Incorporation of Microencapsulated Probiotic Lactic Acid Bacteria in Yoghurt

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ABSTRACT

Background: Probiotics are live microorganisms known for the many health benefits they confer on their host. *Lactobacillus acidophilus* is a lactose fermenting bacterium that is found naturally in the human body and is also found to exhibit probiotic properties. The gastrointestinal tract of humans is however, a harsh environment for microorganisms to survive and render the intended health benefits. Microencapsulation is a technique by which a substance is enveloped by another, forming a capsule that may range up to several hundred microns in size. In this study the probiotic organism *L. acidophilus* was microencapsulated in a hydrocolloid bead (alginate) and then incorporated in yoghurt.

Methods: Probiotic properties of *Lactobacillus acidophilus* was evaluated. It was then microencapsulated in 50 per cent carrot juice and then incorporated in yoghurt. Nutrient composition of this value added yoghurt was studied and its sensory parameters compared with control yoghurt.

Result: In the present experiment the viability of *L. acidophilus* at 90 mins was 10.75± 0.45 log cfu/ml and the tolerance of *L. acidophilus* to 0.4 per cent of bile was significant. Microencapsulation of *L. acidophilus* was attempted by mixing carrot juice at 50 per cent to the alginate beads, thus imparting an appealing orange colour to the beads. The yoghurt thus prepared using microencapsulated *L. acidophilus* had an acidity of 0.7 ± 0.4 and total solid of 21.35 per cent on dry matter basis. Sensory analysis revealed maximum overall acceptability for carrot juice admixed microencapsulated probiotic-enriched yoghurt.

Key words: Carrot microencapsulated, Functional yoghurt, Probiotics.

INTRODUCTION

India’s Ayurvedic writings, dating back to 6000 BC, indicate that regular consumption of dairy products lead to a long and healthy life. Food products such as yoghurt, kefir, aged cheeses, kimchi, sauerkraut, miso, tempeh and some soy beverages naturally contain probiotics. Eating yogurt is often recommended for people who are lactose intolerant. This is because yogurt has less lactose than milk and other dairy products. *Lactobacillus acidophilus* is one of the probiotics in yoghurt that is responsible for “pre-digesting” the lactose, which makes it easier to digest.

Yoghurt is a food produced by bacterial fermentation of milk, particularly a culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. It contains protein and several other nutrients found in dairy foods, like calcium, vitamin B-2, B-12, potassium and magnesium. The Food and Agriculture Organization of the United Nations (FAO) defines probiotics as “live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host.” Probiotics have been associated with well-being for a very long time. The consumption of probiotics is reported to have several beneficial effects such as improvement of intestinal health, amelioration of symptoms of lactose intolerance and increased immunity to various other diseases. Some of the common probiotic organisms are *Lactobacillus, Bifidobacterium, Saccharomyces boulardii, Streptococcus thermophilus, Enterococcus faecium* and *Leuconostoc*.

*Lactobacillus acidophilus* is a form of bacteria that’s found naturally in the body, usually in the intestines and mouth. It produces vitamin K as well as lactase, the enzyme that breaks down lactose (the sugars in milk products). *L. acidophilus* is the most commonly used probiotic organism. Microencapsulation is the process of surrounding or enveloping one substance within another substance on a very small scale, yielding capsules ranging from less than one micron to several hundred microns in size. Substances may be microencapsulated with the intention that the core material be confined within capsule walls for a specific period of time. Alternatively, core materials may be encapsulated so that the core material will be released either gradually through the capsule walls, known as controlled release or diffusion, or when external conditions trigger the capsule walls to rupture, melt, or dissolve (Jothi et al., 2012). The
oral administration of most of the probiotics results in the lack of ability to survive in a high proportion of the harsh conditions of acidity and bile concentration commonly encountered in the gastrointestinal tract of humans. Providing probiotic living cells with a physical barrier against adverse environmental conditions is therefore an approach currently receiving considerable interest. Probiotic encapsulation technology has the potential to protect microorganisms and to deliver them into the gut.

In the present study carrot juice was added to the wall material of the capsules to improve its aesthetic properties and for its nutritional value. Carrots are one of the richest source of beta carotene, a carotenoid compound responsible for giving fruits and vegetables their orange pigment. It is a precursor of Vitamin A and a powerful antioxidant and has been found to help protect against cancer and aging.

With the aim of preparing a functional probiotic yoghurt, the present study was formulated to evaluate the probiotic properties of *Lactobacillus acidophilus* and microencapsulate this probiotic in carrot juice in the preparation of functional yoghurt.

**MATERIALS AND METHODS**

In the present study conducted in (2015-16) at College of Food and Dairy Technology, TANUVAS, Chennai the methodology for preparation of yoghurt with carrot juice microencapsulated probiotic has been standardised. Yoghurt was prepared as per the method adopted by De (1980). Standard yoghurt cultures were used for the preparation. Functionality of the product was enhanced by adding *Lactobacillus acidophilus* and carrot juice. Phenotypic identification of *Lactobacillus acidophilus* was done by gram staining.

Probiotic properties of *L. acidophilus* was assessed for its tolerance to bile salt and acid (5N HCl) as per the procedure adopted by Zinedine and Faid (2007).

**Microencapsulation of *L. acidophilus***

50 percent of carrot juice was added to the wall material and then microencapsulated a sper the procedure of Krasaekoopt *et al.* (2003).

**Physiochemical analysis**

The pH of the yoghurt was measured using a pH meter. The acidity of the yoghurt was estimated as per the protocol in AOAC (2000).

**Estimation of nutritional value**

Fat, moisture, carbohydrate, protein, ash and fibre were estimated as per the protocol in AOAC 18th Edn 2006.

**Sensory analysis**

The 9 point hedonic scale was used to measure the consumer acceptance of the finished product. The product was standardized using sensory evaluation. All the samples were organoleptically evaluated by a panel of persons for acceptability on a scale of 9 point ranging from like extremely to dislike extremely.

**Statistical analysis**

The data obtained were analysed statistically using the procedure of Snedecor and Cochran (1980).

**RESULTS AND DISCUSSION**

**Gram’s staining showing Gram positive pleomorphic cell morphology**

In the present study, as illustrated in Plate 1, Gram positive violet stained rods were observed affirming the characteristic phenotypic nature of *L. acidophilus*.

**Characterization of *L. acidophilus* for Probiotic properties**

**Tolerance of *L. acidophilus* to acidity at pH 3.**

Table 1 shows a highly significant decrease in the viability of the *L. acidophilus* when incubated up to 180 minutes. This shows that there is a gradual decrease in viability in acidic conditions. However a count of 7 log \(_{10}\) cfu/ml was maintained when incubated upto 180 minutes at pH3 .This determines the acid tolerance of the organism which is characterisitc of a probiotic bacteria. The mean ± SE viability at 90 mins was 10.750± 0.553log\(_{10}\)cfu/ml. This was in agreement to Gibson and Roberfroid (1998) that for probiotics strains to survive and colonize in the gastrointestinal tract, they must express tolerance to acidity.

**Table 1: Tolerance of *L. acidophilus* (log\(_{10}\)cfu/ml) to acidity at pH3.**

| Culture      | Incubation period in minutes | F Value |
|--------------|-----------------------------|---------|
|              | 0                           | 90      | 180     |
| *L. acidophilus* | 12.766± 0.402               | 10.750± 0.553 | 7.716± 0.490 |

**Average of six trials.**

Small case superscripts shows difference between treatments.
The findings were also in consonance to the observations of McLauchian et al. (1998) that acid tolerance is an important quality for a probiotic.

The finding of this study meets the recommendation of the FAO / WTO draft guidelines of 2002 and ICMR-DBT (2011) guidelines for evaluation of probiotics in food, that tolerance to gastric acidity is one of the attributes of a potential probiotic.

**Tolerance of L. acidophilus isolates to bile salt**

Goldin et al. (1992) reported that a concentration of 0.3 per cent bile was used in most screening studies for testing viability of bile resistant strains. Garriga et al. (1998) reported that bile concentrations of 0.1 to 0.4 per cent were typical for selection of bile tolerant strains. Table 2 shows a significant difference in the viable count of L. acidophilus in the control and in 0.4 per cent bile. However, it is also seen that the probiotic organism can tolerate 0.4% bile and the prerequisite of 10^7cfu/ml recommended for a probiotic food as suggested by Samona and Robinson (1994) was met in this study. This was further affirmed by JayaPrasad et al. (1999), who reported that one of the criteria for lactic acid bacteria to be called probiotic is its tolerance to bile salt.

Zinedine and Faid (2007) also reported that the lactic acid bacteria showed tolerance to 0.3 per cent bile. In the present study bile concentration of upto 0.4 per cent w/v were used. However the mean intestinal bile concentration is believed to be 0.3 per cent w/v as reported by Gilliland et al., 1984.

Table 2: Tolerance of L. acidophilus (log cfu/ml) to bile salts\(^a\).

| Culture      | Control | Bile 0.4 % | t value |
|--------------|---------|------------|---------|
| L. acidophilus| 12.77±0.341 | 10.90±0.373 | 3.73*   |

\(^a\)Average of six trials.

In the present study tolerance of L. acidophilus to 0.4 per cent of bile was significant. Hence, the tolerance to bile was in tandem to the recommendation by FAO/WHO guidelines of 2002 and ICMR –DBT guidelines for evaluation of probiotics (2011) to fulfill the attributes of a potential probiotic.

**Preparation of microencapsulated L. acidophilus**

Trials conducted to optimize the inclusion of carrot juice to the wall material at concentrations of 10, 20, 30, 40 and 50 percent was based on Sensory perception. An optimized inclusion level of 50 percent carrot juice was taken for further trials.

**Preparation of yoghurt**

Skim milk of 0.1 per cent fat was used for preparation of low fat yoghurt.

**Sensory analysis**

Table 3 shows no significant difference between control and yoghurt containing microencapsulated probiotic in colour and appearance, flavour, sweetness, sourness and overall acceptability. However, the body and texture shows significant difference because of the presence of the hydrocolloid beads in the probiotic capsule incorporated yoghurt.

Table 4 shows no significant difference in the flavour and sweetness of the yoghurts with the plain capsules and carrot juice incorporated capsules. However, there was a highly significant difference in the colour and appearance of the two yoghurts. This is because the carrot incorporated beads was found to be pleasing in appearance over the plain beads by the sensory panellists.

**Estimation of pH and acidity**

The pH and acidity of the yoghurt prepared in the study was found to be 4.65±0.025 and 0.7 ± 0.08 respectively and was lesser than that reported by Bansode et al. (2012).

**Table 3: Hedonic sensory evaluation score card for comparison of control and microencapsulated probiotic yoghurt\(^a\).**

| Sensory parameter          | Control | Yoghurt containing microencapsulated L. acidophilus | t value |
|---------------------------|---------|---------------------------------------------------|---------|
| Colour and appearance     | 7.67±0.210 | 7.67±0.220                                      | 0.54 NS |
| Flavour                   | 8.00±0.257 | 8.00±0.258                                      | 0.68 NS |
| Body and texture          | 8.50±0.223 | 7.67±0.210                                      | 2.71*   |
| Sweetness                 | 8.17±0.167 | 8.17±0.168                                      | 0.00 NS |
| Sourness                  | 7.7±0.127   | 7.6±0.223                                       | 1.02 NS |
| Overall acceptability     | 7.99±0.157 | 7.80±0.136                                      | 2.41 *  |

\(^a\)Average of 6 trials; *Significantly different.

**Table 4: Hedonic sensory evaluation score card for comparison of microencapsulated and carrot juice admixed microencapsulated Probiotic Yoghurt\(^a\).**

| Sensory parameter          | Microencapsulated Probiotic Yoghurt | Probiotic Yoghurt containing admixed with carrot juice | t value |
|---------------------------|-------------------------------------|------------------------------------------------------|---------|
| Colour and appearance     | 7.67±0.220                          | 9.00±0.00                                             | 6.32 ** |
| Flavour                   | 8.00±0.258                          | 8.00±0.231                                            | 0.75 NS |
| Body and texture          | 7.67±0.210                          | 8.17±0.166                                            | 2.39*   |
| Sweetness                 | 8.17±0.168                          | 8.83±0.168                                            | 2.83*   |
| Sourness                  | 7.5±0.0223                          | 8.00±0.00                                             | 2.24 NS |
| Overall acceptability     | 7.72±0.136                          | 8.56±0.271                                            | 2.82*   |

\(^a\)Average of 6 trials; *Significantly different; **Highly significantly different.
**Table 5**: Estimation of pH and acidity of carrot juice microencapsulated probiotic yoghurt.

| Physicochemical parameters of L. acidophilus® |       |
|---------------------------------------------|-------|
| pH                                          | 4.65±0.025 |
| Acidity (% lactic acid)                     | 0.7 ± 0.008 |

*Average of 6 trials.

**Table 6**: Nutritional composition of the microencapsulated probiotics with carrot juice yoghurt.

| Nutrients                  | % of component |
|----------------------------|----------------|
| Moisture                   | 78.65          |
| Carbohydrate and sugars    | 75.47          |
| Protein                    | 19.72          |
| Fat                        | 0.74           |
| Ash                        | 3.93           |
| Fibre                      | 0.14           |

On dry matter basis.

**Nutritional composition of the yoghurt with the carrot juice incorporated microcapsules**

The moisture content of the yoghurt prepared in the study was 78.65 per cent. From Table 6, it can be inferred that the moisture of the yoghurt prepared in the study is in agreement with the work of Olugbuyiro et al. (2011). The total solids content of the yoghurt under study was 21.35 per cent while the maximum TS reported by Olugbuyiro et al. (2011) was only 19.2 per cent. The excess TS of the trial yoghurt may be due to the presence of the hydrocolloid beads.

The fat content of the yoghurt under study was 0.74 per cent on dry basis and 0.16 per cent on wet basis as according to the USDA (2001), it is considered to be fat-free yoghurt.

![Yoghurt with L. acidophilus encapsulated in carrot juice.](image)

The solids non-fat content of the yoghurt prepared was 21.19 per cent and exceeded the corresponding values reported Olugbuyiro et al. (2011). This may be due to the presence of the alginate beads. The ash content of the yoghurt under study was 3.93 per cent on dry basis and 0.839 on wet basis and exceeded the limits reported by Olugbuyiro et al. (2011). This may be due to the minerals present in the carrot juice. Fibre was also found at a minute quantity of 0.14 per cent. This may likely be due to some small fibres present in the carrot juice.

**CONCLUSION**

In this study, *L. acidophilus* showed good probiotic properties and hence can be considered as a potential candidate to be used in the preparation of probiotic dairy products. Carrot juice encapsulated probiotic lactic acid bacteria incorporated in yoghurt, can be commercially viable for developing fermented milk product with good therapeutic properties.

**REFERENCES**

AOAC, (2000). Method 942.15. Acidity (titratable) of Fruit Products. Official Methods of Analyses of the Associate of Official Analytical Chemists (17th ed.) AOAC, Washington D.C., USA.

Association of Official Analytical Chemists, (2006). Official Methods of Analysis, 18th Edn. 2005, Current Revision 1, (2006). Published by AOAC International, Suite 500, 481 North Frederick avenue, Gaithersburg, Maryland 20877-2417, USA.

Bansode, V., Pawar, D., Satpute, P.T., Zakiruddin, M., Jadhao, A.S and Machewad, G.M. (2012). Studies on effect of initial pH, storage temperature and preservatives on shelf life of yoghurt. Indian Journal of Dairy Science. 65(6).

De, Sukumar. (1980). Outlines of Dairy Technology. Oxford University Press, India.

FAO/WHO Working Group Report. (2002). Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada.

Garriga, M., Pascual, M., Montford, J.M and Hugas, M. (1998). Selection of Lactobacilli for chicken probiotic adjuncts. Journal of Applied Microbiology. 84: 125-132

Gibson, G.R. and Roberfroid, M.B. (1998). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. Journal of Nutrition.125:1410-1412.

Gilliland, S.E. (1979). Beneficial interrelationships between certain microorganisms and humans: candidate microorganisms for use as dietary adjuncts. Journal of Food Protection. 42: 164-167.

Goldin, B. R. and Gorbach, S. L. (1992). Probiotics for humans. In: Probiotics. [R. Fuller, (ed)], Chapmanand Hall, London. Pages 355-376

Indian Council of Medical Research –Department Biotechnology. (2011). Guidelines for Evaluation of Probiotics in Food, Published by Indian Council of Medical Research, New Delhi.

Jaya Prasad, Harsharanjit Gill, John Smart and Gopal, P.K. (1999). Selection and characterization of Lactobacillus and Bifidobacterium strains for use as probiotics. International Dairy Journal.8: 993-1002.

Jothi Sri, Surya Prabha, Seethadevi and Muthuprasanna. (2012). Microencapsulation: A review. International Journal of Pharmacology and Bio Science. 3: 509-531.

Joseph P. Salji and Anwar, A. (1983). Effect of initial acidity of plain yoghurt on acidity changes during refrigerated storage. Journal of Food Science. 48(1): 258-259.

Krasaekoorp, W.,Bhandari, B and Deeth H. (2003).Evaluation of encapsulation techniques of probiotics for yoghurt. International Dairy Journal. 13(1): 3-13.

McLauchalan, G., Fullarton, G.M., Crean, G.P. and McColl, K.E. (1998). Comparison of gastric body and antral pH: a 24
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hour ambulatory study in healthy volunteers. Gut. 30: 573-578.
Olugbuyiro and Joy E. Oseh (2011). Physico-chemical and sensory evaluation of market yoghurt in Nigeria. Pakistan Journal of Nutrition. 10(10): 914-918, ISSN 1680-5194.
Samona, A. and Robinson, R.K. (1994). Effect of yogurt cultures on the survival of bifidobacteria in fermented milks.

Journal of the Society of Dairy Technology. 47: 58-60.
Snedecor, G.W. and Cochran, W.G. (1980). Statistical Analytical Methods. Oxford and IBM Publishers Co., New Delhi, India.
Zinedine, A. and Faid, M. (2007). Isolation and characterization of strains of Bifidobacteria with probiotic properties. World J. Dairy and Food Science. 2(1): 28-34.