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Effect of Lecithin and Calcium Chloride Solution on the Microencapsulation Process Yield of Calcium Alginate Beads

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ABSTRACT

The physical protection by microencapsulation is a new method to increase the survival of probiotic bacteria. The size of beads containing probiotics has significant effect on organoleptic properties of foods. Reduction of the sphere size to less than 100 μm would be advantageous for texture considerations and allow direct addition of encapsulated probiotics to a large number of foods. In microencapsulation process, Tween 80 can be replaced by lecithin in order to prevent its detrimental effect on the viability of probiotic bacteria. Calcium alginate as an edible polymer was used for the microencapsulation of probiotic bacteria by emulsion technique. A modified microencapsulation method based on emulsion technique was investigated in this study to produce microcapsules with diameters below 100 μm . A completely randomized design (CRD) experiment was applied in triplicates to evaluate the effect of surfactant (0, 0.2, 0.4 and 1% lecithin with 0.2% Tween 80 as control) and calcium chloride solution (100 and 200 mL) on the microencapsulation process yield of microencapsulated *Lactobacillus casei*. Microsphere characterization was performed by scanning electron microscopy (SEM). The microencapsulation process yield increased with increasing the volume of calcium chloride solution and it decreased with increasing the amount of lecithin. The highest microencapsulation process yield was obtained when 0.2% Tween 80 was used as surfactant. The shape of beads was spherical and their mean diameter was $17.80 \pm 3.55 \mu\text{m}$.

Key Words:

encapsulation;
yield;
lecithin;
calcium chloride solution;
probiotic.

INTRODUCTION

Probiotic bacteria can be defined as live microorganisms which when administered in adequate amounts confer health benefit on the host [1]. The physical protection by microencapsulation is a new method to increase the survival of probiotic bacteria [2]. Encapsulation can isolate the bacterial cells

from the harsh environmental condition, thus potentially reducing cell loss. Other food-grade encapsulation materials, gellan-xanthan and alginate-starch mixtures, have also been proposed for encapsulation of probiotics in spheres with diameters between 1 and 3 mm [2,3]. Preliminary trials revealed

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that this sphere size is too large to allow direct incorporation in food products such as milk, yoghurt and sour cream, without adversely affecting the mouth feel. The reduction of the sphere size to less than 100 μm would be advantageous for texture considerations and allow direct addition of encapsulated probiotics to a large number of foods. Calcium alginate has been used extensively for the microencapsulation of lactic acid bacteria owing to its non-hazardous nature, ease of usage, and low cost [2-8]. The survival of *Lactobacillus casei* NCDC-298 in solution of colonic pH was increased with increasing alginate concentrations [9].

Alginate, a linear heteropolysaccharide of *D*-mannuronic and *L*-guluronic acid can be found in the cell walls and intercellular spaces of brown algae [10]. It provides both flexibility and strength to the plants. Alginic acid, the free acid from alginate, is the intermediate product in the commercial manufacture of alginates and has limited stability. In order to make stable water-soluble alginate products, alginic acid is transformed into a range of commercial alginates by incorporating different salts. This produces Na-, K-, NH_4 -, Mg-, and Ca- alginate [11]. Alginate includes a

variety of products made up of *D*-mannuronic acid and *L*-gluconic acid (Figure 1), which are arranged in regions composed solely of one unit or the other, referred to as M-blocks and G-blocks, and regions where the two units alternate [11]. Both the ratio of mannuronic acid to gluconic acid and the structure of the polymer determine the solution properties of the alginate. Monomeric M- and G-residues in alginates are joined together in sections consisting of homopolymeric M-blocks (MMMMM) and G-blocks (GGGGG) or heteropolymeric blocks of alternating M and G (MGMGMG). In the polymer chain, the monomers will tend to find their most energetically favourable structure. The rather bulky carboxylic group is responsible for a β -1,4 equatorial/equatorial glycosidic bond in M-M, and α -1,4 axial/axial glycosidic bond in G-G, and an equatorial/axial bond in M-G. The consequence of this is a buckled and stiff polymer in the G-block regions and a flexible ribbon-like polymer in the M-block regions. The MG-block regions have intermediate stiffness. Figure 2 shows the schematic representation of sodium alginate molecule $(\text{C}_6\text{H}_7\text{O}_6\text{Na})_n$ [12].

Carbohydrate polymers such as alginate have been

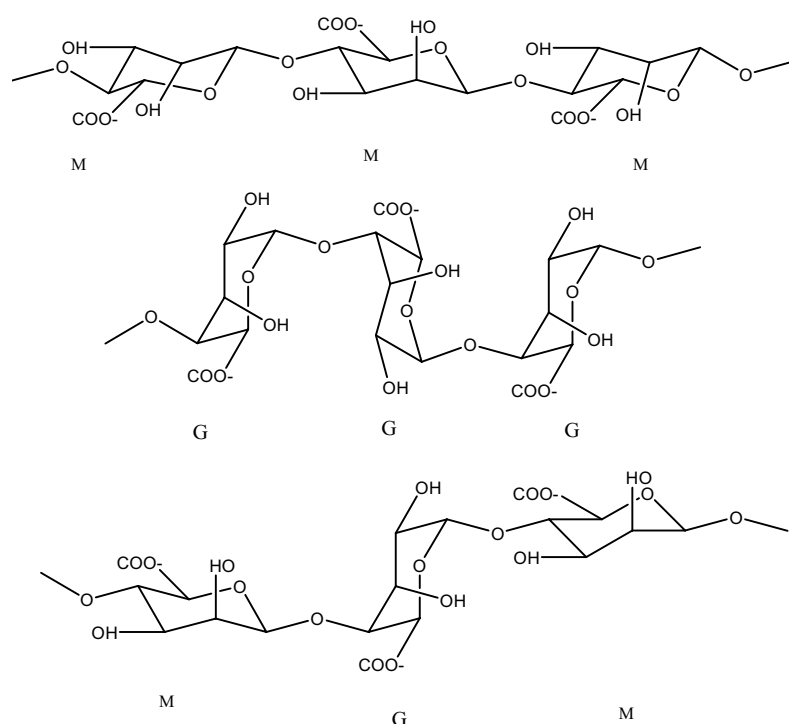


Figure 1. The structure of the chemical units of alginate (M = mannuronic acid and G = guluronic acid).

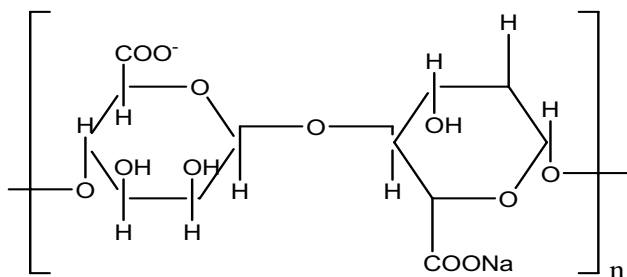


Figure 2. Chemical structure of the sodium alginate molecule.

used in various food applications [13]. For alginate capsules, a number of factors determine the internal structure, including intramolecular distribution and proportion of the guluronic and mannuronic acid residues, concentration and distribution of mono- and divalent cations and pH [14]. Alginates high in guluronic acid form stronger and more compact gels in the presence of Ca^{2+} , but at the same time more sensitive to fluctuations in calcium concentration than the weaker and yet more stable high-mannuronic acid alginate gels [15]. The alginate matrix stays structurally stable in low acid environments, however, as pH is lowered below the pK_a values of mannuronic and guluronic acid (3.6 and 3.7, respectively) alginate is converted to alginic acid with release of calcium ions and the formation of a more dense gel due to water loss [16].

Several methods have been developed for the encapsulation of probiotics. Alginate beads can be formed by both extrusion and emulsion methods [17]. Extrusion is the oldest method for manufacturing capsules with hydrocolloids. It simply involves preparing a hydrocolloid solution, adding microorganisms to it, and extruding the cell suspension through a syringe needle in the form of droplets to free-fall into calcium chloride solution. The size and shape of the beads depend on the diameter of the needle and the distance of free-fall, respectively. The size of the beads varies between 2-5 mm. In emulsion technique, a small volume of the cell-polymer suspension is added to a large volume of a vegetable oil such as soybean oil, sunflower oil, canola oil or corn oil. The mixture is homogenized to form a water-in-oil emulsion. Once the water-in-oil emulsion is formed, the water-soluble polymer must be insolubilized to form small gel par-

ticles within the oil phase. The smaller the internal phase particle size of the emulsion, the smaller the final microparticles would be. The insolubilization method of choice depends on the type of supporting material used. The size of the beads varies between 25 μm and 2 mm [17]. Emulsion technique is a suitable method for encapsulation of probiotic bacteria in the beads smaller than 1 mm [2,18]. The type and amount of surfactant as well as the volume of oil and calcium chloride solution can affect the microencapsulation process yield. The higher ratio of oil to alginate leads to increased bead recovery. It has, however, been suggested to use 2:1 ratio of oil to alginate in the microencapsulation of probiotic bacteria [2,18].

Tween 80 was reported to be used successfully as a surfactant in microencapsulation process [2,18] but it has detrimental effect on the viability of probiotic bacteria. There are yet no systematic reports on application of lecithin as an emulsifier for encapsulation of probiotics. The present paper reports the effect of varying concentrations of lecithin (0, 0.2, 0.4 and 1%) on microencapsulation process yield of alginate beads. This was done due to its non-hazardous nature for live microbial cells and low costs. A modified microencapsulation method based on emulsion technique was investigated in this study to produce microcapsules with diameters below 100 μm . The objectives of the present study were first to develop a method for producing alginate microspheres with viable probiotic bacteria in which diameters below 100 μm and, second, to optimize the microencapsulation process yield by using the varying amounts of lecithin and calcium chloride solution. For this purpose, emulsion technique was used for alginate bead production. In addition, the effect of surfactant type on the microencapsulation process yield was investigated.

EXPERIMENTAL

Bacteria and Growth Conditions

Pure culture of *Lactobacillus casei* was obtained from CHR-Hansen (Denmark) and was activated by inoculating in the MRS-broth (de Man-Rogosa-Sharpe) at 37°C for 24 h. The probiotic biomass in late-log phase

was harvested by centrifugation at 4500 rpm for 10 min at 4°C (Sorvall, model RC-5C, rotor GS-3, Newtown, CT, USA), washed twice in sterile 0.9% saline under the same centrifugation conditions, and used in the microencapsulation process.

Encapsulation Procedure and Yield

The beads produced with emulsion technique are smaller than those produced with extrusion technique. Smaller beads do not have detrimental effect on the mouth feel of food products. So, for producing the beads smaller than 100 µm in diameter, emulsion technique was used in this study. Alginate beads were produced using an encapsulation method which was a further development of the method first described by Sheu et al. [18]. The solutions and glassware required for microencapsulation process were sterilized at 121°C for 15 min. A mixture of 2% alginate (Merck), 2% Hi-maize resistant starch (Merck) and 0.1% culture was prepared. The mixture was added into 200 mL Canola oil (Ladan, Behshahr Oil Industry, Iran) containing 0.02% Tween 80 or 0, 0.2, 0.4 and 1% lecithin (Nazgol Oil Industry, Iran). The mixture was stirred vigorously (400 rpm for 20 min, Heydolph stirrer, Germany) till it was emulsified and appeared creamy. Then, 100 and 200 mL of 0.1 M calcium chloride solution were added quickly along the side of

the beaker, the phase separation of oil/water emulsion occurred. The mixture was allowed to stand 30 min to separate prepared calcium-alginate beads in the bottom of beaker at the calcium chloride layer. The oil layer was drained and beads were collected in calcium chloride solution, washed once with 0.9% saline containing 5% glycerol, and stored at 4°C. The microencapsulation process yield was measured as wet weight of calcium alginate beads (in gram) obtained from 100 g of alginate/starch/bacteria mixture and expressed in percent.

Microsphere Characterization

It is possible to determine the size distribution of the microcapsules by scanning electron microscopy and optical microscopy [19,20]. In this study the diameters of 120 randomly selected beads were measured with an eyepiece micrometer on an optical microscope at a magnification of 100×. The surface morphology of calcium alginate beads was investigated by a scanning electron microscope (SEM Model XL30, Philips, Poland). In this method unfixed samples were placed on the block and frozen in liquid nitrogen and after fracturing, it was coated with gold (sputter coater, Model SCDOOS, Bal-Tec, Switzerland) and examined at an accelerating voltage of 20.0 kV.

Table 1. Experimental design and mean microencapsulation process yield±SD of calcium alginate beads preparation.

Formulation code	Canola oil (mL)	CaCl ₂ 0.1 M (mL)	Tween 80 (%)	Lecithin (%)	Mean±SD microencapsulation process yield
F1	200	100	0.2	-	39.61±0.38*
F2	200	100	-	0.0	36.99±1.30*
F3	200	100	-	0.2	24.71±0.46*
F4	200	100	-	0.4	22.64±0.92*
F5	200	100	-	1.0	0±0.00*
F6	200	200	0.2	-	44.23±1.11*
F7	200	200	-	0.0	42.74±1.47*
F8	200	200	-	0.2	33.31±0.84*
F9	200	200	-	0.4	28.69±1.13*
F10	200	200	-	1.0	4.28±0.59*

(*) represents the statistically significant ($P \leq 0.01$) differences in the same column respectively according to Duncan's multiple range test.

Acidification Kinetics of Microencapsulated *Lactobacillus Casei*

Free and encapsulated cells of *L. casei* were cultivated in MRS broth to determine if the encapsulated cells were still metabolically active and if nutrients and metabolites could permeate bead wall. An experiment was performed comparing the time taken for free and encapsulated cells to acidify MRS broth medium. For that, 0.025 g of free cells and 0.025 g of encapsulated cells (in 10 g beads) were inoculated into the MRS broth medium and incubated at 37°C for 56 h. pH Values and OD (optical density at 525.2 nm wavelength) were measured every 4 h.

Statistical Analysis

Experimental design and formulations used for calci-

um alginate beads preparation are shown in Table 1. The collected data were analyzed by SAS [21]. The mean values and the standard deviation were calculated from the data obtained with triplicate trials. These data were then compared by the Duncan's multiple range method.

RESULTS AND DISCUSSION

Bead Formation and Physical Examination of Calcium Alginate Beads

The method introduced by Sheu et al. [18] was further developed to produce spherical alginate microspheres by controlling the diameter of alginate beads with stirring speed of emulsion. Vigorous stirring (400 rpm for

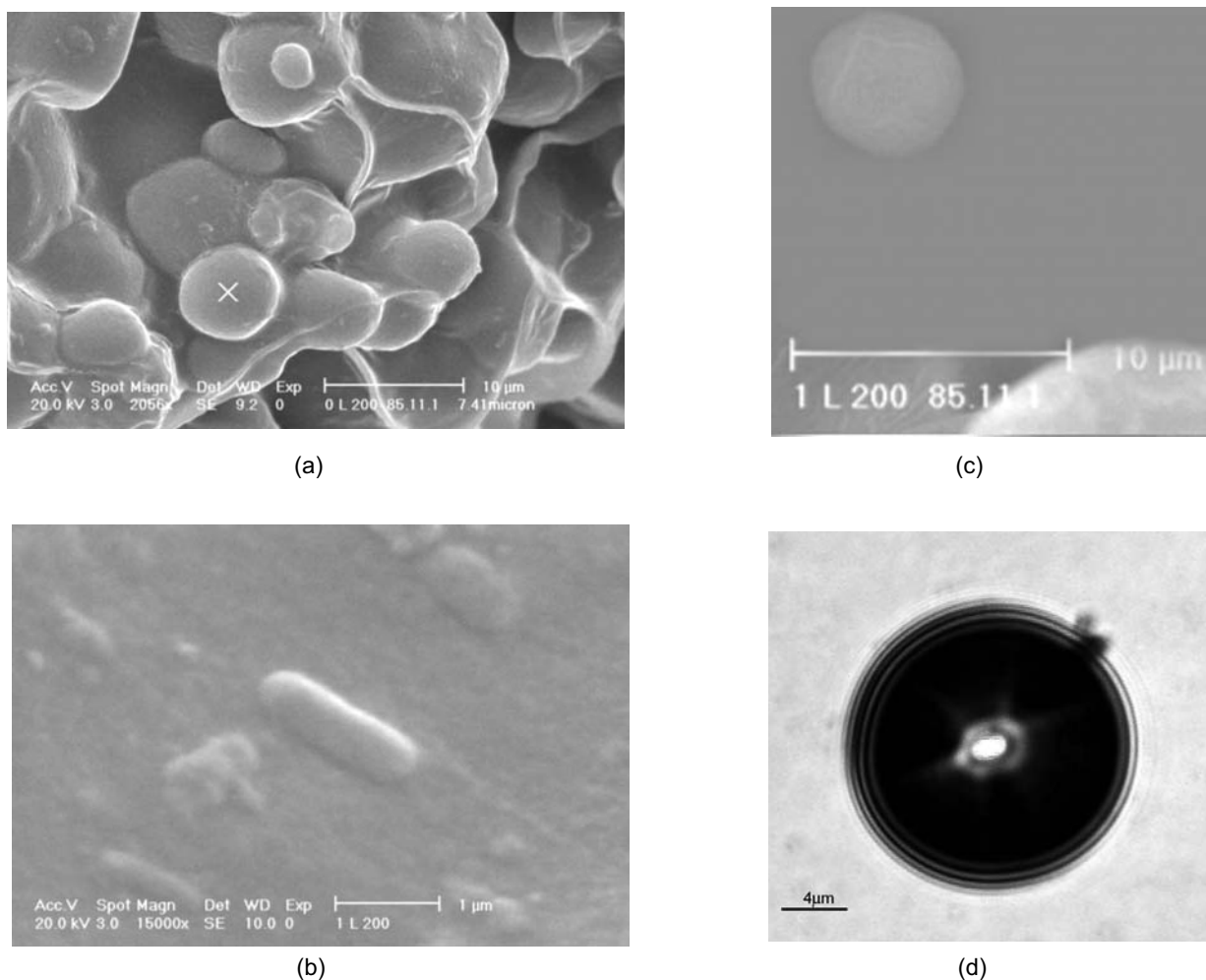
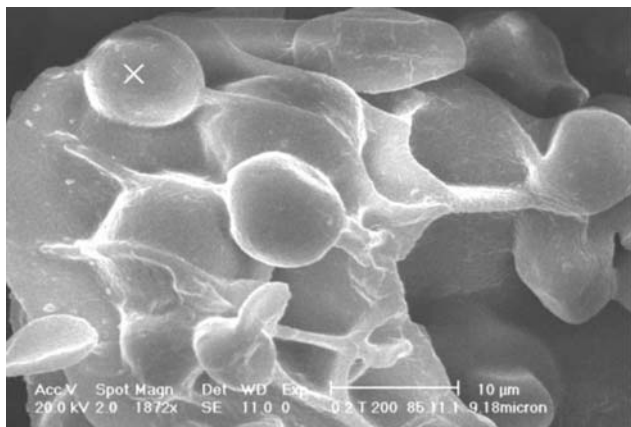
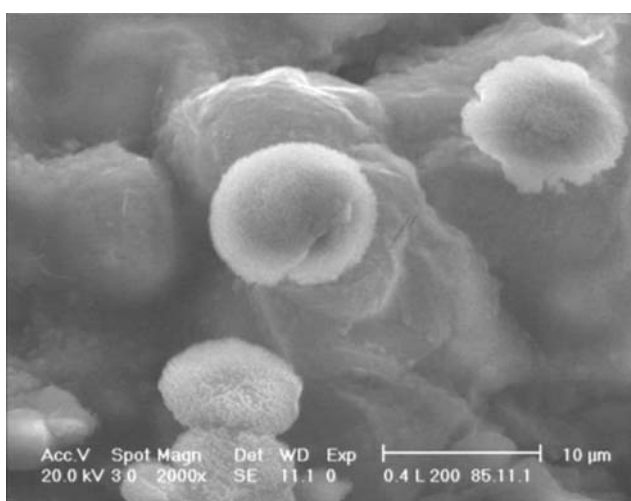


Figure 3. Scanning electron photomicrographs of microcapsules in the sample with 0% lecithin and 200 mL CaCl₂; (a) a microcapsule with 7.41 μm diameter, (b) a bacterial cell with 1.2 μm diameter, (c) a microcapsule with 8 μm diameter and (d) a microcapsule with 20 μm diameter that was determined with optical microscopy.



(a)



(b)

Figure 4. Scanning electron photomicrographs of beads, (a) in the sample with 0.4% lecithin and 200 mL CaCl_2 , and (b) in the sample with 0.2% Tween 80 and 200 mL CaCl_2 . In 0.4% lecithin concentration, beads showed stringy texture but in 0.2% Tween 80 the surface of beads was smooth.

20 min) of emulsion resulted spherical beads in the range of 10-30 μm . As expected, after the addition of calcium chloride into the oil phase containing sodium alginate/starch/bacteria mixture immediately induced ionic cross-linking of alginate chains, thus hydrogel beads were formed [22]. The obtained size and shape of beads by means of optical microscopy were comparable to that obtained by scanning electron microscopy (Figure 3a-d). SEM revealed that the microencapsulation methods used in this study produced a consistent mixture of spherical alginate microspheres with a smooth surface (Figure 3c). The

beads featured in the present study had a similar shape to those shown by Sultana et al. and Wojtas et al. [2,19]. The average size of 120 randomly selected beads was $17.80 \pm 3.55 \mu\text{m}$. The shape of the beads was generally spherical; sometimes elliptical shaped capsules were observed as well. The texture of the capsules produced with lecithin was different from those produced with Tween 80. Lecithin had a detrimental effect on the texture of the beads. In 0.4% lecithin concentration, beads showed stringy texture with varying shapes but in 0.2% Tween 80 the surface of beads was smooth (Figure 4).

Effect of Type and Amount of Surfactant on the Microencapsulation Process Yield

As demonstrated in Figure 5, the size of the droplets produced during homogenization depends on the balance between the time for an emulsifier to adsorb to the surface of a droplet and the time between droplet-droplet collisions. The ease with which a droplet can be disrupted during homogenization increases as the interfacial tension decreases. Thus it is possible to produce droplets with smaller sizes by homogenizing in the presence of an emulsifier which reduces the interfacial tension. Emulsifiers have two functions during the homogenization process: first, they decrease the interfacial tension between the oil and water phases, thereby reducing the amount of energy required to deform and disrupt the droplets and sec-

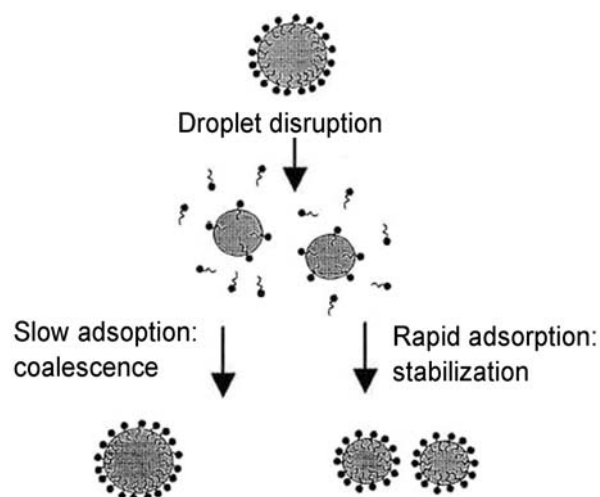


Figure 5. Emulsifier adsorption onto the surface of a droplet during homogenization.

ond, they form a protective coating around the droplets which prevents them from coalescing with each other. The size of the droplets produced during homogenization therefore depends on a number of different characteristics of an emulsifier:

(1) The ratio of emulsifier to dispersed phase: there must be sufficient emulsifier present to completely cover the surfaces of the droplets formed.

(2) The time required for the emulsifier to move from the bulk phase to the droplet surface: the faster the adsorption time, the smaller the droplet size.

(3) The probability that an emulsifier molecule may be adsorbed to the surface of a droplet during its encounter with the droplet: the greater the adsorption efficiency, the smaller the droplet size.

(4) The optimum amount that the emulsifier reduces the interfacial tension: the greater the amount, the smaller the droplet size and.

(5) The effectiveness of the emulsifier membrane in protecting the droplets against coalesce: the better the protection, the smaller the droplet size [23].

For a fixed concentration of oil, water, and emulsifier, there is a maximum interfacial area which can be completely covered by the emulsifier. As homogenization proceeds, the size of the droplets decreases and the interfacial area increases. Once the droplets fall below a certain size, there is an insufficient emulsifier present to completely cover their surface, and so they tend to coalesce with their neighbours. The minimum size of stable droplets that can be produced during homogenization (assuming monodisperse droplets) is therefore governed by the type and concentration of emulsifier present. One must select the most appropriate emulsifier for each type of food product, taking into account its performance during homogenization, solution conditions, cost, availability, legal status, ability to provide long-term stability, and the desired physicochemical properties of the product. Figure 6 shows that in low concentration of lecithin (0.2%), the microencapsulation process yield is high but it is lower than microencapsulation process yield obtained when 0.2% of Tween 80 is used. This may be due to the effectiveness of Tween 80 in reducing the interfacial tension and well protecting the droplets against coalesce. Application of 0.2% Tween 80 has produced smooth surface beads in comparison with lecithin (Figure 4). The microencapsulation

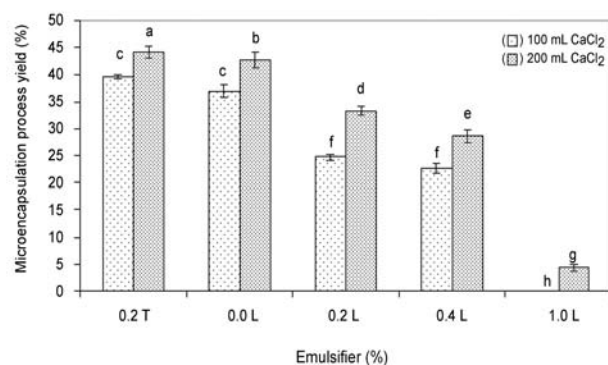
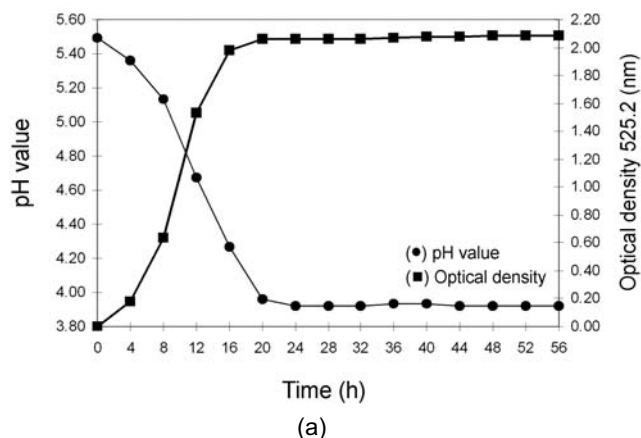


Figure 6. The effect of surfactant concentration and calcium chloride solution volume on the encapsulation process yield of calcium alginate beads. (1) 0.2% Tween 80, (2) 0% lecithin, (3) 0.2% lecithin, (4) 0.4% lecithin, and (5) 1% lecithin. a-h represent the statistically ($p \leq 0.01$) significant difference between all treatments. Error bars indicate the standard deviation.

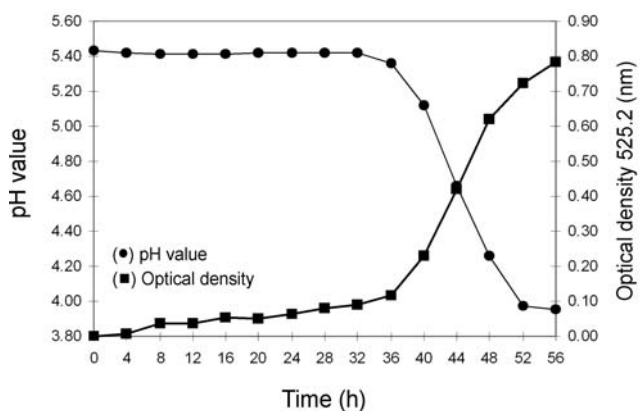
process yield has been decreased with increasing the lecithin concentration because of stringy texture and non-spherical shape of produced particles which make microparticles to coalesce and make the large particles of hairy or chain structure. The resulted large particles were accumulated on the top of calcium chloride solution because of their hairy structure. So, the amount of beads in the bottom of beaker decreased.

Effect of CaCl₂ to Alginate Ratio on the Microencapsulation Process Yield

0.1 M CaCl₂ solution was applied for cross-linking of alginate chains and formation of alginate beads [2,16,17]. In the present study 1:1 and 2:1 ratios of 0.1 M CaCl₂ solution to alginate solution (2%) were used to determine the effect of calcium chloride solution ratio on the microencapsulation process yield. It was demonstrated that the microencapsulation process yield increased when the calcium chloride solution was doubled. Higher bead recovery observed when 200 mL CaCl₂ solution was used in every concentration of surfactant (Figure 6). When calcium chloride solution increases, it may increase the adsorption efficiency of emulsifier molecules to the surface of droplet and so, protecting the droplets against coalesce. This produces droplets with smaller size and increases the microencapsulation process yield.



(a)



(b)

Figure 7. The changes of pH values and optical density (at 525.2 nm wavelength) over 56 h in MRS-broth medium: (a) containing free cells of *Lactobacillus casei*, and (b) containing encapsulated cells of *Lactobacillus casei*.

Metabolic Activity of Microencapsulated *Lactobacillus Casei*

Acidification kinetics and optical density (OD) over a period of 56 h in MRS broth medium containing free and encapsulated cells of *Lactobacillus casei* were compared to show that if the encapsulated bacterial cells are active and could produce acid similar to free cells.

The results (Figure 7a and b) showed that free and encapsulated cell changed the pH value and optical density of MRS broth medium with time, indicating that bacterial cells were remained viable and active in the calcium alginate beads. However the time taken for the encapsulated cells was longer than that by the free cells to arrive at the same pH. The time taken for free cells to decrease the initial pH value of MRS

broth medium to 4 was about 20 h and that of encapsulated cells was about 50 h. This could be due to the slow uptake of nutrients and slow release of the metabolites across the encapsulating alginate shell of beads. A similar pattern was also observed by Larisch et al. (1994) in alginate/poly-*L*-lysine beads containing *Lactococci* cells. It has been suggested that the size of the beads affects the rate of mass transfer and metabolic activity of microencapsulated cells [4].

CONCLUSION

A modified microencapsulation method based on emulsion technique was investigated in this study to produce microcapsules with diameters below 100 μm . Recent study has indicated that it is possible to control the alginate microspheres size by using high stirring speed (400 rpm for 20 min) of emulsion and produce spherical beads in the range of 10-30 μm . It was shown that the microencapsulation process yield of alginate beads may be dependent on the surfactant type and concentration as well as the volume of calcium chloride solution. Tween 80 had better ability than lecithin in alginate bead production and higher volume of CaCl_2 solution resulted high microencapsulation process yield. The improved method allowed us to produce micron size beads rather than millimetre sizes which were reported by previous researchers [20]. It gives a smooth texture when the beads are incorporated into products. Future studies are needed to monitor the effect of other surfactants on the microencapsulation process yield, as well as studying the effect of various surfactants on the viability of probiotic bacteria.

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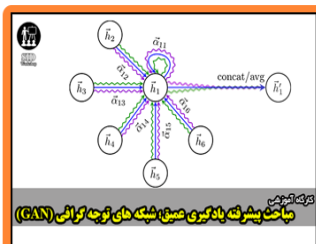


عضویت در
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فیلم های
آموزشی

کارگاه های آموزشی مرکز اطلاعات علمی جهاد دانشگاهی



مباحث پیشرفته یادگیری عمیق؛
شبکه های توجه گرافی
(Graph Attention Networks)



کارگاه آنلاین آموزش استفاده از
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