# 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°C. 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°C) to form an O/W/O double emulsion and then heated at 85°C 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.

## I. 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

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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 mech-

anical 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 micro- capsules 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.

## Ⅱ. EXPERIMENTAL

### 1. 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.

### 2. 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.

### 3. 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.

### 4. Preparation of anhydrous milk fat loaded microcapsules

Microcapsules were prepared using a process consisting of double emulsification and heat gelation. Wall solutions containing 15-30 g/100 g 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℃, 6.7 kPa, 12 hr) and then stored in a desiccator pending analysis.

## 5. 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.).

## 6. 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.

## 7. 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.

## 8. 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).

# Ⅲ. SUMMARY OF RESULTS

## 1. 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



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).

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.

• 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.

2. Theophylline release from microcapsules

Release of theophylline from microcapsules into SIF and SGF at 37°C is shown in Figs 4, 5 and 6. Preliminary studies indicated that the GAST-
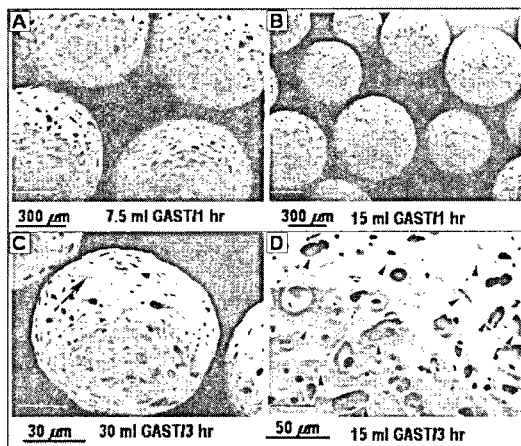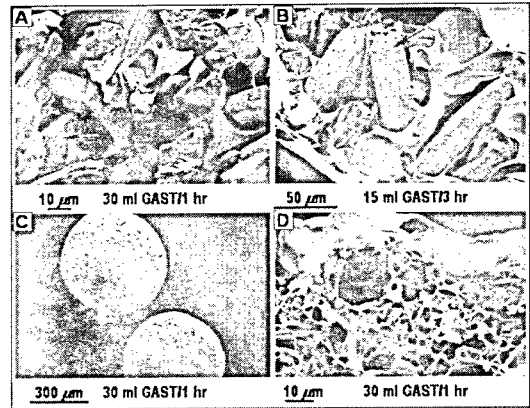


Fig. 1. Outer structure of theophylline-containing WPI microcapsules cross-linked for 1 hr with 7.5 or 15 ml of GAST (A and B, respectively) and for 3 hr with 15 or 30 ml GAST (D, C, respectively).

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.
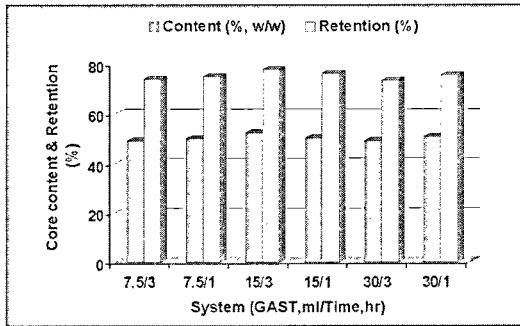


Fig. 3. Theophylline content and core retention efficiency in microcapsules prepared at different cross-linking conditions (7, 15, and 30 ml of GAST and reaction time for 1 or 3 hr).
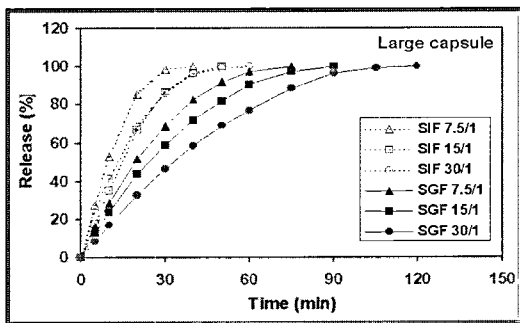


Fig. 4. *In vitro* core release from large micro-capsules cross-linked with 7.5, 15 and 30 ml of GAST for 1 hr into enzyme-free SIF and SGF at 37°C.

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.
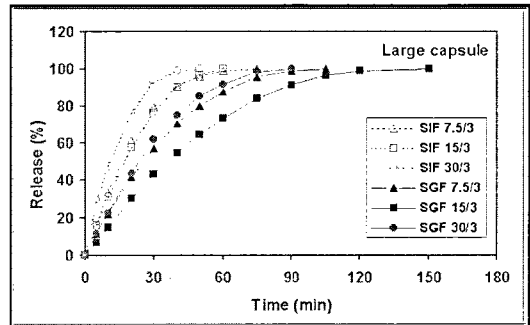


Fig. 5. *In vitro* core release from large micro-capsules cross-linked with 7.5, 15 and 30 ml of GAST for 3 hr into enzyme-free SIF and SGF at 37°C.
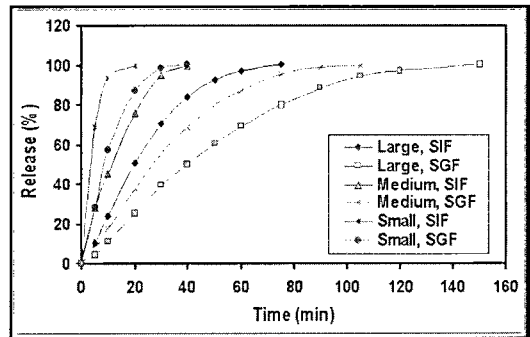


Fig. 6. *In vitro* core release from large, medium and small microcapsules cross-linked with 15 ml of GAST for 3 hr into enzyme-free SIF and SGF at 37°C.

## 3. 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 0.4 um) suggested that they represented footprints of core droplets that were originally present at the surface and were lost
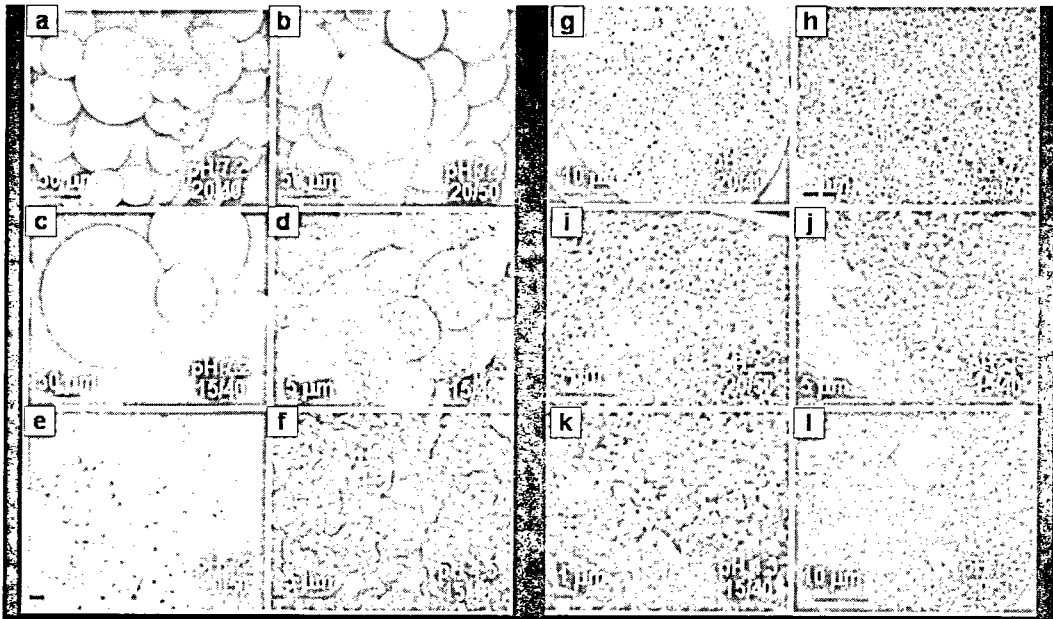
Fig. 7. Outer and inner structures of microcapsules prepared from core-in-wall emulsions (15/40, 20/40, 20/50) with pH 4.5, 5.5 and 7.2.

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.

## 4. 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
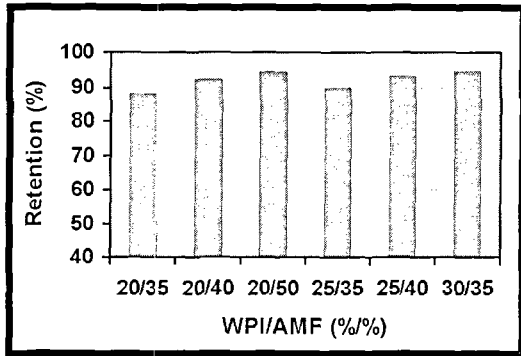
Fig. 8. Core retention in microcapsules prepared from pH 7.2 core-in-wall emulsions with WPI concentration ranging from 20 to 30g/100g and AMF load of 35-50g/100g on dry basis.
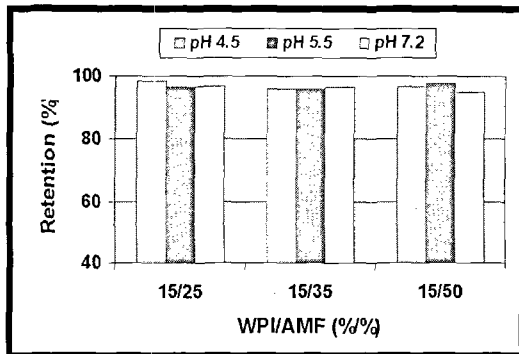


Fig. 9. Core retention in microcapsules prepared at different pH with core-in-wall emulsions containing 15 g/100 g WPI and 25-50 g/100 g (on dry basis) AMF.

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.

## 5. 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.
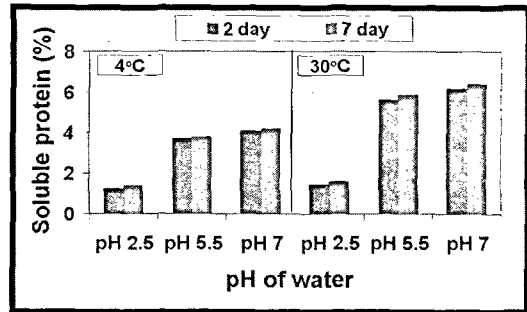


Fig. 10. Soluble protein (% of total protein) in microcapsules suspended in water at pH 2.5-7.0 and incubated at 4 and 30°C for 7 days. Microcapsules were prepared from pH 7.2 core-in-wall emulsion 20/50.

## IV. 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.

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