The comparison of Encapsulated Lactobacillus Acidophilus by Calcium Alginate 4 % , Mixture of Alginate-high Maize Resistant Starch with Added UncapsulatedBacteria to Symbiotic Mayonnaise

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ABSTRACT: This study aimed at producing symbiotic mayonnaise through adding Lactobacillus Acidophilus which had encapsulated by calcium alginate (4%), a mixture of high maize starch (2%) and calcium alginate (2 %) to mayonnaise emulsion and a treatment only by adding uncapsulated bacteria. The experiments on total count, acidity pH and viscosity revealed that the most resistance to change in initial total count, pH and acidity were exhibited in all encapsulated samples by mixture of high maize starch and calcium alginate (each of them was 2% in the mixture), then encapsulated bacteria by calcium alginate (4 %) and treatment containing uncapsulated bacteria respectively.

Keywords: Encapsulation, Probiotic , Lactobacillus , Mayonnaise and Modified Starch

INTRODUCTION

Changing of lifestyle and people habits along with women occupations have caused a growing demand of consuming fast with. In this case, Sauces are the most important groups of seasons and play an important role in fast food. Mayonnaise which defines as a W/O emulsion produces by mixing , egg yolk , vinegar , mustard , vegetable oil (Payan , 1996).Probiotics ability in curing of gastric micro flora and prevent gastrointestinal disorders has been proved(Ranadheera, 2010).Today, consumers expect more being healthy of food and have been sensitive to their healthiness 7, therefore functional foods such as probiotic and prebiotics has become more popular(Saarela, 2006).Probiotic benefits are including, obtaining of micro flora, protecting against pathogens, reducing gastrointestinal disorders occurring by lactose, decreasing of hypercholesterolemia and high blood pressure(Possemiers, 2010).L. acidophilus is a member of hetero fermentative group that ferments sugar to lactic acid and reduce pH less than 5. This variety is naturally occurring in gastrointestinal system in human and animals and known as probiotic (Mokarram, 2009).Encapsulation is physical process in which to entrap a micro stuff (D<1mm-mm).by encapsulation , food flavors covering , adjusting the diffusion of material and improving shelf life may possible(Burgain, 2011).Microencapsulation of probiotic cells is one of the most modern technique which
categorizes in extrusion and emulsion methods. Overall microencapsulation of probiotic can protect them against high acidity, bile salts, deep freezing, freeze drying, oxygen molecule, heat shock (Larousse and Brown, 1997). The aim of this study was the possibility of injection of L acidophilus (uncapsulated and encapsulated with alginate (4% sodium alginate; mixture of 2% sodium alginate and 2% modified starch) in Mayonnaise during 92 days and investigation of its physiochemical properties.

**MATERIALS AND METHODS**

Lyophilized L. acidophilus injected in MRS broth media, cultured at 37°C for 24 hr. these bacteria cultured again in MRS media and centrifuged at 600g for 10 min. Separated cells cleansed by normal saline 0.9% and finally each variety dissolve in normal saline 0.5 ml to 10^10 cfu/ml and prepared for encapsulation (Chávarri, 2010, Wunwisa, 2004 and Mandal, 2006).

**Encapsulation**

Encapsulated alginate seeds prepared base on Sheu and Marshal Method (1993). All stuffs including sodium alginate (4%), modified high maize starch (2%), sodium chloride 0.1 M were sterilized in autoclave at 121°C for 15 min then cooled to 38°C. 20 ml of prepared solutions carried to centrifuge and vortex to become homogenous, then 100ml soybean oil containing 0.2% Tween 80 (emulsifier) mixed with aforesaid solutions on a mixer (180rpm) to reach a n opalescent color. Calcium chloride 0.1 M added to harden microencapsulates and emulsion breaking after 5min and finally the mixture centrifuged by 300 g at 41°C for 5min, twice cleansed by distilled water and centrifuged again.

**Mayonnaise production**

Mayonnaise dressing prepared base on Chen, (20050 recipe 4. the ingredients were vinegar containing acetic acid (5% w/w) 9%, soybean oil 74%, egg 14%, salt 1%, vanillin 0.1%, sugar 1% and white pepper 0.36%. Sugar and vinegar mixed together then mixed to reach a homogenous mixture by a mechanical mixer and all ingredients added except oil, finally soybean oil added gently and mixed (1600rpm, 4 more min by 2000rpm) then cooled to 5-10°C before inoculation.

**Counting of inoculated bacteria**

Pour plate method using MRS-Agar culture media was used to counduct counting of grown bacteria at 5°C during 91 storing days. 1 g of mayonnaise samples weighed and reach to the volume with 10 ml of phosphate buffer (0.1 M pH= 7) and shook 10 min in 120rpm to lyse the capsulations, then resulted homogenous solution which contains distillation of 0.1 released bacteria reached to distillation of 10^-12, pour plated 48 hr and incubated by parafilm at 37°C. The amount of bacteria measured base on the following formula:

\[
N.O \text{ of bacteria} = \frac{10 \times \text{counted colonies}}{\text{distillation of solution of poured plate}}
\]

**Physiochemical experiments**

pH: titrable acidity measured base on national iran standard NO 5222 and in 7 days time intervals using the following formula

\[
W = \frac{V \times 0.9}{M}
\]

In which:
- V: the amount of consumed sodium hydroxide
- M: the sample weight in titration
- W: acidity percent

All results were analyzed by SPSS software, ver 18 by Duncan test.
RESULTS AND DISCUSSION

Comparison of the amount of present L. acidophilus in mayonnaise samples
Results revealed that both capsulated treatment showed no significant differences during 91 days storing at 4 °C while both treatments contained uncapsulated bacteria exhibited (p<0.05).
The number of uncapsulated L.acidophilus reduction was 2.659 log unit in samples containing uncapsulated cells. It was 1.1497 in mixture of high maize resistant starch (2%), calcium alginate (2%) and 1.48 in treatment contains encapsulated bacteria by calcium alginate 4%.

Comparison of acidity
Samples containing encapsulated bacteria with mixture of calcium alginate (2%) and hi maize resistant starch (2%) differed significantly from other samples during 91 days of storing. Acidity changes in treatment were 0.25, 0.142 and 0.21 for treatment containing uncapsulated bacteria, treatment containing encapsulated bacteria by calcium alginate and starch and the one contains only encapsulated bacteria by calcium alginate respectively (figure 1).

Comparison of samples pH
Comparing reduction of pH between treatments showed that treatment containing uncapsulated bacteria 0.3, treatment containing encapsulated bacteria by calcium alginate and starch 0.1 and the one contains only encapsulated bacteria by calcium alginate was 0.244 in pH changing. These results exhibits the significant differences among samples (p<0.05) (figure 2).

Conclusion
Encapsulation cause to change less in product during storage and improving shelf life of it and remained encapsulated bacteria were double than the population of uncapsulated bacteria in product.

Results also revealed that by replacing 2% modified starch hi maize to calcium alginate mixture, the life of probiotic bacteria during storing and less pH and acidity changing in final product. Mortazavaian, (2008) reported that encapsulated bacteria by calcium alginate in Doogh can increase the life of probiotic bacteria and decrease pH changing in Doogh. These results are in agreement with Homayouni, (2008).and Khalida Sultana, (2000).

Table 1. counted live bacteria in 7 days time intervals during 92 days of storing

<table>
<thead>
<tr>
<th></th>
<th>Free</th>
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En-hi& Al= encapsulated with hi maize starch and calcium alginate
En-Al =encapsulated with alginate
Figure 2. pH changes in encapsulated, encapsulated hi maize alginate, encapsulated with alginate samples

Figure 3. Acidity changes in encapsulated, encapsulated hi maize alginate, encapsulated with alginate samples

REFERENCES


