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RESEARCH **P**APER

Microencapsulation of micro-organisms and ginger extract

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Micro-organisms *Lactobacillus* sp. were encapsulated in 3 per cent sodium alginate solution and 0.2M calcium chloride solution. The capsules formed were hardened in calcium chloride solution. To determine the efficiency of bacteria they are released in phosphate buffer. Encapsulation efficiency of *Lactobacillus* was 91 per cent. This shows amount of ethanol production do not vary with encapsulation. Further the immobilization of lactic acid bacteria also shows that *Lactobacillus,* isolated from curd, encapsulated in alginate was further lyophilized for its storage. The ginger juice was also encapsulated. The wet and dry weight of the capsules produced in 100 rpm at 0.2 bar pressure was 53.71g and 4.63g, respectively whereas the wet weight and dry weight of capsules produced in 60 rpm and at 0.2bar pressure was 74.10g and 6.93g. Sensory analysis was done which shows that the capsules retained their odour and flavour. Thus, this study revealed that the encapsulation method helps in the preservation of microorganisms and other flavouring compound.

Key words : Micro-organisms, Lactobacillus, Encapsulation, Ginger

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INTRODUCTION

Microencapsulation is a technology that can improve the retention time of nutrients, whether it is in the form of solid, liquid or gaseous materials, in the food and allow their controlled release at a specific time under specific conditions (Dziezak, 1988). The principle of the microencapsulation is very old as it involves the development of the microencapsulation began with the preparation of capsules containing dyes. These were incorporated into paper for copying purposes and replace carbon paper. So microencapsulation cannot be considered as a product but it is a process of encapsulation of micron sized particles of solid, liquid or gas in an inert shell which protects and isolates them from the external environment. This technology is mainly used for the purpose of protection, controlled release of core material. These properties of the microcapsules make this process very useful for different industries. Microencapsulation has been utilized in the pharmaceutical industry to offer controlled release of the drugs in the body and for the preparation of capsules containing active ingredients. It is relatively new to the food industry and is finding use in maximising the retention of the bioactivity of the components during the processing and storage of the formulated products and their delivering of the formulating components in the target site of the body (Bakan, 1973). Microencapsulation is also used to encapsulate the fish oil, to encapsulate the probiotic bacteria, to encapsulate the major flavours components.Microencapsulation has been widely used in industries for several decades. Its principle is also very old which includes the preparation of capsules containing dyes and then they are incorporated into paper for copying purposes and they replace carbon paper too. The history of microencapsulation can be traced back as 1927, when the capsules were spray dried with oil gum acacia coatings. Microencapsulation of biologically active materials in the form of an artificial cell was reported as early as 1964 (Chang, 1964). However, it is only in the last 10 years that many centres are developing this method extensively (Chang and Zhang, 1995). More recently, we have concentrated on three areas of artificial cells for blood substitutes, enzyme therapy and cell therapy.

Microencapsulation is the process of enclosing micron sized active ingredients into an inert external shell which protects the core material from the environment and release the core material in a controlled manner or it can be defined as the creation of barrier to avoid chemical reactions and enable the controlled release of the ingredients (Vilstrup, 2001). It involves the mass transport between the core (the

ingredients) and the shell (capsule or coating). All the three states of matter (solid, liquid and gas) may be microencapsulated. This allows liquid more easily than solids, and afford some measure of protection to those handling hazardous materials. This technology is mainly used for the purpose of protection, controlled release and compatibility of core material. These properties of the microcapsules make this process very much beneficial for different industries (Kwak *et al*., 2001).The reasons for encapsulation are countless. Sometimes the core must be isolated from its surrounding as in isolating vitamins from the deteriorating effects of oxygen, retarding evaporation of a volatile core, improving the handling properties of a sticky material, or isolating a reactive core from the chemical attack. In other cases, the objective is to control the rate at which it leaves the microcapsule as in the controlled release of drugs or pesticides. The problem may be as simple as masking the taste or odour of the core, or as complex as increasing the selectivity of an adsorption or extraction processes.

Microencapsulation of micro-organisms :

Micro-organism can be encapsulated in different coating material for different purposes, micro-organisms are very sensitive to certain environment condition, and they cannot survive in these conditions so maintain the number of micro-organism they can be encapsulated in a protective coating which protect them from external environmental conditions (Kailasapathy *et al*., 2006). Sometimes micro-organisms release their enzymes in a specific conditions so they should be encapsulated until proper conditions are maintain and after reaching these microbes release their enzymes which diffuses from coating or comes out by dissolving the coat. Microencapsulation techniques have been successfully used to enhance dairy fermentation for the production of concentrated lactic acid bacteria and to improve the survival of microorganisms in dairy products and mayonnaise (Lee *et al.*, 2004 and Krasaekoopt *et al.*, 2006). Among the encapsulation devices, microencapsulation in calcium alginate microparticles has been widely used for the immobilization of lactic acid bacteria owing to its ease of handling, nontoxic nature, and low cost (Mortazavian *et al.*, 2004). It is a method that preserve bacteria from detrimental factors of environments such as high acidity (low pH), bile salts (Chou and Weimer, 1999 and Succi *et al.*, 2005), molecular oxygen in case of obligatory anaerobic microbes, bacteriophages and chemical as well as antimicrobial agents.

Encapsulation of probiotics :

Probiotics are live microbial supplements that beneficially affect the host by improving its intestinal microbial balance (Andersson *et al*., 2001). Beneficial effects of these microbes are its antagonistic and immune effects (Jankowski *et al*.,1997). The use of these microbes stimulates the growth of preferred micro-organisms, crowds out harmful bacteria and reinforces the body's natural defence mechanism (Hamilton-Miller *et al.*, 1999). Lactic acid bacteria (LAB) are the most important probiotic micro-organisms typically associated with the human gastrointestinal tract. These bacteria are Gram positive, odd shaped, non-spore forming, catalase negative organisms that are devoid of cytochromes and are of non- aerobic habit but are aero tolerant, fastidious, acid tolerant and strictly fermentative; lactic acid is the major end product of sugar fermentation (Axelsson, 1993). A few of the known LAB that are used as probiotics are *Lactobacillus acidophilus, Lactbacillus amylovorous, Lactobacillus.*

RESEARCH METHODOLOGY

The present study was carried out at microencapsulation of micro-organism and ginger extract :

Isolation and identification of bacteria :

For isolation, make the dilutions of the curd sample by taking 9 ml of distilled water and 1 ml of curd sample as 10^{-1} dilution and subsequently make 10^{-2}, 10^{-3} upto 10^{-6}. Then do spreading on nutrient agar plates and incubate it for 24 to 48 hours at 37^0C. The next step was preparation of pure culture of the bacteria. For pure culture streaking is done on nutrient agar and incubated for 24 to 48 hours. After getting the results of streaking prepare pure culture of bacteria in nutrient broth. There were three different types of staining to determine whether the bacteria were *Lactobacillus* or not. They were Gram staining, acid fast staining and endospore or spore staining.

Gram staining :

For Gram staining smear was air dried and heat fixed also. Cover the smear with crystal violet for 30 seconds and wash off the slide with water. Then flood the slide with iodine's solution for 60 seconds and wash of the slide with alcohol (add alcohol drop by drop so that all colour flows from the smear. Wash the slide with water and use safranin as a counter stain for 30 seconds then wash the slide with water and dry it and examine it under the microscope. *Lactobacillus* is Gram positive and rod shaped bacteria.

Acid fast staining :

Smear was clean and air dried and heat fixed. Then flood the smear with carbol fuchsin and heat the slide to steam for 3 to 5 minutes (add more stain time to time so that smear do not get dried) and wash the slide with water. Decolorize the smear with acid alcohol for 10-30 seconds until the smear is faint pink in colour, then wash the slide and counter stain with methylene blue for 1 to 2 mins and wash it and dry it and examine it under the microscope.

Spore staining :

Smear was made on clean slide and heat fixed. Smear was flooded with malachite green and heat the slide to steaming and steam for 5 mins by adding more stain side by side so that the smear do not get dried. Wash the slide with water and counter stain it with safranin for 30 seconds and again wash it with water and blot dry the slide and examine it under the microscope.

Microencapsulation of *Lactobacillus* :

The capsules were prepared aseptically using an Encapsulator and laminar air flow with a nozzle size of 2µm, (1.5% (w/v) sodium alginate 10^9 CFU/ml and 30 min hardening in 0.1 M calcium chloride solution(Fig. A).

Fig. A : The experimental set up for encapsulation of *Lactobacillus* sp.

Efficacy of cell release from capsules :

To determine the viable counts of the entrapped bacteria, 0.1 g of capsules were re-suspended in 10 ml of phosphate buffer (0.1 M, pH 7.0) followed by gentle shaking at room temperature.

Microencapsulation of ginger extract :

500g of ginger was procured from the local market and its juice was extracted using a grinder. The juice was then mixed with sodium alginate at the ratio of 1:3. Then this mixture was sprayed over calcium chloride present in the gelling bath with continuous agitation. The capsules thus, formed were kept for about 30 mins for hardening. Then the capsules were dried in hot air oven at 60°C for 24hrs. For the production of two different size capsules, rpm of 60 and 100 was used whereas the 0.2kg/cm^2 pressure was kept constant. The nozzle size used was of 2mm.

RESEARCH FINDINGS AND ANALYSIS

The experimental findings obtained from the present study have been discussed in following heads:

Growth of *Lactobacillus* :

The growth of *Lactobacillus* in free culture and immobilized culture were shown in Table 1, Fig.1 and 2.

Table 1 : Growth of *Lactobacillus*

	Dilution	Vol. in which dissolved	No. of colonies	Colony forming unit
Free culture	10^{-4}	10	111	1.11 x 10^6
Immobilized culture	10^{-4}	30	34	1.02 x 10^6

Fig. 1 : Growth of *Lactobacillus*

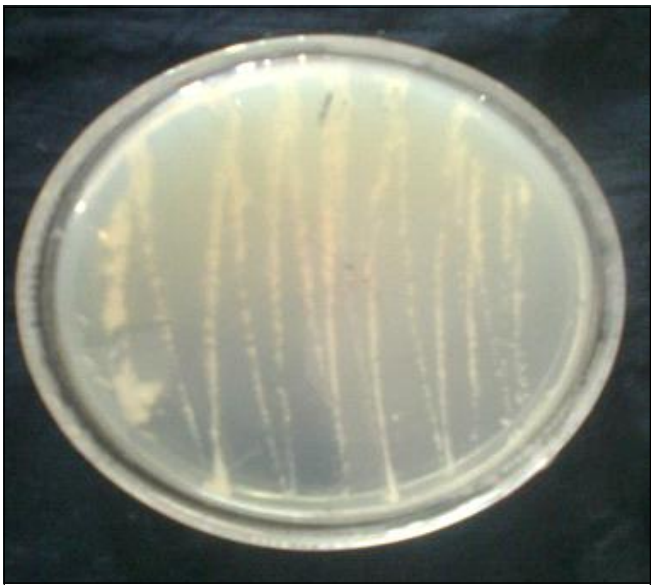

Fig. 2 : Growth of purefree *Lactobacillus*

Encapsulation of lactic acid bacteria :

Capsule production depends on pressure applied .. .. *et al.*, 2006) as at high flow rate, capsules loss has been observed. Increase in pressure show decreasing trend of capsule production. Encapsulation efficiency of *Lactobacillus* was 91 per cent. It means that *Lactobacillus* was encapsulated successfully as there was little loss in calcium chloride (Fig. 3 and 4).

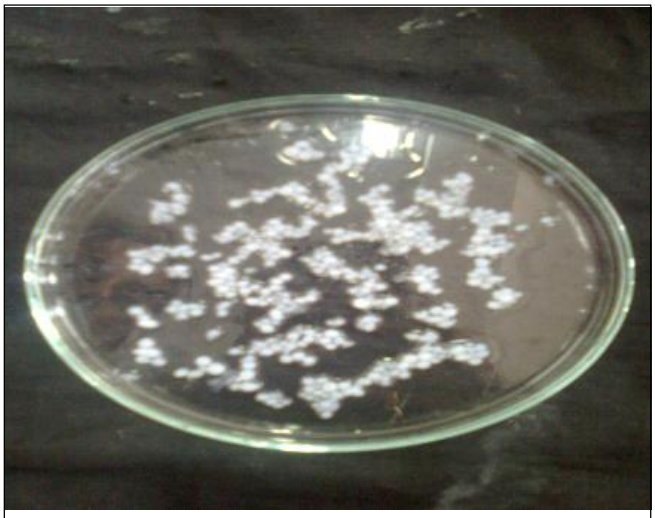

Fig. 3 : Capsules of immobilized *Lactobacillus*

Fig. 4 : Lyophilized capsules

Encapsulation of ginger extract :

Ginger juice was extracted from the ginger. It was then encapsulated with sodium alginate in the ratio 1:3. After the formation of the capsules at different rpm it shows variation in the size by the formation of the capsules larger in size at high rpm (Table 2 amd Fig. 5). Capsules are made at 0.2kg/ cm^2 pressure at different rpm and their wet and dry weight was noted. Capsules were dried in hot air oven. The dry ginger capsules retained their odour.

Table 2 : Dry weight and wet weight of capsules of ginger extract

	Dry weight(grams)	Wet weight(grams)
60 rpm	6.93	74.10
100 rpm	4.63	53.71

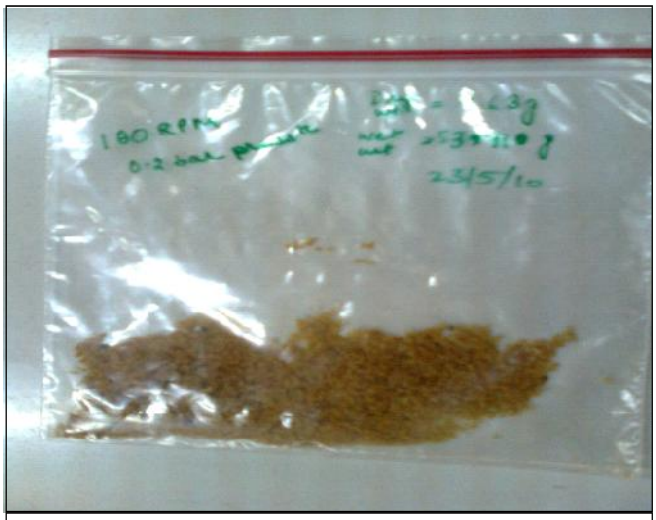

Fig. 5 : Ginger extract encapsulated at 100 rpm

Conclusion :

In this study we conclude that microbes like *Lactobacillus* can be immobilised with the help of sodium alginate and calcium chloride solution. The efficiency of both free and immobilised cultures were found to be equivalent. The encapsulated ginger extract was found to retain their flavour. Thus, with the help of microencapsulation, micro-organisms and other flavouring compounds can be encapsulated which can be preserved for further use.

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