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ASSESSMENT OF COLOUR CHANGES DURING STORAGE OF ELDERBERRY JUICE CONCENTRATE SOLUTIONS USING THE OPTIMIZATION METHOD

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ABSTRACT

Background. Elderberries are a source of dietary supplements and bioactive compounds, such as anthocyanins. These dyes are used in food technology. The aim of the study was to assess the changes in colour parameters, anthocyanin contents and sensory attributes in solutions of elderberry juice concentrates during storage in a model system and to determine predictability of sensory attributes of colour in solutions based on regression equations using the response surface methodology.

Material and methods. The experiment was carried out according to the 3-level factorial design for three factors. Independent variables included pH, storage time and temperature. Dependent variables were assumed to be the components and colour parameters in the CIE $L^*a^*b^*$ system, pigment contents and sensory attributes.

Results. Changes in colour components X, Y, Z and colour parameters L^*, a^*, b^*, C^* and h^* were most dependent on pH values. Colour lightness L^* and tone h^* increased with an increase in experimental factors, while the share of the red colour a^* and colour saturation C^* decreased. The greatest effect on the anthocyanin concentration was recorded for storage time. Sensory attributes deteriorated during storage. The highest correlation coefficients were found between the value of colour tone h^* and anthocyanin contents in relation to the assessment of the naturalness and desirability of colour. A high goodness-of-fit of the model to data and high values of R^2 for regression equations were obtained for all responses.

Conclusion. The response surface method facilitates optimization of experimental factor values in order to obtain a specific attribute of the product, but not in all cases of the experiment. Within the tested range of factors, it is possible to predict changes in anthocyanin content and the sensory attributes of elderberry juice concentrate solutions as food dye, on the basis of the lack of a fit test. The highest stability of dyes and colour of elderberry solutions was found in the samples at pH 3.0, which confirms the advisability of using an anthocyanin preparation to shape the colour of high-acidity food products, such as fruit fillings, beverages, desserts.

Key words: elderberry, juice, colour, anthocyanins, response surface

INTRODUCTION

In recent years both in the food and the pharmaceutical industries great attention has been focused on plant-origin raw materials rich in natural antioxidants. Elderberry is such a raw material due to its relatively high content of polyphenols, including anthocyanins (Bermudez-Soto and Tomas-Barberan, 2004).

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Elderberry (*Sambucus nigra* L.) is a tall shrub naturally found in Europe, north Africa, India and western Asia, as well as being grown in many countries worldwide as an ornamental or medicinal plant. Its flowers and ripe fruits are used as raw herb material (Kołodziej and Drożdżal, 2011). Due to its content of a poisonous glycoside, sambunigrin, its fruits may be consumed only after thermal processing. Elderberry flowers contain relatively low amounts of this glycoside, thus they may be used directly to produce juices, syrups and liqueurs. These flowers are rich sources of flavonoids and phenolic acids (Christensen et al., 2008; Dawidowicz et al., 2006). Ripe fruits are used mainly to produce juices and juice concentrates, as well as jams, preserves and liqueurs (Veberic et al., 2009). Fruits and pomace are sources of anthocyanin pigments for the production of natural food colorants and pharmaceuticals (Seabra et al., 2010). Thanks to their high of polyphenol content, these fruits have long been ascribed anti-inflammatory and anti-influenza properties (Krawitz et al., 2011), antitumour and antiatherogenic properties (reduction in triglyceride concentration and inhibition of platelet aggregation) (Wattenberg, 1990). A model study on the biological activity of anthocyanins from the elderberry plant showed their antioxidant effect and protective role in the inhibition of endothelial cell damage in blood vessels under oxidative stress (Youdim et al., 2000).

Anthocyanins form one of the flavonoid classes, comprising an important group of polyphenols. Anthocyanins provide colour to different parts of plants. Depending on the type of basic molecule of anthocyaninidine and attached substituents, anthocyanins provide colours ranging from orange through red to blue. The colour tone in anthocyanins depends to a considerable extent on the acidity of the medium. Elderberry contains mainly anthocyanins, such as cyanidin-3-glucoside and cyanidin-3-sambubioside, while quercetin 3-rutinoside (rutin) predominates among polyphenols from the flavonol group (Bermudez-Soto and Tomas-Barberan, 2004). The anthocyanin content of these fruits, depending on the variety, amounts to 660–800 mg/100 g (Kaack and Austed, 1998; Veberic et al., 2009). By modifying fruit colour, anthocyanins have a direct effect on their attractive appearance, while indirectly they also influence other sensory attributes. On the basis of a fruit's colour, consumers also evaluate its expected taste, aroma and texture. The more saturated a fruit colour is, the stronger the association with better taste, juicy flesh and the rich aroma of ripe fruits.

Colour is a major sensory attribute of food, particularly fruits and their processed products. Its tone and intensity indicate the degree of ripeness, texture and taste of fresh raw materials or taste, aroma and shelf life of their preserves. Loss of intrinsic colour is typically associated with a deterioration of taste and aroma, while in the case of preserves, it may also indicate the use of inappropriate technology or inadequate conditions and long storage. The application of appropriate technologies facilitates the maintenance of natural colour in semi-processed fruit preserves and final products to a considerable extent.

The response surface methods are effective tools in optimizing the process, in which process parameters as independent variables have a multifaceted, complex effect on the analysed attributes (Koocheki and Azarpazhooh, 2010). It is a set of statistical and mathematical tools used in designing, improving and optimizing these processes (Myers and Montgomery, 2002). Optimization methods facilitate simultaneous determination of the effect of several experimental factors on different characteristics of the object being tested, i.e. the effect of several independent variables on several dependent variables (Gacula, 1996). The application of optimal experimental designs makes it possible to obtain an extensive body of data from a relatively small number of experiments and to determine optimal levels of independent variables in order to provide desirable attributes of a given product. The response surface method facilitates rapid interpretation of results based on calculations using equations as well as 3D graphs plotted on this basis and their 2D projections and sections on response surfaces (perturbation). In order to facilitate comparison of the effect of factors expressed using different measures, curves may be plotted for standardised ('coded') values, where the lowest level of a factor is ascribed the value of –1, the highest the value of +1, while an intermediate level receives the value of 0.

The aim of the study was to assess the changes in colour parameters, anthocyanin contents and sensory attributes in solutions of elderberry concentrates during storage in a model system and to evaluate the predictability of sensory attributes of colour in solutions based on instrumental analysis and regression equations determined by response surface methodology.

MATERIAL AND METHODS

Elderberry juice concentrate

Analyses were conducted on elderberry juice concentrate (Diana Vegetal Inc., NY, US) with an initial anthocyanin content of 1440 mg/100 dm^3 and 65°Bx. The concentrate was used to prepare model solutions with an anthocyanin content of 12.5 mg/100 dm^3, using Mc'Ilvain buffer solutions with a pH of 3.0, 4.0 and 5.0.

Experimental design

The experiment conducted by the response surface method was designed using the Design Expert version 6.0. computer programme (Statease Inc., Minneapolis, USA). The 3-level factorial design was selected for three experimental factors, covering 32 measurement points including the central point coded (0, 0, 0), with measurements taken in 5 replications. The following were assumed as experimental factors (independent variables):

A – pH: 3.0, 4.0 and 5.0

B – storage time: 0, 10 and 20 days

C – storage temperature: 10, 20 and 30°C.

Dependent variables (responses) included colour components (*X*, *Y*, *Z*), colour parameters (*L**, *a**, *b**, *C**, *h**), content of anthocyanins and sensory attributes of tested solutions. The actual values of the three levels of factors correspond to standardised values coded as –1, 0 and +1. The experimental design is presented in Table 1.

The experiment

Based on the experimental scheme, dilutions of elderberry juice concentrate were prepared in phosphate buffer solutions at pH 3.0, 4.0 and 5.0. Glass vials filled with 10 cm^3 solutions each were saturated with nitrogen for 3 min. To provide anaerobic conditions, sealed and heated in a water bath (10 min, 85°C), next cooled and stored in the dark under conditions meeting the experimental scheme. Three vials were prepared for each of the 32 measurement points and the results are means from those measurements.

Samples of solutions after thermal treatment and storage were subjected to sensory examination of colour, then centrifuged for 10 min at 12,000 rpm on a MPW-210 centrifuge and the supernatant obtained was used in measurements of colour parameters and pigment contents.

Measurement of colour parameters

Colour components were measured instrumentally in the CIE XYZ system, followed by the calculations of colour parameters in the CIE *L*a*b** and CIE *L*C*h* systems (Commision Internationale de l'Eclairage 1976). Colour was measured in a Konica-Minolta CM-3600d spectrophotometer equipped with a computer programme (Color Data Sotfware Spectra Magic). Measurements were taken in light transmitted within the visible wavelength range of 380–700 nm at a D65 light source in the SCE system (elimination of the reflection effect of the glass cuvette). Determinations were made in cuvettes with an optic layer of 0.2 cm in thickness, with distilled water being the reference sample.

Determination of anthocyanin content

Anthocyanin content was determined by high-performance liquid chromatography (Escarpa and Gonzalez, 2000) using a Thermoseparation chromatograph with a UV2000 detector and a Waters Spherisorb 5 µm ODS2 4.6×250 mm column Analytical Cartrige Part No. PSS839540. Separation was performed in the following phase gradient: A – 0.01M phosphoric acid (H_3PO_4, Merck) in water, B – 100% methanol, applying within 0–10 min 5–50% B in A, 10–15 min 50–70% B in A, 15–20 min 70–80% B in A and 20–25 min 80–100% B. Anthocyanins were identified at 520 nm based on the spectra and retention time and the content was calculated on the basis of the reference curve for cyanidin-3-glucoside (Sigma-Aldrich).

The index of anthocyanin was determined as the ratio of absorbance for pigment solutions at pH 1.0 and 4.5 (Giusti and Wrolstad, 2001). Absorbance was measured using a Helios Alpha spectrophotometer (Thermo Electron Corporation USA) at a wavelength of 515 and 700 nm in cuvettes with the 10 mm optic layer. The solutions were diluted with buffers at pH 1.0 and 4.5, so that absorbance of the solution at pH 1.0 was 0.3–0.8. A buffer at pH 1.0 was the reference sample.

Table 1. Design of 3-level factorial model for the independent variables (actual and coded levels)

Std	Run	Independent variables – actual level A	actual level B	actual level C	coded level A	coded level B	coded level C
1	29	3	0	10	–1	–1	–1
2	19	4	0	10	0	–1	–1
3	16	5	0	10	1	–1	–1
4	3	3	10	10	–1	0	–1
5	32	4	10	10	0	0	–1
6	21	5	10	10	1	0	–1
7	28	3	20	10	–1	1	–1
8	24	4	20	10	0	1	–1
9	1	5	20	10	1	1	–1
10	20	3	0	20	–1	–1	0
11	23	4	0	20	0	–1	0
12	2	5	0	20	1	–1	0
13	7	3	10	20	–1	0	0
14	30	4	10	20	0	0	0
15	26	5	10	20	1	0	0
16	4	3	20	20	–1	1	0
17	11	4	20	20	0	1	0
18	27	5	20	20	1	1	0
19	15	3	0	30	–1	–1	1
20	22	4	0	30	0	–1	1
21	9	5	0	30	1	–1	1
22	8	3	10	30	–1	0	1
23	17	4	10	30	0	0	1
24	12	5	10	30	1	0	1
25	13	3	20	30	–1	1	1
26	25	4	20	30	0	1	1
27	6	5	20	30	1	1	1
28	18	4	10	20	0	0	0
29	31	4	10	20	0	0	0
30	5	4	10	20	0	0	0
31	14	4	10	20	0	0	0
32	10	4	10	20	0	0	0

A – pH, B – time, C – temperature.

Sensory examination of colour

Sensory examination was performed in a professional laboratory equipped with appropriately lighted stations meeting the respective standards. Analyses were carried out by a panel of 20 experts in colour examination. The solutions were evaluated in terms of the following colour attributes: intensity, naturalness and desirability by applying a numerical scale from 1 to 10 (Baryłko-Pikielna and Matuszewska, 2009).

RESULTS AND DISCUSSION

The effect of independent variables on the responses investigated was determined on the basis of equations and graphs of response surfaces. Tables 2 and 3 present parameters of response equations for components and parameters of colour, pigment contents and attributes of sensory examination.

The row of presented equations corresponds to the row selected by the programme. The goodness of fit for quadratic equations was obtained for all responses. During storage, the values of colour components *X*, *Y*, *Z* increased with an increase in pH, storage time and temperature, as it is indicated by positive equation coefficients (Table 2). An increase in *X*, *Y* and *Z* values is connected with a reduction in anthocyanin content during storage of solutions, i.e. a lightening of their colour. The greatest effect on changes in colour components was found for pH in solutions, as shown by the high values of equation coefficients. The lowest *X*, *Y* and *Z* values were observed at pH 3.0, i.e. at good stability of anthocyanins. The values of components increased with an increase in the levels of all tested factors; however, at the highest pH and storage time a decrease was manifested in *X* and *Z* values, which is connected with a darkening of colour in solutions,

Table 2. Characteristics of response surface equations for colour parameters of solutions of elderberry juice concentrate during storage

Factor	Parameter							
	X	*Y*	*Z*	*L**	*a**	*b**	*C**	*h**
Intercept	64.24	52.48	52.73	77.69	31.02	8.48	32.10	15.23
A	**5.90**	**11.89**	**17.50**	**7.17**	**–18.22**	**–6.67**	**–19.10**	**2.73**
B	0.36	**0.96**	–0.08	**0.81**	**–1.61**	**0.86**	–1.00	**3.89**
C	**0.76**	**1.44**	0.44	**0.64**	**–2.03**	**0.74**	**–1.39**	**4.31**
A^2	**–2.54**	**–3.10**	**–8.18**	**–2.66**	**4.50**	**6.88**	**6.76**	**9.01**
B^2	**–0.90**	–0.98	–1.09	–0.77	0.53	0.12	0.58	–0.59
C^2	0.45	1.04	–0.91	0.46	–1.63	0.03	–1.35	2.27
*A*B*	**–0.57**	–0.43	**–2.38**	–8.3E-03	0.18	**2.04**	1.23	**4.96**
*A*C*	–0.28	–0.20	**–1.77**	–0.50	–0.15	**1.85**	0.92	**5.32**
*B*C*	**0.56**	**1.28**	0.55	**1.12**	**–2.01**	0.52	**–1.41**	**3.65**
Significance of the model ($p > F$)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Lack of fit test	0.0107	0.0011	0.0080	0.0041	<0.0001	<0.0001	<0.0001	<0.0001
R^2	0.9809	0.9810	0.9810	0.9737	0.9826	0.9789	0.9822	0.9019

Values calculated for standardised values of independent variables.
Factors statistically significant at $p \leq 0.05$ are shown in bold type.

Table 3. Characteristics of response surface equations for anthocyanins concentration and sensory analysis of colour of solutions of elderberry juice concentrate during storage

Factor	Parameter				
	anthocyanins contents	$A^{pH1.0}$/ $A^{pH4.5}$	intensity of colour	naturalness of colour	desirability of colour
Intercept	9.64	5.58	8.25	10.01	10.09
A	**–1.32**	**–0.28**	**0.13**	**–0.26**	**–0.60**
B	**–1.92**	**–0.99**	**0.14**	**–0.33**	**–0.64**
C	**–1.18**	**–0.46**	–0.05	–0.11	**–0.26**
A^2	–0.23	**–0.21**	–0.04	–0.18	–0.38
B^2	**0.68**	**0.35**	0.42	**–0.30**	**–0.50**
C^2	–0.18	–0.08	0.01	0.12	0.04
*A*B*	**–1.01**	**–0.48**	0.10	**–0.38**	**–0.83**
*A*C*	**–0.53**	**–0.32**	**–0.15**	–0.15	**–0.33**
*B*C*	**–1.04**	**–0.43**	–0.02	–0.15	**–0.33**
Significance of the model ($p > F$)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Lack of fit test	0.0530	0.0622	0.8129	0.0502	0.0311
R^2	0.9779	0.9577	0.9616	0.9610	0.9913

Values calculated for standardised values of independent variables.
Factors statistically significant at $p \leq 0.05$ are shown in bold type.

as a result of the formation of pigment degradation products (Fig. 1). The highest values of equation coefficients at variable A (pH) were observed for component *Z*, and the same was true for the quadratic effect (A^2) and the interaction effect (*A*B*).

The values of colour parameters changed during storage of solutions. Based on the values of equation coefficients the greatest effect on changes in colour parameters was found for the pH of the solutions, while the effect was markedly smaller, although statistically significant ($p < 0.05$), for storage time and temperature (Table 2). Colour lightness *L** and colour tone *h** increased with an increase in pH, storage time and temperature, while the values of parameter *a** (the proportion of red colour) and *C** (colour saturation) decreased (Figs. 2, 3). The values of parameter b* (the proportion of yellow colour) decreased with an increase in pH, while they increased with increasing storage time and temperature. Such a varied effect of experimental factors on this variable is expressed in the high values of interaction effects (*A*B*, *A*C*). The direction of changes in parameter *b** was dependent on pH, since at low pH levels the value of *b** decreased, while at higher pH levels it increased (Fig. 2). This is connected with a marked change in the colour of stored solutions at pH 5, in which a brownish-red colour tone was observed. This results from the transformation of the flavylium cation into colourless forms and the accumulation of anthocyanin degradation products. A similar shape of the response surface as that for parameter *b**, depending on pH, is observed for the tone angle *h** (Fig. 3). At low pH values solutions assumed a red-pinkish colour, while during storage at a higher pH the colour changed towards orange-red or brown. In the case of parameter *h** the effect of storage time and temperature, determined on the basis of values of equation coefficients, was higher than that of pH, whereas the quadratic effect was marked for

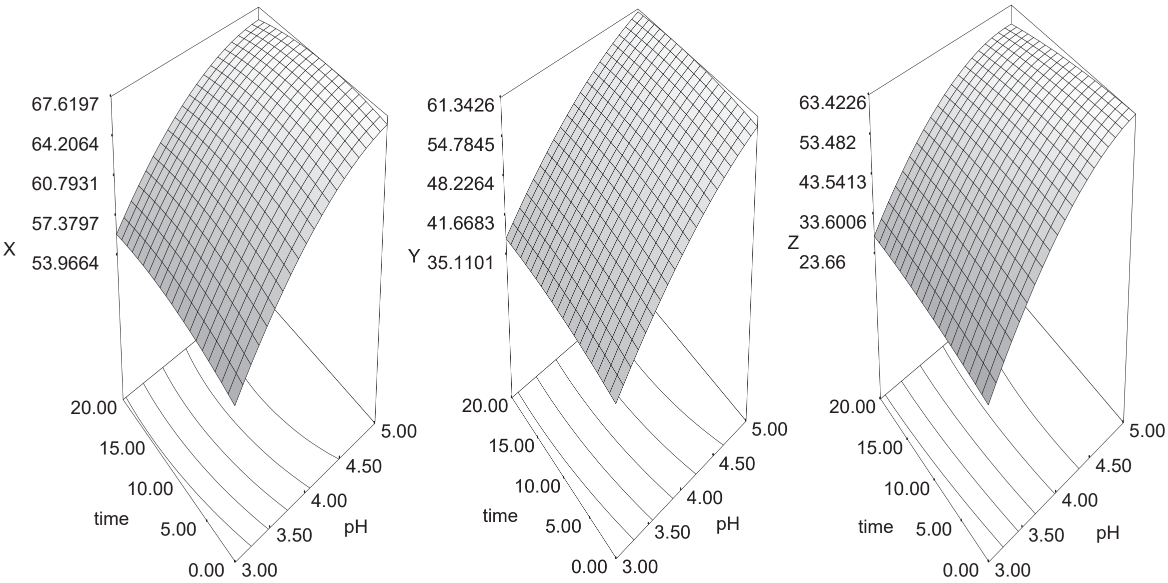

Fig. 1. Response surfaces for colour component changes during storage at a temperature of 20°C

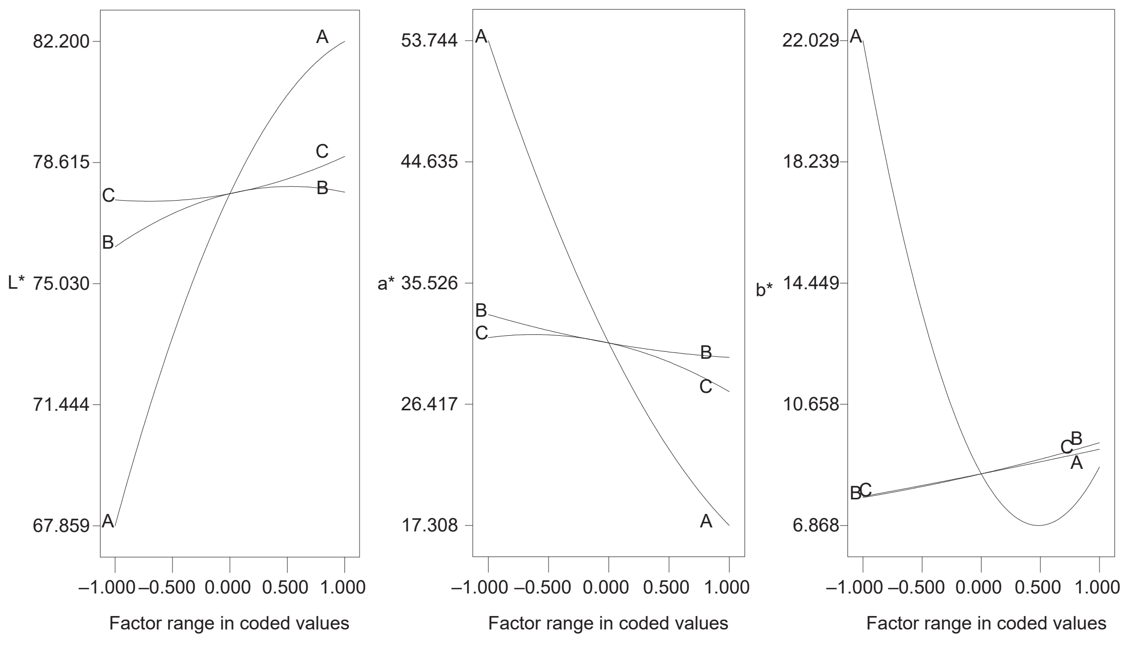

Fig. 2. Cross-sections through response surfaces for changes of colour parameters *L**, *a**, *b** of solutions of elderberry juice concentrate at the code value of the remaining variables equalling 0: A – pH, B – time, C – temperature

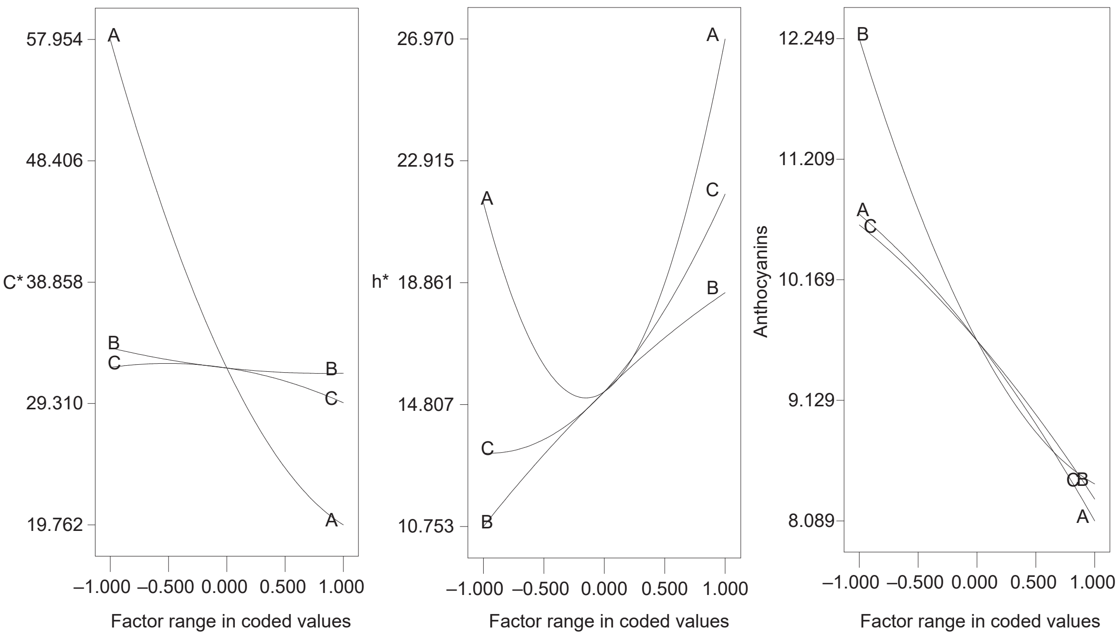

Fig. 3. Cross-sections through response surfaces for changes of colour parameters *C**, *h** and anthocyanins concentration of solutions of elderberry juice concentrate at the code value of the remaining variables equalling 0. Destignations: A – pH, B – time, C – temperature

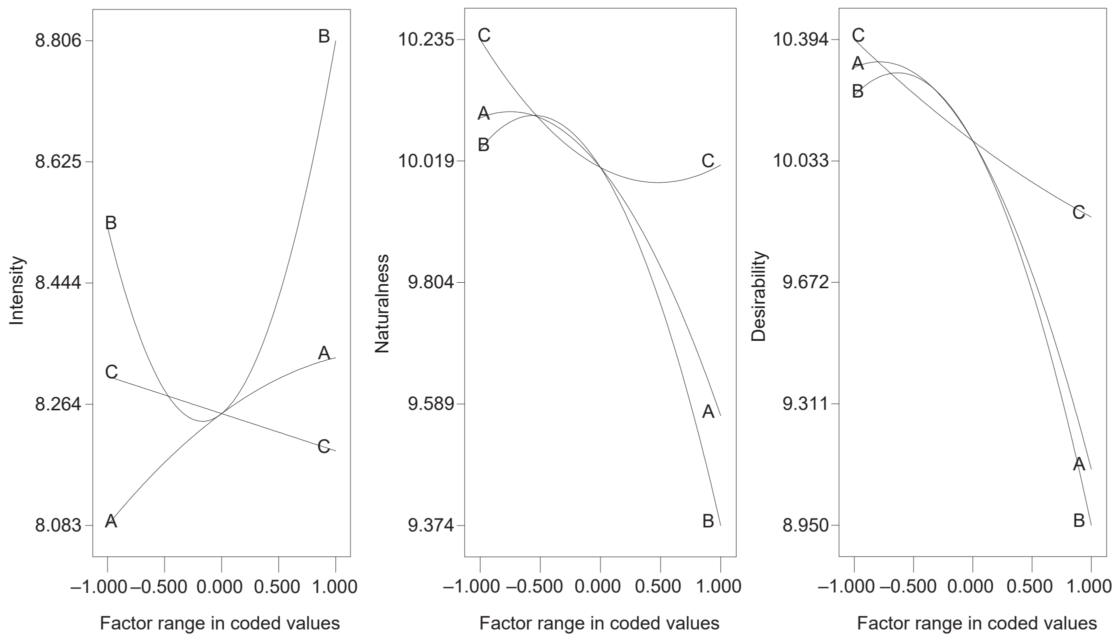

Fig. 4. Cross-sections through response surfaces for changes of sensory evaluation of colour intensity, naturalness and desirability of solutions of elderberry juice concentrate at the code value of the remaining variables equalling 0. Destignations: A – pH, B – time, C – temperature

pH (A^2) and the interaction effect was evident for all independent factors ($A*B$, $A*C$, $B*C$) (Table 2). The effect of pH on thte colour of the solutions was already visible before storage, which results from the presence of different forms of anthocyanins, depending on the pH of the medium. Only in the medium of high acidity (pH 0 to 3.0) are anthocyanins found in the form of a red flavylium cation. The concentration of this pigment form decreases with an increase in pH, while the proportions of the blue chinoid base, colourless pseudo base and yellow or colourless chalcones increase (Delgado-Vargas and Paredes-Lopez, 2003). The lowest values of $h*$ before storage, amounting to 13–15, were recorded for samples with pH 4.0 and 5.0, which corresponds to the pinkish red or purple red colours. At pH 3.0 the initial samples were red in colour ($h*$ of approx. 23). As a result of storage, the tone angle $h*$ changed towards orange and yellow, while samples with a low pH showed high stability of colour tone ($h*$ of approx. 20–25). In the case of solutions with pH 5.0, the values of $h*$ increased considerably, to as much as 56 in the sample stored for 20 days at a temperature of 30°C, which corresponds to the orange-brown colour. Changes in the structure of anthocyanins under the influence of changes in the pH of the medium are observed in the case of pigment extracts from different raw materials. This is particularly evident in the case of preparations from red cabbage, containing anthocyanins acylated with phenolic acids. In model studies on the red cabbage preparation it was found that pH had the greatest effect on changes in colour in these solutions during storage (Walkowiak-Tomczak and Czapski, 2007).

During solution storage anthocyanin pigment content decreased in each sample. On the basis of equation coefficient values the greatest effect on that indicator was recorded for storage time (factor B), although the effect of temperature and pH was also statistically significant ($p < 0.05$) (Table 3). An increase in pH, storage time and temperature caused a reduction in anthocyanin content and in the value of $A^{pH1}/A^{pH4.5}$, as shown by the negative equation coefficients for factors A (pH), B (storage time), C (storage temperature) (Table 3). The $A^{pH1}/A^{pH4.5}$ ratio is an indicator of anthocyanin degradation, since it expresses the ratio of absorbance in the solution containing all colour compounds at pH 1.0 (monomeric and polymerised anthocyanins and non-enzymatic browning products) to absorbance of the solution at pH 4.5, in which monomeric anthocyanins are transformed to colourless compounds (Wrolstad et al., 2005). Thus a reduction in this coefficient indicates that degradation of anthocyanins is occurring, which is accompanied by the accumulation of brown degradation products. For the changes in contents of pigments and the coefficient of their degradation a significant interaction effect was recorded for all independent variables (Table 3).

The colour of the solutions during storage was also evaluated in sensory examination in terms of its applicability as an ingredient of fruit drinks. Scores for the attributes being evaluated deteriorated with an increase in pH, storage time and temperature. Only in the case of colour intensity did scores increase slightly with an increase in pH and storage time, which may be connected with the formation of brown degradation products (Fig. 4). A greater effect on scores in sensory examination was found for pH (A) and storage time (B) than for storage temperature (C) (Table 3). In the descriptive assessment of colour in solutions after storage, all samples with pH 3.0 and 4.0 were denoted as red, and for pH 5.0 as brownish red. In all cases samples with pH 5.0 received the lowest scores in the examination of the naturalness and desirability of colour. In those samples a purple-brown and brown colour tone, considered undesirable in fruit drinks, quickly appeared. In turn, samples with pH 3.0 and 4.0 were given positive scores in this respect. The panel described these solutions on the basis of their colour as blackcurrant juice or drink.

In order to determine the dependence between instrumental and sensory assessment of colour and the applicability of measurements of colour parameters in predicting its sensory attributes, the values of the correlation matrix were calculated for colour parameters measured instrumentally and the attributes of visual examination (intensity, naturalness, desirability) (Table 4). No significant correlation ($p < 0.05$) was found between colour components X, Y and Z and scores in sensory examination. Similarly, in the case of parameters $b*$ and $C*$, no significant correlations were observed with the visual evaluation of colour. For colour lightness $L*$ and the proportion of the red colour $a*$, a significant ($p < 0.05$) but weak correlation was recorded with scores for

Table 4. Correlation between colour parameters *L**, *a**, *h**, anthocyanins concentration and colour sensory analysis of solutions of elderberry juice concentrate during storage

Variables	*L**	*a**	*h**	Anthocyanins	Intensity	Naturalness	Desirability
*L**	1.00	**–0.98**	0.19	**–0.55**	0.26	–0.31	**–0.38**
*a**	**–0.98**	1.00	–0.25	**0.55**	–0.29	0.34	**0.42**
*h**	0.19	–0.25	1.00	**–0.73**	0.08	**–0.66**	**–0.75**
Anthocyanins	**–0.55**	**0.55**	**–0.73**	1.00	–0.19	**0.69**	**0.74**
Intensity	0.26	–0.29	0.08	–0.19	1.00	**–0.37**	**–0.40**
Naturalness	–0.31	0.34	**–0.66**	**0.69**	**–0.37**	1.00	**0.95**
Desirability	**–0.38**	**0.42**	**–0.75**	**0.74**	**–0.40**	**0.95**	1.00

Correlations statistically significant at ≤0.05 are shown in bold type.

colour desirability (r = 0.42). Only in the case of the colour tone angle *h** and contents of anthocyanins were their correlation coefficients much greater with the assessment of naturalness and desirability of colour, amounting to 0.66–0.75. This means that the sensory evaluation was based primarily on colour tone, connected with the value of pH. The values of *C** and *h** are better correlated with the visual assessment of colour than is the case with *a** and *b** values (Wrolstad et al., 2005). None of the instrumentally measurable colour parameters, or the concentration of pigments showed a significant correlation with the score for colour intensity. In turn, a very high, positive correlation (r = 0.95) was observed between the naturalness and desirability of colour, which means that the drinks consumers value highest are of natural colour, corresponding to that in the natural raw material, i.e. fruits with a familiar colour. On the basis of correlations, it was found that in the experiment the instrumentally measured colour tone angle *h** is the parameter which most reliably describes the colour hue of the drink found acceptable by consumers in sensory examination.

CONCLUSIONS

For all responses a goodness of fit with quadratic equations was found for the data. In all cases the value of the F test, evaluating the significance of the model, was lower than 0.0001, which indicates a very good fit of the model to the responses recorded. High values for the R^2 coefficient of determination show the good fit of regression equations to the values of responses. The response surface method facilitates optimization of experimental factor values in order to obtain a specific attribute of the product. However, it is not possible in all cases in this experiment. Due to the low values on the lack of fit test for colour parameters, predicting values for these responses is limited. In contrast, for such variables as pigment content, anthocyanin degradation coefficient, intensity and naturalness of colour, the lack of fit test is non-significant (lack of fit test $F > 0.05$), which makes it possible to apply calculated equations to predict the values of these responses within the range of experimental factors being investigated. The most dyes and colour stability were found in the samples at pH 3.0, which confirms the advisability of using an anthocyanin preparation to shape the red or orange-red colour of high-acidity food products, such as fruit fillings, beverages, desserts. Storage of products with a higher pH brings about undesirable color changes in the brownish-red sample.

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