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Effect of Different pH on Canthaxanthin Degradation

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***Abstract*—**In this research, natural canthaxanthin as one of the most important carotenoids was extracted from *Dietzia natronolimnaea* HS-1. The changes of canthaxanthin 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) was investigated. The effects of different pH (3, 5 and 7), as well as, time treatment (3, 18 and 33 days) in the environmental temperature (24°C) on the degradation were studied by response surface methodology (RSM). The Hunter values (L*, a*, and b*) and the concentration of canthaxanthin (*C*, mg/L) illustrated more degradation of this pigment at low pHs (pH≤ 4) by passing the time (days≥10) with R^2 97.00%, 91.31%, 97.60%, and 99.54% for *C*, L*, a*, and b* respectively. The predicted model were found to be significant ($p<0.05$).

***Keywords*—**Degradation, Emulsion, Response Surface Methodology (RSM)

I. Introduction

CAROTENOIDS are one of the most important and beneficial type of pigments in the nature. Moreover, the diversity of these colors in the vegetables, fruits, marine organizations, and micro organizations is another applicable and useful property of these kinds of pigments. Besides, among over 600 known carotenoids, canthaxanthin plays a vital role in protecting the tissues, against free radicals and oxidizing agents. The Anti-Oxidant activity [1], [2], preventing serious health disorders, such as breast and lung cancers [3]-[5] and any heart and cardiovascular diseases are the major responsibilities of canthaxanthin [6]. The anti-Oxidant ability of canthaxanthin is equal to astaxanthin, higher than α-carotene, zeaxanthin, lutein, β-criptoxanthin, and lycopene; this characteristic of canthaxanthin is 100 times more than tocopherol [7]. Because of some beneficial aspects mentioned above, canthaxanthin 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 color created. Although canthaxanthin ($C_{40}H_{52}O_2$), such as other polyene compounds, is soluble in the oily solutions, a little polarity is observed in this pigment by its terminal groups [8]. In this way, canthaxanthin 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 [9]. Processing and preservation the baked goods, drinks, and tomato products containing these pigments are extremely difficult due to their high sensitivity to operational and environmental conditions such as pH, light, O_2, temperature, and etc. The Hunter color values, L*, a* and b*, and the concentration of canthaxanthin (*C*, mg/L) were measured by spectrophotometer, in order to show and investigate the amount of the color degradation under different situations [10].Response surface methodology (RSM) was used to improve the ability of predicting the amount of canthaxanthin which is degraded under various combinations of different pH and temperature. This method 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 [11], [12]. In addition, the reduction in total number of experiments is another advantage of this method. A central composite design (CCD) was conducted on L*, a*, b*, and *C*.

The intention of this study was to evaluate the effectiveness of different environmental parameters, different pH and time at 24°C, on the degradation of natural canthaxanthin obtained from *Dietzia natronolimnaea* HS-1, and enriched in the oil-in-water emulsion. RSM was applied in order to predict the amount of decreasing trend by measuring L*, a*, b*, and *C*. The stability of carotenoids under different treatment has been reported by several researchers. In instance, the reduction of carotenoids of tomato by thermal treatment was studied by Stahl and Sies, [13]; the stability of carrot carotenoids during thermal treatment was done by Mayer-Miebach [14]; the stability of carotenoids existed in watermelon and green beans reported respectively by [15], [16]. However, the degradation of natural canthaxanthin has not been investigated yet.

II. Materials and Methods

A. Materials

Peotone was purchased from Himedia (Mumbai, India) and Yeast extract, Sorbet Potassium, Arabic gum, the pure Acetone (99.5%), phosphoric acid, and buffer solutions were all obtained from Merck (Darmstadt, Germany). Beet molasses was purchased from Qazvin Sugar Industry (Qazvin, Iran), and the pure Ethanol (96%) obtained from the Taghtir Khorasan Company (Mashhad, Iran). Moreover, the pure Corn Oil was purchase from the domestic shopping center (Golden Maize, Emirates Refining Company Ltd. Sharjah, United Arab Emirate), and the canthaxanthin standard supplied by Dr. Ehrenstorfer GmbH (Germany).

B. Natural Canthaxanthin

The strain of bacterium *Dietzia natronolimnaea* HS-1 (DSM 44860) maintained on YM (containing 5g/L Peptone + 5g/L Yeast extract + 40g/L molasses at pH 7), was cultured in an incubator at 28˚C for 7 days. Afterward, the biomass was

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washed by Physiological Serum (NaCl; 9g/L in deionized water), and centrifuged at 8,000×g for 5 minutes to separated from supernatants [17].

Subsequently, to split the wall of *Dietzia natronolimnaea* HS-1 and extract the high amount of pigments, each 2mL of biomass was suspended in the 10mL of pure Ethanol while was vortexing for 5 minutes. Then, it was centrifuged at 8,000×g for 5 minutes. Finally, Ethanol extracts were collected and filtered through a 0.2µm hydrophobic fluorophore membrane (Sigma-Aldrich Co., United States). In this way, canthaxanthin was extracted at high level and solved in Ethanol.

C. Oil-in-Water Emulsion Preparation

In order to prepare the emulsion, corn oil was added to the canthaxanthin solved in Ethanol, and heated up to 38˚C in RV10D evaporator (IKA, Germany) to remove the Ethanol and leave the canthaxanthin in the oil.

Afterwards, 5g/100mL of the vegetable oil containing the canthaxanthin, 5g/100mL Arabic gum and 0.5g/100mL potassium sorbate (to prevent the growing of fungus), were added to distilled water, reached to 100mL 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, Denmark). The pH of emulsion was adjust to 3, 5, and 7 using phosphoric acid and buffer solutions and kept at 24°C for 30 days. The pH of emulsion was measured with a digital pH meter ETS-D6 (IKA, Germany) while being stirred by a magnetic stirrer.

D. Color Evaluation

The amount of color was measured by spectrophotometer and the Hunter method. For this purpose, the turbidity of emulsion should be removed by the acetone. Vegetable oil containing canthaxanthin 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 canthaxanthin was spread throughout the homogenous solution. Afterwards, L* (lightness/darkness), a* (redness/greenness), b* (yellowness/blueness), and the absorbance of canthaxanthin solution were investigated by spectrophotometer (HACH DR/4000U, USA). To obtain the concentration of natural canthaxanthin in mentioned solution, the Fig. shown the relation between the amounts of canthaxanthin standard and its absorption in the same solution was used and compared with the experimental samples. All experiments were conducted in triplicate.

E. Response Surface Methodology

To demonstrate the degradation of color, L*, a*, b*, and *C* were designed by central composite design (CCD) and investigated by RSM (Minitab 15). In this study, the effect of the independent factors, pH (X_1) and time (X_2, days) on the Hunter parameters and the amount of concentration were investigated. The 14 runs of experimental data based on three levels of these factors were examined while (X_1) was ranged between 3-7, (X_2) between 3-33 days at 24°C (Table I). Experimental data were fitted to a second-order polynomial model (1). The optimal points were predicted according to the following quadratic polynomial model:

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

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.

III. Results and Discussion

A. Modeling Color Degradation of Natural Canthaxanthin

The relationship between pH (X_1) and time (X_2) as the independent variables and each response was determined by analysis of variance (ANOVA). Table II shows the independent variables have the significant effect on the degradation of canthaxanthin (Y) at $p<0.05$. In addition, the accuracy of the model was proved via the regression coefficient (R^2) presented in Table II. Second order polynomial model were predicted the amount of degradation of canthaxanthin. By using the R^2 shown in Table II and this polynomial second – order (2), the amount of degradation will be predicted. As the result, by replacing respectively the favorite pH and time instead of X_1 and X_2 in (3-6), the amount of *C*, L*, a*, and b* could be measured; and the amount of degradation of mentioned canthaxanthin emulsion could be predicte

TABLE I
CENTRAL COMPOSITE DESIGN AND THE RESPONSES FOR THE COLOR VALUES

Run Order	pH	Time (days)	C (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

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_{11} + \beta_{22} X_{22} + \beta_{33} X_{33} + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (2)$$

$$C=5.36193+1.14350X_1-1.58100X_2+ 0.54850X_1^2+0.78800 X_2^2 \quad (3)$$

$$L^*=88.2737-0.7050X_1+2.0100X_2+1.3273X_2^2 \quad (4)$$

$$a^*=2.24329+0.62500X_1-1.34333X_2 \quad (5)$$

$$b^*=43.8554+3.9367X_1-9.4317X_2+2.7403X_2^2+0.8875X_1X_2 \quad (6)$$

Decreasing the amount of *C* (8.958– 4.030), a* (3.83-0.45) and b*(57.63-32.18), and increasing the amount of L* (86.62 - 92.04) was caused by declining the pH from 7 to 3 and passing the time during 30 days. Fig. 1 to 4 presented, demonstrate the effect of pH and time treatments on L*, a*, b*, and *C*. According to Fig. 2-4, the amount of a*, b*, and *C* in pH ≤4 decreased over the time. This result was reversed for *L** (Fig. 1). a* factor was decreased with higher rate than b*. Definitely, the redness was degraded more than yellowness. On the other hand, the amount of L* raised, illustrated the tendency of solution toward whiteness/lightness. Subsequently, some chemical changes occurred, caused the orange-red emulsion changed to white-yellow solution. As mentioned before, pH and time are classified as the significant factors on the degradation of canthaxanthin. Among these, time treatment showed the considerable effect. According to all these Fig. and modules, more degradation in the environmental temperature was occurred approximately in pH ≤ 4 after 10 days. The predicted values were correlated to the experimental data with regression coefficient (R^2) of 97.00%, 91.31%, 97.60%, and 99.54% for *C*, L*, a*, and b* respectively.

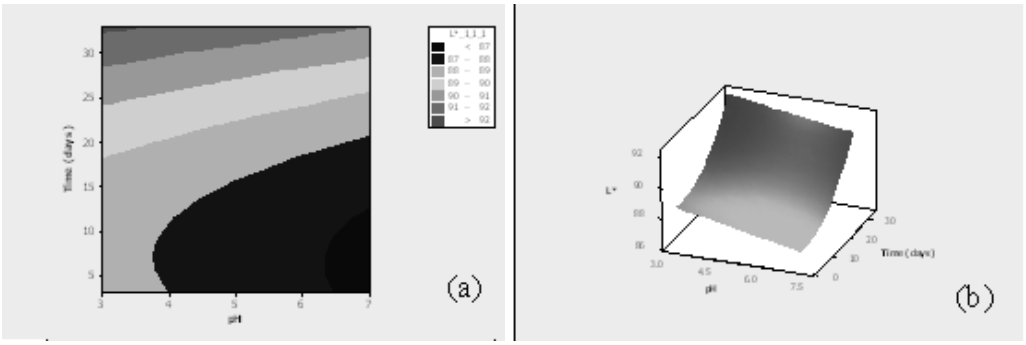

Fig. 1 (a) Counter plot of L*, (b). Surface plot of L*

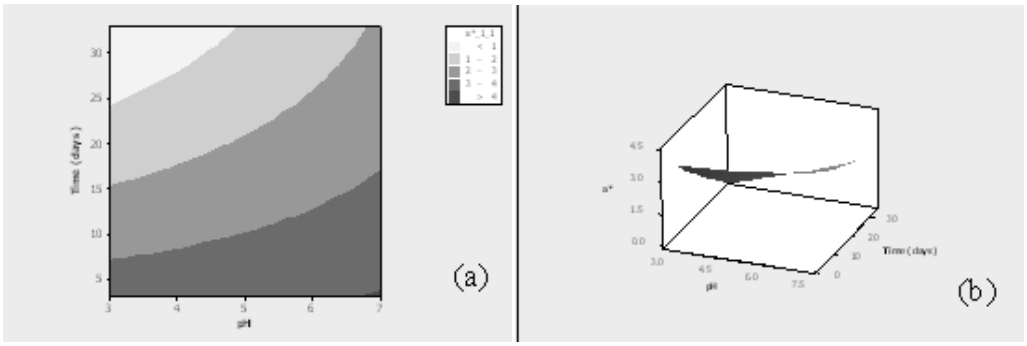

Fig. 2 (a) Counter plot of a*, (b). Surface plot of a*

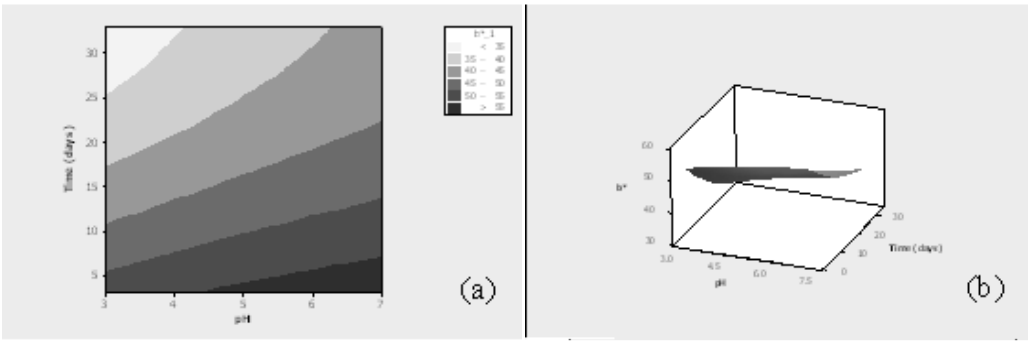

Fig. 3 (a) Counter plot of b*, (b). Surface plot of b*

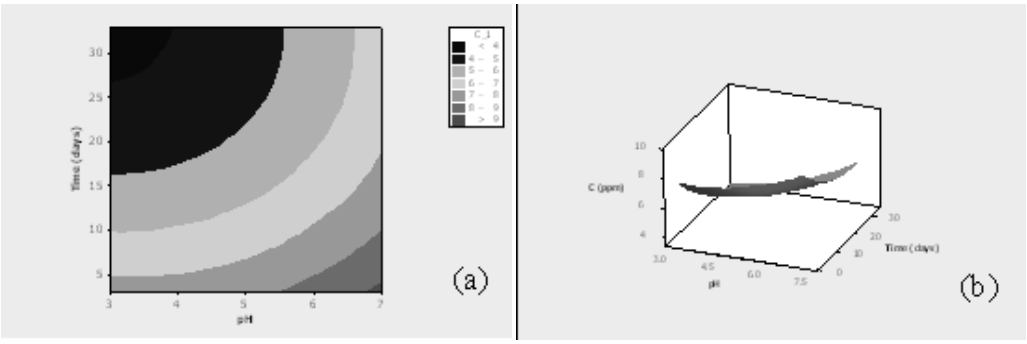

Fig. 4 (a) Counter plot of *C*, (b). Surface plot of *C*

So many double bonds existed in the chain of carotenoids are so sensitive against environmental factors such as temperature, acidic solutions, light, and oxygen. Definitely, some changes observed in their isomers and chain length, are caused by the influence of high temperatures and low pHs over time [18]. The *trans*-isomer transformed to a *cis*-isomer (yielding a slightly lighter color), makes further changes in its chemical and physical properties [19], [20]. These kinds of changes are prejudiced on the wavelength absorbance and on the tendency of color. Some researchers investigated the effect of different parameters and different situations on the degradation of pigments. In instance, the negative effect on lutein of carrot juice was observed in different temperature (4, 25, and 35°C) after 3 months (higher degradation at 35°C) [21]. In saffron, carotenoid degradation was reported under pH, heat, and light treatments [22]. During olive processing, violaxanthin and neoxanthin decreased [23].

IV. Conclusion

In this study, the amount of the degradation of natural canthaxanthin produced by *Dietzia natronolimnaea* HS-1 in the environmental temperature, in different pH 3-7 for 30 days was investigated. Canthaxanthin was used in the oil-in-water emulsions to increase its absorption, transfusion, and bioavailability. Response surface methodology (RSM) was applied to investigate, model and predict the degradation trend. More degradation of canthaxanthin was shown in pH ≤4 after 10 days. As the result, the orange-red emulsion of canthaxanthin was changed toward yellowness and whiteness. L*, a*, and b* as the Hunter color values and the amount of canthaxanthin concentration (*C*) studied, significantly proved this trend ($p<0.05$). High correlation coefficient (R^2) was observed between experimental data and predicted values, R^2 97.00% for *C*, 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 canthaxanthin would attracted more customers.

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