ORIGINAL ARTICLE

Effect of temperature and relative humidity on the stability of betalains encapsulated in cryogels from protein and polysaccharide

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Abstract The stability of betalains (Bet) encapsulated in cryogels made with a mixture of albumin (ALB) and albumin-pectin (ALB-PEC) as wall materials were evaluated during storage at 32% and 83% relative humidity (RH) at several different temperature conditions (4 °C, 30 °C and 40 °C). The retention of betalains (betanin + isobetanin) and phenolic compounds and the antioxidant activity were determined by high-performance liquid chromatography, the Folin–Ciocalteu method and radical ABTS^{*+} capture methodology. The color parameters and images of the encapsulated betalains were obtained. Cryogels prepared with ALB at 32% RH and at 4 °C provided betanin and isobetanin retention of 72% and 82%, with half-life times of 108 and 165 days, respectively. The antioxidant activity and phenolic compounds showed retention greater than 70% during storage at 32% RH at all temperatures. Cryogels prepared with ALB-PEC also conferred high retention percentages of phenolic compounds at 83% RH, but this high RH caused a significant decrease in the retention of betalains. Both ALB and ALB-PEC improved betalain stability during storage compared with the extracts without encapsulating. Therefore, cryogels could be used as protection matrices for betalains.

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Introduction

Betalains are a type of natural, water-soluble pigment found in beetroot (*Beta vulgaris*), which are classified into two major categories, betacyanins and betaxanthins. Betacyanins are the product of a condensation reaction between betalamic acid and cyclo-Dopa [cyclo-3-(3,4-dihydroxyphenylalanine)] residue whereas betaxanthins are the result of a betalamic acid reaction with amino compounds. Betacyanins are categorized as betanin and isobetanin, which causes the characteristic red–purple color in beetroot (Güneşer 2016; Chhikara et al. 2019). Furthermore, betaxanthins, categorized as vulgaxantine-I and vulgaxantine-II, produce a yellow-orange color (Schwartz and Elbe 1983; Herbach et al. 2004; Otálora et al. 2016). The chemical structures of the betacyanins and betaxanthins result in different stability levels (Herbach et al. 2004). However, other factors such as the water activity, oxygen, temperature, and pH can decrease the stability of the pigments (Herbach et al. 2006; Pitalua et al. 2010). Due to hydrolysis reactions, at a water activity level (a_w) higher than 0.64, the betalains can be degraded to form betalamic acid and cyclo-DOPA-5-O-glycoside (Manchali et al. 2013). An increase in the temperature can cause isomerization reactions and decarboxylation (Herbach et al. 2006). Thus, encapsulation processes have been used as an alternative to decrease the effects of the processing conditions and improve the stability of these types of compounds during storage (Janiszewska 2014; Chhikara et al. 2019). Cryogels are matrices that have gained interest because they can protect bioactive compounds against

degradation and allow for prepared systems of controlled delivery (Otálora et al. 2015; Kwan and Davidov-Pardo 2018). Cryogels are obtained from hydrogels prepared from proteins or polysaccharides dried by lyophilization (Betz et al. 2012). We hypothesized that, as in other kinds of matrices, cryogels can be used as wall materials to decrease the effects of relative humidity (RH) and temperature during the storage of betalain extracts. According to Rutz et al. (2013), xanthan and tara gum have been used to produce cryogels that improve the stability of carotenoids at 4 °C and 25 °C. According to the authors, during 85 days of storage at 25 °C, cryogels from xanthan-tara gum and xanthan gum provided the best carotenoid protection, with 41.4% and 44.3% retention, respectively; at 4 °C, xanthan-tara gum revealed the best performance, preserving 55.8% of carotenoids. In other study, Otálora et al. (2016) evaluated the stability of betalain from cactus fruit (*Opuntia ficus-indica*) at 34.6%, 57.6%, 74.8% and 84.3% RH for 25 days, using cryogels prepared from calcium alginate and a mixture of albumin from bovine whey and calcium alginate. They concluded that cryogels protect the pigment, improving its stability at 34.6% and 57.6% RH. Nevertheless, studies about evaluation of the stability of betalain encapsulated using cryogels prepared from mixtures of biopolymers are scarce. In previous studies, we determined the rheological and structural properties of gels from mixtures of albumin and low-methoxyl amidated pectin, which revealed stronger interactions between them when in specific composition ranges (Chaux-Gutiérrez et al. 2019). We found that cryogels prepared from a suitable gel composition of these biopolymers had high encapsulation efficiency when betalain was used as a guest molecule (not yet published). For the reasons mentioned above, it is important to evaluate the stability of betalains under several storage conditions and stablish the capacity of albumin-pectin cryogels to retain and protect bioactive compounds. Therefore, this study aims to evaluate the effects of temperature and relative humidity during storage on the kinetic parameters of color, retention of betalain (betanin and isobetanin), phenolic compounds and antioxidant activity of cryogels prepared with albumin from eggs (ALB) and with a mixture of low-methoxyl amidated pectin (PEC) and ALB.

Materials and methods

The betalain (Bet) powder extract (Beetroot (*Beta vulgaris*) juice; maltodextrin, citric and ascorbic acid) was provided by Sensient® Technologies Corporation (Jundiaí, São Paulo, Brazil). Albumin in powder form was obtained from Naturovos Ltd. (Salvador do Sul, RS, Brazil; 90% protein, 4.98% ash, 0.06% lipids). Low-methoxyl amidated pectin

(PEC) was provided by Danisco Ltd. (GRINDSTED® LA 210, São Paulo, SP, Brazil; degree of sterification 34%, degree of amidation 17%). Sodium hydroxide, calcium chloride, hydrochloric acid, magnesium and potassium chloride were obtained from Panreac (Castellar del Vallès, Barcelona, Spain), 2.2´- Azino-bis (3-ethylbenzenothiazoline6-sulfonic acid); (ABTS), Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Folin–Ciocalteu agent (2.0 N), galic acid and betalain standard were obtained from Merck Chemical Co (Darmstadt, Germany).

Preparation of cryogels

Cryogels from albumin (ALB) and low-methoxyl amidated pectin (PEC) were prepared according Chaux-Gutiérrez et al. (2019) as follows. First, ALB (5 g/100 g) and PEC (5 g/100 g) dispersions were prepared separately in water at 25 °C under constant stirring for 3 h. Then, the pH of the dispersions was adjusted to 8 with HCl (0.1 M) and NaOH (0.1 M). Next, ALB-Bet gel was prepared by heating the ALB dispersion at 85 °C under constant stirring for 15 min and then cooling at 40 °C. Afterwards, 2% (w/w) (based on the total mass of ALB dispersion) of betalain extract (Bet) was added while stirring for 20 min. The resulting ALB-Bet mixture was stored in a petri dish at 4 °C until gel formation. The ALB-Bet gel was protected from light during preparation. ALB-Bet-PEC gel was prepared by heating ALB dispersions as described above, then the PEC dispersion was added at 40 °C at a ratio of ALB:PEC 4:1 (w/w) under constant stirring for 5 min. Subsequently, 2% (w/w) (based on the total mass of the ALB-Bet dispersion) of Bet was added while stirring for 20 min. To promote the gel formation, a sodium chlorite solution (2% w/v) was added at 1% (w/w) (based on the total mass of the PEC dispersion) under constant stirring for 10 min. The ALB-Bet-PEC mixture was stored in a petri dish at 4 °C until gel formation. The ALB-Bet and ALB-Bet-PEC gels were frozen at -18 °C for 24 h and lyophilized (LIOBRAS, São Carlos, SP, Brazil).

Stability of ALB-Bet and ALB-Bet-PEC cryogels

The stability of the cryogels was studied at two relative humidities for 45 days at different temperatures. The cryogels and betalain extract (control) were stored in hermetically sealed glass containers at 32% and 83% RH, which were obtained with saturated solutions of $MgCl_2$ and KCl, respectively. For both treatments, samples were stored at 4 °C, 30 °C and 40 °C. The color parameters (L^*, a^*, b^*), betanin and isobetanin content, phenolic compound content and antioxidant activity were measured for 45 days.

Betanin and isobetanin content

The betanin and isobetanin content were determined according to a methodology proposed by Otálora et al. (2016), with some modifications. Briefly, 120 g of a sample were weighed and dispersed in 15 mL methanol:water (1:1) solution while stirring for 1 h at 300 rpm at 25 °C. This dispersion was centrifuged at 5500 rpm for 20 min, and the supernatant was separated. The betalain content was determined by high-performance liquid chromatography. The Azura ® HPLC system (Knauer, Berlin, Germany) was equipped with a pump (P 6.1L), an autosampler (AS 6.1L) and a UV/vis detector (DAD 6.1L). A C18 column (100 mm × 3 mm) was used for chromatographic separation of the betalains. The column oven was set to 25 °C. The flow rate was 0.5 mL/min and the injection volume was 20 μL. The mobile phases consisted of an aqueous solution of formic acid (2% v/v) (component A) and methanol (component B). The gradient elution was as follows: 0 min (5% B), 12 min (25% B) and 15 min (70% B) (Spórna-Kucab et al. 2015). Betalains were detected at 534 nm. All analyses were performed in duplicate.

Phenolic compounds

The phenolic compounds were determined using the Folin–Ciocalteu method (Singleton et al. 1999). Samples were mixed with a methanol:water (1:1) solution. The absorbance was measured at 725 nm in a spectrophotometer UV/VIS (UV-3000, Shanghai Mapada Instruments Co., Ltd., Shanghai, China). The phenolic compounds were determined using a calibration curve prepared with a gallic acid aqueous solution (0–500 ppm). The result was expressed as mg of gallic acid equivalents (GAEs) per gram (mg GAEs.g-1). All analyses were performed in triplicate.

Antioxidant activity

The antioxidant activity was determined by radical ABTS[*+] capture methodology, according to a method proposed by Re et al. (1999) Samples were mixed with a methanol:water (1:1) solution. The absorbance was measured at 730 nm in a spectrophotometer UV/VIS (UV-3000, Shanghai Mapada Instruments Co., Ltd., Shanghai, China). The antioxidant activity was determined using a calibration curve prepared with an aqueous solution of Trolox (0–200 μmol/L) and expressed as micromoles of Trolox equivalents (TEs) per gram (μmol TEs g^{-1}). All analyses were performed in triplicate.

Retention percentage

The retention percentage (Ret) (Eq. 1) was defined as the ratio of the betalain content (betanin or isobetanin), phenolic compounds or antioxidant activity before (C_b) and after (C_a) storage (45 days) (Kang et al. 2019).

$$Ret(\%) = C_a/C_b \times 100 \tag{1}$$

Color analysis

The color parameters were determined using a spectrophotometer X-Rite 939 (X-Rite, Inc, Michigan, USA). The results were expressed according to CIELAB (L^*, a^*, b^*) systems, and (C^*) and the hue (h^*) parameters were determined using Eqs. 2 and 3, respectively.

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

$$h^* = arctg(b^*/a^*) \tag{3}$$

Degradation kinetics of betalains

The concentrations of betalains (betanin + isobetanin) were adjusted according to Eq. 4 (zero-order reaction) and Eq. 5 (first-order reaction). The reaction rate (k) was determined by the best adjustment to the data that described the behavior of the degradation process of the betalains (betanin and isobetanin) inside ALB-Bet and ALB-Bet-PEC cryogels. The effects of temperature on the degradation process were evaluated through the Q_{10} factor (Eq. 6) and the activation energy (E_a), according to the Arrhenius model (Eq. 7) (De Moura et al. 2018).

$$C = C_0 - kt \tag{4}$$

$$\ln(C/C_0) = -kt \tag{5}$$

$$Q_{10} = k_T/k_{T-10} \tag{6}$$

$$k = k_0 e^{-(E_a/RT)} \tag{7}$$

$$t_{1/2} = (\ln 2)/k \tag{8}$$

where C is the concentration of betalains (betanin or isobetanin) at time t; C_0 is the concentration of betalains (betanin or isobetanin) at time zero; k is the reaction rate constant, Q_{10} is the temperature coefficient; E_a is the activation energy (kJ/mol); k_0 is the pre-exponential factor; R is the universal gas constant (8.314 J/(mol. K); T is the absolute temperature (K); $t_{1/2}$ is the half-life time, k_T is the reaction rate at 40 °C and k_{T-10} is the reaction rate at 30 °C.

Experimental design

The effects of each factor on the stability of betalains encapsulated in cryogels were determined using a factorial design with two numerical factors: the storage temperature (4 °C, 30 °C and 40 °C) and relative humidity (32 and 83%). Statistical analysis of the experimental design was defined using a significance level $\alpha = 5\%$ and was performed with Minitab software 16®, (Minitab, State College, EUA).

Results and discussion

Betanin and isobetanin content and retention percentage

Table 1 shows the results of the response variables. The temperature, RH and the interactions among them significantly affected ($p < 0.05$) the retention of betanin and isobetanin inside ALB-Bet and ALB-Bet-PEC cryogels (Table 2). ALB-Bet cryogels subjected to 32% RH at 4 °C and 30 °C showed retention rates higher than those observed in the control sample (Fig. 1a, b, c). Notably, an increase in temperature from 4 to 40 °C led to a decrease in betanin retention from 72 to 52%. The ALB-Bet-PEC cryogels showed 64% (4 °C), 63% (30 °C) and 58% (40 °C) retention, suggesting that ALB has an important role in the protection of betanin at lower temperatures and that the mixture ALB-PEC used as a wall material improves the stability of betanin at 30° and 40 °C (Fig. 1a, b, c).

Regarding isobetanin, the temperature also affected the retention in both cryogels, resulting in a decrease from 82% (4 °C) to 70% (30 °C) to 66% (40 °C) for the ALB-Bet cryogel and a decrease from 71% (4 °C) to 66% (30 °C) to 60% (40 °C) for the ALB-Bet-PEC cryogel. These values are higher than those observed in the control sample, indicating that ALB improves the stability of isobetanin regardless of the treatment temperature. An increase in the RH to 83% significantly decreased ($p < 0.05$) the retention of betalains (betanin + isobetanin) in both cryogels as well as in the control sample. However, although the cryogels showed higher retentions than those of the control samples, the results were only approximately 24% for betanin and 16% for isobetanin inside the ALB-Bet cryogel, and close to 22% inside the ALB-Bet-PEC cryogel (Fig. 1d). Physicochemical changes observed during storage time at 83% RH prevented the quantification of betanin and isobetanin content at 30 °C and 40 °C (Fig. 1e, f). Notably, although an increase in the temperature led to a decrease in the retention at 32% RH, the values of the cryogels were always higher than 50%, indicating that the

cryogels prepared using ALB and PEC as wall material can protect betanin and isobetanin in these conditions.

The RH significantly affects the retention of betalains (betanin + isobetanin). Increasing temperature and a_w increase the probability of hydrolysis of aldimine bonds and the release of cyclodopa-5-O-glycoside and betalamic acid (Schwartz and Elbe 1983; Herbach et al. 2006; Otálora et al. 2016). Do Carmo et al. (2018) reported that the typical red–purple of beetroot extract encapsulated by spray drying using isolate whey protein from milk (15% w/w) and subjected to a_w of 0.733 changed to a brown color. According to the authors, the betalain hydrolysis or Maillard reaction caused this color change. Serris and Biliaderis (2001) observed that, at a water activity level of 0.64, there was an increase in the degradation rate of pigment from beetroot encapsulated by spray drying using pullulan and two types of maltodextrin. The authors concluded that the porosity of the matrix and structural changes caused by water adsorption affected the barrier properties against oxygen. Juice of beetroot encapsulated with arabic gum stored at 30 °C at several a_w levels showed higher degradation after 20 days at a_w 0.748 and 0.898 (Pitalua et al. 2010).

Phenolic compound retention

Table 2 shows the effects of temperature storage, RH and interaction temperature-RH on the phenolic retention inside ALB-Bet and ALB-Bet-PEC cryogels. Both the ALB and ALBPEC mixtures affected the degradation process of phenolic compounds in the beetroot extract, showing a retention percentage higher than that observed in the control sample (Fig. 1). ALB-Bet cryogel showed a retention percentage greater than 90% at RH 32% regardless of the storage temperature. However, the temperature significantly affected ($p < 0.05$) phenolic compound retention inside ALB-Bet-PEC cryogels, resulting in a decrease from 91% (4 °C) to 83% (40 °C). A relative humidity of 83% significantly decreased ($p < 0.05$) phenolic compound retention in both cryogels (Table 1); ALB-Bet cryogels showed a lower retention than ALB-Bet-PEC. As in the case of betanin and isobetanin, an increase in a_w also increased the molecular mobility and hydrolysis reactions. Rocha-Parra et al. (2016) also reported the effect of a_w in phenolic retention, showing that an increase in RH caused wine encapsulated in maltodextrin and gum arabic to lose all malvidin-3G at an a_w of 0.58.

ALB and PEC can interact with polyphenols through hydrogen bond linkages and there is competition between them for these compounds (Gonçalves et al. 2011; Jakobek 2015; Ozdal et al. 2019). At a higher RH, ALB-Bet-PEC cryogels could interact with the polyphenols present in the beetroot extract, improving the protection compared with

Table 1 Factorial experimental design and response variables obtained during storage time for ALB-Bet and ALB-Bet-PEC cryogels

x_1	x_2	L^*	a^*	b^*	C^*	h^*	Betanin retention (%)	Isobetanin retention (%)	Phenolic compund retention (%)	Antioxidant activity retention (%)
ALB-Bet										
4	32	20.25 ± 0.25	22.53 ± 0.14	− 10.83 ± 0.11	24.99 ± 0.08	337.20 ± 0.70	72.77 ± 0.60	82.66 ± 0.65	92.43 ± 2.08	90.52 ± 6.56
30	32	20.41 ± 0.06	22.72 ± 0.31	− 7.55 ± 0.54	23.95 ± 0.47	340.20 ± 0.00	61.51 ± 0.46	70.56 ± 0.94	90.71 ± 7.12	85.01 ± 1.39
40	32	21.25 ± 0.09	22.37 ± 0.09	− 4.73 ± 0.06	22.86 ± 0.10	346.95 ± 0.07	52.29 ± 1.99	66.88 ± 0.41	93.30 ± 2.77	84.88 ± 2.29
4	83	36.34 ± 0.38	26.23 ± 0.86	2.02 ± 0.01	26.31 ± 0.86	22.63 ± 1.81	24.82 ± 1.69	16.82 ± 0.77	70.01 ± 3.16	63.10 ± 2.24
30	83	53.49 ± 0.13	3.51 ± 0.57	30.41 ± 0.14	30.62 ± 0.08	83.41 ± 1.08	0.00	0.00	46.36 ± 2.81	52.04 ± 2.32
40	83	51.10 ± 0.54	2.84 ± 0.20	31.82 ± 0.23	31.95 ± 0.21	84.89 ± 0.40	0.00	0.00	18.12 ± 2.53	21.44 ± 1.17
ALB-Bet-PEC										
4	32	25.33 ± 0.29	22.31 ± 0.35	− 9.95 ± 0.16	24.42 ± 0.38	336.95 ± 1.34	64.61 ± 1.48	71.78 ± 0.09	91.04 ± 4.09	91.15 ± 3.95
30	32	24.91 ± 0.00	23.60 ± 0.40	− 7.74 ± 0.15	24.83 ± 0.43	339.40 ± 0.00	63.46 ± 0.72	66.71 ± 1.12	86.79 ± 3.09	86.33 ± 1.10
40	32	24.50 ± 0.93	22.55 ± 0.40	− 4.86 ± 0.42	23.07 ± 0.48	346.70 ± 0.85	58.44 ± 0.37	60.27 ± 2.90	83.04 ± 2.67	72.99 ± 2.17
4	83	34.08 ± 1.78	29.63 ± 0.19	4.14 ± 0.79	29.92 ± 0.08	7.95 ± 1.55	24.81 ± 0.66	22.65 ± 1.52	76.32 ± 4.26	58.88 ± 1.09
30	83	54.04 ± 0.61	5.03 ± 0.18	31.88 ± 0.63	32.28 ± 0.60	81.03 ± 0.49	0.00	0.00	63.07 ± 2.85	50.61 ± 3.69
40	83	54.01 ± 1.10	5.71 ± 0.17	34.64 ± 1.18	35.11 ± 1.20	80.64 ± 0.04	0.00	0.00	37.31 ± 5.00	37.17 ± 1.12
Control										
4	32	14.48 ± 0.09	21.74 ± 0.09	− 5.30 ± 0.21	22.37 ± 0,14	345.86 ± 0.09	59.38 ± 0.97	49.94 ± 2.88	69.94 ± 1.48	89.52 ± 3.99
30	32	14.05 ± 0.01	22.80 ± 0.46	− 4.73 ± 0.27	23.28 ± 0.51	345.19 ± 0.11	54.71 ± 2.30	48.13 ± 0.28	58.42 ± 2.15	85.70 ± 5.64
40	32	13.60 ± 0.22	22.91 ± 0.04	− 3.79 ± 0.18	23.23 ± 0,07	346.50 ± 0.28	53.68 ± 0.31	44.65 ± 0.39	48.74 ± 3.00	77.60 ± 1.91
4	83	25.08 ± 0.09	7.32 ± 0.80	0.29 ± 0.04	7.32 ± 0.80	5.08 ± 0.04	19.10 ± 1.08	13.08 ± 0.66	28.80 ± 2.99	27.40 ± 0.63
30	83	25. 54 ± 2.00	1.33 ± 0.01	2.58 ± 0.18	2.91 ± 0.16	62.80 ± 1.90	0.00	0.00	17.21 ± 2.90	21.61 ± 2.89
40	83	26.19 ± 0.01	1.79 ± 1.00	3.47 ± 1.54	3.91 ± 1.82	63.47 ± 3.18	0.00	0.00	8.41 ± 1.01	9.79 ± 0.88

x_1; storage temperature (°C) and x_2; relative humidity (%)

Initial betanin content: 816 ± 9.93 mg g^{-1} (Control), 343.13 ± 2.12 mg g^{-1} (ALB-Bet) and 343.72 ± 1.24 mg g^{-1} (ALB-Bet-PEC). Initial isobetanina content: 991 ± 14.00 mg g^{-1} (Control), 284.17 ± 4.64 mg g^{-1} (ALB-Bet) and 288.81 ± 6.21 mg g^{-1} (ALB-Bet-PEC). Initial phenolic compound: 13.13 ± 0.88 mg GAEs g^{-1} (Control), 3.49 ± 0.23 mg GAEs g^{-1} (ALB-Bet) and 2.51 ± 0.30 mg GAEs g^{-1} (ALB-Bet-PEC). Initial Antioxidant activity: 23.03 ± 0.40 μmol TEs g^{-1} (Control), 15.23 ± 0.80 μmol TEs g^{-1} (ALB-Bet) and 16.32 ± 0.27 μmol TEs g^{-1} (ALB-Bet-PEC)

Table 2 ANOVA of temperature factors, RH and the interaction temperature-RH for ALB-Bet and ALB-Bet-PEC cryogels

| Source of variation	Df	L^* SQ	L^* p value	a^* SQ	a^* p value	b^* SQ	b^* p value	h^* SQ	h^* p value	C^* SQ	C^* p value	Betanin retention (%) SQ	Betanin retention (%) p value	Isobetanin retention (%) SQ	Isobetanin retention (%) p value	Phenolic compund retention (%) SQ	Phenolic compund retention (%) p value	Antioxidant activity retention (%) SQ	Antioxidant activity retention (%) p value
ALB-Bet																			
x_1	2	183.7	0.000	354.4	0.000	769.9	0.000	3107	0.000	7.633	0.002	1146	0.000	637.6	0.000	1.552.3	0.000	855.6	0.000
x_2	1	2081.3	0.000	409.0	0.000	2543.9	0.000	231.525	0.000	97.1	0.000	8722.5	0.000	13.775	0.000	6570.5	0.000	5038.3	0.000
$x_1 x_2$	2	162.8	0.000	354.7	0.000	398.1	0.000	2041	0.000	31.7	0.000	95.9	0.009	12.3	0.003	1.532.5	0.000	845.5	0.000
Pure error	6	0.53		1.23		0.38		5		1.02		25.1		2.1		36.4		9.8	
R^2		0.99		0.99		0.99		1		0.99		0.99		0.99		0.99		0.99	
Adjusted R^2		0.99		0.99		0.99		1		0.99		0.99		0.99		0.99		0.99	
ALB-Bet-PEC																			
x_1	2	180.15	0.000	297.9	0.000	764.0	0.000	2915	0.000	20.608	0.014	553.4	0.000	659.1	0.000	1315.3	0.000	946.22	0.000
x_2	1	1554.5	0.000	374.6	0.000	2617.5	0.000	232.850	0.000	115.9	0.000	8716.6	0.000	10.341	0.000	2731.1	0.000	3618.2	0.000
$x_1 x_2$	2	207.5	0.000	342.5	0.000	464.3	0.000	1936	0.000	33.1	0.005	310.6	0.000	66.5	0.006	626.3	0.515	19.37	0.000
Pure error	6	9.94		5.05		4.55		15		6.6		3.3		9.0		28.6		78.12	
R^2		0.99		0.99		0.98		0.99		0.96		0.99		0.99		0.99		0.98	
Adjusted R^2		0.99		0.99		0.97		0.99		0.93		0.99		0.99		0.98		0.96	

cryogels prepared with ALB only. Cryogels of ALB and PEC subjected to 32% RH showed similar retention percentages to those prepared using xanthan gum and tara gum, which showed phenolic retention approximately 80% after storage for 56 days at 4 °C and 25 °C (Rutz et al. 2013). Although increasing temperature also decreased phenolic retention at 83% RH in both cryogels, it should be noted that the phenolic compounds showed higher stability than that of betanin and isobetanin at a higher relative humidity. Tsali and Goula (2018) also reported a decrease in phenolic retention from extracted grape pomace encapsulated in maltodextrin and skimmed milk with an increase in the storage temperature.

Antioxidant activity

In all treatments, increased temperature decreased the antioxidant activity. Although cryogels at 83% RH showed a lower retention than that at 32%, both cryogels had a higher retention than that observed in the control sample. These results agree with those of Fang and Bhandari (2011), who showed that an increase in RH led to a decrease in antioxidant activity retention. This contrasts with results of Pitalua et al. (2010), who reported that an increase in RH causes betalain degradation and the probable formation or release of new intermediate compounds that increase the antioxidant activity. Notably, when comparing the betalain content, polyphenols and antioxidant activity, the latter two parameters had a similar degradation pattern (Fig. 1) at 32% and 83% RH, which suggests that polyphenols had a greater effect on the antioxidant activity retention than betalain content. Fang and Bhandari (2011) and Rocha-Parra et al. (2016) also reported a correlation between the polyphenol content and antioxidant retention pattern in stability studies of bayberry powder and encapsulated wine subjected to a_w 0.33 and 38 °C, respectively.

Color parameters

After 45 days of storage at 4°, 30° and 40 °C, ALB-Bet-PEC and ALB-Bet cryogels had L^* values of 20–25, and showed an increase in (L^*) at 83% RH (Table 1). At 30° and 40 °C, the increase in RH led to a decrease in redness (a^*). All samples stored at 32% RH showed a bluish tonality ($b^* < 0$) regardless of storage temperature. However, all cryogels lost this coloring during storage at 83% RH, leading to an appearance of a yellowish tonality ($b^* > 0$), which increased at 30° and 40 °C. The Chroma (C^*) results indicate that there is a slight tendency to decrease the vividness of the color at 32% RH when the temperature was increased; however, a vivid color was obtained at 83% RH due to an increase in the color
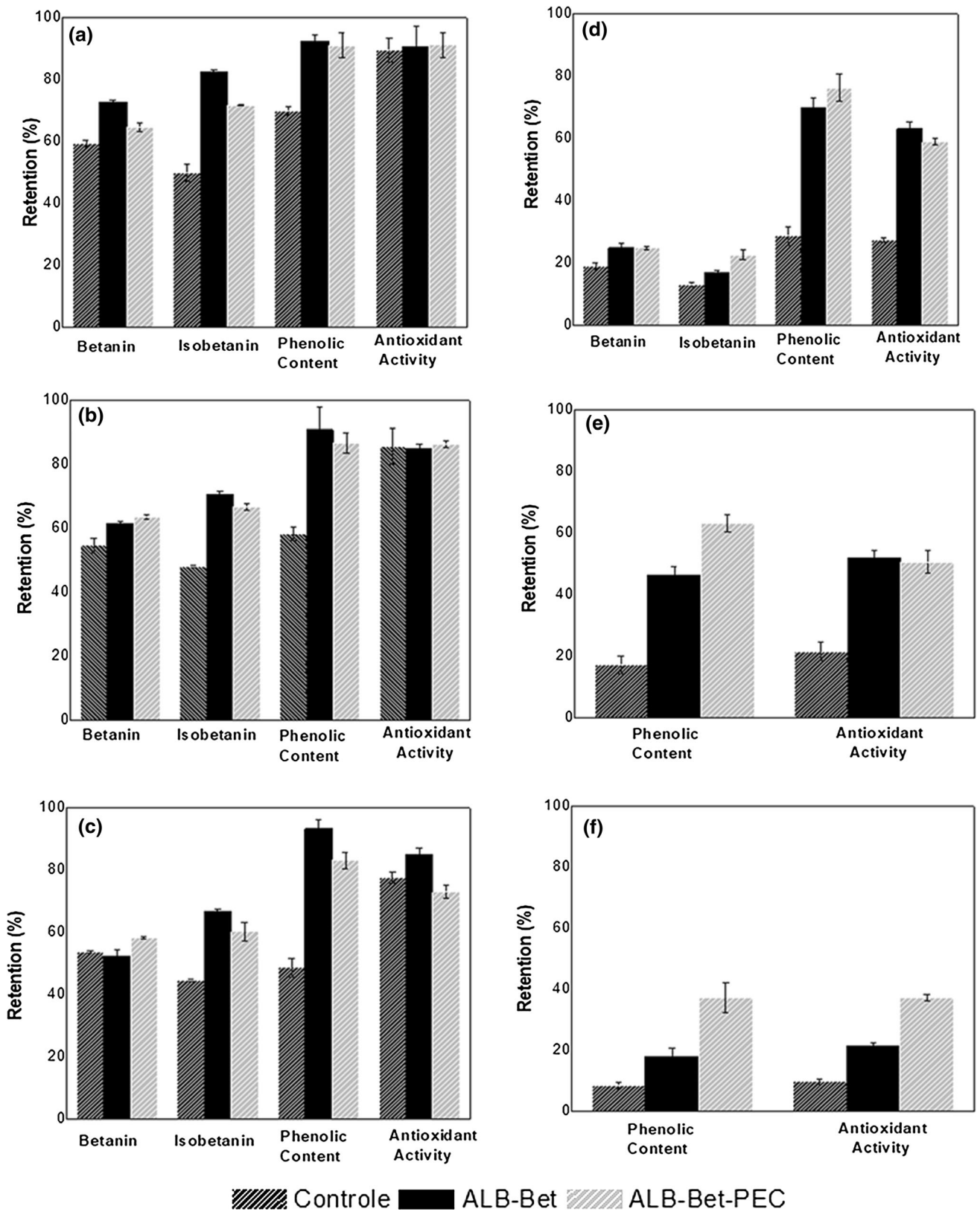
Fig. 1 Betanin and isobetanin retention, phenolic compound retention and antioxidant activity of the control sample and ALB and ALB-PEC cryogels after 45 days of storage at 32% RH and 4 °C (a), 30 °C (b), 40 °C (c) and 83% RH at 4 °C (d), 30 °C (e), 40 °C (f)

saturation. For the tonality analysis, through the polar coordinate system (hue angle, $h*$), both cryogels showed the characteristic red–purple tonality at 32% RH but with variations in the angle from 337 to 347 as a function of the storage temperature. At 83% RH and 4 °C, the tonality appeared more reddish, with a $h*$ of 23 degrees for ALB-Bet and 8 degrees for ALB-Bet-PEC cryogels. At 30° and 40 °C, the tonality became yellowish with a $h*$ of approximately 81. At 83% RH and at 30° and 40 °C, the cryogels showed a decrease in the $a*$ and $b*$ parameters with positive values (Fig. 3), indicating the release of betalamic acid, which has a bright yellow tonality, as suggested by Herbach et al. (2006). At both RH and at 4 °C, all cryogels maintained their red tonality during the whole storage time, which illustrates the effects of the storage temperature on the color parameters. These results agree with those reported by Cejudo-Bastante et al. (2014) for ulluco (*Ullucus tuberosus*) extract, indicating few changes in the tonality at 4 °C and a yellowish color at 20 °C.

Degradation kinetics of betalains (betanin and isobetanin)

The betalains (betanin and isobetanin) degradation kinetics data was fitted to a first-order reaction (Eq. 5) with a coefficient of determination of $R^2 > 0.91$. Table 3 shows the reaction rate constant (k) and half-life time ($t_{1/2}$) of betalains inside the cryogels. Figure 2 shows the betanin and isobetanin content inside ALB-Bet and ALB-Bet-PEC cryogels as a function of the storage time at 32% RH and 4 °C, 30 °C and 40 °C. The degradation rate of betalains in the cryogels subjected to 32% RH increased significantly with the storage temperature, regardless of the wall material used. Cryogels became more stable at 4 °C during the storage and showed a lower degradation rate of betalains than the control sample, with a half-life time higher than those observed with other treatments (Table 3).

Comparing the two wall materials at 4 °C, the results indicate that ALB could protect betalain extract better than the mixture of ALB-PEC. Isobetanin showed a high half-life ($t_{1/2} = 165$ days) when ALB was used as the wall material. ALB, with an isobetanin half-life of 103.5 days and a degradation rate of 6.7×10^{-3} (day^{-1}) at 30 °C, was shown to protect this substance more effectively than ALB-PEC.

Conversely, at 40 °C, betanin showed a lower degradation rate when the ALB-PEC mixture was used than that observed with only ALB as the wall material, resulting in half-lives of 63 days and 53.3 days, respectively. For isobetanin, the ALB cryogel resulted in a lower degradation rate than ALB-PEC, with half-lives of 88.9 and 69.3 days, respectively.

Shaaruddin et al. (2017) reported an increase in the degradation rate of betalain encapsulated by spray-drying, using maltodextrin and resistant maltodextrin as carriers, when the storage temperature was increased from 4 to 40 °C. According to the authors, the degradation rate was 4.1×10^{-3} (4 °C) to 5.1×10^{-3} (40 °C) weeks^{-1} for maltodextrin and 4.1×10^{-3} (4 °C) to 5.3×10^{-3} (40 °C) weeks^{-1} for resistant maltodextrin, which are higher than those observed for the cryogels prepared in this study.

The results in Table 3 also indicate that the increase in RH (83%) significantly affected the degradation rates of betanin and isobetanin; for example, the half-life of approximately 19 days at 4 °C for ALB-Bet and 23 days for ALB-Bet-PEC indicate that the degradation rate was slightly affected by the wall material used.

The effects of the RH on the cryogels can be observed in Fig. 3. At 30° and 40 °C, the cryogels presented a color change from an initial red–purple to a yellow-orange during storage. The results for the degradation rate of betalain in both cryogels are lower than those reported by Güneşer (2016) in the thermal treatment of milk with betalain extract added, which showed a degradation rate of 13.58 10^{-3} min^{-1} to 30.98×10^{-3} min^{-1}. These values are lower than those reported by Otálora et al. (2016) for betalain encapsulated in alginate and a mixture of bovine serum albumin (BSA) and alginate stored at 34% RH and 25 °C, which showed values of 13.55×10^{-2} and 11.14×10^{-2} day^{-1} with half-lives of 5.1 and 6.3 days, respectively. Serris and Biliaderis (2001) studied the effects of RH on the degradation rate of betalains, and betalain encapsulated in pullulan and maltodextrin matrix at RH greater than 50% showed increases in moisture and oxygen that led to an increase in the degradation rate.

The effect of the storage temperature on the degradation rate was determined through the Arrhenius equation (Eq. 7). Table 3 shows the temperature coefficient (Q_{10}) and the activation energy (E_a) for betanin and isobetanin degradation in ALB-Bet and ALB-Bet-PEC cryogels at 32% RH. Cryogels with only ALB showed a higher E_a (13.5 kJ mol^{-1} for betanin and 12.4 kJ mol^{-1} for isobetanin) than that observed for the ALB-PEC mixture (2.3 kJ mol^{-1} for betanin and 5.0 kJ mol^{-1} for isobetanin), indicating that betalain degradation is more dependent on the storage temperature. These results are lower than those reported by Serris and Biliaderis (2001) for betalain encapsulated in pullulan and maltodextrin (30°, 40° and 50 °C, a_w 0.23, $E_a = 18.25$ kJ mol^{-1}), which indicates that the cryogels prepared in this study showed more effective protection against the effects of temperature. Cryogels prepared with ALB showed a higher Q_{10} for betanin than that prepared using ALB-PEC; they were similar for isobetanin. The values suggest that an increase in 10 °C led to an increase in the betalain degradation rate of 10–30%.

Table 3 Kinetic parameters of betalain degradation

| Cryogels	Temperature (°C)	k (day^{-1})	R^2	$t_{1/2}$ (days)	Activation energy (E_a) (kJ mol^{-1})	Q_{10} (30–40 °C)
Betanin						
ALB-Bet	4	6.4×10^{-3}	0.95	108.3	13.5	1.3
(32% RH)	30	9.8×10^{-3}	0.95	70.7		
	40	1.3×10^{-2}	0.95	53.3		
ALB-Bet-PEC	4	9.8×10^{-3}	0.98	70.7	2.3	1.1
(32% RH)	30	1.0×10^{-2}	0.98	68.6		
	40	1.1×10^{-2}	0.96	63.0		
ALB-Bet	4	3.1×10^{-2}	0.97	22.1	–	–
(83% RH)	30	–	–	–		
	40	–	–	–		
ALB-Bet-PEC	4	3.0×10^{-2}	0.96	22.8	–	–
(83% RH)	30	–	–	–		
	40	–	–	–		
Isobetanin						
ALB-Bet	4	4.2×10^{-3}	0.97	165.0	12.4	1.2
(32% RH)	30	6.7×10^{-3}	0.95	103.5		
	40	7.8×10^{-3}	0.91	88.9		
ALB-Bet-PEC	4	7.5×10^{-3}	0.97	92.4	5.0	1.2
(32% RH)	30	8.2×10^{-3}	0.94	84.5		
	40	1.0×10^{-2}	0.94	69.3		
ALB-Bet	4	3.6×10^{-2}	0.98	19.2	–	–
(83% RH)	30	–	–	–		
	40	–	–	–		
ALB-Bet-PEC	4	2.9×10^{-2}	0.95	23.4	–	–
(83% RH)	30	–	–	–		
	40	–	–	–		

Conclusion

Both ALB-Bet and ALB-Bet-PEC cryogels were shown to be useful as a protective matrix for betalains. Increases in the relative humidity and storage temperature increased the degradation rate and decreased the half-life of betalains. The RH had a greater effect on the stability of betalains during storage. At 32% RH and 4 °C, ALB protected the betanin and isobetanin more effectively, with retention percentages of 72% and 82% and half-lives of 108 and 165 days, respectively. The addition of PEC increased the stability of betanin at higher temperatures (30 and 40 °C) and improved the phenolic compound retention at high relative humidity (83% RH). The antioxidant activity and phenolic compounds retention were above 70% at 32% RH at all temperatures. Both revealed a similar degradation pattern at all conditions, which indicates that polyphenols had a greater effect on the antioxidant activity than betalains. At 32% RH, all cryogels showed the characteristic red–purple tonality; at 83% RH and 4 °C, the tonality appeared more reddish and lighter than that at 32% RH. Temperatures of 30 and 40 °C at 83% RH shifted the tonality to yellowish, likely due to formation of yellow degradation products such as betalamic acid. The degradation kinetics of betalains at 32% RH were well represented by a first-order reaction, and a higher activation energy was found using ALB than the ALB-PEC matrix. Cryogels improved the betalains stability during storage more than betalains extract without encapsulation. Future studies for evaluating the stability of betalains at different
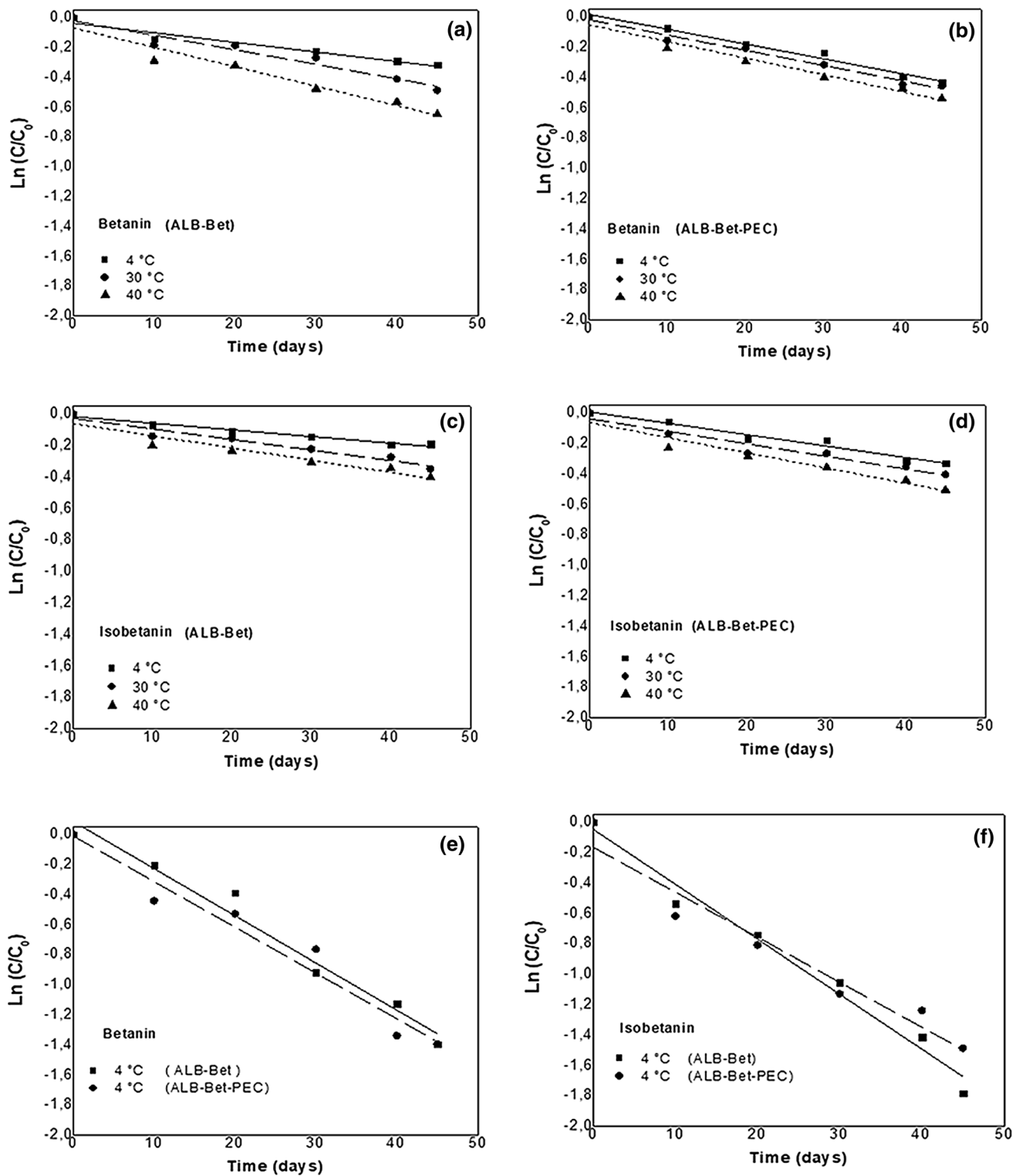
Fig. 2 First-order kinetics of the degradation of betanin and isobetanin inside cryogels subjected to 32% RH at 4 °C, 30 °C and 40 °C: ALB-Bet (**a**, **c**) and ALB-Bet-PEC (**b**, **d**). At 83% RH and 4 °C: ALB-Bet and ALB-Bet-PEC (**e**, **f**)

Fig. 3 ALB-Bet (**a**) and ALB-Bet-PEC (**b**) cryogels during storage
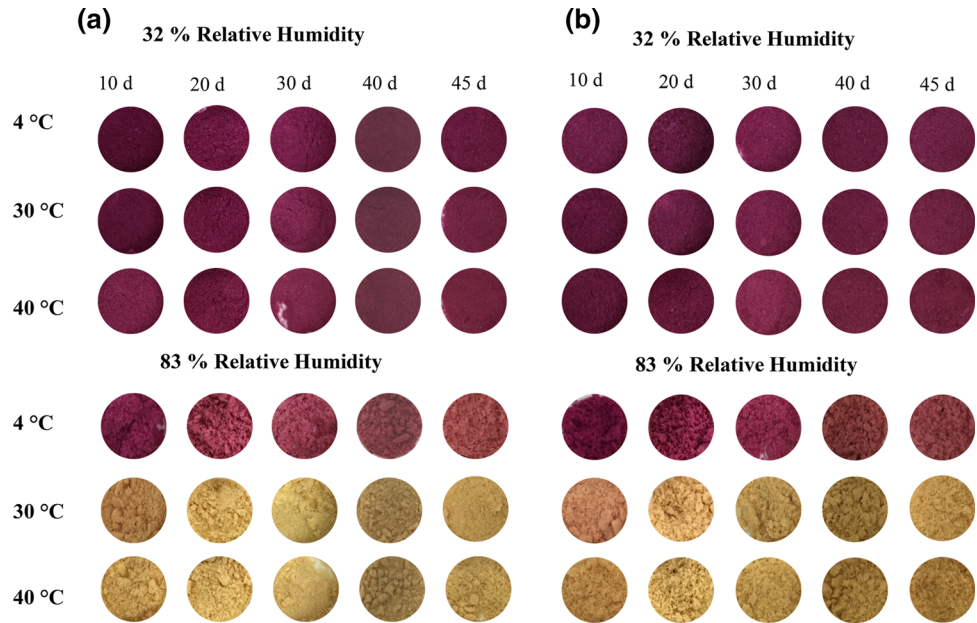
pH conditions and light exposure during the storage as well as their bioavailability are desirable.

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