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Degradation Kinetics and Utilization of Extracted Tomato Skin Carotenoids in Filling Cream and Glazing Jelly Preparation

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Abstract: Stability of total carotenoids, extracted from tomato skin, was studied at different temperatures and pH values. Degradation of extracted carotenoids followed a pseudo-first-order kinetic model. The neutral and alkaline pH provided a great protective effect to the carotenoids with degradation percent 31.9 and 22.6 % after holding for 6 days. The lower degradation rat constants ($6.9\times10^{-2} \pm 3\times10^{-4}$ and $4.7\times10^{-2} \pm 6\times10^{-4}$) and a longer half-life (3.4 and 3.7 day) at neutral and alkaline pH were observed. Calculated thermodynamic showed that the stability of carotenoids was at a temperature lower than 100°C. Activation energy, frequency factor, enthalpy and entropy significantly ($p<0.05$) decreased from 50.9 ± 1.6, 19.8 ± 0.53, 8.9 ± 0.20 and -76 to 38.1 ± 0.11 kj/mol, 16.4 ± 0.03, 1.4 ± 0.05 kj/mol and -104 J/mol K, respectively with increasing thermal treatment time from 20 to 60 min. The filling cream and glazing jelly which prepared using that extracted carotenoids had a high sensory attributes compared to those products which prepared using the sun set yellow as a synthetic color. The received scores showed that the extracted carotenoids are able to compete with synthetic colorants.

Key words: Carotenoids · Tomato · Skin · Thermodynamic · Cream · Jelly · Sensory evaluation

INTRODUCTION

Color is one of the most important quality attributes in food. It is the first impression of the quality and acceptability of a particular food judged upon its appearance [1]. There is a considerable interest worldwide in the development of food colorants from natural sources. The consumer preference for naturally derived colorants is associated with their image of being healthy and of good quality. In addition, some synthetic colorants are considered to be responsible for allergenic and intolerance reactions. The list of natural colors is small and only a few are available in sufficient quantity to be useful to the food manufacturer [2].Tomato (*Lycopersicon esculentum*) is one of the most popular of vegetables, used as a salad, in food preparations and as juice, soup, puree, ketchup or paste. Lycopene is the principal carotenoids, causing the characteristic red hue of tomatoes [3, 4]. Carotenoids are a class of natural pigments occurs widely in nature that synthesized by plants [5]. Epidemiological studies have also shown that the increased consumption of lycopene rich foods is associated with a low risk of cancer, quenching of singlet oxygen, trapping of free radicals, strengthening the immune system and preventing LDL oxidation [6-9]. Commercial processing of tomato produces a large amount of pomace waste [10]. The waste obtained in the form of seeds and skin residues, which could provide a useful source of lycopene [11]. Some factors affecting on carotenoids content and composition in fruits and vegetables may include pH, acidity, processing temperature and duration [12, 13]. Higher processing and storage temperatures lead to losses of carotenoids in tomato juice [14, 15] its lead to structural modifications such as cis-isomerization [16]. The effect of acid on carotenoids was reported in few studies. Carotenoids were found to be mono and diprotonated by nitric acid and moderately strong acids, such as trichloroacetic and trifluoroacetic acids [17]. Organic acids liberated during the processing of fruit juices are strong enough to promote rearrangements of 5, 6-epoxide groups to 5, 8-furanoid groups of arytenoids [18-20]. Heat treatment can facilitate the interaction between acids and carotenoids in the real juice [21]. Most of recent studies have described the kinetics of lycopene degradation in the tomato-based products and not of total carotenoids, which extracted

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from tomato skin. In addition, utilization of extracted tomato skin carotenoids in confectionary products not full studied.

The objective of this study was extraction and determination of total carotenoids in tomato skin. Study the effect of temperatures, pH and holding time on degradation kinetics of total carotenoids in tomato skin extract. Utilize the extracted carotenoids in preparation of filling cream and glazing jelly and its effect on sensory characteristics of these products.

MATERIALS AND METHODS

Materials: Tomato skin was obtained from Kaha Company for Preservative Foods, Kaha, Kalubia, Egypt and stored at 2-8°C. The skin was dried in a cabinet dryer at 40°C until 8 % moisture content. The dried skin was ground and passed through 0.15 mm sieves. Sucrose, shortening, salt, calcium chloride and non-fat milk were purchased from the local market. Commercial soy lecithin was obtained from the Egyptian Company for Food (Biscomisr), Ammeria, Cairo, Egypt. Hexane, ethyl alcohol, potassium sorbate and sorbic acid were obtained from El-Gomhoria Company for Drugs and Chemicals, Cairo, Egypt. Corn syrup was donated from Starch and Glucose Company, Kalubia, Egypt. Caragenan was purchased from sigma-Aldrich Company.

Methods

Extraction of Carotenoids: The total carotenoids were extracted according the method described by Lavecchia and Zuorro [22]. Ten grams of ground dried tomato skin was placed into amber flask and then 250 ml of ethanol and 500 ml of hexane were rapidly added. The flask was magnetically stirred for 15 min. Then, 150 ml of deionized water were added to allow phases separation. Stirring was continued for 5 min, after that the system was transferred to a funnel separator with isolation faraway of the light. The upper hexane layer was taken and evaporated in rotary evaporator at 40°C under vacuum. The oily carotenoids extract was kept in a dark bottle with nitrogen under freezing temperature until analyzed and utilized.

Carotenoids Determination: Carotenoids content was determined by a spectrophotometric method according to Hornero and Mínguez [23]. Tomato skin extract was dissolved in hexane and the absorbance was measured using a spectrophotometer Shimadzu UN-1201 (Shimadzu Co. Ltd. Kyoto, Japan) at 472 and 508 nm. The obtained absorbance values were introduced in the following equations:

$$C^R = (A_{508} \times 2144.0 - A_{472} \times 403.3)/ 270.9\ \mu g/ml$$
$$C^Y = (A_{472} \times 1724.3 - A_{508} \times 403.3)/ 270.9\ \mu g/ml$$
$$C^T = C^R + C^Y\ \mu g\ /ml$$

Where C^R represents the red isochromatic fraction content, C^Y represents the yellow isochromatic fraction content and C^T represents total carotenoids content. The carotenoids determination was carried out in triplicate.

pH Stability: Effect of different pH values on carotenoids stability was measured according to the method described by Huang and Elbe [24]. 250 µl of extracted carotenoids and 250 µl Tween 20 as an emulsifier were mixed with 2.5 ml of 0.1 M phosphate buffer of various pH values from 3 to 9. The absorbance was then measured spectrophotometrically.

Thermal Stability: Three ml of total carotenoids extract divided in a 3 ml glass tubes (1ml in each tube). The tubes placed in thermo statically controlled water bath at different temperatures ranged from 50 to 100°C for different time's intervals from 20 to 60 min. Also, other three tubes treated in autoclave at 121°C for time intervals from 10 to 30 min. The treated samples were cooled immediately in an ice bath. The absorbance values were measured in hexane using spectrophotometer.

Kinetics Analysis: The obtained data was performed according to those equations used by Robert *et al.* [25]. The data were best fit by a first-order kinetic model, $\ln C = \ln C_o - k^{(t)}$. Degradation rate constants (k) were obtained from the slope of a plot of the natural log of the percentage retention of carotenoids vs. time. The activation energy (E_a) and frequency factor (A) were determined from the Arrhenius model $k = Ae^{-(Ea/R)/T}$, where E_a/R is the slope and $\ln A$ is the intercept of the relationship between the natural log k and ($1/T$) in degrees kelvin. For a first-order reaction, the half-life was determined at a specific pH and temperature by the equation $t = \ln 2/k$. The enthalpy of activation (ΔH) was obtained by plotting $\ln (k/T)$ vs. ($1/T$) and the entropy of activation (ΔS) was obtained from Equation 1 based on the transition state theory:

$\ln (k/T) = \ln(k_B/h) + \Delta S/R - \Delta H/RT$

Where k_B is the Boltzmann constant and h is Planck's constant.

Filling Cream Preparation: Filling cream was prepared using the modified formula according to Jeffery [26]. The used ingredients were sucrose, 62.55 %; shortening, 34.78; lecithin, 0.05 %; salt, 0.04 %; non-fat milk 2.48 % and potassium sorbate, 0.10 %. The shortening creamed for 3 min. in Kitchenaid, model KSM 90 St Joseph, Michigan, USA. Then sucrose was added to the creamed shortening and beaten for 5 min. Other ingredients were added to the sugar-fat mixture and beaten for 3 min. Finally different concentrations of tomato skin carotenoids extract (200, 400, 600 and 800 µl/100 g of the mixture) were added. Sunset yellow was also used at concentration of 0.02 % as a synthetic color. Thereafter, the prepared filling cream samples were sensory evaluated.

Glazing Jelly Preparation: The glazing jelly was prepared according the procedure reported by Al Sayed *et al.* [27]. The ingredients, which used were sucrose, 35.85 %; water, 54.35 %; corn syrup, 9.06 %; caragenan, 0.38 %; sorbic acid, 0.08 %; potassium sorbate, 0.13 and calcium chloride, 0.15 %. A mixture of sucrose and caragenan was boiled in the water and then, calcium chloride; sorbic acid and potassium sorbate were added to the mixture. Also, corn syrup was added with continuous stirring. After complete dissolving of the ingredients, the heating was stopped and color was added at 50°C. A synthetic color (sunset yellow) was added at 0.02 % of the mixture whereas natural extracted carotenoids from tomato skin was added at 200, 400, 600 and 800 µl/100 g of the mixture. Jelly samples were cooled in the refrigerator for 5 hr.

Sensory Evaluation: Filling cream and glazing jelly samples prepared by using sunset yellow as a synthetic color and tomato skin extract as a natural color were evaluated for their quality attributes by ten members preference taste panel, from staff of the Department of Food Science, Faculty of Agriculture, Ain Shams University. The panelists were asked to score appearance, color, flavor, grainess and overall acceptability according to Bennion and Bam Ford [28] by using a 9-point numerical scale.

Statistical Analysis: ANOVA and Regression analysis (using PROC REG procedure) of the obtained data were carried out by Statistical Analysis System [29]. The compared between means was exposed by Duncan multiple range at significance difference 5 %. Results followed by different alphabetical letters significantly differed.

RESULTS AND DISCUSSION

Total Carotenoids: The total carotenoids in dry tomato skin found to be 78.9 mg/100 g whereas, oily tomato skin extract contained 162.8 mg of total carotenoids per milliliter of extract. The total carotenoids content for the present study was in the range of previously reported values. In common varieties of tomatoes, total carotenoids are found at a concentration of 1.98 - 346.4 mg/100 g [22, 30].

Effect of pH: The effect of different pH values on degradation rate of carotenoids in tomato skin extract presents in Table 1. The degradation percent of carotenoids increased with decreasing the pH values and increasing the time. The extracted carotenoids characterized by higher stability at pH 9 compared with the other studied pH values (3, 5 and7) through increasing treatment time from 2 to 6 days. The degradation value reached 22.6 % at pH 9 after 6 days, while decreasing the pH values from 9 to 3 the carotenoids instability were gradually increased. It means that carotenoids were more stable at the alkaline pH values than at acidic pH values. The maximum degradation value was 39.9 % at pH 3 after 6 days. Carotenoids are highly susceptible to degradation by external agents, such as low pH, promoting changes of in color due to the rearrangement or formation of degradation compounds such as cis-isomers, epoxides, short chain products and in some cases, volatile compounds. These observations are in agreement with those obtained by Al Sayed *et al.* [27] and Mercadante [31].

Carotenoids Degradation Kinetics at Different pH and Time: Table 2 shows pseudo-first-order degradation rate constants (k) at different pH values, obtained from the slopes of logarithmic plots of the retention percentage vs. time (day). Increasing the pH value from 3 to 9 led to a significantly ($p< 0.05$) decreasing in the rate constant (k). The lowest degradation rate constant ($4.7\times10^{-2} \pm 6\times10^{-4}$) was observed at pH 9 with correlation coefficient 0. 9177. At pH 3 the carotenoids degradation rate constant arrived, to the maximum value ($9.7\times10^{-2} \pm 3\times10^{-4}$) with correlation coefficient 0.8928. For a first-order reaction, the half-life was determined at the studied pH values that

Table 1: Effect of different pH values on carotenoids degradation percent during six days.

	pH			
Time (day)	3	5	7	9
2	$3.8^{aC} \pm 1.2$	$2.1^{bC} \pm 0.1$	$2.5^{abC} \pm 0.1$	$1.2^{bC} \pm 0.3$
4	$34.8^{aB} \pm 0.8$	$26.0^{bB} \pm 0.2$	$21.5^{cB} \pm 0.4$	$16.0^{dB} \pm 0.3$
6	$39.9^{aA} \pm 2.8$	$36.3^{bA} \pm 6.9$	$31.9^{cA} \pm 6.2$	$22.6^{dA} \pm 3.9$

Means in the same row with different small letters are significantly different ($P < 0.05$).
Means in the same column with different capital letters are significantly different ($P < 0.05$).
Values are the average of three replicates in each treatment ± SD.

Table 2: Carotenoids degradation rate constants (k) and half-life at different pH values.

	pH			
Parameter	3	5	7	9
k	$9.7^{a} \times 10^{-2} \pm 3 \times 10^{-4}$	$8.2^{b} \times 10^{-2} \pm 3 \times 10^{-3}$	$6.9^{c} \times 10^{-2} \pm 3 \times 10^{-4}$	$4.7^{d} \times 10^{-2} \pm 6 \times 10^{-4}$
r^2	0.8928	0.9247	0.9357	0.9177
Half-life (day)	3.0	3.2	3.4	3.7

Means in the same row with different letters are significantly different ($P < 0.05$).
Values are the average of three replicates in each treatment ± SD.

Table 3: Effect of different thermal treatments on carotenoids degradation percent in tomato skin extract.

	Heating treatment			Autoclave treatment	
Time (min)	50°C	70°C	100°C	Time (min)	121°C
20	$1.75^{d} \pm 0.16$	$11.87^{c} \pm 0.15$	$23.78^{b} \pm 0.00$	10	$32.53^{a} \pm 0.95$
40	$4.59^{d} \pm 0.16$	$13.94^{c} \pm 0.17$	$38.31^{b} \pm 0.12$	20	$47.11^{a} \pm 1.12$
60	$6.36^{d} \pm 0.04$	$27.88^{c} \pm 0.13$	$45.76^{b} \pm 0.30$	30	$64.39^{a} \pm 1.53$

Means in the same row with different letters are significantly different ($P < 0.05$).
Values are the average of three replicates in each treatment ± SD.

Table 4: Arrhenius and thermodynamic parameters of total carotenoids pigments in tomato skin extract at different time.

Time (min.)	Activation energy[a] (kj/mol)	Intercept(ln A)	r^2	Enthalpy[b] (kj/mol)	Entropy of activation[b] (J/mol K)	r^2
20	$50.9^{a} \pm 1.6$	$19.8^{a} \pm 0.53$	0.89	$8.9^{a} \pm 0.20$	-76	0.76
40	$42.2^{b} \pm 0.40$	$17.3^{b} \pm 0.13$	0.99	$3.7^{b} \pm 0.13$	-95	0.99
60	$38.1^{c} \pm 0.11$	$16.4^{c} \pm 0.03$	0.88	$1.4^{c} \pm 0.05$	-104	0.99

Means in the same row with different letters are significantly different ($P < 0.05$).
Values are the average of three replicates in each treatment ± SD.
[a]Values were obtained from slopes of Arrhenius plots.
[b]Values were obtained from transition state theory equations. A, frequency factor.

Table 5: Carotenoids degradation rate constants (k) and half-life at different temperatures.

	Temperature (°C)			
Parameter	50	70	100	121
k	$1.1^{c} \times 10^{-3} \pm 3 \times 10^{-6}$	$5.0^{bc} \times 10^{-3} \pm 2 \times 10^{-5}$	$1.0^{b} \times 10^{-2} \pm 9 \times 10^{-6}$	$3.7^{a} \times 10^{-2} \pm 6 \times 10^{-3}$
r^2	0.9901	0.9281	0.9761	0.9755
Half-life (min.)	7.5	6.0	5.3	4.0

Means in the same row with different letters are significantly different ($P < 0.05$).
Values are the average of three replicates in each treatment ± SD.

ranged to 3-9. The half-life data in Table 2 appears that the stability of carotenoids of tomato skin extract improved with increasing the pH value. The half-life at pH 9 was superior compared to those at other pH values ranging between 3.0 and 7.0. Organic acids liberated during the processing of fruit juices are strong enough to promote rearrangements of active groups in the carotenoids [19, 20].

Effect of Thermal Treatments: Table 3 presents the effect of different thermal treatment on total carotenoids degradation. The carotenoids degradation significantly ($P < 0.05$) increased when the treatment temperature was increased from 50-100°C and the time from 20 to 60 min. At 50°C for 20 min. the carotenoids degradation was only 1.75 %. With increasing the temperature up to 100°C for 20 min. the degradation was dramatically increased to

23.78 %. On the other hand, rising the treatment time to 40 and 60 min. at all treatment temperatures lead to increase the total carotenoids instability. Carotenoids treated for 60 min. degraded with high percent compared to other treatment time at temperatures ranged between 50 and 100°C. At 50°C for 60 min. the carotenoids loss was 6.36 %. With increasing the temperature up to 100°C the degradation was dramatically significantly ($p<0.05$) increased to the maximum percent of 45.76 %. Chen *et al.* [32] reported that the levels of all the mono-cis forms of lycopene were decreased with increasing heating time. The degradation rate of cis-lycopene might be greater than the formation rate. Similar to â-carotene, it is also possible that the mono-cis forms of lycopene could be converted to di-cis forms of lycopene. The pasteurization treatment lead to reduce the carotenoids pigment contents by 38% and lutein by 20% in Brazilian Valencia orange juices [33]. At high treatment temperature (121°C) for 10 min. the carotenoids degradation significantly ($p<0.05$) arrived to the highest percent of 32.53 % compared to other thermal treatments at 50, 70 and 100°C for 20. Increasing the treatment time to 30 min at 121°C, the carotenoids degradation was arrived to its maximum value of 64.39 % compared to other thermal treatments. The results agree with that found by Lee and Chen [34], who noticed that the largest degradation rate constant was found at 150°C, followed by 100 °C and 50°C.

Kinetics of Carotenoids Degradation at Different Temperatures and Times:

Arrhenius and Thermodynamic Parameters: The degradation kinetics of total carotenoids pigments in tomato skin extract was monitored by storing the carotenoids extract at 50, 70 and 100°C for 20, 40 and 60 min. Arrhenius and thermodynamic parameters of total carotenoids are present in Table 4. The carotenoids held for 20 min. had the highest recorded activation energy being 50.9 kj/mol. With increasing the treatment time the activation energy was decreased. After 60 min. the activation energy arrived to 38.1 kj/mol. The same trend was observed with the intercept. The intercept decreased from 19.8 to 16.4 with increasing the treatment time from 20 to 60 min. It means that when the treatment was increased at the studied temperatures, the activation energy required to destroy the carotenoids decreased. The highest enthalpy of activation (ΔH) and the less negative entropy (ΔS) of activation for treated carotenoids was found at 20 min with values 8.9 kj/mol and -76 J/mol K, respectively. With increasing the treatment time to 60 min. the ΔH was significantly ($p< 0.05$) decreased to its minimum value 1.4 kj/mol. On the other hand, the ΔS was arrived to the negative greatest value -104 J/mol K. Correlation coefficient (r^2) of the linear relationship, which obtained from a plot of the enthalpy of activation vs. the entropy of activation ranged between 0.99 to 0.76. It means that the carotenoids stability decreased with increasing the treatment temperature and time. Lee and Chen [34], Takeoka *et al.* [35], Shi *et al.* [36] and Zanoni *et al.* [37], reported that the lycopene remains relatively stable during typical food processing procedures, except at extreme conditions (for example, very high temperature or very long heating time).

Degradation Rate Constant: Table 5 shows the first-order deterioration rate constants (k), obtained from the slopes of plots of the carotenoids logarithmic retention percentage vs. time (min.). Increased the storage temperature led to significantly ($p<0.05$) increased in the degradation rate constant. The lowest degradation rate constant ($1.1x10^{-3}$) was observed at temperature 50°C. With increasing the treatment temperature up to 121°C the degradation rate constant arrived to the highest value $3.7x10^{-2}$. Correlation coefficient of the linear relationship, which obtained from a plot of the logarithmic retention percentage vs. time (min), ranged between 0.9901 to 0.9281.

The half-life of extracted carotenoids was determined at different temperatures (50, 70, 100 and 121°C). The highest half-life was observed at temperature 50°C with value of 7.5 min (Table 5). With increasing the treatment temperature, the half-life of the carotenoids declined to the minimum value (4.0 min.) at 121 °C. The highest level of carotenoids destruction was found to be 46.6% and 29.3% at 121°C and 100°C, respectively. According to the previous data it could be said that the very high treatment temperature (121°C) was more effective in carotenoids degradation than the treatment temperatures up to 100 °C. The obtained data are in agreement with the findings of Shi *et al.*[38], who reported that at low temperatures such as at 60 and 80°C, the cis-isomers was formed and tend to accumulate. At high temperature treatments such as at 100 and 120°C, the all-trans to cis-lycopene isomerization reaction is expected to be speeded with formation of cis isomers, the unstable artifacts of carotenoids.

Sensory Evaluation of Filling Cream: As shown in Table 6, filling cream prepared using total carotenoids extract at different concentrations received scores ranged between 8.3 to 8.8 and 8.2 to 8.7 for flavor and graineess,

Table 6: Mean score values of sensory evaluation of filling cream prepared using synthetic color (sun set yellow) and tomato skin carotenoids extract at different concentrations.

		Carotenoids extract (%)			
Sensory character	Synthetic color (0.02%)	0.18	0.36	0.54	0.72
Appearance	8.3^{ab}	8.6^{ab}	8.9^{a}	8.9^{a}	8.0^{b}
Color	7.4^{b}	7.9^{b}	8.7^{a}	9.0^{a}	7.6^{b}
Flavor	8.4^{a}	8.4^{a}	8.8^{a}	8.8^{a}	8.3^{a}
Graininess	8.1^{a}	8.3^{a}	8.7^{a}	8.7^{a}	8.2^{a}
Overall acceptability	7.6^{b}	7.8^{b}	8.7^{a}	8.7^{a}	7.6^{b}

Means in the same row with different letters are significantly different ($P < 0.05$)

Table 7: Mean score values of sensory evaluation of glazing jelly prepared using synthetic color (sun set yellow) and tomato skin carotenoids extract at different concentrations.

		Carotenoids extract (%)			
Sensory character	Synthetic color (0.02%)	0.18	0.36	0.54	0.72
Appearance	8.7^{a}	6.5^{d}	7.2^{c}	8.9^{a}	8.1^{b}
Color	8.3^{ab}	6.7^{c}	7.1^{c}	8.8^{a}	8.2^{b}
Flavor	8.9^{a}	8.8^{a}	8.8^{a}	9.0^{a}	8.8^{a}
Graininess	8.3^{a}	8.3^{a}	8.3^{a}	8.7^{a}	8.4^{a}
Overall acceptability	8.2^{b}	6.4^{d}	7.0^{c}	8.9^{a}	8.1^{b}

Means in the same row with different letters are significantly different ($P < 0.05$).

respectively with non-significant difference (P>0.05) compared to the filling cream prepared using synthetic color with score values being 8.4 and 8.1for flavor and graininess, respectively. The samples prepared using 0.18, 0.36 and 0.54 % carotenoids extract had an appearance scores ranged between 8.6 and 8.9 with non-significant difference compared to appearance score value of the sample prepared using synthetic color. The highest overall acceptability and color scores observed in filling cream prepared using carotenoids extract at 0.36 and 0.54 % with significant difference compared to filling cream, which prepared using synthetic color at concentration 0.02 %.

Sensory Evaluation of Glazing Jelly: Sensory attributes of glazing jelly prepared with different levels of carotenoids pigments extracted from tomato skin are shown in Table 7. Non-significant difference (P>0.05) was observed between the flavor and graininess mean score values for glazing jelly prepared using synthetic color or extracted carotenoids. Glazing jelly prepared using 0.54 % carotenoids extract received the highest appearance and color values with non-significant difference (P>0.05) compared to those of glazing jelly prepared using synthetic color (0.02 %). On the other hand, glazing jelly prepared using 0.54 % carotenoids extract had overall acceptability score (8.9) higher than that of the glazing jelly prepared using the synthetic color (8.2) with significant difference (P<0.05).

CONCLUSION

Tomato skin carotenoids extract can be use as a coloring agent in some confectionary products such as filling cream and glazing jelly. Degradation kinetics of carotenoids extract appears the stability at pH 7 and 9. In addition, the thermodynamic parameters proved stability at temperatures less than 100°C. The filling cream and glazing jelly which prepared using that extracted carotenoids had a high sensory attributes with non-significant difference (p>0.05) compared to those products which prepared using the sun set yellow as a synthetic color.

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