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Study on the stability of β-carotene microencapsulated with *pinhão* (*Araucaria angustifolia* seeds) starch

Jordana Corralo Spada[a,*], Caciano Pelayo Zapata Noreña[b], Ligia Damasceno Ferreira Marczak[a], Isabel Cristina Tessaro[a]

[a] *Chemical Engineering Department, Federal University of Rio Grande do Sul (UFRGS), Rua Engenheiro Luiz Englert s/n, Porto Alegre, RS 90040-040, Brazil*
[b] *Institute of Food Science and Technology, UFRGS, Av. Bento Gonçalves, 9500, Porto Alegre, RS 91501-970, Brazil*

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ABSTRACT

Native and hydrolyzed *pinhão* starches were used as coating materials for β-carotene microencapsulation by freeze-drying. The purpose of the present study was to evaluate the stability of β-carotene encapsulated under three different conditions: in the presence of ultraviolet light at 25 ± 2 °C, in the dark at 25 ± 2 °C and in the dark at 10.0 ± 0.2 °C. The color of the samples was also analyzed. Microcapsules prepared with native starch showed the lowest stability during storage. In contrast, microcapsules encapsulated with 12 dextrose equivalent (DE) hydrolyzed starch exhibited the highest stability. First-order kinetic and Weibull models were applied to describe the degradation of β-carotene over time. The R^2 values of the Weibull model were greater than those of the first-order kinetic model. Moreover, multivariate analyses (principal component and cluster analyses) were also conducted.

1. Introduction

Interest in the development of food colorants from natural sources as alternatives to synthetic dyes has increased because of legislative action and consumer concern (Giusti & Wrolstad, 1996). β-Carotene is an important member of the carotenoid family, a group of compounds widely distributed in nature that are responsible for the yellow, orange and red colors of fruits and vegetables (Kandlakunta, Rajendran, & Thingnganing, 2008; Rodriguez-Amaya, 2002). The carotenoids are used in food, nutraceutical and pharmaceutical preparations because of their potential applications as colorants and their provitamin A activity. In addition, the carotenoids display antioxidant activity by scavenging oxygen radicals and reducing oxidative stress (Rao & Honglei, 2002; Young & Lowe, 2001).

The carotenoids are susceptible to isomerization and oxidation, which results in the loss of their properties. To prevent these processes, microencapsulation, a technique capable of enhancing the stability of carotenoids, is often applied (Higuera-Ciapara, Felix-Valenzuela, Goycoolea, & Argüelles-Monal, 2004; Laos, Lõugas, Mandamets, & Vokk, 2007; Loksuwan, 2007; Rascón, Beristain, García, & Salgado, 2011; Shu, Yu, Zhao, & Liu, 2006; Sutter, Buera, & Elizalde, 2007). Microencapsulation by freeze-drying is a method used to preserve compounds by entrapping the ingredient in a coating or wall material. In this manner, reactive, sensitive or volatile agents (vitamins, colorants, flavors, plant extracts, etc.) can be transformed into stable materials (Shahidi & Han, 1993; Vos, Faas, Spasojevic, & Sikkema, 2010).

In the food industry, different wall materials are often used, including gums, celluloses, starches and proteins (Jackson & Lee, 1991). Gum arabic is considered an excellent wall material; however, several problems are associated with the use of this gum for microencapsulation, including its high cost and limited supply (Wilson & Shah, 2007). Thus, the search for complete or partial substitutes for gum arabic has been encouraged. For instance, starch has become a viable alternative because it is abundant and cheap.

Starches are abundant and cheap encapsulating agents that protect encapsulated ingredients from oxidation (Shahidi & Han, 1993). Native starches present several limitations, which restrict their use as encapsulating agents. As a result, modified starches have been used to address these functionality problems. Hydrolyzed starches, which can be obtained using acids or specific enzymes, are common encapsulating agents because these materials are low in cost, have a bland flavor and provide good flavor protection against oxidation (Wagner & Warthesen, 1995); however, hydrolyzed starches present poor emulsification properties (Porrarud & Pranee, 2010).

Pinhão was used in the present study because starch is the main component of the seed (approximately 36%) and can be easily isolated by extraction in water under mild conditions without

* Corresponding author. Tel.: +55 51 3308 3638; fax: +55 51 3308 3277.
E-mail address: jcorralospada@yahoo.com.br (J.C. Spada).

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additives (Cordenunsi et al., 2004). *Pinhão* is the seed of *Araucaria angustifolia*, a pine tree widely distributed in southern Brazil. Due to irrational extraction for commercial purposes or deforestation for traditional agriculture, this important tree is at risk of extinction. Research on the sustainable use of derivatives of *A. angustifolia* could stimulate the preservation of this species. In addition, the nutritional aspects and technological applications of *pinhão* have not been reported in the literature.

The objectives of the present study were to produce a freeze-dried powder containing β-carotene as the core material and native *pinhão* starch, 6 DE hydrolyzed *pinhão* starch and 12 DE hydrolyzed *pinhão* starch as the encapsulating agent and to evaluate the influence of starches with different dextrose equivalents on the loss of β-carotene during storage.

2. Materials and methods

2.1. Materials

The *pinhão* seeds used in the present study were purchased at a local market (Porto Alegre/RS, Brazil). The seeds were cleaned with water, dried under ambient conditions for 48 h, selected, frozen in polyethylene bags and stored at −18 °C. Trans-β-carotene (powder) was purchased from Sigma Chemical Company (St. Louis, USA). All of the other chemicals used in the current investigation were obtained from Vetec (Duque de Caxias, RJ, Brazil) and Dinâmica (Diadema, SP, Brazil) and were of pro analysis grade.

2.2. Starch extraction

The extraction protocol described by Bello-Pérez et al. (2006) was used in the present study. Seeds without internal and external coats were ground in cold water (1 kg/1.5 l) using a domestic mixer. The homogenate was filtered through a steel sieve (Mesh Tyler 100), and the process was repeated twice. The water containing the starch was stored in a refrigerator (approximately 4 °C) overnight and was decanted. Precipitated starch was washed three times with cold water and dried in an oven at 40 °C (TE-381, Tecnal, Brazil) for 48 h. To obtain a fine powder, the dry starch was crushed in a domestic mixer and sieved (Mesh Tyler 100).

2.3. Starch modification

Samples with different dextrose equivalents (DE) were prepared according to the method described by Spada, Marczak, Tessaro, and Noreña (2012). *Pinhão* starch was added to HCl in a 1:4 (w/v) ratio. The mixture was stirred magnetically (CT 712RN, Cientec, Brazil) at 225 rpm for 12 h at a constant temperature. The hydrolysis conditions (temperature and acid concentration) for the preparation of hydrolyzed starches with dextrose equivalents (DE) of 6 and 12 were 37 °C and 3 mol l^{-1} and 44 °C and 5 mol l^{-1}, respectively. After hydrolysis, the starch was cooled and neutralized with NaOH (2 mol l^{-1}), centrifuged at 528 g/10 min/25 °C and washed with three volumes of distilled water. The starch was dried at 40 °C in a conventional oven for 2 days until the moisture content was between 10% and 15% on a dry basis.

The dinitrosalicylic methodology was used to determine the dextrose equivalents (DE) of the modified starch produced by acid hydrolysis (Miller, 1959).

2.4. Microcapsule preparation

The starch (native or hydrolyzed) was suspended in distilled water (29%, w/w) and heated in a thermostatic bath (Q226M, Quimis, Brazil) at 100 °C to obtain a gelatinized starch paste. After cooling the gelatinized starch paste, trans-β-carotene was added in 1:500 (w/w) ratio on a dry starch basis. The mixture was stirred for 15 min until complete homogenization was achieved. The samples were frozen for 12 h at −40 °C (CL 120-40, Coldlab, Brazil) and were freeze-dried for 30 h at 300 μm Hg (LS 6000, Terroni Equipamentos®, Brazil). To observe differences between the starches with different dextrose equivalents, samples using native starch (A), 6 DE hydrolyzed starch (B) and 12 DE hydrolyzed starch (C) as the wall material were prepared.

2.5. Experimental procedure

The degradation of microencapsulated β-carotene in the presence of light was studied using a light chamber with four UV lights parallel to the samples at a distance of 42 cm. In total, 1 g portions of powder were placed into transparent glasses at 25 ± 2 °C. The β-carotene concentration was determined in triplicate at selected times for one month. The initial β-carotene content (C_0) was measured immediately after freeze-drying, and the results are expressed as the percent of β-carotene remaining (C/C_0, %). The same test was performed for free β-carotene.

The samples were stored at 10 °C in the dark to observe differences in β-carotene degradation at temperatures less than 25 °C. Similar to the previous samples, 1 g portions of powder were placed in amber glasses in an oven (10.0 ± 0.2 °C), and the concentration of β-carotene was determined at selected times. The initial β-carotene content (C_0) was measured immediately after freeze-drying, and the results are expressed as the ratio of C/C_0 (%). The same test was performed for free β-carotene.

2.6. Experimental analysis

2.6.1. Determination of the β-carotene content

The procedures used to determine the β-carotene content were based on the fact that β-carotene is lipophilic and soluble in hexane. In contrast, the matrix (starch) is water soluble and insoluble in hexane.

To measure the initial carotene content and carotene retention during storage, the methodology described by Zhou, Gugger, and Erdman (1994) was modified. According to the methodology, 50 mg of the sample was dispersed in 2.5 ml of water and 20 ml of hexane in a test tube, and the tubes were agitated on a shaking table (MA 563, Marconi, Brazil) at 2500 rpm for 20 min until the powder was bleached. After shaking, the absorbance of the hexane fraction was measured at 454 nm with a spectrophotometer (UV-1600, Pró-Análise, Brazil). This wavelength corresponded to the absorbance maximum of the spectrum (from 300 to 600 nm), which is in agreement with the results of previous studies by Desobry, Netto, and Labuza (1997) and Sutter et al. (2007).

The absorption coefficient for β-carotene per gram of dry solids was used to determine the amount of β-carotene (μg), which was obtained from the absorbance units of the hexane extracts. This absorption coefficient was determined using a standard curve constructed from five different known concentrations of β-carotene in hexane.

2.6.2. Color measurement

The color of the samples was assessed using a handheld tristimulus colorimeter (Minolta Chroma Meter CR-400, Osaka, Japan), and CIE color space coordinates including L^*, a^* and b^* were determined with a CIE standard illuminant D65. The lightness value, L^*, indicates the darkness/lightness of the sample (L^* varies from 0 (black) to 100 (white)), a^* is a measurement of the greenness/redness of the sample (a^* varies from −80 to +100), and b^* is the extent of blueness/yellowness (b^* varies from −50 to +70). The color parameters were measured immediately after freeze-drying and were determined periodically during storage (25 days).

Table 1
Kinetic parameters and coefficients of determination of the degradation of free β-carotene under different storage conditions.

Sample	Storage condition	First-order kinetic model			Weibull Model		
		k (days^{-1})	$t_{1/2}$ (days)	R^2	b	n	R^2
Free β-carotene	10 °C (dark)	0.049 ± 0.003	14	0.96	0.041 ± 0.026	1.062 ± 0.200	0.97
	25 °C (dark)	0.069 ± 0.003	10	0.98	0.058 ± 0.025	1.046 ± 0.135	0.98
	UV light	0.136 ± 0.007	5	0.97	0.09 ± 0.049	1.121 ± 0.167	0.98

Mean of three replicates ± standard error.

The total color difference (ΔE^*) was considered for the overall color difference evaluation, and the Hunter–Scotield equation was employed (Minolta, 1994):

$$\Delta E* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (1)$$

The polar coordinate chroma or saturation (C^*) indicates the dullness/vividness of the product (C^* ranges from 0 to 60). Using Eq. (2), C^* can be calculated from a^* and b^*:

$$C* = \sqrt{(a*)^2 + (b*)^2} \quad (2)$$

Color coordinates (a^* and b^*) were used to calculate the hue angle (H^*), and Eq. (3) was applied. Angles between 0° and 90° characterize the quality of color and range from red to yellow. In particular, angles that are closer to 90° (yellow) are indicative of a greater loss of β-carotene.

$$H* = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (3)$$

The colorimeter was calibrated against a standard white reference tile. The samples were placed in a clear glass Petri dish, and the color measurements were conducted in triplicate.

2.7. Kinetic modeling

The degradation of β-carotene and loss of color were calculated using a first-order kinetic model and the Weibull model.

2.7.1. First-order kinetic model

A first-order model (Eq. (4)) was used to fit the thermal degradation data of the carotenoids (Chen, Shi, Xue, & Ma, 2009) and changes in the color data over time (Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009; Topuz, 2008).

$$\ln\left(\frac{C_t}{C_0}\right) = \pm kt \quad (4)$$

$$t_{1/2} = \frac{0.693}{k} \quad (5)$$

In the above equations, the signs (+) and (−) indicate the formation and degradation of the quality parameter, respectively. C_0 and C_t are the quality parameters at time zero and time t (days), respectively, k is the first-order kinetic constant and $t_{1/2}$ is the half-life.

2.7.2. Weibull model

According to Corradini and Peleg (2004), thermal inactivation of enzymes and thermal degradation of compounds such as chlorophyll and thiamine can be fitted to a power law model called the Weibullian model, according to Eq. (6):

$$\ln\left(\frac{C_t}{C_0}\right) = -bt^n \quad (6)$$

where C_0 and C_t are the quality parameters at time zero and time t, respectively, and b and n are temperature-dependent coefficients. The model was used to determine whether the experimental data fit a mathematical model that was different from those traditionally used to model carotenoid degradation and color changes.

2.8. Data analysis

The experimental data were fitted to different kinetic models and processed using the Statistica 7.0 software for Windows (Statistica 7.0, Statsoft @, Tulsa, OK, USA). Similarities and dissimilarities among the color parameters and β-carotene retention data were elucidated by principal component analysis (PCA). Although PCA is generally used for the assessment of interactions among numerous variables, the method can be employed to elucidate relationships between columns and rows of smaller data matrices (Morais et al., 2002). PCA was performed using the SAS 9.2 software (SAS, Institute Inc., Cary, NC). Multivariate analysis and a pattern recognition method called hierarchical grouping (or cluster analysis) were used to identify similarities among the samples.

3. Results and discussion

3.1. β-Carotene degradation

Table 1 shows the kinetic parameters and coefficients of determination (R^2) for free β-carotene stored under different conditions, which were obtained by fitting the experimental data to first-order kinetic and Weibull models. Both models yielded a good fit to the data, and the R^2 values varied between 0.96 and 0.98. However, the b coefficient of the Weibull model was not significant. In this case, the first-kinetic model should be used.

The half-life of free β-carotene corresponded to five days under ultraviolet light at room temperature, 10 days in the dark at room temperature and 14 days at 10 °C in the dark. Henry, Catignani, and Schwartz (1998) evaluated the oxidative and thermal degradation of *trans*-β-carotene, 9-*cis*-β-carotene, lycopene and lutein using an oily model system and concluded that the degradation of these carotenoids followed a first-order kinetic model. Chen et al. (2009) investigated the degradation kinetics of lycopene in water- and oil-based tomato model systems as a function of the thermal treatment and light irradiation and found that both enhanced the degradation of this carotenoid. Moreover, lycopene degradation also followed first-order kinetics.

The kinetic parameters and coefficients of determination of the degradation of β-carotene encapsulated in different materials under different storage conditions are shown in Table 2. The Weibull model yielded a good fit of the data, and the corresponding R^2 values (0.97–0.99) were greater than those of the first-order kinetic model (0.92–0.97). Therefore, the Weibull model provided a good fit and showed better statistical parameters than the first-order kinetic model, which is widely used in the degradation of carotenoids. Native starch did not improve the stability of the core, and the corresponding k values were even greater than those of free β-carotene (Tables 1 and 2). The poor β-carotene retention rates observed during storage by native starch are likely due to the lack of emulsification and low film-forming capacity of this wall material (Loksuwan, 2007). The poor film forming capacity of the native *pinhão* starch was confirmed by Spada et al. (2012), who observed clustering by scanning electron microscopy in the samples prepared with this material, by contrast, the microcapsules from modified *pinhão* starch that did not present clustering.

Table 2
Kinetic parameters and coefficient of determination (R^2) of the degradation of β-carotene microencapsulated in different wall materials stored under different conditions.

Sample	Storage condition	First-order kinetic bmodelmodel		Weibull Model		
		k (days^{-1})	R^2	b	n	R^2
Native starch	10 °C (dark)	0.048 ± 0.001	0.96	0.059 ± 0.015	0.952 ± 0.081	0.99
	25 °C (dark)	0.076 ± 0.006	0.93	0.010 ± 0.004	1.624 ± 0.130	0.99
	UV light (25 °C)	0.149 ± 0.006	0.97	0.264 ± 0.058	0.821 ± 0.070	0.98
6 DE starch	10 °C (dark)	0.030 ± 0.002	0.93	0.079 ± 0.013	0.695 ± 0.053	0.99
	25 °C (dark)	0.031 ± 0.001	0.97	0.050 ± 0.007	0.845 ± 0.046	0.99
	UV light (25 °C)	0.088 ± 0.004	0.96	0.177 ± 0.027	0.771 ± 0.049	0.99
12 DE starch	10 °C (dark)	0.014 ± 0.001	0.95	0.026 ± 0.009	0.821 ± 0.108	0.97
	25 °C (dark)	0.017 ± 0.001	0.92	0.033 ± 0.003	0.786 ± 0.036	0.99
	UV light (25 °C)	0.026 ± 0.001	0.97	0.014 ± 0.006	1.171 ± 0.134	0.98

The freeze-dried samples with native starch did not show formation of particles, while the samples prepared with 6 DE and 12 DE starch had particles with irregular and undefined shape and smooth walls with some lumps. These morphological aspects mentioned above may also explain the better retention of the β-carotene with hydrolyzed starch.

Unlike native starch, capsules with 12 DE hydrolyzed starch showed kinetic constants that were 4–6 times lower than that of free β-carotene.

Capsules prepared with 12 DE hydrolyzed starch also promoted greater β-carotene stability than capsules prepared with 6 DE hydrolyzed starch. Hydrolyzed starches with lower dextrose equivalents contain a greater proportion of saccharides with long chains, which form a barrier that is more inflexible and permeable to oxygen, allowing greater oxidation and degradation of the encapsulated compound (Wagner & Warthesen, 1995).

These results can also be explained by the surface carotene content (or the content of carotene that was not encapsulated) of the microcapsules. Namely, samples with modified 12 DE *pinhão* starch showed relatively low surface carotene contents, while native *pinhão* starch possessed the highest surface carotene content (Spada et al., 2012). Differences in the surface carotene content are related to the composition of the starch after heating. Free soluble amylose tends to form a continuous network through hydrogen bonds, resulting in a gel-like structure that can entrap β-carotene. Amylose possesses a high film forming ability; therefore, the presence of high levels of free soluble amylose in hydrolyzed *pinhão* starch may lead to rapid shell formation around β-carotene, preventing the loss of this compound during the drying process.

Figs. 1 and 2 show the β-carotene content of microcapsules prepared with different wall materials over time. Fig. 1 presents the results of samples stored at 10 °C in the dark, and Fig. 2 presents the samples stored at 25 °C in the dark (a) and under ultraviolet light (b). Solid lines represent the predicted data of the Weibull model.

Similar results were observed by Wagner and Warthesen (1995), who found that the loss of α- and β-carotene encapsulated with 4 DE hydrolyzed starch and 36.5 DE starch in the presence of light was 90% and 70%, respectively. The authors observed the effects of light and showed that the half-life of the encapsulated compounds increased with an increase in the DE, likely due to the formation of a dense oxygen-impermeable matrix.

The samples stored under UV light showed greater β-carotene degradation than those stored in the dark at room temperature. Matioli and Rodriguez-Amaya (2003) also observed an effect of light on carotenoid degradation. These authors encapsulated lycopene in gum arabic and maltodextrin by lyophilization and obtained a half-life of 13 days and 10 days for samples stored in the dark and under ultraviolet light at room temperature, respectively.

β-Carotene degradation increases as the temperature increases. Desobry et al. (1997) encapsulated β-carotene using 25 DE maltodextrin matrices and studied the stability of these powders at 25, 35 and 45 °C. The authors concluded that 80% of spray-dried β-carotene degraded in 7 weeks at 45 °C and 12 weeks at 35 °C. Moreover, after 12 weeks at 25 °C, the β-carotene retention rate was equal to 30%.

3.2. Color changes

The powders had initial L^* values (lightness) ranging from 68.80 ± 0.11 to 72.80 ± 0.09, initial a^* (redness) values ranging from 20.45 ± 0.10 to 23.40 ± 0.35 and initial b^* values ranging from 11.78 ± 0.25 to 12.78 ± 0.18. The initial chroma (C^*) values were 24.10 ± 0.59, 25.08 ± 1.74 and 26.20 ± 0.31, and the initial H^* values were 31.73 ± 3.08, 30.63 ± 3.14 and 26.74 ± 3.26 for microcapsules with native starch, 6 DE starch and 12 DE starch, respectively. The initial variations among the powders were attributed to the structures of the capsules and the amount of total and surface carotene in the material (Desobry et al., 1997). After 25 days of storage, a^* decreased with an increase in L^* because β-carotene oxidation reduced the intensity of the pink color of the powders and increased the white color of starch (Table 3). The parameter b^* was not a good indicator of carotene retention because it characterizes the extent of yellowness to blueness, and these colors were not dominant.Similar results were reported by Desobry et al. (1997) and Desobry, Netto, and Labuza (1999) who colorimetrically evaluated β-carotene encapsulated in maltodextrin systems and found that L^* (lightness) and a^* (redness) were the most sensitive parameters.

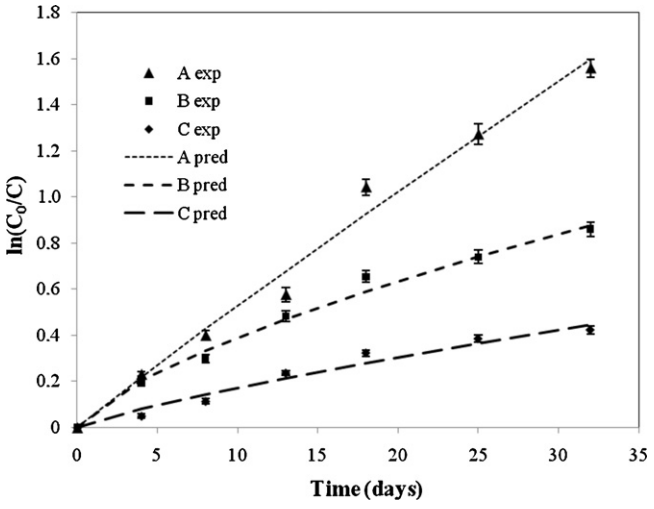

Fig. 1. Degradation rate of β-carotene microencapsulated in native starch (A), 6 DE hydrolyzed starch (B) and 12 DE hydrolyzed starch (C) stored at 10 °C in the dark. The standard error of each point was approximately 5%, and each point represents the average of 3 replicates. Full lines represent the predicted points of the Weibull model.

Table 3
Color of the microcapsules after 25 days of storage.

Sample	Storage condition	Color parameter					
		L^*	a^*	b^*	ΔE^*	H^*	C^*
Native starch	UV light	91.72 ± 1.51	4.84 ± 0.71	9.21 ± 0.96	24.05 ± 1.72	62.33 ± 1.03	10.40 ± 1.18
	Dark	88.11 ± 1.08	9.23 ± 1.02	13.51 ± 0.39	19.02 ± 1.49	55.69 ± 3.72	16.36 ± 0.25
	10 °C	86.42 ± 1.52	12.27 ± 1.03	13.52 ± 0.72	15.93 ± 0.81	47.81 ± 0.87	18.26 ± 1.23
6 DE starch	UV light	81.36 ± 1.02	16.10 ± 0.92	13.76 ± 1.05	13.74 ± 1.22	40.54 ± 3.97	21.18 ± 0.45
	Dark	81.58 ± 1.46	17.49 ± 0.56	14.63 ± 1.18	13.55 ± 1.36	39.94 ± 1.39	22.80 ± 1.19
	10 °C	80.25 ± 1.85	18.37 ± 0.54	15.33 ± 1.34	12.17 ± 0.60	39.86 ± 1.62	23.92 ± 1.27
12 DE starch	UV light	80.99 ± 0.45	16.51 ± 0.74	14.06 ± 1.30	11.32 ± 0.15	40.05 ± 1.34	21.68 ± 1.41
	Dark	80.49 ± 0.84	17.39 ± 1.33	12.58 ± 0.62	10.19 ± 0.16	35.89 ± 3.43	21.47 ± 0.72
	10 °C	79.81 ± 1.61	17.95 ± 0.55	12.76 ± 1.21	9.33 ± 1.13	35.43 ± 2.60	22.02 ± 1.38

Mean of three replicates ± standard error.

Sutter et al. (2007) concluded that coordinated a^* best represented the observed color changes in β-carotene encapsulated in a mannitol-phosphate matrix. Other studies have shown a strong relationship between a^* and the total carotenoid concentration of foods such as sweet potatoes (Bengtsson, Namutebi, Alminger, & Svanberg, 2008; Takahata, Noda, & Nagata, 1993) and paprika (Ramakrishnan & Francis, 1973).The total color difference (ΔE^*), hue angle (H^*) and chroma (C^*), which are a combination of chromatic coordinates, were also analyzed (Table 3). These colorimetric parameters are extensively used to characterize the color variation in foods during processing (Gonçalves, Pinheiro, Abreu, Branda, & Silva, 2007; Karabulut, Topcu, Duran, Turan, & Bülent, 2007; Maskan, 2001; Skrede et al., 1997). Meléndez-Martínez, Britton, Vicario, and Heredita (2007) used H^* to ascertain the influence of the chemical structure on the color of carotenoids.

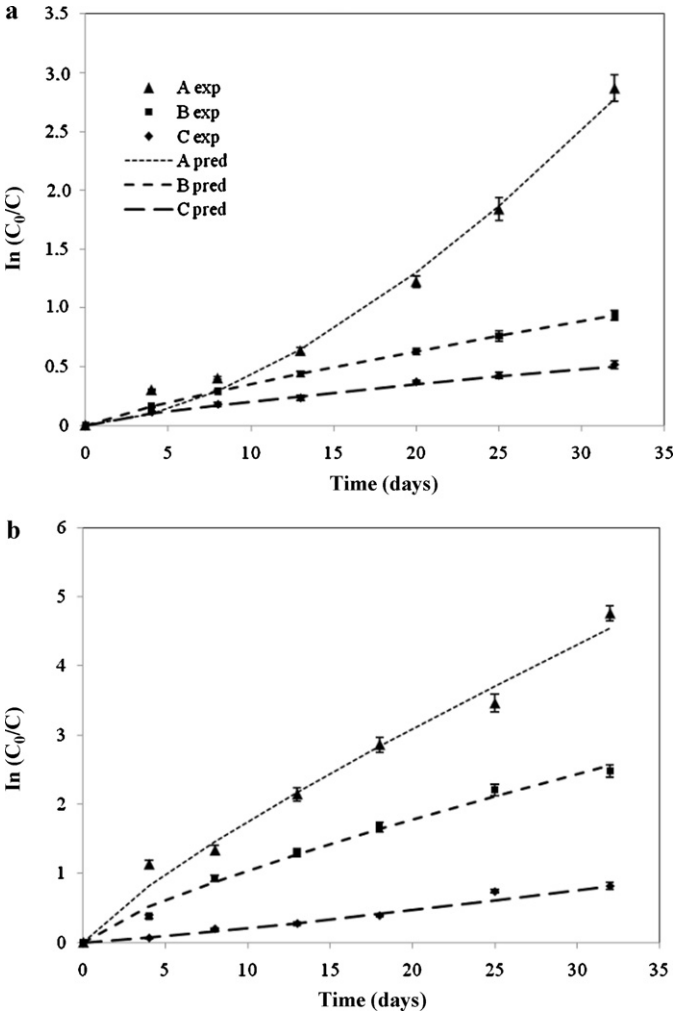

Fig. 2. Degradation rate of β-carotene microencapsulated in native starch (A), 6 DE hydrolyzed starch (B), and 12 DE hydrolyzed starch (C) stored at 25 °C in the dark (a) and stored under ultraviolet light (b). The standard error of each point was approximately 5%, and each point represents the average of 3 replicates. The full lines represent the predicted points of the Weibull model.

Samples with native starch presented higher ΔE^* and H^* values and lower C^* values, which is indicative of a greater loss of red color and significant changes in the original hue or tonality. Hydrolysis increased the potential of starch as an encapsulating agent, and 12 DE hydrolyzed starch produced the least amount of color changes than the other materials. These results are similar to those of the spectrophotometric analysis.

The parameter that best represented the observed color changes was the coordinate chromatic a^* (redness). Changes in this parameter as a function of the storage time were fitted to first-order kinetic and Weibull models, and the kinetic parameters and R^2 values are shown in Table 4. The data reported are the averages of three determinations, and the standard error was less than 5%. The predicted color changes (solid lines) were in good agreement with the experimental points. The Weibull model yielded a good fit of the data, and the corresponding R^2 values 0.97–0.99) were greater than those of the first-order kinetic model (0.71–0.97). Therefore, the Weibull model provided a good fit and showed better statistical parameters than the first-order kinetic model.

The first-order kinetic model constants (k) of degradation under ultraviolet light were 0.060, 0.016 and 0.014 for microcapsules prepared from native starch, 6 DE hydrolyzed starch and 12 DE hydrolyzed starch, respectively. As evidenced in the experimental data, the use of hydrolyzed starch as a wall material decreased the rate of red color loss.

Sutter et al. (2007) also studied the kinetics of a^* for samples containing β-carotene as the core material and mannitol as the wall material. In the aforementioned study, samples stored at 11% and 44% RH (relative humidity) followed a first-order kinetic model, and samples stored at 75% RH presented zero-order kinetics.

3.3. Multivariate analysis

The results obtained through the application of principal component analysis (PCA) to all of the assays were similar. Therefore, only the results from microcapsules with hydrolyzed starch stored under different conditions are discussed.

The two principal components accounted for 93.92% of the variance in the data (Fig. 3). The first principal component (82.70% of the total data inertia) was high for H^*, ΔE^* and L^*, while the chromatic coordinate a^*, C^* and the β-carotene content presented negative values. This component (PC1) was likely related to the

Table 4
Kinetic parameters and coefficient of determination of the color (a^*) of the microcapsules.

Sample	Storage condition	First-order kinetic model		Weibull model		
		k (days^{-1})	R^2	b	n	R^2
Native starch	10 °C (dark)	0.023 ± 0.003	0.71	0.16 ± 0.034	0.356 ± 0.073	0.98
	25 °C (dark)	0.033 ± 0.003	0.86	0.136 ± 0.019	0.527 ± 0.130	0.97
	25 °C (UV light)	0.060 ± 0.004	0.94	0.162 ± 0.035	0.672 ± 0.073	0.99
6 DE starch	10 °C (dark)	0.012 ± 0.001	0.89	0.043 ± 0.009	0.575 ± 0.071	0.99
	25 °C (dark)	0.012 ± 0.001	0.91	0.038 ± 0.003	0.631 ± 0.119	0.97
	25 °C (UV light)	0.016 ± 0.002	0.97	0.009 ± 0.001	1.199 ± 0.150	0.98
12 DE starch	10 °C (dark)	0.005 ± 0.000	0.96	0.002 ± 0.000	1.291 ± 0.211	0.97
	25 °C (dark)	0.009 ± 0.001	0.81	0.048 ± 0.014	0.460 ± 0.100	0.97
	25 °C (UV light)	0.014 ± 0.001	0.92	0.046 ± 0.006	0.611 ± 0.043	0.99

Table 5
Pearson correlation coefficients between the color parameters and β-carotene content of microcapsules prepared with 6 and 12 DE hydrolyzed starch.

	β-Carotene (%)	L^*	a^*	b^*	ΔE^*	H^*	C^*
β-Carotene (%)	1.00						
L^*	−0.83	1.00					
a^*	0.85	−0.86	1.00				
b^*	−0.60	0.54	−0.54	1.00			
ΔE^*	−0.92	0.96	−0.91	0.67	1.00		
H^*	−0.87	0.85	−0.94	0.79	0.93	1.00	
C^*	0.77	−0.79	0.96	−0.30	−0.82	−0.81	1.00

degree of β-carotene degradation. Namely, unlike a^*, C^* and the β-carotene content, which decreased during storage, H^*, ΔE^* and L^* increased over time. The second principal component (PC2) was high for b^* and could be interpreted as the indicative axis of other phenomena or reactions with β-carotene. The b^* values may be related to isomerization and reisomerization. In particular, if auto-oxidation and reversal reactions are competitive and the *trans* form of β-carotene can be degraded, the *cis* form can also undergo degradation (Boscovic, 1979).

Table 5 shows the Pearson correlation coefficients between the color parameters and β-carotene content for microcapsules prepared from hydrolyzed starch. The highest correlation coefficients were observed between L^* and ΔE^* (0.96) and between a^* and C^* (0.96). The β-carotene content showed the highest correlation coefficient with ΔE^* (−0.92) and a negative relationship with L^* (−0.83), b^* (−0.60) and H^* (−0.87). In contrast, C^* (0.77) and a^* (0.85) showed a positive relationship with the β-carotene content.

The chromatic coordinate b^* displayed low correlation coefficients with the other parameters. Specifically, the highest correlation coefficient was observed with H^* (0.79).

The projection of the microcapsules in the first two PCs is displayed in Fig. 4. Two separate groups were observed, as shown in the figure. All of the samples at time zero, the specimens analyzed after 4 days of storage and the microcapsules produced with 12 DE hydrolyzed starch stored for 13 days in the dark were located on the left side of the figure. The samples prepared from 6 DE hydrolyzed

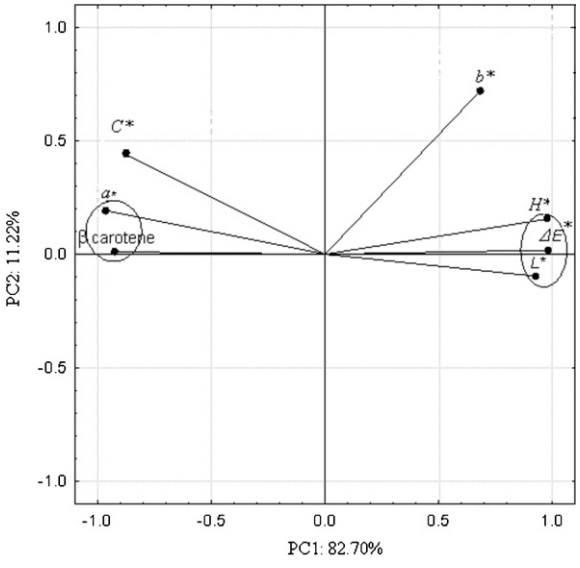

Fig. 3. Projection of the color parameters and β-carotene content in the plane defined by the two principal components.

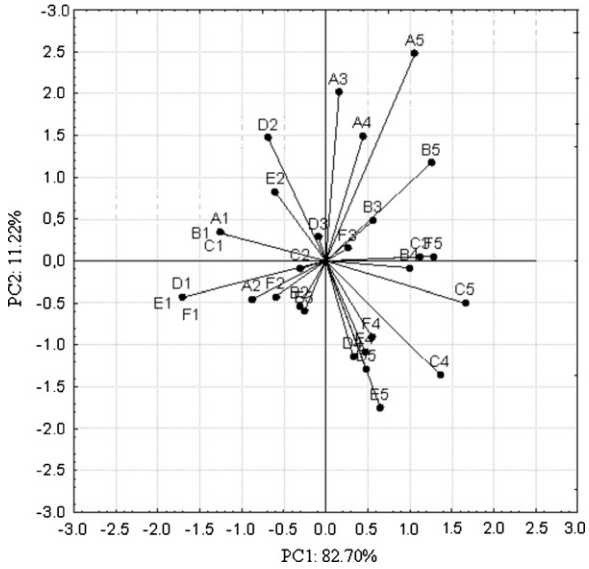

Fig. 4. Results of the principal component analysis: score plots in the PC2 vs. PC1 plane of microcapsules. (A) Microcapsules of 6 DE hydrolyzed starch stored at 10 °C in the dark, (B) microcapsules of 6 DE hydrolyzed starch stored at 25 °C in the dark, (C) microcapsules of 6 DE hydrolyzed starch stored at 25 °C under UV light, (D) microcapsules of 12 DE hydrolyzed starch stored at 10 °C in the dark, (E) microcapsules of 12 DE hydrolyzed starch stored at 25 °C in the dark, and (F) microcapsules of 12 DE hydrolyzed starch stored at 25 °C under UV light. The numbers 1, 2, 3, 4 and 5 represent storage times of 0, 4, 13, 18 and 25 days, respectively.

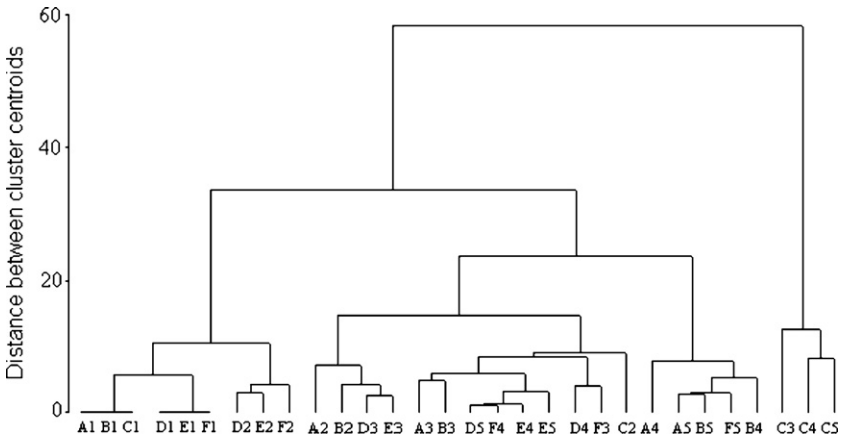

Fig. 5. Dendrogram of distance cluster centroids among microcapsules. (A) Microcapsules of 6 DE hydrolyzed starch stored at 10 °C in the dark, (B) microcapsules of 6 DE hydrolyzed starch stored at 25 °C in the dark, (C) microcapsules of 6 DE hydrolyzed starch stored at 25 °C under UV light, (D) microcapsules of 12 DE hydrolyzed starch stored at 10 °C in the dark, (E) microcapsules of 12 DE hydrolyzed starch stored at 25 °C in the dark, and (F) microcapsules of 12 DE hydrolyzed starch stored at 25 °C under UV light. The numbers 1, 2, 3, 4 and 5 represent storage times of 0, 4, 13, 18 and 25 days, respectively.

starch were preferentially located on the right side of the figure and presented higher values on the PC1 axis.

To better understand the relationships among the samples, cluster analysis was performed. Fig. 5 shows a dendrogram of distance cluster centroids, which are related to the similarity among microcapsules. Namely, smaller distances represent greater similarities. As expected, the greatest similarities were observed among A1, B1 and C1 and among D1, E1 and F1 because these microcapsules correspond to time zero and were prepared with the same wall material. The next greatest similarity and smallest distance were observed between F4 and D5; thus, even after 18 days under ultraviolet light, samples with 12 DE hydrolyzed starch displayed similar behavior to samples stored at 10 °C for 25 days.

Furthermore, a separation between samples C3, C4 and C5 and the other microcapsules was observed, which is in agreement with the results of previous studies. Microcapsules prepared with 6 DE hydrolyzed starch showed lower stability over time under ultraviolet light. As shown in Fig. 5, sample C5 presented the highest PC1 value.

4. Conclusion

The present study shows the stability of microcapsules containing β-carotene as the core material and native and hydrolyzed *pinhão* starch as the wall material.

The stability tests showed that the samples were sensitive to ultraviolet light. Moreover, in the dark, the samples presented higher rate constants at 25 °C than at 10 °C. In addition, hydrolysis greatly increased the potential applicability of starch as an encapsulating agent. Capsules prepared with 12 DE hydrolyzed starch enhanced the stability of β-carotene, while capsules prepared with native starch showed rate constants that were similar to those of the free compound. First-order kinetic and Weibull models were used to fit the experimental data on β-carotene degradation and a^*, and the Weibull model presented the best statistical parameters.

Colorimetric analysis proved to be a useful tool for evaluating carotene degradation, and PCA was conducted to identify periodic measurements of one or more representative parameters of color that could be used to determine β-carotene retention in the microcapsules. Moreover, cluster analysis was a useful tool for the differentiation of samples.

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