

Evaluation of Survival Rate and Physicochemical Properties of Encapsulated Bacteria in Alginate and Resistant Starch in Mayonnaise Sauce

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Abstract

Three types of symbiotic mayonnaise sauces, with free, encapsulated bacteria with calcium alginate (in 4% concentration) and encapsulated with calcium alginate and resistant starch by two strains of *L. acidophilus* and *L. casei* were manufactured in triplicate under the same conditions. The numbers of viable cells, pH, acidity and rheological properties of symbiotic mayonnaise samples during 91 days of storage in refrigerated (4°C) conditions was evaluated. It was observed that the number of viable cells of *Lactobacillus acidophilus* and *L. casei* was reduced significantly ($p < 0.05$) from day 1 to day 91 of storage period in Free State comparing encapsulated bacteria in both type of them. Reduction of viable cells encapsulated with Hi maize-alginate had a slower rate than probiotic cells encapsulated with calcium alginate (4%) in both *L. acidophilus* and *L. casei* mayonnaise samples container types. About changing in pH and increasing acidity of symbiotic mayonnaise sauces, the samples containing free probiotic cells had the highest changing and samples inoculated encapsulated cells with Hi maize-alginate mixture had lowest changing in these factors between all sample types. Also there weren't any significant changes in rheological properties of encapsulated symbiotic mayonnaise sample compared with control samples but in samples containing free cells a significant differences compared with control samples were seen. Finally, proved microencapsulation could help survival probiotic cells enough in therapeutic effects on consumers at the end of storage and modification encapsulation with adding Hi maize starch to calcium alginate improved preserving chemical qualities of symbiotic mayonnaise sauce. cells enough in therapeutic effects on consumers at the end of storage and modification encapsulation with adding Hi maize starch to calcium alginate improved preserving chemical qualities of symbiotic mayonnaise sauce.

Keywords: Calcium alginate; Resistant starch; Microencapsulation; *Lactobacilli*; Survival; Mayonnaise sauce

Introduction

The expansion of new food products turns out to be increasingly challenging, as it has to comply the consumers' expectations for products that are at once relish and healthy [1], in this regard, functional food which have health benefits further than their nutritional contents and especially foods containing probiotics are products that are growing in popularity [2].

Probiotics are defined as alive microorganisms which, when employed in sufficient amounts confer a health benefit to the consumers. Prebiotics are non-digestible substances which provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria [3].

On the protective level, probiotics can act as a 'barricade' against pathogens, by decreasing the luminal pH, producing bacteriocins, competing for limiting nutrients, activate mucosal immune responses and by sticking to the intestinal mucosa, thereby occupying an ideal position at the expense of potentially harmful colonizers [4]. It has been suggested that they must be presented at a minimum level of 106 CFU/g of Probiotic products or 107 CFU/g at point of delivery or be consumed in adequate amounts to comply a daily intake of 108 CFU [5].

Probiotics must be metabolically stable and active in the product, surviving while passing the upper digestive tract in large numbers, and showing the ability to adhere and colonize the intestine system [6]. The physical protection of probiotics by microencapsulation is a new method to improve the probiotic survival. Encapsulation helps to separate the bacterial cells from the effects of the harsh environment and gastrointestinal tract, thus potentially preventing cell from dead

[7]. The benefits of applying alginate as an encapsulating agent include: non-toxicity, formation of suitable matrices with calcium chloride to entrap sensitive materials such as living microbial cells, easiness in trapping living microbial cells and inexpensively [8].

Mandal, Puniya and Singh proved an increasing concentration of calcium alginate from 2% to 4% which had a positive effect on the survival of *L. casei* in simulated harsh conditions of GIT and heat processing, and calcium alginate in 4% concentration had the best effects [9].

The prebiotics, indigestible food components, affects the host by selectively instigating either the growth or activity, or both or a limited number of bacterial species already living in the colon [10]. A food product containing both probiotics and prebiotics is named as symbiotic or functional food. There is a synergy between probiotics and prebiotics in symbiotic products [11]. Combining alginate with starch as a prebiotic leads to better effectiveness of different bacterial cells specifically LAB. Also combination of calcium alginate with starch

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produces beads with good unified structures and prebiotic effect of the bead shell [12].

Food, especially dairy products are Preferred as an ideal mean for delivering probiotic bacteria to the human gastrointestinal tract [13] but restriction of dairy products such as the presence of allergens and the need for cold storage facilities, as well as an increasing request for new foods and tastes have begun a trend in non-dairy probiotic product development. Further, it is important to expand probiotic products with food and drinks that are part of day to day normal diet to maintain minimum beneficial level easily [14]. An alternative strategy to increase the efficacy of a probiotic treatment would be to use a food matrix which naturally contains a higher content of ingredients with protective properties [4]. The development of novel formulations is a challenging task but, regardless of whether the product is newer improved, the product must be stable during storage and transportation, easy to handle and apply, enhance the activity of the organism in the field, be cost-effective and practical.

Mayonnaise is of course not only one of the most favored sauces but also apparently one of the oldest and most greatly used sauce in the today's world [15]. Mayonnaise is a kind of semi-solid, oil-in-water emulsion. It is customarily prepared by carefully mixing egg yolk, vinegar, oil, and spices (especially mustard). Mayonnaise which made in this way typically contains 70–80% fat [16]. Physicochemical properties of food carriers used for probiotic delivery, such as buffering capacity and pH, are important factors that affect survival of the probiotic and therefore potential probiotic effects during gastric passage. Food formulations with acceptable pH ranges and high buffering capacity would increase the pH of the gastric tract and thereby improve the stability of probiotics [17], so mayonnaise due to having some advantages like its high buffering capacity, similar pH to some dairy products and its good matrix to protect of probiotics (because of having a large amount of protective ingredients like oil & egg) could be a new choice to produce a new functional food.

Material and Method

Preparation and enumeration of free and encapsulated probiotics

Pure freeze-dried probiotic culture of *L. casei* (Lc-01) and *L. acidophilus* (La5) were obtained from CHR-Hansen (Harsholm, Denmark) and were 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 collected by centrifugation at 600g for 10 min at 4°C (Microcentrifuges SIGMA; model 1-14K; Nr. 10021), then washed twice in sterile 0.9% saline under the same centrifugation conditions, and used in the microencapsulation process.

Bacterial counts were determined immediately after manufacturing of mayonnaise (at time 0) and during 91 days storage at 5°C. The samples of mayonnaise (10 g) were diluted in 100 ml sterile peptone water (0.1%) and 1 ml aliquot dilution was poured into plates of the MRS agar [18-20].

Counting of probiotic bacteria was achieved as described by Haynes and Playne [21]. All container of mayonnaise was sampled on each week of enumeration. All enumerating plates of *L. casei* and *L. acidophilus* were incubated at 37°C for 72 h under aerobic conditions. The averages of all results were expressed as colony-forming units per gram of sample (CFU/g). To count the encapsulated bacteria in mayonnaise, the entrapped bacteria were released from the beads according to the method of Sheu and Marshall [22]. Ten grams of mayonnaise were re-

suspended in 100 mL of phosphate buffer (0.1 M, pH 7.0), followed by shaking in a stomacher for 10 min. The mayonnaise sample containing free bacteria was treated in a similar way, in order to maintain the same treatment conditions. The counts (CFU/g) were determined by plating on MRS-agar as discussed above. The mayonnaise sample containing free bacteria was treated in a similar way so as to maintain the same treatment conditions. All experiments were done in triplicate.

Microencapsulation procedure

All glassware's and solutions used in the protocols were sterilized at 121°C for 15 min. Alginate beads were produced using a modified encapsulation method originally reported by Sheu and Marshall [22]. All treatments including a Sodium alginate solution 4% (Merck, Darmstadt, Germany) and a 2% alginate mixture in distilled water containing 2% Hi-maize resistant starch (Sigma, Aldrich, Germany) were prepared, sterilized by autoclaving (120°C for 15 min) and cooled to 38-40°C. Twenty milliliters of this solution and 4mL of cell suspension were transferred into a centrifuge tube (40 mL) and the content was vortexed to homogeneity. Soybean oil (100 mL) containing 0.2% Tween 80(emulsifier) was taken in a beaker (500 mL) and to this the alginate-cell mixture was added drop wise while stirring magnetically. After 5 min, a uniformly turbid emulsion was obtained to which 0.1M calcium chloride (100mL) was quickly added for hardening of microcapsules and breaking the emulsion. The capsules were harvested by centrifuging at 350 g for 10 min at 41°C and washed twice with distilled water. The long-term activity of BL21_MlrA in comparison with wild *Sphingomonas* strain confirmed much higher potential of the modified bacteria. Immobilization in alginate allowed forming beads with high activity toward MC. A column packed with alginate entrapped cells eliminated MC efficiently from contaminated freshwater. These promising results will broaden the perspective of practical application of microorganisms in bioremediation of freshwater.

Production of mayonnaise sauce

All ingredients used to prepare the mayonnaise, such as soybean oil, eggs, vinegar, mustard, sugar, and salt were purchased from the local supermarket. mayonnaise recipe was modified from Chen [23] with the following ingredients in percentage (w/w): pure whole egg (yolk + albumen)14%, vinegar (5% (w/v) acetic acid) 9%, soybean oil 74%, salt 1.0%, vanillin 0.1%, mustard 0.54%, sugar 1.0% and ground white pepper 0.36% [23].

The preparation was as follows: firstly egg and vinegar were mixed together and then all other ingredients except oil were added and stirred homogeneously by a mechanical overhead stirrer. Finally oil was added very slowly, while stirring at 1600 rpm for 1 min, followed by 2000 rpm for another 4 min. The mixture was cooled to 5-10°C and stored [16]. The mayonnaise was divided to six parts of 250 g (A, B, C, D, E and F). 1% (w/v) free *L. casei* and *L. acidophilus* were added to batches A and B, respectively. The initial counts of these bacteria in mayonnaise for A and B were about 6×10^9 and 5×10^8 (CFU/g), respectively and D portions of mayonnaise sauce mixed with freshly prepared encapsulated *L. casei* and *L. acidophilus* into 4% calcium alginate beads. The initial counts of these bacteria for C and D were about 3×10^8 and 3×10^9 (CFU/g), respectively. Also, microencapsulated bacteria *L. casei* and *L. acidophilus* into beads containing 2% calcium alginate and 2% Hi maize starch incorporated into E and F samples. The counts of probiotic cells in these types of mayonnaise were approximately 9.8×10^7 and 4×10^9 (CFU/g), respectively. All experiment was conducted in triplicate.

Chemical and physical analysis

The pH of the mayonnaise was measured using a digital pH-meter (Microprocessor pH-meter,). Titratable acidity was determined using the following formula at mill moles per 100 grams [24].

$$\text{Equation 1: } W = V \times 0.9 / M$$

W = acidity at mill moles per 100 grams

V = milliliters of consumed soda

M = sample weight (preferably 100 g)

All chemical measurements were done in triplicate.

Reaction of alginate without borate

One ml of concentrated sulphuric acid and 0.1 ml of 0.5 g/l of alginate solution sample were taken in the test tube. The test tube was cooled in the ice bath and the mixture was shaken and kept in the water bath at 55°C. After 30 min, the sample was cooled in ice bath and 30 µl of carbazole reagent (0.1% in ethanol) was added. The tube was allowed to stand for about 3h and then the absorbance was taken at 546 nm. The color was stable for 2 h.

Rheology analysis

The rheological measurements were evaluated using Brookfield rotational viscometer (DVIII) equipped by ULA. Obtained data analyzed by rheocalc 3.2 software, to determine its rheological properties. The temperature of the test (25 ± 0.1°C) was kept circulating-refrigerating water bath TC502 made by Brookfield Company.

Statistical analysis

The collected data was analyzed by SPSS statistics software, Version 18 edition. The mean values and the standard error were calculated from the data obtained with triplicate trials. These data were then compared by the Duncan's multiple range method.

Result and Discussion

PH changes during mayonnaise sauces storage

The pH changes during mayonnaise sauce storage in experimental mayonnaise sauces during storage at 5°C (per 7-day intervals has been presented) for a period of 91 days are shown in Figures 1 and 2.

The pH changes (at end of 91 days storage) of mayonnaise sauces with encapsulated probiotic bacteria was lesser than the mayonnaise sauces inoculated with free probiotic bacteria, also the mayonnaise sauces containing encapsulated cells with Hi maize-alginate in both of *L. casei* and *L. acidophilus* samples had the minimum of pH changes during storage times.

Therefore pH decreasing in symbiotic mayonnaise samples containing *L. acidophilus* in Free states was about 0.3 and in samples containing encapsulated bacterial cells in Hi maize and alginate mixture and encapsulated in alginate 4% were about 0.1 and 0.244 unit respectively. Also *L. casei* inoculated in symbiotic mayonnaise samples in un-encapsulated forms, decreased pH about 0.3579 and bacterial cells in encapsulated with Hi maize and alginate mixture and encapsulated in alginate 4% states were decreased the pH of their environments about 0.1 and 0.17 unit respectively.

Generally Probiotic bacteria are slow acid producers [25] hence changes of pH at storage period time in symbiotic mayonnaise sauces wasn't great.

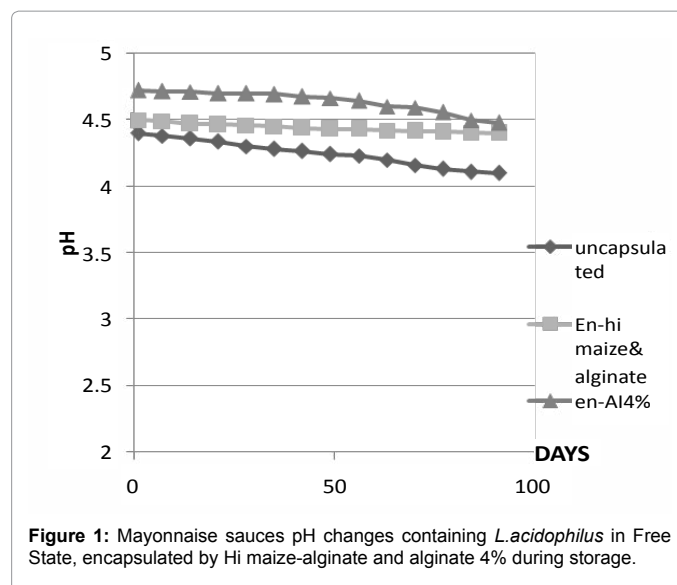


Figure 1: Mayonnaise sauces pH changes containing *L. acidophilus* in Free State, encapsulated by Hi maize-alginate and alginate 4% during storage.

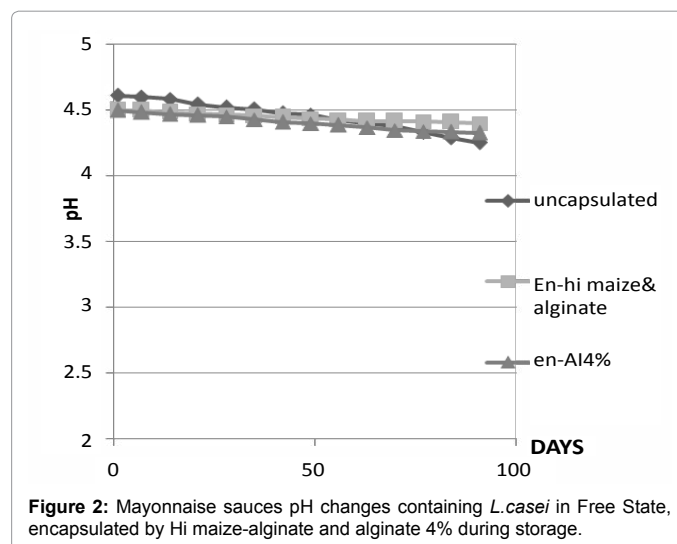


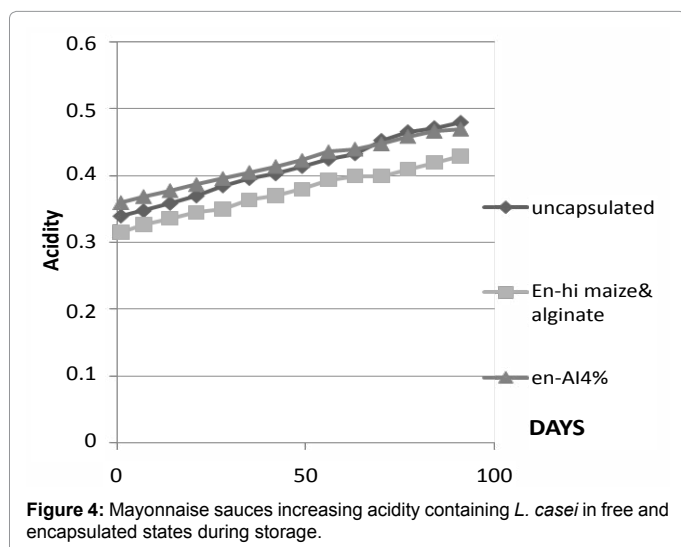
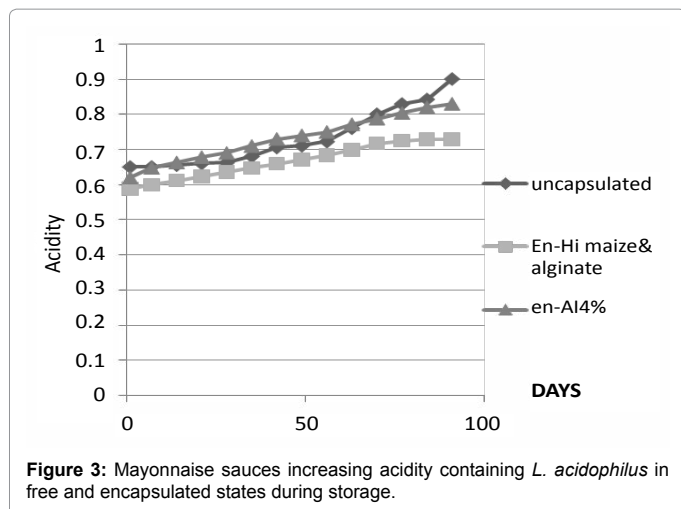
Figure 2: Mayonnaise sauces pH changes containing *L. casei* in Free State, encapsulated by Hi maize-alginate and alginate 4% during storage.

Producing acidity during mayonnaise storage

Measuring the acidity per 7- day intervals (Figures 3 and 4) indicated parallel results that were reached about pH changes during 91 refrigerated in 4°C. According these data's, increasing acidity in mayonnaise samples containing free *L. acidophilus* was approximately 0.25 and increasing acidity in samples containing encapsulated cells (similar strain) in Hi m aize and alginate mixture and sauces samples those blended with encapsulated cells in calcium alginate 4% were 0.142 and 0.21 respectively.

Also increasing acidity in mayonnaise samples containing *L. casei* in free, encapsulated with Hi maize-alginate and calcium alginate 4% were 0.14, 0.1 and 0.11 respectively.

As a result increasing of acidity from starting storage until 91 days in mayonnaise sauces those were containing encapsulated probiotic cells were less than free states, also the probiotic cells in Hi maize-alginate coating is produced the minimum level of acidity in treatment containers in two kind of *L. acidophilus* and *L. casei* probiotic bacteria's.



Therefore, microencapsulation of free cells greatly, but not completely, restricted their metabolic activity [26]. Even though symbiotic mayonnaise samples inoculated with *L. acidophilus* had a little more acid producing compared with samples included *L. casei* bacterial cells.

Survival of free and microencapsulated *L. acidophilus* & *L. casei* in mayonnaise sauce during storage time

The effect of encapsulation with Hi-maize starch and alginate mixtures (2% concentration) and also by alginate (4% concentration) on the viability of probiotic microorganisms comparing with free cells in the mixture of mayonnaise sauces during 91 days of refrigerated storage period (4°C) was investigated. Logarithmic numbers of surviving bacteria (log cfu/mL) were measured at seven-day intervals are shown in the Table 1.

According to Table 1, viable cells of *L. acidophilus* mixture with mayonnaise sauce showed 2.659 log decreases for the Free State after 91 days, while the encapsulated state with alginate (4% concentration) of the same strains showed a decrease of 1.48 logs. In the case of encapsulated bacteria (*L. acidophilus*) with Hi-maize starch and alginate mixtures, the cell numbers dropped substantially about 1.1497

log numbers by 91 days of storage at 4°C. The loss of *L. acidophilus* showed significant differences ($P < 0.05$) between the free and both type of encapsulated states in symbiotic mayonnaise sauce and there wasn't a significant difference between two type of encapsulated types, but as was observed, using the Hi maize starch along with alginate in micro beads cause improving 22.3 percentage the survival of *L. acidophilus* bacteria during storage period than usage alginate (4%) lonely.

Sultana et al., [20], Homayouni et al., [8] and Mirzaei et al., [13], showed that using the Hi maize in encapsulation probiotics can significantly improving protection of viable bacterial counts in symbiotic ice cream and Iranian white brined cheese respectively [7,12,19]. The mayonnaise sauce samples that were contain of *L. casei* also showed the same results. In the case of free *L. casei*, the cell numbers dropped substantially about 2.52 log numbers by 91 days of storage at 4°C, however the decrease of the viable counts of encapsulated bacteria in Hi maize-alginate and encapsulated bacteria in alginate (4%) were 1.1log and 1.377log respectively that indicates 20.11 percentage improving on survival of cells in the case of encapsulated with alginate-Hi maize comparing with cells coated with alginate 4%.

There was a significant difference ($P < 0.05$) between means of the counts of viable cells per 7-days intervals during storage time (91 days) in Free states of *L. casei* and both of encapsulated samples, however there wasn't observed any significant difference in comparison means of viable cells during storage time between Hi maize- alginate and alginate4% encapsulated forms. These results indicate remarkable of increasing viability and resistance of probiotic cells by encapsulation against harsh environmental conditions at storage times.

Increasing cell stability of encapsulated probiotic bacteria with calcium alginate over the refrigerated storage time has been previously reported by Krasakoopt, Kailasapathy and Mortazavian, that which are in agreement with the results obtained from present research [27-29]. Also these data's again confirmed advantages of encapsulation of probiotic cells with Hi maize in combination with alginate that more than twice protection of viable counts of bacteria compared Free State caused. In addition, it was demonstrated that, encapsulated cells required longer time to decrease one log cycle in viable counts and encapsulated bacterial cells with alginate and resistant starch had the most slow rate of loss viable cell rate.

The supporting impact of microcapsules on probiotic bacteria arises from their barrier properties against the detrimental conditions of mayonnaise environment such as low pH, organic acids and antibacterial effects of mustard [30] and the existence of some antibacterial enzymes' from albumen such as lysozyme, conalbumin and avidin.

Overall the problem of sensitivity to acidity of the probiotic culture is compounded by the fact that acidity may increase during storage, a phenomenon known as 'over acidification' [31]. Increasing rate of viability decline of free probiotic bacteria can be attributed to the adverse effects of accumulated organic acid molecules and hydrogen ions after they grew inside the mayonnaise environment at days 49 and 56 in *L. acidophilus* and *L. casei* respectively.

About encapsulated bacteria accumulating of organic acid molecules and hydrogen ions had a lower rate because of limitation accessibility to nutrients'. This fact is indicated by slower rates of pH drop and acidity increase during refrigeration of both encapsulated types of probiotic bacteria which caused a slower rate of viability decline comparing free probiotic bacteria cells. But the greater loss of survival of encapsulated *L. acidophilus* and *L. casei* cells at the end of

storage period time may be due to the gradually increased amount of organic acid molecules inside the capsules after slow diffusion through the capsules pores in mayonnaise environment.

This suggests that the starch grains were presented in the cavities in the alginate matrix [19] may could fulfill the shell surface of beads and due to more restriction of crossing nutrient and metabolites across shell matrix and also reduction of entrance organic acids and other adverse components (such as some antibacterial components of mustard or antibacterial enzymes' of albumen) exist in mayonnaise environment. In this regard, reduction of metabolic activity of probiotic cells in the case of coated cells with resistant starch and alginate caused to a very gentle increasing of acidity and so slowing loss of viable cells during refrigeration period.

With comparing two strains of probiotic cells, it was found that loss of all types of free and coated bacterial cells of *L. casei* from days 1 to 91th was lower than the same type of *L. acidophilus* cells. In this way the survival of bacteria against unfavorable conditions could be species dependent. This finding is in agreement with those of Haynes, Kailasapathy and Homayouni [23,28].

Comparing rheological properties of mayonnaise sauces before and after storage period

The viscosity of Non-Newtonian fluids cannot be calculated using only the base properties, the main calculation should be in the flow model. In order to correlate flow model and medium model with the smallest change possible. Since the flow behavior index is only a function of the states of the fluid such as the temperature, it can be defined and calculated in the medium model. By adding this partial function for the definition of the flow behavior index in the base medium model, implementing the governing equations or tables for all the fluid models is made possible.

In case the fluid is a Newtonian fluid such as water, the flow behavior index should be set to the constant number of "1" and with decline of this index from "1" demonstrate fluid is nearing shear-thinning properties [32,33].

In this work comparing consistency indexes and flow indexes of symbiotic mayonnaise sauces before and after period of storage time were studied.

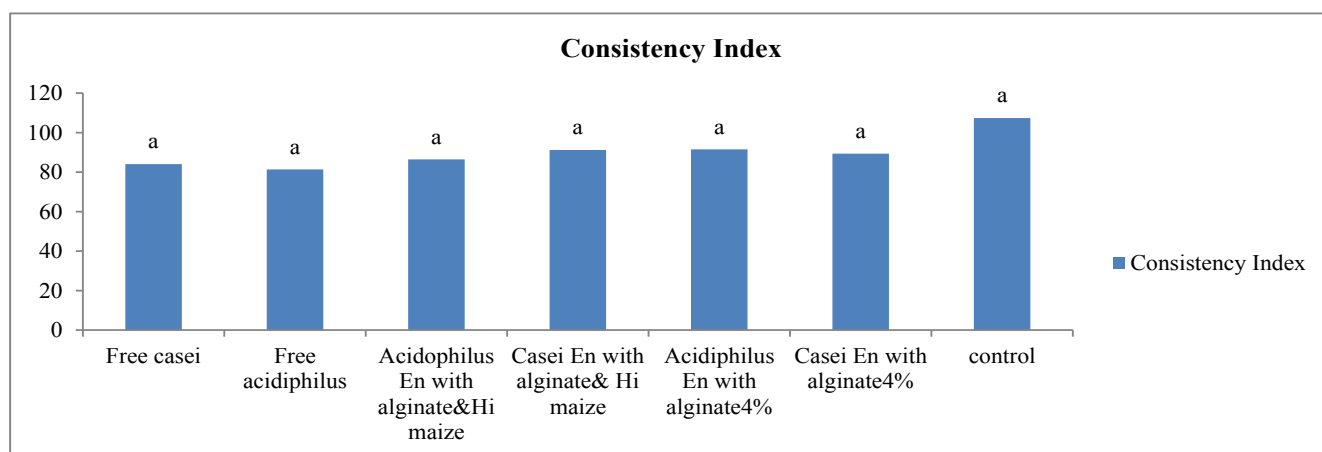


Figure 5: Consistency indexes were compared by the Duncan's multiple range method at the start of storage time ($p < 0.05$).

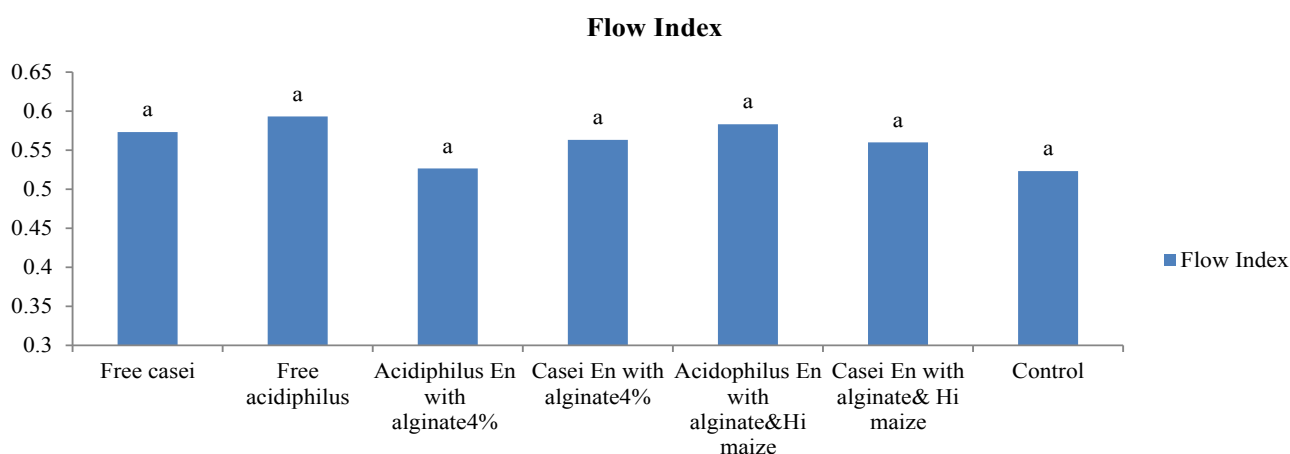


Figure 6: Flow indexes were compared by the Duncan's multiple range method at the start of storage time ($p < 0.05$).

According Figure 5 consistency indexes of symbiotic mayonnaise sauces in neither samples at the start of storage period hadn't significant difference ($p < 0.05$) with control treatments (control samples hadn't any probiotic cells) and this fact indicates that adding free probiotic cells or encapsulation operation hadn't significant changes in rheological properties of mayonnaise sauce.

This fact proved while it wasn't any significant differences ($p < 0.05$) between flow indexes of control and symbiotic mayonnaise sauces in both free and encapsulated probiotic cell samples (both of *L. casei* and *L. acidophilus*) at the start of storage period (Figure 6).

So there weren't significant changes in rheological properties of our symbiotic product by inoculation of probiotic cells in free or encapsulated form in mayonnaise sauces samples (Figure 7).

After 91 days storage period rheological properties of symbiotic mayonnaise sauces samples were measured and compared with control samples to determine any changes in consistency indexes and flow indexes.

The results (Figures 8 and 9) indicated that the consistency indexes in control samples and symbiotic mayonnaise sauces containing encapsulated probiotic bacteria's (both *L. casei* and *L. acidophilus* in two kinds of beads) were not any statistical significant difference

($p < 0.05$). But all symbiotic mayonnaise samples containing free cells (of both species) had a significant differences ($p < 0.05$) in consistency indexes compared with control mayonnaise samples, however there were not any significant differences between mayonnaise samples containing encapsulated bacteria and samples containing free cells (both species in two kinds of beads) in consistency indexes .

About flow index, after period of storage time there weren't significant differences ($p < 0.05$) between control samples and samples containing encapsulated probiotics (both strains, both coating) but we had significant differences ($p < 0.05$) in flow indexes of control samples and mayonnaise containing free cells (both strains). In other wise we saw significant differences ($p < 0.05$) in flow indexes neither of samples containing free *L. acidophilus* and samples containing encapsulated cells though nor about samples containing free *L. casei* cells.

Thus after 91 days storage period we didn't see significant changes in rheological properties of encapsulated probiotic mayonnaise samples (Figure 7).

Conclusion

In this study we proved that mayonnaise sauce could be serving as carriers for delivering the probiotic bacteria into the human gut. Also this work demonstrated that microencapsulation of probiotic bacteria significantly and noticeably improves their viability in mayonnaise sauce throughout the 91 days refrigerated storage at 4°C.

The high total solids level in mayonnaise sauce including the oil and egg solids may provide protection for the probiotic bacteria. A modified method of encapsulation has been reported in this study. There appears to be much potential for using the prebiotic resistant starch with alginate during encapsulation since it does enhance the survival of the probiotic bacteria.

According to the results of this study, microencapsulation of *L. acidophilus* and *L. casei* cells with calcium alginate gel and resistant starch can successfully keep the count of this probiotic bacterium higher than encapsulated cells of them with calcium alginate 4%, but enough in both methods for the therapeutic minimum in the mayonnaise sauce [34].

So at the end of 91 days of storage, the number of viable probiotic

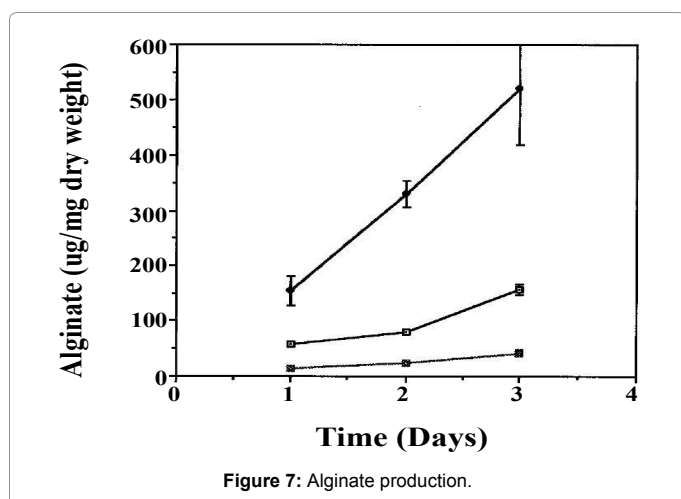


Figure 7: Alginate production.

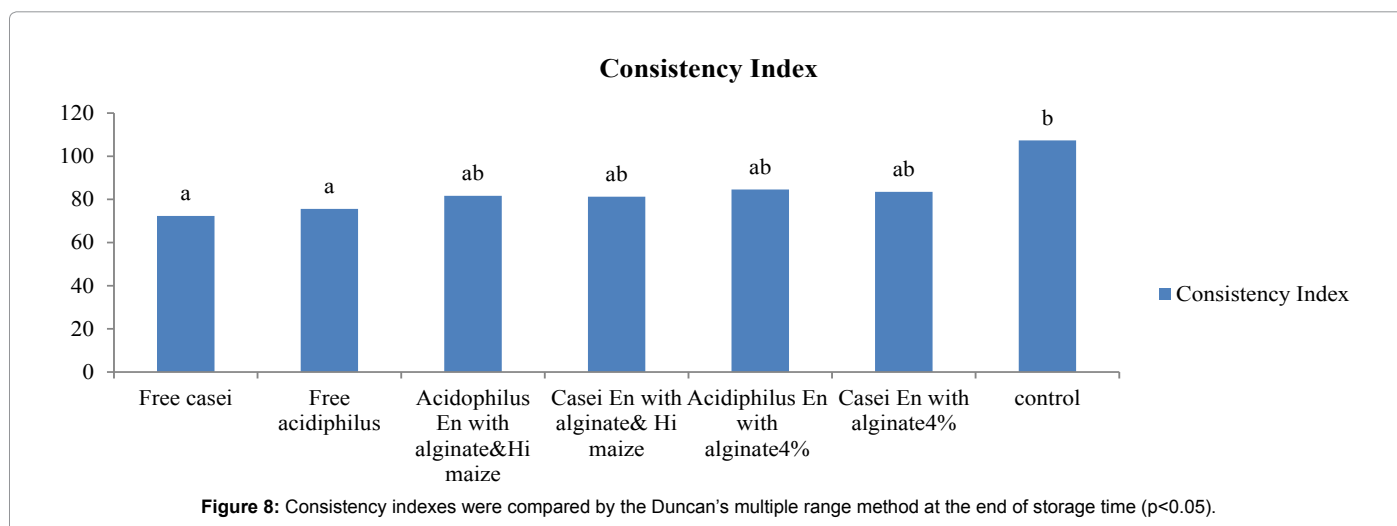


Figure 8: Consistency indexes were compared by the Duncan's multiple range method at the end of storage time ($p < 0.05$).

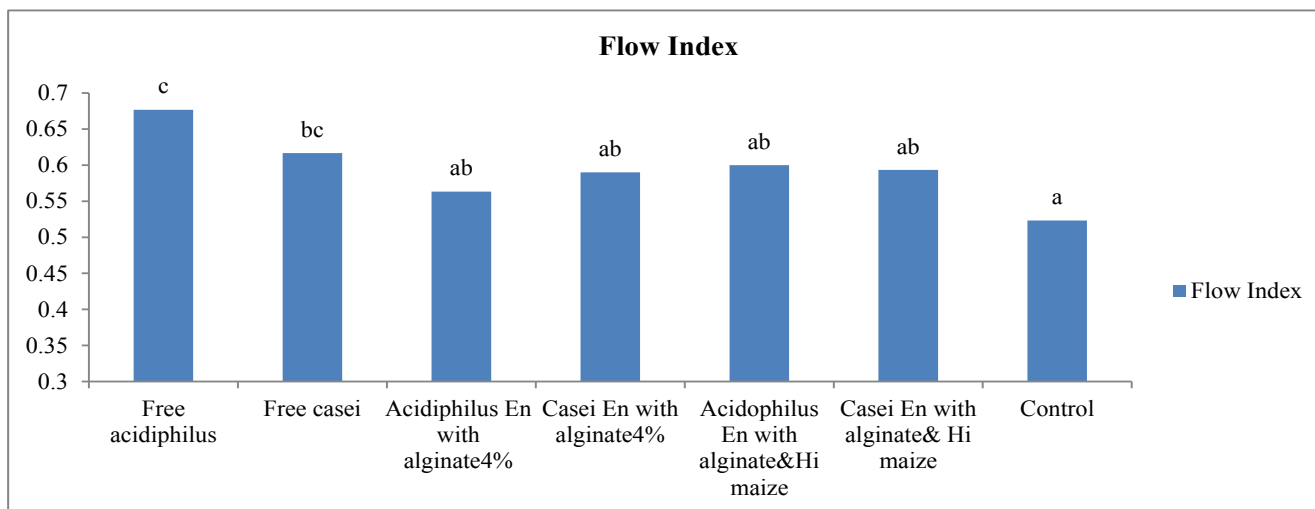


Figure 9: Flow indexes were compared by the Duncan's multiple range method at the end of storage time ($p < 0.05$).

Days	<i>L.acidophilus</i>			<i>L.casei</i>		
	Free	En-Hi&AL ^a	En-AL4% ^b	Free	En-Hi&AL ^a	En-AL4% ^b
0	8.698	9.601	9.42	8.748	8.301	8.477
7	8.6	9.3	9.397	8	8.278	8.384
14	8.505	9.1	9.389	7.778	8.146	8.291
21	8.468	8.9	9.318	7.568	8.1	8.198
28	8.451	8.85	9.24	7.477	8.079	8.105
35	8.301	8.82	9.113	7.486	7.994	8
42	8	8.77	8.845	7.409	7.929	7.919
49	7.9	8.7	8.654	7.358	7.845	7.786
56	7.568	8.66	8.397	7.278	7.69	7.733
63	7.185	8.602	8.38	7.146	7.582	7.6
70	6.948	8.54	8.25	7.095	7.462	7.337
77	6.711	8.486	8.11	6.845	7.304	7.284
84	6.474	8.47	8	6.544	7.227	7.22
91	6.039	8.45	7.94	6.23	7.204	7.1

a: Encapsulated by Hi maize starch and Calcium alginate (2% concentrations)

b: Encapsulated by Calcium alginate (4% concentrations)

Table 1: Viability of probiotic bacteria (log cfu/mL) in encapsulated and un-encapsulated treatments over a 91-day refrigerated storage period (4°C), per 7-day intervals.

bacteria in mayonnaise types containing *L. acidophilus* and *L. casei* that encapsulated (with Hi maize-alginate and alginate 4%) were higher than what was recommended by the International Dairy Federation (107 CFU/g) (Table 1).

The addition of probiotic cultures in encapsulated forms tend to slow down the post acidification during storage of mayonnaise sauces. Thus according our views encapsulation led to limitation accessibility of probiotic bacterial cells to consuming nutrients and caused to restriction to increasing acidity and pH changing comparing the Free State probiotic cells in mayonnaise sauces samples during storage period [35].

The pH changing and increasing of acidity in symbiotic mayonnaise samples containing *L. acidophilus* and *L. casei* encapsulated with Hi maize and alginate were less than mayonnaise samples that contained encapsulated cells with calcium alginate 4% but we've seen the most changing in acidity and pH in samples contains free cells (in both kind of probiotic bacteria's). This may be due to the slower uptake of

nutrients and the slow release of metabolites across the encapsulated alginate shell and particularly in beads those filled with resistant starch particles [19].

Also about rheological changes in symbiotic mayonnaise sauces it's defined that inclusion of probiotic cells in free or coated states couldn't have a significant effect on consistency and flow indexes of mayonnaise sauces but after the period of storage the high ratio of increasing acidity and producing more of other metabolites in mayonnaise containing free probiotic cells (*L. casei* & *L. acidophilus*) comparing with control samples we had a significant changes in flow and consistency indexes and these samples hadn't initial textures.

Even though by the microencapsulation of probiotic cells additional of biological and chemical properties, we could save rheological (flow and consistency indexes) qualities' of symbiotic mayonnaise sauces and so there weren't significant changes in these factors comparing control treatment's after 91 days storage period.

According to these results, microencapsulation with alginate gel and resistant starch had the best efficiency to survive the probiotic cells enough for therapeutic effects and as well as increasing storage time with less chemical changes in symbiotic mayonnaise sauce during refrigeration at 4°C.

Alginates have a clear antibacterial effect as evidenced by the reduction in growth following the addition of alginates. This inhibitory effect of alginates on bacterial growth was found to be bacteriostatic as the cultures grew well when plated onto agar. The bacteriostatic effect is likely in part to be due to the iron chelating capabilities of alginates as the antibacterial effect was lost when the MIC assay was repeated using alginates loaded with iron. Thus in summary we provide evidence that it is likely that alginates mediate these alterations in the gut micro flora through their potential to sequester iron.

Further studies are needed to evaluate the protection effect of microencapsulation on the probiotic survival in the gastrointestinal tract.

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