J. Agric. Food Chem. **1998,** *46,* 4702–4705

Analysis of Biodenitrification Conditions of Red Beet Juice Using the Response Surface Method

Janusz Czapski,*,† Monika Maksymiuk,† and Włodzimierz Grajek‡

Institute of Plant Food Technology, Agricultural University of Poznań, Wojska Polskiego 31, 60-624 Poznań, Poland, and Department of Biotechnology and Food Microbiology, Agricultural University of Poznań, Mazowiecka 48, 60-623 Poznań, Poland

Microbiological denitrification of red beet juice proceeds under conditions that are unfavorable for pigments. In this study the effects of pH, accessible oxygen, and temperature on the stability of red and yellow pigments were examined. All three factors examined affect the content of pigments. The strongest effects were exerted by pH and oxygen access in red beet juice. An interactive effect of these parameters on losses of red pigments was observed. At increased pH of juice, losses of red pigment were very high, reaching 60−80% at pH 8.0, whereas losses of yellow pigments decreased with an increase of pH. An increase of temperature within the range of 25−30 °C did not practically increase losses of pigments and did not influence the color of the juice.

Keywords: *Red beet juice; pigments; betalaines; denitrification*

INTRODUCTION

Excessive accumulation of nitrate in vegetables negatively affects their nutritional value and limits their consumption. Removal of excess nitrate and nitrite from vegetables is difficult. They are extracted in part during hydrothermal treatment. However, in the case of finely disintegrated products, for example, purees and juices, extraction of nitrate is not feasible and has to be replaced by other methods.

One of the most promising methods of denitrification of juices and vegetable homogenates is a biotechnological method based on the reduction of nitrate ions to gaseous nitrogen by denitrifying bacteria. A few descriptions of such solutions are reported in the literature (Kerner et al., 1988; Emig et al., 1990; Gierschner and Hammes, 1991; Reiss, 1992).

Our recent studies on the denitrification of red beet juice have shown that the process changes the organoleptic properties of juice, particularly its color (Grajek et al., 1997). Red beets contain a large amount of red pigments, of which betanine is best known. Betanine is a characteristic pigment of red beets from which it can be isolated and then used for food coloring.

The stability of betacyanines is influenced by temperature, pH, oxygen, light, water activity, enzymes, and some metal ions. The literature data show that pigment decomposition during heating does not proceed according to the kinetics of the first-order reaction and that the reaction order is dependent on the presence of oxygen in the system (Attoe and von Elbe, 1982; Czapski, 1985).

The color of red beet juice is its most characteristic feature. One of the color-measuring methods is the CIE $L^*a^*b^*$ system. The axes L^*, a^*, and b^* determine a three-dimensional color space. Positive and negative a^* values determine the amount of red and green color, respectively, whereas positive and negative b^* values determine the amount of yellow and blue color, respectively (Figure 1). The L^* value is a measure of color lightness, and a^* and b^* values determine color chromaticity, which consists of hue and saturation (chroma).

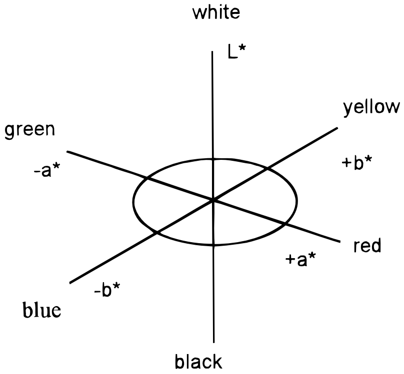

Figure 1. CIE $L^*a^*b^*$ color system.

Microbiological denitrification of juice proceeds under conditions that are unfavorable for pigments. The most destructive factor is high pH, usually >7.0. Considering the cardinal role of pigments in the sensory evaluation of red beet juice, such conditions of juice denitrification should be selected that limit losses of pigments to the maximum. Optimizing computer programs are here extremely effective as they determine quickly and accurately the effect of processing conditions on the attributes of the object examined.

The purpose of this work was to determine the effect of denitrification conditions on the red and yellow pigments and on the color of red beet juice. The importance of the following variables at different levels was determined: pH, accessible oxygen, and temperature.

MATERIALS AND METHODS

Red beet juice was obtained by diluting juice concentrate of the Poparex Co., Nowy Tomysl, Poland, with distilled water at a ratio of 1:4, which corresponds to 15% solids. The juice

---

* Author to whom correspondence should be addressed [fax (+48)61 8 487 314; e-mail czapski@owl.au.poznan.pl].

† Institute of Plant Food Technology.

‡ Department of Biotechnology and Food Microbiology.

10.1021/jf980498c CCC: $15.00

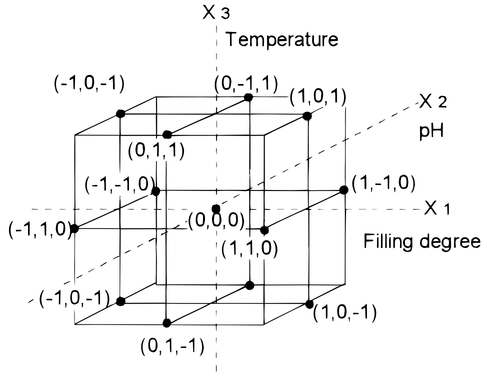

Figure 2. Box−Behnken design.

Table 1. Box−Behnken Experimental Design Arrangement

	coded settings			actual settings		
obsn no.	X_1, filling degree	X_2, pH	X_3, temp (°C)	X_1, filling degree	X_2, pH	X_3, temp (°C)
1	−1	−1	0	0.1	4.0	27.5
2	−1	1	0	0.1	8.0	27.5
3	1	−1	0	1	4.0	27.5
4	1	1	0	1	8.0	27.5
5	0	−1	−1	0.55	4.0	25.0
6	0	1	−1	0.55	8.0	25.0
7	0	−1	1	0.55	4.0	30.0
8	0	1	1	0.55	8.0	30.0
9	−1	0	−1	0.1	6.0	25.0
10	1	0	−1	1	6.0	25.0
11	−1	0	1	0.1	6.0	30.0
12	1	0	1	1	6.0	30.0
13	0	0	0	0.55	6.0	27.5
14	0	0	0	0.55	6.0	27.5
15	0	0	0	0.55	6.0	27.5
16	0	0	0	0.55	6.0	27.5
17	0	0	0	0.55	6.0	27.5

was two-stage ultrafiltered in an Amicon LP-1A apparatus using spiral filters with 100 and 10 kDa cutoff to eliminate microorganisms. The juice was then preserved with sodium azide (1 g/L of juice) and adjusted to a desired pH value with 10% citric acid or 0.1 N NaOH. Aliquots of juice prepared in this way were placed in glass vials of 10 mL volume each. The vials were then sealed and stored at desired temperatures.

The juice was centrifuged at 20000*g* for 10 min, and its content of pigments was determined according to Nilsson's method by measuring the absorbance at 476 nm for yellow pigments, at 538 nm for red pigments, and at 600 nm for disturbing substances (Nilsson, 1975).

The color of the juice was determined in a Hitachi U-300 spectrophotometer at a 1.0 nm slit, 600 nm/min scanning speed, 380−780 nm wavelength range, and C light source. The measurements were carried out in transmitted light. The results are presented in the CIE $L^*a^*b^*$ system.

Experimental Design and Statistical Analysis. The design of experiments and the analysis of results were based on a computer program Design-Expert ver. 4.06 of Stat-Ease Inc., Minneapolis, MN. Designing the experiments by the response surface method assumes that a certain number of experiments will be carried out at certain levels of variables during which the experimenters will at the same time study selected interactions between experimental factors and a given feature of the experimental unit (Gacula, 1993). The number of space dimensions is equal to the number of variables + 1 (response), which in our case corresponded to quadridimensional space.

Upon its calculation, the surface equation allows one to plot the relationship between the factors examined and the response values in three-dimensional space. The surface of each response is represented by a plot, the horizontal axes of which determine the range of two factors and the vertical axis of which is the response examined. In the case of more than two factors being examined, the remaining factors are at a constant level. By cutting the surface along different levels of response, one can obtain a two-dimensional plot, analogical to a topographic map. Each contour represents another response value. Both two- and three-dimensional plots enable one to evaluate the effect of factors under examination or the variable under scrutiny. The contour plot also enables one to select the levels of experimental factors, that is, in our case, the temperature, pH, and filling degree, required to obtain optimal response values.

In the experiments we used three variables at three levels: oxygen access as determined by the degree of filling the vessel with juice (X_1 0.1, 0.55, and 1.0); pH of juice ($X_2 = 4.0$, 6.0, and 8.0); and temperature ($X_3 = 25$, 27.5, and 30 °C). A three-variable Box−Behnken design was arranged. It is a three-level incomplete factorial design formed by combining two-level factorial designs with balanced incomplete block designs in a particular manner (Khuri and Cornell, 1996). The design is displayed in Figure 2. The coordinates of the design points are defined in the coded variables. Table 1 shows coded settings together with the actual settings of independent variables for experiment 1, the results of which are presented in this paper. The other two experiments were conducted for the narrower range of pH, that is, from pH 4.0 to 5.5 and from pH 5.0 to 7.0. All experiments were carried out in a randomized order to minimize any effects of extraneous factors on the responses observed.

The following responses were analyzed: the contents of the red and yellow pigments; coefficient ϕ, defined as the ratio of red pigments to yellow pigments; and color attributes L^*, a^*, and b^*.

RESULTS AND DISCUSSION

Because the contents of red and yellow pigments changed when the pH of the red beet juice was adjusted to the required pH, we analyzed the losses of pigments. Tables 2 and 3 show the estimates of the parameters in surface response equations for pigment losses, and Table 4 shows the estimates for the ratio of contents of red and yellow pigments.

In all cases, pigment losses were best fitted for the second-order surfaces. The models fitted for losses of red pigments were statistically significant by the *F* test at a level below 0.001. The values of lack of fit test were >0.1, indicating a good fit of the models. In the case of most models for yellow pigment losses, the significance of fitting was usually at a level below 0.1, but the predicted values of the determination coefficient R^2 were high, >0.85.

The content of red pigments in all samples decreased during storage. The biggest losses were observed for

Table 2. Regression Coefficients for Fitted Models of Red Pigment Losses[a]

days	intercept	X_1	X_2	X_3	X_1^2	X_2^2	X_3^2	X_1X_2	X_1X_3	X_2X_3
1	21.40	−13.34	22.90	4.34	13.60	−1.27*	−3.18	−11.28	−0.45*	2.73
2	33.14	−20.01	20.34	0.65	1.60	−0.04*	−4.47	−15.45	1.17*	−1.97
3	47.30	−23.74	16.21	1.93	−6.56	−1.16*	−9.89	−16.33	−1.75*	−4.90
4	55.10	−24.78	12.87	3.07	−9.06	−2.76	−11.31	−16.38	−4.57	−6.53
5	55.10	−25.24	10.84	4.43	−9.75	−0.90*	−7.18	−15.90	−0.78*	−4.72

[a] Asterisks (*) indicate nonsignificant coefficients eliminated at $P = 0.1$ by stepwise selections.

Table 3. Regression Coefficients for Fitted Models of Yellow Pigment Losses[a]

days	intercept	X_1	X_2	X_3	X_1^2	X_2^2	X_3^2	X_1X_2	X_1X_3	X_2X_3
1	−18.20	−6.35	−12.96	−2.24	10.35	6.08	9.17	−9.88	−3.53	−0.70*
2	−23.50	−6.70	−12.81	−1.31	13.29	14.81	6.26	−4.75	−2.30*	−5.83
3	−17.88	−5.79	−4.75	−0.16	7.12	5.49	−1.84	−1.22	−1.55*	3.42
4	−19.32	−10.34	2.64	−0.15	16.04	16.04	5.31	−2.60*	−3.28	2.08*
5	−19.38	−13.38	5.3	−0.45	12.47	15.47	8.17	−6.4	−5.20	4.20

[a] Asterisks (*) indicate nonsignificant coefficients eliminated at $P = 0.1$ by stepwise selections.

Table 4. Regression Coefficients for Fitted Models of Red to Yellow Pigments Ratio[a]

days	intercept	X_1	X_2	X_3	X_1^2	X_2^2	X_3^2	X_1X_2	X_1X_3	X_2X_3
1	1.3	0.21	−0.82	−0.15	0.18	−0.15	0.23	0.07*	−0.12	0.01*
2	1.0	0.27	−0.79	−0.01	0.09	−0.01	0.18	0.14	−0.05*	−0.04*
3	0.81	0.34	−0.63	−0.06	0.12	−0.06	0.26	0.14*	0.01*	0.10*
4	0.72	0.33	−0.52	−0.07	0.10	−0.07	0.25	0.21	0.02*	0.12
5	0.72	0.33	−0.45	−0.09*	0.05*	−0.09*	0.23	0.17	0.01*	0.12

[a] Asterisks (*) indicate nonsignificant coefficients eliminated at $P = 0.1$ by stepwise selections.

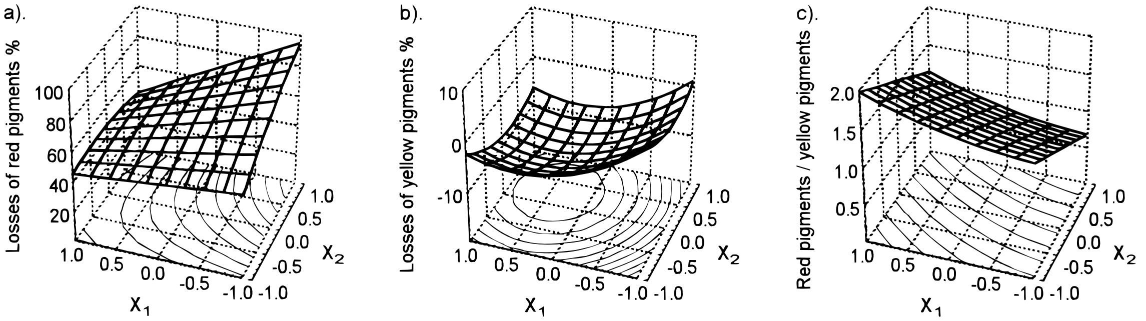

Figure 3. Response surfaces and contour plots for losses of red pigments (a), yellow pigments (b), and red to yellow pigments ratio (c) after 3 days. Coded values: X_1, filling degree; X_2, pH. Temperature (X_3) was fixed at zero coded level.

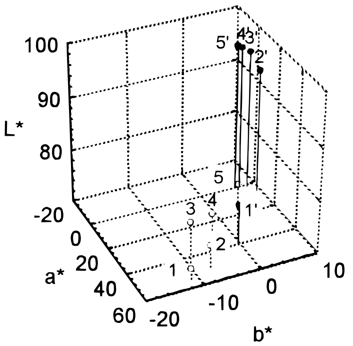

Figure 4. Changes of CIE $L^*a^*b^*$ parameters of red beet juice during storage at 24 °C, filling degree 0.1. (1−5) Days of storage; (○) pH 4; (●) pH 8.

the sample of pH 8 and the filling degrees 0.1 and 0.55. As soon as after 1 day of storage, losses for this sample were 82.4% at 27.5 °C (observation 2) and 61.9% at 30 °C (observation 8). At pH 4 and 6, losses ranged from 1% (observation 10) to 34% (observation 3). The content of red pigments further decreased with storage time.

The content of yellow pigments changed differently; in many cases it increased during storage. At the beginning of storage, that is, during the first 3 days, this phenomenon was particularly evident for the samples of pH 8. Later, the differences among samples decreased markedly.

All three factors examined affected the content of pigments. When parameter estimates in surface response equations are analyzed for losses of red pigments (Table 2), it becomes clear that pH and filling degree, in other words, the amount of available oxygen, had the strongest effect; the effect of temperature, however, remained relatively small. An increase of pH increased pigments losses, whereas an increase of filling degree caused them to decrease (Figure 3a). The effect of these variables changed with storage time: the role of juice reaction was reduced, whereas the role of the amount of air was strengthened. The interaction between pH

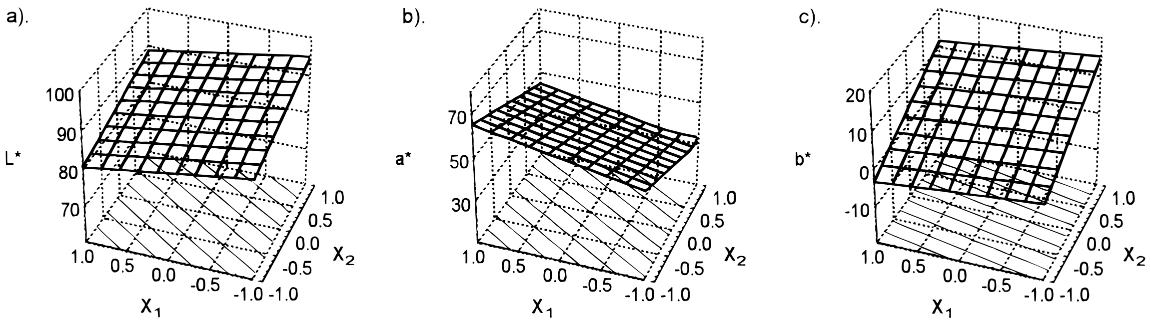

Figure 5. Response surfaces and contour plots for color parameters L^* (a), a^* (b), and b^* (c) after 3 days. Coded values: X_1, filling degree; X_2, pH. Temperature (X_3) was fixed at zero coded level.

value and filling degree was evident, whereas the remaining interactions were considerably weaker, and the interaction between air volume and temperature was frequently insignificant.

pH and filling degree also affected losses of yellow pigments the most. As in the case of red pigments, an increase of filling degree decreased yellow pigment losses (Figure 3b). The opposite was, however, the effect of pH because the losses of yellow pigments decreased with an increase of pH. The increase of yellow pigment content resulted most likely from two factors: With an increase of pH, the content of yellow betaxanthines has been observed to increase (Saguy, 1979; Czapski, 1988). At high pH value, partial decomposition of red pigments occurs, yielding brown pigments. Both groups of pigments are determined simultaneously as their sum in the spectrophotometric method.

The changes of pigment contents also caused the ratio of their content to change (Figure 3c). The ratio of the content of red to yellow pigments, that is, coefficient ϕ, determines in a simplified way the hue of red beet juice color. The value of ϕ for initial juice was 1.4. It decreased during storage, reaching even the value of 0.07 at 30 °C for juice of pH 8 and 0.1 filling degree. The strongest effect on coefficient ϕ was exerted by the pH of juice, followed by filling degree (Table 4). Increases of pH and of the amount of air caused coefficient ϕ to decrease. The effect of temperature was considerably smaller compared to the two factors mentioned above. The hue changed from red-violet to brown-red and brown with a decrease of coefficient ϕ.

Changes in amounts and proportions of pigments were also reflected in the changes of color (Figure 4). Because of degradation of red pigments, lightness L^* and the value of a^*, indicative of red color, decreased and the value of b^* increased, which corresponded to an increased proportion of yellow color.

Similarly as in the case of pigment losses, the changes of color attributes were influenced the most by pH and filling degree, with temperature having a small effect (Figure 5), frequently insignificant at 0.1 confidence level. For color attributes, the significance of the value of lack of fit test was below 0.001; thus, satisfactory approximation was not obtained. Best fitting was obtained for first-order models. Tendencies seen in Figure 4 were characteristic of all days of sample storage.

In the remaining two series of experiments, red beet juices under investigation differed with respect to pigment content. The character of surface responses was the same. The best fitting of the model was obtained also for red pigment losses. Despite a decreased pH range, the fit of the model for yellow pigment losses and color attributes has not been improved.

At increased pH of red beet juice losses of red pigments are significant even at poor access of air. Interaction between pH and oxygen access is observed for losses of red pigments. It is recommended to biodenitrify red beet juice under anaerobic conditions and at possibly low pH values. Red beet juice denitrification under the conditions of no access of air, temperature of 25−30 °C, and pH level close to 6 allows one to obtain juice of well-preserved color.

LITERATURE CITED

Attoe, E. L.; von Elbe, J. H. Degradation kinetics of betanine in solutions as influenced by oxygen. *J. Agric. Food Chem.* **1982**, *30*, 708−712.

Czapski, J. The effect of heating conditions on losses and regeneration of betacyanins. *Z. Lebensm. Unters. Forsch.* **1985**, *180*, 21−25.

Czapski, J. The effect of selected factors on the stability of betacyanins in the juice of red table beets (Polish; English abstract). *Rocz. Akad. Roln. Poznan.* **1988**, *169*, 5−62.

Emig, J.; Meisel, C.; Wolf, G.; Gierschner, K.; Hammes, W. P. Reduction of nitrate and nitrite in vegetable juices prior to lactic acid fermentation. *Food Biotechnol.* **1990**, *4*, 575−577.

Gacula, M. C., Jr. Product optimization. In *Design and Analysis of Sensory Optimization*; Food and Nutrition Press: Trumbull, CT, 1993; pp 105−235.

Gierschner, K.; Hammes, W. P. Mikrobiologische Nitrat Entfernung aus Gemüesaften bzw. Gemüseflüssigprodukten. *Fluess. Obst* **1991**, *58*, 236−239.

Grajek, W.; Walkowiak-Tomczak, D.; Czapski, J. Changes in pigment content in red beet juice denitrified by *Paracoccus denitrificans*. *Polish J. Food Nutr. Sci.* **1997**, *6/47*, 35−42.

Kerner, M.; Mayer-Miebach, E.; Rathjen, A.; Schubert, H. Verringernung des Nitrargehaltes pflanzlicher Lebensmitteln−Verfahrenskonzepte bei Verwendung immobilisierter Mikroorganismen. *Z. Lebensm.-Technol. -Verfahrenstechnik*. **1988**, *39*, 564−570.

Khuri, A. I.; Cornell, J. A. Second-order models and designs. In *Response Surfaces. Designs and Analysis*; Dekker: New York, 1996; pp 107−146.

Nilsson, T. Studies into the pigments in beetroot. *Lantbrukshoegsk. Ann.* **1975**, *36*, 179−184.

Reiss, J. Reduction of nitrate in carrot juice by immobilized cells of *Staphylococcus carnosus*. *Dtsch. Lebensm. Rundsch.* **1992**, *88*, 352−353.

Saguy, I. Thermostability of beet pigments (betanine and vulgaxanthin-I): Influence of pH and temperature. *J. Food Sci.* **1979**, *44*, 1554−1557.

Received for review May 13, 1998. Revised manuscript received August 25, 1998. Accepted August 26, 1998. This work was supported by the State Committee for Scientific Research (Poland) Grant 5 S 307 041 04.

JF980498C