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Development of a New Colorimetric Method Determining the Yield of Microencapsulation of α-Tocopherol

PAHN-SHICK CHANG,* JONGHYUK LEE, AND JAEHWAN LEE

Department of Food Science and Technology, Seoul National University of Technology, Seoul, Korea

Microencapsulation of α-tocopherol effectively protects α-tocopherol from oxidation and produces high-value-added and long-shelf-stable foods. High-performance liquid chromatography (HPLC) has been applied to measure the yield of microencapsulated α-tocopherol with high accuracy; however, it takes long analysis time. An alternative method is required to determine the yield of microencapsulated α-tocopherol in food industry. A new, easy, and sensitive colorimetric method using 5% cupric acetate pyridine and oleic acid was developed. Correlation coefficient (*r*) of colorimetric method on α-tocopherol in microencapsulation system and of results between colorimetric method and HPLC were +0.996 and +0.989, respectively, which indicates that this novel colorimetric method can be successfully applied to evaluate the yield of microencapsulated α-tocopherol instead of HPLC. The optimum storage temperature and pH of microencapsulated α-tocopherol for 7-day storage were 25 °C and pH 9, respectively, determined by this new colorimetric method.

KEYWORDS: α-Tocopherol; microencapsulation; colorimetric method; HPLC

INTRODUCTION

Lipid (fat and oil) in most foods is sensitive to heat energy, light energy, and the presence of oxygen and easily undergoes oxidation. Oxidized lipid is a critical factor in food deterioration during storage (*1*−*4*). Since lipid oxidation produces off-flavors making the foods less acceptable or unacceptable to consumers (*5*−*7*), the prevention of lipid oxidation is considered one of critical steps determining storage period and prolonging shelf life of foods in food industry. Antioxidants are therefore often used in food processing and become one of the important food ingredients (*8*−*10*). Hydrogen-donating antioxidants can protect lipid molecules by donating hydrogen atom to peroxyl or alkoxyl lipid radicals and antioxidants themselves become radicals, which should not be involved in further oxidation process (*11*−*13*). Antioxidants are categorized into natural and synthetic origins while natural antioxidants are much preferred because of the increase of consumers' health concerns. Generally, the cost of natural antioxidants is higher than synthetic ones, and many researches have focused to improve the efficacy of natural antioxidants.

α-Tocopherol is a well-known fat-soluble antioxidant and is widely used in the food industry. One electron reduction potential of α-tocopherol is 500 mV while that of polyunsaturated fatty acids (PUFA) is 600 mV, which suggests that α-tocopherol can donate hydrogen atom to peroxyl or alkoxyl lipid radicals before PUFA do and efficiently prevents lipid oxidation. If α-tocopherol becomes corresponding radicals during storage and transportation, α-tocopherol cannot protect food products anymore and may cause unwanted problems on the stability of foods (*14*−*16*). Therefore, it is required to develop a new technology preventing α-tocopherol from oxidation. Also, the new technology should make proper controlled release of antioxidants when required. Microencapsulation technology was introduced and tested for these required conditions and was reported as a suitable and dependable technology for protecting and releasing antioxidants (*17*−*19*). Until now, microencapsulation has been widely applied in food industry while some disadvantages of encapsulated products in digestion and swallowing were reported (*20*−*23*).

Optimization of processing parameters is a key factor in microencapsulation. An accurate, reliable, and sensitive method should be established to measure the yield of microencapsulation for effective microencapsulation processing. HPLC has been used to determine the yield of microencapsulated α-tocopherol quantitatively with high accuracy, but it requires a long time for analysis and may also cause errors in extraction steps of α-tocopherol from microencapsulated source (*24*−*26*). Consequently, instead of HPLC method, a simpler and relatively sensitive method for evaluating the yield of microencapsulation is demanded especially in industrial fields as well as in academic areas.

The objectives of this study were to establish a new colorimetric method for determining yield of microencapsulated α-tocopherol and to investigate the optimum temperature and pH of the microencapsulated α-tocopherol during storage using a developed new method.

MATERIALS AND METHODS

Materials. Agar was purchased from Junsei Chemical Co. Ltd. (Tokyo, Japan). Waxy corn starch from Samyang Genex Co. (Seoul,

* To whom correspondence should be addressed. Tel: 82-2-970-6437; fax: 82-2-976-6460; e-mail: pschang@snut.ac.kr.

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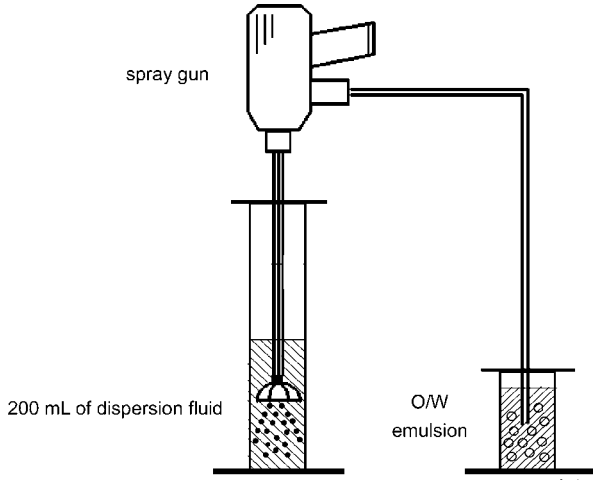

Figure 1. Schematic diagram for the microencapsulation apparatus.

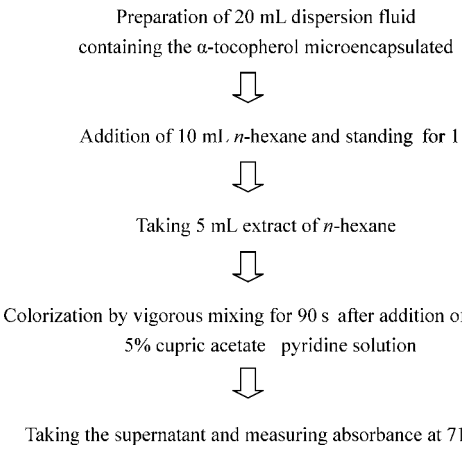

Figure 2. Schematic diagram for the analysis of microencapsulation yield by 5% cupric acetate pyridine colorization.

Korea) and oleic acid from Showa Chemicals Inc. (Tokyo, Japan) were purchased. α-Tocopherol of more than 93% purity was purchased from Korea Goking Co. (Ansan, Korea), and polyoxyethylene sorbitan monolaurate (HLB: 16.7) was purchased from Ilshin Emulsifier Co. Ltd. (Kimpo, Korea). Other chemicals are of reagent grade.

Microencapsulation Process of α-Tocopherol. The microencapsulation of α-tocopherol was carried out by the modification of the method previously reported by Chang and Cho (*27*) for the microencapsulation of docosahexaenoic acid. The mixture of α-tocopherol and oleic acid with the ratio of 4:1 (w/w) was used as core material for microencapsulation derived from previous study (*27*). Oleic acid was used for indirect coloration of α-tocopherol for the spectroscopic analysis of α-tocopherol. A 0.75% (w/v) of the mixture of agar and waxy corn starch with the ratio of 2:1 (w/w) was prepared in 100 mL of distilled water as wall material and was gelatinized at 70−80 °C. Preliminary study on the optimum mixture ratio between an emulsifier and core material was conducted and the ratio of 1:17.4 (w/w) was selected and used in this study. At a water bath of 65 °C, a mixture ratio of 0.49 g polyoxyethylene sorbitan monolaurate and 8.52 g core material was prepared and homogenized with a homogenizer (Ultra-Turrax T-25, Janke and Kunkel Co., Staufen, Germany) at 9000 rpm in 30 s. Ten grams of wall material was added into this homogenized mixture and was further homogenized for 50 s. Twenty grams of the emulsion solution containing wall material and core material with an emulsifier was sprayed into 200 mL of distilled water at 25 °C using a spray gun (W-300, Wagner, Germany) as shown in **Figure 1**.

Development of Colorimetric Method for the Yield of Microencapsulation of α-Tocopherol. A new colorimetric method using 5% (w/v) cupric acetate pyridine (pH 6.01) was applied to determine the yield of microencapsulated α-tocopherol (**Figure 2**). The principle of this colorimetric method is interaction between two cupric agents and four free fatty acids forming colored cupric soaps with the cagelike complex, $Cu_2(C_{18}H_{34}O_2)_4$. Lowry and Tinsley (*28*) developed a rapid free fatty acid determining method and Kwon and Rhee (*29*) measured free fatty acids from lipase activity using this colored cupric soap principle. For UV/vis spectrometry determination, α-tocopherol needs to be colored proportionally to the concentration. However, α-tocopherol is not colored directly by cupric acetate pyridine. Oleic acid can be mixed with α-tocopherol and react with cupric acetate pyridine resulting in color production proportional to the concentration of oleic acid. Therefore, oleic acid was introduced for indirect coloration of α-tocopherol. After the processing of emulsification and microencapsulation of α-tocopherol as described above, 100 mL of *n*-hexane was added into 200 mL of dispersion fluid containing microcapsules and was mixed with shaking for 10 s. The resulting mixture stood for 1 h, and 5 mL of *n*-hexane upper layer was taken and mixed with 1 mL of 5% cupric acetate pyridine solution for color development with vortexing for 90 s. The colored solution stood for 5 min in the dark, and the absorbance, *a*, of *n*-hexane upper layer was measured at 715 nm with UV/visible spectrophotometer (UV-2101PC, Shimadzu Co., Tokyo, Japan). The yield of microencapsulated α-tocopherol can be calculated using the following equation.

$$\text{yield of microencapsulation of } \alpha\text{-tocopherol (\%)} = \frac{b-a}{b} \times 100 \quad (1)$$

Absorbance *a* and *b* indicates the amount of α-tocopherol in solution with or without emulsifiers, respectively.

Evaluation of the Colorimetric Method for the Microencapsulation Yield. The 10 g of wall material was sprayed into 200 mL distilled water with the assumption that microcapsules were not produced. Core materials of α-tocopherol and oleic acid mixtures with the ratio of 4:1 (w/w) without emulsifier were then added into this solution to make concentrations of 2, 4, 6, 8, 10, and 12 μM of α-tocopherol. α-Tocopherol concentration was measured by a newly developed colorimetric method according to the procedure in **Figure 2**.

These samples were treated with 10 mL *n*-hexane and after standing, the extracted upper layers were directly applied into high-performance liquid chromatography (HPLC) (Waters 600, Waters Co., IL) to measure the amount of α-tocopherol. HPLC with a UV detector using a Shimpack CLC−SIL (150 mm × 4.60 mm i.d., Shimadzu, Kyoto, Japan) was used. Mobile phase was a mixture of *n*-hexane:2-propanol (49:1, v/v) and flow rate was 0.8 mL/min velocity. Eluent was monitored at 215 nm and injection volume was 10 μL. The results from HPLC analysis were compared with those from the colorimetric method. Serially diluted standard α-tocopherol in *n*-hexane were prepared and used for the quantification of α-tocopherol using the above HPLC conditions. All the samples were prepared in triplicates. Analysis times in colorimetric method and HPLC method were compared to each other using six skilled researchers. Researchers were trained with both colorimetric method and HPLC method and analysis time taken for each step was measured 10 times.

Temperature and pH Effects on the Storage Stability of Microencapsulated α-Tocopherol. Temperature and pH may affect the accuracy and reliability of this new colorimetric method on quantitative analysis of the microencapsulated α-tocopherol. Effects of temperature and pH during storage were investigated for determining the stability of microencapsulated α-tocopherol. Samples of microencapsulated α-tocopherol prepared from mixtures of 0.49 g polyoxyethylene sorbitan monolaurate and 8.52 g core material were put into an oven at 5, 15, 25, 35, or 45 °C and were stored for 7 days. The concentration of α-tocopherol released from microcapsules was determined and yield of microencapsulated α-tocopherol was calculated according to the above equation. At selected optimal storage temperature, the effects of pH on the stability of microencapsulated α-tocopherol were determined in the pH range from 3, 5, 7, 9, 11, to 13 and the yield of microencapsulated α-tocopherol was also calculated. Preparation of pH 3, 5, 7, 9, 11, and 13 solutions were made by dissolving citric acid-Na_2HPO_4 buffer, citric acid-Na_2HPO_4 buffer, citric acid-Na_2HPO_4 buffer, Clark and Lubs buffer, Tricin-KOH buffer, and KCl−NaOH buffer, respectively. Samples were prepared in triplicates.

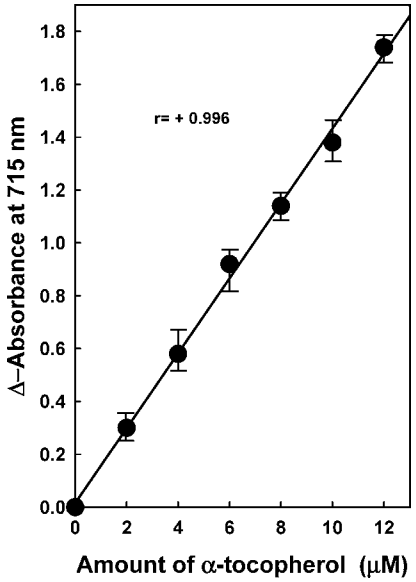

Figure 3. Standard curve for the calculation of the yield of microencapsulation by 5% cupric acetate pyridine colorization.

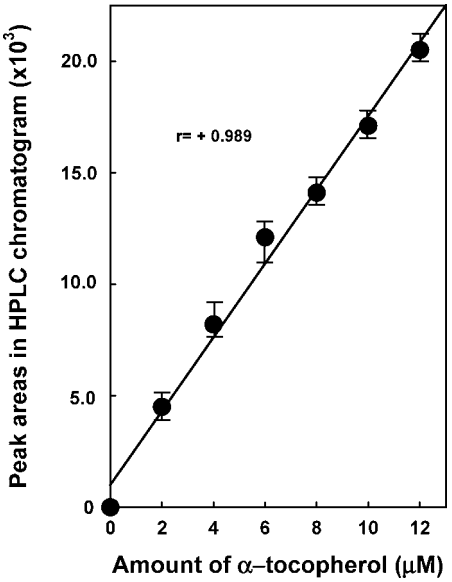

Figure 4. Standard curve for the calculation of the yield of microencapsulation by HPLC.

RESULTS AND DISCUSSION

Establishment of the Colorimetric Method. Standard curve on various concentrations of α-tocopherol mixed with 20% (w/w) of oleic acid colored by 5% cupric acetate pyridine is shown in **Figure 3**. The concentration of α-tocopherol was measured by HPLC analysis. Correlation coefficient *r* determined by a new colorimetric method was +0.996. As the amount of α-tocopherol not incorporated in microcapsules increased, the absorbance at 715 nm was proportionally and positively increased. Therefore, the yield of microencapsulation and the degree of destruction in microcapsule during storage can be calculated on the basis of the standard curve and the yield of eq 1. The colorimetric method using cupric acetate pyridine measures the released α-tocopherol from microcapsules indirectly. The principle of this method is to determine the released oleic acid being mixed with α-tocopherol from microcapsules. As this new colorimetric method does not determine α-tocopherol directly, validity and accuracy of this method need to be evaluated. Standard curve on various concentrations of α-tocopherol determined by HPLC is shown in **Figure 4**. Correlation coefficient *r* determined by HPLC was +0.989. Correlation coefficient *r* between the results from colorimetric method and those from HPLC are +0.989 as shown in **Figure 5**. Therefore, the colorimetric method using 5% cupric acetate pyridine can be used as an alternative assaying method to HPLC for determining the yield of microencapsulated α-tocopherol.

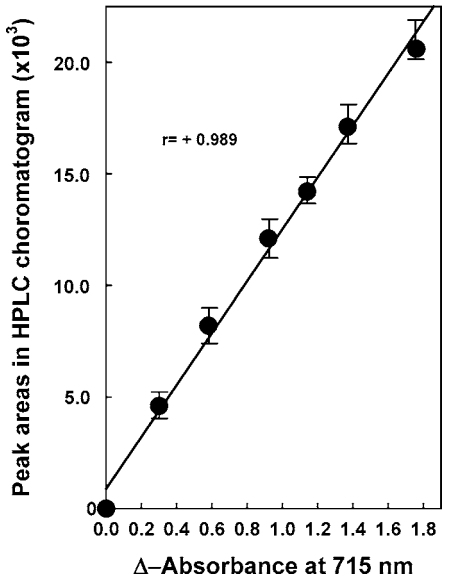

Figure 5. Correlation between peak area by HPLC and absorbance by colorimetric method on α-tocopherol.

Table 1 indicates analysis time for each step in colorimetric method and HPLC method. Total analysis time using a colorimetric method and HPLC was about 5.5 and 18.0 min, respectively. Comparing analysis time for one sample, the colorimetric method takes less than one-third of the time required by HPLC method because of the relatively long sample pretreatment time and elution time in HPLC analysis.

Table 1. Comparison of Analysis Time Taken for the Steps in Colorimetric Method and HPLC Method (Mean ± Standard Deviation, $n = 10$)

colorimetric method			HPLC analysis method		
step	description	time (min)	step	description	time (min)
1	mixing oleic acid with α-tocopherol	0.5 ± 0.03	1	pretreatment of sample	2.0 ± 0.14
2	color development	1.0 ± 0.06	2	addition of internal standard	1.0 ± 0.08
3	preparation of reference cuvette	2.0 ± 0.15	3	chromatographic analysis	15.0 ± 1.1
4	absorbance measurement	1.0 ± 0.08			
	total time (min)	5.5 ± 0.32		total time (min)	18.0 ± 1.32

Temperature and pH Effects on the Storage Stability of Microencapsulated α-Tocopherol. Effects of storage temperature on the stability of microencapsulated α-tocopherol in dispersion fluid determined by new colorimetric method are shown in **Figure 6**. More than 98% of microencapsulated α-tocopherol was stable over the whole range of temperature and more than 99% microcapsules were maintained without destruction for 7 days especially in 25−35 °C.

Below 25 °C, the destruction of microcapsules was increased with the decrease in storage temperature, and it may be due to the retrogradation of waxy corn starch in wall material in response to the temperature down (*30*, *31*). The destruction of microcapsules above 35 °C was supposed to be due to the

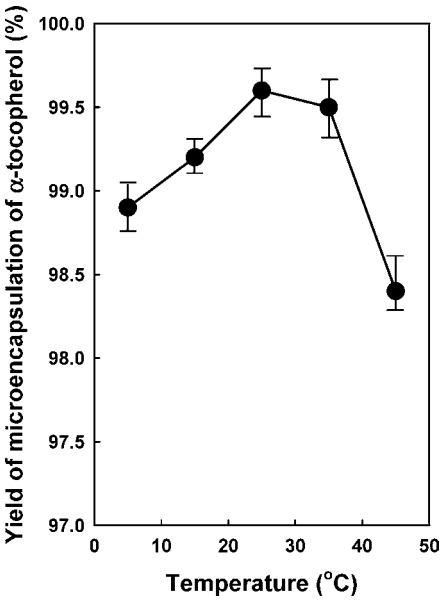

Figure 6. Effect of temperature on the 7-day storage stability of microencapsulated α-tocopherol with waxy corn starch and agar.

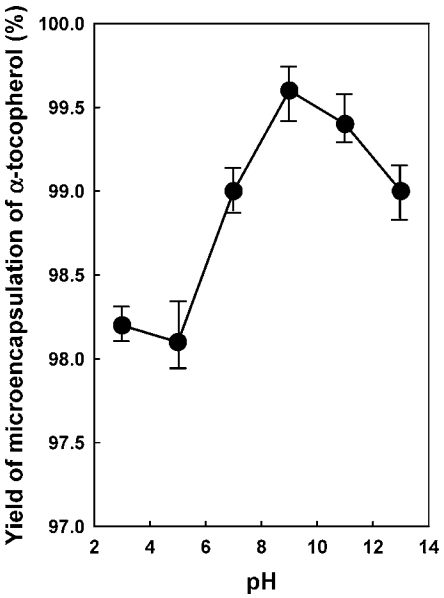

Figure 7. Effect of pH on the storage stability of microencapsulated α-tocopherol with waxy corn starch and agar.

thermal degradation of wall material. These results indicated that the optimal temperature for the storage of microcapsules of α-tocopherol was in the range of 25−35 °C, and a room temperature could be adapted for the long-term storage.

Figure 7 shows the effects of pH on the stability of the microcapsules at 25 °C which was selected as an optimum storage temperature. The microcapsules of α-tocopherol were most stable at pH 9. The stability of wall material was decreased in acidic condition while microcapsules in the alkaline condition were stable even up to pH 11.

CONCLUSION

A simple and sensitive colorimetric method was developed using 5% solution of cupric acetate pyridine and oleic acid for determining the yield of the microencapsulation of α-tocopherol. The reliability of this colorimetric method was evaluated and confirmed by HPLC. By using this novel colorimetric method, the optimum temperature and pH conditions for the storage stability of microcapsules containing α-tocopherol were determined as 25 °C and pH 9.0, respectively. Conclusively, this novel colorimetric method can be applied to analyze the yield for the microencapsulation of α-tocopherol quantitatively with shorter analysis time than HPLC.

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LITERATURE CITED

(1) Lee, J.; Choe, E. Lipid oxidation and tocopherol contents change in full-fat soy flour during storage. *Food Sci. Biotechnol.* **2003**, *12*, 504−507.

(2) Wang, X. J.; Wang, Z. B.; Xu, J. X. Effect of salvianic acid A on lipid peroxidation and membrane permeability in mitochondria. *J. Ethnopharmacol.* **2005**, *97*, 441−445.

(3) Kanatt, S. R.; Chander, R.; Radhakrishna, P.; Sharma, A. Potato peel extract-a natural antioxidant for retarding lipid peroxidation in radiation processed lamb meat. *J. Agric. Food Chem.* **2005**, *53*, 1499−1504.

(4) Punta, C.; Rector, C. L.; Porter, N. A. Peroxidation of polyunsaturated fatty acid methyl esters catalyzed by N-methyl benzohydroxamic acid: a new and convenient method for selective synthesis of hydroperoxides and alcohols. *Chem. Res. Toxicol.* **2005**, *18*, 349−356.

(5) Takahashi, Y. K.; Nagayama, S.; Mori, K. Detection and masking of spoiled food smells by odor maps in the olfactory bulb. *J. Neurosci.* **2004**, *24*, 8690−8694.

(6) Venkateshwarlu, G.; Let, M. B.; Meyer, A. S.; Jacobsen, C. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. *J. Agric. Food Chem.* **2004**, *52*, 311−317.

(7) Im, S.; Hayakawa, F.; Kurata, T. Identification and sensory evaluation of volatile compounds in oxidized porcine liver. *J. Agric. Food Chem.* **2004**, *52*, 300−305.

(8) Ng, T. B.; Gao, W.; Li, L.; Niu, S. M.; Zhao, L.; Liu, J.; Shi, L. S.; Fu, M.; Liu, F. Rose(*Rosa rugosa*)-flower extract increases the activities of antioxidant enzymes and their gene expression and reduces lipid peroxidation. *Biochem. Cell Biol.* **2005**, *83*, 78−85.

(9) Materska, M.; Perucka, I. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum L*). *J. Agric. Food Chem.* **2005**, *53*, 1750−1756.

(10) Ito, M.; Murakami, K.; Yoshino, M. Antioxidant action of eugenol compounds: role of metal ion in the inhibition of lipid peroxidation. *Food Chem. Toxicol.* **2005**, *43*, 461−466.

(11) Decker, E. A. Antioxidant mechanisms. In *Food Lipids-Chemistry, Nutrition, and Biotechnology*; Akoh, C. C., Min, D. B., Eds.; Marcel, Dekker: New York, 1998.

(12) Fujisawa, S.; Kadoma, Y. Kinetic studies of the radical-scavenging activity of estrogens and antiestrogens. *Anticancer Res.* **2004**, *24*, 3931−3937.

(13) Zhao, Y.; Chaiswing, L.; Oberley, T. D.; Batinic-Haberle, I.; St Clair, W.; Epstein, C. J.; St Clair, D. A mechanism-based antioxidant approach for the reduction of skin carcinogenesis. *Cancer Res.* **2005**, *65*, 1401−1405.

(14) Hoppu, U.; Salo-Vaananen, P.; Lampi, A. M.; Isolauri, E. Serum alpha- and gamma-tocopherol levels in atopic mothers and their infants are correlated. *Biol. Neonate* **2005**, *88*, 24−26.

(15) Miwa, K.; Kishimoto, C.; Nakamura, H.; Makita, T.; Ishii, K.; Okuda, N.; Yodoi, J.; Sasayama, S.; Serum thioredoxin and alpha-tocopherol concentrations in patients with major risk factors. *Circ. J.* **2005**, *69*, 291−294.

(16) Pfluger, P.; Kluth, D.; Landes, N.; Bumke-Vogt, C.; Brigelius-Flohe, R. Vitamin E: underestimated as an antioxidant. *Redox Rep.* **2004**, *9*, 249−254.
(17) Zimmermann, M. B. The potential of encapsulated iron compounds in food fortification: a review. *Int. J. Vitam. Nutr. Res.* **2004**, *74*, 453−461.
(18) Hile, D. D.; Pishko, M. V. Solvent-free protein encapsulation within biodegradable polymer foams. *Drug Delivery* **2004**, *11*, 287−293.
(19) Philip, I.; Kaifer, A. E. Noncovalent encapsulation of cobaltocenium inside resorcinarene molecular capsules. *J. Org. Chem.* **2005**, *70*, 1558−1564.
(20) Trotta, M.; Cavalli, R.; Carlotti, M. E.; Battaglia, L.; Debernardi, F. Solid lipid micro-particles carrying insulin formed by solvent-in-water emulsion-diffusion technique. *Int. J. Pharm.* **2005**, *288*, 281−288.
(21) Rehor, A.; Hubbell, J. A.; Tirelli, N. Oxidation-sensitive polymeric nanoparticles. *Langmuir* **2005**, *21*, 411−417.
(22) Liu, H.; Finn, N.; Yates, M. Z. Encapsulation and sustained release of a model drug, indomethacin, using CO_2-based microencapsulation. *Langmuir* **2005**, *21*, 379−385.
(23) Aubert-Pouessel, A.; Venier-Julienne, M. C.; Saulnier, P.; Sergent, M.; Benoit, J. P. Preparation of PLGA microparticles by an emulsion-extraction process using glycofurol as polymer solvent. *Pharm. Res.* **2004**, *21*, 2384−2391.
(24) Mathys, W.; Surholt, B. Analysis of microcystins in freshwater samples using high performance liquid chromatography and an enzyme-linked immunosorbent assay. *Int. J. Hyg. Environ. Health* **2004**, *207*, 601−605.
(25) Lochman, J.; Sery, O.; Jankovsky, L.; Mikes, V. Variations in rDNA ITS of Czech Armillaria species determined by PCR and HPLC. *Mycol. Res.* **2004**, *108*, 1153−1161.
(26) Nedderman, A. N.; Savage, M. E.; White, K. L.; Walker, D. K. The use of 96-well scintiplates to facilitate definitive metabolism studies for drug candidates. *J. Pharm. Biomed. Anal.* **2004**, *34*, 607−617.
(27) Chang, P. S.; Cho, G. B. Optimization of the conditions for the O/W emulsion containing ω3 polyunsaturated fatty acid. *J. Korean Soc. Food Sci. Technol.* **1998**, *30*, 1114−1119.
(28) Lowry, R. R.; Tinsley, I. J. Rapid determination of free fatty acids. *J. Am. Oil Chem. Soc.* **1976**, *53*, 470−472.
(29) Kwon, D. Y.; Rhee, J. S. A simple and rapid colorimetric method for determination of free fatty acids for lipase assay. *J. Am. Oil Chem. Soc.* **1966**, *63*, 89−92.
(30) Shamai, K.; Shimoni, E.; Bianco-Peled, H. Small-angle X-ray scattering of resistant starch type III. *Biomacromolecules* **2004**, *5*, 219−223.
(31) Jobling, S. A.; Westcott, R. J.; Tayal, A.; Jeffcoat, R.; Schwall, G. P. Production of a freeze−thaw-stable potato starch by antisense inhibition of three starch synthase genes. *Nat. Biotechnol.* **2002**, *20*, 295−299.

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