DR. LÍA NOEMÍ GERSCHENSON (Orcid ID : 0000-0002-6663-9697)

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Development of functional pectin edible films with fillers obtained from red cabbage and beetroot.

Carlos M. Otálora González[a,b], Maria D. De'Nobili[a,b], Ana M. Rojas[a,b], María F. Basanta[a,b] Lía N. Gerschenson[a,b] *.

[a]*Universidad de Buenos Aires (UBA), Facultad de Ciencias Exactas y Naturales, Departamento de Industrias. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).*
[b]*Instituto de Tecnología de Alimentos y Procesos Químicos (ITAPROQ). UBA-CONICET. Ciudad Universitaria. Avenida Intendente Guiraldes 2620, (1428) CABA, Argentina.*

* Corresponding author. Tel./ fax: + 54 11 52859014.
E-mail address: lia@di.fcen.uba.ar

ABSTRACT

The present study aims to develop through casting, functional composite edible films based on pectin using beetroot powder (BRP) and red cabbage powder (RCP) as fillers obtained from by-products of plant tissue industrialization. Physico-chemical, mechanical, thermal properties and colour stability of the films along 30 days of storage (5 °C- 45 °C; absence of light) were evaluated. The moisture and the stress at break decreased and the intensity of red colour and the hydrophobicity increased with the presence of fillers. According to the FTIR results, there was a good compatibility between matrix and fillers in the composites. Pectin films generated an enhanced stability of red colour after 30 days of storage at 5° C and 25° C and, in general, after 16 days for 45°C, showing their suitability to be used as colouring agents for edible food packaging applications.

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Keywords: Edible films, pectin, beetroot, red cabbage.

1.Introduction

Food packaging systems have different functions, including those related to containment, food information, and marketing. Recently, active packaging systems based on biopolymers and natural extracts have begun to attract the interest of scientists and industries (Pirsa et al., 2020). This packaging system has the objective of not only ensuring food safety but also extending storage life while diminishing the negative impact of synthetic polymers on the environment (Otoni et al., 2017).

Cellulose, starch, chitosan and pectin are the polysaccharides most used to constitute active and edible packaging systems. Pectin is an easily accessible biopolymer, non-toxic, biodegradable, edible and due to its gelification capacity it is considered a good matrix for edible film production (Espitia et al., 2014). This polysaccharide can be recovered from by-products of plant tissues. The incorporation of fibres, vitamins, pigments and flavors in pectin films gives them antimicrobial, nutritional and antioxidant properties (De'Nobili et al., 2013: Basanta et al., 2018).

Beetroot (BR) and red cabbage (RC) are highly consumed vegetables, easy to cultivate and commercialize at low price. BR contains high betalains concentration, being these pigments used as colourants and additives in food products (Esatbeyoglu et al., 2015). Betalains comprise red/violet betacyanins and yellow/orange betaxanthins (Otálora et al., 2020). Red cabbage contains intense red pigments which have a colouring behavior superior to the one obtained with the harmful synthetic dye E 129 (Red No. 40). The RC presents an anthocyanins complex profile (Wiczkowski et al., 2013), derived from cyanidin 3-diglucoside-5-glucoside conjugated with different sugars and acyl groups (Zhao et al., 2017). Various factors affect anthocyanin and betalains colour and stability, such as temperature, pH, light, oxygen, enzymes, metallic ions, sugars and sulfites (Patras, 2019; Otálora et al., 2020).

The residues from the industrialization of vegetables have become an environmental problem. By-products of plant tissues, such as those of BR and RC, are promising sources of fibres and polyphenols with nutritional, antioxidant and/or antimicrobial properties, and are low-cost natural materials. Their use as food ingredients and additives is a way to alleviate the environmental problem as well as to add value to the raw material. They can also be used for the

development of composite bio-based packaging materials, decreasing the use of fossil-based plastics (Pirouzifard et al., 2019).

Over the last 20 years, numerous studies have been performed concerning the formulation and production of biodegradable films. However, it is necessary to develop new studies that help to improve their properties as well as to explore their potential applications. Our research has as main objective the use of biopolymers that can be obtained from industrial wastes to develop composite biodegradable films and to evaluate their potential applications. As a consequence, we studied the effect on pectin film properties of the incorporation of beetroot and cabbage powders. Also, the colour stability was evaluated at different temperatures and pHs, for the purpose of analyzing the possibility of using the film as coloured packaging.

2.Materials and Methods

2.1.Chemicals

Food grade pectin (GENU^{TM} pectin type LM-12 CG) was a gift from CP Kelco (Denmark) (Supplementary file 1, S.1). Glycerol (Biopack, Argentina) and potassium sorbate (Sigma, USA) used were of analytical grade. Deionized water (Milli-Q^{TM}, USA) was used.

2.2.Red cabbage and beetroot powders obtention

BR (*Beta vulgaris* L. var. *conditiva)* and RC (*Brassica oleracea* var. *capitata* f. *rubra*) were obtained from local markets in Buenos Aires (Argentina). They were washed (potable water with a residual available chlorine level of 1 ppm), cut and blanched.

a) The BR were peeled and cut into slices (1 cm thick, 4.9 cm to 6.0 cm diameter). They were blanched by immersion for 7 minutes in water at 90 °C with a 0.5 kg / L vegetable tissue/water ratio according to Otálora et al. (2020).
b) The RC were cut into strips 13 to 15 cm long and approximately 1 cm wide. 100 g of RC were steam blanched during 7 min over boiling water at 1 atm pressure.

After each blanching treatment, the vegetal tissues were immersed in an ice-water bath for 5 min to stop the heating effect. The samples were freeze-dried (Pennsalt lyophilizer, USA), milled in a domestic blade mill (DeLonghi, Argentina) and sieved (shaker Retsch, Germany) to obtain powders with a size lower than 105 μm.

2.3.Chemical analysis of cell wall components in powders

Cellulose, lignin, uronic acid, total carbohydrates and protein contents were determined as reported by Otálora et al. (2020)

2.4.Betalains and anthocyanins extraction and content determination

An amount of 1 g of powder or edible film was weighed into a falcon conical tube and stirred for 1 min in a vortex (Velp Scientifica, Italy) with 15 ml Milli-Q^{TM} water. It was sonicated (ultrasonic cleaner, Testlab, Argentina) for 30 minutes, at a frequency of 40 Khz and a power of 240 W. It was centrifuged at 5000 rpm for 15 min at 4 °C (centrifuge 5804R, Eppendorf, Germany) and the supernant aqueous extract was used for measurement.

Anthocyanins content of the extracts was determined according to Wrolstad et al. (2005). Betalains content of the extracts was determined according to Otálora et al. (2020) (S.1).

2.5. Film preparation

The elaboration process developed by De'Nobili et al. (2013) was used for simple film (SF) manufacture, 2.67 g of low methoxyl pectin were added to 90.00 g of deionized water into a glass beaker under stirring at 300 rpm in a vertical agitator (LH, Velp Scientifica, Italy). Then, the mixture was heated to 90 °C on the stirrer heating stage at a rate of 5 °C/min and, under constant temperature and stirring, 0.03 g potassium sorbate and 1.67 g of glycerol were added, followed by 0.17 g of $CaCl_2.2H_2O$ pre-dissolved in 2 mL of water. Total weight of film making solution was completed to 100.00 g by addition of deionized water. The final pH of the solution was 2.9. An amount of 12 g of solution was poured into silicone molds of 7 cm diameter (Silikomart, Italy) and dried in a controlled temperature chamber at 60 °C (FAC, Argentina) for 3 h. Film equilibration took place, for 7 days, in a desiccator (Nalgene, Rochester, USA) containing a saturated solution of NaBr (water activity ≅ 0.575; 25 ºC). For composite films (CF), 1.5 % w/w of BR or RC powder was incorporated in the formulation after hydration with a fraction of the formulation total water (powder/water ratio, 1/10). This incorporation was performed, after the addition of $CaCl_2.2H_2O$, under stirring at 300 rpm for 1 minute. Previous assays allowed to determine the maximum quantity of powder that gave origin to a smooth film without protuberances able to be detected through visual or tactil evaluation.

2.6. Characterization of the films

Films were evaluated at least three times with each technique, unless stated.

2.6.1.Thickness, moisture content and water solubility

Film thickness was determined using a digital micrometer model ID-C112M (Mitutoyo Corp. Kawasaki, Japan) with an accuracy of 1 μm. This parameter was measured on each film, at six randomly selected points and mean and standard deviation (SD) were calculated. These values were used for stress calculation. The moisture content of the films was determined by drying in a

vacuum oven (Gallenkamp, UK) at 105 ºC for 24 hours and the results were expressed as % moisture in dry basis (% db). Films water solubility was determined according to Alzate et al. (2017).

2.6.2.Colour

Cielab colour parameters (L*, a*, and b*) and ΔE* were determined on films and powders according to Basanta et al. (2018) (S.1).

2.6.3.Mechanical properties

Tensile properties were evaluated according to the standard method D882-02 (ASTM, 2002) with some modifications using a universal Instron testing machine model 3345 (Instron Corp, USA), equipped with a 100 N load cell and a strain rate of 0.8 mm/s (S.1).

2.6.4.Contact angle and humectability

The contact angle and humectability were evaluated with an interfacial tensiometer (Sinterface, Germany) using the sessile drop method according to Ollé et al. (2014).

2.6.5.Fourier transform infrared spectroscopy

The IR spectra of films were recorded with a Nicolet iS50 FT-IR (Thermo Scientific Nicolet, USA) spectrometer using diamond attenuated total reflectance (ATR) technique with a 45°incident angle (S.1).

2.6.6.Differential scanning calorimetry

Glass transition temperature (Tg, onset initial value) was obtained using a differential scanning calorimeter (DSC, 822e, Mettler Toledo, Switzerland) with a nitrogen flow system (S.1).

2.6.7.Scanning electron microscopy (SEM)

Film microstructure was characterized by means of a ZE122 SEM Supra 40 scanning electron microscope (Carl Zeiss, Germany). The cross sections and the surface of films were metalized with a platinum layer prior to observation.

2.6.8.Colour stability

Films colour stability was evaluated as a function of time, temperature, and pH. Samples were immersed in buffers of pH 4, 5, 6, 7 and 8, dried with filter paper and stored for 30 days at 5 °C, 25 °C, and 45 °C, in the dark. The samples were characterized using CIELab system as well as through photography. The colour change of the films during storage was calculated (ΔE). For comparison purposes, it was also evaluated the change in colour with pH, of the aqueous extracts of the powders.

2.7.Statistical Analysis

Statistical analysis was performed using the Prism 5 utility (GraphPad, California, USA). The significance of the differences between simple and composite films, was carried out using ANOVA with a significance level, α, of 0.05 and the Tukey test was used "a posteriori" (Sokal & Rohlf, 2000).

3.Results and discussion

3.1Powders characterization

Table 1 shows, that BR and RC powders had low moisture content fact that guarantees stability against deterioration during storage (Clemenson et al., 2012). They were mainly constituted by cell wall polysaccharides and proteins and lignin were also present, contributing to the formation of the film network developed by the commercial pectin. BR powder had higher betacyanins than betaxanthins content which is consistent with the high a*value while RC powders presented a total anthocyanin content of 3.68 mg C3G/g of powder. The BR and RC powders presented second order transitions (Tgs) at 13.15 °C for the former and at 5,12 °C and 37.95 °C for the latter, showing that both fillers are in the rubbery state at room temperature trend that might condition the textural behavior of the films. The Tgs observed can be influenced by the different contents of cellulose and non-cellulosic carbohydrates in both powders (Iijima et al., 2000; Szczesniak et al., 2008).

3.2Films characterization

Table 2 shows that addition of BR and RC powders to the pectin based films caused an increase in thickness. These could be a consequence of changes in the film structure due to the formation of new non-covalent interactions between the filler and the matrix macromolecules and/or to the increase in solid contents which increased the density (Kouchak et al, 2015). SF films had the highest moisture content, probably due to pectin hydrophilicity that promoted interactions with water (Tran et al., 2017). The incorporation of the powders reduced the film water content probably due to the hydrogen bonds formed between pectin and –OH groups that were present in the filler, limiting the pectin-water interactions (Cano et al., 2015). Jamróz et al. (2019) also reported a significant decrease in moisture content by the addition of beetroot extract in *furcellaran* films. All the films showed a high solubility which is characteristic of edible polysaccharidic films, and no significant differences could be observed with filler incorporation (Sothornvit & Krochta, 2000).

Table 2 reports betacyanins, betaxanthins and anthocyanins content in the films and colour parameters. Simple films were transparent, slightly yellowish, and with high brightness. The incorporation of BR and RC into the films, caused a decrease in brightness, and an increase in parameter b* and parameter a*. The films turned to be red, and not transparent, due to the powder pigments. This is consistent with the higher concentration of betalains than betaxanthins in beet powder and with the adoption by anthocyanins of an oxonium-type structure at the pH of 2.9 (Patras, 2019), characteristic of the pectin film-forming solution.

Composite films showed a decrease in stress and firmness and a slight decrease in deformation at break when compared with simple films. It is known that, in general, there is an increase in stress with fillers (Kouchak et al, 2015) but Fama et al. (2010) reported a decrease in hardening with the incorporation of garlic powder to a starch matrix. These authors attributed that trend to the presence in the filler of chemical moieties capable of forming hydrogen bonds between the filler and the matrix macromolecules, affecting polymeric network formation and film structure.

Contact angle is a measure of surface hydrophobicity. Composite films had a higher contact angle (Table 2), probably due to structural changes that affected the surface and or to the hydrophobic components of the filler, especially lignin (Azeredo et al., 2016). Wettability is an important property of edible films because it allows to understand the interfacial film-water interaction and is related to water resistance and it decreased significantly with BR powder incorporation . According to results obtained, CBRF and CRCF will be more adequate for covering foods with high lipids content than SF.

It can be observed (Table 2) that the films are in the rubbery state, showing Tg values at -95.58, -84.26 and 87.11 °C, for the glycerol rich phase (Basanta et al., 2018). For SF, an additional Tg was observed at -9.43 °C due to a pectin rich phase. For composite films, values of -10.64 and -13.01 °C, were observed due to the existence, for them, of a pectin/filler-rich phase. Probably, the components of the cell wall present in the powders modified the intra- and inter-molecular hydrogen bonds in the films, producing changes in this temperature.

Simple films SEM images (Figure 1 a, b) show a homogeneous, smooth, non-porous surface and cross-section. In contrast, composite films were highly influenced by the presence of the powders generating a heterogeneous and rough structure with some cracks and pores that can be observed in the cross sections (Figure 1 c,d,e,f). This heterogeneous structure might be, at least, partially responsible for the results obtained in relation to contact angle and wettability of the composite films.

3.3 FTIR spectrum

Films spectra show the following peaks (Figure S1): ≈3279 cm^{-1} (O-H stretching), ≈1739 cm^{-1} (C=O stretching), ≈1645 cm^{-1} (C=O stretching), ≈1329 cm^{-1} (C-H bending), ≈1022 and ≈1100 cm^{-1} (C-H carbohydrate skeleton stretching) and 921 cm^{-1} (Wang et al., 2016). A band at 1417 cm^{-1} is attributed to the formation of ionic bonds between calcium cations and pectin carboxyl anions (Noh et al., 2018). A 2938 cm^{-1} band corresponds to the characteristic signals of glycerol (Perez et al., 2009). In general, the pectin film spectrum is mainly characterized by wave numbers 1022, 1100, and 920 cm^{-1}, as reported by Coimbra et al. (1999), who confirmed the importance of the 1104-1014 cm^{-1} peaks as wave numbers that characterize uronic acid-rich pectin polysaccharides.

The addition of powders to simple films, did not cause the presence of new bands (Figure S1), however, there were increases in transmittance in the regions 1600-1700 cm^{-1} (C=O stretching) and 3279 cm^{-1} (O-H stretching) of the spectrum which might be related to the formation of hydrogen bonds between the chemical components of the powders and the pectin of the matrix (Jamróz et al., 2019; Wang et al., 2016). This trend is indicative of the effective incorporation of the components of the powders into the polymeric matrix. Changes in the intensity of O-H stretching were also observed by Tran et al. (2017) when they added microparticles rich in betalains in starch films.

3.4 Colour stability of the films as a function of time, temperature, and pH

In order to evaluate the stability of the pigments included in the films, the colour change (ΔE) and visual appearance (photographies) of the films were studied at different pHs and storage temperatures, for 30 days.

While the aqueous extracts of the powders (Figure S2) showed a high sensitivity to pH change, the composite films showed stability of the colour (red) for the initial time at pHs between 4 and 8. In Figure 2 the ΔE is reported and it can be observed that at 5 °C, the films colour remained constant during storage at all pHs (A1, A2). At 25 Cº CRCF (B2) showed greater colour stability compared to CBRF (B1) since anthocyanins are more stable to temperature changes (Zhao et al., 2017). At 45 °C, a marked colour change along storage was observed for all films and pHs and the films CRCF (C2) showed the largest ΔE (Figure 2) after 30 days of storage at pH 6, 7 and 8 at 45 °C. The intensification of the yellow colour at 45 °C (Figure S2) for CBRF and CRCF can be attributed to degradation of betalains and anthocyanins due to the effect of high temperature (Ananga et al., 2013; Otálora et al., 2020).

4. Conclusions

Composite pectin films with fillers (BRP, RCP) obtained from plant by-products were developed. The fillers contained betalains (BRP) or anthocyanins (RCP) and were rich in non-cellulosic carbohydrates. The BRP and RCP were effectively incorporated into the pectin matrix producing the modification of the colour to red, decreasing the stress at break and increasing the hydrophobicity of the films.

The inclusion in the films of the powder containing pigments, contributed to the absence of colour change with pH modification after 30 days storage at 5 °C and 25 °C. The pectin films with the red cabbage anthocyanins (CRCF) exhibited the highest colour stability at these temperatures. Accordingly, these films would be promising for the development of active colouring packaging, that might be applied as such in foods with pHs between 4 and 8 and with shelf lives of 30 days.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ETHICAL GUIDELINES

Ethics approval was not required for this research.

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LEGENDS TO THE FIGURES

Figure 1. SEM micrographs of the surfaces (a,c,e,) and cross sections (b,d,f,) of the pectin simple films (SF) (a, b), beetroot powder film (CBRF) (c, d) and red cabbage powder film (CRCF) (e,f) .

Figure 2. Colour change (ΔE) of films with different pHs along 30 days, at 5 °C (A1 and A2), 25 °C (B1 and B2) and 45 °C (C1 and C2). A1, B1 and C1: beetroot powder film (CBRF). A2, B2 and C2: red cabbage powder film (CRCF).

LEGEND TO THE SUPPLEMENTARY FIGURES

Figure S1. ATR-FTIR of simple film (SF), composite film with BRP (CBRF) and composite film with RCP (CRCF).

Figure S2. Photographies of films with different pHs along 30 days, at 5 °C (A1 and A2), 25 °C (B1 and B2) and 45 °C (C1 and C2). A1, B1 and C1: beetroot powder film (CBRF). A2, B2 and C2: red cabbage powder film (CRCF). For comparison purposes, there were also included photographies showing the change in colour with pH, of the aqueous extracts of the powders.

Table 1. Moisture, chemical composition, Tg and color parameters for beetroot and red cabbage powders.

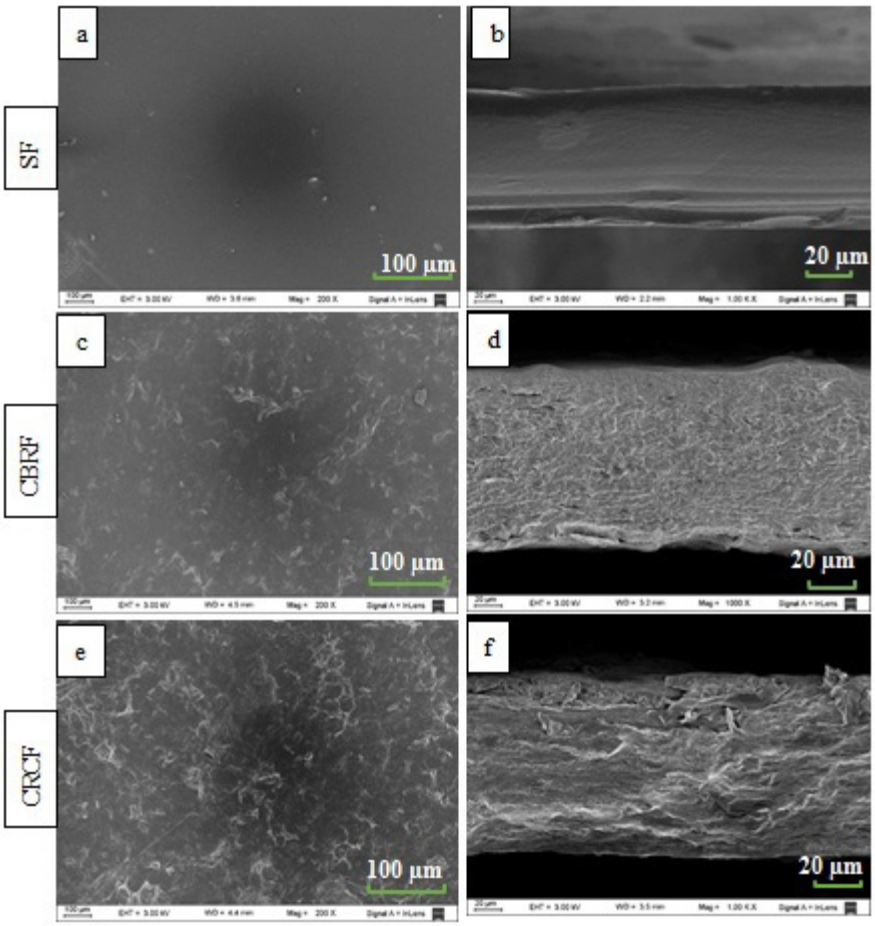
	Beetroot powder	Red cabbage powder
Moisture content (g/100 g)	4.93±0.02 *	6.2±0,3
Cellulose (g/100 g)	7.0±0.1 *	3.7±0.2
Non-cellulosic carbohydrates (g/100 g)	30.9±0.8 *	73±2
Uronic acids (g/100 g)	9.8±0.8 *	8.9±0,3
Proteins (g/100 g)	8.5±0.4 *	9±1
Lignin (g/100 g)	1.72±0.04 *	2.9±0.2
Anthocyanin (mg C3G/ g)	-	3.68±0.02
Betacyanins (mg betanin/g)	0.47±0.01	-
Betaxanthins (mg vulgaxanthin /g)	0.26±0.04	-
Glass transition temperature, Tg (°C)	13.15	5.12, 37.95
Color parameters		
L*	19.72± 0.03*	41±2
a*	36.6± 0.4*	9,89±0,05
b*	4.7± 0.3*	-15±3

Mean and standard deviations for n = 3 are reported. Values are reported per mass of powder. (–). C3G: cyanidin-3-glucoside.* Otálora et al. (2020).

Table 2.Thickness, moisture content, water solubility (WS), contact angle, wettability, mechanical properties, Tg, pigment content and color parameters of the films.

Parameters	SF	CBRF	CRCF
Thickness (μm)	120±6[a]	174±4[b]	190±1[b]
Moisture (g/100 g of film)	44.9±0.2[a]	34.7±0.2[b]	37±1[b]
WS (%)	89±4[a]	81±3[a]	88±3[a]
Contact angle (°) measured at 20 s	0.4±0.2[a]	5.3±0.7[b]	2.5±0.5[c]
Wettability (mN/m)	-0.0004±0.0001[a]	-0.30±0.08[b]	-0.06±0.01[a]
Stress at rupture (MPa)	9.5±0,3[a]	8.3±0,8[b]	7.2±0,2[c]
Deformation at rupture	0.32±0.06[ab]	0.34±0.03[a]	0.27±0,02[b]
Firmness (MPa)	30±4[a]	24±3[b]	26±1[b]
Glass transition temperature, Tg (°C)	-95.58 -9.43	-84.26 -10.64	-87.11 -13.01
Anthocyanin (mg C3G/100g of film)	-	-	70.0±0.9
Betacyanins (mg betanin/100g of film)	-	1.8±0.3	-
Betaxanthins (mg vulgaxanthin/100g of film)	-	0.786±0.007	-
Color parameters			
L*	89.3±0.3[a]	44±1[b]	47.3±0.2[c]
a*	-2.7±0.3[a]	49±1[b]	53.4±0.8[c]
b*	4.7± 0.3[a]	13±1[b]	10.5±0.9[c]
ΔE	0	69±2[a]	70±1[a]

Mean and standard deviations for n = 3 are reported. C3G: cyanidin-3-glucoside. Same letter in a row means non-significant differences ($p < 0.05$) between data reported.



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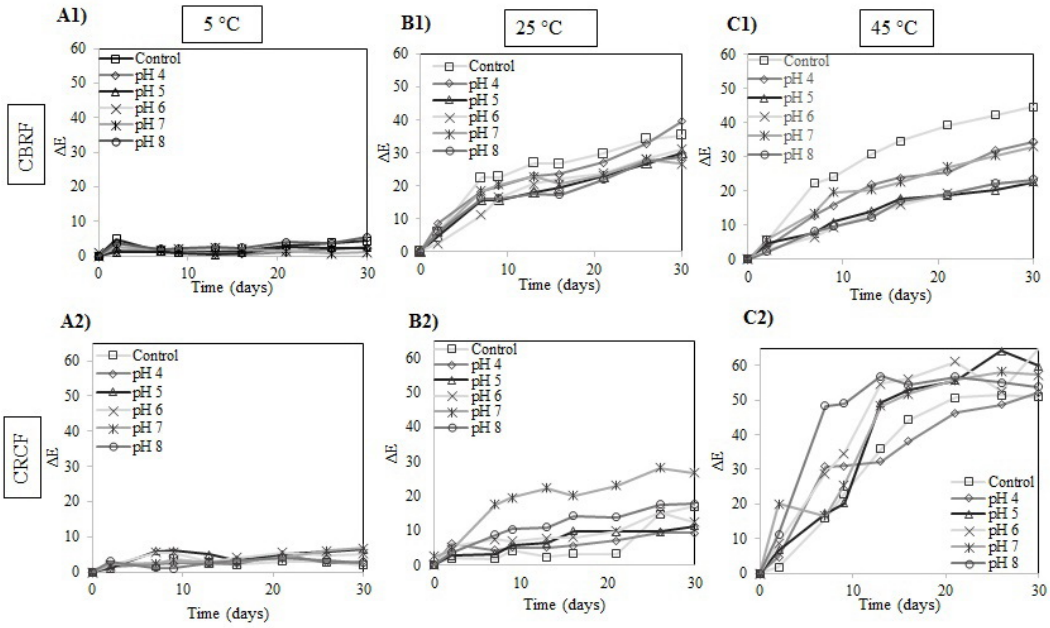

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