Food Sci. Biotechnol. Vol. 15, No. 1, pp. 13 ~ 18 (2006)

Food Science and Biotechnology

Continuous High Pressure Carbon Dioxide Processing of Mandarin Juice

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Abstract Mandarin juice was processed using a continuous high pressure CO_2 system. Response surface methodology was used to investigate the effects of the processing parameters such as temperature, pressure, residence time, and %(w/w) ratio of CO_2 to juice on total aerobic count (TAC), pectinesterase (PE) activity, cloud level, °Brix, pH, and titratable acidity (TA) of the juices. Maximum log reduction (3.47) of TAC was observed at 35°C, 41.1 MPa, 9 min residence time, and 7% CO_2. PE was inactivated by 7-51%. The cloud was not only retained but was also enhanced by 38%. Lightness and yellowness increased, and redness decreased. The processing temperature and % CO_2/juice ratio significantly affected high pressure CO_2 processing of the juice in terms of pasteurization, PE inactivation, cloud increase, and color change. The °Brix, pH, and TA before and after treatment remained unchanged.

Keywords: mandarin juice, high pressure CO_2 processing, sterilization, quality change

Introduction

Mandarin cultivar 'Murcott' is harvested from January to March and has an excellent eating-quality. Even though the processing of juice with heat treatment is particularly safe due to total inactivation of microbes through irreversible cell damage, it causes undesirable changes in quality including degradation of nutrients and organoleptic attributes (1).

High pressure carbon dioxide treatment is receiving a great deal of attention for the inactivation of microorganisms and enzymes in thermally sensitive food processing. Several factors in high pressure carbon dioxide processing are attributed to inactivation of some microorganism and enzymes by a pH decrease and/or by the extraction of intracellular substances from the cell (2). When CO_2, which is non-toxic and environmentally safe compared with organic solvents, is sparged into the juice, it dissolves in the endogenous water of the juice, which acidifies the solution. This lowered pH is likely to irreversibly inhibit essential metabolic functions (3). On release of pressure, CO_2 is evolved and the final pH of the juice remains the same (4). Another possible cause of inactivation is the extraction of intracellular materials from the cell. The CO_2 extracts some of the vital cell constituents and thereby lethally disturbs or unbalances the biological system of the cells and promotes inactivation (5).

Previous work on the processing of orange juice using a continuous high pressure carbon dioxide system in a 12.2 m holding tube was performed by Kincal (6). This system can destroy microorganisms while preserving sensory attributes, and improve cloud retention despite residual pectinesterase (PE) activity. However, no research work on mandarin juice using a continuous high pressure carbon dioxide system has been performed. Mandarin juice has distinctive flavor, color, aroma (7), and natural microbial flora compared with orange juice.

Response surface methodology can be used to comprehensively examine various parameters with minimum experimental effort, and determine the most relevant factors and their influence ranges as well as interactions among the factors. Multiple regressions, a mathematical relationship between responses and independent variables, can be applied to determine the effects of the processing conditions (8, 9).

The objective was to investigate the effects of processing temperature, pressure, residence time, and ratio of CO_2 to juice on pasteurization of microorganisms, inactivation of PE, and quality of mandarin juice using response surface methodology.

Materials and Methods

Mandarin juice Frozen unpasteurized mandarin juice was purchased from Blue Lake Citrus, Inc. (Winter Haven, FL, USA). It was thawed at room temperature for 36 hr and then placed in a refrigerator (4°C) for 12 hr before high pressure CO_2 treatment to increase its microbial count.

Experimental design Twenty-eight experiments were performed according to a Box-Behnken design (8) with 4 variables and 3 levels. The independent variables were processing temperature, processing pressure, residence time, and %(w/w) ratio of CO_2 to juice. The coded and uncoded values are given in Table 1. Log reduction of total aerobic count (TAC), % PE inactivation, % cloud increase, and color were chosen as the dependent variables. For creating response surfaces, the experimental data were fitted into a second order polynomial equation of the following form.

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Received April 19, 2005; accepted November 19, 2005

Table 1. Coded and uncoded values for independent variables

Run	Coded				Uncoded			
No.	X_1	X_2	X_3	X_4	Temperature (°C)	Pressure (MPa)	Residence time (min)	% CO2 /juice ratio
1	-1	-1	0	0	25	13.8	7	7
2	-1	+1	0	0	25	41.4	7	7
3	+1	-1	0	0	45	13.8	7	7
4	+1	+1	0	0	45	41.4	7	7
5	-1	0	-1	0	25	27.6	5	7
6	-1	0	+1	0	25	27.6	9	7
7	+1	0	-1	0	45	27.6	5	7
8	+1	0	+1	0	45	27.6	9	7
9	-1	0	0	-1	25	27.6	7	2
10	-1	0	0	+1	25	27.6	7	12
11	+1	0	0	-1	45	27.6	7	2
12	+1	0	0	+1	45	27.6	7	12
13	0	-1	-1	0	35	13.8	5	7
14	0	-1	+1	0	35	13.8	9	7
15	0	+1	-1	0	35	41.4	5	7
16	0	+1	+1	0	35	41.4	9	7
17	0	-1	0	-1	35	13.8	7	2
18	0	-1	0	+1	35	13.8	7	12
19	0	+1	0	-1	35	41.4	7	2
20	0	+1	0	+1	35	41.4	7	12
21	0	0	-1	-1	35	27.6	5	2
22	0	0	-1	+1	35	27.6	5	12
23	0	0	+1	-1	35	27.6	9	2
24	0	0	+1	+1	35	27.6	9	12
25	0	0	0	0	35	27.6	7	7
26	0	0	0	0	35	27.6	7	7
27	0	0	0	0	35	27.6	7	7
28	0	0	0	0	35	27.6	7	7

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_4+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2+\beta_{44}X_4^2+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{14}X_1X_4+\beta_{23}X_2X_3+\beta_{24}X_2X_4+\beta_{34}X_3X_4$$

where, Y is the log reduction of TAC (log (n_0/n)), % PE inactivation, % cloud increase or color value, X_1 is processing temperature (°C), X_2 is processing pressure (MPa), X_3 is residence time (min), X_4 is % CO_2/juice ratio, and β_0, ... β_{34} represent the estimated regression coefficients. Statistical analysis was performed using Statistical Analysis System software (Version 8.01, SAS Institute Inc., Carry, NC, USA).

High pressure CO_2 treatment The continuous high pressure CO_2 processing system (Praxair, Chicago, IL, USA) was used in this work. The juice was pressurized to 6.89 MPa using a reciprocating pump. CO_2 was also introduced by a reciprocating pump at the same pressure. The CO_2/juice mixture was then passed through a reciprocating pump to reach the operating pressure. This pressure was maintained throughout the holding tube (79.2 m length, 0.635 cm ID) for a specified residence time, which was adjusted by the flow rate of the mixture. The mixture then exited the holding tube, was depressurized by passing through a back pressure regulator, and collected in sterile bottles.

Operating parameters were set for each treatment and 3.5 L of juice were allowed to pass to insure steady state conditions before sample collection. The flow rates of juice and CO_2 were measured by mass flowmeters. TAC, PE activity, cloud, color, °Brix, pH, and titratable acidity (TA) of the juices before and after treatment were analyzed. The detailed experimental procedure can be found elsewhere (10).

Analysis of treated samples

Total aerobic counts All juice samples were plated on 3M Petrifilm (3M, St. Paul, MN, USA) for enumeration of TAC colonies. The juice was diluted serially using 90 mL dilution bottles containing Butterfield's phosphate buffer (Hardy Diagnostics, Santa Maria, CA, USA). One milliliter of dilutions was plated in duplicate. All the plates were incubated at 35°C for 2 days and enumerated. The log reduction is expressed as log (n_0/n), where n_0 and n are the initial and posttreatment microbial counts, respectively. All the values reported are the arithmetic means of two or four replicates.

Pectinesterase activity PE activity was measured by the method of Rouse and Atkins (11). After pouring 25 mL of 1% pectin solution into a 100 mL beaker, the mixture was stirred continuously while 1 mL of juice was added and then the pH was adjusted to 7.50 with 0.1 N and/or 0.01 N NaOH solutions. The titration process was started by initiating the automatic titrator (Brinkman Instruments Co., Herisau, Switzerland) and computer. The computer software was used to calculate the slope of NaOH titrated (y-axis) versus reaction time (x-axis) necessary to maintain the pH at 7.50 and determined the PE activity according to the following definition of % PE inactivation:

$$\% \text{ PE inactivation} = \frac{PE_{original}-PE_{treated}}{PE_{original}}\times 100$$

Cloud determination A sample of 1.50 mL of juice was pipetted into a 1.5 mL microcentrifuge tube and placed in an Eppendorf centrifuge (Model 5415, Brinkman Instruments, Inc., Westbury, NY, USA). The sample was then centrifuged at 320×g for 10 min at room temperature. Absorbance of the supernatant at 660 nm was recorded as the cloud value with distilled water serving as blank (12). Percentage cloud increase was calculated from the following equation:

$$\% \text{ Cloud increase} = \frac{cloud_{treated}-cloud_{original}}{cloud_{original}}\times 100$$

pH, °Brix, Titratable acidity, and color After placing 20 mL of juice in a 50 mL beaker with a magnetic stirrer bar, the pH and °Brix level of the juice were measured using a pH meter (Metrohm 632, Brinkman Instruments

Co., Herisau, Switzerland) and a digital refractometer (range 0-18°Brix, Fisher Scientific, Pittsburgh, PA, USA) with temperature correction, respectively. For measurement of TA, 20 mL of juice was placed into a 50 mL flask and titrated against standardized 0.1 N NaOH to the phenolphthalein end point (pH 8.2). The volume of NaOH was recorded and converted to g citric acid per 100 mL. Color analysis was performed using a Colorgrad 14 system (Colorgrad system 105, BYK-Gardner Inc., Columbia, MD, USA). The reflectance was measured with a 50 mL sample of juice in a sample cup (glass, 57 mm diameter).

Results and Discussion

Total aerobic counts TAC results for untreated and treated juices are shown in Table 2. In order to analyze the data, a response surface regression model was applied. The statistical analysis showed that the quadratic model fit was statistically significant ($p<0.05$), and that there was a satisfactory correlation between the actual and fitted values ($R^2 = 0.83$) (Table 3). Dropping the terms that were not significant at the 0.05 level, the following regression equation was obtained:

$$\text{Log reduction of TAC} = -14.3627 + 0.5297\ X_1 + 0.8329\ X_4 - 0.0052\ X_1^2 - 0.0265\ X_4^2$$

The terms in the model represent the linear effects and the second-degree effects of processing temperature and % CO_2/juice ratio. Temperature and percent CO_2 had a significant impact on log reduction of TAC.

Meanwhile, the coefficients for pressure and residence time were not statistically significant in this work. Pressure independence can be an advantage because a higher pressure processing system can lead to the problem of extracting flavor from the juice, due to the enhanced solvating power of supercritical CO_2 under higher pressure. A higher pressure system also requires high investment cost. The independent residence time also suggested that equilibrium was reached quickly when CO_2 was mixed with the juice in the processing hold-coil of the system tested.

The absence of any significant effect of treatment pressure and residence time on the log reduction of TAC in the experimental results indicates that one of the major mechanisms in the inactivation of microorganism was the expansion of a large quantity of CO_2 in the juice during decompression. Lin *et al.* (5) assessed the effect of pressure alone on sterilization of *Leuconostoc dextranicum* at 69 and 207 MPa for 20 and 40 min at 35°C without CO_2, and found that there was no lethal effect with a series of pressure release and re-pressurization cycles during an experiment. This was because the expansion volume of the liquid alone during decompressions was smaller than that of the gas.

Based on the above results, the high pressure CO_2 processing system reduced the TAC of the natural flora in mandarin juice by about three orders of magnitude. Maximum log reduction (3.47) of TAC was observed at the conditions of 35°C, 41.1 MPa, 9 min residence time and 7% CO_2/juice ratio. However, a complete sterilization could not be obtained in any of the cases. Thus, inactivation of microorganisms was not satisfactory because at least a 5-log pathogen reduction was required to prevent disease outbreaks (13).

Table 2. Total aerobic counts, pectinesterase, and cloud in the juice after high pressure CO_2 treatment

Run	Total aerobic counts		Pectinesterase		Cloud	
No.	CFU /mL	Log (n_0/n)	unit /mL	% inactivation	OD at 660 nm	% increase
Control	3.3×10^5	-	1.30	-	1.38	-
1	1.4×10^4	1.37	1.15	11.5	1.40	1.4
2	1.1×10^3	2.47	1.11	14.6	1.28	-7.2
3	2.7×10^2	3.08	0.79	39.2	1.65	19.5
4	6.8×10^2	2.68	0.64	50.7	1.55	12.3
5	6.7×10^3	1.69	1.21	6.9	1.07	-22.4
6	9.4×10^2	2.54	1.16	10.7	1.41	2.1
7	4.5×10^3	1.86	0.82	36.9	1.78	28.9
8	5.4×10^2	2.78	0.70	46.1	1.77	28.2
9	5.7×10^4	0.76	1.21	6.9	1.70	23.1
10	5.6×10^3	1.77	1.17	10.0	1.06	-23.1
11	9.5×10^2	2.54	1.15	11.5	1.91	38.4
12	3.2×10^3	2.01	0.76	41.5	1.63	18.1
13	1.3×10^3	2.40	1.07	17.6	1.82	31.8
14	3.6×10^2	2.96	1.09	16.1	1.77	23.1
15	4.0×10^2	2.91	1.17	10.0	1.59	15.2
16	1.1×10^2	3.47	1.08	16.9	1.62	17.3
17	1.2×10^4	1.43	1.22	6.1	1.86	34.7
18	1.9×10^2	3.23	1.11	14.6	1.50	8.6
19	7.0×10^3	1.67	1.21	6.9	1.79	29.7
20	2.4×10^2	3.13	1.05	19.2	1.39	0.7
21	2.3×10^4	1.15	1.21	6.9	1.79	29.7
22	5.1×10^2	2.81	1.11	14.6	1.43	3.6
23	1.7×10^4	1.28	1.22	6.1	1.90	37.6
24	6.7×10^2	2.69	1.02	21.5	1.49	7.9
25	1.7×10^2	3.28	1.06	18.4	1.64	18.8
26	6.9×10^2	2.67	1.03	20.7	1.70	23.1
27	5.5×10^2	2.77	1.03	20.7	1.69	22.4
28	4.7×10^2	2.84	1.03	20.7	1.65	19.5

ABC research Corp. (Gainesville, FL, USA) identified the residual colonies after treatment of mandarin juice at 45°C, 41.4 MPa, 9 min residence time and 12% CO_2/juice ratio. They found that *Stenotrophomona maltophilia* (a kind of *Pseudomonas spp.*) was the only organism growing on the petrifilm after high pressure CO_2 treatment. Further work is needed to investigate the inactivation kinetics of this microorganism.

Figure 1 shows the simultaneous effects of processing

Table 3. Regression coefficients of the 2nd order polynomial model for log reduction of total aerobic counts, pectinesterase, and cloud

Factors	Total aerobic counts		% PE inactivation		% Cloud increase	
	Coeff	*p*-value	Coeff	*p*-value	Coeff	*p*-value
Constant	-14.3627	0.0177	88.8672	0.1561	-175.6462	0.0334
X_1	0.5297	0.0045	-5.5606	0.0067	10.0280	0.0005
X_2	0.0782	0.4293	-0.9091	0.4106	-0.4131	0.7623
X_3	0.6793	0.3959	3.0275	0.7313	10.4133	0.3522
X_4	0.8329	0.0057	-0.7860	0.7844	-6.9518	0.0699
X_1^2	-0.0052	0.0102	0.0729	0.0027	-0.1052	0.0009
X_2^2	0.0006	0.4858	-0.0031	0.7669	-0.0107	0.4193
X_3^2	-0.0389	0.3935	-0.5583	0.2771	-0.0166	0.9788
X_4^2	-0.0265	0.0024	-0.3118	0.0016	0.0273	0.7858
$X_1 X_2$	-0.0027	0.1065	0.0152	0.3996	0.0025	0.9094
$X_1 X_3$	0.0008	0.9367	0.0675	0.5851	-0.3150	0.0570
$X_1 X_4$	-0.0077	0.0984	0.1345	0.0153	0.1295	0.0513
$X_2 X_3$	0.0000	1.0000	0.0760	0.3996	0.0978	0.3870
$X_2 X_4$	-0.0012	0.7006	0.0137	0.7000	-0.0105	0.8138
$X_3 X_4$	-0.0062	0.7771	0.1925	0.4390	-0.0900	0.7701

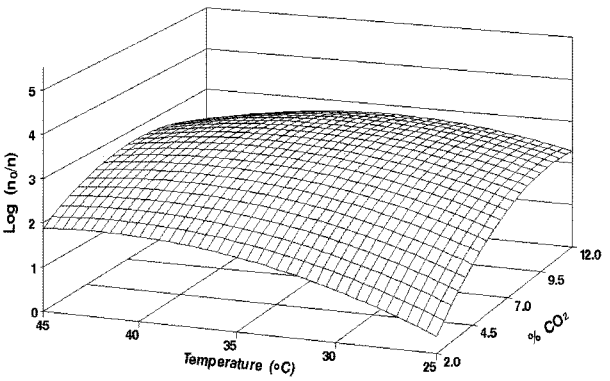

Fig. 1. Fitted response surface for log reduction of TAC with respect to processing temperature and % CO_2/juice ratio (pressure: 27.6 bar, residence time: 7 min).

temperature and % CO_2/juice ratio for log reduction of TAC by response surface with pressure and residence time fixed at their medium values. Log reduction was increased with increasing processing temperature and % CO_2/juice ratio at a processing pressure of 27.6 bar and a residence time of 7 min. However, the effects of processing parameters on log reduction differed depending on various combinations of the four parameters.

Pectinesterase activity Table 2 shows PE activities for untreated and treated samples. The inactivation of PE ranged from 6.1 to 50.7%, with the maximum inactivation being achieved at 45°C, 41.4 MPa, 7 min residence time and 7% CO_2/juice ratio.

The experimental data were fitted into the regression equation. The statistical analysis showed that the quadratic

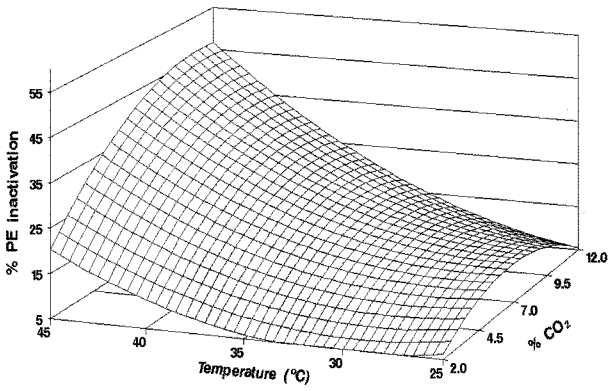

Fig. 2. Fitted response surface for % PE inactivation with respect to processing temperature and % CO_2/juice ratio (pressure: 27.6 bar, residence time: 7 min).

model fit was statistically significant ($p<0.05$), and that there was a satisfactory correlation between the actual and fitted values ($R^2 = 0.93$) (Table 3). Eliminating all insignificant terms ($p>0.05$), the following regression equation was obtained:

$$\% \text{ PE inactivation} = -5.5606\ X_1 + 0.0729\ X_1^2 - 0.3118\ X_4^2 + 0.1345\ X_1X_4$$

The terms in the model represent the linear effect of processing temperature, the second-degree effects of temperature and % CO_2/juice ratio, and an interaction between processing temperature and % CO_2/juice ratio. Inactivation of PE was possible with a combination of higher temperature and higher ratio of CO_2 to juice. Balaban *et al.* (14) demonstrated that the use of supercritical carbon dioxide can inactivate PE and render a stable cloud in orange juice without the use of extremely high pressure. It has also been demonstrated that PE activity is decreased with decreasing pH (15).

Figure 2 shows the effects of processing temperature and % CO_2/juice ratio for % PE inactivation by response surface with pressure and residence time fixed at their medium values. Maximum % PE inactivation was observed at the highest temperature and maximum % CO_2/juice ratio at a processing pressure of 27.6 bar and a residence time of 7 min. However, the effects of processing parameters on % PE inactivation differed depending on various combinations of the four parameters, as shown in Table 2.

Cloud Table 2 shows cloud values for untreated and treated samples. Experimental data showed not only cloud retention but also cloud enhancement. The highest cloud increase was 38.4% at 45°C, 27.6 MPa, 7 min residence time, and 2% CO_2/juice ratio. This finding of enhancement was noteworthy as it was achieved despite the presence of active PE in the juice.

One mechanism of cloud retention by the high pressure CO_2 processing system may involve precipitation of calcium ions in the juice. When CO_2 dissolves in the juice, it combines with water and forms carbonic acid that dissociates into bicarbonate and hydrogen ions at high concentration. When the pressure is released, bicarbonate

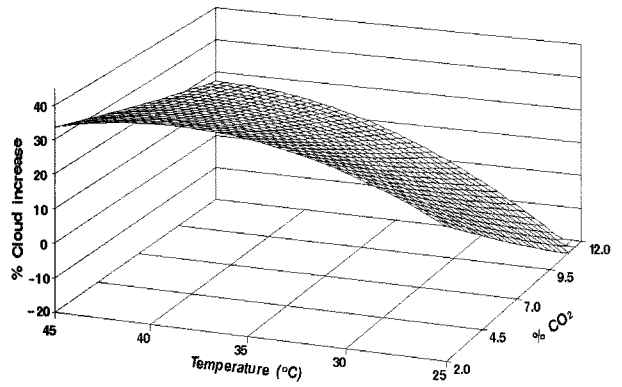

Fig. 3. Fitted response surface for % cloud increase with respect to processing temperature and % CO_2/juice ratio (pressure: 27.6 bar, residence time: 7 min).

is converted to carbonate and may precipitate calcium as calcium carbonate, thus preventing it acting as a bridge between pectic acid chains that leads to cloud loss (6).

The experimental cloud data were fitted into the regression equation. The statistical analysis showed that the quadratic model fit was statistically significant ($p<0.05$), and that there was a satisfactory correlation between the actual and fitted values (R^2 =0.93) (Table 3). Dropping the terms that were not significant at the 0.05 level, the following regression equation was obtained:

$$\% \text{ Cloud increase} = -175.6462 + 10.0280\ X_1 - 0.1052\ X_1^2$$

The terms in the model represent the linear effect and the second-degree effect of processing temperature. Only processing temperature had a significant effect on % cloud increase.

Figure 3 shows the effects of processing temperature and % CO_2/juice ratio for % cloud increase by response surface. Temperature played a major role in increasing cloud in the juice. Maximum % cloud increase was observed at a processing temperature of 45°C and CO_2/juice ratio of 2.0%. The reason why maximum % cloud increase was observed at minimum % CO_2/juice ratio was not clear. However, this phenomenon also differed at different processing conditions.

Color Table 4 shows color values for untreated and treated samples. There was a significant difference in color values between untreated and treated juices. The lightness and yellowness increased and the redness decreased after high pressure CO_2 treatment. This was attributed to the dissolution of pigments by high pressure CO_2 because most of them are non-polar and therefore miscible with CO_2. de Ancos *et al.* (16) and Lim *et al.* (17) reported that high pressure treatment increased the amount of extractable carotenoids in orange juice due to the release of more carotenoids from the food matrix after the irreversible denaturation of protein-carotenoid complexes induced by pressure.

The following equations were proposed by fitting the experimental data of color values. The statistical analysis showed that the quadratic model fit was statistically significant ($p<0.05$), and that there was a satisfactory correlation between the actual and fitted values (R^2 = 0.97, 0.94 and 0.98 for L, a and b values, respectively) (Table 5). The terms in the model represent the linear effects, the second-degree effects and an interaction of each independent variable.

$$L = 62.8173 - 0.6433\ X_1 + 0.6492\ X_4 + 0.0096\ X_1^2 + 0.0014\ X_2^2 - 0.0245\ X_4^2 - 0.0100\ X_2X_4$$

$$a = 17.7612 - 0.2986\ X_1 - 0.6582\ X_4 + 0.0031\ X_1^2 + 0.0246\ X_4^2 - 0.0124\ X_2X_3$$

$$b = 41.6524 - 0.3879\ X_1 - 0.5781\ X_3 + 0.4947\ X_4 + 0.0059\ X_1^2 + 0.0012\ X_2^2 + 0.0382\ X_3^2 - 0.0192\ X_4^2 -$$

Table 4. pH, °Brix, titratable acidity, and color changes for untreated and treated mandarin juices after high pressure CO_2 treatment

Run No.	pH	°Brix	Titratable acidity	Color L, a, b
Control	4.48	14.0	0.356^{g}	50.38, 15.30, 32.79
1	4.49	14.0	0.394^{abc}	53.44, 13.88, 35.40
2	4.49	14.0	0.383^{bcde}	53.09, 13.40, 35.23
3	4.49	14.0	0.381^{cdef}	54.34, 13.32, 36.08
4	4.49	14.1	0.380^{cdef}	53.79, 13.19, 35.70
5	4.49	14.0	0.380^{cdef}	53.41, 13.32, 35.46
6	4.49	14.0	0.383^{bcde}	53.09, 13.51, 35.18
7	4.50	14.0	0.382^{cde}	53.93, 13.06, 35.82
8	4.49	14.1	0.376^{ef}	54.04, 13.76, 35.82
9	4.49	14.0	0.383^{bcde}	52.49, 14.86, 34.57
10	4.49	14.1	0.385^{bcde}	52.45, 13.54, 34.60
11	4.50	14.1	0.380^{cdef}	53.63, 14.70, 35.39
12	4.49	14.1	0.379^{cdef}	53.02, 13.66, 35.13
13	4.48	14.0	0.383^{bcde}	53.16, 12.30, 35.37
14	4.49	14.0	0.378^{def}	53.70, 13.83, 35.63
15	4.48	14.0	0.385^{bcde}	52.61, 12.84, 34.99
16	4.48	14.0	0.387^{abcde}	52.39, 13.00, 34.82
17	4.49	14.0	0.381^{cdef}	52.17, 14.60, 34.37
18	4.49	14.0	0.383^{bcde}	53.06, 12.78, 35.32
19	4.48	14.0	0.384^{bcde}	52.69, 14.41, 34.84
20	4.48	14.0	0.401^{a}	50.81, 12.92, 33.80
21	4.48	14.0	0.392^{abcd}	52.41, 14.40, 34.68
22	4.48	14.0	0.390^{abcde}	51.59, 12.92, 34.35
23	4.49	14.0	0.384^{bcde}	52.25, 14.38, 34.52
24	4.49	14.0	0.387^{abcde}	51.53, 13.45, 34.24
25	4.48	14.0	0.390^{abcde}	52.49, 12.86, 34.90
26	4.48	14.0	0.389^{abcde}	52.35, 13.45, 34.68
27	4.48	14.1	0.367^{gf}	52.35, 13.41, 34.71
28	4.48	14.0	0.397^{ab}	52.78, 13.29, 34.93

The same superscripts in titratable acidity are not significantly different at the 5% level by Duncan's multiple range test.

Table 5. Regression coefficients of the 2nd order polynomial model for color values

Factors	L		a		b	
	Coeff.	*p*-value	Coeff.	*p*-value	Coeff.	*p*-value
Constant	62.8173	0.0000	17.7612	0.0000	41.6524	0.0000
X_1	-0.6433	0.0000	-0.2986	0.0032	-0.3879	0.0000
X_2	0.0224	0.6080	0.0946	0.0882	0.0056	0.8381
X_3	-0.5058	0.1665	0.5529	0.2049	-0.5781	0.0194
X_4	0.6492	0.0001	-0.6582	0.0003	0.4947	0.0000
X_1^2	0.0096	0.0000	0.0031	0.0055	0.0059	0.0000
X_2^2	0.0014	0.0034	-0.0007	0.1317	0.0012	0.0004
X_3^2	0.0346	0.1016	-0.0286	0.2471	0.0382	0.0086
X_4^2	-0.0245	0.0000	0.0246	0.0000	-0.0192	0.0000
$X_1 X_2$	-0.0003	0.6129	0.0006	0.4633	-0.0003	0.4020
$X_1 X_3$	0.0053	0.2853	0.0063	0.2908	0.0035	0.2689
$X_1 X_4$	-0.0028	0.1634	0.0014	0.5559	-0.0014	0.2530
$X_2 X_3$	-0.0068	0.0706	-0.0124	0.0111	-0.0038	0.0995
$X_2 X_4$	-0.0100	0.0000	0.0011	0.4888	-0.0072	0.0000
$X_3 X_4$	0.0025	0.7996	0.0137	0.2563	0.0012	0.8398

0.0072 X_2X_4

pH, °Brix and titratable acidity Table 4 shows the pH, °Brix, and TA of untreated and treated juices. No estimate of variation was given because very little variation was noted between the values of the subsamples. There was no difference in pH of untreated and treated samples, despite the presence of CO_2 dissolved in the juice, because most of the CO_2 was separated from the juice and the pH returned to its original value when the pressure was released from the system. °Brix of the treated juice also was not significantly different from that of untreated juice. Duncan multiple comparison test was applied for statistical analysis of the TA data. Treated samples had a higher TA than the untreated one due to the presence of soluble CO_2. However, there was no difference between treated samples. Arreola (4) and Kincal (6) also showed that use of a high pressure CO_2 system did not change pH and °Brix of orange juice.

Acknowledgments

The authors are indebted to Cheju National University (CNU) for supporting Professor Lim through a grant from the CNU Development Foundation in Korea.

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