207	0.0447	0.0113
r^2	0.92	0.80	0.93	0.89	0.95	0.91	0.98	0.83
$t_{1/2}$ (days)	4	25	4	55	21	33	15	61

4 Conclusion

The process allowed manufacture of particles carrying a large amount of paprika oleoresin and was shown to be an interesting alternative for developing a hydrophilic ingredient to be used as natural color in candy industry. The co-crystallization was a good alternative to handle this material for further applications in food products converting an oil (hydrophobic active compound) into granular, dry and free-flowing particle. The agglomerates obtained showed low moisture content and water activity, suggesting good microbiological stability. The kinetics of color degradation presented a good fit for first order models and was dependent on both temperature and the incidence of light, emphasizing the importance of the storage conditions on color retention. The co-crystallization process improved the retention of β-carotene in the sugar matrix, making it less susceptible to losses due to the degradation of color at higher temperatures and exposure to light.

References

Alvim, I. D., & Grosso, C. R. F. (2010). Microparticles obtained by complex coacervation: Influence of the type of reticulation and the drying process on the release of the core material. *Food Science and Technology (Campinas)*, 30(4), 1069-1076. http://dx.doi.org/10.1590/S0101-20612010000400036.

Alvim, I. D., Stein, M. A., Koury, I. P., Dantas, F. B. H., & Cruz, C. L. C. V. (2016). Comparison between the spray drying and spray chilling microparticles contain ascorbic acid in a baked product application. *Lebensmittel-Wissenschaft + Technologie*, 65, 689-694. http://dx.doi.org/10.1016/j.lwt.2015.08.049.

Astolfi-Filho, Z. (2003). *Encapsulação de sucos de frutas por co-cristalização com sacarose* (Dissertação de mestrado). São José do Rio Preto: Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista.

Barbosa-Cánovas, G. V., Fontana, A. J., Schmidt, S. J., & Labuza, T. P. (2007). *Water activity in foods: fundamentals and applications*. Ames: Blackwell Publishing. http://dx.doi.org/10.1002/9780470376454.

Bhandari, B. R., Datta, N., D'arcy, B. R., & Rintoul, G. B. (1998). Cocrystallization of honey with sucrose. *Lebensmittel-Wissenschaft + Technologie*, 31(2), 138-142. http://dx.doi.org/10.1006/fstl.1997.0316.

Chen, J., Shi, J., Xue, S. J., & Ma, Y. (2009). Comparison of lycopene stability in water-and oil-based food model system under thermal- and light-irradiation treatments. *Lebensmittel-Wissenschaft + Technologie*, 42(3), 740-747. http://dx.doi.org/10.1016/j.lwt.2008.10.002.

Colle, I. J., Lemmens, L., Knockaert, G., Van Loey, A., & Hendrickx, M. (2015). Carotene degradation and isomerization during thermal processing: A Review on the kinetic aspects. *Critical Reviews in Food Science and Nutrition*, 56(11), 1844-1855. http://dx.doi.org/10.1080/10408398.2013.790779. PMid:25830560.

Deladino, I., Anbinder, P. S., Navarro, A. S., & Martino, M. N. (2007). Co-crystallization of yerba mate extract (*Illex paraguaiensis*) and mineral salts within a sucrose matrix. *Journal of Food Engineering*, 80(2), 573-580. http://dx.doi.org/10.1016/j.jfoodeng.2006.06.016.

Desai, K. G. H., & Park, H. J. (2005). Recent developments in microencapsulation of food ingredients. *Drying Technology*, 23(7), 1361-1394. http://dx.doi.org/10.1081/DRT-200063478.

Elizalde, B. E., Herrera, M. L., & Buera, M. P. (2002). Retention of β-carotene encapsulated in a trehalose-based matrix as affected by water content and sugar crystallization. *Journal of Food Science*, 67(8), 3039-3045. http://dx.doi.org/10.1111/j.1365-2621.2002.tb08856.x.

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols - a review. *Trends in Food Science & Technology*, 21(10), 510-523. http://dx.doi.org/10.1016/j.tifs.2010.08.003.

Hartel, R. W. (2001). *Crystallization in foods*. Gaithersburg: An Aspen Pub.

Hassani, B., & Sharifi, A. (2012). Application of anthocyanin extracted from barberry in food processing. *International Journal of Agriscience*, 2, 522-528.

Koncsek, A., Daood, H. G., & Helyes, L. (2016). Kinetics of carotenoid degradation in spice paprika as affected by storage temperature and seed addition. *Acta Alimentaria*, 45(4), 459-468. http://dx.doi.org/10.1556/066.2016.45.4.1.

Lopes, T. J., Xavier, M. F., Quadri, M. G. N., & Quadri, M. B. (2007). Antocianinas: uma breve revisão das características estruturais e da estabilidade. *Revista Brasileira Agrociência*, 13(3), 291-297.

López-Córdoba, A., Deladino, L., Agudelo-Mesa, L., & Martino, M. (2014). Yerba mate antioxidant powders obtained by co-crystallization: Stability during storage. *Journal of Food Engineering*, 124, 158-165. http://dx.doi.org/10.1016/j.jfoodeng.2013.10.010.

López-Córdoba, A., Gallo, L., Bucalá, V., Martino, M., & Navarro, A. (2016). Co-crystallization of zinc sulfate with sucrose: A promissory strategy to render zinc solid dosage forms more palatable. *Journal of Food Engineering*, 170, 100-107. http://dx.doi.org/10.1016/j.jfoodeng.2015.09.024.

Mahfoudhi, N., & Hamdi, S. (2015). Kinetic degradation and storage stability of β-carotene encapsulated by freeze-drying using almond gum and gum Arabic as wall materials. *Journal of Food Processing and Preservation*, 39(6), 896-906. http://dx.doi.org/10.1111/jfpp.12302.

Munawar, N., & Jamil, H. M. T. H. (2014). The Islamic perspective approach on plant pigments as natural food colourants. *Procedia: Social and Behavioral Sciences*, 121, 193-203. http://dx.doi.org/10.1016/j.sbspro.2014.01.1120.

Munin, A., & Edwards-Lévy, F. (2011). Encapsulation of natural polyphenolic compounds: a review. *Pharmaceutics*, 3(4), 793-829. http://dx.doi.org/10.3390/pharmaceutics3040793. PMid:24309309.

Oriani, V. B., Alvim, I. D., Consoli, L., Molina, G., Pastore, G. M., & Hubinger, M. D. (2016). Solid lipid microparticles produced by spray chilling technique to deliver ginger oleoresin: Structure and compound retention. *Food Research International*, 80, 41-49. http://dx.doi.org/10.1016/j.foodres.2015.12.015.

Queiroz, M. B., & Nabeshima, E. H. (2014). Naturalidade e autenticidade. In G. C. Queiroz, R. A. Rego & D. C. P. Jardim. *Brasil bakery & confectionery trends 2020* (pp. 159-195). Campinas: ITAL.

Sardar, B. R., & Singhal, R. S. (2013). Characterization of co-crystallized sucrose entrapped with cardamom oleoresin. *Journal of Food Engineering*, 117(4), 521-529. http://dx.doi.org/10.1016/j.jfoodeng.2012.12.011.

Sardar, B. R., Tarade, K. M., & Singhal, R. S. (2013). Stability of active components of cardamom oleoresin in co-crystallized sugar cube during storage. *Journal of Food Engineering*, 117(4), 530-537. http://dx.doi.org/10.1016/j.jfoodeng.2013.03.035.

Sartori, T., Consoli, L., Hubinger, M. D., & Menegalli, F. C. (2015). Ascorbic acid microencapsulation by spray chilling: Production and characterization. *Lebensmittel-Wissenschaft + Technologie*, 63(1), 353-360. http://dx.doi.org/10.1016/j.lwt.2015.03.112.

Silva, L. B., Queiroz, M. B., Fadini, A. L., Fonseca, R. C. C., Germer, S. P. M., & Efraim, P. (2016). Chewy candy as a model system to study the influence of polyols and fruit pulp (açai) on texture and sensorial properties. *Lebensmittel-Wissenschaft + Technologie*, 65, 268-274. http://dx.doi.org/10.1016/j.lwt.2015.08.006.

Spada, J. C., Noreña, C. P. Z., Marczak, L. D. F., & Tessaro, I. C. (2012). Study on the stability of β-carotene microencapsulated with *pinhão* (*Araucaria angustifolia*) seeds starch. *Carbohydrate Polymers*, 89(4), 1166-1173. http://dx.doi.org/10.1016/j.carbpol.2012.03.090. PMid:24750928.

Vázquez, A., & Beristain, C. I. (1998). Co-crystallization of cucumber concentrate. *Archivos Latinoamericanos de Nutricion*, 48(3), 247-249. PMid:9951539.