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Principal component analysis of the factors involved in the extraction of beetroot betalains

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Abstract

Betalains represent a natural and safe alternative to colour synthetic additives that are used currently in food industry. In addition to the colouring property, betalains are of particular interest because of their importance in health: antimicrobial and antioxidant activity, radio-protective, antiviral, anti-inflammatory and detoxifying effect. This study compares different classics methods of betalains extraction, using water and weak acid solutions from fresh, dried and lyophilized beetroot. The highest content of betanin (20 mg /g) has been obtained from lyophilized beet using 0.5% citric acid and 0.1% ascorbic acid as solvents. In this study temperature, pH, liquid/solid ratio were considered the main factors which influence the performance of extraction process and stability of betanin. The extraction is better at high temperatures (70°C), regardless of the other two parameters. At low temperature it is necessary to increase the liquid/solid ratio and decrease the pH for getting a good yield of extraction. Conditions to ensure the stability of the betalains preservation are: low-temperature and humidity, lack of light and air. The experimental results have been interpreted through the Principal Component Analysis (PCA), using The Unscrambler soft.

Keywords: beetroot, extraction, betanin, betalains preservation

1. Introduction

The global market for additives was estimated to 940 millions of dollars in 1990, from which 42% are synthetic additives, 27% natural additives, 20% identical natural additives and 11.4% caramel colours. In 1994 the European Union authorized only 43 colouring additives for food industry: 17 were synthetic additives, 13 natural and 13 identical natural additives [7]. The number of colouring agents used in USA dropped from 700 in 1970 to only 36 nowadays. From these, 7 are synthetic colouring agents and 26 are natural [7]. The pigments extracted from plants are widely used nowadays as substituent to synthetic colorants in food, pharmaceutical and cosmetics industries.

Betalains are plant pigments, water soluble, that contain nitrogen and whose colours are variables, from red-purple in the case of betacyanins to yellow for betaxanthins [27]. Betalains are a natural and secure alternative for the synthetic colouring agents used nowadays. It does not exist a superior limitation for the recommended daily intake.

The betalains are situated in cells vacuoles of different plant organs: in flowers (*Portulacaceae, Caryophyllales*), in *Cactaceae* fruits, in beet root (*Beta vulgaris*) and in some big mushrooms like *Amanita*, *Hygrocybe* and *Hygrosporus* [21]. Betalains are also found in bracts, like in *Bougainvillea* (*Nyctaginaceae*) which has a wide

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variety of colours [20]; in *Amaranthus* seeds and *Teloxis* leaves [1].

One of the most promising sources of betalains is *Cactaceae*, from which the cactus pearls (*Opuntia* genus) and pitayas (*Cereus, Hylocereus* şi *Selenicereus* genera) are often cultivated and used as betalains sources for food colouring [16,22,23]. Betalains from cactus fruits have a larger colouring spectrum than those from beetroot, from yellow-orange (*Opuntia sp*.) to purple red (*Hylocereus sp*.). Another advantage of cactus fruits is the soil needed for cultivation and the minimum water requirements [6]. Though, the most commercially available betalains are those extracted from beetroot (*Beta vulgaris*), that contain two important colouring agents soluble in water: betanin (red pigment) and vulgaxanthin I (yellow pigment). According to Nilsson (1970) [17] the betacyanins and betaxanthins content in beetroot is 0.04-0.21% and 0.02-0.14% respectively, depending on beetroot variety [29], although the new varieties can produce a higher content of betalains.

In 2001 Gasztonyi et al. [11] analyzed the pigment amount in 5 varieties of beetroot *(Bonel, Nero, Favorit, Rubin* and *Detroit*). In all the analyzed samples the most important purple red pigments were betanin, isobetanin, betanidin and isobetanidin, and the yellow pigments were vulgaxanthin I and vulgaxanthin II. The *Bonel, Favorit* and *Rubin* varieties had the highest betacianine content (approximated 0.08%) while the *Nero* variety had a lower betacianine content (0.06%). The *Rubin* variety had the highest betacianine/betaxanthine ratio (2.08) so it was considered the appropriate variety for food colouring production. Using DPPH (1,1-diphenyl-2-picrylhydrazyl) method to measure the antioxidant capacity, some betacyanins (medium concentration of 3.7 mM) and betaxanthins (medium concentration of 4.2 mM) demonstrated a 3-4 fold higher antioxidant capacity than this of ascorbic acid (13.9 mM). The antioxidant activity of betalains was also higher than of polyphenols (6.1 mM) and catechins (7.2 mM) [5]. In other studies [12,13], the betanin antioxidant capacity using TEAC (*Trolox Equivalent Antioxidant Capacity*) method was determined. Some works demonstrated the antiradicalic capacities of *in vitro* betalains [3,5,8,9,14,15,18,31]. Some studies based on *in vitro* tests concluded that beetroot is classified in the first ten vegetables with a powerful antioxidant capacity [28], and betalains are responsible for these properties [19,31].

2. Materials and methods

Materials. The betanin was extracted from beetroot, *Beta vulgaris* specie, purchased from local market. The beetroot was carefully washed and scarped and the spent beetroot pulp was obtained. These were used for betanin extraction in fresh, dried (oven dried at 40-45°C for 10-12 hours) and lyophilized state (using CHRIST ALPHA 1-4 LD plus lyophilizator).

Betanin extraction. The solid-liquid extraction was made to obtain a maximum yield with minimum pigment degradation and it is a widely spread method for food colouring agents obtaining. In this work, the ability of weak acid solutions and hydro-alcoholic solutions were tested for betanin extraction from the spent beetroot pulp. The liquid/solid ratio used in this study was 5:1. The experimental extraction variants are presented in table 1.

Table 1. Experimental variants for colouring agents' extraction from the spent beetroot pulp

Variants	Solvent
V1	Distilled water
V2	1% citric acid
V3	0.5% citric acid
V4	0.2% citric acid
V5	0.1% ascorbic acid
V6	50 % ethanol
V7	20% ethanol
V8	0.5% citric acid and 0.1% ascorbic acid
V9	0.2% citric acid and 0.1% ascorbic acid
V10	20% ethanol and 1% citric acid
V11	20% ethanol and 0.5% citric acid

Experimental conditions. The stability of the obtained extracts was measured after 30 days, in different environment conditions: at room temperature (20°C) and lighting, refrigeration (4°C) and darkness and freezing (-18°C in Ultrafreezer SANYO MDF- U 5411) in darkness.

The betanin content was determinate by spectrophotometry, using the JENWAY 6505

UV/Vis spectrophotometer. Betanin has maximum absorption at $\lambda = 535$ nm. The betanin content extracted from 1 g of beetroot was calculated using Eq. 1.

$$m_i = \frac{A_i \cdot F_d \cdot \overline{M}}{\varepsilon \cdot l} \cdot \frac{V_e}{1000 \cdot m_s} \quad \text{(Eq. 1)}$$

m_i - quantity of extracted betanin from 1 g of beetroot;

A_i - absorbance;

$\overline{M}$ - medium molecular mass, g/mol;

ε – molar extinction coefficient (1120 $L \cdot cm^{-1} \cdot mol^{-1}$ for betanin) [24,25];

V_e – extract volume, ml;

m_s - mass of beetroot used in extraction procedure;

F_d - dilution factor.

Knowing that the principal factors that affect the extraction yield and betanin stability are temperature, pH, liquid/solid ratio, this study was performed using an experimental trifactorial algorithm, as presented in Table 2. Water bath RAYPA, INOLAB pH 720 pH-meter and METTLER TOLEDO XS 403 SM analytical balance were used for experimental procedure. All the extractions for this experiment were performed using distilled water as solvent.

Table 2. Experimental matrix

Experiment	T, (^0C)	$R_{L/S}$	pH
0	20	5	2.5
1	70	5	2.5
2	20	10	2.5
3	20	5	8
4	70	10	2.5
5	70	5	8

Statistical analysis. The experimental results were analyzed using Principal Component Analysis (PCA) with full cross-validation. PCA constitutes the most basic statistical method of all multivariate data analysis, and involves decomposing one "data matrix" into a structural part (model) and a "noise" part (error). The main purpose of all multivariate data analysis is to decompose the data in order to detect and model "hidden phenomena".

PCA was assessed using the Unscrambler X 10.1 software version from CAMO Software AS (Oslo, Norway).

3. Results and discussion

In this study, the betanin extraction from beetroot in different states and different experimental conditions was performed. The experimental results, in mg betanin/g beetroot, are presented in figures 1 and 2.

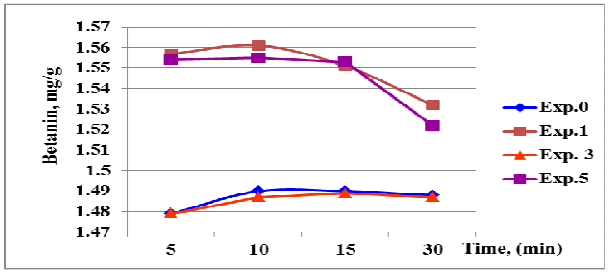


Figure 1. Variation of extracted betanin from fresh beetroot, as a function of temperature and pH

Figure 1 presents the experimental results obtained when liquid/solid ratio 5:1 and distilled water were used. By comparing Experiment 0 (t = 20°C, pH = 2.5) to Experiment 1 (t =70°C, pH = 2.5) and Experiment 3 (t = 20°C, pH = 8) with Experiment 5 (t =70°C pH = 8) it can be observed that temperature greatly influences the extraction yield for betanin. Also, by comparing the results of Experiment 1 (t =70°C pH = 2.5) with Experiment 5 (t =70°C pH = 8) it can be observed that at high temperatures the pH does not significantly influences the extraction yield. On the other side, at low temperatures the acidic environmental conditions have positive influence (Experiments 0 and 3).

The results are comparable to those obtained by von Elbe et al., in 1974 [29] on beetroot juice and paste at variable pH between 2.0 and 9.0. They showed that the absorption spectra of the solutions with pH between 4.0 and 7.0 are similar.

In figure 2 it can be observed that the high temperature has a positive influence on the extraction (Experiment1 (t°=70°C, L/S ratio = 5) and Experiment 4 (t°= 70°C, L/S ratio = 10) independent of the liquid/solid ratio. When the extraction is performed at 20°C and the liquid-solid ratio is higher, the extraction is significantly positively influenced (Experiment 0 (t°= 20°C, L/S ratio = 5) and experiment 2 (t°= 20°C, L/S ratio = 10). So, when

the extraction is performed at low temperatures, the liquid/solid ratio should be raised.

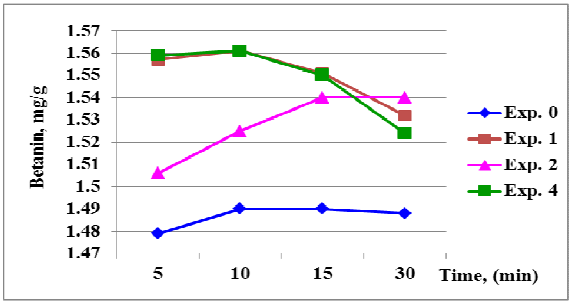


Figure 2. Variation of extracted betanin quantity as a function of temperature and liquid/solid ratio

Another factor that increases the betanin degradation speed is light exposure (Figure 3). To demonstrate the effect of light exposure, the betanins were extracted with 0.5% citric acid and 0.1% ascorbic acid (V8) and the extracts were kept for 6 days at 20°C in light and in darkness, respectively. It could be observed that light exposure raised the betanin degradation speed, the betanin concentration was approximately half of that obtained in the samples maintained in dark.

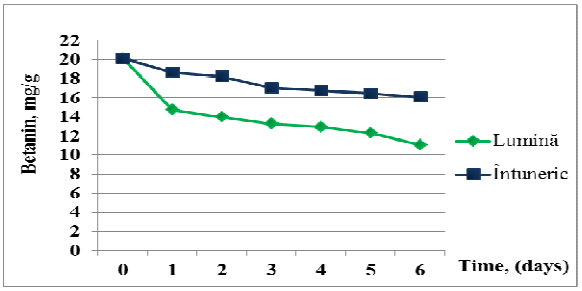


Figure 3. Light exposure influence on betanin stability; the betanin was extracted with 0.5% citric acid and 0.1% ascorbic acid (V8) from lyophilized beetroot

Similarly, the research of de Von Elbe et al., 1974 [29]; Attoe von Elbe, 1981 [2] and Cai et al., 1998 [4] showed that after storage of betanin solution with pH=7, in air or N_2, for 6 days at 15°C with light exposure conducted to the increase of degradation speed.

The main aim of this research was to study the relations between the variables (raw material type, extraction solvent, storage temperature and duration and light exposure during storage) and the response (the betanin quantity) in the data set, at the same time, through the analysis of the principal components (PCA).

In order to detect potential errors in the input of data, the data set was graphically represented, and it was observed that the data input was accurate, as no samples were found outside the parameters under evaluation. At first, the analysis of the critical points was carried out by using the number of components deemed optimal. The system recommends a number of 5 main components which explain 100% the data variation, for calibration, as well as for the validation of results. A number of 4 main components may explain over 96% of the data variation, with a result validation of 80%.

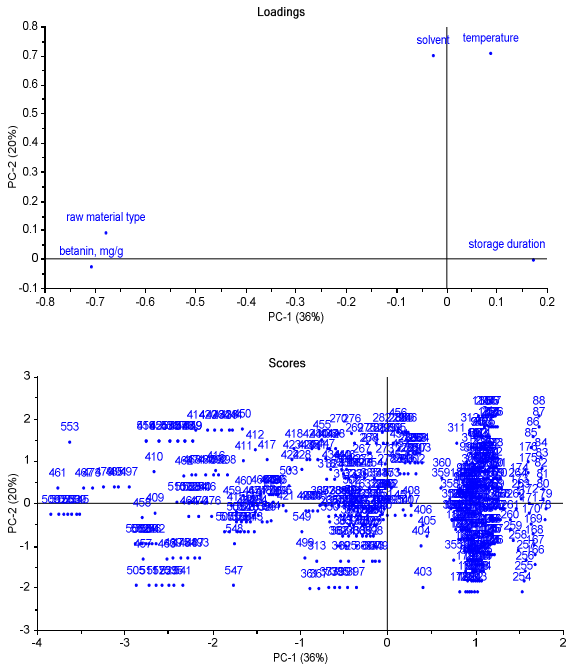


Figure 4. Loadings and Scores plots for PC-1 and PC-2

The PCA model was interpreted after outliers removing, by evaluating the scores and loadings plots simultaneously.

From figure 4 it can be observed that PC-1 is given by the variation of betanin quantity and the type of raw material used for extraction (fresh, dried or lyophylized) and it explains 36% of data variation.

In figure 4 it can be observed that the raw material used in experiment and its state (fresh, dried or

lyophilized) has a great positive influence on extracted betanin (figures 4 and 5). Therefore, the fresh beetroot has a lower betanin concentration, while the lyophilized beetroot is more concentrated in this compound.

PC-2, which explains 20% from data variation, is influenced by the storage temperature which is very important for betanin stability (figure 6). It can be observed that the samples kept at freezing temperature (-18°C) contain higher betanin concentrations.

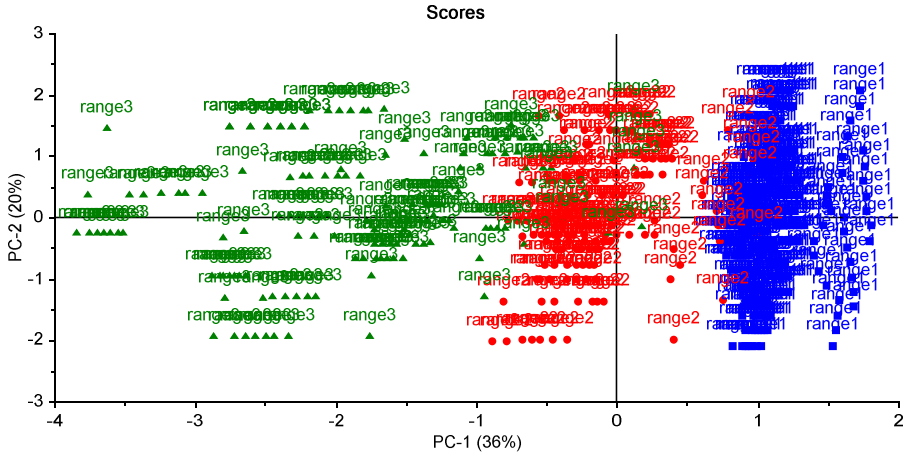


Figure 5. Scores plot for PC-1 and PC-2 with samples grouping after raw material type (*range* 1 (blue) – fresh beetroot; *range* 2 (red) – dried beetroot; *range* 3 (green) – lyophilized beetroot).

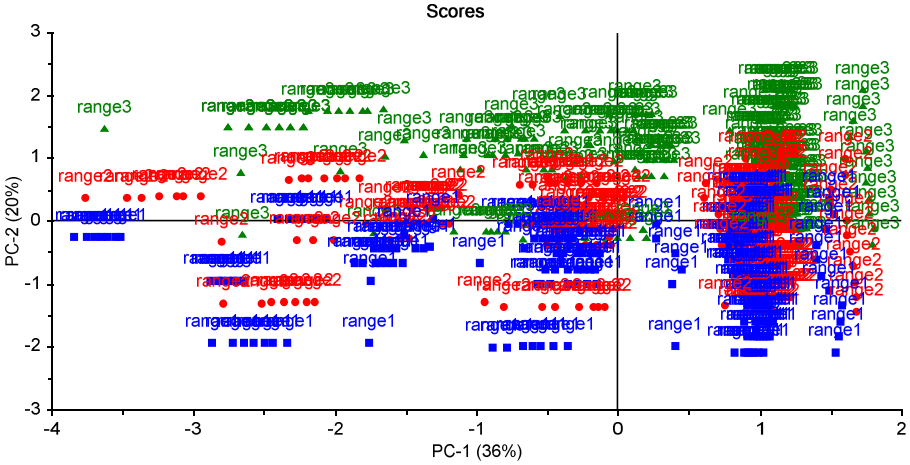


Figure 6. Scores plot for PC-1 and PC-2 with samples grouping as function of storage temperature (*range* 1 (blue) – room temperature; *range* 2 (red) – refrigeration temperature, *range* 3 (green) – freezing temperature).

Some studies reported that the temperature increase conducts to the increasing of the betanin degradation rate [26]. The thermal degradation of betacyanins from beetroot and cactus fruits respects the first order kinetics [29].

During thermal treatment betanin can be degraded by isomerisation, decarboxylation or cleavage and the red colour is reduced step-by-step and a brown colour will appear.

PC-3, which explains 20% from data variation, is given by the storage duration, in days. Figure 7 shows that during short-term storage the betanin concentration is not significantly influenced, but when the storage arises to 30 days the betanin concentration in the extracts is lowered.

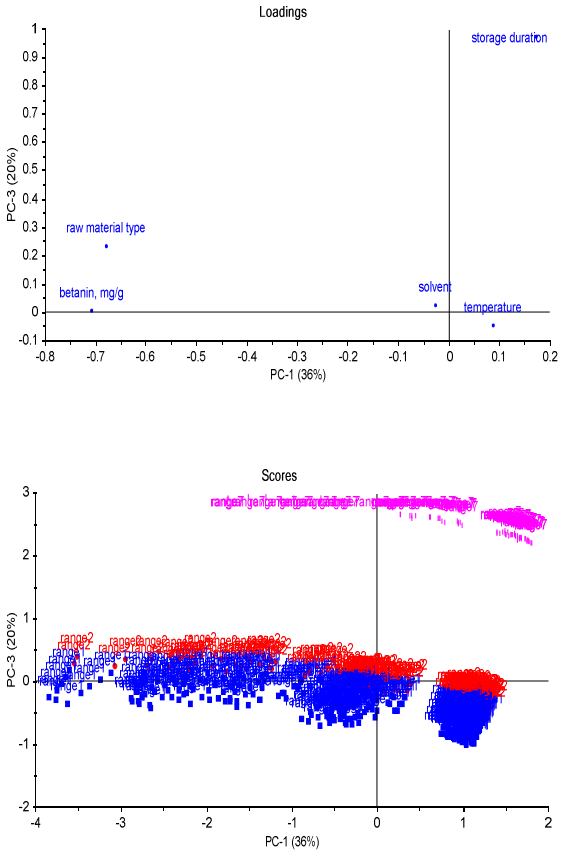
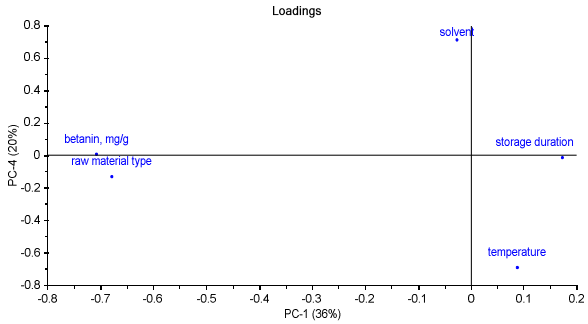


Figure 7. Scores and loadings plots for PC-1 and PC-3 with samples grouping in function of storage duration



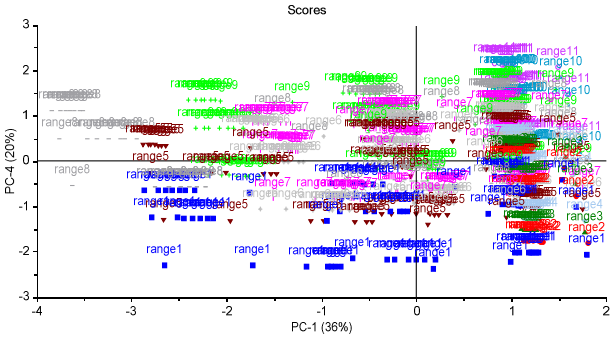


Figure 8. Scores and loadings plots for PC-1 and PC-4 with samples grouping as a function of solvent type used for extraction (*range* 1- V1, *range* 2- V2, *range* 3- V3, r*ange* 4- V4, *range* 5- V5, *range* 6- V6, *range* 7- V7, *range* 8- V8, *range* 9- V9, *range* 10- V10, *range* 11- V11)

PC-4, which explains also 20% of data variation, is given by the solvent type used in betanin extraction. From figure 8 it can be observed that the solvent has also a great influence on betanin extraction and the V8 coded variant (0.5% citric acid and 0.1% ascorbic acid) conducted to the extraction of the highest betanin quantity. The obtained results are in concordance with the industrial practice where betanins from beetroot are currently extracted under acidic conditions (usually citric acid) [30].

4.Conclusions

The betanin, a red colouring agent widely used in food industry, was extracted from beetroot. The highest betanin content (20 mg/g of beetroot) was found from lyophilized spent beetroot pulp using 0.5% citric acid with 0.1% ascorbic acid as solvent. Important amounts of beetroot betanins were obtained by ascorbic acid extraction also.

If the extraction is made at low temperatures, the acidic medium has a positive influence. The high temperature has a positive influence no matter of the liquid/solid ratio.

Betanin is better preserved using freezing temperatures, after 30 days of storage the betanin lost was lower than 25%. Therefore, the conditions that have a positive effect on betanin preservation are low temperature, darkness, low air admission and low humidity.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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