Membrane Formation by Interfacial Cross-Linking of Chitosan for Microencapsulation of *Lactococcus lactis*

A. F. Groboillot,[1] C. P. Champagne,[2] G. D. Darling,[3] D. Poncelet,[4] and R. J. Neufeld[1]*

[1]*Department of Chemical Engineering, McGill University, Montreal, Quebec, H3A 2A7, Canada;* [2]*Food Research and Development Centre, Saint-Hyacinthe, Quebec, Canada;* [3]*Department of Chemistry, McGill University, Montreal, Quebec, Canada; and* [4]*Flamel Technologies, Vénissieux Cedex, France*

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Lactic acid bacteria were microencapsulated within cross-linked chitosan membranes formed by emulsification/interfacial polymerization. The technique was modified and optimized to provide biocompatible conditions during encapsulation involving the use of mineral oils as the continuous phase and chitosan as the membrane material. Chitosan cross-linked with hexamethylene diisocyanate or glutaraldehyde resulted in strong membranes, with a narrow size distribution about a mean diameter of 150 μm. Cell viability and activity was demonstrated by the acidification of milk. Loss of acidification activity during microencapsulation was recovered in subsequent fermentations to levels similar to that of free cell fermentations. © 1993 John Wiley & Sons, Inc.

Key words: chitosan microcapsules • lactic acid bacteria, microencapsulation • interfacial cross-linking

INTRODUCTION

Immobilization of lactic acid bacteria may provide improved productivity in dairy fermentations due to retention and protection of the cells and through the recovery and reuse of the starter cultures. Among immobilization methods, cell entrapment in gelled biopolymer is commonly used with κ-carrageenan or Ca–alginate as matrix. The main advantage of gel immobilization is the biocompatibility; although large-scale formulation is difficult, the beads are often permeable to cells[11], mass transfer limitations are often encountered, and the reactor volume occupied by the beads is generally significant. Finally, alginate gels containing lactic acid bacteria tend to be liquefied by lactic acid.[27]

In order to reduce mass transfer effects, the poly-L-lysine (PLL) membrane coating of alginate beads followed by liquefaction of the alginate core was demonstrated by Lim[14] in a multistage process. Leakage of lactic cultures from the matrix was still evident in the case of PLL–alginate beads.[1] In continuous manufacture of yogurt, some cell release is desirable for the continuous inoculation of the final product. However, to acidify cream for the production of cottage cheese dressing and in the manufacture of cultured butter, loss of cells from the immobilized starter may result in continued acidification during storage.[2]

Microcapsule formation by emulsification/interfacial polymerization results in liquid droplets envelopped within a strong, ultrathin, semipermeable polymeric membrane. Compared to the gels, the membrane should have the potential to retain cells, minimize mass transfer limitations,[22] and better protect the cells against phage contamination.[28] Microencapsulation should also reduce the volume occupied by the immobilized cells. Whereas nylon membrane microcapsules have been applied to enzyme immobilization,[5,10] applications in live cell immobilization have been overlooked due to toxicity of the reagents and harsh conditions of encapsulation. As an example, lactic acid bacteria microencapsulated within nylon or cross-linked polyethyleneimine membranes[12] were either nonviable or inactive. More biocompatible materials and formulation conditions may reduce toxic effects while providing thin but resistant membranes. As examples, membranes constructed of cross-linked polysaccharide[4] or protein[25] have been used to immobilize enzymes.

The present study involves microencapsulation within chitosan, a positively charged linear polysaccharide formed by deacetylation of chitin[15] (Fig. 1). Chitosan, which is water soluble below pH 6, is used in food technology[8] and for immobilization of plant cells in gel form.[7] Microspheres have been produced by an emulsification method in soya oil to encapsulate cisplatin within chitosan cross-linked with glutaraldehyde.[7] The objective of the present study was to microencapsulate lactic acid bacteria within a cross-linked chitosan membrane (Fig. 1) formed at the oil–water interface. Biocompatible reagents were used together with oil-soluble cross-linking agents at low concentration to minimize cell contact.

MATERIALS AND METHODS

Chitosan Solution

Chitosan (Protan, Portsmouth, NH; Novachem, Halifax NS Canada; Sigma, St. Louis, MO) was dissolved (2 to 6 g) in

* To whom all correspondence should be addressed.

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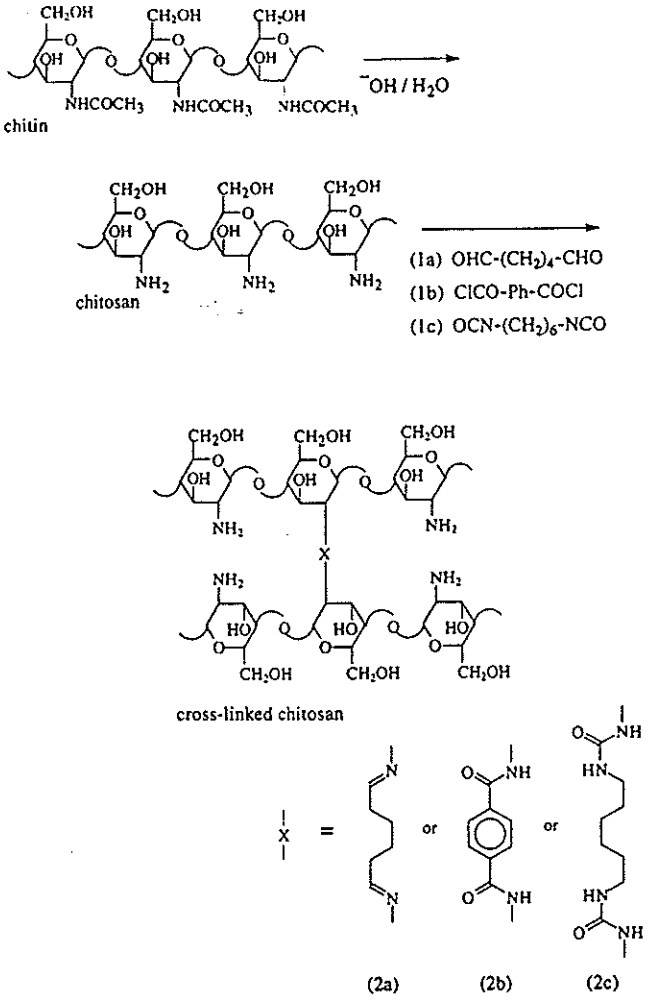

Figure 1. Deacetylation of chitin to chitosan and the subsequent cross-linking with different bifunctional agents: (1a) glutaraldehyde (GA), (1b) terephthaloyl chloride (TC), (1c) hexamethylene diisocyanate (HDI). Structures (2a), (2b), and (2c) are formed when GA, TC, and HDI are respectively used to cross-link chitosan.

0.35 to 1.05 *M* acetic acid solution, buffered with 10 mL of 0.9 *N* sodium acetate, and the pH was set to 5.6 with 5 *N* NaOH. The final volume was adjusted to 100 mL, and the solids were removed by filtration (Whatman No. 1 filter paper).

Lactic Acid Bacteria

Lactococcus lactis cremoris was maintained on slants of Elliker broth (Difco) with 15 g/L Bacto-agar (Difco). Inoculum was prepared by transfer from slant to 50 mL Elliker broth for 48 h (23°C), then to 1 mL to 100 mL Elliker broth for 22 h (23°C). Cells were then centrifuged at 9500*g* for 15 min, washed with 0.1 g/L peptone water (Difco), and recentrifuged. A pellet of about 0.5 g (wet weight) and containing 5×10^{10} colony forming units (CFU) is obtained.

Microencapsulation

Ten milliliters of 4% chitosan solution or chitosan–cell suspension (cell pellet mixed into 10 mL of 4% chitosan solution) was dispersed into 50 mL of organic phase facilitated by 1 mL of sorbitan trioleate (Span 85 from Atkemics, Brandford, Ontario). The continuous organic phase consisted of cyclohexane (American Chemicals, Montreal), sunflower seed oil (Queen), canola oil (Crisco), light mineral oil (American Chemicals, Montreal), Carnation mineral oil, or Klearol mineral oil (Witco, New York). The emulsion was formed in a 150-mL reactor by stirring at 200 rpm with a frame lattice impeller[21] for 5 min with cyclohexane or 1 min with the various oils.

Membrane formation was initiated by the addition of 0.6 g terephthaloyl chloride (TC; Aldrich), 0.3 mL of glutaraldehyde (GA; 25 wt % solution in water, Aldrich), or 0.5 mL hexamethylene diisocyanate (HDI; American Chemicals, Montreal) mixed with 10 mL of the respective continuous phase. The reaction times were 3 min for terephthaloyl chloride or glutaraldehyde and 15 min for hexamethylene diisocyanate. The reaction was stopped by dilution with 50 mL of the respective organic or oil phase. In the case of glutaraldehyde, *p*-aminohippuric acid (Sigma; 2 g/mL of glutaraldehyde) mixed with 10 mL of continuous phase was also added at this point.

Microcapsules were recovered by settling and decantation or by centrifugation, washed with 50% polysorbate (Tween 20 from American Chemical, Montreal) and either sodium chloride (0.9 g/L) or peptone (1 g/L) solution, and filtered on Whatman No. 1 filter paper.

Fermentation

Fermentations were performed in thermostatted (30°C), agitated bioreactors (marine impeller, 75 rpm) containing 100 mL sterilized (121°C, 8 min) reconstituted skim milk (Agropur) at 9% solids. The microencapsules containing cells were either added directly to milk after formulation or were first incubated 18 h at 23°C in 100 mL Elliker broth prior to incubation. The inoculation level is equivalent to 5×10^8 CFU/mL assuming 100% full cell viability following microencapsulation. After clearing the curdled milk with sodium citrate (0.5 g/L) and adjusting the pH to 8, microcapsules were recovered by filtration (Whatman filter No. 1). The filtered microcapsules were washed with peptone water before reuse for fermentation on fresh milk.

Analysis and Measurements

Viscosity of dissolved chitosan and oil was measured with a Brookfield digital LVTDV-1 viscometer using a UL adapter for small samples.

The volumetric size distribution of microcapsules was determined by laser light scattering (Malvern Instrument, series 2600C). The diameter corresponding to the highest frequency was used as mean diameter (d_m).

The relative membrane toughness was evaluated as the time (τ) to break all the microcapsules with a diameter higher than 50 μm in a 15-mL microcapsule suspension using a tissue homogenizer (SDT Tissumizer with shaft SDT100EN, Cincinnati, OH).

Acidification of milk by lactic bacteria was monitored by pH measurement. The time $t_{5.5}$ required to reduce the pH from 6.5 to 5.5 was determined to measure the activity of the free or encapsulated bacteria.

The bacteria concentration expressed in colony forming units per milliliter (CFU/mL) of milk or medium was determined by successive dilutions in peptone water and cell plate count conducted on Elliker broth with 15 g/L Bacto-agar.

RESULTS

Optimization of Microencapsulation Procedure

Microencapsulation by interfacial cross-linking of chitosan was optimized to achieve gentle formulation conditions, as evidenced by cell viability and activity, and to obtain strong and spherical microcapsules. The effects of the type of chitosan, solvent, and cross-linking agent were evaluated on microcapsule characteristics: diameter, ease of recovery, and strength. Regarding chitosan, data are only presented for Protan Seacure 123 low viscosity, chitosan because microcapsules could not be achieved with Sigma, Novachem, or Protan Seacure 123 high viscosity chitosan.

Bimodal size distributions were evident, as seen in Figure 2 for two different types of mineral oils as the organic phase. Irrespective of the cross-linking agent, the higher viscosity oil resulted in smaller diameters and size distributions (Table I). For example, microcapsules cross-linked with GA had a mean diameter of 220 μm when formed in sunflower seed oil (63 cP) and 120 μm for A&C mineral oil (68 cP), compared with 465 μm for Klearol mineral oil (12.5 cP).

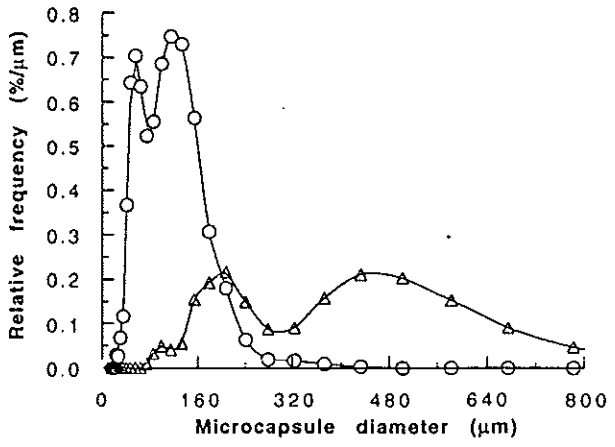

Figure 2. Volumetric size distribution of chitosan/HDI microcapsules formed in A&C mineral oil (O) and Klearol mineral oil (Δ). The initial concentration of chitosan was 4%.

Table I. Organic phases tested and their influence on microcapsule size and toughness.[a]

Type of organic phase	Viscosity at 23°C (cP)	Microcapsule size (μm)	Relative strength (min)
Cyclohexane	1		
Sunflower seed oil	63		
Canola oil	58		
Mineral oil (A&C)	68	123	11.2
Carnation mineral oil (Witco)	20	400	
Klearol mineral oil (Witco)	12.5	465	4

[a] Microcapsules were produced with HDI (0.5 mL) as cross-linking agent and 4% Protan (Seacure 123) chitosan solution.

By increasing the chitosan concentration, the viscosity of the discontinuous phase was enhanced, resulting in larger microcapsule diameter (Table II). Depending on the difference of viscosity between the aqueous and organic phases, mean diameters of the microcapsules covered a broad range from 100 μm to 1 mm. The chitosan microcapsules were osmotically sensitive, as other solutes resulted in a large variation in diameter. As an example, 120-μm chitosan microcapsules decreased in diameter to 70 μm when added to milk.

Microcapsule recovery after membrane formation was facilitated by the use of lower viscosity organic solvents such as cyclohexane. However, more viscous solvents such as vegetable or mineral oils were preferable because of improved biocompatibility. Microcapsules formed from a 4% chitosan solution (60 cP) dispersed within low viscosity oils (Klearol or Carnation mineral oil) could be decanted from the continuous phase within a short period of time (2 min). More viscous oils (A&C mineral oil or sunflower seed oil, 68 and 63 cP, respectively) prolonged separation and often required centrifugation.

Microcapsules produced with a 2% chitosan solution (10 cP) were difficult to separate from A&C mineral oil (68 cP) and from Klearol mineral oil (12.5 cP). Thus oil viscosities lower than or equal to that of the chitosan solution were necessary to recover the microcapsules, a phenomenon that appeared to be related to the size of the microcapsules. When the mean diameter was higher

Table II. Mean diameter and strength of microcapsules provided with varying concentrations of Protan Seacure 123 (low viscosity) chitosan.[a]

Concentration (%)	Viscosity at 23°C (cP)	Microcapsule size (μm)	Relative strength (min)
1	5	no microcapsule	
2	10	220	2
4	60	540	5
6	256	620	1

[a] The microcapsules were produced with glutaraldehyde (0.3 mL) as cross-linking agent in Witco Klearol mineral oil.

than 250 μm, the separation of the microcapsules from the oil phase was largely facilitated. In a comparison of oils with similar viscosity to that of the chitosan solution, A&C mineral oil permitted a better recovery than sunflower seed or canola oil in the case of HDI. With nonfresh vegetable oil, the recovery became impossible when chitosan was cross-linked with HDI. Thus, the choice of cross-linking agent also affected the ease of separation, with GA or TC cross-linked membranes being easier to decant then those formed with HDI.

Low molecular weight chitosan (Protan, Seacure 123) provided stronger membranes since higher concentrations were possible for the same low viscosity. Low chitosan concentration resulted in fragile membranes and high solution viscosity with increasing chitosan concentrations produced brittle microcapsules (Table II). A 4% concentration resulted in the optimum balance between solution viscosity and membrane strength. Values of membrane toughness τ varied from 4 to 11 min for chitosan/HDI microcapsules formed in Klearol mineral oil and A&C mineral oil, respectively (Table I). This difference was related to microcapsule diameters which varied with differences in viscosity of the aqueous and oil phases. The influence of the type of cross-linking agent was also observed on τ: 10 or 11 min for chitosan/HDI or chitosan/GA and 5 min for chitosan/TC (Table III). Thus, the strongest membranes were formed when cross-linked with GA or HDI.

Fermentation with Encapsulated Lactic Acid Bacteria

The time to reduce milk pH to 5.5 ($t_{5.5}$) is commonly used to assess the acidification activity of lactic cultures. The $t_{5.5}$ values (mean of two replicates) were 12.5, 16.5, and 19.5 h for cells encapsulated within chitosan cross-linked with TC, GA, and HDI, respectively. The onset of acidification by encapsulated cells was observed after 10 h of fermentation following microencapsulation resulting in an extended acidification period compared to that of a similar concentration of nonencapsulated cells ($t_{5.5} \approx 2.5$ h).

The $t_{5.5}$ was also determined for nonencapsulated cells ranging in concentration from 10^4 to 10^9 CFU/mL of milk. An extended initial delay period was observed at low cell concentration and a linear relationship was obtained between $t_{5.5}$ and the initial cell concentration (X_0) plotted on a logarithmic scale ($r = 0.99$):

$$t_{5.5} = 22.5 - 2.32 \times \log X_0$$

In comparison, the encapsulated lactic cultures demonstrated an activity equivalent or lower than 10^4 CFU/mL, representing a decrease in viability of at least 4 log levels.

Acidification activity was seen to increase following incubation of the microencapsulated cultures or during successive fermentations. Microcapsules cross-linked with HDI resulted in $t_{5.5}$ values of 13, 5.5, and 4.5 h for the first, second, and third fermentations, respectively, following an initial incubation on Elliker broth (Fig. 3). When cross-linked with GA, the $t_{5.5}$ values were 16.5 and 5.5 h for the first and second fermentations, respectively, without preliminary incubation. Even though the initial delay period became shorter from one fermentation to the next, the slope of the linear drop in pH appeared to remain constant, as seen in Figure 3. The generation time estimated on the first fermentation of cells immobilized in HDI or GA microcapsules ranged between 1 h and 1 h 12 min.

The buffering ability of chitosan due to its amine groups may reduce the effect of pH reduction as determined in the activity assay. Chitosan solution added to milk, resulting in a final concentration of 0.04%, dampened the pH when titrated with lactic acid solution.

The influence of chitosan solution on the acidification activity of lactic bacteria was also examined. The pH of milk in a free cell fermentation dropped from 6.5 to 5.8 over 18 h of fermentation at 30°C when the chitosan concentration in milk was 0.04%. During the same period, the cell count dropped from 5×10^8 to 7×10^7 CFU/mL. In the absence of chitosan, the pH dropped to 5.5 within 2.5 h, concomitant with a cell increase to 1.5×10^9 CFU/mL. The pH value measured during free bacterial fermentations in the presence of chitosan were corrected by the buffering effect of chitosan measured

Table III. Types and proportions of cross-linked agent and reaction time tested to optimize the microencapsulation procedure.[a]

Type of cross-linking agent	Tested amount	Tested reaction time (min)	Microcapsule size (μm)	Relative strength (min)
Glutaraldehyde	0.1,0.2,0.3.0.4, 0.5,0.6 (mL) 0.1,0.2,0.3.	1,2,3,10	165	10
Terephthaloyl chloride	0.4,0.5,0.6, 0.7 (g)	1,2,3,5,6,10	123	5
Hexamethylene diisocyanate	0.25,0.5.1 (mL)	10,15,30	123	11.2

[a] The underlined values correspond to the optimum amount of cross-linking agent or reaction time. Microcapsules were produced with Protan (Seacure 123) chitosan solution and A&C mineral oil.

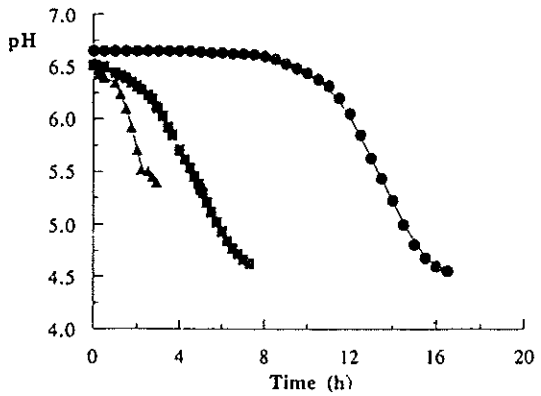

Figure 3. Milk acidification profile during successive fermentations with chitosan/HDI encapsulated cells during first (●), second (■) and third fermentations (▲). Cells were encapsulated in 4% chitosan solution cross-linked with 0.5 ml HDI in A&C mineral oil.

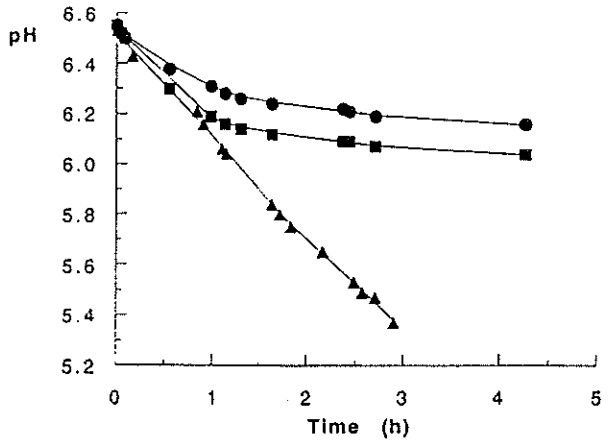

Figure 4. Acidification activity of lactic acid bacteria (▲), compared to that with a chitosan concentration in milk of 0.04% (●). The pH values were corrected (■), taking into account the buffering effect of the chitosan.

during the milk titration (Fig. 4). This correction did not permit recovery of the measured pH during fermentation without chitosan.

Chitosan/HDI microcapsules without cells were added to a free cell fermentation. Similar pH response curves to that of the control (free cell fermentation without microcapsules) indicated that the chitosan microcapsules did not buffer the solution and demonstrated also that the cross-linked chitosan membranes did not have an inhibitory effect on lactic acid bacterial activity and growth of free cells.

DISCUSSION

The objective of the present study was to microencapsulate lactococci by emulsification and interfacial cross-linking of the biopolymer chitosan. This is the first report of the membrane encapsulation of cells using an interfacial cross-linking technique. Benefits achieved in the formation of strong, resistant, cross-linked membranes may be offset by the harsh conditions encountered in cross-linking reactions. The interface was created by the dispersion of an aqueous solution or suspension of the reagent within an immiscible organic phase.

Solvents such as cyclohexane are often used as the organic solvent but are incompatible with live cells.[12] Replacement with biocompatible materials such as vegetable and mineral oils provides an interesting alternative, particularly for applications in biotechnology, medicine, and the food industry. Larisch et al.[12] demonstrated that mineral oils were nontoxic in contact tests with lactic acid cultures. The problem then is to find an effective yet nontoxic cross-linking agent. Lactic acid bacteria are not tolerant of sebacoyl chloride, a polymerization monomer used to form polyamide membranes.[12] Reduced concentrations of water-immiscible reactants may minimize toxic effects, due to reduced contact with the cells. Finally, it is necessary to ensure that the conditions for membrane formation fall within a pH range compatible with biological cells.

The optimization of the microencapsulation procedure involved compromises between the oil selected and the types and proportions of cross-linking agent and polymer. Microcapsules were preferred with mean diameters ranging from 200 to 500 μm in a narrow size distribution to facilitate recovery, and the membrane should be strong, retaining high levels of cell activity and retention.

The strong influence of the formulation conditions on the microcapsule diameter may provide a means to control this important parameter. Microcapsules with a mean diameter of 200 to 500 μm would result in minimal mass transfer limitations[16] and volume occupied by the encapsulation material, while facilitating recovery of the immobilized cells at the end of fermentation. A bimodal size distribution of the microcapsules was observed, contrary to the monomodal distribution generally obtained for microcapsules or beads produced by an emulsification technique.[21,25]

Due to the high viscosity of the oils and chitosan solutions, impeller rotational speeds greater than 200 rpm should promote a more homogeneous disruption of the droplets throughout the entire volume of the reactor, resulting in a narrower and unimodal diameter distribution. However, elevated mixing intensities may also lead to a reduction in mean diameter to levels which may not be suitable for many applications. Poncelet De Smet et al.[21] observed that a low concentration of span [1–2% (v/v)] for production of cellulose nitrate microcapsules resulted in a polymodal type distribution. In the case of chitosan membranes, span concentration was optimized at 2% in order to obtain spherical microcapsules and to facilitate the recovery step.

As the impeller speed was maintained at 200 rpm for all the experiments, the mean diameter of the microcapsules was mainly controlled by the difference of viscosity between the aqueous and organic phase. When the viscosity of the oil phase increased or when the viscosity of the aqueous phase decreased, the aqueous phase is more finely dispersed during the emulsion formation. The difference of viscosity affected the shear stress and shear rate applied on the liquid droplet. As the dispersion of 4% chitosan solution (60 cP) in A&C mineral oil (68 cP) resulted in a more uniform size distribution compared to that achieved with Klearol mineral oil (12.5 cP), the difference of viscosity between the two phases also affected the size dispersion. It appears that a more uniform dispersion may be obtained when the viscosities of the respective phases are similar.

Recovery of the microcapsules from the oil phase and subsequent washing procedures following membrane formation must be efficient and rapid to minimize the time of contact between the cells and the organic reagents. Chitosan itself is used in the food industry as a lipid-binding food additive.[6] Due to its affinity to oil, difficulties may be anticipated during microcapsule recovery. Decantation was easier when the oil was less viscous than the chitosan solution. Under these conditions, larger microcapsules were obtained facilitating decantation. The type of cross-linking agent and oil appeared to influence the ease of separation from the oil

due to the interactions between oil, cross-linking agent, and chitosan. In the case of nonfresh vegetable oils, oxydation of oil can result in formation of radicals susceptible to reaction with HDI.[13]

The strength of the membrane is also an important characteristic of the microcapsules, particularly if cell release is to be avoided. The toughness τ of the microcapsules depends upon the strength of the membrane and the diameter of the microcapsules. Larger diameters lead to more fragile microcapsules.[20] At mean diameters lower than 300 μm, the microcapsules were stronger. The strength of the membrane is related to chitosan concentration (Table II) and to the type of cross-linking agent. The membrane formed with 4% chitosan cross-linked with HDI or GA resulted in stronger microcapsules than with TC, probably because of a higher degree of cross-linking. The reaction of the bifunctional reagent with chitosan resulted in bridge formation linking the chitosan molecules (Fig. 1). The length of the bridge depends on the type of cross-linking agent. Longer and more flexible bridges as those formed with HDI enhanced reactivity with chitosan, in comparison to the shorter bridges formed with the other cross-linking agents (Fig. 1).

The reactivity between the cross-linking agent and chitosan is related also to the comparative speed of reaction of the cross-linking agent with water and with the amine group. In the case of glutaraldehyde, the reaction with water is reversible, resulting in better reactivity for GA than for TC. Also, the polycondensation reaction between TC and the amine group liberates hydrochloric acid, protonating the amino groups and reducing the amount of reactive NH_2 on the chitosan.

The viability and activity of the encapsulated cells were compared on the basis of acidification rates. Loss of cell viability following microencapsulation appeared significant, since the activity of freshly encapsulated cells corresponded to that of a small inoculum. Growth occurring within the microcapsules was apparent as the culture activity increased substantially with time. The activity was associated with the encapsulated cells as observed when the encapsulated cells were recovered and recycled. According to Cogan,[3] the generation time for *Lactococcus lactis* at 30°C in milk is 1 h 13 min, which agrees with the values found in the present study for microencapsulated cells. Significant improvements in cell viability and activity were demonstrated for cells encapsulated by interfacial cross-linking, in comparison to previously published work. Larisch et al.[12] did not observe acidification with encapsulated lactococci in nylon or polyethyleneimine membranes. Activity loss was due primarily to contact between cells and cross-linking agents or other reagents during microencapsulation. Also the more reactive cross-linking agents resulted in stronger membranes but reduced activity of the cells. Glutaraldehyde, due to its water solubility, may be potentially more toxic than less water-soluble reactants. However, in the present study, cells encapsulated within chitosan/GA were more active (reduced $t_{5.5}$), likely due to a shorter reaction and recovery time and lower concentration of reactive agent needed.

Free chitosan in solution appeared to dampen the activity response of cells due to its buffer capacity and inhibitory effect on lactic bacterial growth. However, with microcapsules it was found that the buffering capacity of cross-linked chitosan and that of residual free chitosan within the microcapsules is negligible. The growth inhibition may be due to binding of chitosan to the cells, as polycations generally exhibit antimicrobial properties.[23]

Yeast growth is also affected by chitosan.[9,24] Regarding lactic cultures, population reductions because of chitosan have been observed with both *Pediococcus*[9] and *Lactococcus*.[23] The sensitivity of the cells depends on the type of chitosan, acid or water soluble.[18,19] Chitosan affects transport mechanisms through the cell walls by binding membrane macromolecules but not the integrity of the cell wall itself.[23] Rha et al.[26] reported that *Bacillus subtilis* within chitosan–polyphosphate or chitosan–alginate gel beads exhibited 34% and 98% of the growth rate of free cells, respectively. The difference was attributed by the authors to transfer limitation in the case of chitosan–polyphosphate.

In the present study, it was seen that cross-linked chitosan did not inhibit cell growth, suggesting that only soluble chitosan is inhibitory. Residual soluble chitosan within the microcapsules is likely in low concentration or is absent, as higher activity was observed for encapsulated cells than for free cells in the presence of chitosan. This was confirmed by the fact that no buffer effect was observed with fermentation of free cells in the presence of uninoculated chitosan microcapsules. Finally, the type of cross-linking agent affected the encapsulated cells to a greater extent than that of chitosan on the observed decrease in cell activity.

CONCLUSION

Lactococcus lactis was microencapsulated by interfacial cross-linking of chitosan. The use of cross-linked chitosan membranes in microencapsulation was described, together with the first report of live cell microencapsulation by an interfacial cross-linking technique. Though loss of cell activity was observed following microencapsulation, activity was later recovered during successive fermentations to levels similar to that of free cells. Toxicity of the cross-linking agent reduced cell viability during encapsulation. A more biocompatible cross-linking agent may improve cell viability during membrane formation.

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