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RESEARCH ARTICLE

# A PRELIMINARY STUDY ON STABILITY AND VIABILITY OF ENCAPSULATED PROBIOTIC YEAST *SACCHAROMYCES BOULARDII AND SACCHAROMYCES CEREVISIAE* AND ITS ABILITY TO BIND MYCOTOXIN.

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**Abstract**

Microencapsulation can be defined as the process of entrapment or enclosure of microorganisms or cells by means of coating them with proper hydrocolloid(s) in order to segregate the cells from the surrounding environment. The objective of the study was to microencapsulate the probiotic yeast *Saccharomyces boulardii* and *S. cerevisiae* by different methods namely water in oil (w/o) emulsion method, Water in oil in water (w/o/w) emulsion method and microencapsulation using sodium alginate. The optimum pH and temperature and was deduced for all the three techniques. The stability, viability and Aflatoxin binding ability of the probiotic yeast were also done. It was found that the Optimum pH and Temperature for all the three technique were 5 and 40°C respectively. Water in oil in water (w/o/w) emulsion method was found to be better method when compared with the other two methods. It also was able to bind with the Aflatoxin.

**Keywords:** Microencapsulation, *Saccharomyces boulardii, Saccharomyces cerevisiae,* Aflatoxin.

## Introduction

Probiotics are microorganisms which settle in the intestine medium and render healthful effects on the host (humans or animals), substantially via maintenance and improvement of the microbial balance (between the healthful and harmful microorganisms) of the intestine (Fuller 1989; 1991; Goldin 1998; Gismondo et al.1999) over the years the Word probiotic has been used in several different ways. It was originally used to describe substances produced by one protozoan which stimulated another but was later used to describe animal feed supplements which had a beneficial effect on the host animal by affecting its gut flora (Parker 1974). Direct-fed microbials (DFM) are a category of probiotics that are used in the animal industry in the United States (Fuller 1989; Schrezenmeir and De Vrese 2001). Typically, DFM as a category includes: traditional “probiotics” (live bacterial, fungal or yeast cultures), non-viable bacterial, fungal or yeast cultures, or end-products of bacterial, fungal or yeast fermentations. Some of these products include cultures that utilize a mechanism of action similar to Competitive Exclusion Cultures, but are not included in that FDA definition (CVM 1997).

The probiotic concept is open to lots of different applications in a large variety of fields relevant for human and animal health. Probiotic products consist of different enzymes, vitamins, capsules or tablets and some fermented foods contain microorganisms which have beneficial effects on the health of host. They can contain one or several species of probiotic bacteria. Most of products which destine human consumption are produced in fermented milk or given in powders or tablets.

These capsules and tablets do not used for medicinal applications. They are just used as health supporting products. The oral consumption of probiotic microorganisms produces a protective effect on the gut flora. Lots of studies suggest that probiotics have beneficial effects on microbial disorders of the gut, but it is really difficult to show the clinical effects of such products. The probiotic preparations use for traveller's diarrhoea, antibiotic associated diarrhoea and acute diarrhoea which is showned that they have positive therapeutic effect (Gismondo, et al. 1999, Çakır 2003). More than 400 bacterial species exit in human intestinal tract. It is an enormously complex ecosystem that includes both facultatively anaerobic and anaerobic microorganisms (Naidu, et al. 1999).

Microencapsulation, as one of the newest and most efficient methods, has recently been under especial consideration and investigation. From a microbiological point of view, microencapsulation can be defined as the process of entrapment/enclosure of microorganisms cells by means of coating them with proper hydrocolloid(s) in order to segregate the cells from the surrounding environment; in a way that results in appropriate cell release in the intestinal medium (Sultana et al., 2000; Krasaekoopt et al., 2003; Picot and Lacroix, 2003a).

Emulsion technique has been successfully applied for the microencapsulation (Audet et al., 1988; Lacroix et al., 1990). In this method, small volume of cell/polymer slurry (dispersed phase) is added to the large volume of vegetable oil (as a continuous phase) such as soy oil, sunflower, corn, and light paraffin oil. In contrary with the extrusion technique, it can be easily scaled up and the diameter of produced beads is considerably smaller (25 μm-2 mm). The probiotic can be microencapsulated by this emulsion technique. The main advantages of encapsulating a probiotic are it protects and enhances survival of bacteria in foods. It allows entrapped probiotic microorganisms to be incorporated into dairy products such as yogurt, cheese, and frozen milk product. About 40% of *Lactobacilli* survive in frozen ice cream when entrapped in calcium alginate sphere than free cells .The encapsulation of *Bifidobacterium* significantly improves survival, compared to free cells, throughout storage from 43% - 44% to 50% - 60% in frozen dairy product. Microencapsulated form of *B. pseudolongum* exhibits improvement of survival in a simulated gastric environment when compared to free viable microorganisms.

The emulsion method of microencapsulation is generally done by three methods they are water in oil (w/o) emulsion method, Water in oil in water (w/o/w) emulsion method and microencapsulation using sodium alginate.

A mycotoxin is a toxic secondary metabolite produced by organisms of the fungi kingdom, commonly known as molds. The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops. One mold species may produce many different mycotoxins, and the same mycotoxin may be produced by several species. Most fungi are aerobic (use oxygen) and are found almost everywhere in extremely small quantities due to the minute size of their spores. They consume organic matter wherever humidity and temperature are sufficient. Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high. The reason for the production of mycotoxins is not yet known; they are not necessary for the growth or the development of the fungi. Mycotoxicosis is the term used for poisoning associated with exposures to mycotoxins. The symptoms of mycotoxicosis depend on the type of mycotoxin; the concentration and length of exposure; as well as age, health, and sex of the exposed individual. The major groups of mycotoxins are Aflatoxin**,** Ochratoxin, Citrinin, Ergot Alkaloids, patulin, Fusarium.

The aim of the study is to encapsulate the probiotics cultures of *S.boulardii* and *S.cerevisiae* by using three different techniques (water in oil (w/o) emulsion method, Water in oil in water (w/o/w) emulsion method and microencapsulation using sodium alginate) and checked for their viability, survival of the encapsulated probiotics under the stimulated gastric conditions and checked the ability of probiotic bind towards the mycotoxin.

The Objective of the present study is to encapsulate the probiotics cultures of *Saccharomyces boulardii* and *Saccharomyces cerevisiae* by using three different techniques (water in oil (w/o) emulsion method, Water in oil in water (w/o/w) emulsion method and microencapsulation using sodium alginate) and checked for their viability, survival of the encapsulated probiotics under the stimulated gastric conditions and checked the ability of probiotic to bind with the mycotoxin.

## Materials and Methods

**Culture collection**:

The culture used for the studies was obtained from T.Stanes & company limited, Coimbatore.

**Simple staining:**

The fresh culture of *Saccharomyces boulardii* and *Saccharomyces cerevisiae* was taken and thin smeared over a clean glass slide; crystal violet was added to the smear for 2 minutes and washed the slide, examined under microscope.

**Carbohydrate fermentation test:**

Carbohydrate fermentation test detect the ability of microorganisms to ferment a specific sugar. Here the fermentation test was done for *Saccharomyces boulardii* and *Saccharomyces cerevisiae*. The phenol red broth was prepared and ten different types of sugars namely glucose, galactose, fructose, lactose, cellobiose, sucrose, mannitol, maltose, sorbitol and xylose were added to it, Durham's tubes were introduced into it and were sterilised. The cultures were inoculated into the respective tubes and were incubated at 30°C for 24-48 hours. After the incubation results were observed.

**Standardisation of encapsulation procedures**:
The encapsulation procedures were standardised by three methods namely:

1. Water in oil (w/o) emulsion.
2. Water in oil in water (w/o/w) emulsion.
3. Encapsulation by sodium alginate method.

**Water in oil (w/o) emulsion:**

The water/oil emulsion was prepared by mixing 42.2g of water and 5.4g of isolated soya protein was mixed and stirred at 200 rpm for 2 mins in a magnetic stirrer (Remi, Mumbai). And 1ml of culture was added to it. Afterwards 52.6g of oil was slowly added and gently stirred in order to obtain a stable emulsion. (15mins, 800 rpm and magnetic stirrer (Remi-Mumbai) the emulsion formed and was viewed under the microscope for the stability.

**Water in oil in water (w/o/w) emulsion:**

The water in oil in water emulsion were carried out by two step protocol that consisted on the preparation of a simple water oil emulsion and a further addition of this emulsion to a second aqueous phase. The simple water oil emulsion was prepared by adding the aqueous phase containing 14g of water that include 1% of carrageenan and 1ml of culture was added. To the oil phase 40g of oil that contained the hydrophobic surfactant: 0.03% polysorbate 80. Both the phases were previously heated separately at 70°C and mixed. After the homogenisation process (15mins 800rpm, spinix vortex) the emulsion were cooled down to the room temperature, allowing the k-carrageenan to polymerize. In the second step 42.2g of water and 5.4g of isolated soya protein for 2mins in a magnetic stirrer (magnetic stirrer remi, Mumbai). .afterwards 52.6g of the previously prepared water in oil in water emulsion was incorporated. The water oil emulsion was slowly added and then gently stirred in order to obtain stable water in oil water emulsion (12mins, 800rpm – remi Mumbai). The emulsion formed and was viewed under the microscope for the stability. (Candelaria et al, 2013).

**Encapsulation by sodium alginate method:**

The emulsion method of microencapsulation was used to encapsulate probiotics. 120ml of sterile 3% sodium alginate was mixed with 30ml of the probiotic suspension and 50ml of the suspension of sodium alginate was gently dispensed using a pipette into a beaker containing 200ml of vegetable oil and stirred at 200rpm using a magnetic stirrer (remi-Mumbai). Calcium chloride (0.1M) was gently added to the sides of the beaker until the emulsion was broken. The emulsion formed and was viewed under the microscope for the stability. (Shah and Ravula, 2000).

**Optimision of condirions for encapsulation:**

**optimisation of ph for encapsulation:**

The optimisation of pH was done for Water in oil (w/o) emulsion, Water in oil in water (w/o/w) emulsion and Encapsulation by sodium alginate method.In this method the emulsions formed were maintained at different pH namely 3, 5,7,8,9. After that the emulsion with different pH were observed under microscope for its stability.

**Optimisation of temperature for encapsulation:**

The optimisation of temperature was done for Water in oil (w/o) emulsion, Water in oil in water (w/o/w)

emulsion and Encapsulation by sodium alginate method. In this method the emulsions formed were maintained at different temperatures namely 4°C, 25°C 28°C, 40°C, 50°C. After that the emulsion with different temperatures were observed under microscope for its stability.

**Optimisation of hydrophobic surfactant for water in oil in water emulsion**:

The water in oil in water emulsion was prepared by using different hydrophobic surfactant namely Tween 20, Tween 40, Tween 80 and Triton 100 x and was viewed under microscope for the determination of stability.

**Determination of the viability of encapsulated probiotic:**

The viability of the encapsulated probiotic was identified by dissolving 1gm of microcapsule to 99ml of sterile saline solution. Then it was serially diluted up to $10^{10}$ dilutions. The dilutions were poured into a YMA plates (YMA plate containing yeast extract, mannitol, dipotassium phosphate, magnesium sulphate, sodium chloride, calcium chloride and agar) and spread plate technique was performed by adding 0.1ml of serially diluted saline and it was plated to all the dilution and was incubated at 30°C for 24-48 hours and the viability was identified by the number of colonies formed. Then the colony forming unit was identified by

$$cfu = \frac{total\ no\ of\ colonies}{volume\ of\ sample\ taken\ X\ Dilution\ factor}$$

**Determination of survival of encapsulated probiotic in stimulated gastric conditions:**

The survival of the encapsulated probiotic in stimulated gastric conditions was identified by preparing sabouraud dextrose broth 9ml of different gastric ph namely 3, 5, 8, 10 and was sterilised. 1gm of the culture was added to it and was diluted. Ox bile 0.03% for 15ml was prepared and the pellets obtained by centrifuging all three emulsion methods were collected and added to it. Now the bile with the different encapsulated probiotic and the broth with the different gastric conditions were spread plated and were checked for the viability by the number of colonies formed. Then the colony forming unit was identified by

$$cfu = \frac{total\ no\ of\ colonies}{volume\ of\ sample\ taken\ X\ Dilution\ factor}$$

**Determination of aflatoxin binding by the encapsulated probiotic**:

The aflatoxin binding by the encapsulated probiotic was identified by taking the lyophilised cells of the probiotic organisms and it was suspended in water of about 5 - 10 mg/ml. Dispense the 5mg of the suspension to the centrifuge tubes. Now add 50ng of aflatoxinB1 and incubate it for 15-180 mins. After incubation the eppendorf tubes were centrifuged at 8000rpm for 20mins (remi-Mumbai). The supernatant was collected and was run in the thin layer chromatography and checked for the aflatoxin band. The pellet was then collected washed twice with water of about 2*0.5ml and centrifuged with the portion B (portion B-2*5ml of chloroform and methanol in the ratio of 9:1) and then the pellet was collected and 0.5ml of water is added along. The residues were collected and checked for the presence of aflatoxin. All the three potion (a, b, c) were extracted twice and run in the thin layer chromatography and was checked for the aflatoxin band.

$$cfu = \frac{total\ no\ of\ colonies}{volume\ of\ sample\ taken\ X\ Dilution\ factor}$$

**Results and Discussion**

**Simple staining:**

*Saccharomyces boulardii* and *Saccharomyces cerevisiae* were simple stained by crystal violet and it was observed to be budding yeast.The plate 1 shows the colony morphology and growth of *saccharomyces boulardi* and *saccharomyces cerevisiae.*

**Carbohydrate fermentation test:**

The carbohydrate fermentation shows the difference between the two species *Saccharomyces boulardi* and *Saccharomyces cerevisiae by* their ability to ferment sugar. The fermentation of the sugar is observed by the change of colour from red to yellow indicating the acid production due to the fermentation of sugar and the gas production was observed by the presence of bubbles in Durham's tube. The results of carbohydrate fermentation were given in table 1 and plate 2.

**Standardisation of encapsulation procedures optimization of ph for encapsulation:**

The optimisation of pH was done for Water in oil (w/o) emulsion, Water in oil in water (w/o/w)

emulsion and Encapsulation by sodium alginate method by calculating the diameter of the encapsulation. In all the three methods of encapsulation, the encapsulated probiotic was more stable at pH 5. Other pH such 3,7,8,9 the diameter of the capsules were either too high or too low which may cause the encapsulated probiotic to disrupt from its form. Similar method was followed to encapsulate probiotics by Su et al., (2006) and stable emulsion was obtained at pH 5. The results are depicted in Plate 3 and Figure 1.

**Optimisation of temperature for encapsulation:**

The emulsions were standardised under different temperatures and the diameter of the encapsulation was identified and the optimum range was calculated for all the thee methods namely Water in oil (w/o) emulsion, Water in oil in water (w/o/w) emulsion and Encapsulation by sodium alginate. It was observed in all the three methods that the encapsulated probiotic was more stable in a temperature of about 40°C. In the other temperatures like 4°C, 25°C ,28°C, 50°C the capsules were either too high or too low which may cause the encapsulated probiotic to disrupt from its form. Stable emulsion was formed at 20°C by Su et al., (2006).The results are recorded in Plate 4 and Figure 1.

**Optimisation of hydrophobic surfactant for water in oil in water emulsion**

The emulsions were standardised under different hydrophobic surfactants and the diameter of the encapsulation was identified and the optimum range was calculated. It was observed that the probiotic encapsulated with tween80 has more stability than the other emulsifiers because while using the other three emulsifiers the capsules were either too large or too small which may cause destruction of the capsules (Plate 5). Similarly another organism was encapsulated using tween80 along with the span 80 in order to obtain a stable emulsion (Schmidts T et al 2009).

**Determination of the viability of encapsulated probiotic:**

The viability of the encapsulated probiotic was identified by spread plate technique and their colony forming unit per gram was noted. The viability of the encapsulated probiotic was checked using the pellets obtained by water in oil emulsion, water in oil in water emulsion and encapsulation using sodium alginate techniques and it was found to be the cells which are encapsulated by water in oil in water emulsion technique is more viable than the cells obtained by other two methods (Table 2 and Figure 3). This shows water in oil in water emulsion method is more effective. This viability count is similar to that of (Shah and Ravula, 2000) method of encapsulating organism.

**Determination of survival of encapsulated probiotic in stimulated gastric conditions:**

The survival of the encapsulated probiotic was checked under different stimulated gastric conditions and their viability was noted. It was viable in all the three methods. The viability was better in Water in oil in water emulsion method than the other two methods. This shows water in oil in water emulsion methods has more stability and viability in gastric conditions and also it was tolerant in bile **(**Table 3 and Figure 4, 5). Guerin et.al, (2003) reported Similar results of survival of different encapsulated organism.

**Determination of aflatoxin binding by the encapsulated probiotic**:

The encapsulated probiotic was checked for its effect on binding of mycotoxins. It was found that *saccharomyces boulardii* has more ability of binding aflatoxin than *saccharomyces cerevisae*, thus showing that the *saccharomyces boulardii* has more effect on binding to mycotoxins. The Plate 6 clearly shows that both the organism *saccharomyces boulardii* and *saccharomyces cerevisiae* has the ability of binding mycotoxins but when comparing to *saccharomyces cerevisiae*, *saccharomyces boulardii* has more capacity of binding aflatoxin and neutralise its mycotoxity. Several other organisms were checked for their ability to bind aflatoxin and comparisons were made (El Nezami H et al., 1998). The present study has shown that the probiotic yeast which is microencapsulated by water in oil in water emulsion technique has more stability and viability than the other two methods of microencapsulation and also significantly has more viability even under the gastric environmental conditions when compared to the other to techniques. This helps the probiotic to safely reach the target without any disturbances of gastric environmental conditions. The ability of binding to mycotoxins was checked and it was found that *Saccharomyces boulardii* has more binding activity than the *saccharomyces cerevisiae,* which enhances the activity of the probiotic without any harmful ill effects.

**Table 1:** Carbohydrate Fermentation.

| Sugars | *Saccharomyces boulardi* | *Saccharomyces cerevisiae* |
|---|---|---|
| **Dextrose** | Positive | Positive |
| **Maltose** | Positive | Positive |
| **Xylose** | Negative | Negative |
| **Fructose** | Positive | Positive |
| **Lactose** | Negative | Negative |
| **Galactose** | Positive | Positive |
| **Celloboise** | Negative | Negative |
| **Sucrose** | Positive | Positive |
| **Mannitol** | Negative | Negative |

**Table 2:** Viability Count Using Different Emulsion Techniques.

| Encapsulation Methods | Colony Forming Unit Per Gram |
|---|---|
| Water In Oil | $4.7\times10^{7}$ |
| Water In Oil In Water | **$6\times10^{7}$** |
| Using Sodium Alginate | $3.4\times10^{7}$ |

**Table 3:** Determination of survival under stimulated gastric conditions.

| pH Values | Water In Oil (Cfu/Gm) | Sodium Alginate(Cfu/Gm) | Water In Oil In Water (Cfu |
|---|---|---|---|
| pH 3 | $2.8\times10^{5}$ | $1.6\times10^{5}$ | **$2.0\times10^{5}$** |
| pH 5 | $1.4\times10^{5}$ | $1.3\times10^{5}$ | **$2.0\times10^{5}$** |
| pH 8 | $1.6\times10^{5}$ | $2.2\times10^{5}$ | **$2.5\times10^{5}$** |
| pH 10 | $2.8\times10^{5}$ | $1.8\times10^{5}$ | **$2.8\times10^{5}$** |

**Plate 1**: **Simple Staining**

***Saccharomyces boulardi*** ***Saccharomyces cerevisiae***

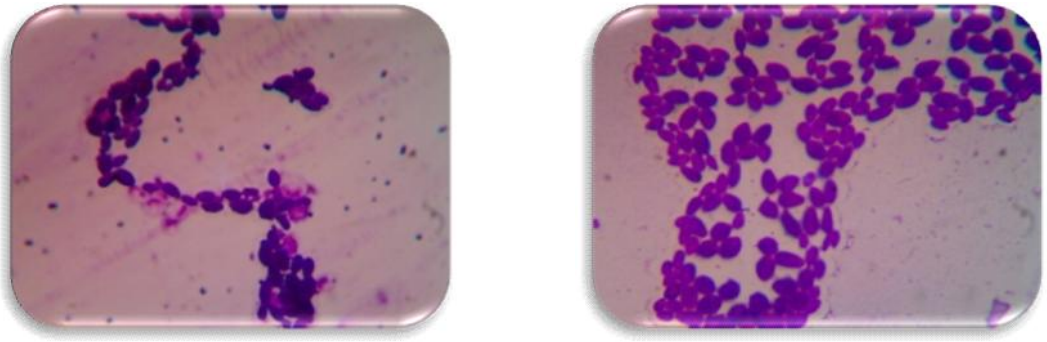
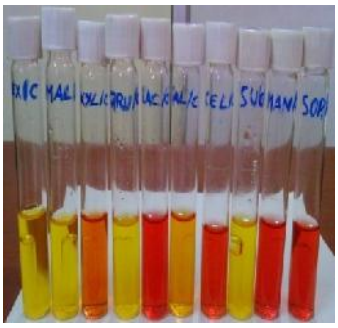


**Plate 2: Carbohydrate Fermentation Test**

***Saccharomyces boulardi*** ***Saccharomyces cerevisiae***



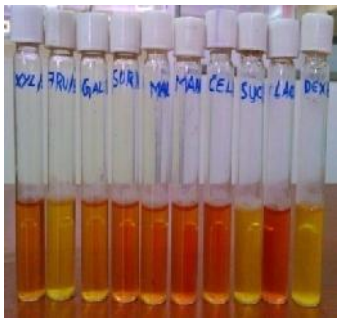
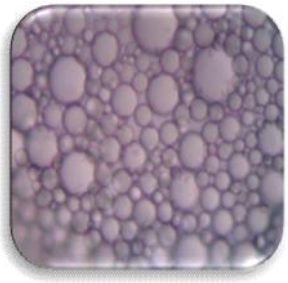


**Plate 3: *Optimisation Of pH For Encapsulation***

**A** **B** **C**

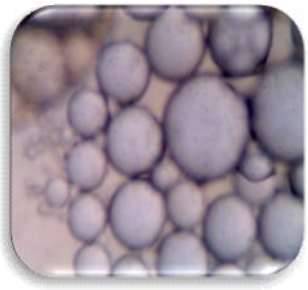


A. Water in oil (w/o) B. Water in oil in water emulsion (w/o/w) C.

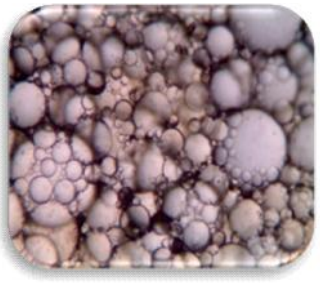
Encapsulation by sodium alginate.

**Plate 4:** Optimisation of the Temperature for Encapsulation

**A** **B** **C**



A. Water in oil (w/o) Water in oil in water emulsion (w/o/w) B. C.



Encapsulation by sodium alginate

**Plate 5:** Encapsulation with the optimal emulsifier

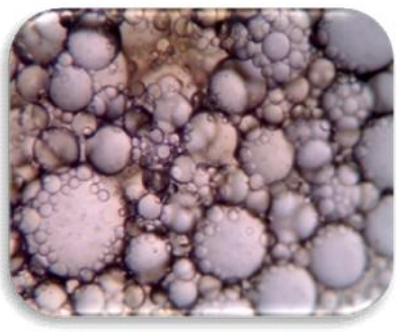


**Plate 6:** Aflatoxin binding ability of *Saccharomyces boulardii*

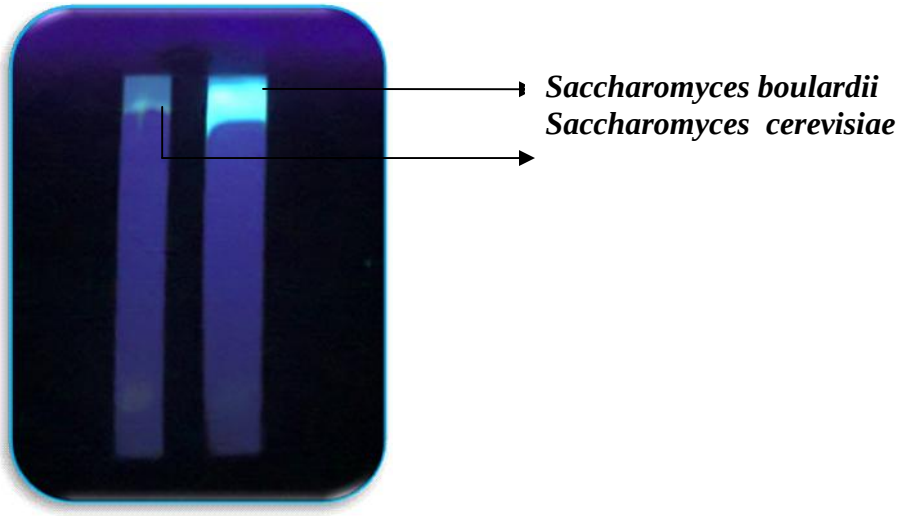
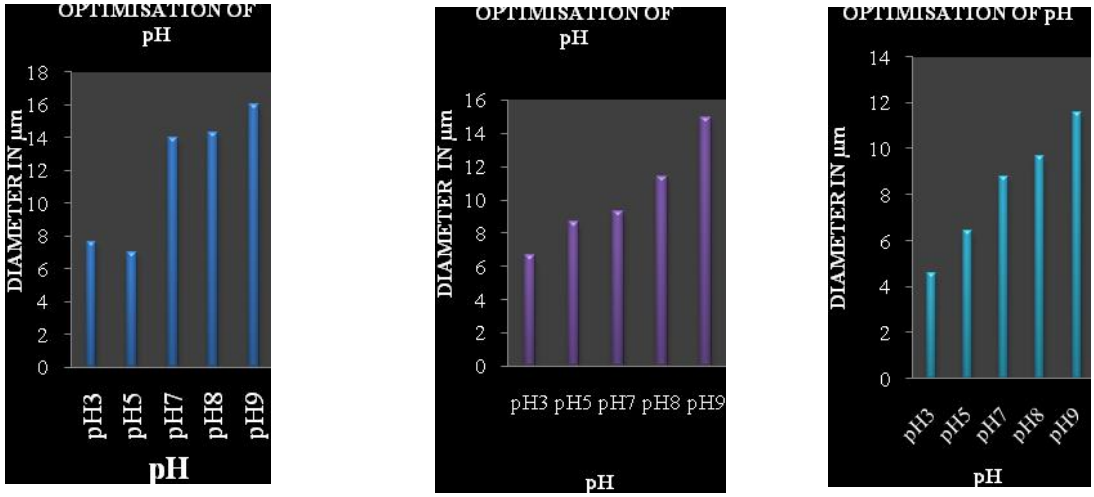



**Figure1: Optimization Of pH For Encapsulation**

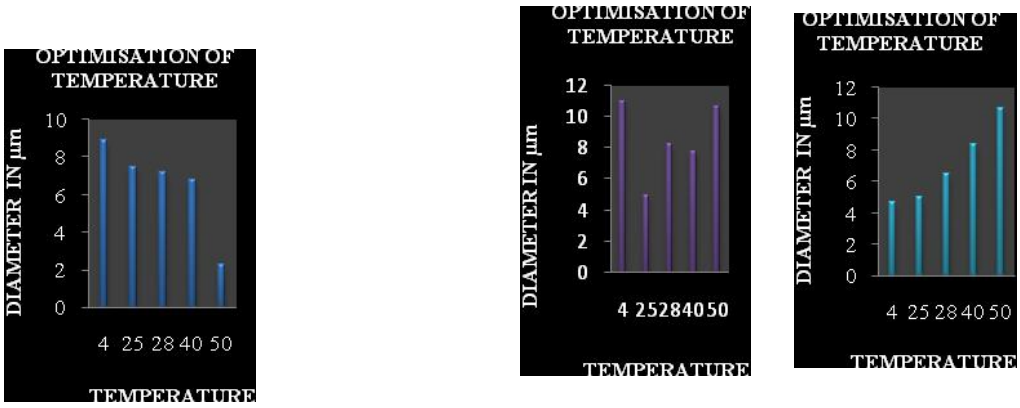
**A** **B** **C**




A. Water in oil (w/o) emulsion B. Water in oil in water (w/o/w) emulsion C. Encapsulation by sodium alginate.

**Figure 2: Optimization Of pH For Encapsulation**

**A** **B** **C**




A. Water in oil (w/o) emulsion B. Water in oil in water (w/o/w) emulsion
C. Encapsulation by sodium alginate.

**Figure 3:** viability count by different emulsion techniques.

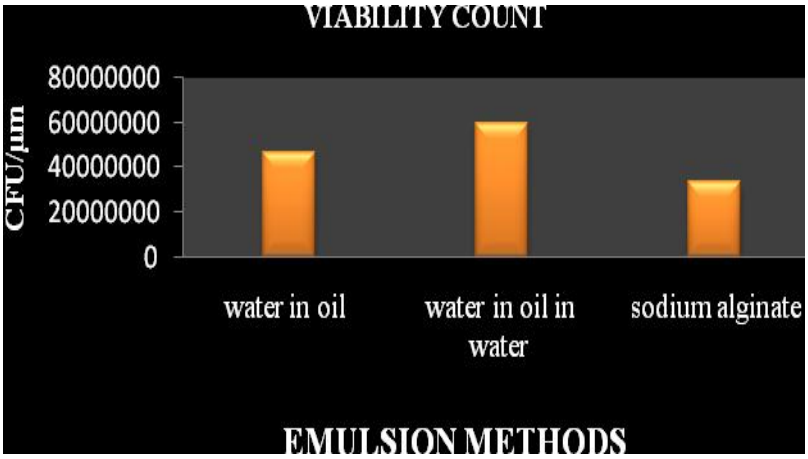



**Figure 4:** survival of encapsulated probiotic under stimulated gastric conditions.

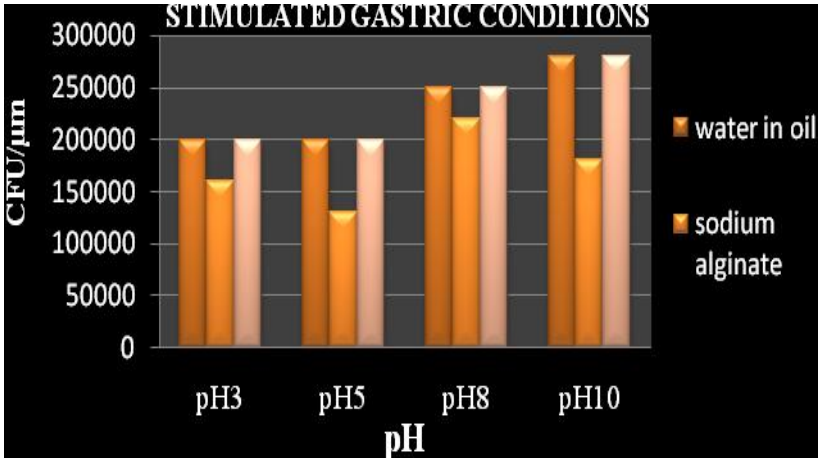
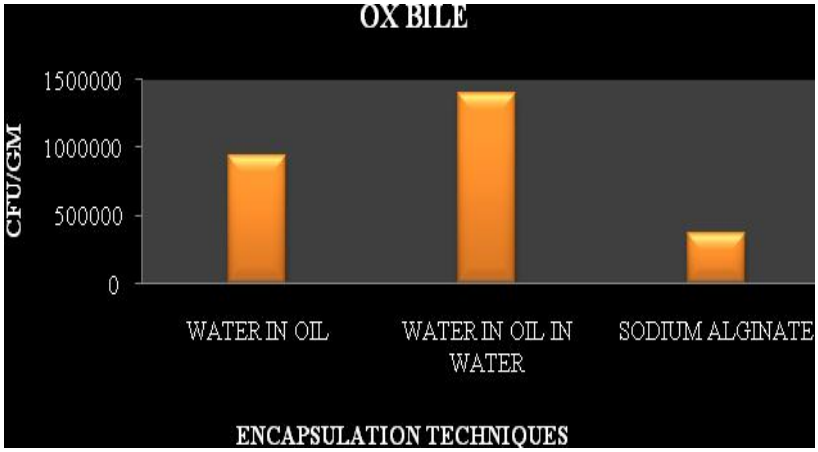



**Figure 5:** survival of encapsulated probiotic under stimulated gastric conditions using ox bile.



**References**

Audet P, Paquin C, Lacroix C (1988). Immobilized growing lactic acid bacteria with κ-carrageenan-locust bean gum gel. App lMicrobiol Biotechnol.29: 11-18.

Çakır, İ. 2003. Determination of some probiotic properties on Lactobacilli and Bifidobacteria. Ankara University Thesis of Ph.D.

Candelaria Poyato a, Iñigo Navarro-Blasco b, Maria Isabel Calvo c, Rita Yolanda Cavero d, Iciar Astiasarán a, Diana Ansorena Oxidative stability of O/W and W/O/W emulsions: Effect of lipid composition and antioxidant polarity. Food Research International 51 (2013) 132–140.

CVM. 1997. VM Policy on Competitive Exclusion Products. (FDA, Centre for Veterinary Medicine, Office of Management and Communications, Rockville

El-Nezami H., Kankaanpää P., Salminen S., Ahokas J. (1998a): Ability of dairy strains oflactic acid bacteria to bind a common food carcinogen, aflatoxin B1. Food Chem Toxicol36:321-326.

Fuller R (1989). Probiotics in man and animals. J Appl Bacteriol.66: 365-378

Fuller R (1991). Probiotics: the scientific basis. London: Chapman& Hall.

Gismondo, M.R., Drago, L., Lombardi, A. 1999. Review of probiotics available to modify gastrointestinal flora. International Journal of Antimicrobial Agents.12:287-292.

Goldin S (1998). Microencapsulation-industrial appraisal of existing technologies and trend. Trends Food Sci Technol.15: 330-347

Gouin, D.; Vuillemard, J.C.; Subirade, M. Protection of Bifidobacteria encapsulated in polysachharide-protein gel beads against gastric juice and bile. J. Food Prot. 2004, 66, 2076–2084.

Krasaekoopt W, Bhandari B, Deeth H (2004). The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. Int Dairy J14: 737-743.

Naidu, A. S., Bidlack, W. R. and Clemens, R. A. 1999. Probiotic spectra of lactic acid bacteria (LAB). Critical Reviews in Food Science and Nutrition

Parker, R.B. (1974). Probiotics, the other half of the antibiotics story. Animal Nutrition and Health 29, 4-8

Picot A, Lacroix C (2004). Encapsulation of bifidobacteria in whey protein microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. Int Dairy J.14: 505-515

Schmidts T et al. Influence of hydrophilic surfactants on the properties of multiple W/O/W emulsions. Journal of colloid and interface science .2009; 338:184-192.

Schrezenmeir, J., and M. De Vrese. 2001. Probiotics, prebiotics, and synbiotics-approaching a definition. American Journal of Clinical Nutrition73 (Suppl.):354s-361s.

Shah N.P. and Ravula, R.R. 2000.Microencapsulation of probiotic bacteria and their survival in frozen fermented dairy dessert. The Australian journal of dairy technology. Vol: 55, pg: 139-144.

Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P,Kailasapathy K (2000). Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Int J Food Microbiol. 62: 47-55.

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