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ORIGINAL ARTICLE

Relationship between color and betalain content in different thermally treated beetroot products

Virginia Prieto-Santiago[1] · M. Mar Cavia[1] · Sara R. Alonso-Torre[1] · Celia Carrillo[1]

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Abstract Several health benefits are currently attributed to natural pigments that give fruit and vegetables their inherently colorful properties. Color measurements might therefore serve as quick indicators of the potential health-promoting properties of such foods. Nevertheless, the relationship between color and pigment content depends on the type of matrix and pigment, as well as the factors affecting their interaction, which calls for further investigation. Hence, the aim of the present study is to investigate the relationship between color parameters and betalain content in three commonly consumed beetroot products (beetroot juice, beetroot puree and whole beetroot), subjected to thermal treatment. Our results showed a negative correlation between the total betalain content and the color parameters L*, a*, b*, chroma, and hue angle in beetroot juice, beetroot puree and whole beetroot. Two chromatic parameters, a* and chroma, are proposed as the best descriptors for the betalain concentrations of these products. Likewise, the tristimulus L/ab combination for the juice is also suggested as a good descriptor. Our findings highlighted that the relationship between color and total betalain content depended on the beetroot product under assessment, with the strongest correlations found for the juice. Squeezed beetroot was therefore suggested as an alternative to improve this relationship in more complex matrices such as whole cooked beetroots. Useful information from color determination sheds light on the relationship between color and betalain pigments in beetroot, suggesting that color determination could be used as an indicator of betalain content.

Keywords Betalains · Color · Beetroot products · Correlation

Introduction

Phytochemicals and their bioactive properties have been widely studied during the last decade and much of that research has examined the content and the kinds of bioactive compounds in vegetables as a marker of the health-related benefits associated with their consumption (Rodriguez-Casado 2016).

Extraction and subsequent determination of the bioactive compounds are the procedures usually applied to assess the bioactive value of a food product. Extraction procedures usually involve the disintegration of the food matrix together with the use of solvents with different polarities, followed by agitation, sonication or centrifugation steps, which implies costly and time-consuming procedures that are not always eco-friendly (Azmir et al. 2013). Interest is therefore increasing in establishing the bioactive value of food through quick, easy and non-destructive approaches. Since many of the bioactive compounds present in vegetables are natural pigments, color determination may be considered as a good indicator of the pigment content, which in turn is often an index related to the potential health-promoting properties of those sorts of food (Leong et al. 2018).

The color of an object can be described by several color coordinate systems, which differ with regard to the symmetry of the color space and in the coordinate system used to define points within that space (Pathare et al. 2013). The

✉ Celia Carrillo
ccarrillo@ubu.es

[1] Nutrición y Bromatología, Facultad de Ciencias, Universidad de Burgos, 09001 Burgos, Spain

most popular systems are RGB (red, green and blue), Hunter L a b, Commission Internationale de l'Eclairage's (CIE) L*a*b*, CIE XYZ, CIE L*u*v*, CIE Yxy, and CIE LCH; CIELAB color scales are the most widely used system for color quantification in the food industry (Pathare et al. 2013).

There are many intense food colors, among which the bright purple-red color of beetroot is highly distinctive. Red beetroot (*Beta vulgaris*) has a large and fleshy root that is edible. It belongs to the Chenopodiaceae family and is rich in polyphenols and water-soluble nitrogen pigments known as betalains, that give the color to this vegetable. To date, the structures of 75 different betalains have been detailed. Betalain pigments are composed of a nitrogenous core structure of betalamic acid [4-(2-oxoethylidene)-1,2,3,4-tetrahydropyridine-2,6-dicarboxylic acid]. Betalamic acid can either condense with imino compounds (cyclo-DOPA and/or its glucosyl derivatives) to form violet betacyanins (e.g. betanin), or with amines and their derivatives to form yellow betaxanthins (e.g. indicaxanthin) (Azeredo 2009).

Several health-related biological activities have been associated with betalain-rich foods, such as free radical scavenging, inhibition of DNA-damage, prevention of lipid peroxidation, gene regulation, antiproliferative, anti-inflammatory and antimicrobial activities (Esatbeyoglu et al. 2015; Gengatharan et al. 2015; Gandía-Herrero et al. 2016; Carrillo et al. 2017, 2019). In vivo studies suggest that supplementation with betalains could be a promising alternative to inflammation-, dyslipidemia- and oxidative stress-related diseases such as hypertension, stenosis of the arteries, atherosclerosis and cancer. Moreover, beetroot betalains could improve exercise performance independently of any physiological effects of nitrate (Lechner and Stoner 2019; Rahimi et al. 2019a, b, c; Swartz et al. 2019). Therefore, the retention of pigments in beetroot is important not only for its visual appeal but also as a guarantee of its potential health benefits (Chandran et al. 2014).

Beetroot is usually consumed as salads, purees or soups and as pasteurized juices. It shows that, the vast majority of beetroot products currently consumed are submitted to thermal treatments. Betalains are known to be very sensitive to several factors including low pH, high-water activity and elevated temperatures (Herbach et al. 2006b). As a consequence of heat, several betalain degradation reactions occur, such as hydrolysis, dehydrogenation and decarboxylation (Herbach et al. 2005), resulting in a gradual reduction of reddishness and the eventual appearance of a light yellowish-brown color (Herbach et al. 2004a).

In this respect, the effect of thermal treatment on anthocyanin and carotenoids in relation to the visual color in a range of fruit and vegetable purees has previously been described (Ahmed et al. 2002, 2004), although very few studies have focused on betalains. Therefore, the aim of the present work is to investigate the relationship between color parameters and betalain content in three beetroot products (beetroot juice, beetroot puree and a whole beetroot) subjected to a thermal treatment, in order to establish a quick way to monitor the betalain content in commonly consumed beetroot products.

Materials and methods

Plant material

Fresh beetroots (*Beta vulgaris, cv. Monti*) were used in the experiment within twenty-four hours after purchasing. Three beetroot products were assessed, in order to elucidate the relationship between color and betalain content in thermally treated beetroot: beetroot juice, beetroot puree, and whole beetroots.

Whole beetroots were washed and peeled before use in subsequent experiments. Beetroot puree was prepared by grinding peeled beetroot pieces in a domestic blender. The puree was packed into glass bottles and immediately subjected to thermal treatment. Beetroot juice was extracted from whole peeled beetroot, cut into pieces, and passed through a domestic juicer. The extracted juice was filtered through a cheesecloth to remove the remaining pomace, poured into glass bottles, and immediately processed.

Thermal treatment

Beetroot juice, beetroot puree and whole peeled beetroots were treated in an autoclave (120 °C) for 10, 20, 30, 40, 50, and 60 min. The purpose of this thermal treatment was to obtain beetroot products with different betalain contents, which was achieved by applying a constant temperature over incrementally lengthier treatment times. In this way, a range of thermally treated beetroot products over different lengths of time were subjected to pigment quantification and color determination, together with the subsequent correlation and regression analyses. The samples were cooled before color and betalain determination.

Betalain extraction and determination

The extraction of betalains was performed as described elsewhere (Ravichandran et al. 2013). Briefly, 0.5 g of grinded fresh sampled beetroot was placed in a tube to which 5 ml of ethanol–water solution (50:50 v/v) was added. The tube was thoroughly shaken (RT, 15 min), centrifuged (5500 rpm, 10 min, 4 °C), and the supernatant recovered. This procedure was repeated twice, and the

three extracts were combined and stored at − 40 °C until analysis.

The betaxanthin and betacyanin contents of the extracts were spectrophotometrically determined following the method described elsewhere (Nilsson 1970). The extract containing betalains was diluted with a phosphate buffer (pH 6.5) until reaching an absorbance of 0.4–0.5 at 538 nm. The samples were measured at 538 nm, 476 nm, and 600 nm (UV-6300PC spectrophotometer). The measurement at 538 nm yielded a quantification of the betacyanins, while the betaxanthins were quantified at 476 nm. The reading at 600 nm was used to correct the absorbance of the impurities. The results were expressed in mg of betacyanin (in terms of betanin) per kg and mg of betaxanthin (in terms of vulgaxanthin) per kg (mg/kg). The total betalain content was calculated as the sum of both betacyanins and betaxanthins and the results were expressed as mg of total betalains (TB) per kg.

Color measurement

A color evaluation was performed with a Hunter Lab colorimeter (Hunter Lab Color Flex EZ 45/0° color spectrophotometer, USA). The instrument was calibrated with a standard black and white ceramic tile before the measurements. The results were expressed in accordance with the CIELAB system with a reference to illuminate D65 and with a visual angle of 10°. Values of L*, a*, and b* were measured to describe a three-dimensional color space and interpreted as follows: L* indicates lightness read from 0 (completely opaque or "black") to 100 (completely transparent or "white"); a positive a* value indicates redness (-a* is greenness) and a positive b* value indicates yellowness (-b* is blueness) on the hue-circle (Pathare et al. 2013).

Two derived color parameters, the hue angle (h_{ab} = arctan(b°/a°)) and the chroma value ($C^* = \sqrt{a^{*2} + b^{*2}}$) were calculated. The hue angle (h_{ab}) expresses the color nuance (Pathare et al. 2013) and the values are defined as follows: red–purple: 0, yellow: 90, bluish-green: 180, and blue: 270 (Pathare et al. 2013). The chroma is a measure of chromaticity (C*), which denotes the purity or saturation of the color (Pathare et al. 2013). Chroma (C*), the quantitative attribute of colorfulness, is used to determine the degree of difference of a hue in comparison to a grey color with the same lightness: the higher the chroma values, the higher the perceived color intensity of the samples to the naked eye (Pathare et al. 2013).

The data of each measurement were constituted of the averaged quadruplicate measures on equidistant points of each sample. The measures were taken on the surface of whole beetroots and on a standardized glass recipient in the case of puree and juice.

Data analysis

All analyses were carried out in triplicate (n = 3) and the results expressed as mean values ± standard deviation. One-way analysis of variance (ANOVA), the LSD test, and the Pearson correlation and linear regression analysis were computed with the Statgraphics Centurion XVI software package (StatPoint Technologies, Inc., USA) at a minimum significance level of $p < 0.05$.

Results and discussion

Analysis of beetroot betalains

The betalain content of beetroot juice, beetroot puree and whole beetroot subjected to incrementally lengthier thermal treatment times is presented in Table 1.

The three beetroot products under study (whole beetroot, beetroot puree and beetroot juice) showed a decreased amount of betalains as the treatment times increased (Table 1). Temperature was the most important factor on betalain stability in food processing (Azeredo 2009). Our findings were consistent with previous studies, demonstrating that red beet subjected to thermal treatments (blanching, boiling, drying or roasting) (Ravichandran et al. 2013; Paciulli et al. 2016; Carrillo et al. 2017) lost 6–81% of their betalain content, depending on the treatment time and the applied temperature.

It is important to highlight that betacyanins and betaxanthins showed different sensitivities towards treatment (Table 1). Regardless of the treatment, betacyanins were the predominant group of compounds, although the betacyanin/betaxanthin ratio was not stable throughout the treatment. The highest betacyanin/betaxanthin ratios were observed after 10, 20, and 30 min of treatment for the juice, the whole beetroot, and the puree, respectively. The lower ratios corresponded to lengthier treatments in the three products under assessment. Contradictory findings regarding the thermal stability of both families of betalains, in beetroot, have been reported. According to some authors, the structural stability of betacyanin is greater than the structural stability of betaxanthin (Singer and von Elbe 1980; Herbach et al. 2004a) and betaxanthin pigments usually degrade faster than betacyanin pigments (Singer and von Elbe 1980; Ravichandran et al. 2013). However, according to other authors, betaxanthins are more stable than betacyanins (Herbach et al. 2006c). Based on our findings, such contradictory results may be related to differences in the treatment conditions applied, since a different trend was observed during the short and long treatments. Although betacyanins are structurally more stable, which may explain their higher stability at the

Table 1 Betalain content (mg/kg) of thermally treated beetroot juice (BJ), beetroot puree (BP) and whole beetroot (WB)

Sample	Treatment (min)	Betacyanins (mg/kg)	Betaxanthins (mg/kg)	Total betalains (mg/kg)
BJ	10	543.6 ± 5.3*a*	292.8 ± 8.2*a*	836.3 ± 8.5*a*
	20	416.2 ± 7.7*b*	232.4 ± 8.8*b*	648.6 ± 9.2*b*
	30	350.5 ± 7.3*c*	196.8 ± 3.1*c*	547.2 ± 5.2*c*
	40	219.3 ± 0.5*d*	120.2 ± 3.5*d*	339.5 ± 4.0*d*
	50	145.0 ± 0.7*e*	84.1 ± 2.2*e*	229.1 ± 2.6*e*
	60	88.0 ± 0.9*f*	59.8 ± 4.4*f*	147.8 ± 5.3*f*
BP	10	529.7 ± 9.1*a*	339.1 ± 6.9*a*	868.8 ± 15.4*a*
	20	428.7 ± 4.8*b*	230.8 ± 9.2*b*	659.5 ± 11.3*b*
	30	350.3 ± 4.3*c*	182.5 ± 2.8*c*	532.8 ± 4.8*c*
	40	228.8 ± 5.0*d*	147.9 ± 4.9*d*	376.7 ± 9.7*d*
	50	174.3 ± 3.7*e*	133.3 ± 3.5*e*	307.6 ± 7.1*e*
	60	138.2 ± 7.2*f*	136.2 ± 9.2*e*	274.4 ± 16.3*f*
WB	10	419.8 ± 5.5*a*	263.3 ± 4.7*a*	683.1 ± 3.3*a*
	20	403.3 ± 3.2*b*	210.2 ± 2.8*b*	613.5 ± 5.9*b*
	30	261.0 ± 5.3*c*	157.3 ± 1.9*c*	418.3 ± 7.1*c*
	40	203.8 ± 6.2*d*	128.0 ± 3.4*d*	331.8 ± 9.4*d*
	50	125.1 ± 8.1*e*	95.3 ± 6.5*e*	220.4 ± 14.5*e*
	60	102.7 ± 7.9*f*	75.8 ± 2.7*f*	178.5 ± 10.5*f*

Values are expressed as mean ± SD (n = 3). Different letters indicate significant differences between treatments for each sample

beginning of the treatment, they can also suffer degradative reactions as a consequence of heating. Such degradation mechanisms, which according to our findings, may begin after several minutes of treatment, have been reported to be mainly hydrolysis or cleavage (formation of betalamic acid and cyclo-DOPA 5-O-glucoside), dehydrogenation and decarboxylation (Alard et al. 1985; Huang and Elbe 1985). However, the degradation of betacyanins is not the only consequence of the thermal treatment. Betaxanthin formation from betacyanins was also observed in purple pitaya (*Hylocereus polyrhizus*) juice (Herbach et al. 2007), which might also explain the increased percentages of betaxanthins observed over lengthier treatment times. Betaxanthin formation may occur due to the condensation of free amino acids with the betalamic acid generated by betacyanin hydrolysis (Herbach et al. 2006a).

Color analysis in beetroot

The chromatic characteristics of the three beetroot products under study are shown in Table 2.

There were significant differences between the color parameters L*, a*, and b* of the juices treated at different times (Table 2). As the treatment time increased, higher values of L*, a*, b* were observed. Thus, the color difference for each parameter (ΔL*, Δa*, Δb*) was always positive. These observations indicate a color shift towards lighter and more yellow juices, as a consequence of thermal treatment, which are in agreement with previous findings for other betalain rich juices (Herbach et al. 2004a, 2007) and explainable by the aforementioned betacyanin degradation reactions. The hydrolysis reactions not only produce a decrease in tinctorial strength, but also a considerable color shift towards yellow (Herbach et al. 2006c). Betalamic acid and cyclo-DOPA 5-O-glucoside are, respectively, bright yellow and colorless. Betacyanin dehydrogenation is also of special interest in terms of the color change of betacyanin-containing solutions, as red products are converted into yellow degradation products, among which neobetanin can be considered the most determinate (Alard et al. 1985). Dehydrogenation has been also reported as the main reason for the noticeable color shift observed during the thermal treatment of red beet juice, purple pitaya juice, and a pigment purified solution (Herbach et al. 2004a, b, 2006a, b). Betacyanins with different decarboxylation levels were also identified, together with their corresponding neo-derivatives as heating degradation products of betacyanins from red beetroot juice, shifting the color towards orange/red (Wybraniec 2005), and purple pitaya extract (Wybraniec and Mizrahi 2005). Moreover, neoformation of betaxanthins may have contributed as well to the yellowishness of the samples that was observed, although the degree of contribution from each reaction cannot be established based on our data.

However, the positive color difference also found for a* (Δa*) would indicate that samples were becoming redder as the treatment time increased, which would be incongruent with the betacyanin degradation discussed above. In

Table 2 Colour parameters of thermally treated beetroot juice (BJ), beetroot puree (BP) and whole beetroot (WB)

Sample	Treatment (min)	L	a	b	C*	h_{ab}
BJ	10	0.73 ± 0.01*f*	4.03 ± 0.04*f*	0.96 ± 0.10*f*	4.14 ± 0.04*f*	13.43 ± 1.36*c*
	20	0.98 ± 0.03*e*	5.29 ± 0.06*e*	1.31 ± 0.03*e*	5.45 ± 0.05*e*	13.88 ± 0.34*bc*
	30	1.14 ± 0.03*d*	6.49 ± 0.19*d*	1.66 ± 0.06*d*	6.70 ± 0.18*d*	14.37 ± 0.74*bc*
	40	1.85 ± 0.02*c*	9.86 ± 0.03*c*	2.53 ± 0.07*c*	10.17 ± 0.02*c*	14.37 ± 0.39*bc*
	50	2.62 ± 0.03*b*	13.59 ± 0.21*b*	3.61 ± 0.18*b*	14.06 ± 0.23*b*	14.85 ± 0.62*b*
	60	3.25 ± 0.02*a*	15.47 ± 0.22*a*	4.51 ± 0.07*a*	16.11 ± 0.22*a*	16.24 ± 0.24*a*
BP	10	8.59 ± 0.12*e*	27.58 ± 0.13*c*	8.93 ± 0.11*e*	28.99 ± 0.15*e*	17.94 ± 0.13*d*
	20	8.42 ± 0.15*e*	27.36 ± 0.35*c*	8.10 ± 0.26*f*	28.53 ± 0.40*e*	16.50 ± 0.34*e*
	30	10.00 ± 0.14*d*	29.32 ± 0.15*b*	9.45 ± 0.27*d*	30.81 ± 0.22*d*	17.86 ± 0.40*d*
	40	11.36 ± 0.30*c*	29.45 ± 0.27*b*	10.77 ± 0.38*c*	31.35 ± 0.38*c*	20.08 ± 0.49*c*
	50	12.49 ± 0.17*b*	29.61 ± 0.49*b*	12.21 ± 0.30*b*	32.02 ± 0.45*b*	22.41 ± 0.65*b*
	60	14.62 ± 0.12*a*	31.70 ± 0.19*a*	15.29 ± 0.10*a*	35.19 ± 0.21*a*	25.74 ± 0.06*a*
WB	10	22.79 ± 1.52*c*	14.81 ± 2.14*c*	3.37 ± 0.66*c*	15.19 ± 2.18*c*	12.84 ± 1.93*d*
	20	22.80 ± 0.61*c*	15.69 ± 2.05*c*	3.47 ± 0.53*c*	16.08 ± 2.01*c*	12.66 ± 2.35*d*
	30	25.26 ± 1.04*b*	20.47 ± 1.49*ab*	5.97 ± 0.70*b*	21.33 ± 1.53*b*	16.26 ± 1.64*c*
	40	25.77 ± 1.45*b*	19.67 ± 1.35*b*	5.63 ± 0.44*b*	20.47 ± 1.35*b*	16.00 ± 1.23*c*
	50	30.19 ± 1.64*a*	21.52 ± 1.09*a*	8.69 ± 0.61*a*	23.22 ± 1.03*a*	22.03 ± 1.73*a*
	60	29.77 ± 1.06*a*	20.54 ± 1.02*ab*	8.92 ± 0.61*a*	22.40 ± 0.87*a*	23.52 ± 2.09*a*

Values are expressed as mean ± SD (n = 4). Different letters indicate significant differences between treatments for each sample

this regard, interpretation of any qualitative color difference between both samples, based only on the representation a*-b*, would have to be treated with caution. A sample with a higher a* value on the red–green axis is not necessarily perceived as a redder color, as hue is not only defined by a* or b* values. According to Little (1975) and McGuire (1992), hue angle and chroma parameters provide more information on the spatial distribution of colors.

In this regard, the hue angle of the juices gradually increased with lengthier treatment time (Table 2), which indicates a trend towards yellowish and less red samples, although significant differences were only observed after 60 min of thermal treatment. These results are in line with previous findings in purple pitaya juice (Herbach et al. 2007), beetroot juice (Herbach et al. 2004a) and purified betanin, phyllocactin, and hylocerenin solutions (Herbach et al. 2006a).

A significant increase in the chromaticity of the juice was observed throughout the treatment (Table 2). As higher chroma parameter values indicate an increase in the color purity of the sample, our results would appear to show that juices with a lower betalain content resulted in more intensely colored beverages. Thus, based on our findings, the a* and chroma parameters showed similar behaviors that were contrary to the expectations in beetroot juice. Previous reports also presented controversial results between instrumental and subjective/visual analysis of color in other pigmented samples. Even though a trained panel identified redder samples as the pigment concentration increased, that same trend was not reflected by the colorimeter (Eagerman et al. 1973).

Eagerman et al. (1973) described the difficulty of colorimeter photocells when adjusting to low luminosity, in a similar way to the human eye, in order to explain the behavior of color in relation to pigment content. Color analysis in dark liquids is related more to a lightness/darkness measurement than to a color judgement, so some of the color parameters (a*, b*, chroma and hue angle) might not be properly calculated. At high concentrations of pigment, some of those parameters might not behave as expected and, depending on the luminosity level, can even show an opposite trend in what is known as the "area of confusion" or the "area of inversion". The area of inversion depends on the predominate color and thus, in samples where the higher value corresponds to a* (red), the inversion area could appear in that color parameter. The same phenomenon may explain our observations in beetroot: as the red pigments were lost, higher values of a* were registered. The inversion area for the chroma parameters was precisely at the inversion point of the scale parameter of the predominate color; when squared, the smaller factor contributes a relatively insignificant amount to the overall function (Eagerman et al. 1973). Other authors also found higher a* and chroma in samples with lower pigment contents, as dark pigmented compounds mask color and, in consequence, less pigmented samples result in higher color parameter values (Gonçalves et al. 2007a; Ubeira-Iglesias et al. 2019).

No significant differences between beetroot puree treated during 10 and 20 min were observed for the color parameters L*, a* and chroma (Table 2). When the samples were heated for more than 20 min, a trend towards increased values of L*, a*, b*, chroma and hue angle was observed, although insignificant in the case of a*. A similar trend towards increased a* and b* values was reported in literature for beetroot puree treated at 120 °C (Chandran et al. 2014).

A trend towards increased values in all the color parameters assessed was still noted in whole beetroot, although no significant differences between samples treated for 10 or 20 min, 30 or 40 min, and 50 or 60 min were observed for any of those parameters (Table 2), which may be explained by the heterogeneity of this matrix. Thus, the measurement of L*, a*, b*, chroma and hue angle on the surface of a whole red beetroot was insufficient to distinguish beetroots treated over incrementally lengthier treatment times.

Correlations and regressions between betalain content and color

The aim of the present work was to study the relationship between color and betalain content in beetroot. Accordingly, a correlation and a linear regression analysis between both variables were developed. Taking into account that positive physiological effects have been attributed to both betacyanins and betaxanthins (Kanner et al. 2001; Azeredo 2009; Gengatharan et al. 2015), the variable "total betalain content" was used in the statistical analysis as a marker of the pigment content in beetroot.

The color parameters L*, a*, b*, chroma and hue angle correlated negatively with the total betalain content in the three beetroot products under assessment (Table 3). The negative correlation between color parameters and pigment content was also evident in previous studies on cherry anthocyanins (Gonçalves et al. 2007b). It is logical that lightness (L*) and yellowness (b*) may correlate negatively with total betalain content, as betacyanin levels diminish and are degraded into yellowish compounds as a consequence of the thermal treatment. However, the reason why a decrease in the pigments that cause redness should result in higher redness value readings is more difficult to understand. As discussed in the previous section, this phenomenon appears when a high pigment concentration darkens the sample, and has previously been discussed in connection with different red fruit products (Eagerman et al. 1973; Herbach et al. 2006b; Gonçalves et al. 2007b).

Chroma and a* showed high correlation coefficients with the total betalain content in the three beetroot products under assessment, although it is important to highlight that the strongest correlations were found in beetroot juice ($r > 0.96$). The correlation coefficient between hue angle and pigment concentration was weaker in the three beetroot products ($r < 0.8$) and cannot therefore be considered a good descriptor for monitoring beetroot pigments.

Some authors (Paciulli et al. 2016) have reported that combinations of L*a*b* parameters correlated better with pigments than each single parameter alone, although the best combination depended on the food. Different combinations were therefore calculated and included in the correlation analysis (Table 3), in order to evaluate whether such combinations add valuable information to the color analysis of beetroot and which are the most representative to monitor pigment changes in this product. Regarding the combinations of the different color parameters in the three products under assessment, it may be highlighted that the correlations between Lab, La/b, and Lb/a, and the pigment contents were negative, while L/ab and a/b showed positive correlations with the content of total betalains.

The combination that showed the stronger correlation with total betalains depended on the beetroot product. L/ab presented a higher correlation with total betalains in the juice. Lb/a was the strongest in the case of the puree and Lab was the best for the whole beetroot. However, it is important to highlight that the tristimulous combinations only improved the correlation between the single-color

Table 3 Pearson correlation coefficients ($p < 0.05$) between total betalain (TB) content and colour parameters (CP) in beetroot juice (BJ), beetroot puree (BP) and whole beetroot (WB)

Sample	CP	TB	Sample	CP	TB	Sample	CP	TB
BJ	L*	− 0.9510	BP	L*	− 0.8913	WB	L*	− 0.8853
	a*	− 0.9678		a*	− 0.8402		a*	− 0.8965
	b*	− 0.9545		b*	− 0.7998		b*	− 0.9320
	C*	− 0.9672		C*	− 0.8340		C*	− 0.9230
	h_{ab}	− 0.6957		h_{ab}	− 0.7867		h_{ab}	− 0.7824
	Lab	− 0.8545		Lab	− 0.8074		Lab	− 0.9253
	La/b	− 0.9557		La/b	− 0.8101		La/b	0.5191
	L/ab	0.9725		L/ab	0.4752		L/ab	0.9145
	Lb/a	− 0.9351		Lb/a	− 0.8243		Lb/a	− 0.8073
	a/b	0.7011		a/b	0.8014		a/b	0.7965

parameters and the total betalain content in the case of beetroot juice.

Although the Hunter ratio a/b has been reported by some authors to be closely correlated with pigments such as carotenoids (Ahmed et al. 2002), it could not be highlighted in the case of betalains for any of the three beetroot products that were assessed ($r < 0.8$). The same ratio was also considered a good indicator of color losses in beetroot puree (Chandran et al. 2014). It might perhaps be used in samples with constant L* values, which is not the case of our thermally treated beetroot products.

Different tristimulous color combinations have already been suggested as good color descriptors in several foods. Ahmed et al. (2004) reported that the Lab combination could describe the variation of total visual color with the anthocyanin content of plum puree during thermal processing. Rodrigo et al. (2007) found that the La/b combination was the best descriptor of the color change of tomato and strawberry puree during thermal and high-pressure thermal treatments, although those authors established no correlation with pigment content. Our results suggested that the best combination in beetroot depended on the beetroot product and showed the highest correlation with total betalains in the case of beetroot juice. When the beetroot matrices were of greater complexity, such as puree or whole beetroot, the correlation between color and pigment content decreased.

Finally, a linear regression analysis was performed for each relationship between total betalain content (X-variable) and the color parameters (Y-variable) a* and chroma (which showed the highest correlations with pigments). Therefore, the total betalain content in each beetroot product could be estimated using the linear regression equations shown in Table 4.

Table 4 Simple linear regression analysis of x-variable (X-var) versus y-variable (Y-var) ($p < 0.05$) for beetroot juice (BJ), beetroot puree (BP) and whole beetroot (WB)

Sample	X-var	Y-var	Coefficient	Intercept	R^2
BJ	TB	a*	− 0.0172	17.004	0.9367
	TB	C*	− 0.0179	17.653	0.9355
BP	TB	a*	− 0.0093	32.109	0.7059
	TB	C*	− 0.0087	35.547	0.6956
WB	TB	a*	− 0.0182	25.983	0.8037
	TB	C*	− 0.0208	28.062	0.8520

From whole cooked beetroot to squeezed beetroot: an approach to improve the betalain–color relationship

As the relationship between color and betalain concentration appeared to be dependent on the matrix, we finally checked whether the color measurement of a whole thermally treated beetroot processed into a squeezed sample might be a better approach to elucidate the betalain content in cooked beetroots.

As already discussed in a previous section, when the color was measured on the surface of a whole beetroot, no significant differences were observed between every single treatment for any of the parameters under assessment. However, having squeezed each of the thermally treated beetroots, prior to color measurement, significant differences between treatments were observed for all the color parameters and the tristimulus combinations (Table 5).

Table 5 Colour parameters (and their combinations) of squeezed whole beetroot

Treatment (min)	L	a*	b*	C*	h_{ab}
10	2.65 ± 0.01*f*	14.22 ± 0.06*f*	2.93 ± 0.05*f*	14.52 ± 0.06*f*	11.64 ± 0.22*f*
20	3.05 ± 0.03*e*	16.10 ± 0.07*e*	3.68 ± 0.03*e*	16.51 ± 0.06*e*	12.89 ± 0.15*c*
30	3.96 ± 0.03*d*	19.27 ± 0.03*d*	4.17 ± 0.09*d*	19.71 ± 0.04*d*	12.20 ± 0.25*e*
40	4.58 ± 0.02*c*	22.44 ± 0.03*c*	5.00 ± 0.06*c*	22.99 ± 0.02*c*	12.55 ± 0.17*d*
50	5.84 ± 0.03*b*	25.24 ± 0.03*b*	6.38 ± 0.03*b*	26.03 ± 0.03*b*	14.17 ± 0.07*b*
60	7.26 ± 0.02*a*	27.63 ± 0.04*a*	8.04 ± 0.06*a*	28.77 ± 0.03*a*	16.22 ± 0.13*a*

Treatment (min)	a/b	Lab	La/b	L/ab	Lb/a
10	4.86 ± 0.10*a*	110.09 ± 2.18*f*	12.85 ± 0.21*f*	0.06 ± 0.00*a*	0.54 ± 0.01*f*
20	4.37 ± 0.05*d*	180.94 ± 1.29*e*	13.35 ± 0.28*e*	0.05 ± 0.00*b*	0.70 ± 0.01*e*
30	4.63 ± 0.10*b*	317.72 ± 4.98*d*	18.32 ± 0.50*d*	0.05 ± 0.00*c*	0.86 ± 0.01*d*
40	4.49 ± 0.06*c*	513.62 ± 4.29*c*	20.59 ± 0.36*c*	0.04 ± 0.00*d*	1.02 ± 0.01*c*
50	3.96 ± 0.02*e*	940.08 ± 6.56*b*	23.13 ± 0.19*b*	0.04 ± 0.00*e*	1.48 ± 0.01*b*
60	3.44 ± 0.03*f*	1611.43 ± 13.32*a*	24.95 ± 0.17*a*	0.03 ± 0.00*f*	2.11 ± 0.02*a*

Values are expressed as mean ± SD (n = 4). Different letters indicate significant differences between treatments

Processing whole cooked beetroots into their squeezed samples improved the correlations between color parameters and total betalain contents; La/b, L/ab, a*, and chroma were the best color descriptors of pigment concentration in beetroot ($r = -0.9932$, $r = 0.9587, -0.9872$ and -0.9844, respectively).

The linear regression analysis between the total betalain content (X-variable) and the color parameters (Y-variable) for the squeezed cooked beetroot gave a coefficient of -0.0251 and -0.0264 (for a* and chroma, respectively); an intercept of 31.0346 and 32.1813 (for a* and chroma, respectively); and a R^2 of 0.9747 and 0.9690 (for a* and chroma, respectively).

These findings suggest that the measurement of color in a squeezed cooked beetroot could be considered a good indicator of its betalain content and, therefore, an acceptable and efficient way of monitoring the potential health-promoting properties of cooked beetroot.

Conclusion

Our results have established that the chromatic parameters L*, a*, b*, chroma and hue angle showed negative correlations with the total betalain content in thermally treated beetroot, beetroot puree and beetroot juice. Chroma and a* values have been suggested as the best descriptors of betalain changes in these products, although some tristimulus Lab combinations have also been proposed as good tools in that respect, mainly in the case of L/ab for the juice. Our findings have highlighted that the relationship between color and total betalain content depends on the beetroot product under assessment, with the strongest correlations found in the juice. Thus, squeezed beetroot is suggested as an alternative to improve this relationship in more complex matrices such as whole cooked beetroots.

Our investigation has added useful information for a better understanding of the relationship between color and betalain pigments in beetroot. It has suggested that color determination could be used as a marker of the pigment concentration.

References

Ahmed J, Shivhare US, Sandhu KS (2002) Thermal degradation kinetics of carotenoids and visual color of papaya puree. J Food Sci 67:2692–2695. https://doi.org/10.1111/j.1365-2621.2002.tb08800.x

Ahmed J, Shivhare US, Raghavan GSV (2004) Thermal degradation kinetics of anthocyanin and visual colour of plum puree. Eur Food Res Technol 218:525–528. https://doi.org/10.1007/s00217-004-0906-5

Alard D, Wray V, Grotjahn L et al (1985) Neobetanin: isolation and identification from *Beta vulgaris*. Phytochemistry 24:2383–2385. https://doi.org/10.1016/S0031-9422(00)83046-0

Azeredo HMC (2009) Betalains: Properties, sources, applications, and stability-a review. Int J Food Sci Technol 44:2365–2376. https://doi.org/10.1111/j.1365-2621.2007.01668.x

Azmir J, Zaidul ISM, Rahman MM et al (2013) Techniques for extraction of bioactive compounds from plant materials: a review. J Food Eng 117:426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Carrillo C, Rey R, Hendrickx M et al (2017) Antioxidant capacity of beetroot: traditional vs novel approaches. Plant Foods Hum Nutr 72:266–273. https://doi.org/10.1007/s11130-017-0617-2

Carrillo C, Wilches-Pérez D, Hallmann E et al (2019) Organic versus conventional beetroot. Bioactive compounds and antioxidant properties. LWT Food Sci Technol 116:108552. https://doi.org/10.1016/j.lwt.2019.108552

Chandran J, Nisha P, Singhal RS, Pandit AB (2014) Degradation of colour in beetroot (*Beta vulgaris* L.): a kinetics study. J Food Sci Technol 51:2678–2684. https://doi.org/10.1007/s13197-012-0741-9

Eagerman BA, Clydesdale EFM, Francis FJ (1973) Comparison of color scales for a dark colored beverages. J Food Sci 38:1051–1055. https://doi.org/10.1111/j.1365-2621.1973.tb02146.x

Esatbeyoglu T, Wagner AE, Schini-Kerth VB, Rimbach G (2015) Betanin-a food colorant with biological activity. Mol Nutr Food Res 59:36–47. https://doi.org/10.1002/mnfr.201400484

Gandía-Herrero F, Escribano J, García-Carmona F (2016) Biological activities of plant pigments betalains. Crit Rev Food Sci Nutr 56:937–945. https://doi.org/10.1080/10408398.2012.740103

Gengatharan A, Dykes GA, Choo WS (2015) Betalains: natural plant pigments with potential application in functional foods. LWT Food Sci Technol 64:645–649. https://doi.org/10.1016/j.lwt.2015.06.052

Gonçalves B, Silva AP, Moutinho-Pereira J et al (2007a) Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium* L.). Food Chem 103:976–984. https://doi.org/10.1016/j.foodchem.2006.08.039

Gonçalves EM, Pinheiro J, Abreu M et al (2007b) Modelling the kinetics of peroxidase inactivation, colour and texture changes of pumpkin (*Cucurbita maxima* L.) during blanching. J Food Eng 81:693–701. https://doi.org/10.1016/j.jfoodeng.2007.01.011

Herbach KM, Stintzing FC, Carle R (2004a) Impact of thermal treatment on color and pigment pattern of red beet (*Beta vulgaris* L.) preparations. J Food Sci 69:C491–C498. https://doi.org/10.1111/j.1365-2621.2004.tb10994.x

Herbach MK, Stintzing FC, Carle R (2004b) Thermal degradation of betacyanins in juices from purple pitaya [*Hylocereus polyrhizus* (Weber) Brittonv & Rose] monitored by high-performance liquid chromatography-tandem mass spectometric analyses. Eur Food Res Technol 219:377–385. https://doi.org/10.1007/s00217-004-0948-8

Herbach KM, Stintzing FC, Carle R (2005) Identification of heat-induced degradation products from purified betanin, phyllocactin and hylocerenin by high-performance liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 19:2603–2616. https://doi.org/10.1002/rcm.2103

Herbach KM, Rohe M, Stintzing FC, Carle R (2006a) Structural and chromatic stability of purple pitaya (*Hylocereus polyrhizus* [Weber] Britton & Rose) betacyanins as affected by the juice matrix and selected additives. Food Res Int 39:667–677. https://doi.org/10.1016/j.foodres.2006.01.004

Herbach KM, Stintzing FC, Carle R (2006b) Stability and color changes of thermally treated betanin, phyllocactin, and

hylocerenin solutions. J Agric Food Chem 54:390–398. https://doi.org/10.1021/jf051854b
Herbach KM, Stintzing FC, Carle R (2006c) Betalain stability and degradation-structural and chromatic aspects. J Food Sci 71:41–50. https://doi.org/10.1111/j.1750-3841.2006.00022.x
Herbach KM, Maier C, Stintzing FC, Carle R (2007) Effects of processing and storage on juice colour and betacyanin stability of purple pitaya (*Hylocereus polyrhizus*) juice. Eur Food Res Technol 224:649–658. https://doi.org/10.1007/s00217-006-0354-5
Huang AS, Elbe JHV (1985) Kinetics of the degradation and regeneration of betanine. J Food Sci 50:1115–1120. https://doi.org/10.1111/j.1365-2621.1985.tb13024.x
Kanner J, Harel S, Granit R (2001) Betalains-a new class of dietary cationized antioxidants. J Agric Food Chem 49:5178–5185. https://doi.org/10.1021/jf010456f
Lechner JF, Stoner GD (2019) Red beetroot and betalains as cancer chemopreventative agents. Molecules 24:1602. https://doi.org/10.3390/molecules24081602
Leong HY, Show PL, Lim MH et al (2018) Natural red pigments from plants and their health benefits: a review. Food Rev Int 34:463–482. https://doi.org/10.1080/87559129.2017.1326935
Little AC (1975) A research note off on a a tangent. J Food Sci 40:410–411. https://doi.org/10.1111/j.1365-2621.1975.tb02213.x
McGuire RG (1992) Reporting of objective color measurements. Hort Sci 27:1254–1255. https://doi.org/10.21273/hortsci.27.12.1254
Nilsson T (1970) Studies into the pigments in beetroot (*Beta vulgaris* L. ssp.*vulgaris* var. *rubra* L.). Lantbrukshoegskolans Ann 36:179–219
Paciulli M, Medina-Meza IG, Chiavaro E, Barbosa-Cánovas GV (2016) Impact of thermal and high pressure processing on quality parameters of beetroot (*Beta vulgaris* L.). LWT Food Sci Technol 68:98–104. https://doi.org/10.1016/j.lwt.2015.12.029
Pathare PB, Opara UL, Al-Said FAJ (2013) Colour measurement and analysis in fresh and processed foods: a review. Food Bioprocess Technol 6:36–60. https://doi.org/10.1007/s11947-012-0867-9
Rahimi P, Abedimanesh S, Mesbah-Namin SA, Ostadrahimi A (2019a) Betalains, the nature-inspired pigments, in health and diseases. Crit Rev Food Sci Nutr 59:2949–2978. https://doi.org/10.1080/10408398.2018.1479830
Rahimi P, Mesbah-Namin SA, Ostadrahimi A et al (2019b) Betalain- and betacyanin-rich supplements' impacts on the PBMC SIRT1 and LOX1 genes expression and Sirtuin-1 protein levels in coronary artery disease patients: a pilot crossover clinical trial. J Funct Foods 60:103401. https://doi.org/10.1016/j.jff.2019.06.003
Rahimi P, Mesbah-Namin SA, Ostadrahimi A et al (2019c) Effects of betalains on atherogenic risk factors in patients with atherosclerotic cardiovascular disease. Food Funct 10:8286–8297. https://doi.org/10.1039/c9fo02020a
Ravichandran K, Saw NMMT, Mohdaly AAA et al (2013) Impact of processing of red beet on betalain content and antioxidant activity. Food Res Int 50:670–675. https://doi.org/10.1016/j.foodres.2011.07.002
Rodrigo D, van Loey A, Hendrickx M (2007) Combined thermal and high pressure colour degradation of tomato puree and strawberry juice. J Food Eng 79:553–560. https://doi.org/10.1016/j.jfoodeng.2006.02.015
Rodriguez-Casado A (2016) The health potential of fruits and vegetables phytochemicals: notable examples. Crit Rev Food Sci Nutr 56:1097–1107. https://doi.org/10.1080/10408398.2012.755149
Singer JW, von Elbe JH (1980) Degradation rates of vulgaxanthine I. J Food Sci 45:489–491. https://doi.org/10.1111/j.1365-2621.1980.tb04082.x
Swartz MC, Allen K, Deer RR et al (2019) A narrative review on the potential of red beetroot as an adjuvant strategy to counter fatigue in children with cancer. Nutrients 11:1–13. https://doi.org/10.3390/nu11123003
Ubeira-Iglesias M, Wilches-Pérez D, Cavia MM et al (2019) High hydrostatic pressure processing of beetroot juice: effects on nutritional, sensory and microbiological quality. High Press Res 39:691–706. https://doi.org/10.1080/08957959.2019.1666842
Wybraniec S (2005) Formation of decarboxylated betacyanins in heated purified betacyanin fractions from red beet root (*Beta vulgaris* L.) monitored by LC–MS/MS. J Agric Food Chem 53:3483–3487. https://doi.org/10.1021/jf048088d
Wybraniec S, Mizrahi Y (2005) Generation of decarboxylated and dehydrogenated betacyanins in thermally treated purified fruit extract from purple pitaya (*Hylocereus polyrhizus*) monitored by LC–MS/MS. J Agric Food Chem 53:6704–6712. https://doi.org/10.1021/jf050700t