The influence of *Alginat_Whey protein* encapsulation on survivability of *Lactobacillus casei*

ır naeemi[1], S. Ali. Mortazavi[2] , Elnaz_Milani[2],soode zolfaghari[1]
1-Azad university of Sabzevar.
2- Research instructor, Iranian Academic Center for Education Culture and Research (ACECR), Azadi Square.
P. O. Box 91775-1376
Mashhad,Iran
E. mail: hajar_naeemi@yahoo.com

Abstract **In this study,used of encapsulating technique to investigate its effect on survivability of probiotic bacteria.the number of viable cells (lactobasillus casei(lafti-L26)) before encapsulation with sodium alginate-whey protein concentration was 10.14- 10.30 log cfu/ml, that after encapsulation decreased to 9.38-9.44 log cfu/ml.**

Observation by a SEM electron microscopy showed that the beads were globular in shape after encapsulation with sodium alginate-whey protein. Totally ,obtained results showed that encapsulation of lactobacillus casei in Alginat-Whey protein capsules,could significantly improve survivability of L.casei.($p<0.05$) that the viable number of this bacteria in frozen yogurt containing encapsulated probiotic,was in the range of investigated levels by the International Dairy Federation(106-107 cfu/g).

Keywords- sodium alginat; whey protein; encapsulation;, electeron microscopy

I. INTRODUCTION *(HEADING 1)*

probiotics, are living microorganisms in gastrointestinal condition [1],which during last decade, have become more popular.at the time of consumption ,in order to produce health and therapeutic benefits ,amount of live microorganisms should be at least 10^6 per *g* or *ml*[2,3,4].This benefits of probiotic bacteria such as lactobacilli and bifidobacteria lead to increase incorporation these bacteria in dairy product.[5]. Encapsulation is the way for probiotics physical protection from the effects of the hostile environment and gastrointestinal tract [6]. Shah and Ravula(2000)reported that the encapsulation ,protected of probiotic bacteria in fermented frozen desserts[7]. Alginates are hetro polysaccharides that formed with D-mannuronic an L-guluronic acid residue joined linearly by (1-4)-glycosidi linkages [8].The use of alginate is favored, because of its characteristics; low cost, simplicity, and bio compatibility [9]. Whey proteins could form gels that this characteristic can be generated by heating the protein solution with subsequent cooling and acidification [10] .Whey proteins are using as food ingredients, because of their ability to form gels, emultion and gelled emulsion, and their nutritional value [10]. The objective of this study was to investigate tha impact of encapsulation on lactobacillus casein survivability.

II. MATERIALS AND METHODS

A. Preparation of microorganisms

Lactobacillus casei *LAFTI-L26 DSL*(batch number*: 246833*)was purchased from *DSM* in lyophilized form.A vial of freeze dried L.casei was inoculated into 5 *mL MRS(De Man ,Rogosa ,Sharpe*)broth(*Merk, KGaA Germany*) and incubated at *37°c* for 12-14 h(activation time of *L.casei*) under aerobic conditions. Then the culture was sub-cultured into 95 mL broth and incubated under previous condition[11].The cells were harvested by using cold centrifuging(4°C) at 4500g for 10 min and washed twice with sterile peptone water 0.1% (Merk KGaA Germany).

B. Microencapsulation of microorganism

The method for preparation of micro capsule wall compoundes :*WPC(wpc 80%* was purchased from kian meshkat organization of Iran) powder was prepared at *8% w/v* in deionized water with agitation for 1h at room temperature and then allowed to stand for *2h* to complete hydration of the proteins. *WPC* solution was adjusted to *pH 8.0* with *1 N NaOH* and heated at *80°C* for *30 min* to denaturate proteins completely, then cooled and held at room temperature for *2h* [12]. Sodium alginate powder ,medium viscosity and high *mannuronic acid*,(*Sigma Aldrich,Pool,UK*)was dispersed in distilled water to make *2%(g/100m*l) solution ,then stirred for 30 min and stand overnight in refrigerator . WPC and alginate solution were combined to form solution with *WPC/Alginat* ratio of *2:1(v/v*).*Sodium alginate-WPC* microcapsules were prepared using an external gelation technique described by Truelstrup et.al(2002) and Allan-Wojtas et.al(2008) and R.Mokarram et.al(2009)[13,14,11].Briefly ,*sodium alginate-wpc* microcapsules were produced by mixing *18g alginate-wpc* solution(1:2 v/v),with *1-1.5 g* washed bacteria suspention .The *alginate-wpc* bacterial mix was subsequently emulsified in *100g* vegetable oil containing *5 gL^{-1} Tween80(Sigma-Aldrich,Pool,UK*)by using of a magnetic stirrer(*Heidolph*) set at *900 rpm* for 20min.Gelation was started by addition of

32 mL Ca^{+2}(*Merk,Germany*) containing emultion(*60 g* vegetable oil,*5 g* L^{-1} *Tween* 80 and *62.5 mM* $CaCl_2$).The alginate-wpc microcapsuls were formed during continuous stirring for 20 min.The beads were allowed to stand for 30 min to complete gelification process , then rinsed ,and subsequently kept in sterile peptone solution0.1% at *4?C*.Then centrifuged at *500 rpm* for *5 min* at *4?c*[11] .

C. numbers of entrapped cells

Freshly capsules(*1g*),were liquefied in *99 mL* of *1%(w/v)* sterile sodium citrate (*Merk, KGaA,Germany*) solution (*pH=6.0)* using a magnetic stirrer at room temperature for 10 min and enumerated triplicate in *MRS* agar at *37?c* for *46-72h*.For free cells ,used of sodium citrate instead of sterile peptpne water[11].

D. Shape of alginate-wpc capsuls

The shape of the microcapsules was observed using scanning electron microscope(*SEM*)(*LEO 1450 vp , Germany*) as previously ,described by Allan et.al(2008)[14].

E. Statistical analysis

In this study , probiotic culture was produced in two forms: free and encapsulated .A completely randomized factorial design was used for the analysis and the results were means of three replicates .*MINITAB (version 13.1*) was used to conduct analysis of variance(*ANOVA*) to determine differences between means of treatments. *exel* tests method, at *5%* level of significance was used to verify the significance of differences among means of treatments.

III .Results and discussion:

A. The number of cells entrapped and shape of alginate-wpc beads

The number of viable cells before encapsulating process with sodium alginate-whey protein concentration was 10.14-10.30 log cfu/ml that it decreased to 9.38-9.44 log cfu/ml, after encapsulation that it showed in the figure I.

encapsulation ,that it showed in the *figure I*.

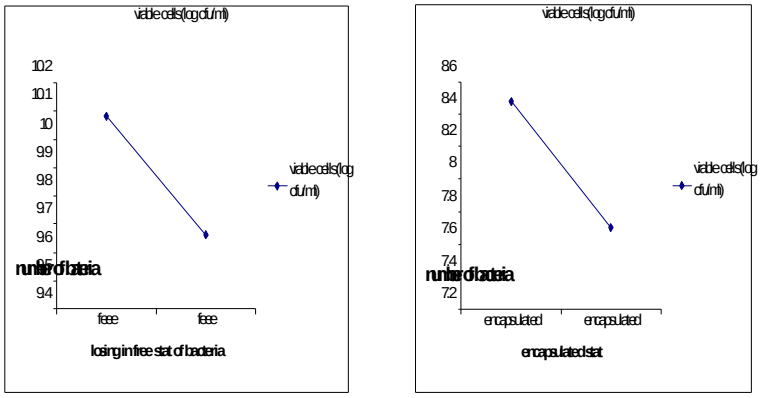

Figure I-enhancement in the number of probiotic cells after encapsulation

Thus, with comparison between this two conditions (before and after encapsulation), the results showed that, encapsulation process by emulsion technique, could have high efficiency of probiotic cell entrapment. The results of this study agree with previous research (truelstrup (2002) and Allan (2008)) [14]. In this way, microbeads produced with <100 diameter. R.R. Mokarram et al. (2009) showed that the micro capsuls were globular in shape after observing by a SEM electron microscopy [11]. The results in this study agrees with R.R.Mokarram (2009) calcium alginate beads (> 1mm) cause a good texture in live microbial feed supplements. [11]As illustrated in *figure II*, using emulsion technique cause to produce globular and spherical capsuls. Sultana et al. (2000) reported improvement in the encapsulation efficiency of L. casei in alginate beads as the biopolymer concentration was increased by addition of Hi-maize resistant starch [18].

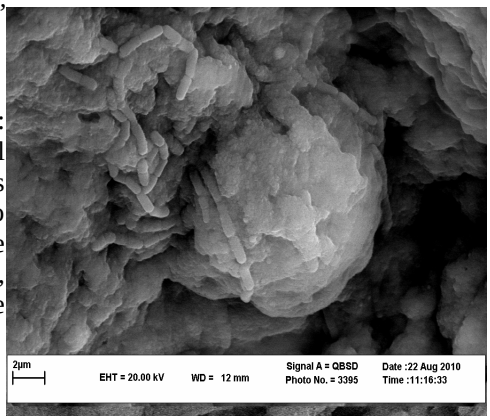

*Figure II-*the shape of micro capsuls

Probiotic cells survived 30% more when they were encapsulated [5]. Researchers showed controlling alginate bead sizes by using of emulsion agitation speed (400 rpm for 20 min) ,lead to produce beads in the range of 10-30 [5].

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