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Microencapsulated Ascorbic Acid for Milk Fortification

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The present study was designed to develop a microencapsulated L-ascorbic acid and iron that could be used to fortify milk and to determine the sensory properties of milk fortified with microencapuslation. Coating material was medium-chain triacylglycerol (MCT), and selected core material was ferric ammonium sulfate and L-ascorbic acid. The highest efficiency of microencapsulation was 95.0% in the ratio of 15:1 as coating to core material. Ascorbic acid release was increased sharply up to 5 d storage as 6.5%. TBA value was the lowest when both capsulated iron and ascorbic acid were added during 12 d storage, compared with other treatments. In sensory analysis, most aspects were not significantly different between control and capsulated ascorbic acid fortified milk at 5 d storage. The present study indicated that the use of microencapsulated ascorbic acid with MCT is effective for fortifying milk. In addition, these results suggest that acceptable milk products can be prepared with microencapsulated ascorbic acid and iron.

Key words: Microencapsulation, L-Ascorbic acid, Iron, Fatty acid esters, Milk

INTRODUCTION

Milk is the universal and nutritious food, however, it contains an extremely low content of iron (Heugenauer *et al.*, 1979). According to the recent nutrition surveys, iron deficiency anemia is a highly prevalent and seemingly considerable problem, resulting from inadequate intake of iron, particularly among young children, adolescents, and women of menstrual age all over the world (HANES, 1974; Nutrition Canada 1973).

Recently, pediatricians and nutritionists universally recommend the addition of iron to milk-based formulas and foods to improve the hematological status (Hegenauer *et al.* 1979). However, iron fortification is difficult in food processing due to potential oxidized off-flavors, color changes, and metallic flavors (Jackson and Lee, 1991), probably as a result of lipid peroxidation of milk fat (Edmanson *et al.*, 1971).

Ascorbic acid is known to be involved in the metabolism of iron in animals (NRC, 1993). Ascorbic acid enhances the absorption of iron from the intestine by reducing ferric iron to the ferrous state, a more soluble form that is easily absorbed (Monsen, 1983). Ascorbic acid is also involved with adenosine triphosphate (ATP) in the release and reduction with the ferric iron from ferritin and its subsequent incorporation with iron-binding protein, apoferritin and transferring into tissue ferritin. (Lim *et al.*, 2000).

Uddin *et al.* (2001) indicated that microencapsulation of ascorbic acid could prevent the color change by ascorbic acid, retard its core release rate, and generally mask its acid taste. Environmental factors, such as temperature, pH value, oxygen, metal ion, UV and X-ray affect the stability of ascorbic acid (Liao and Seib, 1988). In order to overcome some of these shortcomings of ascorbic acid, the microencapsulation technique is applied for encapsulating ascorbic acid.

Microencapsulation, which shows potential as carriers of enzymes in the food industry, could be a good vehicle for the addition of ascorbic acid and iron to dairy products (Jackson and Lee, 1991; Berseneva *et al.*, 1990). Currently, there is a considerable interest in developing encapsulated flavors and enzymes. Among several factors to be considered, choice of coating material is the most important aspect, and depends on the chemical and physical properties of the core material, process used to form microcapsules and the ultimate properties desired in microcapsules.

For the microencapsulation although several researches have been used coating materials such as milk fat, agar and gelatin etc. responsible for enzyme, flavor and iron

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microencapsulation in foods (Braun and Olson, 1986; Magee and Olson, 1981a; Magee and Olson, 1981b.), no study has indicated a possibility of using emulsifier as coating material to coat ascorbic acid to improve its absorption.

Therefore, the objectives of this study were to examine microencapsulation efficiency of ascorbic acid microcapsules with medium-chain triacylglycerol (MCT) and to measure the chemical and sensorial properties of milk fortified with microencapsulated ascorbic acid and iron during storage.

MATERIALS AND METHODS

Materials

For the microencapsulation of ascorbic acid, medium-chain triacylglycerol (MCT) was used as a coating material, while polyacylglycerol monostearate (PGMS) was used for iron complex microencapsulation. MCT and PGMS were purchased from Il-Shin Emulsifier Co., LTD. (Seoul). As a core material, L-ascorbic acid and water-soluble iron complex ferric ammonium sulfate ($FeNH_4(SO_4)_2 \cdot 4H_2O$) were purchased from Sigma Chemical Co., (St. Louis, MO, USA) and Shinyo Pure Chemical Co. LTD. (Osaka, Japan) and were in food grade.

Preparation of microcapsule

Microcapsules of ascorbic acid were made by medium-chain triacylglycerol (MCT). The ratios of coating material to core material were 5:1, 10:1, 15:1, and 20:1 to maximize ascorbic acid content and stability of microcapsules, and mixed at 1,200 rpm for 1 min with a stirrer. An airless paint sprayer (W-300, Wagner Spray Tech. Co., Markdorf, Germany) nebulized a coating material-ascorbic acid emulsion at 45°C into a cyclinder containing a 0.05% polyethylene sorbitan monostearate (Tween 60) solution at 5°C. The diameter of the nozzle orifice was 0.4 mm. The chilled fluid was centrifuged at 450×g for 10 min to separate microcapsules, which were formed as lipid solidified in the chilled fluid.

For iron microencapsulation, PGMS was used as a coating material. For PGMS, distilled water was additionally added because PGMS is highly viscous. The mixture of PGMS and distilled water (50 mL) was heated to 55°C for 20 min, and stirred with 1,200 rpm for 30 s for spraying. Both MCT and PGMS microencapsulation were done in triplicate.

Efficiency of microencapsulation

The dispersion fluid was assayed for untrapped ascorbic acid. Five mL of the dispersion fluid was mixed with 100 mL metaphosphoric acid/glacial acetic acid, and centrifuged, and supernatant was collected.

Total ascorbic acid was analyzed spectrophotometrically using DNP (2,4-dinitrophenyl hydrazine) test (Korea Food Code, 2002). Samples were prepared immediately before analysis and kept cold and protected against by dissolving during analysis. An ascorbic acid stock solution was prepared daily by dissolving 10 mg of ascorbic acid in 100 mL of deionized water (100 μg/mL). It was diluted with deionized water to obtain the final concentration of 10, 20, 30, 40 and 50 μg/mL. Total ascorbic acid was determined using the calibration graph based on concentration (μg/mL) vs absorbance and prepared daily running fresh standard solutions.

For iron measurement, the dispersion fluid was assayed for untrapped iron during microencapsulation. One milliliters of the dispersion fluid was taken and diluted ten times, and total iron content was measured at 259.94 nm by inductively coupled plasma spectrometer (ICP). Lactam 8440 Model Spectrometer (Plasmalab, Victoria, Australia) was used. A sample measurement was run in triplicate.

Microscopic observation

The micro-structural image of the capsules was magnified by 1000-folds with a light microscope (Olympus Optical Co., LTD., Japan) and photographed.

Stability of microcapsules

Thiobarbituric acid (TBA) test

The absorbance change by addition of iron into milk was measured using TBA test at 4°C for 12 d (Hegenauer *et al.*, 1979). Oxidation products were analyzed spectrophotometrically. The reagent for TBA test was prepared immediately before use by mixing equal volumes of freshly prepared 0.025 M TBA, which was neutralized with NaOH and 2M H_3PO_4/2 M citric acid. Reactions of TBA test were started by pipetting 5.0 mL of milk containing iron capsulated or uncapsulated into a glass centrifuge tube and mixed thoroughly with 2.5 mL TBA reagent. The mixture was heated immediately in a boiling water bath for exactly 10 min, and cooled on ice. Ten mL cyclohexanone and 1 mL of 4 M ammonium sulfate were added and centrifuged at 2,490×g for 5 min at room temperature. The orange-red cyclohexanone supernatant was decanted and its absorbance at 532 nm was measured spectrophotometically in a 1-cm light path. All measurement were run in triplicate.

Ascorbic acid release during storage

To measure the stability of ascorbic acid microcapsules, 10 mL of milk was added into the same amount of microcapsule solution (1 mg ascorbic acid /1 mL), stored at 4°C for 12 d, and measured the stability at 3 d interval. The samples were centrifuged and the collected supernatant was analyzed for determination of ascorbic acid content released from microcapsules. All measurement were triplicate.

Sensory evaluation

For the sensory test, whole milk containing capsulated ascorbic acid (100 and 250 ppm) was stored at 4°C for 1, 3, 5, 8 and 12 d. A ten panels, semi-experienced in judging dairy products were recruited from faculty and graduate students in the Department of Food Science and Technology at Sejong University and evaluated the milk samples throughout the study.

The intensity of off-flavor and sourness were scored on a 5-point scale (1 = none, 2 = slight, 3 = moderate, 4 = strong and 5 = very strong), and overall preference was scored on a 5-point scale (1 = dislike extremely, 2 = dislike moderately, 3 = neither like or dislike, 4 = like moderately, and 5 = like extremely). A randomized, balanced, complete block design was used (Cochran and Fox, 1957) that resulted in two replications for all samples.

Statistical analysis

Data from each experiment were analyzed by analysis of variance (ANOVA) using a SAS program (1985) and differences among treatments were determined by LSD at $p<0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Microencapsulation

The efficiency of ascorbic acid microencapsulation made by MCT is shown in Table I. Efficiency of microencapsulation increased proportionally up to coat to core ratio 15:1. The efficiency was the greatest (95.0%) when the coat to core ratio was 15:1, which was significantly different, compared with other groups ($p<0.05$). In the case of 20:1, MCT was left over in the upper layer of dispersion fluid after centrifugation. Therefore, the optimum ratio of MCT to ascorbic acid was found to be 15:1, even though left over MCT was still found in the upper layer.

Similar studies (Kwak *et al.*, 2001; Kim *et al.*, 1999; Jackson and Lee, 1991; Magee and Olson, 1981b) have reported the optimum ratios of coating (agar, gelatin, soluble starch, and milk fat) and core material (ω-3 fatty acid, iron, and flavor etc.) for an efficient microcapsule formation. When ω-3 fatty acid was microencapsulated by milk fat, the ratio of coating to core material was 8:2 and the efficiency was 95.6% (Kim *et al.*, 1996). In addition, Sankarikutty *et al.* (1988) indicated that the 7:3 ratio of cardamon oil to the mixture of gum acacia and maltodextrin showed the highest efficiency among other ratios. Those studies indicated that the optimum conditions including the ratio of coating and core materials, the viscosity of spray solution, the method of microencapsulation varied with kinds of coating, core materials and food to be applied.

Table I. Microencapsulation efficiency of ascorbic acid with different ratios of MCT to ascorbic acid[1]

Ratio (w/w)		Microencapsulation efficiency (%)
MCT[2]	L-ascorbic acid	
5	1	88.9[d]
10	1	90.4[c]
15	1	95.0[a]
20	1	91.5[b]
SEM		3.1

[1]Means of triplicate. Means in a column without the same letter are significantly different ($p<0.05$).
[2]Medium-chain triacylglycerol

Microscopic observation

Photomicrograph of microencapsulated ascorbic acid with MCT is shown in Fig. 1. The size of microcapsules was irregular, and the average size was in the range of 2 to 5 μm. Microscopic examination of microcapsules revealed spherical particles. Microcapsules containing ascorbic acid had smooth surfaces and evenly distributed pockets. The shape of the microcapsules was likely affected by encapsulating conditions. Magee and Olson (1981a), and Braun and Olson (1986) found that lipid and coating fluid temperatures affected the shape of microcapsule by controlling the cooling rate of lipid.

During storage

Ascorbic acid release

To examine the stability of microencapsulation during storage, ten mL microcapsule solution (1 mg/mL) was mixed with 10 mL commercial milk, and stored at 4°C for 12 d. The release of ascorbic acid from microcapsules was then determined at 1, 3, 5, 8 and 12 d as shown in Fig. 2.

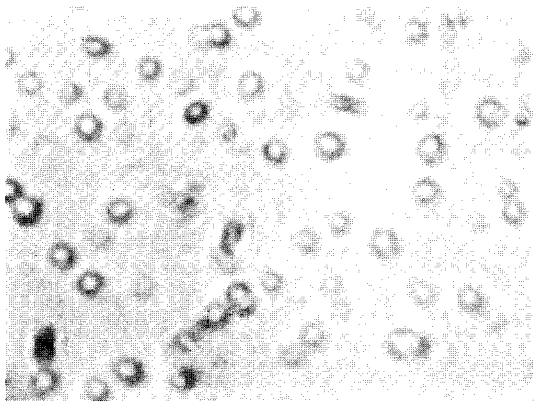

Fig 1. Photograph of microencapsulated ascorbic acid with medium-chain triacylglycerol (MCT). The photograph was taken at 1,000× magnification.

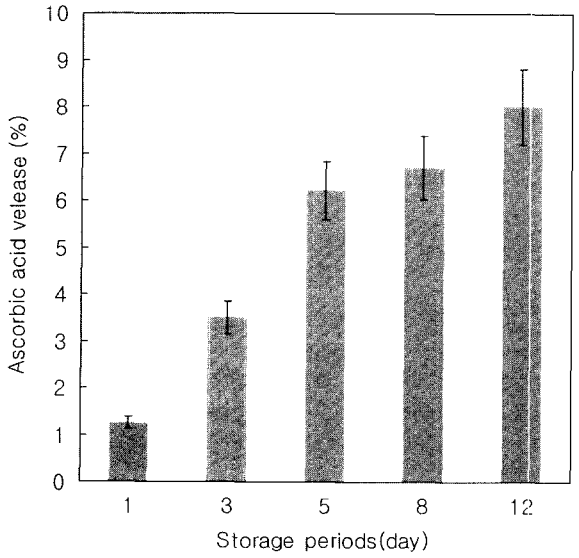

Fig 2. The release of ascorbic acid from microcapsules at 4°C during 12 d storage

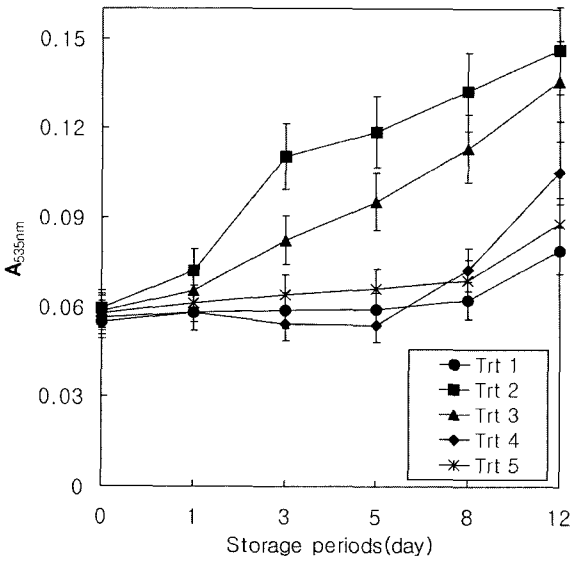

Fig 3. The change of TBA value of milk with 5 different treatments stored at 4°C for 12 d. Trt 1, control (no addition); Trt 2, 100 ppm uncapsulated iron; Trt 3, 100 ppm microencapsulated iron; Trt 4, 100 ppm microencapsulated iron and 100 ppm uncapsulated ascorbic acid; and Trt 5, 100 ppm microencapsulated iron and 100 ppm microencapsulated ascorbic acid.

The stability of microencapsulation was adversely affected during storage. In all samples, the release of ascorbic acid (%) was increased sharply up to 5 d storage, and increased slowly up to 12 d. At 3 d storage, 4% ascorbic acid was released, while 6.8% at 8 day.

TBA test

The effect of ascorbic acid fortification in milk on preventing lipid oxidation by iron (as measured by the TBA test) at 4°C during 12 d storage is shown in Fig. 3. The treatment was divided into 5 different groups as follows: 1) Trt 1, raw milk (control), 2) Trt 2, 100 ppm uncapsulated iron added, 3) Trt 3, 100 ppm microencapsulated iron, 4) Trt 4, 100 ppm microencapsulated iron and 100 ppm uncapsulated ascorbic acid, and 5) Trt 5, 100 ppm microencapsulated iron and 100 ppm microencapsulated ascorbic acid.

In all groups, TBA absorbance increased proportionally to storage period. In 100 ppm uncapsulated iron added group, TBA absorbance increased dramatically as 0.06 to 0.14 from 0 to 12 d. TBA absorbance was significantly lower in ascorbic acid added group, regardless of encapsulation, that those in ascorbic acid unadded group at 5 d. In Trt 4, which containing uncapsulated ascorbic acid, TBA absorbance was sharply increased after 5 d storage, which may be due to partly destruction of ascorbic acid by light. In Trt 5, the TBA absorbance was 0.07 at 5 d and 0.075 at 10 d, while 0.055 at 5 d and 0.10 at 10 d in Trt 4. After 10 d storage, Trt 5 showed a sharp increase in TBA absorbance. It may be explained that lipid oxidation process would be more rapid than ascorbic acid release to prevent milk lipid oxidation enhanced by iron.

The change of TBA value with 250 ppm ascorbic acid and 100 ppm iron in milk is shown in Fig. 4. Higher amount of ascorbic acid reduced an increase of TBA values in Trts 4 and 5. Especially, when uncapsulated ascorbic acid was added along with iron (Trt 4) TBA value was significantly lower, compared with that of 100

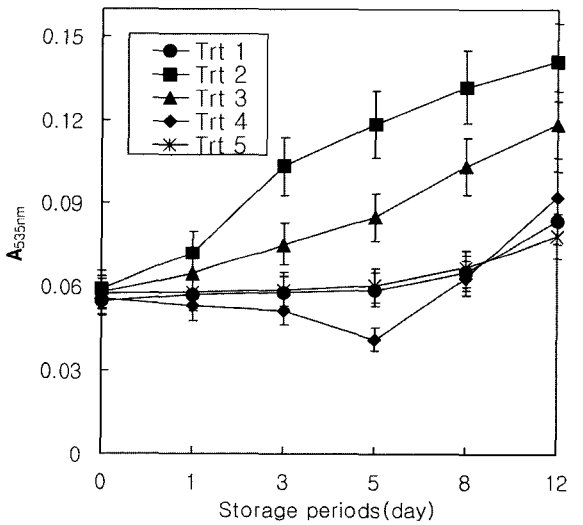

Fig 4. The change of TBA value of milk with 5 different treatments stored at 4°C for 12 d. Trt 1, control (no addition); Trt 2, 100 ppm uncapsulated iron; Trt 3, 100 ppm microencapsulated iron; Trt 4, 100 ppm microencapsulated iron and 250 ppm uncapsulated ascorbic acid; and Trt 5, 100 ppm microencapsulated iron and 250 ppm microencapsulated ascorbic acid.

Table II. Sensory scores of milk containing microencapsulated ascorbic acid at 4°C for 12 d storage

Sensory description	Treatment	Storage period (d)				
		1	3	5	8	12
Sourness	Control	1.0[a]	1.0[a]	1.0[a]	1.0[a]	1.0[a]
	100 ppm	1.2[a]	1.4[a]	1.6[ab]	2.0[b]	3.2[c]
	250 ppm	1.4[a]	1.4[a]	1.8[b]	2.4[bc]	3.2[c]
	SEM			0.3		
Off-taste	Control	1.0[a]	1.0[a]	1.0[a]	1.0[a]	1.0[a]
	100 ppm	1.4[a]	1.6[ab]	2.0[a]	2.0[b]	3.0[c]
	250 ppm	1.4[a]	1.6[ab]	2.2[b]	2.6[bc]	3.6[c]
	SEM			0.3		
Overall Preference	Control	1.0[a]	1.0[a]	1.6[ab]	1.8[b]	2.0[b]
	100 ppm	1.0[a]	1.4[a]	1.8[b]	3.0[c]	3.6[c]
	250 ppm	1.2[a]	1.8[b]	2.4[bc]	3.6[c]	4.2[d]
	SEM			0.3		

[1]Sweetness and off-taste scoring: 1, none; 2, slight; 3, moderate; 4, slightly strong; 5, strong. Means of 8 replicates. Means in a column without the same letter are significantly different ($p>0.05$).

ppm uncapsulated iron added group (Fig. 3).

Sensory analysis

The sensory properties of milk were evaluated at 4°C for 12 d storage (Table II). When 100 ppm microencapsulated ascorbic acid was added into milk, the sourness score was not significantly different from that of control until 5 d ($p>0.05$), but increased thereafter until 12 d. When 250 ppm of microencapsulated ascorbic acid was added, sourness increased and showed a significant difference at 5 d and thereafter.

For off-taste, in both ascorbic acid groups showed a significant difference at 3 d storage, compared with that of control. For overall preference, control and 100 ppm microencapsulated ascorbic acid group showed a high consumer preference up to 5 d storage. However, 250 ppm addition showed an adverse effect on consumers milk preference even at 3 d storage and thereafter. The sensory quality of ascorbic acid-fortified milk has shown to be well maintained by the encapsulation of both ascorbic acid and iron.

CONCLUSION

The present study indicated that the ratio of 15:1 as coating (MCT) to core (L-ascorbic acid) material showed a satisfied efficiency of microencapsulation such as 95.0%. TBA results indicated that the rate of lipid oxidation process decreased significantly in capsulated iron with L-ascorbic acid, regardless of microencapsulation, compared with that in uncapsulated groups. In sensory aspect, there was no significantly adverse effect on milk up to 5 d storage even 250 ppm of L-ascorbic acid microcapsules. Therefore, we suggested that the results of this study provide an important evidence of microencapsulation of L-ascorbic acid applying into milk and acceptable milk products can be prepared with microencapsulated iron and L-ascorbic acid.

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REFERENCES

Berseneva, E. A., Inanov, A. A., Sansonova, T. P., Chernova, E. M., and Oragvelidze, N. I., Microencapsulated aromatizers for tea. *Pishchevaya Paomyshlennost, USSR*, 1, 57-59 (1990).

Braun, S. D. and Olson, N. F., Encapsulation of proteins and peptides in milk fat. Encapsulation efficiency and temperature and freezing stability. *J. Microencapsulation*, 3(2), 115-126 (1986).

Edmondson, L. F., Douglas, F. W. and Avants, J. K., Enrichment of pasteurzied whole milk with iron. *J. Dairy Sci.*, 54, 1422-1426 (1971).

Hegenauer, J., Saltman, P., Ludwig, D., Ripley, L., and Bajo, P., Effects of supplemental iron and copper on lipid oxidation in milk. 1. Comparison of metal complexes in emulsified and homogenized milk. *J. Agric. Food Chem.*, 27(4), 860-867 (1979).

HANES, Preliminary findings of the first health and nutrition examination survey, United States, 1971-1972: Dietary intake and biochemical findings, U. S. Department of Health, Education and Welfare Publications No. (HRA) 74-1219-1 (1974).

Jackson, L. S. and Lee, K., Microencapsulated iron food fortification. *J. Food Sci.*, 56, 1047-1050 (1991).

Kim, C. H., Lee, K. W., Baick, S. C., Kwak, H. S., and Kang, J. O., Studies on the microencapsulation of ω-3 polyunsaturated fatty acid. *Korean J. Food Sci. Technol.*, 28(4), 743-749 (1996).

Korea Food Code. pp. 321-323 (2002).

Kwak, H. S., M. R. Ihm, and Ahn, J., Microencapsulation of β-galactosidase with fatty acid esters. *J. Dairy Sci.*, 84, 1576-1582 (2001).

Liao, M. L. and Seib, P. A., Chemistry of L-ascorbic acid related to foods. *Food Chemistry*, 30, 313-317 (1988).

Lim, C., Kiesius, P. H., Li, M. H., and Robinson, E. H., Interaction between dietary levels of iron and vitamin C on growth, hematology, immune response and resistance on channel catfish (*Ictalurus* punctatus) to *Edwardsiella ictalun*

challenge (2000).

Magee, E. L. Jr. and Olson N. F., Microencapsulation of cheese ripening systems: Formation of microcapsules. *J. Dairy Sci.*, 64, 600-610 (1981a).

Magee, E. L. Jr. and Olson, N. F., Microencapsulation of cheese ripening systems: Stability of microcapsules. *J. Dairy Sci.*, 64, 611-615 (1981b).

Monsen, E. R., Ascorbic acid; an enhancing factor in iron absorption In: Kies, C. (Ed.), National Bioavailability of iron. American Chemical Society, Washington, DC (1982).

NRC (National Research Council); Nutrient Requirement of fish, National Academy Press, Washington, DC (1993).

Nutrition Canada National Survey, Canadian Department of National Health and Welfare Publication No. H58-36-1973 (1973).

Sarikutty, B. M., Sreekumar, M., Nrayanan, C. S., and Mathew, A. G., Studies on microcapsulation of cardamon oil by spray drying technique. *J. Food Sci. Technol., India.*, 25(6), 352-356 (1988).

SAS. *Users Guide: Statistics, Version 5 Edition.* SAS Institute, Inc., Cary, NC (1985).

Uddin, M. S., Hawlader, M. N. A., and Zhn, H. J., Microencapsulation of ascorbic acid: effect of process variables on product characteristics. *J. Microencapsulation*, 18(2), 199-209 (2001).