Implications of the red beet ripening on the colour and betalain composition relationships

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ABSTRACT

The evolution during ripening of *Beta vulgaris* (var. Pablo) on colour and betalain composition, not previously conducted in conjunction in red beets, has been examined. According to the results, it could be asserted that the ripening stage significantly ($p < 0.05$) influenced on all the studied parameters. On the basis of the betalain content, the optimum ripening stage corresponded to a medium weigh-to-calibre ratio, in the light of the significantly ($p < 0.05$) higher content of betalains, especially betanin and vulgaxanthin I. Moreover, colour attributes also differed during ripening, having the medium-ripened beetroots a significantly ($p < 0.05$) more reddish hue (h_{ab}) and lower lightness (L^*), probably due to the higher content of betaxanthins in this stage. The colour differences among red beets in the stage II and the rest of stages were visually appreciable ($\Delta E^*_{ab} > 3$) and mainly qualitative. A new range of opportunities for diversification of colorant market, from a nutritional and colorimetric point of view, could be possible by employing red beets with different stages of ripening.

KEYWORDS

Beta vulgaris L.; ripening; betalains; colour; differential tristimulus colorimetry

ABBREVIATIONS

W/Cal. Weigh-to-calibre ratio

HPLC. High performance liquid chromatography

RID. Refractive index detector

DAD. Diode-array detector

LDA. Linear discriminant analysis

INTRODUCTION

Red beet (*Beta vulgaris* L. sp) belongs to the *Chenopocidiaceae* family and it is cultivated around the world, with usages as food, colorant or medicine. It is native to the Southern Europe, where traditionally is consumed [1]. Beetroot is a red taproot with green leaves and red veins, rich in polyphenols and water-soluble pigments called betalains, which are present in a restricted number of families of the plant order Caryophyllales and the genus Amanita of the Basidiomycetes [2].

The chemical structure of betalains presents a nitrogenated chromophore group based on resonant systems with double conjugated bonds [3]. Betalains are structurally classified as (i) red-violet betacyanins, conjugating betalamic acid with *cyclo*-Dopa and an additional residue of hydroxicinnamic acid derivates or sugars (being betanin and isobetanin the predominant pigments in beetroot), and (ii) the yellow-orange betaxanthins, result of the condensation among betalamic acid and amines or aminoacids, being vulgaxanthin I the main betaxanthin present in red beets. Due to their particular colorimetric characteristics, betalain-rich products represent a promising source of natural colorants, responsible for several beneficial properties for human health such as free radical-scavenging, anti-inflammatory and anticarcinogenic effects [4].

Colour is an important attribute of foods, considered as a quality and acceptability indicator [3]. Up to now, many products are coloured by synthetic dyes, but nowadays consumers are reluctant to artificial sources and their preferences are turning towards natural products that could positively influence their health. In fact, the consumption of natural colorants is growing in the European markets [2]. In connection with betalain-rich products, betanin (extracted from *Beta vulgaris*) is a natural colorant approved by the

Unites States and the European Union as food additive (E-162), and it is extensively used in many products with a wide pH range such as desserts, meat products and drinks [3].

The most apparent external signs of ripening in fruits and vegetables are changes in colour [5]. However, this practice is unsuitable for roots, because their physical appearance is similar from the very early stages of the ripening. That is the case of red beets, which precise moment to be collected for marketing purposes is not based on the colour but when the maximum growth of the root has been achieved [6]. This fact could provoke that beets may not be harvested in an optimum moment from a colorimetric and pigment content point of view. Besides, as far as we know, there are no scientific evidences that point out this criterion as the optimal maturity stage for red beet harvest. Therefore, due to red beets have bioactive compounds important in human nutrition and are used commercially as a dye to colour processed food, it is needed to establish the optimal harvest moment to maximize the colour and pigment yield to obtain a top-quality product. Based on these considerations, this research sought not only the colour and betalain characterization of the red beets at different stages of growth, but also to evaluate the ripening behaviour on colour and betalain profile of *Beta vulgaris* L. in order to establish the optimum moment of harvesting. Our interest was focused on the study of the betalain profile by high performance liquid chromatography-diode array detection (HPLC-DAD) and colorimetric characteristics by applying differential tristimulus colorimetry, which have not been previously considered in conjunction with that purpose in beetroots.

MATERIAL AND METHODS

Samples

A total of 82 fresh red beetroots (*Beta Vulgaris* L., sp. Var. Pablo) were collected in the province of Sevilla (Spain) at different stages of ripening. The temperature ranged

between 9 and 21 °C, establishing the medium value around 14 ºC. Moreover, the average relative humidity was around 76%, although it ranged between 49 and 97%. The average rainfall was established under 2 mm/day. These data have been provided by Instituto de Investigación y Formación Agraria y Pesquera (IFAPA), Junta de Andalucía, Spain.

Instrumental texture analysis

The compression test was performed using a TA.XT Plus Texture Analyzer (Stable Micro System, Godalming, UK) equipped with a HDP/90 platform. Weigh calibration of equipment was carried out with a 5 Kg load cell, developing previous calibrations of force and distance with a 30 Kg load cell. The compression test was performed for each entire beetroot in the equatorial position, using a 50 mm cylindrical aluminium probe. A desired rate of deformation was adapted to each sample in order to avoid the overload of the discharge cell. The test parameters were as follows: speed pre-test, 0.50 mm/s; speed test, 0.50 mm/s; speed post-test, 10 mm/s; distance, 5.0 mm; activation force, 35.0 g; trigger type and auto force, and 500 points per seconds. Texture Exponent software was used for data acquisition to obtain the firmness, i.e. the resistance to the deformation (N/mm) (expressed as the slope of the force-distance curves). To face this task, the inferior point (origin) was set to 25 N and the superior point (maximum strength) was taken corresponded to the 80% of the force-distance curve. Texture analyses were performed using MATLAB R2012b (The Mathworks, Natik, MA, USA, 2012).

Preparation of extracts

Each red beet was cut in half, obtaining two portions for each sample: the upper half (close to leaf) and the bottom half (near the root). Each portion of sample was peeled, cut into strips, and subsequently lyophilized (Labconco, MO, USA). Lyophilized samples were stored at 4 °C until their analysis. 3 ml of methanol and water (50:50) containing 50

mM sodium ascorbate were added to 0.1 g of lyophilized sample. Then, solutions were stirred at 200 rpm for 10 min in darkness, and extracted according to the method proposed by Cejudo-Bastante et al. [7]. All analysis were carried out in triplicate.

HPLC-RID analysis of sugars

Monosaccharides and disaccharides were analysed by HPLC in an Agilent 1100 chromatography system equipped with a quaternary pump, a 1260 RID detector, an automatic injector and Chemstation software (Palo Alto, CA, USA). Prior to direct injection, the samples were filtered through a 0.45 µm nylon filter. Individual sugars were isocratically separated using 5 mM sulphuric acid as eluent, at a flow rate of 0.5 ml/min at 30 ºC. The injection volume was 20 µL and a HI-Plex H column (300 x 7.7 mm, 8 µm particle size) was used. The identification of each chromatographic peak was assigned by their refraction characteristics and retention times of commercial standards. All analysis were performed in triplicate.

HPLC-DAD analysis of betalains

Separation, identification, and semiquantification of betalains were carried out in an Agilent 1200 chromatographic system equipped with a quaternary pump, an UV-Vis diode-array detector, an automatic injector, and ChemStation software (Palo Alto, USA). Zorbax C18 column (250 x 4.6 mm, 5 µm particle size) were used to separation of betalains. Betacyanins and betaxanthins were monitored at 535 and 482 nm, respectively [7]. Semi-quantification was done based on the mean areas of individual betalains. All analysis were carried out in triplicate.

Colour measurements

A Hewlett-Packard UV-Vis HP8454 spectrophotometer (Palo Alto, CA) was used to perform colour measurements. The entire visible spectrum (380-770 nm) was collected at constant intervals ($\Delta\lambda$=2nm) using distilled water as reference and 2 mm path length glass cells. Colorimetric characteristics of red beet extracts were determined by tristimulus colorimetry and expressed in the CIELAB parameters (L^*, a^*, b^*, C^*_{ab}, h_{ab}) with the original Software Cromalab® [8], following the Commission Internationale de L'Eclairage's recommendations (CIE, 2004): the CIE 1964 10º Standard Observer and the Standard Illuminant D65, corresponding to the natural daylight. Euclidean distance between two points in the three-dimensional CIELAB space by L^*, a^* and b^* were used for calculating colour differences (ΔE^*_{ab}): $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

Statistical analysis

All statistical analyses (univariate analyses of variance, Tukey *post hoc* test and ANOVA, linear regressions analysis and linear discriminant analysis (LDA), $p < 0.05$) were carried out using Statistica v.8.0 software [9].

RESULTS AND DISCUSSION

The ripening evolution of the red beet samples was carried out by the measurement of the weigh and calibre, establishing three groups or ripening stages according to the weigh-to-calibre ratio (W/Cal) (Online Resource 1). The first group (n = 12) was formed by beetroots in the stage I (weigh < 50 g and calibre < 4.25 cm), followed by stage II (n = 42) (weigh and calibre between 50-150 g and 4.25-6.5 cm, respectively), being the last aggrupation (n = 28) defined as stage III (weigh > 150 g and calibre between 6.5-9 cm).

The weigh and calibre attributes have been assigned as markers of ripening for being an easily-measured and non-destructive index of harvest maturity. Anyway, with the aim of

assuring the correct classification of the ripening stages based on the weigh and calibre, analyses of texture and sugars were also developed [10, 11]. Subsequent correlation analyses between these parameters and W/Cal were underwent in order to corroborate the weigh-to-caliber ratio as a good marker of ripening.

Instrumental texture analysis

According to the mean scores of slopes (stage I, 44.05 ± 7.09; stage II, 65.93 ± 14.63, stage III, 80.92 ± 13.62), it could be possible to affirm that the firmness of the red beets in the stage III was significantly ($p < 0.05$) higher in comparison with that the rest of the stages (Online Resource 2). That fact states that it was necessary to exert more force for achieving the same degree of deformation, i.e, samples with high W/Cal displayed higher resistance to the advance of the probe. As a result, notwithstanding the behaviour of fruits and vegetables [12, 13], the firmness of red beets increased during ripening. To gain an insight into the possible relationship between them, simple correlation analysis (Pearson coefficient) was performed, considering firmness and weigh-to-calibre ratio as dependent and independent variable, respectively. As a result, a positive and significant ($p < 0.000$) correlation between them was observed (R coefficient = 0.8712), confirming the strong relationship between both parameters (Firmness = 36.92 + 1.56 · W/Cal).

Soluble sugars content

Three monosaccharides and one disaccharide were identified in red beetroots by HPLC, whose content, according to the growth of the tuber, is summarized in Table 1. The monosaccharides identified were glucose, fructose and arabinose, and sucrose as disaccharide. It is noteworthy that arabinose has been scarcely previously reported in red beets [14, 15]. Sucrose was the most abundant soluble sugar quantified in all the beetroots regardless of the stage of ripening (Table 1), in agreement with other authors [16, 4].

Table 1. Mean values and standard deviations of sugars (g/100 g dry weigh) of beetroot extracts according to the tuber ripening stage.

	Stage I	Stage II	Stage III
Sucrose	20.32 ± 21.44 [a]	44.14 ± 15.20 [bc]	44.13 ± 9.56 [b]
Glucose	0.56 ± 0.51 [a]	0.87 ± 0.62 [c]	0.56 ± 0.54 [ab]
Fructose	0.21 ± 0.41 [ab]	0.28 ± 0.62 [bc]	0.04 ± 0.14 [a]
Arabinose	0.13 ± 0.31 [a]	14.41 ± 17.11 [b]	37.68 ± 6.38 [c]
Sum of sugars	21.21 ± 21.38 [a]	59.70 ± 20.69 [b]	82.41 ± 13.84 [c]

Different letters in the same row indicate significant differences according to Tukey test ($p < 0.05$).

Not all individual sugars evolved in the same way during ripening. Thus, the content of sucrose, glucose and fructose reached the maximum and significative ($p < 0.05$) level when red beets reached the stage II of ripening, maintaining and dropping their content afterwards, respectively. An exception was arabinose, whose content significantly ($p < 0.05$) continued to increase as the tuber growth did. That fact could be explained because, according to Zhuang et al. [17], arabinose is one of the most dynamic glycosylic residues during ripening, so the enzymatic activity may release arabinose units from polysaccharides of the tuber cell wall. As a result, sucrose went from representing virtually 100% of the total content of sugars in the stage I to only 50% in the stage III (together with arabinose).

As can be seen, the amount of the total sugars, especially arabinose, increased in parallel to the growth of the red beets, similarly to the behaviour of the firmness. In order to check this relationship, a simple correlation analysis (Person coefficient) between total sugars and W/Cal was performed, being considered as dependent and independent variables, respectively. Significant values of R coefficient ($R = 0.67$, $p < 0.000$) were found, establishing the following model: Total sugars = $20.62 + 2.24 \cdot$ W/Cal. Better scores of R coefficient were obtained when arabinose was correlated with W/Cal ($R = 0.72$, $p < 0.000$; arabinose = $-9.58 + 1.62 \cdot$ W/Cal).

Betalain content

A large extent of individual betalains were identified and semiquantified in red beet extracts, belonged to betacyanins and betaxanthins (Table 2).

Table 2. Mean areas and standard deviations of betalains of beetroot extracts according to the tuber ripening stage.

	Stage I		Stage II		Stage III	
Betacyanins						
Betanin	8303.82 ± 4082.41	a	14123.33 ± 3243.26	c	12252.56 ± 3078.07	b
Isobetanin	4239.44 ± 2314.67	c	1142.00 ± 570.27	a	1415.91 ± 903.22	ab
Gomphrenin I	113.62 ± 85.47	ab	82.23 ± 98.56	a	305.36 ± 236.89	c
Sum of betacyanins	12656.89 ± 3516.23	a	15347.56 ± 3501.09	bc	13973.83 ± 3565.01	ab
Betaxanthins						
Histidine-betaxanthin (muscaarin)	8.35 ± 14.81	a	18.58 ± 17.66	c	8.36 ± 10.99	ab
Glutamine-betaxanthin (vulgaxanthin I)	1968.19 ± 2319.67	a	3881.40 ± 2303.18	c	2216.58 ± 1441.12	ab
Aminobutyric acid-betaxanthin	39.83 ± 24.24	ab	70.13 ± 46.69	c	32.48 ± 32.72	a
Proline-betaxanthin (indicaxanthin)	61.54 ± 36.46	ac	49.05 ± 23.88	a	31.66 ± 13.99	b
Isoleucine-betaxanthin	24.38 ± 11.15	bc	12.19 ± 13.17	b	3.78 ± 15.00	a
Leucine-betaxanthin (vulgaxanthin IV)	148.71 ± 100.98	c	111.87 ± 61.34	ab	96.31 ± 33.81	a
Phenylalanine-betaxanthin	140.01 ± 72.77	c	93.99 ± 65.82	b	67.30 ± 30.68	a
Sum of betaxanthin	2384.81 ± 2470.45	a	4237.21 ± 2384.28	c	2456.47 ± 1488.10	ab
Sum of total betalains	15041.70 ± 5221.19	a	19584.77 ± 4850.91	c	16430.30 ± 4482.23	ab

Different letters in the same row indicate significant differences according to Tukey test ($p < 0.05$).

Among betacyanins, betanin and its isomer isobetanin, and gomphrenin were accurately identified. Betanin and isobetanin are well-known betacyanins present in red beetroots [18] and gomphrenin I in different fruits and flowers [19, 20]. However, to the best of our knowledge, this is the first report identifying gomphrenin I in red beets. Besides, an in-depth identification of betaxanthins were carried out, identifying the well-described in red beets vulgaxanthin I (glutamine-betaxanthin) and other betaxanthins derived from histidine, aminobutyric acid, proline, isoleucine, leucine and phenylalanine, some of them scarcely reported in this feedstock. Betanin was, by far, the major betalain found in all red beetroots, followed by isobetanin and vulgaxanthin I, and representing around 95% of the total betalains [21].

In general, betalains reached the highest amounts when beetroots underwent the ripening stage II, being significantly ($p < 0.05$) different from those beets at the stages I and III (Table 2). This fact could suggest that the synthesis of betalains was dependent on the growth of the beetroots, but until reaching a limit, because red beets in the stage III did not involve higher values of betalains. Therefore, the content of pigments increased according the ripening stage, being the stage II-beets the most abundant in betalains, dropping sharply and significantly ($p < 0.05$) when maturity continued (stage III).

Taking into account the individual betalains, it could be observed that betanin and the majority of betaxanthins (muscaarin, vulgaxanthin I, and aminobutyric acid-betaxanthin) evolved in the same way, exhibiting the medium-ripened red beets significantly ($p < 0.05$) higher values. The rest of betaxanthins (indicaxanthin, vulgaxanthin IV and isoleucine- and phenylalanine-betaxanthin) and isobetanin showed significantly ($p < 0.05$) higher quantities in the early stages of maturation (stage I). The fact that the content of the majority of betalains was significantly ($p < 0.05$) higher in red beets with medium stage of ripening could be of special interest for the industry, taking into account that the usual harvest moment could not be the optimum from a pigment amount point of view.

Colorimetric characteristics

CIELAB colour parameters (L^*, a^*, b^*, C^*_{ab}, h_{ab}) were evaluated in all red beets and computed according to the ripening stage (Table 3). Although colour changes during ripening have been also reported in other raw materials such as banana [5] and tomatoes [12], this is the first attempt to study the colour evolution over ripening in red beets.

It could be observed that hue (h_{ab}) of red beets significantly ($p < 0.05$) increased during the growth, reaching the maximum value in the medium stage. Lightness (L^*) evolved contrarily, with significantly ($p < 0.05$) higher values in the stage I. Consequently, beets

in the stages I and III presented a brighter purple tonality than those in the stage II, which had a more reddish tonality and lower lightness. That fact could be related to the higher content of betaxanthins found in the stage II. Therefore, it could be affirmed that the colour of the red beetroots is strongly dependent on the ripening stage of the root.

Table 3. Mean values and standard deviations of colour parameters (L^*, C^*_{ab}, h_{ab}) of beetroot extracts according to the tuber ripening stage.

	Stage I	Stage II	Stage III
L^*	59.79 ± 7.92 ^{bc}	54.53 ± 6.90 ^{a}	57.38 ± 5.54 ^{b}
C^*_{ab}	67.32 ± 11.97 ^{a}	69.33 ± 8.01 ^{a}	69.58 ± 6.35 ^{a}
h_{ab}	2.82 ± 10.42 ^{a}	12.94 ± 7.39 ^{c}	6.33 ± 8.46 ^{ab}

Different letters in the same row indicate significant differences according to Tukey test ($p < 0.05$).

In an attempt to evidence the colorimetric differences among the maturity stage of the roots and highlight its eventual visual differentiation, colour differences (ΔE^*_{ab}) among pairs of samples according to the ripening stage were calculated. Taking into account that approximately ΔE^*_{ab} of up to 3 CIELAB units indicates colour differences appreciable to the human eyes [22], it was confirmed that visually colour differences ($\Delta E^*_{ab} > 3$) were achieved among pair of samples (data not shown). Among them, the most perceptible difference was found when red beets at the ripening stage II were implicated, above all between stages I and II ($\Delta E^*_{ab} = 13.07$), and to a lesser extent between stages I and III ($\Delta E^*_{ab} = 5.29$).

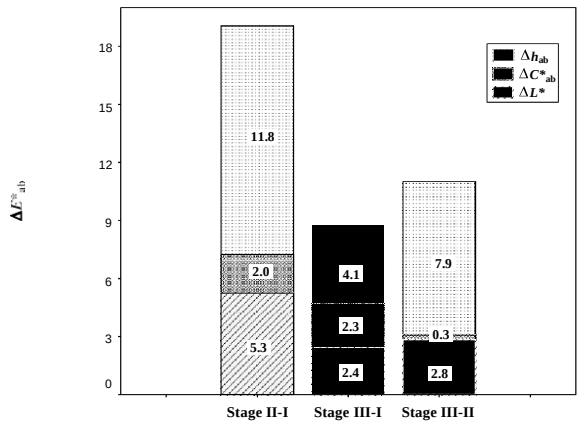

Fig. 1.

In order to compare the effect of maturity, lightness, chroma and hue absolute differences (ΔL^*, ΔC^*_{ab}, Δh_{ab}) among pair of samples according to the ripening stage were calculated (Fig. 1). As can be observed, visually appreciable differences were mainly qualitative, especially higher when ripening stage II was involved. Moreover, colour difference among stages I and III was also mainly due to hue variations. On the other hand, the quantitative colour changes (ΔC^*_{ab} and ΔL^*) were less marked.

Linear Discriminant Analysis (LDA)

Taking into account the appreciable relationship between betalains and colour characteristics, a multivariate analysis (linear discriminant analysis, LDA) was carried out. A forward stepwise LDA was performed to differentiate the three sample groups (stage I, II and III), based on individual betalains and the angular coordinates of the CIELAB space (L^*, C^*_{ab} and h_{ab}). This statistical analysis was performed according to the Wilks' λ statistic to choose the descriptors that best distinguished the different red beets. A F statistic is computed from the partial λ values, leading to a p level. The minimum p level values corresponds to the maximum discriminatory power. According to p-levels and F-values, betanin, isobetanin, gomphrenin and aminobutyric acid-betaxanthin, vulgaxanthin I and IV were the variables included in the discriminant functions. Furthermore, the colorimetric characteristic of L^* and h_{ab} was also considered as discriminant function. Therefore, those variables reached to discriminate samples by ripening stage with high levels of significance ($p < 0.05$). According to the classification functions, the three groups were clearly differentiated (Fig. 2). 87.1% of the samples were correctly assigned, so a successful classification of red beets according the ripening stage has been demonstrated. Moreover, the percentage of prediction was 78.3% for stage I, 91.7% for stage II and 83.9% for stage III.

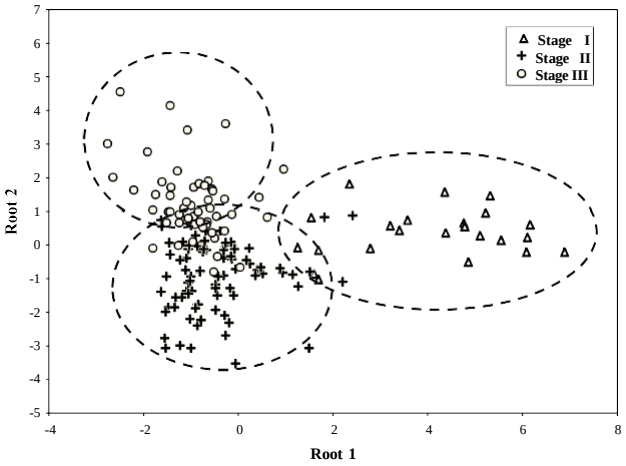

Fig. 2.

CONCLUSIONS

According to the forgoing discussion, it might be affirmed that growth influenced on the chemical composition and colour characteristics of red beets. The fact that the tonality of the red beets depends on the maturity stage could represent a useful tool for industry in order to develop natural colorants *á la carte*. The medium ripening stage is the optimum based on the betalain content, providing red beets with a more reddish tonality and lower lightness. Moreover, this is the first attempt to study the evolution during ripening of the colour characteristics and betalain profile of red beets, fact that could be a greater step forward in agro-alimentary industry, cosmetics, and drug industries, among others.

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Conflicts of interest

Authors declare no conflicts of interest.

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FIGURE CAPTIONS

Fig. 1 Plot of colour differences (ΔE^*_{ab}) with relative contribution of lightness, chroma and hue (%$\Delta^2 L$, % $\Delta^2 C$, % $\Delta^2 H$) according to the ripening stage

Fig. 2 Scatterplot of the canonical variate obtained by LDA according to the ripening stage

Conflict of Interest

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Prof. Octavio Paredes-López
Editor-in-Chief of Plant Foods for Human Nutrition

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Dear Editor,

Authors declare no conflict of interest regarding the manuscript entitled "**Implications of the red beet ripening on the colour and betalain composition relationships"** by Sandra Montes-Lora, Francisco J. Rodríguez-Pulido, María Jesús Cejudo-Bastante, and Francisco J. Heredia.

Yours faithfully,

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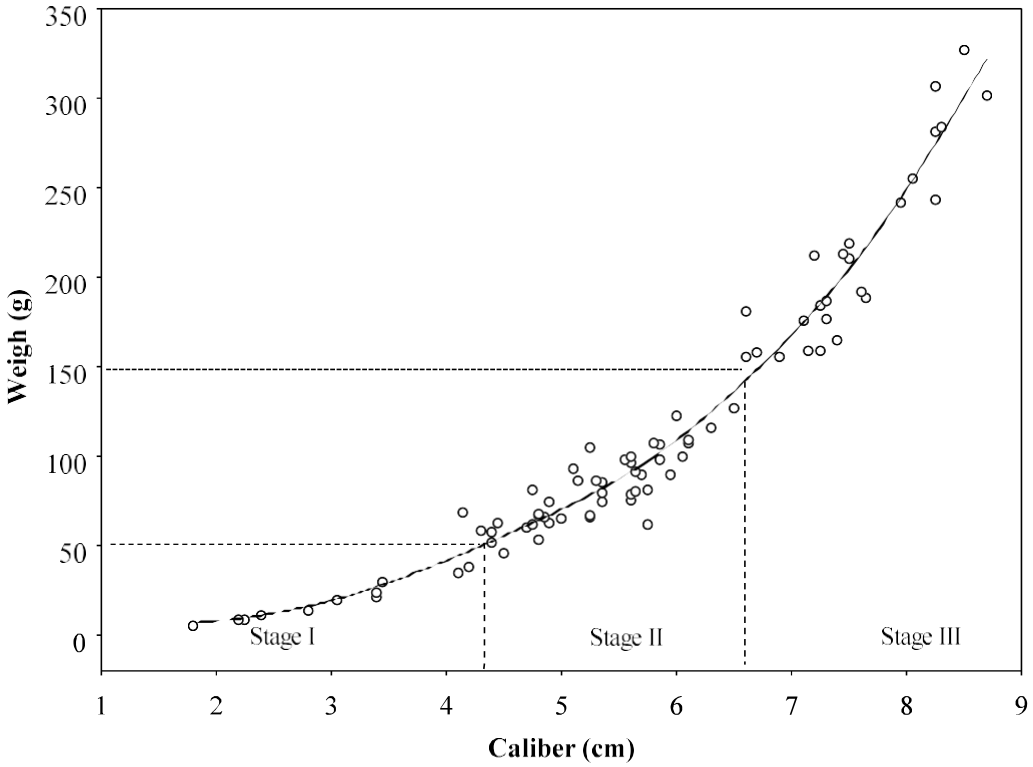
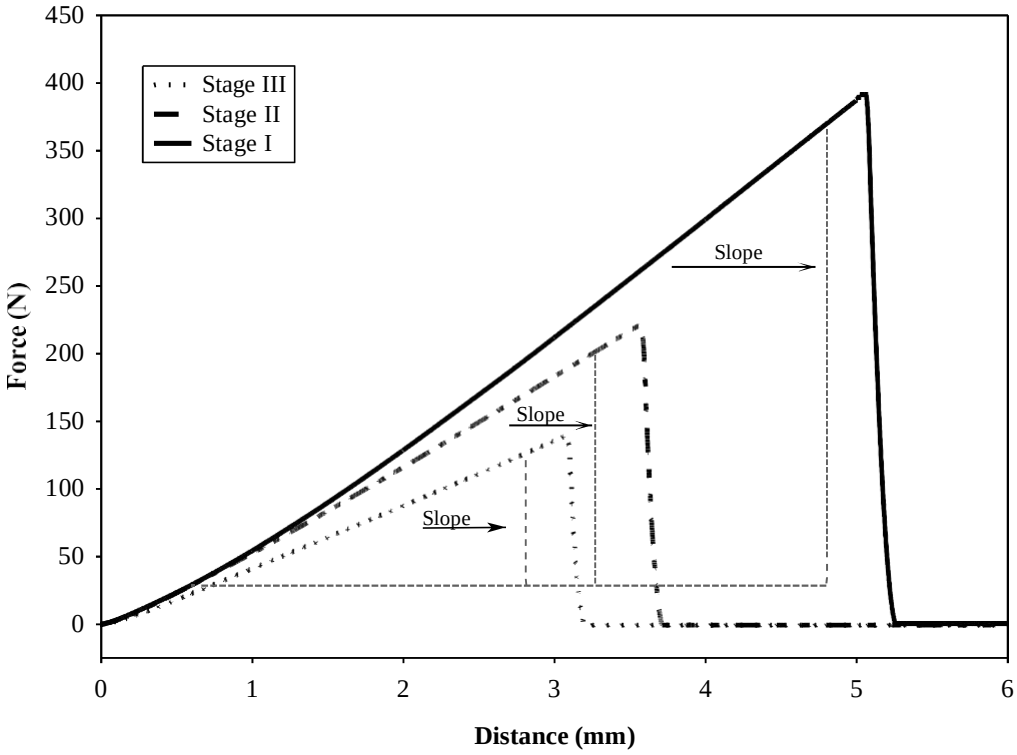


Online Resource 1. Classification of red beets during ripening according to weigh and calibre



Online Resource 2. Force-deformation curves and plot of the slopes according to the ripening stage

Table 1. Mean values and standard deviations of sugars (g/100 g dry weigh) of beetroot extracts according to the tuber ripening stage.

	Stage I		Stage II		Stage III	
Sucrose	20.32 ± 21.44	a	44.14 ± 15.20	bc	44.13 ± 9.56	b
Glucose	0.56 ± 0.51	a	0.87 ± 0.62	c	0.56 ± 0.54	ab
Fructose	0.21 ± 0.41	ab	0.28 ± 0.62	bc	0.04 ± 0.14	a
Arabinose	0.13 ± 0.31	a	14.41 ± 17.11	b	37.68 ± 6.38	c
Sum of sugars	21.21 ± 21.38	a	59.70 ± 20.69	b	82.41 ± 13.84	c

Different letters in the same row indicate significant differences according to Tukey test ($p < 0.05$).

Table 2. Mean areas and standard deviations of betalains of beetroot extracts according to the tuber ripening stage.

	Stage I		Stage II		Stage III	
Betacyanins						
Betanin	8303.82 ± 4082.41	a	14123.33 ± 3243.26	c	12252.56 ± 3078.07	b
Isobetanin	4239.44 ± 2314.67	c	1142.00 ± 570.27	a	1415.91 ± 903.22	ab
Gomprhenin I	113.62 ± 85.47	ab	82.23 ± 98.56	a	305.36 ± 236.89	c
Sum of betacyanins	12656.89 ± 3516.23	a	15347.56 ± 3501.09	bc	13973.83 ± 3565.01	ab
Betaxanthins						
Histidine-betaxanthin (muscaarin)	8.35 ± 14.81	a	18.58 ± 17.66	c	8.36 ± 10.99	ab
Glutamine-betaxanthin (vulgaxanthin I)	1968.19 ± 2319.67	a	3881.40 ± 2303.18	c	2216.58 ± 1441.12	ab
Aminobutyric acid-betaxanthin	39.83 ± 24.24	ab	70.13 ± 46.69	c	32.48 ± 32.72	a
Proline-betaxanthin (indicaxanthin)	61.54 ± 36.46	ac	49.05 ± 23.88	a	31.66 ± 13.99	b
Isoleucine-betaxanthin	24.38 ± 11.15	bc	12.19 ± 13.17	b	3.78 ± 15.00	a
Leucine-betaxanthin (vulgaxanthin IV)	148.71 ± 100.98	c	111.87 ± 61.34	ab	96.31 ± 33.81	a
Phenylalanine-betaxanthin	140.01 ± 72.77	c	93.99 ± 65.82	b	67.30 ± 30.68	a
Sum of betaxanthin	2384.81 ± 2470.45	a	4237.21 ± 2384.28	c	2456.47 ± 1488.10	ab
Sum of total betalains	15041.70 ± 5221.19	a	19584.77 ± 4850.91	c	16430.30 ± 4482.23	ab

Different letters in the same row indicate significant differences according to Tukey test ($p < 0.05$).

Table 3. Mean values and standard deviations of colour parameters (L^*, C^*_{ab}, h_{ab}) of beetroot extracts according to the tuber ripening stage.

	Stage I	Stage II	Stage III
L^*	59.79 ± 7.92 bc	54.53 ± 6.90 a	57.38 ± 5.54 b
C^*_{ab}	67.32 ± 11.97 a	69.33 ± 8.01 a	69.58 ± 6.35 a
h_{ab}	2.82 ± 10.42 a	12.94 ± 7.39 c	6.33 ± 8.46 ab

Different letters in the same row indicate significant differences according to Tukey test ($p < 0.05$).

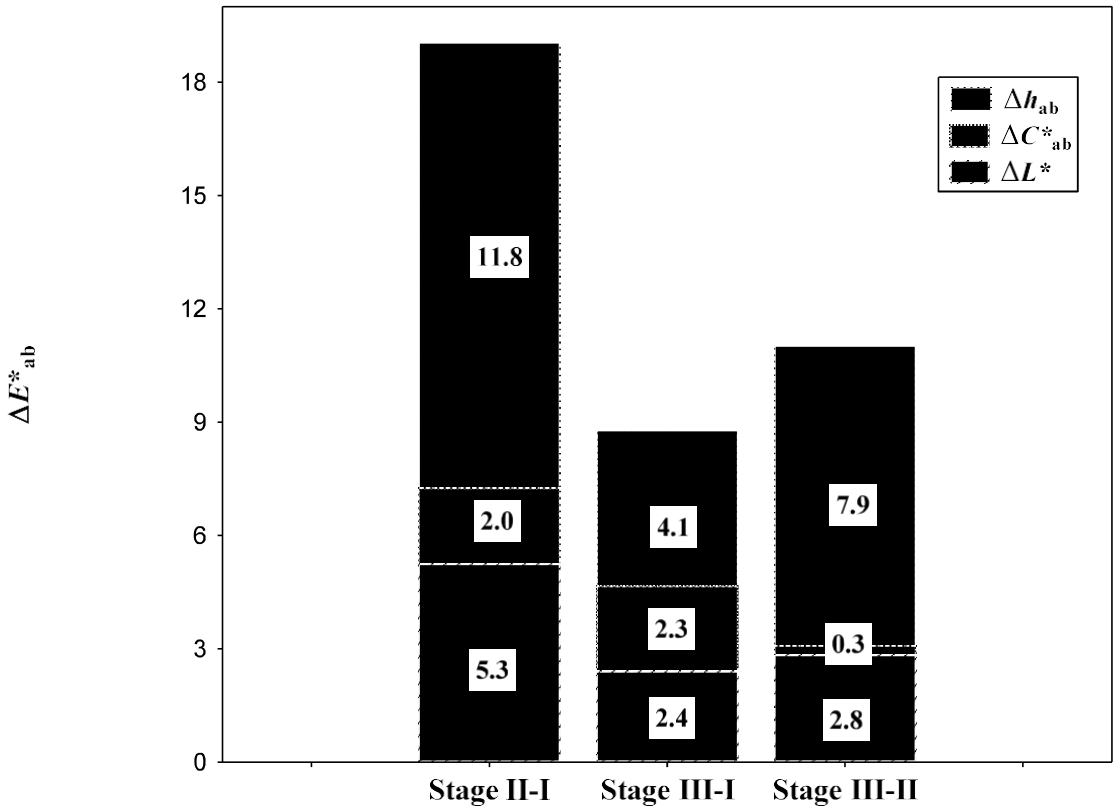


Fig. 1.

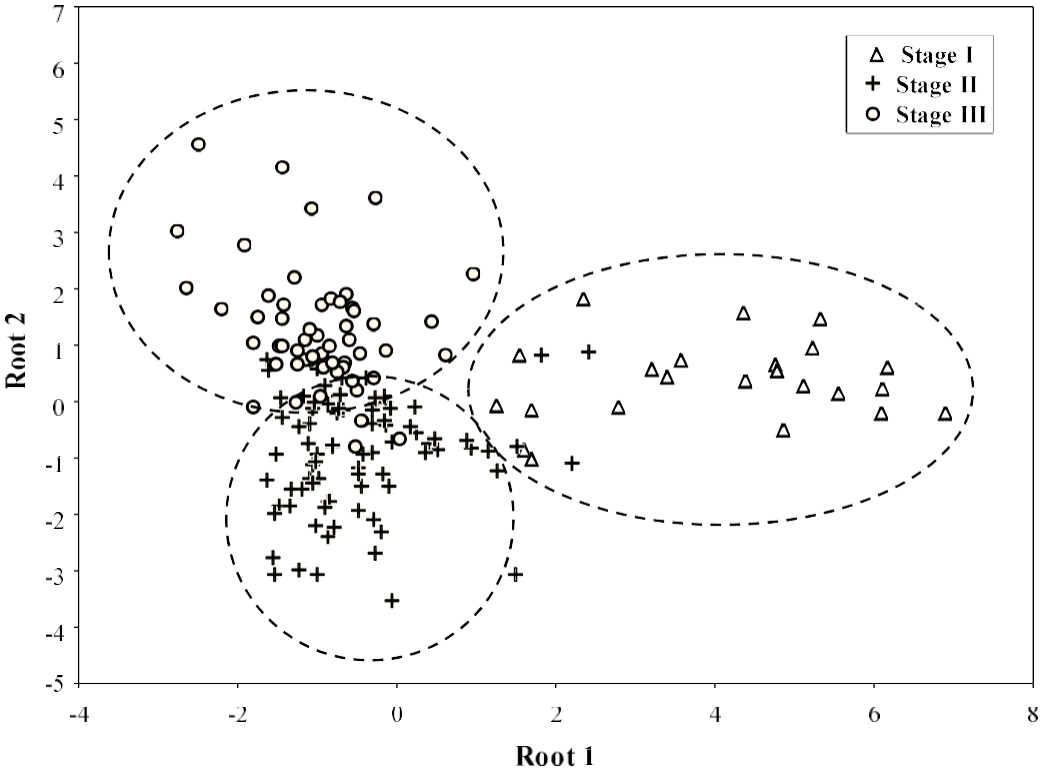

Stage I
Stage II
Stage III
Root 2
Root 1
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-4
-2
0
2
4
6
8

Fig. 2.