

Modeling Colour Degradation of Canthaxanthin Produced by *Dietzia natronolimnaea* HS -1 using Response Surface Optimization: Effect of pH and Treatment Time

Nina Seyedrazi*, Seyed Hadi Razavi and Zahra Emam-Djomeh

Department of Food Science and Engineering, Faculty of Agricultural Engineering and Technology, University of Tehran, P.O. Box 31587-78659, Karaj, Islamic Republic of Iran.

(Received: 20 January 2013; accepted: 04 April 2013)

In this study, natural canthaxanthin (CTX) as one of the most important carotenoids was extracted from *Dietzia natronolimnaea* HS-1. The changes of CTX enriched in oil-in-water emulsions with vegetable oil (5 mg/100 ml), Arabic gum (5 mg/100 ml), and potassium sorbate (0.5 g/100 ml) were investigated. The effects of different pH (3, 5 and 7) and time treatment (3, 18 and 33 days) in the room temperature ($24 \pm 1^\circ\text{C}$) on the colour degradation were studied by response surface methodology (RSM). A second-order polynomial equation fitted to the data was used to predict the responses in the optimal region. The responses were Hunter values (L^* , a^* , and b^*) and CTX concentration (mg/l). Results illustrated more degradation of this pigment at low pHs ($\text{pH} \leq 4$) by passing the time ($\text{days} \geq 10$) with high R^2 of 97.00%, 91.31%, 97.60%, and 99.54% for CTX, L^* , a^* , and b^* respectively. The predicted values were in good agreement with experimental data and thus the model was found to be significant ($p < 0.05$).

Key words: Canthaxanthin degradation, *Dietzia natronolimnaea* HS-1, Emulsion, Response surface methodology (RSM).

Carotenoids are one of the most important and beneficial type of pigments in the nature. Moreover, the diversity of these colours in the vegetables, fruits, marine organizations, and micro organizations is another applicable and useful property of these kinds of pigments (Furr and Clark, 1997). Besides, among over 600 known carotenoids, canthaxanthin [CTX] plays a vital role in protecting the tissues, against free radicals and oxidizing agents. The antioxidant activity, preventing serious health disorders such as breast and lung cancers and cardiovascular diseases are the major responsibilities of CTX (Huang *et al.*, 1992; Furr and Clark, 1997; Liu, 2003; Patel *et al.*, 2004; Mohd Fadzelly *et al.*, 2009; Vitek *et al.*, 2009). The

antioxidant ability of CTX is equal to astaxanthin, higher than α -carotene, zeaxanthin, lutein, β -cryptoxanthin, and lycopene. Also, this characteristic of CTX is 100 times more than tocopherol (Palace *et al.*, 1999). Because of some beneficial aspects mentioned above, CTX is used as an additive in processed food, fruit drinks, baked food and different kind of sausages. Likewise, it is applied in some tan creams for both the beneficial properties and for golden bronze colour created (Gharibzahedi *et al.*, 2012).

Regarding to restriction of synthetic carotenoids in food and cosmetic industries, production of CTX from biological resources has developed recently (Gharibzahedi *et al.*, 2012). Khodaiyan *et al.* (2007) reported that *Dietzia natronolimnaea* HS-1 bacterium is one of the most important sources for microbial production of carotenoids especially CTX. They showed that *D. natronolimnaea* HS-1 compared to the levels of

* To whom all correspondence should be addressed.
Tel: +98-21-88029177;
E-mail: nina.seyedrazi@gmail.com

CX produced by other main wild CTX producing strains.

Although, CTX pigment such as other polyene compounds is soluble in the oily solutions, a little polarity is observed in this pigment by its terminal groups (Higuera–Ciapara *et al.*, 2004). In this way, CTX can be bonded with two phases existed in the oil-in-water emulsions. As a result, the digestion, transfusion, absorption, and ultimately the increasing the bio-availability of the given pigment will be improved (Helmar *et al.*, 2003).

Processing and preservation the baked foods, drinks, and tomato products containing these pigments are extremely difficult due to their high sensitivity to operational and environmental conditions such as pH, light, O₂, temperature and etc. The stability of carotenoids under different treatment has been reported by several researchers. In instance, the stability of carotenoids of tomato and carrot during thermal treatment was studied by Stahl and Sies (1992) and Mayer- Miebach *et al.* (2005), respectively. The carotenoids stability existed in watermelon and green beans were also reported by Perkins-Veazie and Collins (2003) and Sanches-Mata *et al.* (2002) respectively. The Hunter colour values, L^* , a^* and b^* , and the pigment concentration were usually measured in order to show and investigate the amount of the colour degradation under different situations (Giese, 2003; Eren and Kaymak-Ertekin, 2007).

Response surface methodology [RSM] concern as an optimizing technique which is investigating the effect of significant factors, optimal conditions for each response and the relationship existed between the independent variables and the responses (Tiware *et al.*, 2008). In addition, the reduction in total number of experiments is another advantage of this method.

To the best our knowledge, detailed measurements of the degradation of natural CTX have not been investigated. Therefore, the aim of this research was to determine the effectiveness of different environmental parameters, different pH and time at room temperature ($24 \pm 1^\circ\text{C}$) on the degradation of natural CTX obtained from *D. natronolimnaea* HS-1, and enriched in the oil-in water emulsion. RSM in terms of a central composite design [CCD] was used to improve the ability of predicting the amount of CTX which is degraded under various combinations of different

pH and temperature. It also was applied in order to predict the amount of decreasing trend by measuring Hunter values (L^* , a^* , b^*).

MATERIALS AND METHODS

Materials

Peotone was purchased from Himedia (Mumbai, India). Yeast extract, potassium sorbate, Arabic gum, the pure acetone (99.5%), phosphoric acid, and buffer solutions were obtained from Merck Chemical Co. (Darmstadt, Germany). Beet molasses was purchased from Qazvin Sugar Industry (Qazvin, Iran), and the pure ethanol (96%) provided from the Taghtir Khorasan Co. (Mashhad, Iran). Moreover, the pure corn oil was purchased from the domestic shopping center (Golden Maize, Emirates Refining Company Ltd. Sharjah, United Arab Emirate), and the CTX standard supplied by Dr. Ehrenstorfer GmbH (Germany).

Production and extraction of natural canthaxanthin

The strain of bacterium *D. natronolimnaea* HS-1 (DSM 44860) maintained on YM (containing 5 g/l peptone + 5 g/l yeast extract + 40 g/l molasses at pH 7) was cultured in an incubator at 28°C for 7 days. Afterward, the biomass was washed by physiological serum (NaCl; 9 g/l in deionized water), and centrifuged at 8,000×g for 5 min to separated from supernatants (Khodaiyan *et al.*, 2007). Subsequently, to split the wall of *D. natronolimnaea* HS-1 and extract the high amounts of pigments, each 2 ml of biomass was suspended in the 10 ml of pure ethanol while was vortexing for 5 min. Then, it was centrifuged at 8,000×g for 5 min. Finally, ethanol extracts were collected and filtered through a 0.2 µm hydrophobic fluorophore membrane (Sigma-Aldrich Co., United States). In this way, CTX was extracted at high level and solved in ethanol.

Oil-in-water emulsion preparation

In order to prepare the emulsion, corn oil was added to the CTX solved in ethanol, and heated up to 38°C in RV10D evaporator (IKA, Germany) to remove the ethanol and leave the CTX in the oil. Afterwards, 5 g/100 ml of the vegetable oil containing the CTX, 5 g/100 ml Arabic gum and 0.5 g/100 ml potassium sorbate (to prevent the growing of fungus), were added to distilled water, reached to 100 ml and mixed with Ultra-Turrax T-25 (IKA, Germany). Subsequently, the given solution

was pressurized twice in 8 and 15 MPa by means of the homogenizer (APV model, Denmark). The pH of emulsion was adjust to 3, 5, and 7 using phosphoric acid and buffer solutions and kept at room temperature (24±1°C) for 33 days. The pH of emulsion was measured with a digital pH meter (ETS-D6 model, IKA, Germany) while being stirred by a magnetic stirrer.

Colour evaluation

The amount of colour was measured by spectrophotometer and the Hunter method. For this purpose, the turbidity of emulsion should be removed by the acetone. Vegetable oil containing CTX was dissolved in acetone; however, the acetone was already dissolved in the water. Based on these, Arabic gum and the turbidity existed in the emulsion was settled and the clarified homophased solution was obtained. As the result, the CTX was spread throughout the homogenous solution. In the next step, L^* (lightness/darkness), a^* (redness/greenness), b^* (yellowness/blueness), and the absorbance of CTX solution were investigated by spectrophotometer (HACH DR/4000U, USA). To obtain the concentration of natural CTX in mentioned solution, the relation between the amounts of CTX standard and its absorption in the same solution was used and compared with the experimental samples. All experiments were conducted in triplicate.

Experimental design and statistical analysis

A CCD-RSM using the statistical software package Design-Expert (trial version 7.1.6, Stat-Ease Inc., Minneapolis, USA) in order to demonstrate the degradation of colour, L^* , a^* , b^* , and CTX concentration were designed. The effects of two independent factors including pH (X_1) and treatment time (X_2 , day) on the Hunter parameters and the amount of CTX concentration were investigated. The 14 runs of experimental data based on three levels of 126 these factors were examined while (X_1) was ranged between 3 to 7 and, (X_2) was between 3 to 33 days at 24±1°C (Table 1). Experimental data were suitably fitted to a second-order polynomial model. The optimal points were predicted according to the following quadratic polynomial model (Equation 1):

$$Y = \beta_0 + \sum_{i=1}^2 \beta_i X_i + \sum_{i=1}^2 \beta_{ii} X_i^2 + \sum_i \sum_{j=i+1} \beta_{ij} X_i X_j \dots(1)$$

where Y is the predicted value; β_0 is the constant coefficient, β_i is the linear coefficient, β_{ii}

is the quadratic coefficient, β_{ij} is the cross product coefficient, and X_i and X_j are independent variables. The suitability of the fit of the polynomial model equation was tested by the R^2 (coefficient of determination), adjusted- R^2 [R^2 -adj], the prediction error sum of squares [PRESS] and adequate precision. The $PRESS$ statistic is calculated as Equation (2) (Bas and Boyaci, 2007):

$$PRESS = \sqrt{\sum_{i=1}^N (y_{pre,i} - y_{exp,i})^2} \dots(2)$$

Adequate precision compares the range of the predicted values at the design points to the average prediction error. The definition of adequate precision is in Equations (3) and (4) (Bas and Boyaci, 2007):

Adequate precision

$$= \text{Adequate precision} = \frac{\max(\bar{y}) - \min(\bar{y})}{\sqrt{\bar{V}(\bar{y})}} \dots(3)$$

$$\bar{V}(\bar{y}) = \frac{1}{n} \sum_{i=1}^n V(\bar{y}) = \frac{p\sigma^2}{n} \dots(4)$$

In Equations (2)-(4), $y_{i,exp}$ is the experimental responses, $y_{i,pre}$ is the predicted responses, \bar{y} is the predicted value, p is the number of model parameters, σ^2 is the residual mean square from analysis of variance [ANOVA] table, and n is the number of experiments.

The significances of each of the coefficients in the empirical model were selected or rejected based on the p -value. The terms statistically found non-significant ($p > 0.05$) were removed from the initial models and the experimental data were refitted to produce the final reduced model. It should be noted that some non-significant variables ($p < 0.05$) were added again to the model due to quadratic or interaction effects. The correlation between the response and independent variables can be readily seen in the response surface and contour plots. These plots show the simultaneous interaction of two factors on the responses and find the location of optimum experimental variables (Gharibzahedi et al., 2012).

RESULTS AND DISCUSSION

Model fitting

The relationship between pH (X_1) and time (X_2) as the independent variables and each response was determined by ANOVA (Table 2). The results show the independent variables have the significant effect ($p < 0.05$) on the bacterial CTX concentration and, L^* , a^* , and b^* values. The polynomial equations, describing the CTX amount (Y_1) and Hunter L^* (Y_2), a^* (Y_3), and b^* (Y_4) values as a simultaneous function of amount of pH (X_1) and treatment time (X_2), is shown in Equations (5)-

(8), respectively.

$$CTX = 5.36193 + 1.14350X_1 - 1.58100X_2 + 0.54850X_{11} + 0.78800X_{22} \dots(5)$$

$$L^* = 88.2737 - 0.7050X_1 + 2.0100X_2 + 1.3273X_{22} \dots(6)$$

$$a^* = 2.24329 + 0.62500X_1 - 1.34333X_2 \dots(7)$$

$$b^* = 43.8554 + 3.9367X_1 - 9.4317X_2 + 2.7403X_{22} + 0.8875X_1X_2 \dots(8)$$

The lack of fit is an indication of the failure for a model representing the experimental data at which points were not included in the regression or variations in the models cannot be accounted for random error (Bas and Boyaci, 2007). If there is a significant lack of fit which could be indicated by

Table 1. Central composite design (CCD) and the responses for the colour values

Run order	Independent variables			Response variables		
	pH	Time (day)	CTX (ppm)	L^*	a^*	b^*
1	3	18	4.773	89.80	1.20	39.01
2	5	3	8.258	87.84	3.80	57.00
3	5	33	4.072	91.23	0.82	36.62
4	5	18	5.219	88.11	2.29	44.24
5	7	18	7.078	86.62	3.27	47.94
6	5	18	5.368	88.18	2.37	43.51
7	5	18	5.262	87.73	2.03	44.38
8	5	18	5.538	88.87	2.45	43.78
9	7	3	8.958	87.18	3.83	57.63
10	3	3	7.099	87.91	3.72	52.06
11	7	33	6.727	91.72	2.02	41.30
12	5	18	5.432	88.61	2.23	43.61
13	3	33	4.030	92.04	0.45	32.18
14	5	18	5.272	87.97	2.14	43.59

Table 2. Regression coefficients, R^2 , probability values and lack of fit for the final reduced models for the colour degradation

Terms	CTX (ppm)		L^*		a^*		b^*	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
Model	5.36193	-0.0001	88.2737	-0.0001	2.24329	-0.0001	43.8554	-0.0001
X_1 (pH)	1.14350	<0.0001	0.7050	0.040	0.62500	0.002	3.9367	<0.0001
X_2 (Time)	-1.58100	<0.0001	2.0100	<0.0001	-1.34333	<0.0001	-9.4317	<0.0001
X_{11}	0.54850	0.036	0.0023	0.996	0.06367	0.748	-0.5947	0.179
X_{22}	0.78800	0.007	1.3273	0.015	0.13867	0.490	2.7403	<0.0001
$X_1 \cdot X_2$	0.20950	0.269	0.1025	0.737	0.36500	0.034	0.8875	0.038
Lack of Fit		0.056		0.106		0.077		0.066
R^2		97.00%		91.31%		97.60%		99.54%
R^2 - adj		94.42%		83.85%		95.55%		99.14%
PRESS		2.56		6.7		4.04		6.11
Adequate precision		21.99		10.79		22.45		54.02

a low probability value, the response predictor is discarded. The lack of fit illustrated in Table 2, did not result in a significant p -value for selected variables, meaning that these models were sufficiently accurate for predicting the relevant responses. Table 2 also indicates the accuracy of the second-order polynomial models via the R^2 , R^2 -adj, PRESS and adequate precision. The predicted values were correlated to the experimental data with R^2 of 97.00%, 91.31%, 97.60%, and 99.54% for CTX amount, L^* , a^* , and b^* , respectively. However, a large value of R^2 does not always imply that the regression model is a good one. R^2 -adj is a modification of R^2 that adjusts for the number of explanatory terms in a model. Unlike R^2 , the R^2 -adj increases only if the new term improves the model more than would be expected by chance (Ghasemlou *et al.*, 2012). The values of R^2 -adj were 94.42%, 83.85%, 95.55% and 99.14% for CTX, L^* , a^* , and b^* , respectively (Table 2). The low PRESS (2.56-6.71) values suggest for the adequacy of the fitted quadratic models for predictive applications (Table 2). Adequate precision measures the signal-to-noise ratio. A ratio greater than four is desirable. The proposed models for CTX, L^* , a^* , and b^* values had very good signal-to-noise ratios in the range of 10.49 to 54.02 (Table 2). Thus, it can be concluded that the models were statistically sound.

Colour degradation

As shown in Table 2, the CTX (Y_1) was significantly ($p < 0.0001$) influenced by the linear

effect of pH and treatment time. The mutual interaction between pH and treatment time and also their quadratic effects were found to be insignificant ($p > 0.05$). pH and time are classified as the significant factors on the degradation of CTX. The time treatment in compared to pH showed the more considerable effect. According to the obtained results in this work, more degradation in the room temperature was occurred approximately in $\text{pH} \leq 4$ after 10 days. From the optimization results, a condition including pH of 6.97 and treatment time of 3.10 days was predicted to be the individual optimum region that resulted in highest CTX stability (9.166 ppm). Nevertheless, the least CTX stability (3.960 ppm) was estimated to be at the combined level of pH of 3.5 and treatment time of 29.06 days (Fig. 1a and b). CTX (4,4'-diketo- β -carotene), a keto-carotenoid, is characteristically included eight isoprenoid groups and eleven double bonds as conjugated polyene called chromophore along with carbonyl group substitution at each end at positions C-4 and C-4' on the molecule ring. It seems that low pH and long treatment time probably led to generate free radicals in the aqueous solution that may open these aromatic rings and lead to partial oxidation of products such as organic acids, aldehydes, and ketones (Tiwari *et al.*, 2008). Chen *et al.* (1996) reported high storage temperature (35°C) during three months can significantly lead to decrease lutein pigment in carrot juices. Tsimidou and Tsatsaroni (1993) and Queiroz Zepka and

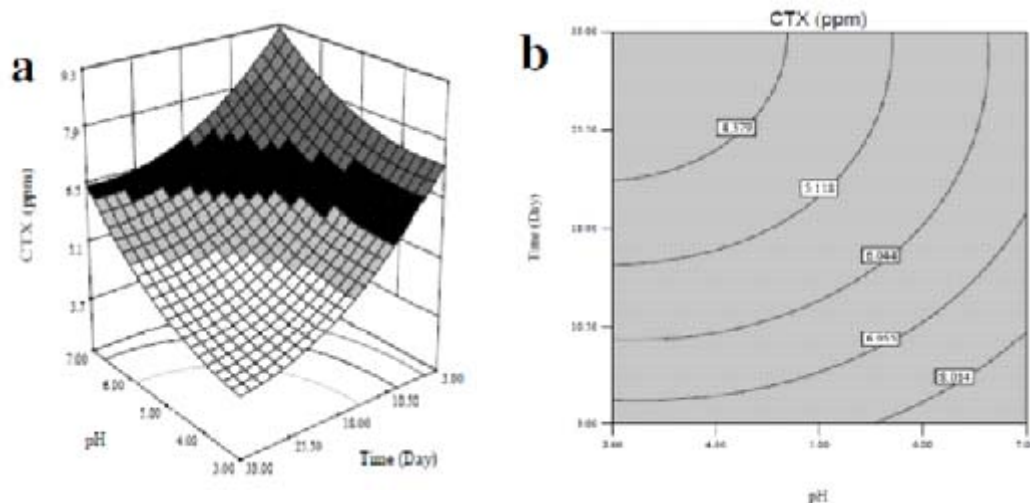


Fig. 1. 3D response surface (a) and counter (b) plots for the effects of pH (X_1) and treatment time (X_2) on the CTX concentration during the colour degradation

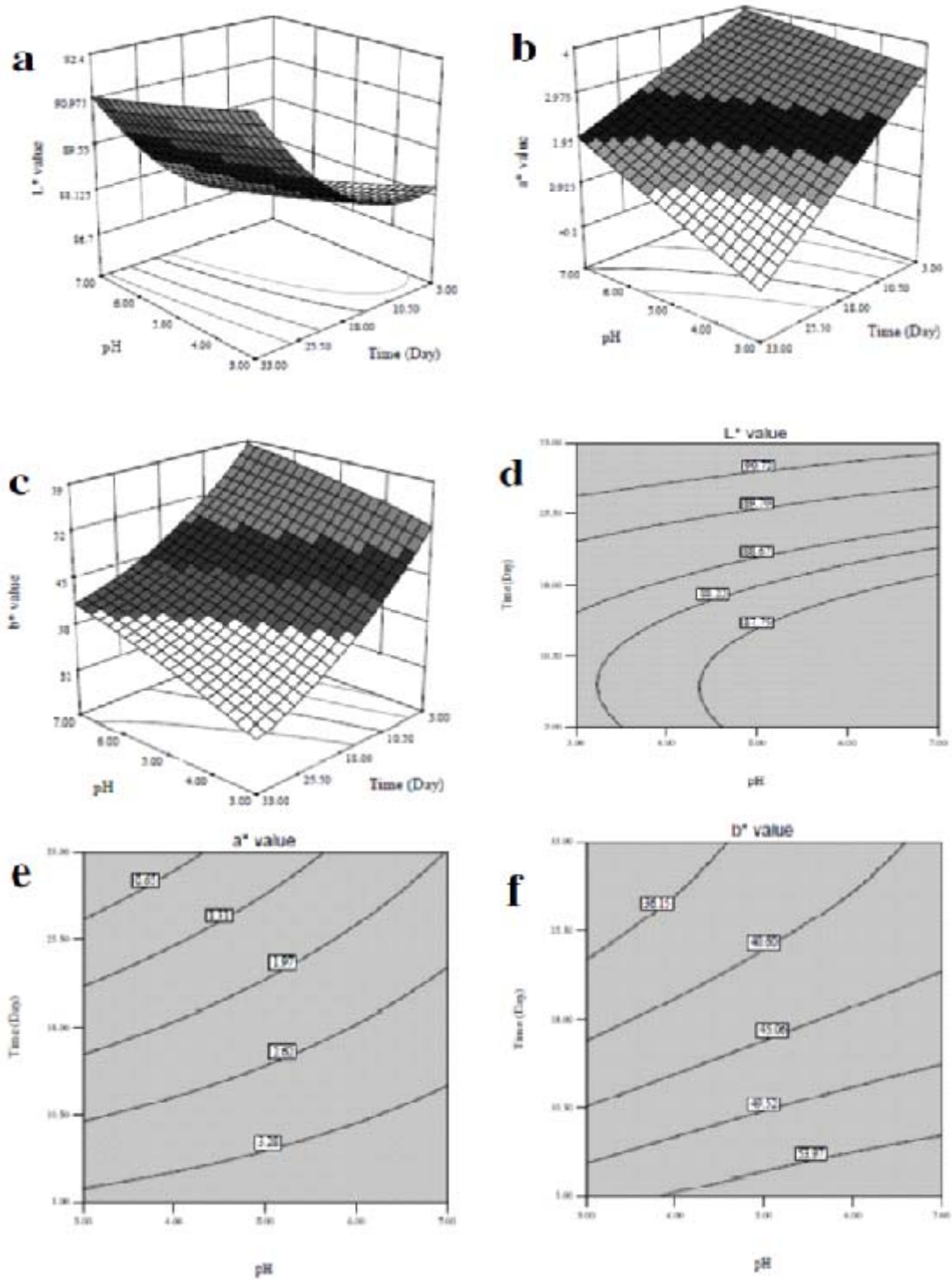


Fig. 2. 3D response surface and counter (d-f) plots for the effects of pH (X_1) and treatment time (X_2) on the Hunter L^* (a and d), a^* (b and e) and b^* (c and f) values

Mercadante (2009) also observed the high degradation of saffron carotenoids under low pH and the presence of prooxidants such as temperature and light.

As shown in Table 2, the linear effect of all independent variables on Hunter L^* (Y_2), a^* (Y_3) and b^* (Y_4) values was highly significant ($p < 0.0001$; $p < 0.01$; $p < 0.05$). The quadratic term of treatment time was significant ($p < 0.0001$; $p < 0.05$) on the L^* and b^* values. The results showed that interaction of pH and treatment time was only significant on the b^* value ($p < 0.05$). The three-dimensional (3D) response surface and counter graphs was plotted to better visualize the interaction effects of independent variables on the Hunter color values (Fig. 2a–f).

Overall, the emulsions with higher turbidity values would be considered as more stable emulsions. Thus, the desirable emulsions has high a^* and b^* values and a low L^* value. The individual optimization results according to this fact indicated that the optimum conditions for the highest turbidity value were pH of 7 and treatment time of 3.87 days. The corresponding predicted response values under the optimum conditions for Hunter L^* , a^* and b^* values of the CTX emulsions were 86.80, 3.87 and 57.63, respectively. Queiroz Zepka and Mercadante (2009) believed that some of chemical changes such as changes in CTX isomer and chain length can cause the colour changes of an emulsion from orange-red to white-yellow. *Trans*-CTX isomer has much better stability and coloring effects than *cis*-CTX (Giese, 2003; Mayer-Miebach *et al.*, 2005). Moraru and Lee (2004) also reported that lycopene isomerization occurs under the simulated gastric digestion and low pH during long treatment time. Therefore, it can be concluded by declining the pH from 7 to 3 and passing the time during 33 days likely the *trans*-isomer to *cis*-isomer were transformed and this fact led to a slightly lighter colour.

Optimization and verification of the models

Optimum level for independent variables was determined to obtain maximum CTX, a^* and b^* values with minimum L^* value. The optimization procedures indicated the overall optimum region to be at pH of 7 and treatment time of 3.87 by overlaying all the responses and response optimizers. The corresponding response values predicted for CTX, a^* , b^* and L^* values were

estimated to be 9.050 ppm, 86.80, 3.87 and 57.63, respectively. The suitability of the presented models for predicting the optimum response values was tested using the recommended optimum conditions. Then, the adequacy of the response surface models was checked by the comparison of experimental and predicted values. The results demonstrated that the corresponding experimental values for CTX, a^* , b^* and L^* values with the optimum conditions were 9.032 ± 0.008 ppm, 87.19 ± 0.32 , 3.66 ± 0.28 and 56.22 ± 1.45 , respectively. No significant difference ($p > 0.05$) was found between the experimental and predicted values.

CONCLUSIONS

In this study, the amount of the degradation of natural CTX produced by *D. natronolimnaea* HS- 1 in the room temperature, in different pH (3-7) for 33 days was investigated. CTX was used in the oil-in-water emulsions to increase its absorption, transfection, and bioavailability. RSM was applied to scrutinize, model and predict the degradation trend. More degradation of CTX was shown in pH 4 after 10 days. As the result, the orange-red emulsion of CTX was changed toward yellowness and whiteness. L^* , a^* , and b^* as the Hunter colour values and the amount of CTX concentration studied, significantly proved this trend ($p < 0.05$). High R^2 was observed between experimental and predicted values. These values were 97.00% for CTX, 91.31% for L^* , 97.60% for a^* , and 99.54% for b^* . Because of the health beneficial of this emulsion, it should be applied in some foods, drinks, and even in tan creams. Likewise, the attractive appearance of food products and tan creams containing CTX would attracted more customers.

ACKNOWLEDGMENTS

This study has been partially supported by a grant provided by the Council for Research at the Campus of Agriculture and Natural Resources of the University of Tehran and the Research Council of the University of Tehran. Gratitude is expressed to the *ZamZam* Iran Co. (Tehran, Iran) for financial assistance.

REFERENCES

1. Furr, H.C., Clark, R.M. Intestinal absorption and tissue distribution of carotenoids. *The Journal of Nutritional Biochemistry*, 1997; **8** (7), 364-377.
2. Huang, D.S., Odeleye, O.E., Watson, R.R. Inhibitory effects of canthaxanthin on in vitro growth of murine tumor cells. *Cancer Letters*, 1992; **65**, 209-213.
3. Liu, R.H. Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. *American Journal of Clinical Nutrition*, 2003; **78**, 517S-520S.
4. Patel, A.M.R., Berces, A., Kerekgyarto, T., Ronto, G., Lammer, H., Zarnecki, J.C. Annual solar UV exposure and biologically effective dose rates on the martian surface. *Advances in Space Research*, 2004; **33** (8), 1247-1252.
5. Mohd Fadzelly, A., Mohamed, M., Rahmat, A., Fry, J. Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and trap (*Artocarpus odoratissimus*). *Food Chemistry*, 2009; **113** (2), 479-483.
6. Vitek, P., Osterrothova, K., Jehlicka, J. Beta-carotene- a possible biomarker in the Martian evaporitic environment: Raman micro-spectroscopic study. *Planetary and Space Science*, 2009; **57**(4), 454-459.
7. Palace, V.P., Khaper, N., Qin, Q., Singal, P.K. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. *Free Radical Biology and Medicine*, 1999; **26** (5-6), 746-761.
8. Gharibzahedi, S.M.T., Razavi, S.H., Mousavi, S.M., Moayedi, V. High efficiency canthaxanthin production by a novel mutant isolated from *Dietzia natronolimnaea* HS-1 using central composite design analysis. *Industrial Crops and Products*, 2012; **40**, 345-354.
9. Khodaiyan, F., Razavi, S.H., Emam-Djomeh, Z., Mousavi, S.M.A., Hejati, M.A. Effect of culture conditions on canthaxanthin production by *Dietzia natronolimnaea* HS-1. *Journal of Microbiology and Biotechnology*, 2007; **17**(2), 195-201.
10. Higuera-Ciapara, I., Felix-Valenzuela, L., Goycoolea, F.M., Arguelles-Monal, W. Microencapsulation of astaxanthin in a chitosan matrix. *Carbohydrate Polymers*, 2004; **56** (1), 41-45.
11. Helmar, S., Ax, K., Behrend, O. Product engineering of dispersed systems. *Trends in Food Science & Technology*, 2003; **14**(1-2), 9-16.
12. Stahl, W., Sies, H. (1992) Uptake of lycopene and its geometrical isomers is greater from heat processed than from unprocessed tomato juice in humans. *Human Clinical Nutrition*, 1992; **122**(11), 2161-2166.
13. Mayer-Miebach, E., Behsnilian, D., Regier, M., Schuchmann, H.P. Thermal processing of carrots: Lycopene stability and isomerisation with regard to antioxidant potential. *Food Research International*, 2005; **38**(8-9), 1103-1108.
14. Perkins-Veazie, P., Collins, J.K. Flesh quality and lycopene stability of fresh-cut watermelon. *Postharvest Biology and Technology*, 2003; **31**(2), 159-166.
15. Sanches-Mata, M.C., Camara, M., Diez-Marques, C. Extending shelf-life and nutritive value of green beans (*Phaseolus vulgaris* L.), by controlled atmosphere storage: macronutrients. *Food Chemistry*, 2002; **80**(3), 309-315.
16. Giese, J. Color measurement in foods. *Food Technology*, 2003; **54**(2), 62-65.
17. Eren, I., Kaymak-Ertekin, F. Optimization of osmotic dehydration of potato using response surface methodology. *Journal of Food Engineering*, 2007; **79** (1), 344-352.
18. Tiwari, B.K., Muthukumarappan, K., O'Donnell, C.P., Cullen, P.J. Modelling color degradation of orange juice by ozone treatment using response surface methodology. *Journal of Food Engineering*, 2008; **88** (4), 553-560.
19. Bas, D., Boyaci, I.H. Modeling and optimization I: Usability of response surface methodology. *Journal of Food Engineering*, 2007; **78** (3), 836-845.
20. Ghasemlou, M., Khodaiyan, F., Jahanbin, K., Gharibzahedi, S.M.T., Taheri, S. Structural investigation and response surface optimisation for improvement of kefir production yield from a low-cost culture medium. *Food Chemistry*, 2012; **133**, 383-389.
21. Chen, H.E., Peng, H.Y., Chen, B.H. Stability of carotenoids and vitamin 261 A during storage of carrot juice. *Food Chemistry*, 1996; **57**(4), 497-503.
22. Tsimidou, M., Tsatsaroni, E. Stability of saffron pigments in aqueous extracts. *Journal of Food Science*, 1993; **58**(5), 1073-1075.
23. Queiroz Zepka, L., Mercadante, A. Degradation compounds of carotenoids formed during heating of a simulated cashew apple juice. *Food Chemistry*, 2009; **117**(1), 28-34.
24. Moraru, C., Lee, T.C. Lycopene isomerization at gastric pH. In: *Nutraceuticals & Functional Foods Session of IFT Annual Meeting*, Las Vegas, NV, USA, 12-16 July, 2004.