Water-insoluble, Whey Protein-based Microcapsules for Controlled Core Release Application

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ABSTRACT

Microcapsules consisting of natural, biodegradable polymers for controlled and/or sustained core release applications are needed. Physicochemical properties of whey proteins suggest that they may be suitable wall materials in developing such microcapsules. The objectives of the research were to develop water-insoluble, whey protein-based microcapsules containing a model water-soluble drug using a chemical cross-linking agent, glutaraldehyde, and to investigate core release from these capsules at simulated physiological conditions. A model water soluble drug, theophylline, was suspended in whey protein isolate (WPI) solution. The suspension was dispersed in a mixture of dichloromethane and hexane containing 1% biomedical polyurethane. Protein matrices were cross-linked with 7.5-30 ml of glutaraldehyde-saturated toluene (GAST) for 1-3 hr. Microcapsules were harvested, washed, dried and analyzed for core retention, microstructure, and core release in enzyme-free simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) at 37℃. A method consisting of double emulsification and heat gelation was also developed to prepare water-insoluble, whey protein-based microcapsules containing anhydrous milkfat (AMF) as a model apolar core. AMF was emulsified into WPI solution (15-30%, pH 4.5-7.2) at a proportion of 25-50% (w/w, on dry basis). The oil-in-water emulsion was then added and dispersed into corn oil (50 ℃) to form an O/W/O double emulsion and then heated at 85℃ for 20 min for gelation of whey protein wall matrix. Effects of emulsion composition and pH on core retention, microstructure, and water-solubility of microcapsules were determined. Overall results suggest that whey proteins can be used in developing microcapsules for controlled and sustained core release applications.

INTRODUCTION

Microencapsulation is a technology or process of forming small capsules (particles) by which small solid particles and liquid droplets are surrounded by a protective thin layer formed from a polymer or embedded within a matrix of a wall material (Rosenberg et al., 1985; Shahidi and Han, 1993). In areas of microencapsulation, the substances that are coated or encapsulated are referred to as core, internal phase, fill, or active. The encapsulating materials are called shell, coating, carrier, encapsulant, or wall materials. Microencapsulation technology provides a variety of functions, including protection of labile materials from environments (oxygen, moisture, light, temperature), prevention of evaporation of volatiles, separation of reactive compounds from other incompatible materials, conversion of a liquid into a solid form, thus easier handling and storage and better uniform mixing into dry mixes, controlled and/or sustained release applications, targeted delivery for enhanced efficacy, and masking and preservation of tastes and flavours (Dziezak, 1988; Rosenberg and Young, 1993; Schrooyen et al., 2001; Shahidi and Han, 1993).

Techniques for microencapsulating food ingredients and the potential use of the technology in the food industry have been reviewed (Dziezak, 1988; Gibbs et al., 1999; Schmitt et al., 1998). Important applications of microencapsulation for foods include flavours, vitamins, minerals, colours, enzymes, essential oils, probiotics, and other nutritional compounds, most of which are very sensitive to oxidation and undergo deterioration readily when exposed to oxygen, light and temperature (Dziezak, 1988; Shahidi and Han, 1993; Schrooyen et al., 2001). Microencapsulation makes it possible to transform these sensitive substances into a free flowing particles or powders in which they are protected by wall materials against evaporation, oxidation and other chemical reactions (McNamee et al., 1998). The particles or powders are then used as ingredients in a dry beverage mix or added into liquid or solid food systems.

Among many different microencapsulation techniques, spray drying is the most commonly used encapsulation method in the food industry, although it is most often considered as a dehydration process. The spray drying process is rather easy and inexpensive, uses equipment readily available, and produces large volume of microcapsules (or powder). Other encapsulation methods (liposome entrapment, freeze drying, and coacervation) have the capability of forming microcapsules with specific properties and functions, however, their practical use in food application and implementation of large scale-up production in the food industry are relatively difficult at the present due to high production cost and food safety concerns because of some solvents or chemicals involved in encapsulation processes.

During the last two decades, microcapsules for controlled-release of pharmaceuticals and other active compounds have been investigated and developed (Langer, 1989; Levy and Andry,

1991). Controlled release is a method by which one or more active core materials are released or made available under the influence of a specific stimulus at a specified state. Controlled release mechanisms of encapsulated cores are based on one or a combination of the following stimuli: a change in temperature, pH or moisture rendering core release by diffusion, dissolution or swelling through wall matrices of microcapsules, an application of mechanical forces such as pressure or shear leading to disintegration of microcapsules, an addition of enzymes causing gradual degradation of wall materials (Karel and Langer, 1988; Pothakamury and Barbosa-Canovas, 1995). Critical to the success of achieving these mechanisms is maintaining the physical integrity of the microcapsule wall matrix until a specific core release mechanism is triggered. This requires, in most cases, developing microcapsules with a wall matrix that is water-insoluble or at least with limited water solubility. In pharmaceutical applications, encapsulating wall materials are commonly made from synthetic polymers. A need to develop natural, biodegradable microencapsulating agents for controlled core release applications exists, in particular for use in food applications. Biopolymers have been used in developing microcapsules for controlled release of a variety of core materials and include albumins, gelatin, polysaccharides such as dextran, derivatives of cellulose and starch, and different carbohydrate-based hydrocolloids (Gupta and Hung, 1989; Levy and Andry, 1990, 1991). Among these, protein microcapsules or microspheres have attracted significant attention as a potential delivery system for controlled core release applications (Arshady 1990: Latha et al., 1995). Most of the studies reported have been focused on albumin or gelatin microcapsules and microspheres (Longo et al., 1982; Saleh et al., 1989).

In recent years, there has been interest in milk proteins as microencapsulating agents and bioactive carriers. The concept of using whey proteins as microencapsulating agents has been investigated and developed (Rosenberg, 1997). A series of studies has indicated that whey proteins exhibit excellent microencapsulating properties and are suitable for microencapsulation of volatile and non-volatile core materials (Moreau and Rosenberg, 1993, Rosenberg and Sheu, 1996; Rosenberg and Young, 1993). However, research effort regarding applications for whey proteins as microencapsulating agents has been focused on developing water-soluble microcapsules using spray drying process. Applications for whey proteins as a wall material in developing water-insoluble microcapsules for controlled and sustained core release have been investigated relatively to a very limited extent.

EXPERIMENTAL

Preparation of theophylline loaded microcapsules

Whey protein isolate (WPI) containing 95.6% (w/w on dry basis) protein was used as a wall

material. Theophylline served as a model water-soluble core. Wall solution containing 20% (w/w) WPI was prepared in deionized water. In all cases, the pH of wall solution was 7.2. Theophylline (1.6 g) was dispersed in 4 g of wall solution and this mixture was then suspended (at 25℃) in a dispersion mixture consisting of 80 ml of dichloromethane and 50 ml of hexane containing 1% biomedical polyurethane in a 250-ml round-bottom flask by stirring at 900 rev./min for 3 min. Then, 7.5, 15, or 30 ml of glutaraldehyde-saturated toluene (GAST) prepared according to the method reported by Longo et al. (1982) was added to the suspension and cross-linking was carried out for 1 or 3 hr. Wet microcapsules were separated from the dispersing solvent mixture by filtration and then washed with a 1:1 mixture of dichloromethane/hexane. Microcapsules were then washed with 1% sodium bisulfite, with distilled water, and finally with acetone. Washed microcapsules were dried in a vacuum oven at 50℃ overnight. Dry microcapsule powders were separated, by sieving, into large (diameter >700 um), medium (diameter 450-700 um) and small (diameter <450 um) microcapsules.

Determination of total theophylline content

Theophylline content in microcapsules included in each size category was determined by extracting with methanol and measuring absorbance of the filtered extract at 274 nm. Core retention was expressed as the ratio (in %) of core content determined in microcapsules to a theoretical core content assuming 100% core retention during the microencapsulation process.

Release of theophylline from microcapsules

Microcapsules were placed in a double wall glass beaker containing 180 ml of either enzyme-free simulated intestinal fluid (SIF) or enzyme-free simulated gastric fluid (SGF). The suspension was stirred at 37℃ using a floating stir bar. Aliquots were withdrawn periodically, using a syringe equipped with a 0.2 um syringe filter and theophylline concentration was determined using spectrophotometer at 274 nm. In all cases, an equal volume of dissolution medium was immediately added to maintain a constant volume. Samples were withdrawn until three successive aliquots showed no increase in optical absorbance (274 nm). The amount of theophylline released from the microcapsules, at a given time, was calculated using standard curves of theophylline in SGF and SIF and expressed as percentage of total theophylline content of the investigated microcapsules.

Preparation of anhydrous milk fat loaded microcapsules

Microcapsules were prepared using a process consisting of double emulsification and heat gelation. Wall solutions containing 15-30g/100g WPI were prepared in deionized water.

Effects of wall solution pH on the encapsulation process and on microcapsule properties were investigated using WPI solutions at pH 4.5, 5.5 and 7.2. Anhydrous milk fat (AMF) was emulsified into the wall solutions at a proportion of 20-50g/100g (on dry basis) using a high pressure homogenizer operated at 50 MPa. Core-in-wall emulsions were denoted by their specific WPI concentration and AMF load. For example, the core-in-wall emulsions 25/50 consisted of 25 g/100 g WPI and 50 g/100 g (on dry basis) AMF.

Corn oil (900 g) containing 1g/100g Span 65 was placed in a double-walled glass beaker connected to a temperature controlled circulating water bath. Temperature of the corn oil (stirred at 900 rpm) was adjusted to 50℃. When the temperature of the corn oil reached 50℃, core-in-wall emulsion (75 g) was slowly added to the stirred corn oil. The mixture was stirred at 900 rpm for 10 min to form an O/W/O double emulsion. Temperature of the mixture was then adjusted to 85℃ and gelation was carried out for 20 min.

Microcapsules were separated from corn coil by filtration and then washed free from corn oil. For washing, microcapsules were suspended in petroleum ether at a microcapsules-to-solvent ratio of 1:3 (w/w), the mixture was stirred for 1 min at 500 rpm and then microcapsules were separated from the solvent. This procedure was repeated three times and then microcapsules were washed, once, in absolute ethanol in the above conditions. Washed microcapsules were dried (55℃, 6.7kPa, 12 hr) and then stored in a desiccator pending analysis.

Particle size distribution

Particle size distribution and mean particle size (d_{32}) in core-in-wall emulsion were determined using a particle size analyzer (Malvern Mastersizer MS20, Malvern Instruments, Malvern, U.K.).

Chemical analyses

Moisture content of the microcapsules was determined gravimetrically by air oven method. Anhydrous milk fat content of the dry capsules was determined using a modification of the Ross-Gotlieb fat determination method.

Water-solubility

Water solubility of microcapsules prepared from pH 7.2, 20/50 core-in-wall emulsion was investigated. Microcapsule samples (0.3 g) were placed in glass vials containing 25 ml water at pH 2.5, 5.5, and 7.0. In order to prevent microbiological-related proteolysis, 0.02% sodium azide was added to the water. Capped vials were incubated at 4 and 30℃ and the

concentration of soluble protein in the aqueous phase was determined after 2 and 7 days of incubation. A sample (0.75 ml) of the aqueous phase was filtered through UniPrep Syringeless Filter Device (0.45 um, Whatman Laboratory Division, Clifton, NJ, U.S.A.) and protein content in the filtrate was determined using the Bio-RadDC Protein Assay kit (BioRad, Hercules, CA, U.S.A.). Bovine serum albumin (BSA) was used as standard protein. Soluble protein was expressed as the proportion (%) of total protein content included in the incubated microcapsules.

Scanning electron microscopy (SEM)

Structural features of microcapsules were investigated using SEM. In all cases, specimens were coated with gold using a model E-5050 Polaron Sputter Coater, and analyzed using an ISI DS-130 scanning electron microscope (International Scientific Instrument Inc., Pleasanton, CA) operated at 10 kV. Micrographs were prepared using a Type 55 Polaroid film (Polaroid Corp., Cambridge, CA).

SUMMARY OF RESULTS

Microstructual feature of theophylline-loaded, whey protein microcapsules

Theophylline-loaded, whey protein microcapsules were spherical and ranged in size from 300 to 800 um in diameter. Outer and inner structures of microcapsules determined by SEM are presented in Figs. 1 and 2. Outer surfaces of microcapsules were not smooth and exhibited irregularities of different shapes (Fig. 1). The size and shape of these structural features suggested that they represented a footprint of theophylline crystals that were originally present at the surface of the wet microcapsules but removed from the surface during the washing procedure of encapsulation process. Results of the SEM indicated that, in some cases, microcapsules exhibited some surface cracks (Fig. 1C). The presence of such cracks was especially evident when microcapsules were cross-linked for 3 hr with 30 ml of GAST. Formation of these cracks could thus be attributed to high cross-linking density that rendered microcapsules fragile.

The inner structure of microcapsules (Fig. 2) indicated that theophylline crystals were embedded physically throughout the cross-linked protein matrix and no indications for interactions between core and wall components were evident. The whey protein matrix of the microcapsules had a very dense appearance that indicated good efficiency of the cross-linking reaction.

Effect of core release on structural features of theophylline-loaded microcapsules was

investigated and results are presented in Fig. 2C and D. Outer structure of microcapsules was not affected in any way during core release and was similar to that observed for freshly prepared microcapsules (Fig. 1). These results thus indicated that core release was due to diffusion through the wall rather than being associated with degradation or solubilization of the protein matrix. Examining the inner features of capsules after core has been released (Fig. 2D) revealed that the wall matrix maintained its structural features and a multitude of voids from which theophylline has been released (through diffusion) was evident

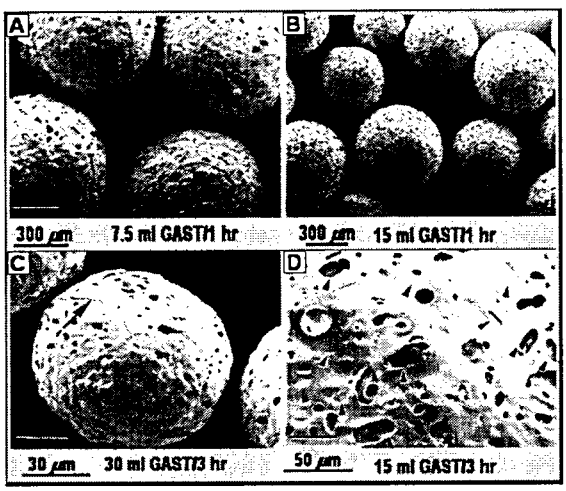

Fig. 1. Outer structure of theophylline-containing WPI microcapsules cross-linked for 1 hr with 7.5 or 15 ml of GAST (A and B, respectively) and for 3 hr with 15 or 30 ml GAST (D, C, respectively).

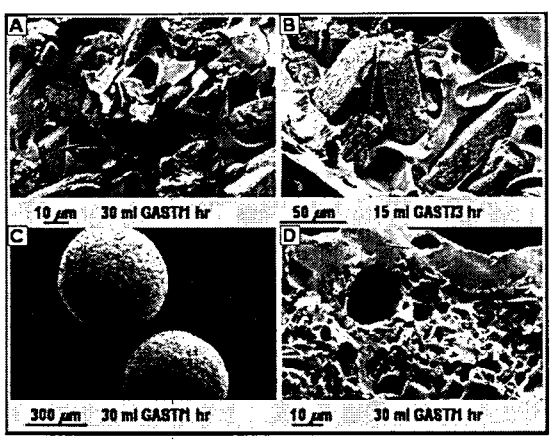

Fig. 2. Inner structure of theophylline-containing microcapsules (A and B); outer and inner structures of microcapsules after complete core release into SGF at 37°C (C and D, respectively). Microcapsules were cross-linked for 1 hr with 30 ml GAST (A, C and D) or for 3 hr with 15 ml of GAST (B).

• Core content and retention in microcapsules containing theophylline

Overall core content and retention efficiency in microcapsules ranged from 49.5 to 52.5% (w/w) and from 73.5 to 78%, respectively, and were not affected by cross-linking conditions, indicating that the microencapsulation process was efficient allowing high core retention. Core losses during the process could be mainly attributed to effects of the washing stage.

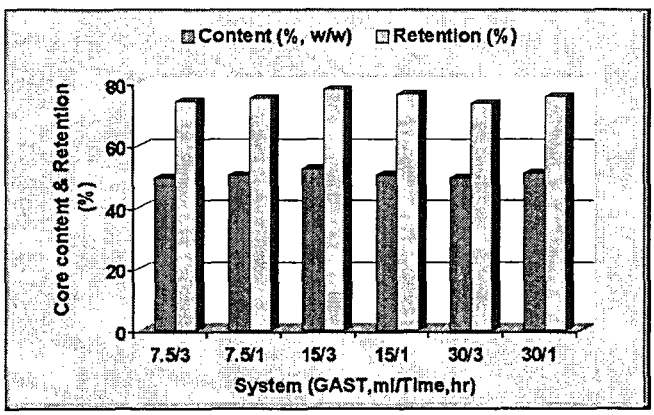

Fig. 3. Theophylline content and core retention efficiency in microcapsules prepared at different cross-linking conditions (7, 15, and 30 ml of GAST and reaction time for 1 or 3 hr).

Theophylline release from microcapsules

Release of theophylline from microcapsules into SIF and SGF at 37℃ is shown in Figs 4, 5 and 6. Preliminary studies indicated that the GAST-cross-linked WPI particles were insoluble in both fluids. This and the results of structure analysis indicated that core release from theophylline-loaded microcapsules could be attributed to diffusion-driven mechanism rather than dissolution of the protein matrix.

Results obtained with microcapsules from the three size categories indicated that in all cases, core release was time-dependent and was affected, to varying extent, by type of simulated digestive fluid, cross-linking conditions, and by microcapsule size. In all cases, the rate of core release from given microcapsules into SIF was significantly higher than that into SGF. Core release was inversely proportional to cross-linking density. Core release from small microcapsules was significantly faster than that from larger capsules.

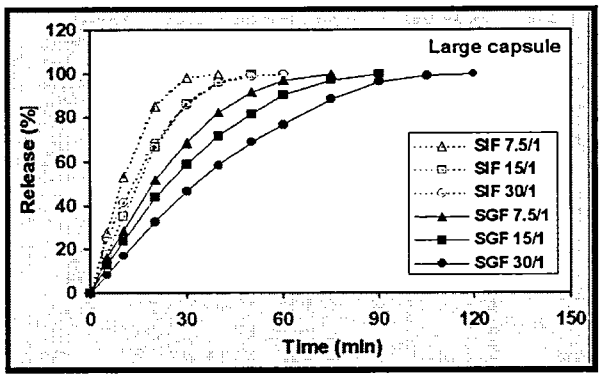

Fig. 4. *In vitro* core release from large microcapsules cross-linked with 7.5, 15 and 30 ml of GAST for 1 hr into enzyme-free SIF and SGF at 37°C.

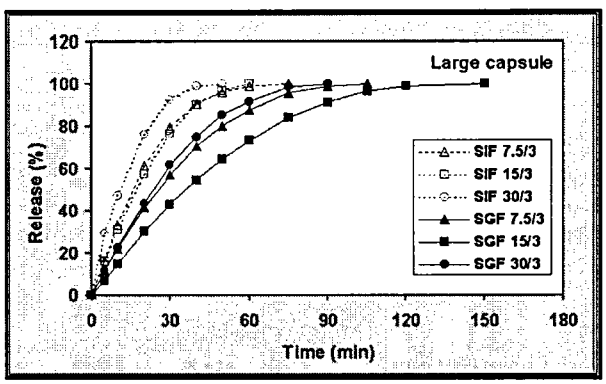

Fig. 5. *In vitro* core release from large microcapsules cross-linked with 7.5, 15 and 30 ml of GAST for 3 hr into enzyme-free SIF and SGF at 37°C.

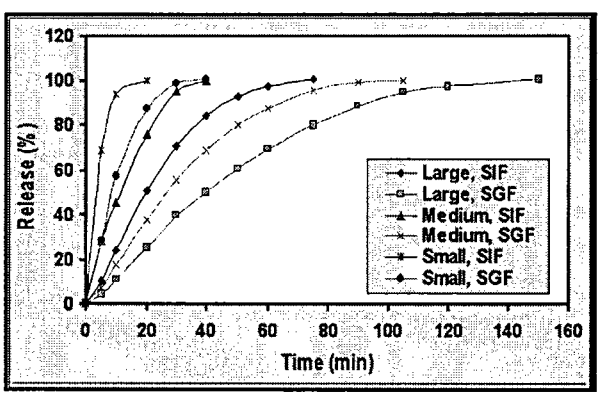

Fig. 6. *In vitro* core release from large, medium and small microcapsules cross-linked with 15 ml of GAST for 3 hr into enzyme-free SIF and SGF at 37°C.

Microstructure of AMF loaded microcapsules

Microcapsules prepared from core-in-wall emulsions with pH 7.2 were spherical, varied in size (10-100 um in diameter) and their outer surface was smooth and dent-free (Fig. 7a, b and c), regardless of WPI or AMF concentration but exhibited some spherical surface pores (Fig. 7e). The diameter of these spherical pores (0.2 to 0.4um) suggested that they represented footprints of core droplets that were originally present at the surface and were lost during the microencapsulation process.

Structural features of microcapsules prepared with pH 4.5 or pH 5.5 emulsions exhibited similar structural features that differed significantly from those of microcapsules prepared from pH 7.2 emulsion. Outer surfaces of these capsules were very porous, wrinkled and exhibited many irregularities (Figs 7d and f) and inner surface a network of large aggregates of proteins separated by many voids of different sizes and shapes (Fig. 7j and k). These structural features suggest aggregation of protein matrices rather than formation of continuous films. The structural features of microcapsules prepared by pH 4.5 or 5.5 were probably influenced by effect of low pH on extent of protein-protein interactions and by the pH-dependent association state of β-lactoglobulin.

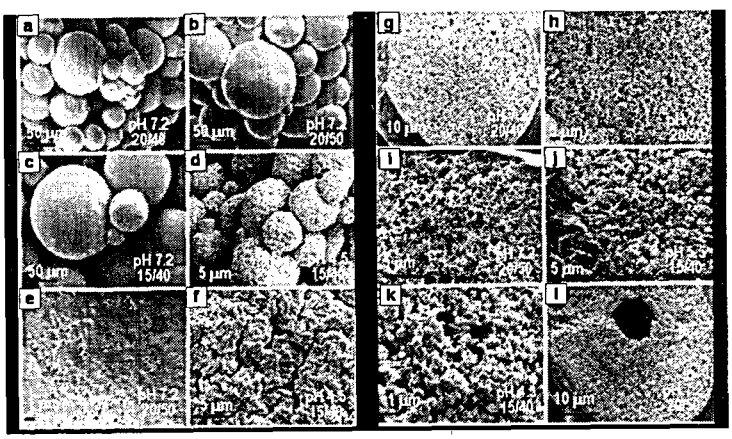

Fig. 7. Outer and inner structures of microcapsules prepared from core-in-wall emulsions (15/40, 20/40, 20/50) with pH 4.5, 5.5 and 7.2.

Core retention in microcapsules containing AMF

Achieving high core retention during microencapsulation is important to the overall efficiency of the microencapsulation process and to the functionality of microcapsules. In all cases, core retention during microencapsulation higher than 88% was obtained (Figs 8 and 9). For microcapsules prepared with pH 7.2 emulsions, core retention ranged from 88 to 94% and increased, at a given wall solids concentration, with initial core load. At a given core load,

core retention was not significantly affected by WPI concentration in the core-in-wall emulsion (Fig. 8).

Core retention in microcapsules prepared with emulsions containing 15% WPI at different pH (Fig. 9) ranged from 96.2 to 98.7%, from 96.6 to 98.4% and from 94.7 to 97.4% for microcapsules prepared at pH 4.5, 5.5 and 7.2, respectively. Although at a given pH core retention was affected by core load, no common trend relating these variables was detected. Overall, results indicated that regardless of core-in-wall emulsion composition and pH, the microencapsulation process, consisting of double emulsification and heat gelation, was very efficient and allowed high core retention to be attained. Core loss during washing could be attributed both to removal of core droplets from the outer surfaces of the wet microcapsules and to some core extraction from interior parts of the capsules by a diffusion-driven mechanism.

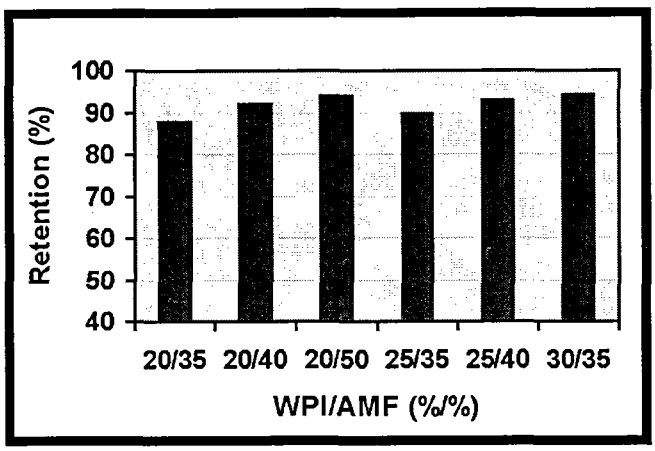

Fig. 8. Core retention in microcapsules prepared from pH 7.2 core-in-wall emulsions with WPI concentration ranging from 20 to 30g/100g and AMF load of 35-50g/100g on dry basis.

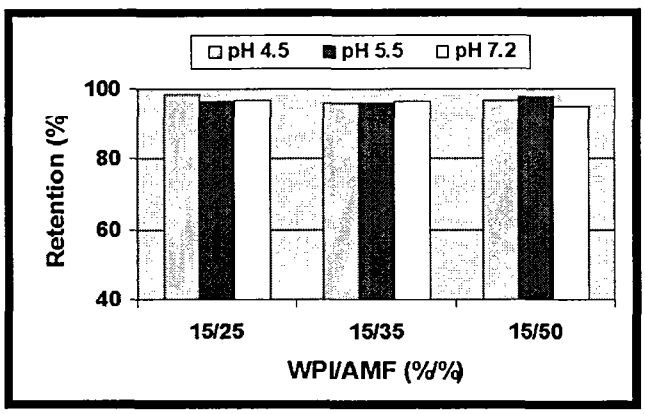

Fig. 9. Core retention in microcapsules prepared at different pH with core-in-wall emulsions containing 15 g/100 g WPI and 25-50g/100g (on dry basis) AMF.

Water solubility of whey protein microcapsules containing AMF

Water-solubility of microcapsules for controlled and sustained core release in an aqueous environment is of great importance to the functionality of these microcapsules. In designing such microcapsules, limited or delayed water-solubility is needed. Water solubility of microcapsules prepared from pH 7.2 emulsions is presented in Fig. 10. Results indicate that microcapsules had only a very limited water-solubility that was affected, to different extents, by time, pH of dispersion medium and temperature.

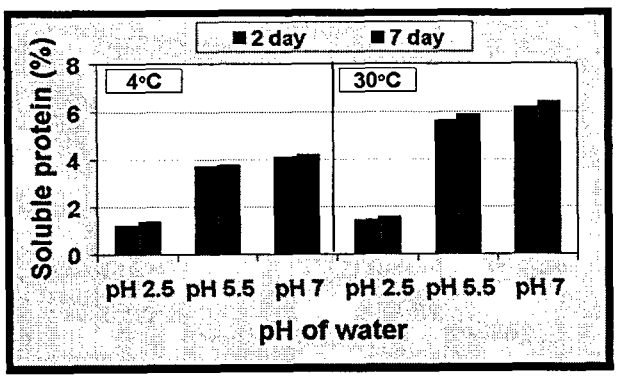

Fig. 10. Soluble protein (% of total protein) in microcapsules suspended in water at pH 2.5-7.0 and incubated at 4 and 30°C for 7 days. Microcapsules were prepared from pH 7.2 core-in-wall emulsion 20/50.

CONCLUSIONS

Cross-linking of the whey protein-based wall matrices of microcapsules by glutaraldehyde-saturated toluene via organic phase was effective in influencing rate of core release. Microcapsules were practically water-insoluble. The developed capsules may be suitable for controlled and sustained core release in application fields where chemical cross-linking is acceptable. A microencapsulation process consisting of double emulsification and subsequent heat gelation was used successfully to prepare water-insoluble, whey protein-based microcapsules containing a model apolar core AMF. The microcapsules had a very limited water-solubility and high core retention, indicating high efficiency of the process. The characteristics of the developed whey protein capsules also suggest that they may be suitable for controlled and/or sustained core release application.

REFERENCES

1. Arshady, A. 1990. Albumin microspheres and microcapsules: methodology of manufacturing techniques. Journal of Controlled Release, 14:111-131.
2. Dziezak, J. D. 1988. Microencapsulation and encapsulated ingredients. Food Technology, April 136-151.
3. Gibbs, B. F., Kermasha, S., Alli, I. and Mulligan, C. N. 1999. Encapsulation in the food industry: a review. International Journal of Food Science and Nutrition, 50:213-224.
4. Gupta, P. K. and Hung, C. T. 1989. Review. Albumin microspheres II: applications in drug delivery. Journal of Microencapsulation, 6:463-472.
5. Karel, M. and Langer, R. 1988. Controlled release of food additives. In: Risch, S. J. and Reineccius, G. A. (Eds), *Flavor Encapsulation*, ACS Symposium Series No. 370. Washington, DC: American Chemical Society, pp. 177-191.
6. Langer, L. 1989. Biomaterials in controlled drug delivery: new perspectives from biotechnological advances. Pharmaceutical Technology, 13:18-30.
7. Latha, M. S., Rathinam, K., Mohanan, P. V. and Jayakrishnan, A. 1995. Bioavailability of theophylline from glutaraldehyde cross-linked microspheres in rabbits following oral administration. Journal of Controlled Release, 34:1-7.
8. Levy, M. C. and Andry, M. C. 1990. An evaluation of gelatin microcapsules prepared using interfacial cross-linking process. Life Science Advances. 19:219-227.
9. Levy, M.C. and Andry, M. C. 1991. Mixed-walled microcapsules made of cross-linked proteins and polysaccharides: preparation and properties. Journal of Microencapsulation, 8: 335-347.
10. Longo, E. W., Iwata, H., Lindheimer, A. and Goldberg, E. P. 1982. Preparation of hydrophilic albumin microspheres using polymeric dispersing agents. Journal of Pharmaceutical Sciences, 71:1323-1328.
11. McNamee, B. F., O'Riordan, E. D. and O'Sullivan, M. 1998. Emulsification and Microencapsulation Properties of Gum Arabic. Journal of Agricultural and Food Chemistry, 46(11):4551-4555.
12. Moreau, D. L. and Rosenberg, M. 1993. Microstructure and fat extractability in microcapsules based on whey proteins or mixtures of whey proteins and lactose. Food Structure, 12:457-468.
13. Pothakamury, U. R. and Barbosa Canovas, G V. 1995. Fundamental aspects of controlled release in foods. Trends in Food Science and Technology, 6:397-406.
14. Rosenberg, M. 1997. Milk derived whey protein-based microencapsulating agents and a method of use, US Patent 5,601,760, February 11.
15. Rosenberg, M., Kopelman, I. J. and Talman, Y. 1985. A scanning electron microscopy

study of microencapsulation. Journal of Food Science, 50:139-144.

16. Rosenberg, M. and Sheu, T. Y. 1996. Microencapsulation of volatiles by spray-drying in whey protein-based wall systems. International Dairy Journal, 6:273-284.
17. Rosenberg, M. and Young, S. L. 1993. Whey proteins as microencapsulating agents. Microencapsulation of anhydrous milkfat - Structure evaluation. Food Structure, 12:31-41.
18. Saleh, A. M., El-Kordagui, L. K., Robb, D. A. and Florence, A. T. 1989. Effect of some drugs and additives on the cross-linking of bovine serum albumin by glutaraldehyde. International Journal of Pharmaceutics, 57:205-210.
19. Schmitt, C., Sanchez, C., Desobry-Banon, S. and Hardy, J. 1998. Structure and technofunctional properties of protein-polysaccharide complexes: a review. Critical Review in Food Science and Nutrition, 38(8):689-753.
20. Schrooyen, P. M. M., Van der Meer, R. and De Kruif, C. G. 2001. Microencapsulation: its application in nutrition. Proceedings of the Nutrition Society, 60:475-479.
21. Shahidi, F. and Han, X. Q. 1993. Encapsulation of food ingredients. Critical Review in Food Science and Nutrition. 33(6):501-547.

유청단백질을 이용한 미세캡슐의 응용

이 성 제
뉴질랜드, Massey 대학교 교수

요 약

미세캡슐(microencapsulation)은 내부 물질(core material)의 방출을 제어하기 위하여 여러 천연 및 생분해성 물질이 사용된다. 유청단백질은 이러한 목적에 아주 적합한 것으로 여겨지고 있는데 이는 독특한 이화학적 특성에 기인되는 것이다. 본 연구팀은 drug이나 생리활성물질을 피복하기 위하여 cross-linking 물질로 glutaraldehyde를 사용하여 수용성 이면서 유청단백질을 근간으로 하는 미세캡슐 제조기술을 개발하였다. 또한 생리적 조건에서 이들 캡슐의 분해 및 포집물질의 방출에 대한 연구를 수행하였는데 수용성 drug으로 사용된 theophylline은 유청단백질에 잘 분산되는 것으로 확인되었다. 이 분산액은 !%의 생리활성물질인 polyurethane을 함유하는 dichloromethane과 hexane 혼합물에 잘 확산이 되었다.

미세캡슐공정에서 피복물질로 사용되는 여러 물질중에서 유청단백질은 생리적 효능이 뛰어나고 여러 물리적 작용이 있기 때문에 새로운 피복소재로써 그 효용성이 매우 높다. 지금까지의 연구결과 유청단백질을 이용한 미세캡슐의 제조는 유청단백질이 가지는 기능적 효과와 내부물질이 지니는 약리효과를 동시에 이용할 수 있다는 점에서 향후 고부가 식품 의약품 첨가물질로써 유용성이 기대된다고 하겠다.

서 론

캡슐화 기술은 고체, 액체, 기체상의 물질을 특정 조건하에서 조절된 속도로 내용물을 방출할 수 있도록 어떤 물질(material)이나 조직(system) 내부에 포장하는 기술로 이 미세포장 단위를 미세캡슐(microcapsule) 이라고 하며 직경이 수 ㎛에서 수 ㎜로 다양하다.

미세캡슐(microencapsulation)은 최근에 유용하게 이용되는 기술로 작은 고형입자와 액상입자로 이루어진 미세한 캡슐을 형성하는 방법을 의미한다. 미세한 입자들은 polymer로부터 형성된 아주 가는 막 또는 층을 만들어 피복물질의 matrix 내에 고정되어 존재한다.

미세캡슐 방법은 피복되는 물질과 피복시키는 물질로 구별되는데 통상적으로 core, internal phase, fill, active로 불려지며 캡슐물질은 shell, coating, carrier, encapsulent, wall material 등 다양하게 불려진다. 미세캡슐기술은 산소, 수분, 광선 및 온도와 같은 외부환경에 민감한 물질을 보호하는 작용, 휘발성화합물의 휘발의 억제, 생리활성물질의 분리 및 액상(liquid)을 고형(solid)으로의 전환 뿐 아니라 여러 기능적 특징을 부여할 수 있다. 따라서 보다 취급이 용이하고 혼합

분말에서 균일하게 분포되게 할 수 있다. 또한 미세캡슐화는 독성, 이미, 이취 등의 바람직하지 않은 요소를 은폐시키거나, 향미나 영양성분 등 불안정한 물질을 외부환경으로부터 보호하거나, 또는 핵물질을 고형화시켜 취급을 간편하게 하고 내용물의 용출속도를 조절하는 등의 목적으로 사용되어 진다. 최근에는 핵물질의 단순한 보호기능에서 벗어나 체내에서의 기능성 향상에 목적을 둔 기능성 미세캡슐화 분야로 그 응용이 확대되고 있다.

미세캡슐화 기술은 풍미성분, 비타민, 미네랄, 색소, 효소, 유산균 및 다른 영양물질 등에 적용되며 이러한 물질들이 외부환경에 노출되었을 때 산화, 변질 등으로부터 보호될 수 있다.

미세캡슐화 기술은 작은 입자나 파우더 형태로도 가능하며 이들은 농축, 산화, 화학반응 등으로부터 피복물질에 의하여 보호된다.

많은 미세캡슐화 방법이 개발되어 왔는데 분무건조(spray drying)는 식품산업에서 가장 보편적으로 이용되는 캡슐화 방법으로 탈수공정으로도 잘 알려져 있다. 분무건조공정은 다루기 쉽고 저렴하며 기존의 장비를 이용할 수 있다는 장점이 있고 파우더 형태의 비교적 큰 입자를 만든다. 미세캡슐화에 적용된 다른 방법은 리포좀(liposome), 동결건조(freeze drying), 코아세르베이트(coaservation) 등을 들 수 있으며 특별한 기능을 부여할 목적으로 사용된다. 그러나 이러한 기술의 응용은 대량생산 설비가 필요하며 생산단가가 비싸고 캡슐화공정에서 유기용매를 사용하기 때문에 안전성 문제로 인하여 식품에 응용에는 한계가 있다.

최근 20 년동안 미세캡슐화 기술은 많은 발전을 해 왔으며 특히 약물의 방출제어(controlled release)를 위한 미세캡슐은 집중적으로 연구되어 왔다. 방출제어는 하나 또는 그 이상의 활성 내부물질이 특정 조건하에서 방출되도록 고안된 방법이다. 방출제어 작용은 피복된 내부물질이 적절한 상태에 방출이 되도록 하는 것이 매우 중요한데 온도, pH 및 수분의 변화가 방출을 촉진시키는데 캡슐을 구성하는 피복제의 확산, 용해, 팽창을 유도하기 때문이다.

이러한 캡슐화 작업은 내부물질의 방출이 유도되기 전까지 피복제가 캡슐의 물리적 성질을 유지하도록 한 것이 매우 중요하다. 대부분의 경우에서 이러한 요건을 충족시키기 위해서는 피복물질이 최소한 수용성이 되어야 하는데 피복제의 개발이 우선적으로 이루어져야 한다.
제약산업에서 캡슐화에 사용되는 피복제는 주로 합성 고분자 (synthetic polymer)가 사용된다. 천연고분자(biopolymer) 물질 역시 미세캡슐화에 사용되는데 알부민(albumin), 젤라틴(gelatin), 데스트란(dextran)과 같은 다당류(polysaccharides), 셀룰로스 또는 전분 유도체들, 탄수화물이 함유된 여러 콜로이드성 물질 등이 대표적으로 사용된다. 이들 중에서 단백질성 물질들이 방출제어에 매우 적합하며 가장 많이 사용된다. 대부분의 연구는 알부민 또는 젤라틴을 이용한 캡슐에 중점되어 왔다.

최근, 미세캡슐 술에 코팅물질로써 우유 단백질에 대한 관심이 증대되었다. 우유 단백질의 하나인 유청단백질을 피복물질로 사용하는 여러 방법이 개발되었는데, 유청단백질은 지질, 휘발성 또는 비휘발성 내부물질을 코팅하기에 아주 적절한 물질로 인정되었다.

유청단백질을 피복물질로 해서 코팅한 경우 분무 건조시에 다공성의 molecular sieve와 같은 특성을 가지고 내부물질로 사용된 지질의 산화를 방지하는데 아주 효과적인 것으로 나타났다. 유청단백질이 가지는 물리학적 특성 즉, 물에 용해가 잘되고 유화시 gel을 형성한다는 점 이외에도 유청단백질은 우유에 존재하는 단백질이기 때문에 소장에서 쉽게 분해되어 여러 기능성 효과

를 나타낼 수 있을 뿐아니라, 내부물질을 효과적으로 방출할 수 있는 특성을 지닌다.

연구결과

유청단백질 미세캡슐의 구조

유청단백질과 theophylline을 혼합하여 미세캡슐을 하면 직경이 약 300-800 um의 구형의 캡슐이 만들어지는데 이를 주사전자현미경(Scanning electron microscopy)으로 관찰해보면 Fig. 1에 보는 바와 같다.

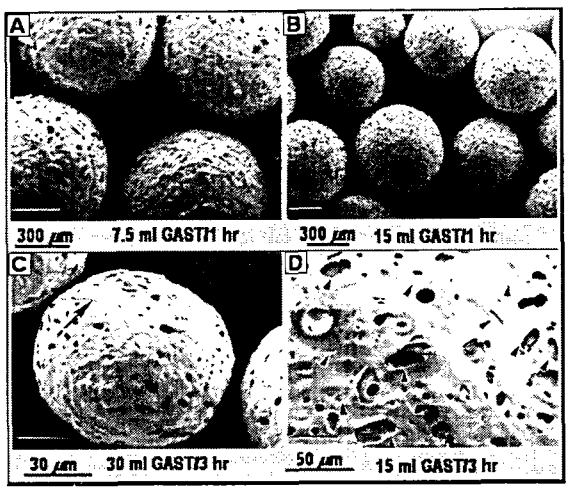

Fig. 1. Outer structure of theophylline-containing WPI microcapsules cross-linked for 1 hr with 7.5 or 15 ml of GAST (A and B, respectively) and for 3 hr with 15 or 30 ml GAST (D, C, respectively).

캡슐외부 표면은 매끄럽지 않고 불규칙적으로 캡슐마다 다소 차이가 있었다. 이러한 표면 구조는 젖은상태의 표면에 존재하였던 theophylline 결정(crystal)의 흔적으로 생각되는데, 미세캡슐화 공정에서 수세과정시 존재하였던 theophylline이 제거되면서 공간처럼 비어 있는 것과 같은 형태를 지니게 된 것이다.

전자현미경 관찰에서 한가지 흥미로운 것은 Fig. C에서 보는 것과 같이, 표면에 금이 가있는 것이 관찰되었다는 점이다. 이러한 crack은 미세캡슐이 만들어 질 때 30 ml의 GAST로 3시간 동안 cross-linking된 결과라 생각되는데 crack은 미세캡슐이 고농도로 cross linking되면 캡슐이 쉽게 부서질 수 있음을 암시하는 것이다.

캡슐내부의 모습은 Fig. 2에 보는 바와 같이 theophylline 결정이 서로 결합된 단백질 matrix에 물리적으로 박혀 있는 것이 확인 되었다. 내부물질과 코팅물질들간의 어떠한 interaction도 관찰되지 않아 유청단백질은 매우 적절한 코팅 물질로 생각되었다.

우청단백질로 이루어진 protein 지지체(matrix)은 매우 촘촘한 모습이며 가교반응에 있어서 우수한 효율을 보였다.

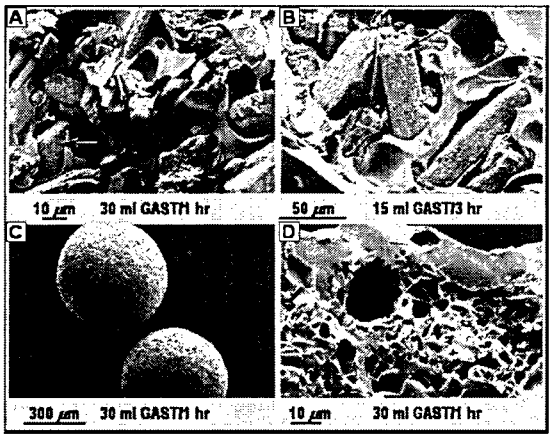

Fig. 2. Inner structure of theophylline-containing microcapsules (A and B); outer and inner structures of microcapsules after complete core release into SGF at 37°C (C and D, respectively). Microcapsules were cross-linked for 1 hr with 30 ml GAST (A, C and D) or for 3 hr with 15 ml of GAST (B).

내부물질의 방출은 theophylline이 함유된 미세캡슐의 모습에 영향을 주는가를 알아본 결과 Fig 2C와 D에 나타난 것 처럼, 미세캡슐의 외부구조는 내부물질이 방출될 때의 모습과 처음 캡슐이 제조된 직후의 모습과 별 차이가 없었다. 이러한 결과는 내부물질의 방출은 단백질 matrix의 분해나 용해에 의한 것보다는 캡슐을 통한 확산에 의한 것임을 입증하는 것이다.

Core 물질이 모두 방출된 후 캡슐 내부를 관찰한 결과 내부구조가 그대로 유지되어 있어 theophylline이 확산에 의하여 유리된 결과를 확인 할 수 있었다.

전반적인 내부물질의 함량은 49.5~52.5%로 나타났고 보존 효율(retention efficiency)은 73.5~78%로 각각 나타났다. Cross-limking 조건에 영향을 받지 않는 것으로 확인 되었다. 이러한 결과로 볼 때 유청단백질을 이용한 미세캡슐 공정은 매우 높은 효율을 지님을 알 수 있었고 내부물질의 손실은 주로 수세 과정에서 일어나는 것으로 밝혀졌다.

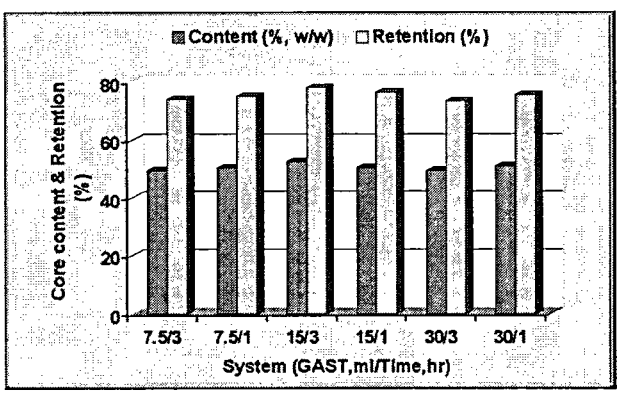

Fig. 3. Theophylline content and core retention efficiency in microcapsules prepared at different cross-linking conditions (7, 15, and 30 ml of GAST and reaction time for 1 or 3 hr).

미세캡슐에서 Theophylline의 방출

Fig. 4와 5는 37C에서 미세캡슐이 SIF (simulated intestinal fluid; 인공장액)와 SGF(simulated gastric fluid; 인공위액)에 대한 theophylline의 방출을 나타낸 것이다. 예비실험에서 GAST가 결합된 유청단백질 캡슐은 SIF, SGF에서 용해되지 않는 것으로 나타났는데, Theophylline의 방출은 위에서 설명한 대로 protein-matrix의 분해가 아니라 확산 mechanism에 의한 것이다.

미세캡슐의 크기를 다양하게 제조하여 내부물질의 방출정도를 비교한 결과, 시간에 영향을 받으며 인공소화액의 종류, cross-linking 조건, 캡슐의 크기에 따라 방출정도가 다르게 나타났다.

모든 경우에 있어서 SGF 보다 SIF가 더 높게 나타났는데, 유청단백질 캡슐은 위장 보다는 소장이나 대장에서 더 잘 방출되는 것을 의미한다. 따라서 유청단백질을 이용한 미세캡슐은 소장이나 대장을 taget으로 하는 약물전달 시스템에 매우 적합하다.

방출 정도는 cross-linking density가 적을수록 증가되는 것으로 나타났고 캡슐 크기가 큰 것보다 작은 캡슐이 더 방출 속도가 빠른 것으로 확인되었다.

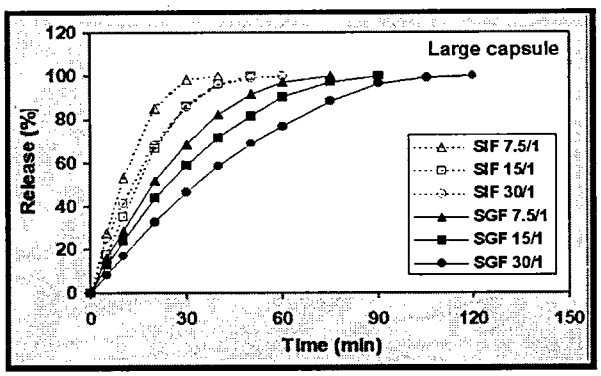

Fig. 4. *In vitro* core release from large microcapsules cross-linked with 7.5, 15 and 30 ml of GAST for 1 hr into enzyme-free SIF and SGF at 37°C.

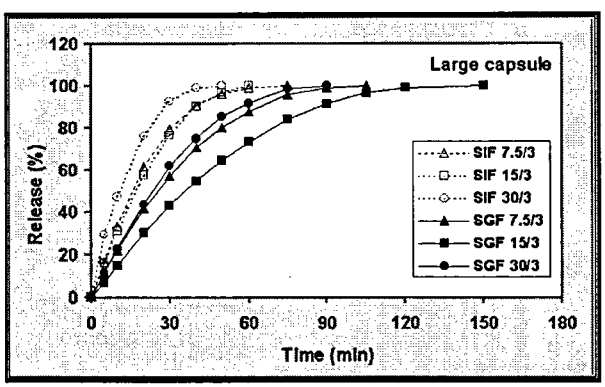

Fig. 5. *In vitro* core release from large microcapsules cross-linked with 7.5, 15 and 30 ml of GAST for 3 hr into enzyme-free SIF and SGF at 37°C.

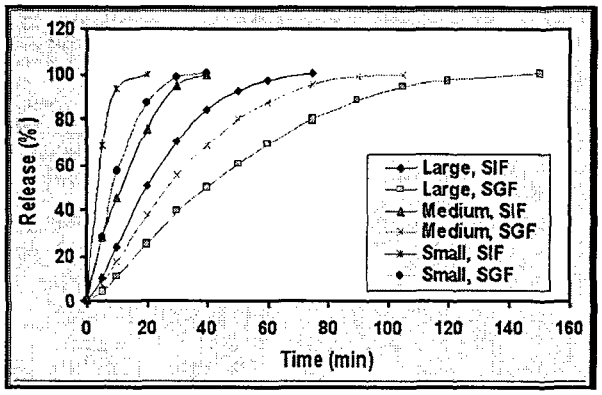

Fig. 6. *In vitro* core release from large, medium and small microcapsules cross-linked with 15 ml of GAST for 3 hr into enzyme-free SIF and SGF at 37°C.

유지방(anhydrous milk fat; AMF)이 함유된 캡슐의 구조

미세캡슐은 pH 7.2의 유화상태에서 제조되는데 직경이 10~100 um의 다양한 크기가 구형으로 제조되었다. WPI(whey protein isolate)나 AMF의 농도와 관계없이 움푹 드러간 자국이 없었고 매끄러운 표면을 지닌 미세캡슐이 제조되었으나 일부의 표면에 구형의 기공이 있었다. 이러한 구형 기공의 평균 직경이 0.2~0.4μm인 사실로부터 비추어 볼 때에 미세캡슐 제조공정 중에 미세캡슐의 표면에 부착되어 있던 내부물질이 손실되어서 구형 기공이 생성된 것으로 보인다.

pH 4.5 또는 5.5 emulsion에서 캡슐을 제조하여 구조를 관찰한 결과 pH 7.2 emulsion에서 제조된 것과 매우 차이가 있었다. 캡슐 표면은 다공성의 구조와 주름이 많이 있었으며 내부 안쪽에는 크기가 비교적 큰 단백질 응고물 등이 있었다 (Fig. 7). 미세캡슐 제조 중에 연속적인 단백질로 된 막구조가 형성되기 보다는 단백질 응고물이 형성되었음을 알 수 있었다. 이는 낮은 pH가 단백질의 상호작용에 영향을 미치고 β-lactoglobulin의 분자간 결합이 pH에 영향을 받기 때문이다.

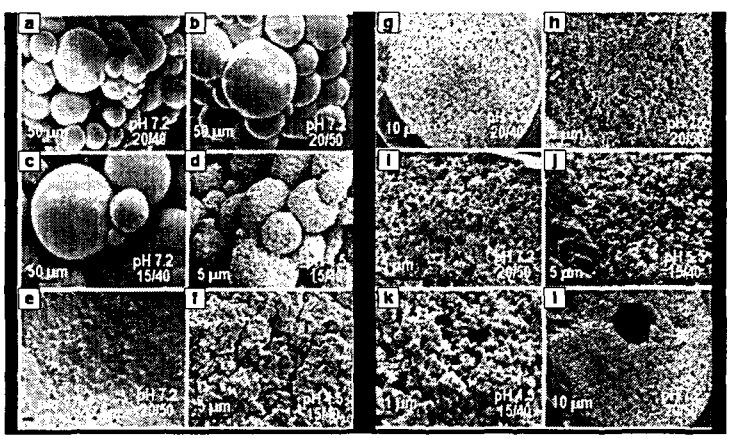

Fig. 7. Outer and inner structures of microcapsules prepared from core-in-wall emulsions (15/40, 20/40, 20/50) with pH 4.5, 5.5 and 7.2.

유지방 함유 미세캡슐의 내부물질의 보존(core retention)

미세캡슐화 공정 중에 내부물질의 보존 정도를 높이는 노력은 전체적인 미세캡슐화의 수율을 높이는 것이기 때문에 매우 중요하다. 유청단백질을 이용한 경우 모든 실험에서 88% 이상의 내부물질 보존 효과가 나타났다.

캡슐에서 내부물질을 효과적으로 보존하기 위하여 15%의 농축 유청단백질을 함유하는 emulsion을 이용하였고 pH는 여러 단계에서 제조하여 관찰한 결과 pH 4.5에서 96.2~98.7%, pH 5.5에서 96.6~98.4%, pH 7.2에서 94.7~97.4%의 높은 수율을 나타내었다.

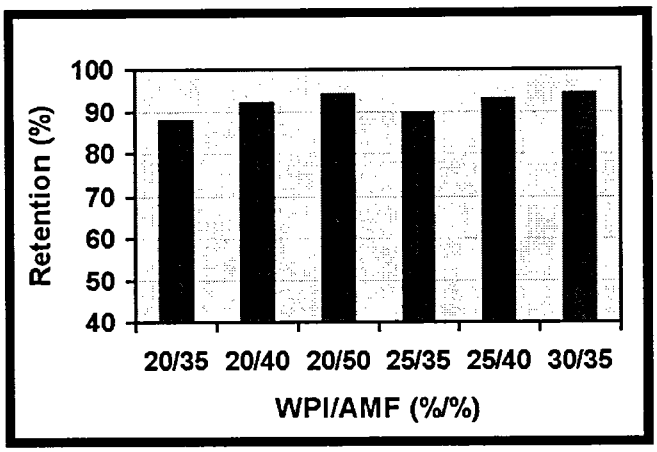

Fig. 8. Core retention in microcapsules prepared from pH 7.2 core-in-wall emulsions with WPI concentration ranging from 20 to 30g/100g and AMF load of 35-50g/100g on dry basis.

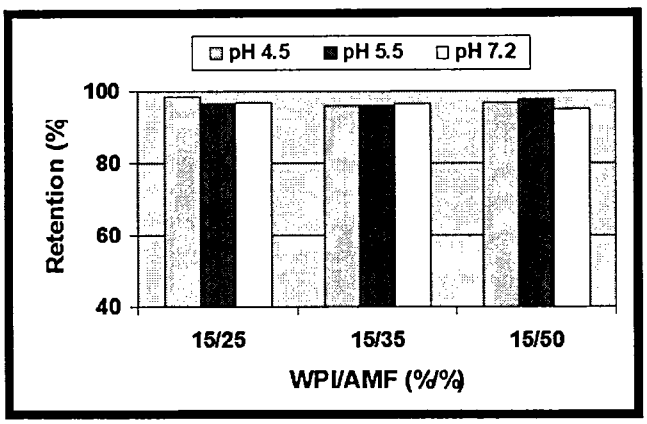

Fig. 9. Core retention in microcapsules prepared at different pH with core-in-wall emulsions containing 15g/100g WPI and 25-50g/100g (on dry basis) AMF.

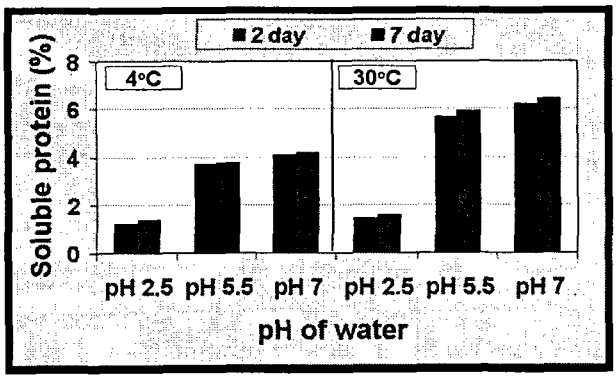

Fig. 10. Soluble protein (% of total protein) in microcapsules suspended in water at pH 2.5-7.0 and incubated at 4 and 30°C for 7 days. Microcapsules were prepared from pH 7.2 core-in-wall emulsion 20/50.

인 용 문 헌

1. Arshady, A. 1990. Albumin microspheres and microcapsules: methodology of manufacturing techniques. Journal of Controlled Release, 14:111-131.
2. Dziezak, J. D. 1988. Microencapsulation and encapsulated ingredients. Food Technology, April 136-151.
3. Gibbs, B. F., Kermasha, S., Alli, I. and Mulligan, C. N. 1999. Encapsulation in the food industry: a review. International Journal of Food Science and Nutrition, 50:213-224.
4. Gupta, P. K. and Hung, C. T. 1989. Review. Albumin microspheres II: applications in drug delivery. Journal of Microencapsulation, 6:463-472.
5. Karel, M. and Langer, R. 1988. Controlled release of food additives. In: Risch, S. J. and Reineccius, G. A. (Eds), *Flavor Encapsulation*, ACS Symposium Series No. 370. Washington, DC: American Chemical Society, pp. 177-191.
6. Langer, L. 1989. Biomaterials in controlled drug delivery: new perspectives from biotechnological advances. Pharmaceutical Technology, 13:18-30.
7. Latha, M. S., Rathinam, K., Mohanan, P. V. and Jayakrishnan, A. 1995. Bioavailability of theophylline from glutaraldehyde cross-linked microspheres in rabbits following oral administration. Journal of Controlled Release, 34:1-7.
8. Levy, M. C. and Andry, M. C. 1990. An evaluation of gelatin microcapsules prepared using interfacial cross-linking process. Life Science Advances. 19:219-227.
9. Levy, M.C. and Andry, M. C. 1991. Mixed-walled microcapsules made of cross-linked proteins and polysaccharides: preparation and properties. Journal of Microencapsulation, 8: 335-347.
10. Longo, E. W., Iwata, H., Lindheimer, A. and Goldberg, E. P. 1982. Preparation of hydrophilic albumin microspheres using polymeric dispersing agents. Journal of

Pharmaceutical Sciences, 71:1323-1328.

11. McNamee, B. F., O'Riordan, E. D. and O'Sullivan, M. 1998. Emulsification and Microencapsulation Properties of Gum Arabic. Journal of Agricultural and Food Chemistry, 46(11):4551-4555.
12. Moreau, D. L. and Rosenberg, M. 1993. Microstructure and fat extractability in microcapsules based on whey proteins or mixtures of whey proteins and lactose. Food Structure, 12:457-468.
13. Pothakamury, U. R. and Barbosa Canovas, G V. 1995. Fundamental aspects of controlled release in foods. Trends in Food Science and Technology, 6:397-406.
14. Rosenberg, M. 1997. Milk derived whey protein-based microencapsulating agents and a method of use, US Patent 5,601,760, February 11.
15. Rosenberg, M., Kopelman, I. J. and Talman, Y. 1985. A scanning electron microscopy study of microencapsulation. Journal of Food Science, 50:139-144.
16. Rosenberg, M. and Sheu, T. Y. 1996. Microencapsulation of volatiles by spray-drying in whey protein-based wall systems. International Dairy Journal, 6:273-284.
17. Rosenberg, M. and Young, S. L. 1993. Whey proteins as microencapsulating agents. Microencapsulation of anhydrous milkfat - Structure evaluation. Food Structure, 12:31-41.
18. Saleh, A. M., El-Kordagui, L. K., Robb, D. A. and Florence, A. T. 1989. Effect of some drugs and additives on the cross-linking of bovine serum albumin by glutaraldehyde. International Journal of Pharmaceutics, 57:205-210.
19. Schmitt, C., Sanchez, C., Desobry-Banon, S. and Hardy, J. 1998. Structure and technofunctional properties of protein-polysaccharide complexes: a review. Critical Review in Food Science and Nutrition, 38(8):689-753.
20. Schrooyen, P. M. M., Van der Meer, R. and De Kruif, C. G. 2001. Microencapsulation: its application in nutrition. Proceedings of the Nutrition Society, 60:475-479.
21. Shahidi, F. and Han, X. Q. 1993. Encapsulation of food ingredients. Critical Review in Food Science and Nutrition. 33(6):501-547.