

## SOME FUNCTIONAL PROPERTIES OF PIGMENT EXTRACTS FROM RED CABBAGE (*BRASSICA OLERACEA*) AND REDBEET (*BETA VULGARIS*)

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**Abstract**— The purpose of the present work was to obtain natural pigments from red cabbage and redbeet. The antioxidant and antimicrobial activities of the fresh pigments were determined. Furthermore, the encapsulation capability and release of the bioactive compounds were analyzed. Pigment extraction was conducted in distilled water with 1% HCl media. The anthocyanin content obtained from cabbage was 8.9 mg/ml and 8.8 mg/ml of betalains from redbeet. The polyphenol content for red cabbage and redbeet were 0.0128 and 0.0123 mg of gallic acid equivalent/g in dry basis, respectively, as determined by Folin-Ciocalteu method.

The stability of the extracts to UV light and temperature was assessed; polyphenolic and pigment contents decreased after exposure. Pure extracts from these vegetables showed an antimicrobial effect under ambient conditions, as well as an inhibitory activity against *Staphylococcus aureus*. However, no antimicrobial effect was detected for the pathogen *Aspergillus niger*. The extracts were successfully encapsulated in calcium alginate beads; an additional coating of chitosan was applied.

**Keywords**— Red cabbage; Redbeet; Anthocyanins; Betalains; Encapsulation.

### I. INTRODUCTION

Synthetic additives, which include antimicrobials, stabilizers, antioxidants and pigments, are commonly found in food formulations. However, consumer preferences favor natural substances. Thus, there is a continuous search for additives from natural origin obtained by non-contaminating technologies (Sapers *et al.*, 1981; Giusti and Wrolstad, 2003; Valenzuela *et al.*, 2003). Particularly, the use of synthetic red dyes are under discussion; therefore, finding red pigments from natural sources become necessary. Natural pigments include several chemical compounds, like anthocyanins, betalains and carmine.

Some of them have additional properties like antioxidant or antimicrobial activities. Within the polyphenolic compounds, anthocyanins have several beneficial effects such as antioxidant and anti-inflammatory capacity; also they may increase blood vessel health, among other helpful aspects (Heo and Lee, 2006). According to Hall (2001) and Giusti and Wrolstad (2003), the betacyanins and betaxanthins present in redbeet (*Beta vulgaris L. var esculenta*) and

anthocyanins found in red cabbage (*Brassica oleracea L. var capitata*) have antioxidant activity.

From *Brassica* specie, Brussel sprouts, broccoli and red cabbage have some of the highest antioxidant activity (Podsdek, 2007; Sikora *et al.*, 2008). Podsdek (2007) reported that the hydrophilic antioxidants in *Brassica* vegetables are responsible for more than 89% of the total antioxidant capacity. Besides, some polyphenolic compounds used as food additives inhibit microbial growth (Estévez and Cava, 2006). To improve the pigment handling, in the present work an encapsulation technique of the extracts was implemented. Encapsulation is a process where a polymer covers small solid particles, liquid droplets or gases (Shahidi and Han, 1993; Deladino *et al.*, 2008). These systems have the property of protecting the core substance and allow it to be released in a controlled manner. Additionally, they can solve some functional issues such as manipulation, increase solubility, etc. (Abreu *et al.*, 2008).

Therefore, the aims of the present work were i) to obtain pigments from red cabbage and redbeet, ii) to explore additional properties of the pigments like antioxidant, antimicrobial activity as well as stability of the fresh pigments and iii) to encapsulate these vegetal extracts.

### II. METHODS

#### A. Pigment Extraction from Red Cabbage and Redbeet

Red cabbage (*Brassica oleracea*) and redbeet (*Beta vulgaris*) were obtained from the local market; they were washed, cut and later placed individually in a flask with a vegetable:solvent ratio of 1:10.

Extraction was carried out using different solutions a) water, b) water and HCl 1%, c) HCl 1% aqueous solution:ethanol (1:1). Cut vegetables were processed using magnetic agitation for 16-18 hours at ambient temperature. Then, they were filtered with Whatman #1 paper and kept in a colored glass flask at 0°C.

#### B. Extraction yield

The total dry matter content was determined by drying in an oven at 100 °C until constant weight (AOAC, 2004). Assays were performed in triplicates.

The extraction yield of the process was calculated considering the extract weight in dry basis and the initial weight of the fresh vegetables.

$$Y(\%) = 100 \frac{\text{Weight of extracted pigments (g.db)}}{\text{Weight of fresh vegetables (g)}} \quad (1)$$

### C. Content of the Extracts

#### *Anthocyanins and Betalains content determination*

The red cabbage's anthocyanins and redbeet's betalains content were determined using a Shimadzu Double Beam Spectrophotometer UV-150-02 (Seisakusho Ltd., Kyoto, Japan).

Spectra were run between 400 and 800 nm to determine the maximum absorbance. Obtained values were used to characterize the pigments. The Lambert-Beer law was applied to calculate compound concentrations, as follows:

$$A = \varepsilon b C \quad (2)$$

where  $A$  is the absorbance,  $\varepsilon$  is the extinction coefficient,  $b$  is the path length through the sample and  $C$  is the concentration of the sample. The extinction coefficient was calculated using calibration curves for each pigment (Fig. 1a and b). Then, concentration of anthocyanins in the red cabbage and betalains in redbeet could be expressed as mg/ml.

#### *Surface Color Measurements*

Aliquots of fresh samples were placed in Petri Dish of 5 cm diameter and 1 cm height. Surface color was measured in a Minolta CR-300 Colorimeter (Minolta CR-300, New Jersey, USA). The parameters defined by the CIE (Commission International de l'Eclairage) luminosity ( $L^*$ ) and chroma (defined by  $a^*$  and  $b^*$  parameters) were used.

Each extract was measured considering 5 replicates. A correlation between surface color and spectrophotometric measurements of pigment content was obtained.

#### *Determination of total polyphenol content*

The Folin-Ciocalteu method (Schlesier *et al.*, 2002) was used to measure the total polyphenolic content of the extracts. This method is based on the oxidative capacity of the phenolic groups with phosphomolibdic and phosphotungstic acid reagents. As a result of the oxidation a blue-green compound appears, which has a maximum absorbance between 725 and 750 nm. Extract (0.2 ml) was mixed with 2 ml of  $\text{Na}_2\text{CO}_3$  2% (w/v), after 2 min rest, 0.2 ml of the Folin-Ciocalteu (1:1) reagent was added. The absorbance was measured in the spectrophotometer at 725 nm after 30 min. Gallic acid (Sigma, USA) was used as a standard substance for the calibration curve. Total polyphenolic concentration units were expressed as mg of gallic acid equivalent/mg of dry extract (mg GAE/mg extract).

#### *Determination of the Antioxidant Activity using DPPH<sup>•</sup>*

The antioxidant activity of both fresh extracts maintained in dark flasks was measured using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>) radical scavengers method (Cai *et al.*, 2003). A 2.5 mg of DPPH<sup>•</sup> (Sigma, USA) solution was prepared in 100 ml of ethanol; 0.1 ml of each extract was added to aliquots of 3.9 ml of the mentioned solution. The mixtures were left at ambient temperature for 40 min and then the absorbance was measured using a spectrophotometer at 517 nm. Distilled water was used as blank.

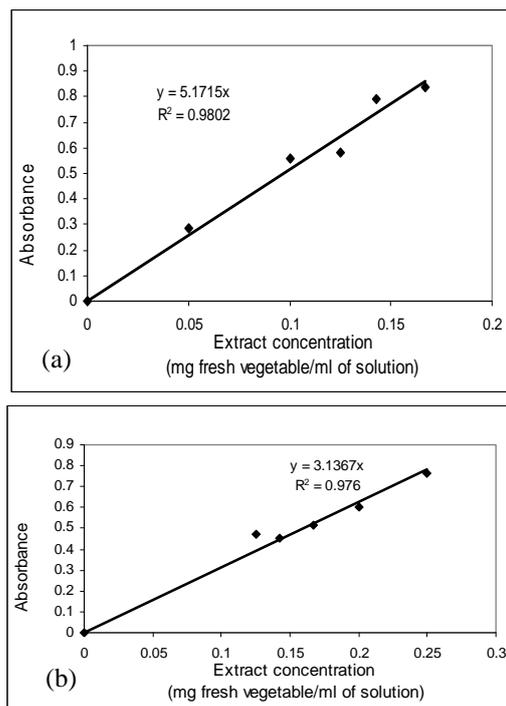


Figure 1: Calibration curves of the extracts obtained for a) Red cabbage and b) Redbeet.

The antioxidant capacity was expressed as the percentage of DPPH<sup>•</sup> radical scavenging activity given by the following equation (Vendramini *et al.*, 2008; Dudonné *et al.*, 2009):

$$AA = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \quad (3)$$

where  $AA$  is the antioxidant activity,  $A(\text{control})$  is the absorbance of the control substance and  $A(\text{sample})$  is the asymptotic absorbance value of the sample.

Additionally, results were calculated using absorbance values of remaining DPPH<sup>•</sup> concentrations ( $\text{DPPH}_{\text{REM}}^{\bullet}$ ) at each time ( $t$ ):

$$\text{DPPH}_{\text{REM}}^{\bullet} = \frac{\text{DPPH}_t^{\bullet}}{\text{DPPH}_{t=0}^{\bullet}} \quad (4)$$

In order to compare these results with literature data, the slope of the linear function, percentage of  $\text{DPPH}_{\text{REM}}^{\bullet}$  versus time, was estimated using a logarithmic scale.

#### **D. Stability of the fresh extracts against temperature and UV light**

To analyze the effect of UV light on the extract stability, 20 ml of fresh extract were placed in Petri dishes of 9 cm diameter. Then, they were kept in a radiation chamber equipped with four lamps UV-C (TUV G30T8, 30W, Philips) at a 30 cm distance. The samples were irradiated with doses of 6.9  $\text{kJ}/\text{m}^2$  for 7 min. The radiation intensity was measured using a digital UV radiometer, Cole-Parmer Instrument Company, Illinois, USA (Dyrby *et al.*, 2001).

To check stability against temperature, 20 ml of fresh extract were placed in a 9 cm diameter Petri dish and were submitted to heating at 120 °C in an oven for 15 min.

The stability of the extracts was estimated consider-

ing the total pigment by surface color, spectrometric measurements and the polyphenolic content by Folin-Ciocalteu method, before and after temperature and UV light treatments.

Fresh extracts were placed in dark flasks (20 ml) for 10 days at 0 °C to evaluate the stability of the color along refrigerated storage.

#### E. Antimicrobial activity of the extracts

Antimicrobial activity was determined on extracts from both vegetables (redbeet and red cabbage) and water-HCl 1% solution (control). Aliquots (12 ml) were placed in sterilized Petri dishes and stored open at room temperature for 10 days, applying the Kirby-Bauer technique (National Committee for Clinical Laboratory Standards, 2000). The antimicrobial activity was also tested using Petri dishes with Plate Count Agar (12 ml) and inoculated with two pathogenic microorganisms: *Staphylococcus aureus* and *Aspergillus niger*. Sterilized filter paper disks, 1cm in diameter, were dipped in fresh and diluted solutions (1/10, 1/100 and 1/1000 v/v) of redbeet and cabbage extracts. Four sample disks were placed on top of the inoculated plates, for each extract type. Plates were incubated at 35°C for 24 hours. The experiment was carried out in triplicate.

#### F. Encapsulation

##### *Encapsulation with Sodium Alginate and Calcium Chloride*

Sodium alginate 2% (w/v) (Protanal, Norway) was added at approximately 1 drop per second to a beaker that contained the fresh extract with CaCl<sub>2</sub> 0.05M (Laboratorio Cicarelli, Argentina). Beads formed were maintained in the solution for 30 min., filtered and washed with distilled water.

##### *Alginate-Chitosan Beads*

To obtain an additional protection, some washed capsules were dipped in a 1% (w/v) Chitosan (Sigma, USA) solution for 30 min. Then, coated beads were filtered and washed.

##### *Release velocity of the encapsulated bioactive compounds*

The assay consisted in placing 20 coated with chitosan and uncoated beads in test tubes with 5 ml of distilled water. Absorbance of liquid samples of each tube was determined until steady state was reached. The effect of the chitosan coating was calculated through the release velocities (RV):

$$RV = \frac{C_{final, accumulated} - C_{initial}}{time} \quad (5)$$

where  $C_{final, accumulated}$  is the accumulated concentration,  $C_{initial}$  is the initial released concentration and time corresponds to 17 days.

Besides, the relative velocity of release (RVR) was determined by the following equation:

$$RVR = 100 \frac{RV_{uncoated} - RV_{coated}}{RV_{uncoated}} \quad (6)$$

All experiments were performed by duplicate.

#### G. Statistical Analysis

Analysis of variance (ANOVA) was performed with

Systat-software (SYSTAT, Inc., Evanston, IL, USA, 2001) version 10.0. Significant differences ( $p < 0.05$ ) between the means were determined using Tukey Test.

### III. RESULTS AND DISCUSSION

#### A. Extract solution selection for the vegetable pigments. Betalains and Anthocyanins determination

The absorbance spectra of each extract, from cabbage and redbeet, were found to be similar for each of the three extract solutions tested. Equivalent wavelengths at the maximum absorbance were found regardless the extract solution (water, water-HCl, water-ethanol-HCl). The extract solution water-HCl 1% and water-ethanol-HCl gave non significant differences in absorbance values, thus the first one was chosen since it is more convenient extract solution. In this medium, the anthocyanins were quantified at 525 nm for cabbage and 534 for the betalains in redbeet. Moreno Alvarez *et al.* (2002), working on degradation of betalains from redbeets, attributed the maximum of absorbance value at 537 nm to betacyanins. The concentration of these compounds was determined through the Lambert-Beer law (Eq.2); 8.9 mg of anthocyanins/ml for cabbage and 8.8 mg of betalains/ml for redbeet were obtained. Considering the fresh vegetable weight, the obtained yield was 0.89 % (w/w) for cabbage and 0.88 % (w/w) for redbeet (Eq. 1). Vrchovska *et al.* (2006) working on tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC), with different agronomic practices and collecting periods, obtained yield values between 0.033 and 0.064 g lyophilized extract per g of plant material.

#### B. Total polyphenol content and antioxidant activity in both vegetable extracts (redbeet and cabbage)

Cai *et al.* (2003) studied the phenolic compound distribution and found that the majority of the polyphenolic substances are present in the external layer of the root (*Beta vulgaris* L. var. *esculenta*). In the present work the outer layer and core of the redbeet were used.

The polyphenolic concentration in red cabbage was 0.013 mg GAE/g db and 0.012 mg GAE/g db for redbeet. These values are in agreement with the ones obtained by Vrchovska *et al.* (2006), although cabbage used was grown under different agronomic conditions. The presence of phenolic compounds in plant extracts significantly contributes to their antioxidant capacity and various authors agree, in many cases, exists a direct relationship between the total phenolic compounds determined by Folin-Ciocalteu method and the antioxidant power (Atoui *et al.*, 2005; Deladino *et al.*, 2008). Dudonné *et al.* (2009) stated that  $DPPH^{\bullet}$  and  $ABTS$  (2, 2'-azinobis (3-ethylbenzthiazoline 6-sulfonate)) assays are the easiest methods to implement and gain the most reproducible results, based on an interlaboratory comparison for measuring antioxidant activity. In this work,  $DPPH^{\bullet}$  was implemented.

Figure 2 shows the residual values of absorbance obtained by the  $DPPH^{\bullet}$  method for the red cabbage extract, the same behavior was observed for redbeet pigment. Using Eq. (3) the antioxidant activity (AA) was

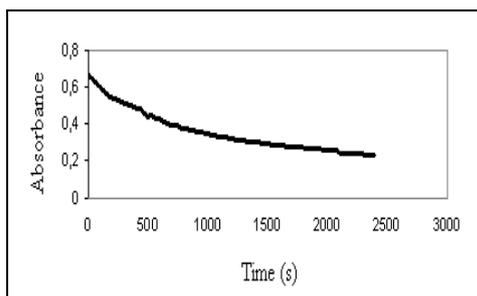


Figure 2: Residual values of absorbance obtained by the DPPH method for red cabbage.

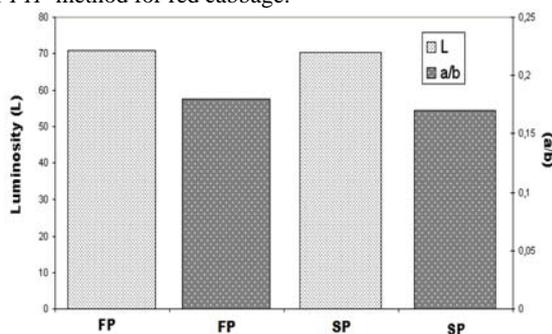


Figure 3: Surface color changes along refrigerated storage (FP: fresh pigment, SP: storage pigment).

calculated and the values obtained were 67 % and 63 % for cabbage and redbeet, respectively. These values are in the range with those obtained in other products such as yerba mate (*Ilex paraguayensis*) with 71% and cinnamon bark 84 % (Dudonné *et al.*, 2009). However, Vendramini *et al.* (2008) declared a value of 10 % for yerba mate. The literature data highly depends on genotype, maturity degree, vegetable parts used for extraction, storage and processing conditions.

To compare the kinetic behavior of the antioxidants, the logarithmic value of  $[DPPH^*]_{REM}$  versus the logarithmic value of time were plotted. The slope values were -0,637 ( $r^2=0.993$ ) for redbeet and -0,207 ( $r^2=0.995$ ) for cabbage. These slopes corresponds to those obtained with concentrations of 90 g BHA/kg  $DPPH^*$  and 50 g of ascorbic acid/kg  $DPPH^*$  in the case of cabbage and 540 g of BHA/kg  $DPPH^*$  and 139.15 g of ascorbic acid/kg  $DPPH^*$  in the case of redbeet (Sánchez-Moreno *et al.*, 1998). These authors reported data for different natural and synthetic antioxidants, comparing their antioxidant activity and kinetics, characterizing them as fast, medium and slow action antioxidants. According to this classification and the time that the stationary value was reached, our cabbage and redbeet extracts can be considered as slow action antioxidants.

### C. Color

To evaluate the stability of the extracts along refrigerated storage, surface color changes were measured. The color parameters ( $a^*$ ,  $b^*$ ,  $L^*$ ) of redbeet showed significant differences for 10 days of storage ( $p < 0.05$ ). For red cabbage,  $a^*$  and  $b^*$  parameters were significantly different, but the luminosity parameter was not influenced by storage time (Fig. 3).

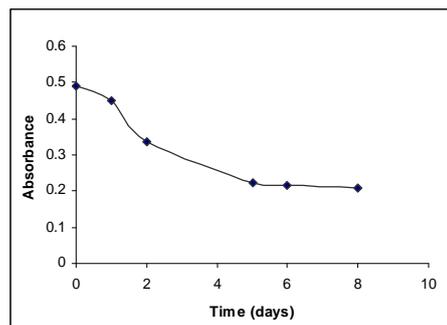


Figure 4: Absorbance values as a function of time for redbeet extract.

Also, the color was evaluated spectrophotometrically by means of the pigment absorbance. In literature, a relationship between surface color and the pigment concentration was determined in strawberries (García *et al.*, 1998). Kayodé *et al.* (2012) optimized the extraction of antioxidant pigments from leaf sheaths of dye sorghum by analyzing the effect of temperature and the concentration of extraction solvent. They evaluated total phenolics and total anthocyanins, and the color parameters of the extracted pigment, as the present work.

A typical curve of absorbance measurements for redbeet is shown in Fig. 4. The decrease in the absorbance value and the color parameters  $a^*$ ,  $b^*$  followed a similar behavior.

### D. Stability under temperature and UV light

Several chemical and physical factors affect negatively dye stability, being temperature and light the most important ones (Dyrby *et al.*, 2001). Podsedek (2007) points out that industrial processing of vegetables such as blanching, canning, sterilization, freezing and domestic cooking, directly affects the content and composition of the antioxidant, which in turn affects the antioxidant activity and their bioavailability.

The studies showed that the cabbage and redbeet extracts under ultraviolet light decreased their polyphenolic concentrations in 9.8 and 22.4 %, respectively and the decrease for the anthocyanins was 23.3 % in cabbage and for the betalains 33.3 % in redbeet.

The temperature had a negative influence over the polyphenol content since the concentration reduction was 8 % for both cabbage and redbeet extracts. The anthocyanins exposed to heat suffered a 46 % reduction for cabbage while the betalain content decreased 75.5% in redbeet.

Hence, extracts obtained from cabbage were more stable than redbeet ones when exposed to either temperature or UV radiation. Other authors found the decrease of natural pigments submitted to UV light and heat (Attoe and Von Elbe, 1985; Dyrby *et al.*, 2001).

### E. Antimicrobial activity of the extracts

After 10 days at ambient temperature, extracts from both vegetables showed no microbial growth; while control plates did. Therefore, it can be suggested that both pigments have a natural antimicrobial effect. A previous work on redbeet roots, reported that betacyanins were responsible for the antimicrobial activity (Stintzing *et al.*, 2000).

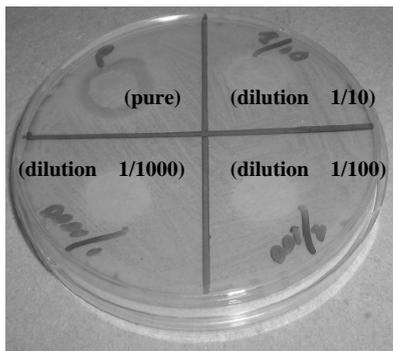


Figure 5: Inhibitory activity of fresh pure and diluted redbeet extract on *Staphylococcus aureus*

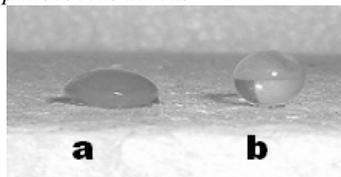


Figure 6: Beads of pigment extracted with water (a) and encapsulated extracts obtained from an acid medium (water+HCl 1%) (b).

With respect to the plates inoculated with the pathogen *Staphylococcus aureus*, an inhibitory activity was observed when an impregnated filter paper with pure pigment extracts for both products (redbeet and cabbage) was used (Fig. 5). Nevertheless, no antimicrobial effect was observed with either pure or diluted pigments when the pathogen *Aspergillus niger* was inoculated.

#### F. Encapsulation

The stability of the bioactive compounds can be greatly enhanced using an encapsulation technique during the processing and storage of food, with the advantage of the reduction of undesired aroma and flavor (Chigurupati *et al.*, 2002; Dyrby *et al.*, 2001). Under usual practices of encapsulation, the active compound is solubilized in sodium alginate, however in this work an alternative technique had to be implemented, because the extract formed a soft gel with sodium alginate inside the burette. In this work, calcium chloride was solubilized in the extracts, and then, the sodium alginate solution was dripped over this mixture. In the case of red cabbage, the shape and color of beads were affected by pH of the solution. Beads of pigment extracted with water presented a spherical shape and blue color, while the beads containing the extracts from an acid medium (water + HCl 1%) were flat shaped and maintained the original red color (Fig. 6). However, the pH medium did not affect either color or shape of the beads containing redbeet extract, being spherical and red. Chitosan additional coating did not modify either shape or color of the beads.

In order for the antioxidant compounds to become active, they must migrate or diffuse into the external media. To study this behavior the release kinetics of total polyphenols and pigments in water was analyzed for both coated and uncoated beads. Until reaching steady state, a 13.5 and 34.7% reduction of total polyphenols and pigment release (*RVR*) were observed for the beads containing chitosan for red cabbage compared to un-

coated ones. In the case of redbeet a rapid release was observed in both cases coated and uncoated before 24 hours. The addition of the chitosan coating did not give an extra protection for redbeet extract. The observed behavior could be explained taking into account the chemical structure of the compounds. Alginate has free carboxylic groups which react with divalent cations, to form a stable gel (King, 1983). Antocyanins and betalains have different chemical structures. The first are derivatives of the basic flavylum cation structure; while betalains come from diazoheptamethin (Francis, 1985). Antocyanins, positively charged, could interact more easily with alginate than betalains that are negatively charged. Therefore, alginate-betalains bounds could be more labile leading to a faster release in water.

#### IV. CONCLUSIONS

In this work, pigments from red cabbage and redbeet were obtained and experimentally studied. Red cabbage and redbeet extracts showed antioxidant properties measured by *DPPH*<sup>•</sup> radical; compared to other antioxidant reported in literature, their activity exhibited a slow action behavior.

During refrigerating storage at 0 °C, the anthocyanins and betalains pigments suffered a reduction in their concentration. Besides, the extracts decreased their concentration in pigments as well as in polyphenols due to UV light and temperature exposure. Extracts obtained from red cabbage were more stable than redbeet ones, when exposed to either temperature or UV radiation.

An additional property explored was the antimicrobial activity in pure extracts of red cabbage and redbeet at ambient temperature. No microbial growth took place during 10 days, in contrast with the control sample, which did not show this inhibitory action. Moreover, when *Staphylococcus aureus* was inoculated an inhibitory action was observed. However, no antimicrobial effect was detected with either pure or diluted pigments when the pathogen *Aspergillus niger* was inoculated.

Finally, extracts have been encapsulated in calcium alginate beads by means of a modification of the traditional method. The pH of the extraction medium showed an influence on the shape and color of red cabbage beads. The addition of the chitosan coating slowed down the release velocity of red cabbage extract. However, this extra protection was not evidenced for the release of redbeet extract. The effect of the encapsulation technique on pigment stability is under analysis in our laboratory.

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