JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2007,** *55,* 5123–5130 **5123**

Encapsulation of Lycopene Extract from Tomato Pulp Waste with Gelatin and Poly(γ-glutamic acid) as Carrier

Y. T. CHIU,† C. P. CHIU,† J. T. CHIEN,† G. H. HO,‡ J. YANG,‡ AND B. H. CHEN*,†

Department of Nutrition and Food Sciences, Fu Jen University, Taipei 242, Taiwan, and Vedan Enterprise Corporation, Taichung 400, Taiwan

Tomato pulp waste, a byproduct obtained during the processing of tomato juice, has been shown to be a rich source of lycopene. The objectives of this study were to use gelatin and poly(γ-glutamic acid) (γ-PGA) as coating materials for the encapsulation of lycopene extract from tomato pulp waste. Initially, lycopene was extracted with supercritical carbon dioxide, followed by microencapsulation using an emulsion system consisting of 4.5% gelatin, 10% γ-PGA, and 4.8% lycopene extract. Analysis of differential scanning calorimetry revealed that the thermal stability of the coating material could be up to 120 °C, with a mean particle size of 38.7 μm based on Coulter counter analysis. The total weight of microencapsulated powder was 617 μg with the yield of lycopene being 76.5%, indicating a 23.5% loss during freeze drying. During storage of microencapsulated powder, the concentrations of *cis*-, *trans*-, and total lycopene decreased along with increasing time and temperature. A fast release of lycopene in the powder occurred at pH 5.5 and 7.0, while no lycopene was released at pH 2.0 and 3.5.

KEYWORDS: Lycopene; tomato pulp waste; encapsulation; gelatin; poly(γ-glutamic acid)

INTRODUCTION

Lycopene, an important biological compound that is mainly present in tomato and tomato products, has received great interest in the past decade because of its possible roles in the prevention of chronic diseases such as atherosclerosis, skin cancer, and prostate cancer (*1*, *2*). However, lycopene can be susceptible to oxidation and isomerization during heating or illumination, especially when stored in the presence of oxygen (*3*−*5*). It has been well-documented that both oxidation and isomerization of lycopene can proceed simultaneously, and the dominant reaction should depend on heating or storage conditions (*3*). As a result, it is beneficial to encapsulate lycopene for commercial use as functional foods. Encapsulation has been used widely to entrap functional components in a carrier to impart protection against oxidation, isomerization, and degradation during storage of healthy foods for an extended period of time (*6*−*8*). In addition, encapsulation can be used to control the release of functional components when ingested in the body; that is, the unstable constituents should remain intact in the stomach and then release in the intestine over a range of physiological pH values (*9*, *10*). Thus, the type of carrier and method of encapsulation determine the extent of protection obtained, and the stability of encapsulated lycopene powder during storage needs to be further explored.

New carriers and encapsulation techniques continue to emerge to meet market challenges. Traditional carriers such as gelatin, modified starch, maltodextrin, and Arabic gum have been widely used as encapsulation substances (*6*, *11*). However, each carrier has advantages and disadvantages in terms of cost and encapsulation efficiency. For instance, gelatin is a popular encapsulation material because of its low cost and nontoxic nature. However, the net charge of gelatin is positive at pH < PI (isoelectric point), and with this characteristic, gelatin should be more proned to form complexes with compounds such as anionic polysaccharide through electrostatic interactions (*12*−*14*). Poly(γ-glutamic acid) (γ-PGA), a biodegradable and nontoxic polymer composed of many glutamic acid units connected by α-amino and γ-carboxyl groups and synthesized from microorganism *Bacillus* species (*15*, *16*), tends to ionize completely because of the dissociation of carboxylic acid groups at pH > 4 (*17*−*19*). The possible applications of γ-PGA in the industrial fields of food, cosmetics, medicine, and the environment have been well-documented (*15*, *17*−*19*). However, the potential of using γ-PGA as a carrier for encapsulation remains unknown.

Tomato pulp waste, a byproduct obtained during the processing of tomato juice, is also a rich source of lycopene (*20*). It would be a great advantage to the health food industry if tomato pulp waste could be used as a raw material for the production of lycopene powder. The objectives of this study were to encapsulate lycopene extract from tomato pulp waste and process it into powder with both gelatin and γ-PGA as carriers.

* To whom correspondence should be addressed. Tel: 886-2-29053626. Fax: 886-2-29021215. E-mail: 002622@mail.fju.edu.tw.
† Fu Jen University.
‡ Vedan Enterprise Corporation.

10.1021/jf0700069 CCC: $37.00
Published on Web 06/02/2007

MATERIALS AND METHODS

Materials. γ-PGA in sodium form (Na-γ-PGA; molecular mass, 990 kDa) was provided by Vedan Enterprise Co. (Taichung, Taiwan). All-*trans*-lycopene standard (purity >98%) was purchased from Sigma Co. (St. Louis, MO). Gelatin (type A, procine skin, PI 8) was also obtained from Sigma Co. The high-performance liquid chromatography (HPLC)-grade solvents acetonitrile and dichloromethane were from Mallinckrodt Co. (Barcelona, Spain). The analytical-grade solvent hexane was from Grand Chemical Co. (Taipei, Taiwan), and 95% ethanol was from Taiwan Tobacco Co. (Taipei, Taiwan). Deionized water was made using a Milli-Q water purification system (Millipore Co., Bedford, MA).

Instrumentation. The differential scanning calorimeter (DSC) (model 910) was from DuPont Co. (United States). The Coulter counter analyzer (model ZM) was from Phoenix Equipment Inc. (United States). The supercritical extractor (model SFE 7010) was from Applied Separation Co. (Allentown, PA). The HPLC system is composed of a Jasco PU-980 pump, a Jasco MD-915 photodiode array detector (Jasco Co., Tokyo, Japan), and an online DP-410 degassing system (Sanwa Tsusho Co., Tokyo, Japan). The high-speed centrifuge (model 5810) was from Eppendorf Co. (Hamburg, Germany). The rotary evaporator (model N-1) was from Eyela Co. (Tokyo, Japan). The freeze dryer (model FD-24) was from King-Ming Co. (Taipei, Taiwan). The low temperature incubator (model TL 520 R) was from Sheng-Long Co. (Taipei, Taiwan). The sonicator (model 2210R-DTH) was from Branson (Danbury, CT). A polymeric C30 column (250 mm × 4.6 mm i.d.; particle size, 5 μm) was from Waters Co. (Milford, MA).

Methods. *Extraction of Lycopene from Tomato Pulp Waste.* Tomatoes (*Lycopersicon esculentum* Mill.) were purchased from a tomato plant in the central part of Taiwan. After they were processed into juice, the tomato pulp waste was cut into pieces and subjected to freeze drying. After the waste was ground into powder, approximately 100 g of tomato powder was poured into a 300 mL stainless steel tube for supercritical carbon dioxide extraction, with the pressure at 350 bar and the temperature at 70 °C (*20*). The extraction was carried out by using a static mode for 90 min initially, followed by 50 min in a flowing mode, 30 min in a static mode for four times, and finally 10 min in a flowing mode. The lycopene extract was collected at intervals with a flow rate at 5 mL/min. A total of about 8 mL of lycopene extract was obtained and stored at −70 °C until further use.

Processing of Encapsulated Powder. Three percentage solutions of aqueous Na-γ-PGA, 10, 15, and 20, were prepared separately, and 3 g of each was mixed with 3 g of 3, 6, and 9% aqueous gelatin solution, respectively. Each mixture was dissolved in a 40 °C water bath, and then, 0.1 g of lycopene extract was added, after which the solution was stirred gently to form a homogeneous emulsion and placed in a water bath at 40 °C for 24 h to evaluate the stability of each emulsion system.

For evaluation of the thermal stability of each emulsion by DSC, 3 g of 15 and 20% aqueous Na-γ-PGA solution was mixed with 3 g of 6 and 9% aqueous gelatin solution, respectively. Each mixture was dissolved in a 40 °C water bath, and the thermal stability of the emulsion was determined using a method as described by Chien et al. (*21*). Briefly, 8 mg of each emulsion was hermetically sealed in a coated sample pan, while a sealed empty pan was used as a reference. Nitrogen was purged with a flow rate of 20 mL/min to maintain a pressure at 500 psi. The sample was heated from 25 to 120 °C at 10 °C/min, and the characteristic transitions were recorded for evaluation of the thermal stability of the encapsulated material. A maximum deviation in T_m of ±0.1 °C was allowed, and the instrument was calibrated with indium ($T_{m,onset}$ = 156.6 °C and ΔH = 28.45 J/mol) by heating from 25 to 200 °C at 10 °C/min.

Likewise, 3 g of each 15 and 20% aqueous Na-γ-PGA solution was mixed with 3 g of 6 and 9% aqueous gelatin solution, respectively. Next, each solution was mixed with 0.1, 0.15, 0.2, 0.25, and 0.3 g of lycopene extract separately so that the final mixture contained 1.6, 2.4, 3.2, 4.0, and 4.9% of lycopene. Each mixture was stirred in a 40 °C water bath to form a homogeneous emulsion, after which the emulsion stood in a 40 °C water bath for 24 h for the stability evaluation. Then, the particle size and distribution of the most stable emulsion were measured using a Coulter counter analyzer by mixing 0.2 g of aqueous lecithin solution (0.01%) and 0.05 g of lycopene emulsion and diluting the solution to 10 mL with distilled water.

For the processing of encapsulated powder, 3 g of 20% aqueous solution Na-γ-PGA solution was mixed with 3 g of 9% aqueous gelatin solution and 0.3 g of lycopene extract. Then, the mixture was stirred to form a homogeneous emulsion, followed by freezing at −30 °C for 30 min and freeze drying for 30 h under a vacuum of 55 mTorr. After the dried sample was ground into a powder, the powder was poured into a brown vial and stored in a desiccator for further use.

Analysis of Lycopene in the Powder. A modified method based on Lin and Chen (*22*) was used to extract lycopene from encapsulated powder. A 0.5 g amount of powder sample was mixed with 40 mL of distilled water in a flask, and the mixture was sonicated for 30 min, after which 40 mL of ethanol−hexane (4/3, v/v) was added and the solution was again sonicated for 10 min. After it was centrifuged for 10 min at 4000 rpm (25 °C), the supernatant was collected. The residue was extracted using the same procedure, and the supernatants were combined, followed by addition of 10 mL of 10% sodium chloride solution and 15 mL of distilled water. After the mixture was sonicated for 5 min, the upper layer was collected and the residue was repeatedly extracted. The upper layers were also pooled, evaporated to dryness under vacuum, dissolved in 2 mL of dichloromethane, and filtered through a 0.2 μm membrane filter for HPLC analysis. A mobile phase developed by Lin and Chen (*22*) was used to separate the various carotenoids in the lycopene powder by using a C30 column with the following gradient elution: 99% *n*-butanol and acetonitrile (3/7,v/v) (A) and 1% dicholoromethane (B) initially, increased to 4% B in 20 min, 10% B in 50 min, and returned to 1% B in 55 min. All-*trans*-lycopene and its six isomers, including di-*cis*-lycopene (I), 15-*cis*-lycopene, 13-*cis*-lycopene, di-*cis*-lycopene (II), 9-*cis*-lycopene, and 5-*cis*-lycopene, were resolved within 52 min with a flow rate at 2 mL/min and detection at 476 nm.

For the identification of cis isomers of lycopene, a concentration of 100 μg/mL of all-*trans*-lycopene in dichloromethane was prepared. The solution was poured into a transparent vial and illuminated under 2000−3000 lux for 24 h at 25 °C, after which the solution was filtered through a 0.2 μm membrane filter and 20 μL was injected for HPLC. The retention time and absorption spectrum of each isomerized lycopene peak were compared with those in the powder sample. In addition, the isomerized lycopene standard was added to the powder sample for cochromatography for further identification of cis isomers of lycopene.

The identification of all-*trans*-lycopene was carried out by comparing the retention times of unknown peaks with reference standards and cochromatography with added all-*trans*-lycopene standards. For tentative identification of cis isomers of lycopene, they were based on spectral characteristics as described above and in several previous studies (*4*, *5*, *20*, *22*). Quantitation was carried out using an absolute calibration method. Seven concentrations of 5, 20, 40, 60, 80, 100, and 160 μg/mL all-*trans*-lycopene standard in dichloromethane were prepared separately, and 20 μL of each was injected into HPLC. The calibration curve was drawn by plotting the concentration against the area, and the linear regression equation and correlation coefficient (r^2) were calculated. Because of the difficulty in obtaining standards of cis isomers of lycopene, they were quantified using the same regression equation as all-*trans*-lycopene (*4*, *5*, *17*). The yields (%) of lycopene in the powder and total lycopene (μg) in the emulsion were calculated using the following formulas:

$$\text{yield (\%)} = \frac{\text{total lycopene in the powder}}{\text{total lycopene in the emulsion}} \times 100$$

$$\text{total lycopene (}\mu\text{g) in the emulsion} = [\text{lycopene concentration (}\mu\text{g/g) in the extract}] \times [\text{amount (g) of lycopene extract added to the emulsion}]$$

Stability of Lycopene Powder during Storage. A 1 g powder sample was poured into a 4 mL brown vial and stored in a temperature-controlled incubator at 4, 25, and 35 °C for 5, 10, 15, 20, 25, and 30 days. Duplicate experiments were performed, and a total of 36 vials were used. After the desired temperature and storage time were reached, a 0.5 g sample in each vial was collected and extracted for HPLC

analysis. The degradation rate constant of lycopene during storage was calculated using a first-order degradation equation as described by Sharma and Maguer (*23*) and is expressed as

$$L = L_0 \times [\exp(-kt)]$$

where L = amount (μg/g) of lycopene at time t, L_0 = initial amount (μg/g) of lycopene, k = apparent reaction rate constant (day^{-1}), and t = days of storage.

The activation energy was calculated using the Arrhenius equation:

$$\ln(k) = -\frac{E_a}{R}\left(\frac{1}{T}\right) + \ln(A)$$

where E_a = activation energy (kJ/mol) and R = ideal gas constant (8.314 J/mol K).

Controlled Release of Lycopene in the Encapsulated Powder. Concentrations of 0.1 M citric acid solution and 0.2 M disodium hydrogen phosphate solution were prepared by dissolving 14.5 g of the former and 14.2 g of the latter in deionized water, respectively, and they were diluted to 500 mL. An appropriate volume of citric acid solution and disodium hydrogen phosphate solution was collected to obtain a ratio of 99:1, 70:30, 45:55, and 18:82 (v/v), with the final pH at 2.0, 3.5, 5.5, and 7.0, respectively.

A 0.4 g of powder sample was mixed with 50 mL of each buffered solution in a flask and shaken at 37 °C (70 rpm) for 5, 15, 30, 60, or 120 min. After the sample was filtered through a Whatman #1 filter paper, a 40 mL filtrate was collected for extraction of lycopene using the same procedure described above. The amount of lycopene released as affected by different pH was monitored by HPLC.

Statistical Analysis. Duplicate experiments were performed, and the mean values plus standard deviations were calculated. All of the data were subjected to analysis of variance and Duncan's multiple range test using SAS (*24*).

RESULTS AND DISCUSSION

HPLC Analysis of Lycopene in Tomato Pulp Waste. On the basis of a previous study by Wang and Chen (*20*), who reported that with supercritical carbon dioxide extraction at 70 °C and 350 bar, a high amount of total lycopene was obtained from tomato pulp waste. Following this approach and using an HPLC method developed by Lin and Chen (*22*), tomato pulp waste was found to contain di-*cis*-lycopene (I), 15-*cis*-lycopene, 13-*cis*-lycopene, di-*cis*-lycopene (II), 9-*cis*-lycopene, and 5-*cis*-lycopene. By using an absolute calibration method and assessing the extinction coefficient of *cis*-lycopene to be equivalent to all-*trans*-lycopene for quantification (*20*), tomato pulp waste was found to contain 525.3 μg/g of total lycopene (all-trans plus cis) in supercritical carbon dioxide extract.

Stability Evaluation of Microencapsulated Emulsion. Three percentage solutions of aqueous γ-PGA, 10, 15, and 20, were mixed with 3, 6, and 9% of aqueous gelatin solutions to obtain the following nine different combinations: (i) 5% γ-PGA and 1.5% gelatin, (ii) 5% γ-PGA and 3% gelatin, (iii) 5% γ-PGA and 4.5% gelatin, (iv) 7.5% γ-PGA and 1.5% gelatin, (v) 7.5% γ-PGA and 3% gelatin, (vi) 7.5% γ-PGA and 4.5% gelatin, (vii) 10% γ-PGA and 1.5% gelatin, (viii) 10% γ-PGA and 3% gelatin, and (ix) 10% γ-PGA and 4.5% gelatin. A 6 g sample from each mixture was collected and mixed with 0.1 g of supercritical carbon dioxide lycopene extract, and the solution was stirred in a 40 °C water bath to form a homogeneous emulsion. All nine emulsions were stored at 40 °C for 24 h for stability comparison, in which only four emulsions (treatments 5, 6, 8, and 9) were shown to be the most stable, as no phase separation of oil and water occurred. Then, the four emulsions were subjected to thermal stability determination by using a DSC. **Figure 1** shows the DSC curve of the coating material

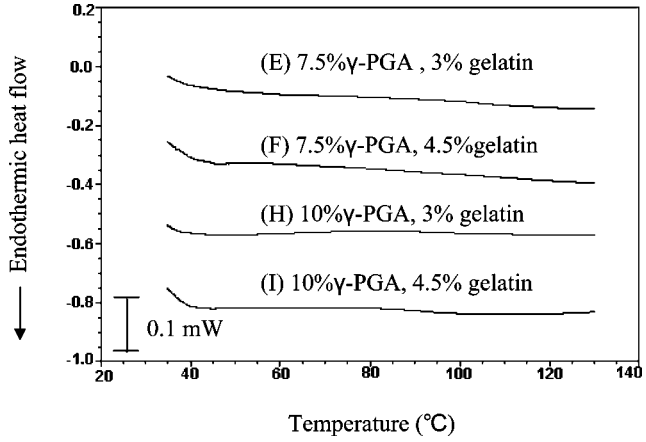

Figure 1. DSC curves of the coating material composed of γ-PGA and gelatin.

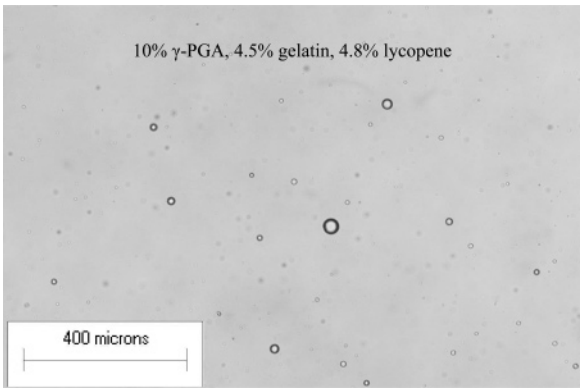

Figure 2. Microphotograph of the lycopene emulsion.

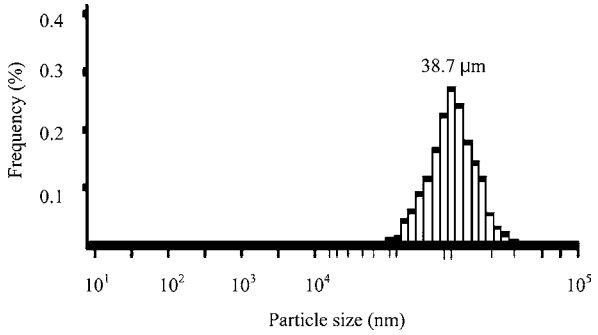

Figure 3. Particle size distribution of the microencapsulated emulsion by Coulter counter analysis.

containing γ-PGA and gelatin at different levels. All four emulsions were found to possess high thermal stability even up to 120 °C, which may be due to the formation of a thermally stable complex between anionic γ-PGA and cationic gelatin through electrostatic interaction.

To raise the lycopene concentration in the four emulsions, five levels of 0.1, 0.15, 0.2, 0.25, and 0.3 g of lycopene extract were added to each emulsion separately for comparison of stability. Results showed that with the lycopene level of 0.3 g, an emulsion containing 10% γ-PGA and 4.5% gelatin was the most stable, while the other three emulsion systems showed a phase separation of oil and water. A microphotograph of this lycopene emulsion shows the core material (lycopene) to be encapsulated in spherical microcapsules (**Figure 2**), which should protect lycopene from being degraded in the presence of light and oxygen. **Figure 3** shows the particle size distribution of the microencapsulated emulsion containing 3 g of 20%

Table 1. Concentration (μg/g) Changes of *cis*-, *trans*-, and Total Lycopene in Microencapsulated Lycopene Powder during Storage[a]

lycopene[b]	temp (°C)	storage time (days) 0	5	10	15	20	25	30
cis[c]	4	45.5 ± 1.0 abx	45.2 ± 4.7 abcx	46.1 ± 0.6 ax	46.5 ± 3.3 abcx	41.7 ± 3.5 bcx	38.8 ± 5.3 cx	38.4 ± 5.1 cx
	25	45.5 ± 1.0 ax	43.3 ± 3.5 abx	40.7 ± 1.3 by	38.4 ± 3.8 bcy	34.9 ± 2.7 cxy	35.0 ± 1.1 cx	29.1 ± 0.3 dy
	35	45.5 ± 1.0 ax	40.3 ± 1.6 bx	34.1 ± 3.6 cz	31.6 ± 1.8 cz	27.4 ± 0.5 dz	29.5 ± 1.1 cy	25.2 ± 0.5 ez
trans	4	88.7 ± 6.7 acx	73.9 ± 2.0 dx	85.0 ± 1.0 ax	74.4 ± 8.7 bcdx	75.1 ± 6.2 bdx	78.4 ± 2.2 bx	70.9 ± 2.1 dx
	25	88.7 ± 6.7 ax	71.5 ± 2.9 bx	73.5 ± 4.1 by	64.7 ± 1.4 cx	68.0 ± 1.8 bx	57.9 ± 3.9 dy	42.2 ± 4.0 ey
	35	88.7 ± 6.7 ax	63.6 ± 0.5 by	59.5 ± 1.1 cz	51.3 ± 5.2 dy	50.6 ± 0.5 dy	48.6 ± 3.4 dz	39.8 ± 0.5 ey
total	4	134.2 ± 2.3 ax	119.1 ± 1.5 cx	131.1 ± 1.1 abx	120.9 ± 9.5 bcdx	116.8 ± 8.9 cdx	117.2 ± 5.5 cdx	109.3 ± 5.9 dx
	25	134.2 ± 2.3 ax	114.8 ± 4.8 bx	114.2 ± 5.3 by	103.1 ± 3.5 cy	102.9 ± 4.2 cx	92.9 ± 4.6 dy	71.3 ± 4.3 ey
	35	134.2 ± 2.3 ax	103.9 ± 1.5 by	93.6 ± 1.1 cz	82.9 ± 6.9 dz	78.0 ± 0.8 dz	78.1 ± 4.3 dz	65.0 ± 0.9 ez

[a] For letters a–e, each value of means bearing different letters in the same row is significantly different ($p < 0.05$). For letters x–z, each value of means bearing different letters in the same column within each lycopene variety is significantly different ($p < 0.05$). [b] Concentration expressed as mean of duplicate analyses ± standard deviation. [c] A mixture of cis-isomers of lycopene.

γ-PGA, 3 g of 9% gelatin, and 0.3 g of lycopene extract by using a Coulter counter analyzer. An average particle size of 38.7 μm in the emulsion system was obtained, with 95% distribution being in the range of 37.6−39.9 μm. Bustos et al. (*25*) used chitosan as the coating material to encapsulate carotenoid-containing shrimp oil, and an average particle size of the microcapsule ranging from 20 to 100 μm was produced with a proportion of oil to water phase at 2:1 (v/v). Our study demonstrated that γ-PGA may replace anionic polysaccharide to be the main coating material for encapsulation.

Production of Lycopene Powder. Of the various drying methods, freeze drying was chosen in our study since it can maintain the highest quality of lycopene powder by minimizing oxidative degradation during drying (*20*). To further increase the yield of lycopene powder, an emulsion containing 15 g of 20% Na-γ-PGA, 15 g of 9% gelatin, and 1.5 g of lycopene extract was placed in a freezer (−30 °C) for 30 min, followed by freeze drying for 30 h and then grinding into powder. The average weight of lycopene emulsion was 29.95 g before freeze drying and dropped to 5.53 g after freeze drying, in which the lycopene level showed the same trend by decreasing from 809.02 to 617.26 μg. The amount of 809.02 μg was calculated by multiplying the weight of lycopene extract (1.54 g) for emulsion preparation and lycopene concentration (525.34 μg/g) in supercritical carbon dioxide extract. Likewise, the level of 617.26 μg was obtained by multiplying the powder weight and lycopene concentration in the powder. The average yield (76.5%) of lycopene was obtained by dividing the amount of lycopene in the powder with that in the emulsion. This result implied that a 23.5% lycopene loss did occur during drying, which should be caused by oxidative degradation. In a previous study dealing with the production of lycopene powder, Wang and Chen (*20*) prepared four emulsions by mixing supercritical carbon dioxide extract and various solvents including chloroform, ethyl acetate, hexane, and ethanol/hexane (4/3, v/v) separately, with both gelatin and sugar as carriers. After spray drying, the powder was found to contain lycopene concentrations at 35.0, 59.6, 238.6, and 111.1 μg/g, respectively. As compared to our study, this method posed a major safety problem as organic solvents were used in preparing emulsions. In addition, a low lycopene concentration in the powder was obtained with chloroform or ethyl acetate as the solvent.

Stability of Lycopene Powder during Storage. Table 1 shows the concentration changes of lycopene in the encapsulated powder during storage. Overall, a decreasing trend was found following the increase of both storage time and temperature (**Figure 4**). After storage at 4, 25, and 35 °C for 30 days, the cis isomers of lycopene showed a decline by 7.1, 16.4, and 20.3 μg/g, respectively, whereas a loss of 17.8, 46.3, and 48.9 μg/g occurred for all-*trans*-lycopene. As for total lycopene (trans plus cis), it showed the same tendency with a drop by 24.9, 62.9,

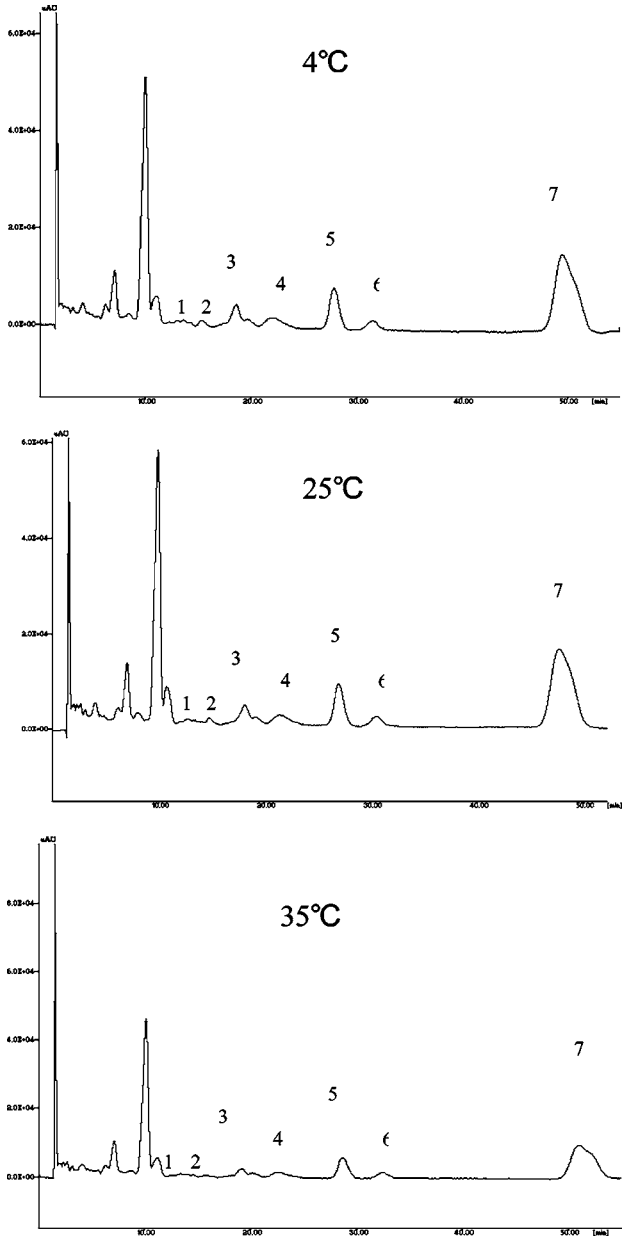

Figure 4. HPLC chromatogram of microencapsulated lycopene powder stored at different temperatures for 30 days. Peaks: 1, di-*cis*-lycopene (I); 2, 15-*cis*-lycopene; 3, 13-*cis*-lycopene; 4, di-*cis*-lycopene (II); 5, 9-*cis*-lycopene; 6, 5-*cis*-lycopene; and 7, all-*trans*-lycopene.

Table 2. Degradation Rate Equations and Rate Constants (Day^{-1}) as Well as Activation Energy of Lycopene Powder

lycopene	temp (°C)	first-order degradation	k (day^{-1})	r^2	activation energy (kJ/mol)	ln(k)
cis[a]	4	$L = 47.30[-\exp(0.0062)t]$	6.20×10^{-3}	0.7049		–5.08
	25	$L = 46.13[-\exp(0.0133)t]$	1.33×10^{-2}	0.9567	26.89	–4.32
	35	$L = 44.07[-\exp(0.0199)t]$	1.99×10^{-2}	0.9354		–3.92
trans	4	$L = 89.88[-\exp(0.0077)t]$	7.70×10^{-3}	0.7932		–4.87
	25	$L = 86.06[-\exp(0.0181)t]$	1.81×10^{-2}	0.8418	26.37	–4.01
	35	$L = 80.55[-\exp(0.0247)t]$	2.47×10^{-2}	0.8767		–3.70
total	4	$L = 137.15[-\exp(0.0071)t]$	7.17×10^{-3}	0.9406		–4.94
	25	$L = 132.18[-\exp(0.0163)t]$	1.63×10^{-2}	0.9008	26.52	–4.12
	35	$L = 124.56[-\exp(0.0229)t]$	2.29×10^{-2}	0.9134		–3.78

[a] A mixture of cis isomers of lycopene.

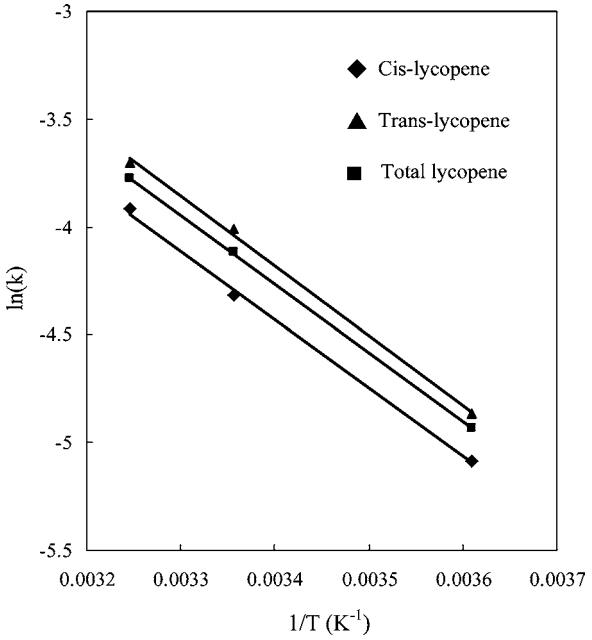

Figure 5. Plot of ln(k) vs 1/T for cis isomers of lycopene and trans as well as total lycopene degradations during storage.

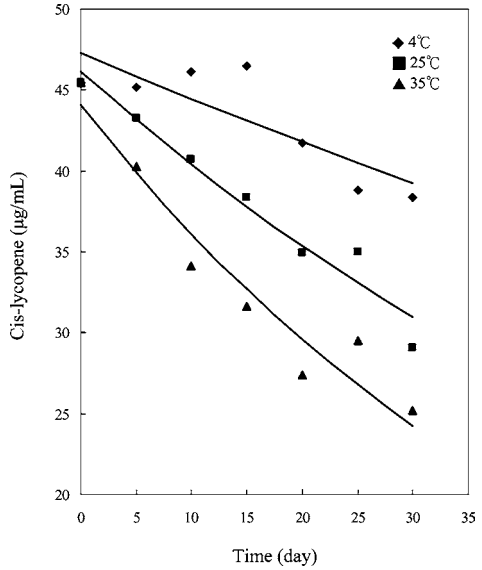

Figure 6. Degradation curve of cis isomers of lycopene during storage at 4, 25, and 35 °C.

and 69.2 μg/g. A similar outcome was observed by Sharma and Maguer (*23*), who reported a high loss of 90% lycopene during storage of freeze-dried tomato samples at room temperature for 4 weeks. Lisiewska and Kmiecik (*26*) also found a lycopene loss of 48 and 26%, respectively, in freeze-dried tomatoes when stored at −20 and −30 °C for 12 months. Likewise, a 50% lycopene loss occurred for tomato powder over a storage period of 30 days at 37 °C (*27*). All of these results further proved that lycopene is susceptible to degradation and can still undergo a considerable loss even when stored at frozen temperature.

With storage temperature at 4 °C, the contents of both *trans*-lycopene and total lycopene showed a diminishing trend over a period of 15 days, whereas the cis isomers of lycopene did not show a significant loss, which may be attributed to degradation and isomerization of *trans*-lycopene proceeding simultaneously during storage. It has been reported that in addition to degradation, *trans*-lycopene may be converted to its cis isomers of lycopene, which would account for an insignificant loss of cis isomers of lycopene during storage (*3*−*5*). Interestingly, a slight loss of cis isomers of lycopene followed after 15 days of storage, which may be due to degradation or conversion to the corresponding *trans*-lycopene (*3*). Several studies have suggested that *cis*-lycopene may be converted to another cis form of lycopene only through the intermediate *trans*-lycopene (*3*−*5*). A similar phenomenon was observed by Lin and Chen (*4*), who determined the lycopene stability during the storage of tomato juice at 4, 25, and 35 °C and reported an inconsistent change of both *trans*- and *cis*-lycopene levels. As explained above, the reversible conversion between trans and its cis isomers of lycopene during storage may account for this effect.

Degradation Rate Constant of Lycopene Powder during Storage. In the kinetic study of lycopene powder stability during storage, the first-order reaction was used to determine the degradation rate constant of lycopene as a high correlation coefficient was obtained. **Table 2** shows the degradation rate equations and rate constants as well as activation energy of lycopene in the powder during storage. With storage temperatures of 4, 25, and 35 °C, the degradation rate constants (day^{-1}) for cis isomers of lycopene were 6.20×10^{-3}, 1.33×10^{-2}, and 1.99×10^{-2}, respectively, while for *trans*-lycopene and total lycopene, the degradation rate constants (day^{-1}) were 7.70×10^{-3}, 1.81×10^{-2}, and 2.47×10^{-2} for the former and 7.17×10^{-3}, 1.63×10^{-2}, and 2.29×10^{-2} for the latter. Overall, the rate constant increased along with increasing storage temperature, with the fastest reaction proceeding at 35 °C followed by 25 and 4 °C. As mentioned before, both *trans*-lycopene and its cis isomers can be liable to degradation and isomerization simultaneously during storage, which should account for a higher degradation rate constant at elevated temperature (35 °C). Moreover, *trans*-lycopene showed a larger degradation rate constant than its corresponding cis isomers, which may be due to the slow conversion of the latter to another

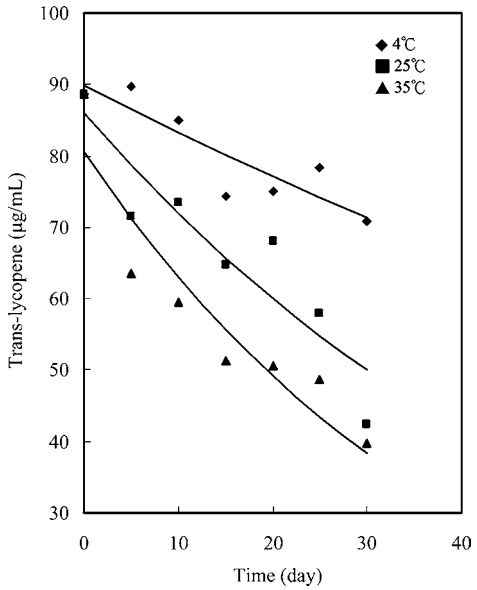

Figure 7. Degradation curve of *trans*-lycopene during storage at 4, 25, and 35 °C.

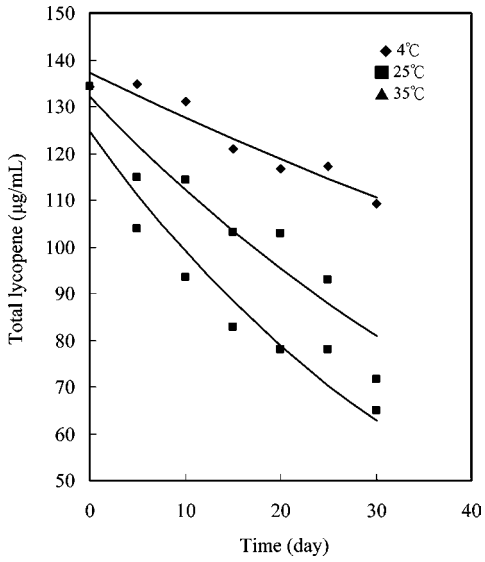

Figure 8. Degradation curve of total lycopene during storage at 4, 25, and 35 °C.

cis form of lycopene. A similar outcome was observed by Sharma and Maguer (*23*), who reported the degradation rate constant (day^{-1}) to be 6.7×10^{-3} and 1.8×10^{-2}, respectively, for dried tomato pulp waste when stored at 5 and 25 °C under vacuum. Comparatively, the smaller rate constant may be explained by the difference in storage condition, especially under vacuum in the dark. According to the Arrhenius equation, the activation energies for cis isomers of lycopene, *trans*-, and total lycopene were 26.89, 26.37, and 26.52 kJ/mol, respectively (**Table 2**). This result is also similar to a report by Sharma and Maguer (*23*), who found the activation energy to be 27.74 kJ/mol for dried tomato pulp waste stored for 60 days under vacuum in the dark. **Figure 5** shows the Arrhenius plot of ln(k) vs $1/T$ for cis isomers of lycopene, *trans*-lycopene, and total lycopene degradations during storage. A high correlation coefficient (r^2) was obtained for cis isomers of lycopene, *trans*-, and total lycopene, which amounted to 0.9974, 0.9987, and 1.0000, respectively. **Figures 6−8** show the degradation curves of cis isomers of lycopene, *trans*-lycopene, and total lycopene during storage at 4, 25, and 35 °C. A first-order reaction curve was shown for the concentration changes of cis isomers of lycopene, *trans*-, and total lycopene in the powder; that is, the amount of total lycopene followed a decreasing trend for the increase of storage temperature. In a study dealing with lycopene stability during heating at 50, 100, and 150 °C, Lee and Chen (*3*) concluded that the degradation reaction also followed a first-order kinetics, with the rate constant (min^{-1}) being 7.50×10^{-4}, 1.24×10^{-2}, and 1.65×10^{-1}, respectively. Theoretically, both isomerization and degradation of lycopene should proceed simultaneously during heating and the latter may be favored only under drastic conditions (*3*). Accordingly, because only mild storage conditions were used in our experiment, the overall rate should depend mainly on the isomerization reaction instead of the degradation reaction. Nevertheless, we have to point out that low correlation coefficients of 0.7049 and 0.7932 obtained for cis isomers of lycopene and *trans*-lycopene, respectively, when stored at 4 °C, indicate a possible reversible reaction between trans and cis forms of lycopene, as reported by Lin and Chen (*4*, *5*).

Controlled Release of Lycopene in the Powder. Table 3 shows the concentration changes of cis isomers of lycopene, *trans*-, and total lycopene released from microencapsulated powder and release rates as affected by pH. Four pH values, 2.0, 3.5, 5.5, and 7.0, were selected to monitor the lycopene release in a simulated stomach and intestine system. Encapsulated powder was found to contain 34.8 μg/g of cis-isomers of lycopene, 71.7 μg/g of *trans*-lycopene, and 106.5 μg/g of total lycopene. These concentrations were not the same as those in the powder used for the stability study during storage (**Table 1**), mainly because the processing time of the powder used for controlled release was different. No lycopene was released at pH 2.0 and 3.5, even after the reaction time was extended to 2

Table 3. Concentration (μg/g)[a] Changes of *cis*-, *trans*-, and Total Lycopene Released from Microencapsulated Lycopene Powder and Release Rate as Affected by pH[b]

	pH									
			5.5				7.0			
time (min)	2.0	3.5	cis[c]	trans	total	release rate (%)	cis[c]	trans	total	release rate (%)
5	ND	ND	29.4 ± 0.5 b	47.1 ± 2.6 b	76.5 ± 1.6 b	71.8	31.2 ± 0.7 a	46.6 ± 1.5 b	77.8 ± 1.5 b	73.1
15	ND	ND	30.3 ± 0.9 b	70.1 ± 1.3 a	100.4 ± 1.4 a	94.3	30.5 ± 0.6 a	69.6 ± 0.3 a	100.1 ± 0.9 a	94.0
30	ND	ND	34.7 ± 1.2 a	69.9 ± 0.8 a	104.6 ± 1.8 a	98.2	30.2 ± 0.1 a	72.3 ± 1.5 a	102.5 ± 1.6 a	96.2
60	ND	ND	32.6 ± 0.5 a	69.9 ± 1.8 a	102.5 ± 1.9 a	96.2	29.9 ± 0.2 a	71.3 ± 3.4 a	101.2 ± 1.8 a	95.0
120	ND	ND	34.5 ± 0.5 a	67.6 ± 1.5 a	102.1 ± 2.3 a	95.9	29.8 ± 0.1 a	73.0 ± 2.1 a	102.8 ± 1.9 a	96.5

[a] Mean of duplicate analyses ± standard deviation. [b] For letters a and b, each value of means bearing different letters in the same column are significantly different ($p < 0.05$). ND, not detected. [c] A mixture of cis isomers of lycopene.

h. In a study dealing with the encapsulation of isoflavone with middle-chain triglycerides and monostearic acids as coating materials, Kim et al. (*28*) reported that the release rate ranged from 6.3 to 9.3% for pH 2−5 and reaction time 1 h, and a plateau at 87.8% was reached for pH 8 and the same reaction time. This result indicated that a low pH would make the core material difficult to release, probably because of the formation of a tight structure through an electrostatic interaction between gelatin and γ-PGA. Lee et al. (*29*) also used chitosan and sodium alginate as a coating material for the encapsulation of guaidenesin and found that the highest release rate was shown for pH 8.8, followed by pH 6.8 and 4.8. This may be explained as follows: most carboxyl groups of sodium alginate and amino groups of chitosan remain ionized at pH 4.8, which may lead to the formation of a tight structure through electrostatic interaction, which in turn prevents the release of guaidenesin from capsules. In contrast, in our study, a pronounced release of lycopene occurred at pH 5.5 and 7.0. With a reaction time of 5 min, the amounts of cis isomers of lycopene, *trans*-, and total lycopene released at pH 5.5 were 29.4, 47.1, and 76.5 μg/g, respectively, while the released amounts were 31.2, 46.6, and 77.8 μg/g at pH 7.0. The release contents further increased to 30.3, 70.1, and 100.4 μg/g for pH 5.5 and a reaction time of 15 min and 30.5, 69.6, and 100.1 μg/g for pH 7.0 and the same reaction time. A maximum was reached after the reaction time was extended to 30, 60, or 120 min for both pH 5.5 and 7.0. With a reaction time of 5 min, the release rates were 71.8 and 73.1%, respectively, for pH 5.5 and 7.0. A high release rate of 94% was attained after the reaction time reached 15 min and above. It may be inferred that the carboxyl groups of γ-PGA remain unionized at pH 2.0, and a tight α-helix structure would make γ-PGA more difficult to be soluble in water. However, following a rise in pH, the α-helix structure can be extended to form a linear and more hydrophilic polyanionic structure, accounting for a fast release of lycopene from the encapsulated powder at high pH (5.5 and 7.0). From a physiological point of view, lycopene powder could remain intact when passing through stomach (pH 2.0−3.5), but a fast release would occur in the intestinal system (pH 5.5−7.0), which should enhance the bioavailability of lycopene. In conclusion, the encapsulation of lycopene extract from tomato pulp waste was accomplished by using a mixture of gelatin and γ-PGA as carriers, with a thermal stability of up to 120 °C and a mean particle size of 38.7 μm obtained. The concentrations of *cis*-, *trans*-, and total lycopene followed a decreased tendency for the increase of both time and temperature during the storage of lycopene powder. No lycopene in the powder was released at pH 2.0 and 3.5, whereas a fast release of lycopene was shown at pH 5.5 and 7.0. Further research is needed to study the bioavailability of lycopene in the encapsulated powder in both animal and human models.

LITERATURE CITED

(1) Rao, A. V.; Agarwal, S. Role of lycopene as antioxidant carotenoid in the prevention of chronic disease: A review. *Nutr. Res.* **1999**, *19*, 305−323.

(2) Giovannucci, E. A review of epidemiologic studies of tomatoes, lycopene and prostate cancer. *Exp. Biol. Med. (Maywood)* **2002**, *227*, 852−859.

(3) Lee, M. T.; Chen, B. H. Stability of lycopene during heating and illumination in a model system. *Food Chem.* **2002**, *78*, 425−432.

(4) Lin, C. H.; Chen, B. H. Stability of carotenoids during processing of tomato juice. *Eur. Food Res. Technol.* **2005**, *221*, 274−280.

(5) Lin, C. H.; Chen, B. H. Stability of carotenoids during storage of tomato juice. *Food Chem.* **2005**, *90*, 837−846.

(6) Zeller, B. L.; Saleeb, F. Z.; Ludescher, R. D. Trends in development of porous carbohydrate food ingredients for use in flavor encapsulation. *Trends Food Sci. Technol.* **1999**, *9*, 389−394.

(7) Cano-Chauca, M.; Stringheta, P. C.; Ramos, A. M.; cal-Vidal, J. Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. *Innovation Food Sci. Emerging Technol.* **2005**, *6*, 420−428.

(8) Krishnan, S.; Bhosale, R.; Singhal, R. S. Microencapsulation of cardamom oleoresin: evaluation of blends of gum Arabic, maltodextrin and a modified starch as wall materials. *Carbohydr. Polym.* **2005**, *61*, 95−102.

(9) Dziezak, J. D. Microencapsulation and encapsulated ingredients. *Food Technol.* **1988**, *42*, 136−148.

(10) Pszczola, D. E. Encapsulated ingredients: providing the right fit. *Food Technol.* **1998**, *52*, 70−76.

(11) Godshall, M. A. The role of carbohydrates in flavor development. *Food Technol.* **1988**, *42*, 71−78.

(12) Dickinson, E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids* **2003**, *17*, 25−39.

(13) Martinez, K. D.; Baeza, R. I.,; Millan, F.; Pilosot, A. M. R. Effects of limited hydrolysis of sunflower protein on the interactions with polysaccharides in foams. *Food Hydrocolloids* **2005**, *19*, 361−369.

(14) Michon, C.; Vigouroux, F.; Boulenguer, P.; Cuvelier, G.; Launay, B. Gelatin/ioto-carrageenan interactions in non-gelling conditions. *Food Hydrocolloids* **2000**, *14*, 203−208.

(15) Shih, I. L.; Van, Y. T. The production of poly-(γ-glutamic acid) from microorganisms and its various applications. *Bioresour. Technol.* **2001**, *79*, 207−225.

(16) Schallmey, M.; Singh, A.; Ward, O. P. Development in the use of Bacillus species for industrial production. *Can. J. Microbiol.* **2004**, *50*, 1−17.

(17) Inbaraj, B. S.; Chien, J. T.; Ho, G. H.; Yang, J.; Chen, B. H. Equilibrium and kinetic studies on sorption of basic dyes by a natural biopolymer poly(γ-glutamic acid). *Biochem. Eng. J.* **2006**, *31*, 204−215.

(18) Inbaraj, B. S.; Chiu, C. P.; Ho, G. H.; Yang, J.; Chen, B. H. Removal of cationic dyes from aqueous solution using an anionic poly-γ-glutamic acid-based adsorbent. *J. Hazard. Mater.* **2006**, *137*, 226−234.

(19) Inbaraj, B. S.; Chiu, C. P.; Chiu, Y. T.; Ho, G. H.; Yang, J.; Chen, B. H. Effect of pH on binding of mutagenic heterocyclic amines by the natural biopolymer poly(γ-glutamic acid). *J. Agric. Food Chem.* **2006**, *54*, 6452−6459.

(20) Wang, C. Y.; Chen, B. H. Tomato pulp as source for the production of lycopene powder containing high proportion of cis-isomers. *Eur. Food Res. Technol.* **2006**, *222*, 347−353.

(21) Chien, J. T.; Lien, Y. Y.; Shoemaker, C. F. Effect of polarity of complexing agents on thermal and reological properties of rice starch gels. *Cereal Chem.* **1999**, *76*, 837−842.

(22) Lin, C. H.; Chen, B. H. Determination of carotenoids in tomato juice by liquid chromatography. *J. Chromatogr. A* **2003**, *1012*, 103−109.

(23) Sharma, S. K.; Maguer, M. L. Kinetics of lycopene degradation in tomato pulp solids under different processing and storage conditions. *Food Res. Int.* **1996**, *29*, 309−315.

(24) *SAS/STAT Guide for Personal Computers*, Version 6; SAS Institute: Cary, NC, 2001.

(25) Bustos, R.; Romo, L.; Yáñez, K.; Díaz, G.; Romo, C. Oxidative stability of carotenoid pigments and polyunsaturated fatty acids in microparticulate diets containing Krill oil for nutrition of marine fish larvae. *J. Food Eng.* **2003**, *56*, 289−293.

(26) Lisiewska, Z.; Kmiecik, W. Effect of storage period and temperature on the chemical composition and organoleptic quality of frozen tomato cubes. *Food Chem.* **2000**, *70*, 167−173.

(27) Zanoni, B.; Peri, C.; Nain, R.; Lavelli, V. Oxidative heat damage of tomato halves as affected by drying. *Food Res. Int.* **1998**, *31*, 395−401.

(28) Kim, N. C.; Jeon, B. J.; Ahn, J.; Kwak, H. S. In vitro study of microencapsulated isoflavone and β-galactosidase. *J. Agric. Food Chem.* **2006**, *54*, 2582−2586.

(29) Lee, K. Y.; Park, W. H.; Ha, W. S. Polyelectrolyte complexes of sodium alginate with chitosan or its derivatives for microcapsules. *J. Appl. Polym. Sci.* **1996**, *63*, 425−432.

Received for review January 2, 2007. Revised manuscript received April 16, 2007. Accepted April 20, 2007.

JF0700069