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Assessment the possibility of probiotic jelly production using microencapsulation technique of *Lactobacillus acidophilus* bacteria

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Abstract

The aim of this study is to improve the pH and thermal sensivity of probiotics for using them in new functional foods, to this *Lactobacillus acidophilus La5* was encapsulated with calcium alginate and some of capsules coated by chitosan. The probiotics bacteria were inoculated to jelly in their free and two microencapsulated forms and stored for 6 weeks at 7 and 25˚c. The morphology and size of microcapsules were measured by optical microscopy, scanning electron microscopy (SEM) technique and particle size analyzer.The thermal resistance in exposing to 70˚c-20min and survival of free and both two microencapsulated bacteria during storage were measured. the pH changes in jelly were also monitored during storage. Sensory evaluation was performed. the pH changes of jelly with microencapsulated *L.acidophilus* were slower than jelly containing free bacteria during storage. The survival rate of microencapsulated *L. acidophilus* was significantly higher than free bacteria and in low temperature ($P < 0.05$) due to the protective property of calcium alginate capsules. Viability of bacteria in this capsules was about 10^7 cfu/ml after 6 weeks. The effect of microencapsulation on thermal resistance property of probiotics was significant ($P<0.05$). The inoculation of free probiotic had significant effect on texture, color, taste, odor, transparency and overall sensory characterization of jelly over the storage period at 4°C ($P<0.05$) as it was unacceptable. The microencapsulation with alginate-chitosan effectively protected the *L. acidophilus* from heat and acid treatment in the product.

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Introduction

In recent years, most of cunsumers have been interested not only in safty and nutritional Value of foods but also in their healthful effects. Such properties can be found in a new class of products know as functional foods containing probiotic. Probiotics bacteria are living microbial supplements when consumed my improve intestinal microbial flora balance thereby exerting their healthful effects as microbial balance of dijestive system may be influenced by various factors including diseases, stress, age, diet, geographic conditions, etc resulting in some healh disorders. Common used probiotics include different species of *bifidobacteria* and *lactobacilli* belonging to lactic bacteria family and because they have been in use for many years, their safty has been confirmed. Their healthful effect appear through producing acid or bacteriocines, compound having antibiotic effect, and boosting immune system, and they may control the level of blood cholesterol, prevent intestine infections, constipation and acute diarrhea as well as growth and reproduction of harmful bacteria induce anticarcinogenic activity of colon, small intestine and liver and help digesting and absorbing of mineral and vitamins (Gilliland, 1990; Shah and Jelen, 1990).

A number of intrinsic and extrinsic factors influence the survival of probiotics in foods during processing and storage. It is important to consider these factors at all stage from additions of the probiotic in the food to delivery of the probiotic to the gut of consumer. These include manufacturing processes, food formulation and matrices, packaging materials and environmental conditions in the supply chain and storage (Govin babu and Nithyalakshmi, 2011). The Food and Agriculture Organization–World Health Organization report also specifies that the standard for any food sold with health claims from the addition of probiotics is that it must contain per gram at least 10^6-10^7 CFU of viable probiotic bacteria (latha sabikhi *et al.*, 2008).

Probiotics could be protected using different method such as microencapsulation. Microencapsulation, microbiologicaly consists of microscopic covering small living cells with a hydrocolloid layer and surrounding them in order to separate from the environment so that provides considerable viability of cells and protection from mild heat treatment in food processing and harmful environments including moisture, pH and unfavorable conditions (Gong *et al.*, 2009). Microencapsulation using emulsion method is widespread because it does not damage the bacteria and is applicable at large scale (Ding and Shah, 2007). Usually, small sizes (micron) can be obtained by this method (Kailasapathy, 2002). Alginate is the most widespread used encapsulating material due to its non-toxity, light weight and biocompatibility. Alginate is a linear heteropolysaccharide consisting of 1-4,D-manoronic and L-glucoronic extracted from algae and classified as unsoluble fiber. Bacteria (1–3 μm size) are well retained in the alginate gel matrix which is estimated to have a pore size of less than 17nm (Klein *et al.*, 1983). Alginate gel does not thermoreversible and is susceptible to disintegration in the presence of excess monovalent ions, Ca2+ chelating agents and harsh chemical environments (Smidsrod and Skjak-Braek, 1990). A crosslinked alginate matrix system at very low pH is reported to undergo a reduction in alginate molecular weight causing a faster degradation and release of active ingredients. Thus, coating alginate beads with poly-cations can improve the chemical and mechanical stability of the alginate beads, consequently improving the effectiveness of encapsulation.

Biological adhesion characteristic of alginate facilitates coating it with other compounds. For example coating alginate bead with poly-cations, such as chitosan that form strong complexes with anionic charg of alginates which are stable in the presence of Ca2+ chelators and reduce the porosity of the gel (Krasaekoopt *et al.*, 2004), also affected the release speed of capsulated active compounds.

in recent research report that Fruit juices may be an alternative vehicle for the incorporation of probiotics because they are rich in nutrients and do not contain starter cultures that compete for nutrients with probiotics. Furthermore, fruit juices are often

supplemented with oxygen scavenging ingredients such as ascorbic acid, thus promoting anaerobic conditions. Fruit juices contain high amounts of sugars which could encourage probiotic growth (Ding and Shah 2008). Given this fact, therefore,fruit jellies and gelatinous desserts may act as conveying media for probiotics. Science majority of people specially children are the main consumers of jellies using these products as nutritional one may promote health and improve cells growth or renovation and maintain body general condition.

We examined the studies conducted by other researchers and found that today, research on producing non-dairy and unfermented probiotic products are expanding, but there were not any studies on jelly. Also, one of the less investigated aspects is heat intolerance of probiotics, so these bacteria are added to the product after heat processing posing food contamination. probiotics ideally should be added prior to pasteurization to maintain their viability, therefore selection of proper species and providing favorable conditions to increase heat tolerance as well as selection of suitable materials and method for encapsulation so that probiotics could tolerate heat processing during production or preparation are of enormous importance. Also applying these bacteria is impossible in some products because of low pH and aw, duth providing a proper coating for probiotics and surrounding them against unfavorable conditions such as low pH and aw while protecting the gel against changes of textural and sensory properties are crucial. To do this different coating such as alginate, chitosan with emulsion technique may be used to improve probiotics tolerance.

The main objective of this study was to increase stability of bacteria to increase products diversity in form of functional and probiotics foods so that the new products contain alive bacteria that have sufficient tolerant in all processes of production and consumption. There were not any studies on probiotic jelly that can be eaten on a daily basis and will contribute to increasing health.

Material and methods

Bacterial strain and culture preparations

Lactobacillus acidophilus La5 was used as probiotic strain supplied by CHR-Hansen, Denmark in freeze dried and DVS form, actived freeze dried cell was used for prepearingmicrobial suspension and microencapsulation process (Govin babu and V.Nithyalakshmi, 2011; Mokrram *et al.,* 2009).

To obtaining a suspension with proper microbial load using MC Farland solution turbidometery method. MC Farland turbidometry method was used to determine the number of bacteria in liquid media. In this way, the turbidity of liquid media containing microbial suspension was compared to a standard with a turbidity propotional to a certain number of bacteria. MC Farland 0.5 standard was obtained by addition of 9.95ml of 1% pure sulfuric acid (0.036 normal) to 0.05ml 1.75% barum chloride (0.048 molar) MC Farland 0.5 standard generates turbidity equivalent to a bacteria suspension as 1.5×10^8 CFU/ml. MC Farland 0.5 solution absorbed 0.1 at 600nm wavelength (Brinques and Ayub, 2011). To obtain proper microbial load for encapsulation, a relationship between suspension microbial absorption and microbial count was calculated using a spectrophotometer. First, some of probiotic bacterium cells added to 0.9% normal saline solution resulted in a concentrated microbial suspention. The concentrated suspension, then, partially diluted by addition of normal saline solution and then transfer to coats of spectrophotometer so that absorption of suspension at 600nm wavelength becomes equal to absorption of MC Farland 0.5 standard solution. To do so, first, different suspension of probiotics bacteria at 0.1-3.5 absorption were made and then cultured to obtain the relationship between absorption and bacterial count. Thus, for each suspension with certain absorption, microbial count was made using MRS Agar at 37˚c for 48h and finally colonies counts were recorded.

Microencapsulation of Probiotics

In this study, *L. acidophilus* was inoculated to jelly in three forms: free or non-encapsulated, encapsulated

with calcium alginate, and encapsulated with calcium alginate coted by chitosan. Probiotic organisms were microencapsulated using emulsion method. 3% solution of alginate was prepeared by blending medium viscosity sodium alginate (Sigma Aldrich, USA) and distilled water using magnetic stirrer then outocluved 121˚c-15min then allowed to cooled at room temperature. 100 mL of sterile 3% sodium alginate solution was mixed with probiotic organisms suspension, Then alginate and bacteria suspension was dispensed using a pipette into a beaker containing 500 mL of vegetable oil (Sunar, Turkey) and 1g (0.2%) of Tween 80 (Merck, Germany). This emulsion was mixed thoroughly at 350 rpm for 20min, with a magnetic stirrer (Heidolph,Germany). A solution of calcium chloride 0.1 M (MP Biomedicals,Netherlands) was slowly added to the side of the beaker until the emulsion was broken and continued mixing at 200 rpm for 20min. After 30 min, the calcium alginate beads were removed from the aqueous phase with dekantor then centrifuging at 350g for 10 min. capsules were washed with distilled water and 5% glycerol to removed remain oil. capsules were refrigerated at 4˚C for 10 h to allow the beads to fully harden (Ding and Shah, 2008).

Low-molecular-weight chitosan (0.4g) (Sigma Aldrich, USA) was dissolved in 90ml distilled water acidified with 0.4ml of glacial acetic acid to achieve a final concentration of 4g/l. The pH was then adjusted to between 5.7 and 6.0 by adding NaOH (1M) (Merck,Germany). The mixture was filtered through Whatman #4 filter paper and was autoclaved at 121˚C for 15min. Then 15 g of washed beads were immersed in 100 ml of chitosan solution with gentle shaking at 100 rpm for 40min on a magnetic stirrer. The chitosan-coated capsules were washed and kept at 4˚C for not more than 1 h. The beads were then used on the same day (Krasaekoopt *et al.*, 2004).

Physical examination of microcapsules

Size analysis

The size of the capsules was determined by using the particle size analyzer (Master sizer 2000, Malvern, UK) with the standard deviation calculated from the cumulative distribution curve.The average bead volume and diameter was then calculated. Size determination was performed for three lots of manufactured beads. The measurements were made in triplicate.

Morphology

The morphology of the microcapsules was observed by optical (Motic BA300, UK) and scanning electron microscope (SEM). The samples were mount on the stub to aid of coated with Au by sputter coater (SC 7610, England) for 3 min. Observations were made using the scanning electron microscope (LEO440i,UK) at an accelerating voltage of 15 Kv.

Bacterial enumeration

Non-encapsulated *L.acidophilus La5* were enumerated in the MRS agar. Saline solution 0.9% was used to prepare the serial dilutions and culture was plated by the pour plate technique. The plates were incubated at 37˚C for 48 h. In brief, for the enumeration of microencapsulated probiotic organisms, the bacteria were released from the alginate capsules by sequestering calcium ions with a phosphate buffer 0.1M at pH 7.0 by gently shaking at room temperature for 10min, Complete release of bacteria from the capsule occur in 10 min. The colony forming units (CFU/ml) were determined by aerobic plating on MRS agar plate and incubating at 37˚C for 48 h. The plating procedures were carried out in duplicates (Se-Jin Kim *et al.*, 2008).

Freshly prepared Chitosan coated alginate micro beads encapsulating probiotics (1g) were broken down in 9 mL of sterile sodium citrate solution 0.1M at pH 6.3 were blended in a stomacher for 1 min prior to breakdown in sodium citrate solution. Dilutions were made in 0.1% peptone and were plated on MRS agar. Colonies of *L. acidophilus* were enumerated following incubation at 37 °C for 48 h under anaerobic conditions.

Probiotic Jelly production

For jelly production a mixture of sugar (<30%), fruit iuice and carrageenan (<1.5%) was prepeared in water

fallowed by heating of mixture at 80-85˚c for 20-30min as cooking stage. when heating was finished, the mixture was Cooled possible then appropriate essence and colorant were added to product. On the next step the pH was adjusted to 3.5-4 by adding citric acid then jelly was poured into the jelly cups at temperature minimum 60˚c. Probiotic bacteria were inoculated to jelly in their free and two microencapsulated forms at hot filling step then jelly was cooled and stored for 6 weeks at 7 and 25˚c. All jelly samples had 10^{11} CFU/ml.

Assessment the heat tolerance of free and microencapsulated bacteria in jelly production

The aim of this study is production of probiotic jelly adding free and microencapsulated forms bacteria before hot filling step of production. Heat tolerance was studied by exposing jelly samples to 70˚c. After heat treatment, the jelly containing free and microencapsulated probiotic bacteria with alginate and alginate coated with chitosan were cooled then buffers were used that helped release the encapsulated probiotic bacteria as described before. Then serial dilutions were prepeared and culture was plated on MRS-Agar and were incubated at 37˚c for 48h. Heat tolerance was determined by comparing the final plate count after 20min of heat treatment with the initial plate count at 0min. All heat tolerance tests were repeated 3 times.

Assessment the viability of free and microencapsulated L. acidophilus under acidic Conditions and low a_w of jelly during storage

To determine the survival of bacteria cells under acidic conditions and low a_w of jelly, samples were stored at 7 and 25˚c for 4-6 weeks. The viable count of cells were determined at 1, 2, 3, 7, 10, 15, 30, 35, 42 days. Releasing and enumeration of bacteria was performed as described previously.

Influence of bacteria on pH changes of jelly during storage

The pH was measured according to the Institute of Standards and Industrial Research of Iran (ISIRI) regulation (ISIRI, No 214). The pH value was determined using 10% dispersion of samples in distilled water using a pH meter (Metrohm 827 pH lab). The pH meter was calibrated by standard buffer solutions 4 and 7. pH of control samples (without bacteria) and jellies containing free and encapsulated bacteria was measured during a storage period of 4-6 weeks. All experiments were performed in triplicate to determine an average and standard error of the mean.

sensory evaluation

Sensory evaluation of jellies was performed after 6 weeks of storage. A panel consisting of 10 trained panelists, assed the color and appearance, mouth texture, ador and taste of jellies at room temperature. Scoring was carried out on a scale of 1 to 5, with 5 being most desirable.

Statistical analysis

A complete randomized factorial design was used for all analysis and all results were means of three replicates. Data analysis was carried out using Statistical Package for Social Sciences (SPSS) Inc. software (20: SPSS Inc., Chicago, IL). The mean differences were analyzed by Duncan's multiple range test.

For fitting the relationship between absorption of microbial suspension and bacterial count, regression method as well as SPSS software, version 20 are used. For fitting the best function of absorption of microbial suspension and bacterial count, different models including observed, linear, logarithmic, inverse, quardratic, cubic, compound, power, S, growth, exponential and logistic are determined and compared according to explanation coefficient (R^2). Results of sensory evaluation analized using friedman non parameteric tests by SPSS 20 software.

Results and discussion

Assessment the relationship between microbial suspension absorption and micro organism count

Basis of explanation coefficient (R^2) and assessment of different functions, cubic was choosed as the best model with explanation coefficient (R^2 = 0.971).

Diagram 1 shows the absorption of microbial suspension and their counts at each absorption. As shown in the diagram, with an increase in absorption of microbial suspension, bacterial count per ml of suspension increases. According to the results for fitting to load of 10^{11} CFU/ml bacteria for inoculation, absorption of microbial suspension must be 3 at 600nm wavelength.

Table 1. Diameter of capsules measured by particle size analyser.

	Calcium alginate (µm)	Calcium alginate-chitosan (µm)
Mean diameter of 10% of capsules d(0.1)	20.386	67.016
Mean diameter of 50% of capsules d(0.5)	86.874	263.020
Mean diameter of 90% of capsules d(0.9)	236.299	663.250
Mean volume diameter of capsules	116.307	322.422

Table 2. viability and heat tolerance of bacteria in jelly.

jelly containing:	heat treatment	
	70˚c-20min	70˚c then cooling at room temperatur
free bacteria	2.10E+04	1.70E+9
alginate capsuls	1.99E+08	5.80E+10
alginate-chitosan capsuls	5.68E+08	7.40E+10

Size distribution and morphology of microcapsules

According to the morphological analysis of the microcapsule, they were globular in shape and a thick skin around the strains was detectable. However, no significant difference in capsule shapes was observed with respect to the different capsules produced with and without coating layers by a optical microscopy but the size and thickness of capsules was increased by coating layer. Also SEM showed that the shape of microcapsules was generally spherical (figure1) and the surface of capsules was smooth (figure2). Covering negative-charged calcium alginate capsules by chitosan (a multicathionic) results in coated capsules with more physicochemical stability and reduced destructive effect of anti-gel and agents involving calcium ions in the stracture of capsule. In this study, using chitosan, a compound protecting calcium alginate capsules, resulted in moreconsistent and thicker capsules thereby increasing probiotics viability.

Table 3. pH changes of jelly during storage.

sample	temperature	24h	48h	72h	1week	10days	2weeks	1month	5weeks	6weeks
control	7˚c	4.400	4.410	4.410	4.423	4.430	4.433	4.436	4.440	4.446
	25˚c	4.366	4.406	4.413	4.433	4.436	4.443	4.443	4.443	4.446
Free bacteria	7˚c	4.406	4.366	4.356	4.280	4.253	4.220	4.110	3.920	3.503
	25˚c	4.400	4.320	4.150	3.976	3.956	3.933	3.883	3.510	3.200
Alginate capsules	7˚c	4.396	4.403	4.393	4.403	4.403	4.416	4.403	4.400	4.402
	25˚c	4.406	4.403	4.393	4.403	4.396	4.396	4.403	4.400	4.410
Alginate-chitosan capsules	7˚c	4.446	4.453	4.436	4.436	4.440	4.450	4.440	4.436	4.436
	25˚c	4.446	4.443	4.436	4.436	4.440	4.446	4.433	4.436	4.433

The size distribution of capsules was analysed by particle size analyser. The average size of the microcapsules was showed in table 1. The mean value of uncoated beads was 116.307µm which was significantly ($P < 0.05$) lower than that coated beads (322.422µm) for all encapsulated probiotics.

Furthermore, the uncoated capsules had the narrowest size distribution followed by chitosan coated capsules. In the particle size distribution data graph, the horizontal axis represents particle diameter (μm) while the vertical axis represents the percent of the relative particle amount or volume fraction (figure3). This is an improved technique to produce micron size beads rather than millimetre size produced by many as it gives a smooth texture when the beads are incorporated into products. This agrees with reported that very large calcium alginate beads (>1 mm) cause a coarseness of texture in live microbial feed supplements (Truelstrup Hansen *et al.*, 2002). while other researchers produced millimeter size capsules which gave gritty texture (Hyndman *et al.*, 1993).

Table 4. sensory evaluation of probiotic jellies.

sample	Taste 1-5	Odor 1-5	Color & appearance 1-5	Mouth texture 1-5	Total acceptibility 1-5
Control (without bacteria)	4.2	3.9	4.9	4.9	4.3
Free bacteria	1.5	1.4	1.9	2.4	1.6
Calcium alginate capsule	4.4	4.3	3.9	4.4	4.4
Calcium alginate-chitosan capsule	4.6	4	4.2	4.8	4.6

Different studies have shown that size reduction of the capsules to less than 100 μm would not offer any significant increase in survival rate of the probiotics on the gastric secretion condition (Truelstrup-Hansen *et al.,* 2002; Hyndman *et al.,* 1993). Despite the fact that the micron size capsules had minor barrier effect, it created less alternation in composition of food product and further inhibit the sandy texture (Mokarram *et al.,* 2009; Truelstrup-Hansen *et al.,* 2002).

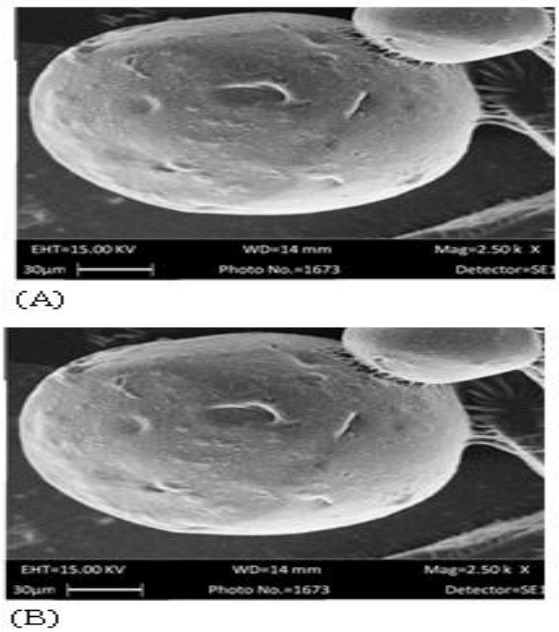

Fig. 1. Scanning electron photomicrograph showing: (A) calcium alginate, and (B) calcium alginate-chitosan capsules.

The larger size of capsules, the more viable probitics, because the bacteria use sufficient water activity inside the capsules under different condition(Possemiers *et al.*, 2010).

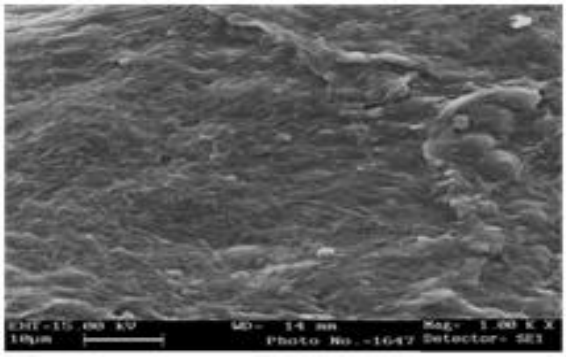

Fig. 2. Surface structure of microcapsules observed by SEM.

Viability of L. acidophilus in heat treatment

As the primary goal of thermal processing is to inactivate the spoilage and pathogenic microorganisms and produce a safe product with enhanced shelf life (Lee & Kaletunc, 2002), it is inevitable to kill non-pathogenic organisms that provide health benefit. Therefore, it may be important to search for a new method to selectively control such organisms under thermal processing. Researchers reported that heat treatment is the method usually employed to reduce the number of bacteria and pasteurization (62.5 ˚C for 30 min) has been recommended as a suitable form of heat treatment

(Se-Jin Kim *et al.*, 2007). When nonencapsulated and encapsulated with alginate and alginate coated by chitosan *L. acidophilus La5* were exposed to 70˚c for 20 min, the bacterial counts decreased from 10^{11} to 2.175×10^{4} and 1.99×10^{8} and 5.68×10^{8} CFU/ml respectively (table 2). This means that *L. acidophilus* loaded in microparticles showed significantly ($P<0.05$) higher heat stability than non-encapsulated *L.acidophilus*. Our results suggested that microencapsulation using alginate coated by chitosan may enhance thermal resistance of the bacteria (diagram 2). Science under real conditions free and encapsulated bacteria are added to the products at hot filling then cooled. Bacteria do not have to tolerate such acute heat processing. Respective data is given in the fallowing table showing that the products containing free, encapsulated with calcium alginate, and calcium alginate coated with chitosan only reduced 1.83, 0.42 and 0.26 log cycle, respectively (table 2).

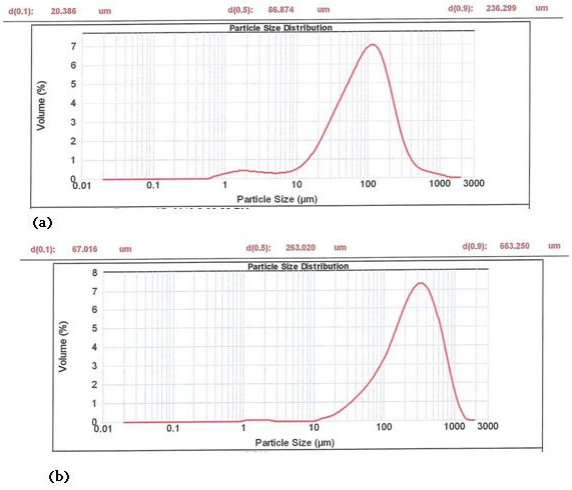

Fig. 3. Particle size distribution of calcium alginate capsules (a) Particle size distribution of calcium alginate-chitosan capsules(b).

viability in two kind of heat treatments showed significant difference ($P<0.05$), but in second form of heating, samples had no significant difference in viability as compared with initial count of bacteria($P>0.05$).

A temperature of 65˚C was found to be lethal to all free probiotic strains tested. However, after 1h of incubation the survival of free and microencapsulated probiotic bacteria was similar. Results indicate that after 1h of heating the alginate matrix offered little protection for the probiotic bacteria (Ding and Shah, 2007). viability of probiotics decrease with higher temperature and time. Bacteria in alginate capsules coated by chitosan had the best heat tolerance then microencapsulation is a way to improve heat resistant of probiotics.

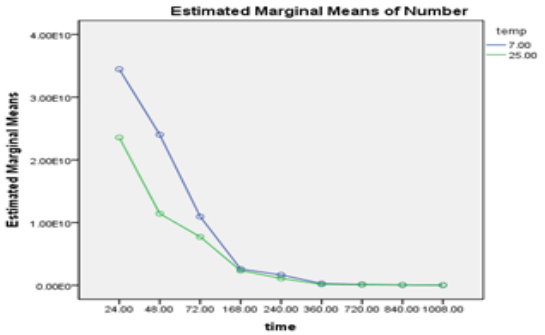

Fig. 4. Effect of storage temperature on *L. acidophilus* viability.

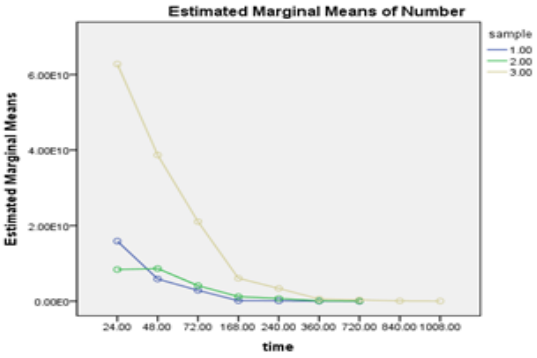

Fig. 5. Effect of microencapsulation on *L. acidophilus* viability (sample1: free bacteria, sample2: calcium alginate capsule, sample3: calcium alginate-chitosan capsul)

Survival of free and encapsulated L. acidophilus in jelly during storage

The survival rate of *L. acidophilus* in the product was monitored during 4-6 weeks storage period at two different temperatures: 7 and 25°C (diagram 2). Water activity and pH of jelly was reported 0.75-0.8 and 4.3 respectively whereas optimum a_w and pH for *lactobacilli* are 0.95 and 6-7 respectively and all strain of *lactobacilli* are sensitive to a_w less than 0.8. because of low pH and a_w in jelly, it can not be a suitable matrice for probiotics therefore

microencapsulation improve survivability of probiotics during storage and bacteria can use free water that available in capsules. The cell number in free *L. acidophilus* samples decrease about 4.81, 5.02 log cycle after 1 month of storage at 7 and 25°C, whereas in microencapsulated samples with calcium alginate and calcium alginate-chitosane, the cell number reduction was about 3.35 , 3.59 and 2.33 , 2.47 log, respectively at the similar condition. There was a rapid loss of free probiotic bacteria in the jellies during four weeks period. The difference between the final cell numbers of free and encapsulated bacteria were significant ($P<0.05$). The encapsulated probiotic bacteria which were protected from the acidic environment of the jelly did not lose their viability as rapidly as the free probiotic bacteria and $>10^7$CFU/mL were still present after six weeks of storage. Comparison of survival rate during storage revealed that storage time and temperature had significant effect on the viability of probiotics ($P<0.05$). this finding is in agreement with those those of Possemier *et al.* (2010) and Aragone *et al.(2007)*. Furthermore, our findings displayed that, encapsulated *L. acidophilus* required longer time to decline 1 log cycle in live cells. Thus, the survival of bacteria in jelly at 4°C is better than at 25°C. Probiotics survived significantly greater in microencapsulated form as compared to free form. In this study, the results determined that encapsulation with alginate-chitosan improved cells viability when compared with encapsulation without chitosan then Calcium alginate-chitosan can significantly increase the survival of *L. acidophilus* in jelly during storage ($P<0.05$). Other researchers showed that the microencapsulation of *L. gasseri* and *B. bifidum* with alginate and a chitosan coating offers an effective means of delivery of viable bacterial cells to the colon and maintaining their survival during simulated gastric and intestinal juice. This complex reduces the porosity of alginate beads and decreases the leakage of the encapsulated probiotic and, moreover it is stable at broad pH ranges (Chavarri *et al.*, 2010). Also was reported the survival of encapsulated probiotic bacteria was higher than free cells by approximately 1 log cycle (Krasaekoopt *et al.*, 2006).

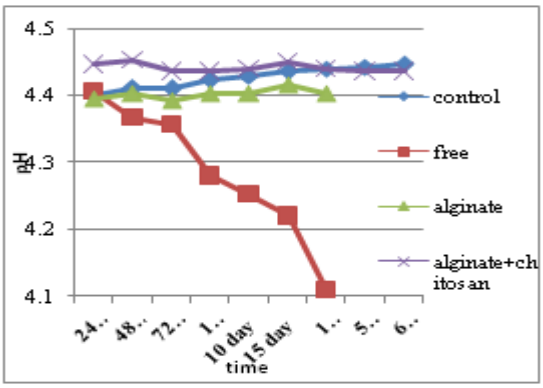

Fig. 6. pH changes of jelly samples during storage at 7°c

pH Changes During the Storage of Probiotic jellies

pH changes in jellies containing free and microencapsulated probiotic bacteria during a storage period of six weeks are shown in table 3. A similar trend in the decline in pH was seen in both microencapsulated jelly.The final pH at the end of the six week storage period of jelly with encapsulated probiotic bacteria was higher than that inoculated with free probiotic bacteria. The average pH at 7 , 25°c decreased from 4.406 , 4.400 to 3.503 ,3.200 respectively in jelly containing free probiotic bacteria after six weeks of storage, also the pH had no significant decline in the jelly containing encapsulated probiotics after the storage period ($P>0.05$). This may be due to slow absorption of nutrients and sluggish release of metabolites through the calcium alginate-chitosan matrix (Homayouni *et al.*, 2009; Sultana *et al.*, 2000). It has been suggested that one of the main factor in metabolic activity of microencapsulated probiotic bacteria is the size of the capsules. Larisch *et al.* (1994) fined that calcium alginate coated by poly-L-lysine need longer time to making same pH changesas free bacteria. This result suggests that probiotic bacteria in an immobilized microencapsulated state have a more stable environment. pH changes of alginated chitosan microencapsulated bacteria showed that there was no significant difference ($P>0.05$). also results showed that storage temperature had no significant effect on pH changes. Free probiotic bacteria may have utilized carbohydrates and produced small amounts of organic acids thus lowering the pH of the product during storage. Many of the free bacteria were not

viable at later stages of storage; although the dead probiotic cells could release enzymes for hydrolyzing sugars in the fruit juice, thus lowering the pH. These results demonstrated that microencapsulation of probiotic bacteria would make a more stable product over a longer storage period. Studies have shown that encapsulated probiotic bacteria make more stable functional food products (Kailasapathy 2005; Saarela *et al.,* 2005).

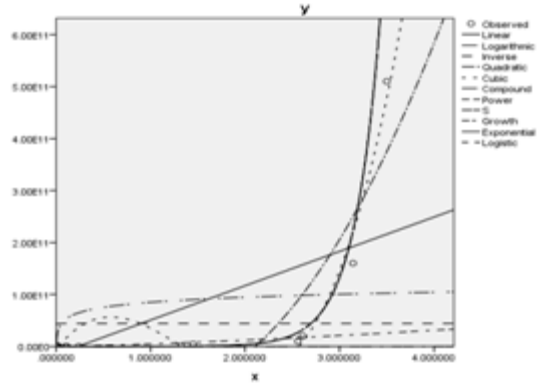

Diagram 1. x:absorbance, y:number of bacteria.

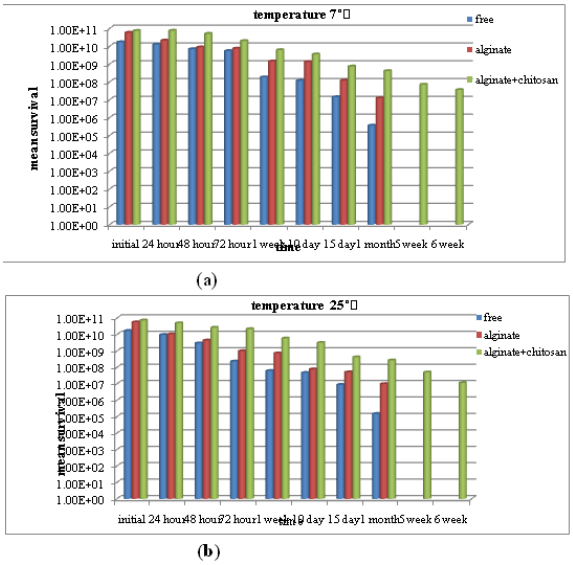

Diagram 2. Survival of probiotic bacteria in jelly stored at 7˚C over a period of 6 weeks (a) Survival of probiotic bacteria in jelly stored at 25˚C over a period of 6 weeks (b).

Sensory evaluation

Average of sensory scores obtained from 10 panelists are represented in Table 4. According to the results, free bacteria had severe undesired distasteful effects on sensory properties of jelly. but the panelist could not identify the difference in the texture, taste and odor between jelly with encapsulated *L. acidophilus,* and the control.

Rather even jelly containing microencapsulated bacteria had more scores in taste, odor and total acceptability but they have less appearance. From statistical analysis there was significant difference ($P<0.05$) in all assesed factors beetwin jelly samples. Also there was no sandiness in none of sample.

Microencapsulated probiotic organisms showed a much higher survival in lowering jelly pH and moderate heat treatment compared to jelly containing free probiotic bacteria. Furthermore, microencapsulation appeared to be effective in protecting cells from mild heat treatment and thus could stimulate research in functional food products that receive a mild heat treatment. In general, probiotic jelly containing free *l. acidophilus* showed a decrease in pH during storage but other samples had no significant pH changes. The pH changes of jelly with microencapsulated bacteria were slower than product containing free *L.acidophilus* during storage due to a slow release of metabolites through the capsul shell. There were no statistically significant differences between the scores received during evaluation of sensory properties of jelies ($P>0.05$) unless free bacteria that they were unacceptable. The capsules are micron size and deliver smooth texture to the product. The natural acidity and lack of nutrients may not make jelly a suitable functional food. Thus, further optimization of microencapsulation techniques is needed to make jellies a novel functional food.

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