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Colour changes of a preparation from red cabbage during storage in a model system

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Abstract

The objective of our research was to determine, using model studies, changes in anthocyanin concentrations in solutions of red cabbage preparation during storage, in relation to pH, time and temperature of storage, oxygen availability, and ascorbic acid concentration. Pigment degradation increased with increasing pH, storage time and temperature. The addition of ascorbic acid and oxygen availability also contributed to the decomposition of anthocyanins. Pigment concentration was affected most by storage time and temperature. However, colour parameters were affected most by pH and the concentration of ascorbic acid.

Keywords: Red cabbage preparation; Anthocyanins; Pigment degradation

1. Introduction

Anthocyanins, as natural colorants, are widely used in the food industry as an alternative to synthetic colorants, e.g., they can replace FD&C Red No. 40 (Allura red). They are characterised by a wide spectrum of colour tones, ranging from orange through red, to purple and blue, depending on the molecular structure and pH value. The interest in anthocyanins derives not only from their colouring effect but also from their beneficial properties, including antioxidising activity, improvement in the tightness of capillary blood vessels and prevention of thrombocyte aggregation, all of which reduce the risk of circulatory diseases (Degenhardt, Knapp, & Winterhalter, 2000; Giusti & Wrolstad, 2003; Tsai, Mclntosh, Pearce, Camden, & Jordan, 2002).

Chemically speaking, anthocyanins are glycosides of one of several forms of anthocyanidins (aglycone), which differ from one another in the position of substitution of hydroxyl and methoxy-groups in the β ring of the flavylium cation. In plant products, anthocyanins occur in the form of mono-, di- and triglycosides. Anthocyanin glycoside residues are, in turn, frequently acylated with phenolic acids. Both glycosidation and acylation of glycoside residues increase anthocyanin stability (Bridle & Timberlake, 1997; Brouillard, 1982; Giusti & Wrolstad, 2003).

The principal aglycone of red cabbage is cyanidin, which occurs as cyanidin 3-sophoroside-5-glucoside and cyanidin 3,5-diglucoside, acylated with sinapic, ferulic, malonic and *p*-coumaric acids (Hrazdina, Iredale, & Mattick, 1977; Sapers, Taffer, & Ross, 1981; Tanchev & Timberlake, 1969). Red cabbage colouring is currently used, to colour various beverages, candies, dry mixed concentrates, chewing gums, yoghurts, and sauces. Investigations have been carried out to find out if it is possible to use it as an indicator of changes in the pH value in pharmaceutical preparations (Chigurupati, Saiki, Gayser, & Dash, 2002). Unlike the majority of the anthocyanins manufactured from berry fruits, the colorant obtained from red cabbage can be used to colour food articles over a wide pH range, not only acidic products but also neutral ones. It can therefore replace synthetic blue dyes (Dyrby, Westergaard, & Stapelfeldt, 2001).

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There are numerous physical and chemical factors which can have a negative impact on the stability of anthocyanins, the most important of which are increased temperature, light, oxygen, pH, ascorbic acid and metal ions. Numerous reports can be found in the literature concerning anthocyanin stability, including red cabbage anthocyanins (Dyrby et al., 2001; Chigurupati et al., 2002; Sapers et al., 1981; Shi, Francis, & Duan, 1992). In this study, the authors investigated the effect of pH, time and temperature of storage, oxygen availability and ascorbic acid on anthocyanin stability and colour parameters in red cabbage colorant preparations (Red Cabbage 30 WSP) employing response surface methodology.

2. Materials and methods

2.1. Materials

The performed experiments employed solutions of a red cabbage colorant preparation (Red Cabbage 30 WSP, Chr. Hansen, Denmark). In order to prepare solutions of the experimental colorant preparation, the authors used buffers (Na_2HPO_4 – citric acid) of pH 3.0, 4.0 and 5.0 (McKenzie & Dawson, 1974). Anthocyanin concentrations were determined (Fuleki & Francis, 1968) using buffers of pH 1.0 (KCl–HCl) and pH 4.5 (NaOH, –citric acid, –HCl).

2.2. Experimental design

The experimental design and result analysis was established using the Design-Expert software (Ver. 5.0, Stat-Ease Inc.). The applied experimental design was that of Box-Behnken for four factors (comprising 29 measurement points) and three levels of independent variables: A – pH (3.0; 4.0; 5.0), B – storage temperature (10, 20 and 30 °C) and C – storage time (0, 15 and 30 days), D – ascorbic acid concentration (0, 100 and 200 mg/100 g). The concentration of anthocyanin and constituent values X, Y, Z, as well as colour parameters in the CIE system ($L^*C^*h^*$) were assumed as dependent variables (responses). The experiment was optimised with response surface methodology.

2.3. Sample preparation and carrying out the experiment

The colorant preparation was dissolved in buffers of pH 3.0, 4.0 and 5.0 and the obtained solutions were characterised using anthocyanin concentrations of 20 mg/100 g. Samples of identical volume of 10 cm^3 were pasteurised in sealed glass vials at 85 °C for 15 min. Two volumes of vial were used: 10 cm^3 (relatively anaerobic conditions) and 20 cm^3 (aerobic conditions). Pasteurised samples were stored under in conditions complying with the experimental design. At the end of the storage period samples were assessed on the basis of anthocyanin concentrations and values of colorant constituents X, Y, Z and colour parameters L^*, C^*, and h^*.

2.4. Determination of the total anthocyanin concentrations

The concentration of anthocyanins was determined employing the Fuleki and Francis method of differential spectrophotometry (1968). Absorbance measurements were performed using the Hitachi U 3000 spectrophotometer, at 520 nm. Samples were diluted with pH 1.0 and 4.5 buffers, so that the absorbance value for the pH 1.0 value did not exceed the range of 0.3–0.8. Buffer with pH 1.0 was used as the reference sample.

2.5. Instrumental colour measurement

Colour measurement was carried out using the Hitachi U 3000 spectrophotometer over the range of visible wavelength of 380–780 nm at a scanning speed of 600 nm/min, 2 mm. Distilled water was treated as the zero sample.

The colour was determined using the CIE system $L^*C^*h^*$, where L^* designates the colour lightness, C^* the colour saturation and h^* the angle of colour tone.

2.6. Sensory evaluation

Sensory evaluation was carried out using a numerical scale (Baryłko-Pikielna, 1975). Colour intensity, naturalness and desirability were evaluated on a 10-point scale. In addition, a descriptive evaluation of the colour tone was carried out, which comprised the general name of the colour tone (e.g., red, yellow-red), as well as the comparative name associated with a food product (e.g., cherry, tea-like).

2.7. Statistical analysis of results

On the basis of the obtained results, the authors ascertained the significance of the effect of the determined factors on the examined responses as well as the significance of interactions occurring between the examined factors. The fitting of the model to data was carried out using the Lack-of-Fit Test. Correlations between the examined factors were expressed as equations of the response surface. This allowed the creation of 3-dimensional diagrams showing the parallel impact of two factors with the remaining factors constant (Gacula, 1993). Actual and code values of levels of the examined factors are shown in Table 1.

Table 1
Levels of examined factors according to Box-Behnken plan

Experimental factor	Code values			Actual values		
(A) pH	−1	0	+1	3.0	4.0	5.0
(B) Storage temperature [°C]	−1	0	+1	10	20	30
(C) Storage time [days]	−1	0	+1	0	15	30
(D) Ascorbic acid concentration [mg/100 g]	−1	0	+1	0	100	200

3. Results and discussion

In the course of storage of prepared solutions from red cabbage, degradation of anthocyanin colorants occurred. Colorant losses increased with increasing pH, temperature, the length of sample storage time and the concentration of ascorbic acid (Fig. 1). Moreover, colorant losses were higher in samples stored under aerobic conditions than under relatively anaerobic ones. Under anaerobic conditions, in samples without the addition of ascorbic acid, anthocyanin losses, after 30-day storage at 20 °C, amounted to 7% at pH 3.0 and 33% at pH 5.0, whereas in samples with the addition of ascorbic acid (100 mg/100 g) losses were 55% and 75%, respectively. Under aerobic conditions, of anthocyanin losses in samples without the addition of ascorbic acid ranged from 46% at pH 3.0 to 70% at pH 5.0, while in samples with the addition of ascorbic acid (100 mg/100 g) 66% and 77%, respectively. These results are in keeping with data published by other researchers who reported that anthocyanins showed the highest stability at pH 3.0 or lower and above this level increased degradation of colorants occurred, especially during heating or storage at non-refrigeration temperatures (Dyrby et al., 2001; Sapers et al., 1981; Shi et al., 1992). The negative impact of ascorbic acid on the anthocyanin content was probably associated with the development of H_2O_2 during the oxidation of ascorbic acid to dehydroascorbic acid. Ascorbic acid acts as an activator for molecular oxygen, leading to the development of free radicals, which disrupt the pyrylium ring (Iacobucci & Sweeny, 1983; Garcia-Viguera & Bridle, 1999).

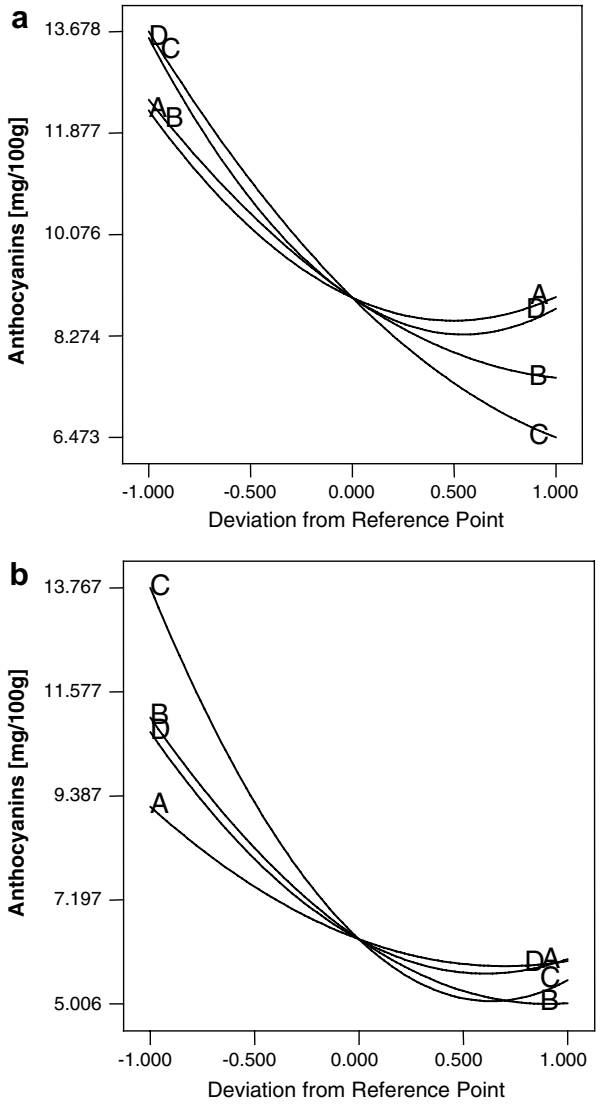

Fig. 1. Cross-sections through response surfaces for changes in anthocyanin concentrations during storage of preparation solutions from red cabbage at the code value of the remaining variables equalling 0. Designations: A – pH, B – storage temperature, C – storage time, D – ascorbic acid concentration. (a) Relatively anaerobic conditions and (b) aerobic conditions.

Despite high colorant losses during storage of red cabbage preparation solutions, their colour intensities were scored from 7.3 to 10.0 during sensory evaluation. However, in relation to storage conditions, the solutions possessed very diverse colour tones, which were described as cherry or blackberry at pH 3.0 and 4.0 and tea-like only at high ascorbic acid concentrations, and as blueberry at pH 5.0. After long storage at high temperature, the tones were described as typical for cooked red cabbage or even herbal tea. The latter samples received the lowest desirability scores for colouring fruit juices (at the level of 2–3 scores), while samples with pH 3.0 were given scores above 8 and frequently 10, showing the potential for the application of red cabbage preparation in cherry, blackberry and blueberry juices and beverages, which are stored at low temperature.

On the basis of coefficient values of response surface equations, it was found that storage time and temperature exerted the strongest impact on the concentration of anthocyanins, while the influence of pH, as well as the concentration of ascorbic acid were less apparent, albeit statistically significant (Table 2). In the majority of samples, it was possible to observe a significant influence of the interaction of temperature and ascorbic acid concentrations with storage time.

Changes in the anthocyanin content were reflected in value changes of colour constituents and parameters. During the storage of solutions of the red cabbage colorant, values of the X, Y and Z colour components increased, which were associated with the decline in the colour intensity (Fig. 2). The only exception were samples at pH 5.0 supplemented with ascorbic acid and stored at 30 °C, in which X, Y and Z values declined. This drop could probably be attributed to the accumulation of brown products of anthocyanin degradation. A definite drop in the Z-value of many samples was observed in aerobic conditions. On the basis of response surface equations it was found that all the examined factors exerted a statistically significant impact on the X and Y constituents, whereas the Z constituent was affected only by pH and ascorbic acid concentration.

Table 2
Characteristics of response surface equations for anthocyanin concentrations

Oxygen availability	Model	Intercept	A	B	C	*D*	A^2	B^2	C^2	D^2	AB	AC	AD	BC	BD	CD
Anaerobic	2	10.67	**−1.88**	**−2.18**	**−5.15**	**−2.43**	0.52	0.16	0.68	**1.45**	**−0.06**	−0.43	0.07	**−1.77**	**−1.38**	**−2.31**
	2	8.90	**−1.65**	**−2.47**	**−3.60**	**−2.40**	**1.67**	1.04	1.12	**2.21**	0.56	0.46	0.80	−1.54	−1.29	**−2.09**
Aerobic	2	6.37	**−1.63**	**−3.01**	**−4.13**	**−2.39**	1.16	1.66	**3.26**	**1.97**	0.94	−0.07	−0.24	**−2.72**	−1.20	**−2.90**
	2	6.25	**−1.83**	**−3.20**	**−4.03**	**−2.55**	1.15	1.76	**4.41**	**1.89**	1.27	−0.24	0.64	**−3.36**	−1.60	−2.25

Anthocyanins concentration = $A + B + C + D + A^2 + B^2 + C^2 + D^2 + AB + AC + AD + BC + BD + CD$.
Factors statistically significant at $p = 0.05$ are shown in bold type.
Model-degree of polynominal.
A – pH.
B – storage temperature [°C].
C – storage time [day].
D – ascorbic acid concentration [mg/100g].

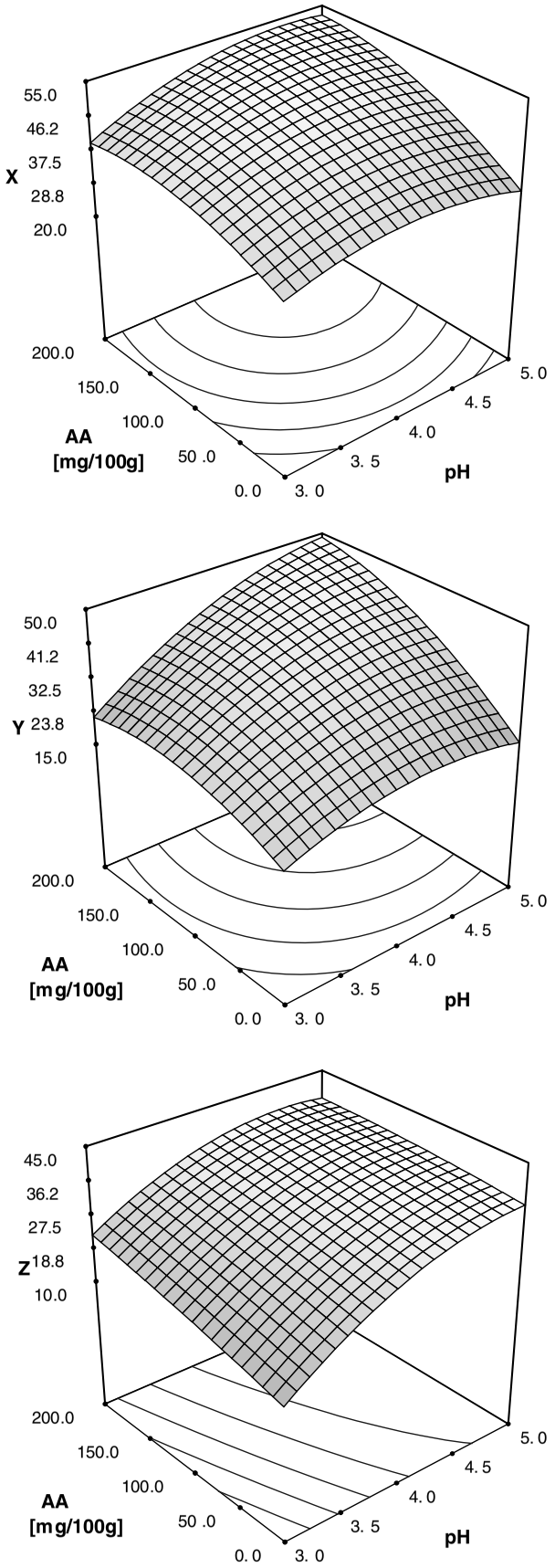

Fig. 2. Response surfaces in relatively anaerobic conditions for colour component changes during storage of preparation solutions from red cabbage at the code value of the remaining variables equalling 0.

The colour of the examined solutions was also determined using the $L^*C^*h^*$ system. During storage of the red cabbage preparation, the value of L^* parameter increased together with the increase in the value of all the examined factors. However, in the case of samples stored at the highest level of all the examined factors, a drop in L^* was recorded under aerobic conditions, which reflected changes in the constituent Y. During storage, the colour saturation (C^*) decreased with the increase in values of the examined factors, with the exception of some samples characterised by high pH values in which the C^* value increased. Changes in the h^* parameter describe the direction of changes of the colour tone. During storage, the h^* value increased with an increase in the level of independent variables (storage time and temperature, as well as ascorbic acid concentration Fig. 3), This indicates the change of the colour towards yellow, which is associated with the development of products of anthocyanin degradation, especially in the presence of ascorbic acid (Garcia-Viguera & Bridle, 1999). However, when considering the influence of pH it was found, on the basis of the coefficient values of the surface response equations and diagrams (Fig. 3), that there was a decline in the angle value of the colour, within the range of pH from 3.0 to 5.0, indicating a colour change towards red-violet with an increase of pH. Similar data were reported by Cevallos-Casals and Cisneros-Zevallos (2004) in their studies on the stability of colorants derived from purple corn and red-fleshed sweet potato. The bathochromic effect, i.e., the shift of the absorption maximum to a higher of wavelength, in a solution of red cabbage colorant was also reported by Sapers et al. (1981). The same direction of changes of the colour tone was corroborated by results of sensory evaluation, which showed that in the initial samples after pasteurisation, the tone changed from pink-red at pH 3.0, through pink-violet at pH 4.0 to violet at pH 5.0, as reflected by the angle drop of the colour tone h^*.

When analysing the results of organoleptic assessment it was found that naturalness and desirability scores declined during the storage of solutions. However, with the exception of some samples at pH 5.0, those changes were small because scores always exceeded 7 points, while for samples at pH 3.0, the scores were often as high as 10 points. Coefficient values of surface response equations for colour naturalness and desirability indicated that it was pH value that exerted the strongest effect on these variables.

In the performed experiment, for the majority of responses (dependent variables), the authors obtained data fit to quadratic equations, which, on the basis of the F-test, were significant below 0.0001 (Table 3). High correlation coefficients for the expected values ($r^2 > 0.9$) confirm a good fit of the model to data, although low values of the Lack-of-fit test limit the possibilities of predicting response values on the basis of the obtained equations.

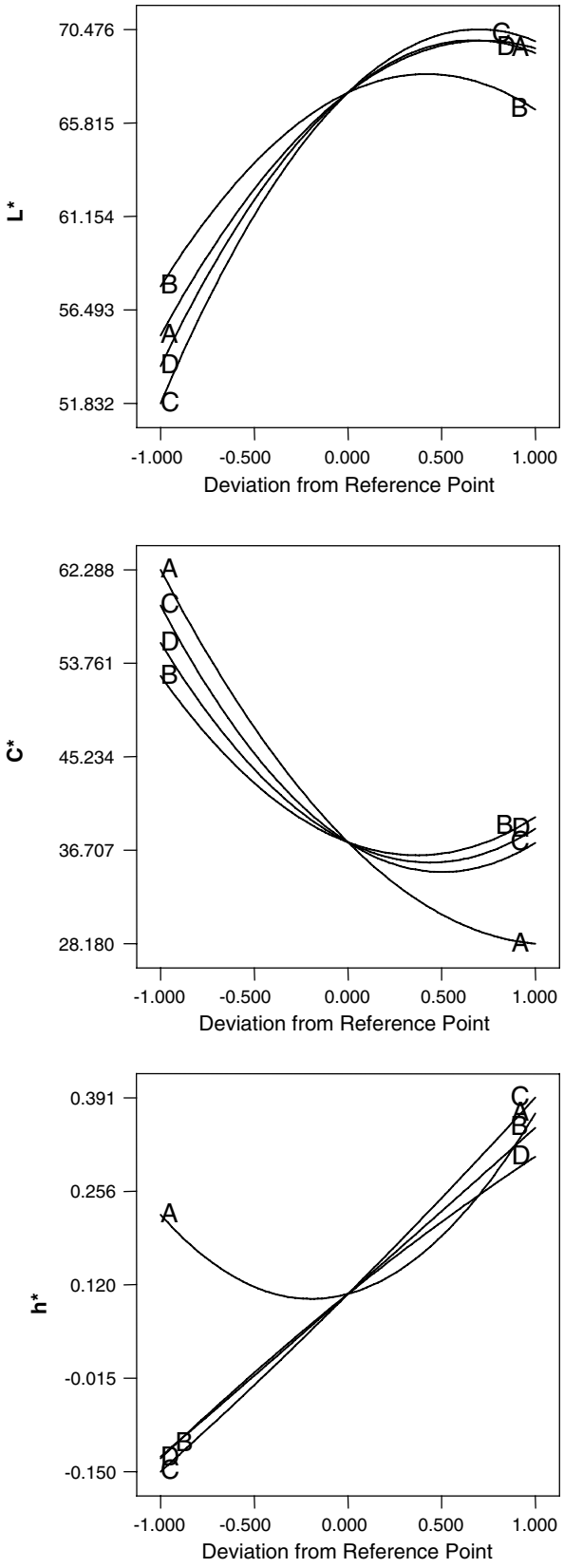

Fig. 3. Cross-sections through response surfaces for changes in parameters L^*, C^*, h^* during storage in relatively anaerobic conditions of preparation solutions from red cabbage at the code value of the remaining variables equalling 0. Designations: A – pH, B – storage temperature, C – storage time, D – ascorbic acid concentration.

Table 3
Results of statistical analysis for samples analysed under relatively anaerobic conditions

Parameter	Model	Significance of *F*-test	Lack-of-Fit test	r^2	ROOT MSE (%)
X	2	<0.0001	0.0677	0.96	2.55
Y	2	<0.0001	0.1087	0.97	2.74
Z	2	<0.0001	0.0214	0.92	3.33
L^*	2	<0.0001	0.0622	0.97	2.40
a^*	2	<0.0001	0.0684	0.98	3.13
b^*	2	<0.0001	0.0036	0.99	2.01
C^*	2	<0.0001	0.0197	0.97	3,68
h^*	2	<0.0001	0.0145	0.98	0.07
Anthocyanins	2	<0.0001	0.0409	0.94	1.28

Model-degree of data fit to the response surface.

4. Conclusions

During the storage of solutions of red cabbage preparations at 20 mg anthocyanins/100 ml, the content of anthocyanins was found to drop with increases in pH, ascorbic acid concentration, as storage time and temperature. Colorant degradation was greater under aerobic conditions. Anthocyanin losses, considerable at times, were not reflected in the sensory evaluation, as all the samples received high scores of colour intensity and only the tone changed. Depending on pH, the colours of the solutions from the red cabbage preparation were described either as cherry, blackberry or blueberry juice. Colour was stable during storage, especially at lower temperatures. Changes in colour parameters were, in many cases, also dependent on pH.

Because of its wide range of colour tones in relation to pH, as well as high anthocyanin stability, the preparation from red cabbage is a highly recommended food colorant, especially for the production of fruit beverages, as it perfectly imitates the colour of many berry fruits. Its characteristic, rather unpleasant smell of red cabbage constitutes a certain limitation.

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