Degradation Kinetics of Capsanthin in Paprika (*Capsicum annuum* L.) as Affected by Heating

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ABSTRACT: Diluted juice of paprika was heated at 80, 90 and 100 °C with holding times of 0, 2, 4, 8 and 16 min. Capsanthin in each sample was determined by HPLC. The degradation kinetics of capsanthin was studied by two groups of reaction models including elementary reaction rate models and those of modified square root-based pseudo (MRBP-). The MRBP-1st order reaction rate model was proven as an appropriate model in this study. The pseudo Q_{10} (PQ_{10}) value was 1.045 and the predicted half-life (capsanthin 2,850 mg/L) was 27.47, 21.23 and 15.23 min, respectively, at 80, 90 and 100 °C.

Key words: capsanthin, paprika, degradation, pseudo Q_{10}, half-life

Introduction

PAPRIKA (*CAPSICUM ANNUUM* L.) IS A KIND OF PEPPER GROWN for the edible fruits. It is an annual shrub belonging to the nightshade family Solanaceae. It is native to tropical areas of the Western Hemisphere, including Mexico, Central America, South America, and the West Indies. The fruits are bell- or tomato-shaped in appearance. They have a mild flavor and can be used in salads, pickles, cooking or as a food colorant (Gross 1991). A coloring agent, oleoresin of paprika, is extracted from the ground pods and used to impart a bright red color to meat and sausage products and also to other processed foods. The beautiful color of Capsicum fruits is mainly due to three kinds of ketocarotenoids: capsanthin, capsorubin, and cryptocapsin. Capsanthin was first isolated from the red pepper fruit in crystalline form (Zechmeister and Cholonoky 1927). Philip and Francis (1971) reported that the capsanthin in paprika amounted to 35% of the total carotenoids. There have been many other reports about capsanthin, namely, on the biosynthetic steps leading to capsanthin and related compounds (Valadon and Mummery 1977), quantitative changes of the main carotenoids (capsanthin, capsorubin, β-carotene and zeaxanthin) in red pepper powder and dried red pepper during storage (Malchev and others 1982), and kinetics of the main carotenoid pigments of red pepper in the de-esterification reaction (Mínguez-Mosquera and Pérez Gálvez 1998). However, the degradation kinetics of carotenoids, especially capsanthin, during heating has not been studied in detail. In this study we investigated the effect of heating on the stability of capsanthin in paprika and applied a newly modified reaction rate model. The objective of this study was to determine degradation kinetics of capsanthin in paprika, information of use in the food industry.

Materials and Methods

Samples

The study was carried out on paprika (*Capsicum annuum* L.) purchased from the grocery section of a local Galleria department store.

Preparation of standards

Internal standard β-apo-8′-carotenal (20% suspension in vegetable oil) was from Hoffman-LaRoche (Basel, Switzerland). The capsanthin standard was obtained from paprika using the methods developed by Chen (1992) and Mínguez-Mosquera and Pérez-Gálvez's (1998) reports. The fruit part of paprika was deseeded and ground in a Waring blender (Dynamics Corp. of America, New Hartford, Conn., U.S.A.) and filtered through a sieve (150 μm, #100). The filtered solution was homogenized with the Brinkmann homogenizer PCA11 (Brinkmann Instruments Co., Westbury, U.S.A.). The homogenized solution (80 g) was extracted with 0.8 g of $MgCO_3$ and 240 mL extractant (hexane-acetone-absolute alcohol-toluene, 10:7:6:7) in a separatory funnel (500 mL). The mixture was allowed to stand for 30 min with periodic shaking. Saponification was carried out by adding 8 mL of KOH-MeOH (40%) and standing in the dark under nitrogen for 2 h at room temp. Fifty mL of the supernatant (crude extract) of the mixture was transferred to another separatory funnel (500 mL). For the removal of water, 80 mL hexane was added to the crude extract, followed by 8 g of Na_2SO_4, which was then allowed to stand for 15 min with periodic shaking. Sixty mL of the supernatant was taken from the dehydrated crude extract and filtered through a 0.45-μm nylon membrane. The filtered solution was evaporated to dryness in a rotary evaporator at below 35 °C. Pigments were collected with acetone in a volume of 3 mL for thin-layer chromatography (TLC). Plates of silica gel 60 GF_{254} (20 cm × 20 cm plates, thickness 1 mm; Merck, Darmstadt, Germany) were used for TLC, with light petroleum ether-acetone-diethylamine (10:4:1) as the solvent system. The capsanthin standard was selected from bands on the TLC plate using the identification test of Mínguez-Mosquera and Hornero-Méndez (1993). The capsanthin standard was dissolved in 2 mL of acetone and stored in vials at –25 °C. The concentration of capsanthin was determined spectrophotometrically by using the corresponding values of ε_o (Davies and Köst 1988)

Heat treatments

The deseeded fruit part of paprika (100 g) was ground in a Waring blender and filtered through a sieve (150 μm, #100). The filtered solution was homogenized with a Brinkmann homogenizer and mixed with distilled water (4:6, wt/wt) and 10 g each filled into test tubes (screw-capped, 16 mm × 125 mm). Heat treatments were carried out at different temper-

atures (80, 90 and 100 °C) for 0, 2, 4, 8 and 16 min. An oil bath was used as the heating device. Heated samples were cooled to room temperature prior to the extraction step.

Extraction of pigments from heated samples

The heat-treated samples (10 g) were transferred to screw-capped test tubes (25 mm × 180 mm) and extracted with 0.1-g $MgCO_3$ and 40 mL of extractant (hexane-acetone-absolute alcohol-toluene, 10:7:6:7). Each tube was allowed to stand for 120 min with periodic shaking. Saponification was done by adding 2 mL of KOH-MeOH (40%) and standing in the dark under nitrogen for 2 h at room temp. Twenty mL of supernatant (crude extract) of each mixture was transferred to a test tube (screw-capped, 25 mm × 180 mm). Twenty mL of hexane was added to the supernatant extract followed by 2-g Na_2SO_4 and allowed to stand for 15 min with periodic shaking. Five mL of the supernatant from dehydrated crude extract was taken and evaporated to dryness in a rotary evaporator at below 35 °C. The pigments were collected with acetone in a volume of 1 mL and filtered through a 0.45 μm nylon membrane prior to HPLC analysis. All assays were performed in triplicate and averages of the assay results were recorded as mg/L. An internal standard (β-apo-8'-carotenal) was added at the beginning of the extraction process in a known quantity (300 μg/10 g of samples).

Quantification

The high-performance liquid chromatography (HPLC) instrument consisted of a HP1100 pump (Hewlett Packard, Inc., Waldbronn, Germany) with a linear photodiode-array detector (Hewlett Packard) and a stainless steel column (25 cm × 4.6 mm i.d.) packed with Hypersil ODS C_{18}(5 μm) (Hypersil, Needham Heights, Minn., U.S.A.). The data were processed with a HP chemstation Data System (Hewlett Packard, Inc., Wilmington, Del., U.S.A.). A sensitivity of 0.01 absorbance unit full scale (AUFS) was used. Spectrophotometric determinations were made with a Beckman DU-650 spectrophotometer (Fullerton, Calif., U.S.A.).

The following HPLC conditions were chosen: flow rate 1.5 mL/min; injection volume of 20 μL (loop); and detection at 450 nm. The gradient program was as follows:

Time	Acetone (%)	Water (%)	Curve
10(EQ)	75	25	
5	75	25	
5	95	5	Linear
7	95	5	
5	100	0	Convex
5	75	25	Linear

All HPLC-grade solvents were purchased from Merck Ltd. (Taipei, Taiwan, ROC) and filtered through a 0.2-μm membrane filter under vacuum prior to use.

Statistical analysis

Results were analyzed by regression analysis. Linear regression correlation coefficients were calculated to determine appropriate kinetic order (SAS 1990).

Results and Discussion

Quantification and degradation kinetics of capsanthin

The capsanthin standard obtained from paprika and the internal standard β-apo-8′-carotenal were used in HPLC. The chromatogram of the saponified extract from the nonheated control is shown as C in Figure 1. To confirm the purity of the standard, an aliquot of capsanthin standard was added to the control; however, no extra peak occurred. Capsanthin in the treated samples was determined by HPLC. Capsanthin contents in samples treated at all temperatures and heating times are shown in Figure 2 (averages of triplicates). The control had 5,700 mg/L. Temperature increases shortened the time of degradation without irregular data. Comparing the findings of Malchev and others (1982), there seemed to occur more degradation of capsanthin with wet heat than with dry heat. In their study the quantitative changes of the main carotenoids, capsanthin, capsorubin, β-carotene, and zeaxanthin, in red pepper powder and dried red pepper varied only slightly with dry heat; they experimented with drying temperatures of 60, 70, 80 and 90 °C during a 6-mo storage. Capsanthin and capsorubin were the most stable.

For the model fitting on the degradation kinetics of capsanthin, zero and first order reaction rate models were adopted in the beginning (Labuza and Riboh 1982; Arabshahi and Lund 1985). Linear regression correlation coefficients were calculated to get more appropriate kinetics order (SAS 1990). Rate constant k was obtained from the slope of linear

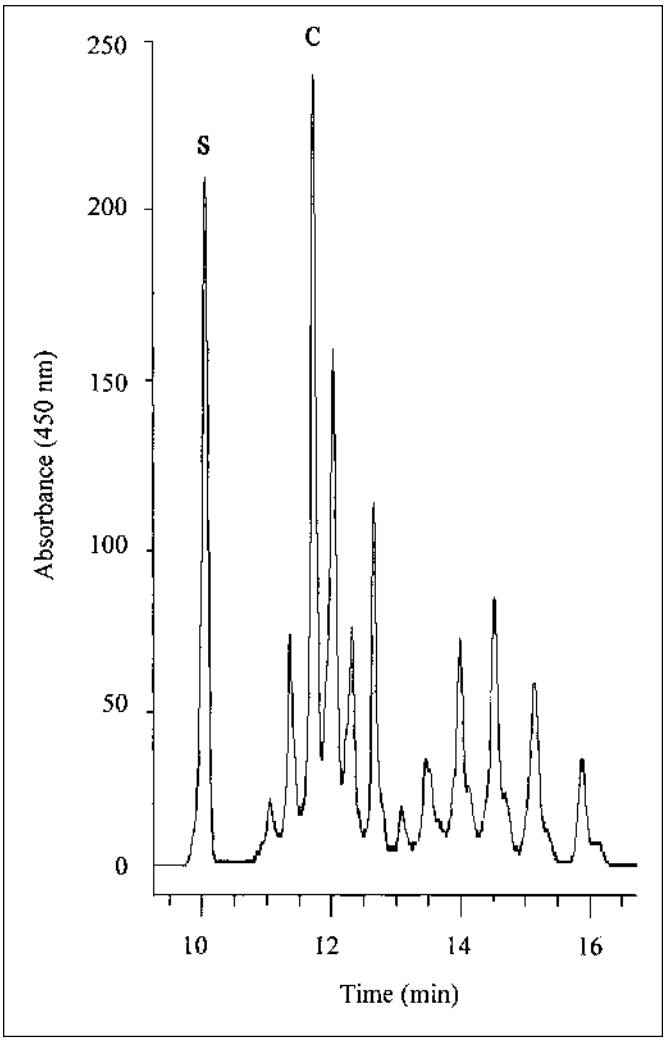

Figure 1—HPLC of saponified extract from Paprika, S, internal standard (β-apo-8′-carotenal)

Table 1—Regression models adapted for capsanthin degradation

Temp (°C)	Regression model	R^2	Temp (°C)	Regression model	R^2
	Zero order rate reaction ($C^b = -k^c \cdot t^d + C_o^e$)			MRBP[a]−Zero order rate reaction ($G^f = -z^g W^h + G_o^i$)	
80	$C = -146.4t + 5461.5$	0.88	80	$G = -644.7\ W + 5903.8$	0.97
90	$C = -144.5t + 5186.5$	0.83	90	$G = -665.9\ W + 5683.3$	0.99
100	$C = -144.5t + 4882.6$	0.66	100	$G = -714.9\ W + 5479.8$	0.91
	1st order rate reaction ($\ln C = -kt + \ln C_o$)			MRBP−1st order rate reaction ($\ln G = -zW + \ln G_o$)	
80	$D^j = -0.033t + 8.611$	0.92	80	$H^k = -0.143\ W + 8.7046$	0.96
90	$D = -0.034t + 8.557$	0.90	90	$H = -0.154\ W + 8.6648$	0.99
100	$D = -0.036t + 8.483$	0.74	100	$H = -0.170\ W + 8.6185$	0.95
	2nd order rate reaction ($1/C = kt + 1/C_o$)			MRBP−2nd order rate reaction ($I/G = zW + I/G_o$)	
80	$E^i = 8\ 10^{-6}\ t + 2\ 10^{-3}$	0.95	80	$I^m = .3\ 10^{-5}\ W + 2\ 10^{-3}$	0.95
90	$E = 9\ 10^{-6}\ t + 2\ 10^{-3}$	0.95	90	$I = .4\ 10^{-5}\ W + 2\ 10^{-3}$	0.99
100	$E = 9\ 10^{-6}\ t + 2\ 10^{-3}$	0.80	100	$I = .4\ 10^{-5}\ W + 2\ 10^{-3}$	0.97
	3rd order rate reaction ($1/C^2 = 2kt + 1/C_o^2$)			MRBP−3rd order rate reaction ($1/G^2 = \cdot 2z\ W + 1/G_o^2$)	
80	$F^n = 4\ 10^{-9}\ t + 3\ 10^{-8}$	0.97	80	$J^o = .2\ 10^{-8}\ W + 2\ 10^{-8}$	0.93
90	$F = 4\ 10^{-9}\ t + 3\ 10^{-8}$	0.98	90	$J = .2\ 10^{-8}\ W + 2\ 10^{-8}$	0.96
100	$F = 5\ 10^{-9}\ t + 4\ 10^{-8}$	0.86	100	$J = .2\ 10^{-8}\ W + 3\ 10^{-8}$	0.98

[a]MRBP− = Modified square root-based pseudo-
[b]C = concentration of capsanthin (mg/L) at time t (min)
[c]k = reaction rate (min^{-1})
[d]t = holding time (min)
[e]C_o = initial concentration (mg/L)
[f]G = concentration of capsanthin (mg/L) at W
[g]z = pseudo reaction rate (W^{-1})
[h]W = square root of t (min)
[i]G_o = initial concentration (mg/L)
[j]D = lnC
[k]H = lnG
[l]E = 1/C
[m]I = 1/G
[n]F = $1/C^2$
[o]J = $1/G^2$

lines plotted for kinetics.

$$C = C_o - kt \quad (1)$$

$$C/C_o = e^{2kt} \quad (2)$$

$$1/C^{n-1} - 1/C_o^{n-1} = (n-1)kt \quad (3)$$

where C is the concentration of capsanthin at time t (min), C_o is initial concentration, n is the reaction order, and k is the rate constant.

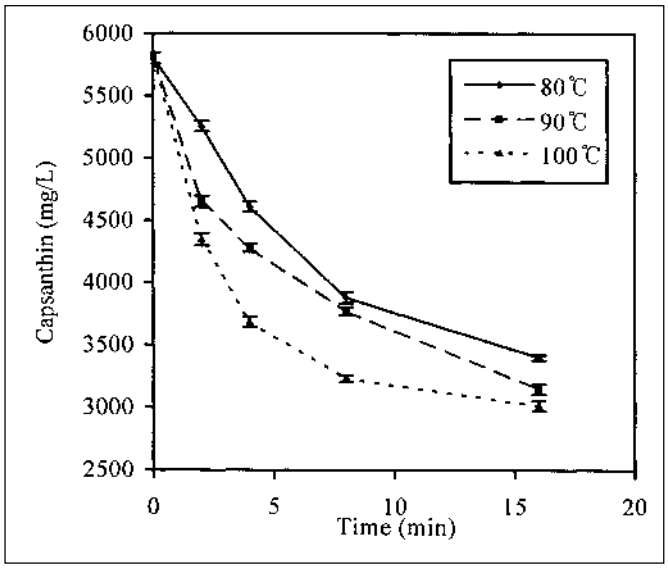

Figure 2—Degradation of capsanthin during heat treatment at different temperature

At the fitting of the zero order reaction rate model Eq. (1) (Table 1), the plot of the capsanthin (mg/L) remaining against time showed 0.88, 0.83, and 0.66 as correlation coefficients (R^2) at 80, 90 and 100 °C, respectively. To obtain higher R^2 , first order reaction rate model, Eq. (2), was chosen in place of the zero order reaction rate model (Cemeroglu and others 1994). There also have been other kinetics studies of pigments suggesting first order reaction rate models (Meschter 1953; Wrolstad and ohters 1970; Debicki-Pospisil and others 1983). Although the first order reaction rate model was more suitable to the results of capsanthin degradation reaction than that of the zero order rate, it was not so desirable at 100 °C in view of R^2 (0.74). Therefore higher order reaction rate models were considered according to the general order reaction rate model Eq. (3) (Steinfeld and others 1989). By the time of 3rd order development, the R^2 was increased to 0.97, 0.98 and 0.86 at 80, 90 and 100 °C, respectively.

We also tried more suitable reaction rate modeling through modification. There have been many trials for the modification of reaction rate models (Darling 1980; Clark 1992; Strecker and others 1995; Pauletti and others 1996). Pauletti and others (1996) reported a pseudo order reaction rate during the kinetics of heat coagulation of concentrated milk proteins at high sucrose concentrations. In that report rheological measurements were used to follow changes in adopting modified model and pseudo order reaction rate. In a report by Strecker and others (1995) there were model developments for polymerization with and without shear. In our study, through many trials of modification, modified square root-based pseudo (MRBP-) order reaction rate models were made, which were using W ($W^2 = t$) as an independent variable in place of t (min). Once MRBP-order reaction rate models were adopted, the R^2 were all above 0.90 at all temperatures. Through successive calculations, the

MRBP-1st order reaction rate model was chosen as an appropriate model in this degradation kinetics study in view of the high R^2 selection (above 0.95) and simplification of model fit.

Pseudo Q_{10} (PQ_{10}) and degradation prediction

To explain the effects of heating temperature on the degradation of capsanthin, the Arrhenius model, Eq. (4), was applied. Because of the application of the square root of t (holding time), named W, pseudo rate constant (z) was substituted for the rate constant (k) in this study. The pseudo rate constant (z) was calculated (Table 1) from MRBP-1st order reaction rate model using linear regression at different temperature conditions. Using least squares linear regression, the relation between the logarithm of the pseudo rate of constants against reciprocal of absolute temperature was predicted (Figure 3). The relationship could be characterized as $Y = -1129.5X + 1.250$ ($R^2 = 0.99$). The high R^2 confirmed that the degradation rate of capsanthin was temperature-dependent.

$$k = K_o\, e^{-Ea/RT} \quad (4)$$

$$\log k = -T^{-1}(E_a/R) + \log K_o \quad (5)$$

$$Q_{10} = k \text{ at } (T+10)\ ^oK / k \text{ at } T\ ^oK \quad (6)$$

$$\log Q_{10} = 2.189\, E_a / T\,(T+10) \quad (7)$$

where k is the reaction rate constant (min^{-1}), K_o is the frequency factor, E_a is the activation energy (cal M^{-1}), R is the gas constant (1.987 cal $M^{-1}\,{}^oK^{-1}$), and T is the absolute temperature.

As in the case of rate constant (z), the term pseudo-Q_{10} (PQ_{10}) value and pseudo-activation energy (PE_a) had to be substituted for the Q_{10} value and E_a. Because of the application of pseudo rate constant (z), this kind of nomenclature was inevitable. The PE_a was calculated with the Arrhenius model, Eq. (4). Eq. (5) was reset by using logarithms on both sides of model Eq. (4). With the help of Eq. (5) and the slope of the graph in Figure 3, PE_a was revealed to be 2.244 kcal/mol. The PQ_{10} value (Table 2) was obtained according to the relationship of Eq. (6) to Eq. (7) (Cemeroglu and others 1994; Lee and others 1995). The PQ_{10} value, that is, total average PQ_{10}, was taken from the mean of the average zQ_{10} value and the average aQ_{10} value. The average zQ_{10} value was the mean of each zQ_{10} value, calculated by Eq. (6) with pseudo rate constant (z) from two sets of different temperature conditions (80 to 90 °C and 90 to 100 °C). The average aQ_{10} value was also the mean of each aQ_{10}, calculated with pseudo rate constant (z) from three different temperature conditions using Eq. (7). To minimize error in the PQ_{10} determination, those complex calculations were carried out. As a result, the PQ_{10} was determined to be 1.045. Little difference between the average zQ_{10} value and the average aQ_{10} value was noticed.

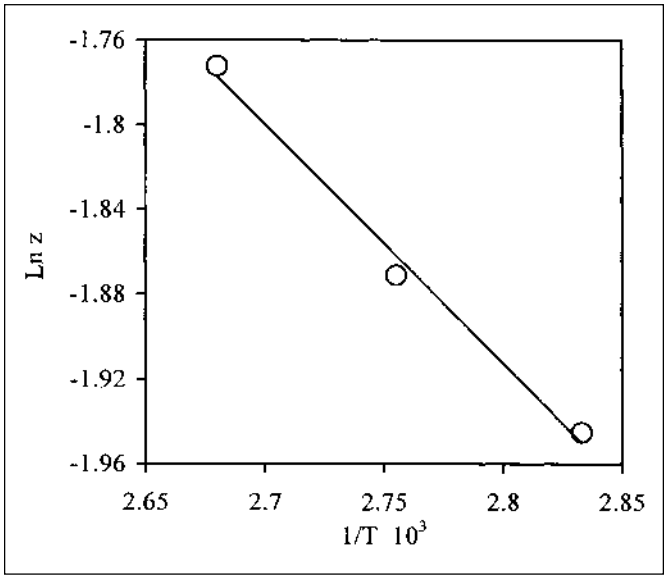

Figure 3—Arrhenius plot for capsanthin in paprika at differnet temperature in the range of 80 to 100 °C. z, pseudo rate constant (W^{-1})

Table 2—Effect of temperature on reaction rate

Temp (°C)	$z^a \cdot W^{-1\,b}$	Average $zQ_{10}{}^c$	Average $aQ_{10}{}^d$	Total $PQ_{10}{}^e$
80	0.143 (0.96)[f]			
90	0.154 (0.99)	1.090	1.001	1.045
100	0.170 (0.95)			

[a] z = pseudo constant rate (W^{-1})
[b] $W^{-1} = 1/W$, $W^2 = t$
[c] Average zQ_{10} = Mean of zQ_{10} value calculated from two different teperatures
[d] Average aQ_{10} = Mean of aQ_{10} value calculated from pseudo-E_a (PE_a)
[e] Total PQ_{10} (pseudo-Q_{10}) = Mean of Average zQ_{10} and Average aQ_{10}
[f] ( ) = Correlation coefficients (R^2)

Predicted half-life ($t_{1/2}$) of capsanthin at different temperatures was calculated. MRBP-1st order reaction rate model was used. In predicting $t_{1/2}$, the time-converting process was used, which means the conversion of the concept of time variable (W to t). At this time the one-half capsanthin concentration (2850 mg/L) of nonheated control (5,700 mg/L) was based in calculation. The pseudo half-life ($pt_{1/2}$: the half-life in the concept of W) was 5.24, 4.60 and 3.90 min at 80, 90 and 100 °C, respectively. For the conversion of time concept the square of $pt_{1/2}$, in other words, $t_{1/2}$ was produced, which was 27.47, 21.23 and 15.23 min at 80, 90 and 100 °C, respectively. The relationship between temperature (y; °C) and $t_{1/2}$ (x; min) is represented in the Eq.: $y = -6.12\,x + 33.55$ ($R^2 = 0.99$), which meant temperature dependency of half-life was dominant.

Conclusion

IN THIS STUDY WE INVESTIGATED THE DEGRADATION KINETICS of capsanthin in paprika when it was affected by heating. Data produced in this experiment should be useful in the food industry whenever concerned with paprika. In other respects, our study would be useful to explain chemical kinetics which could be difficult to determine with the general order reaction rate model in normal time concept. The modification of independent variable, especially concerning time, should be the next aspect of these kinetics studies.

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