Sensory Evaluation of Dahi Supplemented with Co-Encapsulation of Pre- and Probiotics during Refrigerated Storage

K.B Vivek, K. Kondal Reddy, P.V. Mallikarjuna Reddy, L.V. Rao and K. Narsaiah

Abstract--- *The present study was done to evaluate the effect of co-encapsulation of certain probiotics namely, Lactobacillus paraplantarum 321 and Bifidobacterium bifidum 235 along with commercial prebiotic sugars (FOS) on the sensory characteristics in dahi samples at refrigerated condition for a storage period of 20 days. The co-encapsulation of probiotics @ 1×10^7cfu/g along with 3% prebiotic sugars (FOS) was done by extrusion method using 2% sodium alginate. Five different dahi samples were prepared i.e. control, dahi (T1) supplemented with encapsulated L. paraplantarum 321 along with prebiotics, dahi (T2) supplemented with non encapsulated L. paraplantarum 321 along with prebiotics, dahi (T3) supplemented with encapsulated B. bifidum 235 along with prebiotics and dahi (T4) supplemented with non encapsulated B. bifidum 235 along with prebiotics to study the sensory characteristics of dahi. The results indicated that T1 group flavour scores were significantly ($p<0.05$) higher than control group scores on 16th and 20th day whereas, T2 flavour scores were significantly ($p<0.05$) higher than control group scores on 20th day of storage only. T4 scores for flavour were significantly ($p<0.05$) higher than control group on 12th to 20th day of storage. T3 and T4 groups had body and texture scores significantly ($p<0.05$) higher than control group during 12th to 20th day of storage period. T3 group (4th to 20th day) and T4 group (12th to 20th day) had overall acceptability scores significantly ($p<0.05$) higher than control group during storage period. The supplementation of co-encapsulated B. bifidum 235 along with prebiotic (FOS) sugars improved certain sensory characteristics in dahi treatments during refrigerated storage.*

Keywords--- *Sensory Evaluation, Prebiotics, Probiotics Co-Encapsulation, Dahi*

I. INTRODUCTION

DAHI is a traditional Indian fermented milk product known for its refreshing taste, palatability and therapeutic values. It is prepared by fermentation of milk by using lactic acid bacteria. Dahi differs from yogurt in its use of mixed starters of mesophilic *lactococci*. Probiotics are referred to as "live microorganisms, which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). *Lactobacillus* and *Bifidobacteria* species are the most common types of probiotics used. Prebiotics are classified as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health" (Gibson and Roberfroid, 1995).

As the popularity of dahi products continue to grow, manufacturers are constantly evaluating value-added ingredients such as utilisation of co-encapsulated prebiotics and probiotics to entice health-consciousness of consumers.

Sensory analysis represents a critical step at various stages during food product development (Cruz *et al*., 2010). Inclusion of probiotics does not significantly alter the sensory properties of dairy products (Hekmat and Reid, 2006). Sensory analysis of dahi with co-encapsulated prebiotics and probiotics is required for manufacturers so as to have healthful ingredients into their products. Jayamanne and Adams, (2004)

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evaluated sensory properties of probiotic curd using bifidobacteria prepared from buffalo milk and showed improved sensory properties. Although dahi is widely consumed, its sensory profile has received less attention.

No investigation has been reported on sensory attributes of dahi incorporated with co-encapsulated pre- and probiotics during refrigerated storage in dahi. The aim of the present study was to evaluate the sensory characteristics of dahi supplemented with encapsulated pre and probiotics during refrigerated storage.

II. MATERIALS AND METHODS

- *Probiotic Cultures*

The probiotic organisms used in the present study are *Lactobacillus paraplantarum* 321 and *Bifidobacterium bifidum* 235, obtained from National Collection of Dairy Cultures, NDRI, Karnal, and Haryana. *L. paraplantarum* 321 and *B. bifidum* 235 strains were reconstituted in MRS broth and incubated for 24 h at 37^0C. Cells were then cultured in the same conditions for three successive transfers in MRS broth at 37^0C for 20-24 h to get pure colonies. Then, they were grown in fermenter using MRS broth for production of freeze dried *L. paraplantarum* 321 and *B. bifidum* 235. The cells were harvested by centrifugation at 5000 rpm for 15 minutes at 4^0C and washed with 0.9% normal saline, lyophilised and stored at 4^0C.

- *Culture Activation and Maintenance of Dahi Starter Culture*

The mixed dahi starter culture NCDC-167 (*Lactococcus lactis ssp. lactis*, *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis biovar. diactetylactis* in 1:1:1 ratio) was procured from National Collection of Dairy Cultures, NDRI, Karnal, Haryana. The starter culture was maintained in sterilised reconstituted skimmed milk (12 g/100 ml) by subculturing once in a fortnight for attaining high activity (Raju and Pal, 2009). The subcultured dahi starter was used as inoculum, grown in fermenter using MRS broth incubated for 48 h at 37^0C and then centrifuged at 5000 rpm for 15 minutes at 4^0C and washed with normal saline and harvested to get starter culture.

- *Micro-Encapsulation*

The micro-encapsulation of *L. paraplantarum* 321 and *B. bifidum* 235 using 1% sodium alginate as coating material was carried out according to the method of Chen *et al*. (2005), with some modification using micro-encapsulator.

- *Preparation of Probiotic Dahi*

Probiotic dahi was prepared according to the method of Yadav *et al*. (2005) with some modifications. Toned milk was heated to 90^0C for 15 minutes and cooled to 37^0C and divided into different lots. Then, it was inoculated with dahi starter culture NCDC-167 (1×10^7 cfu/g) along with probiotic bacteria with the same concentration and commercial prebiotic (FOS) added one gram/litre in non encapsulated dahi groups, and encapsulated dahi groups added with encapsulated powder added @ one gram/litre of dahi and control containing only dahi starter culture. All dahi treatment groups were packed and stored at 4^0C.

- *Sensory Evaluation*

The properties such as appearance, body and texture, flavour and overall acceptability of different treatments of dahi samples were evaluated by 5 untrained panellists using a 9 point hedonic scale. Randomly, each cup of treatment was drawn from the refrigerator and was served to panellists for judging.

III. RESULTS AND DISCUSSION

The results of the dahi supplemented with encapsulated and non encapsulated pre- and probiotics are presented in the tables 1-3.

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- *Flavour Scores of Dahi Samples*

There was a general decrease in mean flavour scores (Table 1) of all dahi samples, but T3 and T4 the decrease in scores was slower than T1 and T2. On initial day, there was no significant ($p<0.05$) difference in mean flavour scores of all dahi samples. On 16th and 20th day, T1 group flavour scores were significantly ($p<0.05$) higher than control dahi group scores.Mortazavian *et al*. (2007) reported that micro-encapsulation of probiotics leads to flavour fixation of fermented products because encapsulated cells are relatively or totally inactive in metabolism and do not influence flavour profile of the products, especially during storage time.

On 20th day, T2 flavour scores were significantly ($p<0.05$) higher than control dahi scores. It may be due to slight decline in pH and proportionate increase in acidity in control dahi group affecting flavour compared to dahi supplemented with T2 group.The scores of T3 group were significantly ($p<0.05$) higher from 8th to 20th day of storage than control dahi group. It may be due to dormant state of probiotic bacterial metabolism within the microcapsule and during fermentation process and not affecting the flavour. The results is similar to the findings of Adhikari *et al*. (2000) who reported that off flavour produced by *Bifidobacterium* spp. with acetic acid production in yogurt during fermentation had overcome upon micro-encapsulation and flavour also improved. The result is in agreement with the findings of Noland and Aryana (2012) who reported that flavour scores in yogurt with micro-encapsulated bacteria were significantly higher compared to yogurt with non encapsulated bacteria.

The scores for T4 group were significantly ($p<0.05$) higher from 12th to 20th day of storage than control, T1 and T2 groups, but significantly ($p<0.05$) lower than T3 group for the corresponding period of storage. This result is in agreement with Jayamanne and Adams, (2004) who reported that the higher sensory properties associated with probiotic curd could be because of the production of total volatile fatty acids diacetyl and acetyl methyl carbinol by added *Bifidobacteria* in curd. This result is also in agreement with Kebary, (1996) who reported that the flavour of frozen yogurt made with *B. bifidum* was improved due to increase in diacetyl and acetyl methyl carbinol.

The published reports suggest that beads of capsules with the range of 1-3 mm in diameter can adversely affect both texture and flavour of the final product (Chandramouli *et al*., 2004). The alginate beads (Fig. 1) were in the range of 35.7-96.7 µm, which might have not adversely affected the flavour profile in dahi. By 16th and 20th day, the control dahi samples scored lower than all other treatment groups. It might be due to decline in pH and increased level of acid metabolites, in dahi by the starter bacteria. Proteolysis in control dahi group during storage period may also have affected the flavour scores.

- *Body and Texture Scores of Dahi Samples*

There was a general decrease in body and texture scores of all dahi samples, but T3 and T4 samples showed slower rate of decrease in scores than in T1 and T2 groups (Table 2). The mean scores for body and texture (out of 9 points) recorded among treatment dahi samples on initial and 8th day showed no significant ($p<0.05$) difference though some differences were observed on 4th day. T1 group samples showed no significant ($p<0.05$) difference from control dahi group from 4th to 20th day for body and texture scores. It may be due to controlled release of probiotic and fermentation without rapidly decreasing the pH in dahi during refrigerated storage. The body and texture scores of dahi samples supplemented with T1 and T2 groups showed no significant ($p<0.05$) difference from control dahi samples during 4th to 20th day of storage.

T3 group body and texture scores were significantly ($p<0.05$) higher than control dahi group during 12th to 20th day of storage period. The results are consistent with the findings of Khalil and Mansour, (1998) who reported that encapsulated mayonnaise had improved texture due to the production of exopolysaccharides produced by *Bifidobacteria* and/or with the presence of calcium alginate. Beads size in the range of 1-3 mm in diameter can adversely affect both texture and flavour of the final product (Chandramouli *et al*., 2004). Small capsules or beads under controlled conditions may not affect the texture of food products (Rokka and Rantamaki, 2010). The alginate bead (Fig. 1) sizes ranged from 35.7 to 96.7µm which therefore, might have not adversely affected on body and texture profile in T3 dahi group.

T4 group had lower scores for body and texture than control dahi group on 4th day, but T4 scores were significantly ($p<0.05$) higher than control dahi group on 12th to 20th day of storage. It might be due to

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production of exopolysaccharides by probiotic bacteria during fermentation process during refrigerated storage. The result is similar with the findings of Seydin *et al*. (2005) who reported that yogurts fortified with inulin as a prebiotic had good flavour and smooth texture.

- *Overall Acceptability Scores of Dahi Samples*

A general gradual decrease was observed in overall acceptability scores of all dahi treatment samples over a storage period of 20 days. T3 and T4 samples showed a slower rate of decrease in scores than in T1 and T2 group. The mean scores for overall acceptability recorded (Table 3) in dahi samples on initial day, showed no significant ($p<0.05$) difference in mean overall acceptability scores among the dahi treatment groups. Control dahi scores for overall acceptability was significantly ($p<0.05$) lower than T1 on 20th day. It might be due to encapsulation causing *L. paraplantarum* 321 to be in inert state, with less acid production during fermentation process without affecting acceptability during the storage period. The results are consistent with the findings of Khalil and Mansour (1998) who reported that encapsulated mayonnaise had improved texture due to the production of exopolysaccharides produced by *bifidobacteria* and/or with the presence of calcium alginate.

Control dahi scores for overall acceptability were comparable to T2 group up to 16th day and control dahi group showed significantly ($p<0.05$) lower scores than T2 on 20th day of storage. It might be due to higher acidity and pH decline in control group with lower scores than non encapsulated group.

T3 group overall acceptability scores were significantly ($p<0.05$) higher than control group from 4th to 20th day of storage. It may be due to controlled release of probiotic and fermentation without rapidly decreasing the pH in dahi during refrigerated storage. The results are consistent with the findings of Khalil and Mansour, (1998). This result is also in agreement with the observations of An-Erl King *et al*. (2007) who stated that the sensory quality (overall acceptance) of fermented tomato juice showed improvement upon incorporation of encapsulated cells compared to free cells.

T4 scores for overall acceptability were comparable to control group on 4th, 8th day. But, T4 group overall acceptability scores were significantly ($p<0.05$) higher than control dahi group during 12th to 20th day of storage period. It might be due to production of metabolites by probiotic bacteria during fermentation process during refrigerated storage. But control dahi samples with higher decline in pH and increased fermentation process gave lower score than T4 group. The result is in agreement with Jayamanne and Adams, (2004).

IV. CONCLUSION

It may be concluded that co-encapsulation of pre- and probiotics improved flavour, body and texture and overall acceptability in dahi. The addition of encapsulated *Bifidobacterium bifidum* 235 along with prebiotic sugars influenced positively, on its overall acceptability during the storage period and received higher sensory scores compared to other treatments and control group. Future studies are necessary to find the effect of probiotics with different coating materials on the organoleptic properties of dairy based products.

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REFERENCES

[1] Adhikari, K., Mustapha, A., Gruˇn, I.U. and Fernando L. 2000. Viability of microencapsulated *Bifidobacteria* in set yogurt during refrigerated storage. *J. Dairy Sci.* 83:1946-1951.

[2] An-Erl King, V., Huang, H. Y. and Tsen J. H. 2007. Fermentation of tomato juice by cell immobilized *Lactobacillus acidophilus*. *Mid-Taiwan Journal of Medicine,* 12(1):1-7.

[3] Chandramouli, V., K.Kalasapathy, P.Peiris and M.Jones, 2004. An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. *J Microbiol Meth.,* 56: 27-35.

[4] Chen, K.N., M.J.Chen, J.R.Liu, C.W.Lin and H.Y.Chiu, 2005. Optimization of incorporated prebiotics as coating materials for probiotic microencapsulation. *J. Food Sci*., 70: 260-266.

[5] Cruz, A.G., R.S.Cadena, E.H.M.Walter, A.M.Mortazavian, D.Granato, J.A.F.Faria and H.M.A.Bolini, 2010. Sensory analysis: relevance for prebiotic, probiotic, and synbiotic product development. *Comp. Rev. Food Sci. Food Saf.* 9: 358-373

[6] FAO/WHO Experts' Report. 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba, Argentina.

[7] Gibson, G.R. and M.B.Roberfroid, 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr.* 125:1401-1412.

[8] Hekmat, S. and G.Reid, 2006. Sensory properties of probiotic yogurt is comparable to standard yogurt. *Nutr. Res. Rev.* 26:163-166.

[9] Jayamanne, V.S. and Adams, M.R. (2004) Survival of probiotic bifidobacteria in buffalo curd and their effect on sensory properties. *Int. J Food Sci. & Technol.* 39:719-725.

[10] Kebary, K.M.K. 1996. Viability of *Bifidobacterium bifidum* and its effect on quality of frozen zabady. *Food Research International,* 29(5-6):431-437.

[11] Khalil, A.H. and Mansour, E.H. 1998. Alginate encapsulated *bifidobacteria* survival in mayonnaise. *Journal of Food Science* 63: 702-705.

[12] Mortazavian, A., Razavi, S.H., Ehsani, M.R. and Sohrabvandi, S. 2007. "Principles and methods of microencapsulation of probiotic microorganisms". *Iranian Journal of Biotechnology*, 5(1):1-18.

[13] Noland, E. and K.J.Aryana, 2012. Influence of micro-encapsulated probiotic *Lactobacillus acidophilus* R0052 on the characteristics of plain yogurt. *Advances in Microbiology* 2: 364-367.

[14] Raju, P.N. and D.Pal, 2009. The physico-chemical, sensory and textural properties of misti dahi prepared from reduced fat buffalo milk. *Food and Bioprocess Technology,* 2(1):101-108.

[15] Rokka, S. and Rantamaki, P. 2010. Protecting probiotic bacteria by microencapsulation: Challenges for industrial applications. *Eur. Food Res. Technol.* 231:1-12.

[16] Yadav, H., Jain, S. and Sinha, P.R. 2005. Preparation of low fat probiotic Dahi. *Food Technol.,* 55(11):46-53.

Table 1: Effect of Different Treatments and Storage Periods on Mean Flavour Scores of Dahi

Treatment	Initial day	4th day	8th day	12th day	16th day	20th day
Control	8.6±0.24	8.0^{ab}±0.20	7.4^{b}±0.24	6.8^{c}±0.20	6.0^{d}±0.20	5.2^{d}±0.20
T1	8.4±0.24	8.0^{ab}±0.20	7.8^{ab}±0.20	7.0^{c}±0.20	6.6^{c}±0.24	6.0^{c}±0.20
T2	8.2±0.20	7.8^{b}±0.20	7.4^{b}±0.24	6.8^{c}±0.20	6.4^{cd}±0.24	5.8^{c}±0.20
T3	8.8±0.20	8.4^{a}±0.24	8.4^{a}±0.24	8.2^{a}±0.20	8.0^{a}±0.20	7.4^{a}±0.24
T4	8.6±0.24	8.0^{ab}±0.20	8.0^{ab}±0.20	7.6^{b}±0.24	7.2^{b}±0.20	6.6^{b}±0.24

Each mean value is obtained from four replications

^{abc}Values with same superscripts in a column do not differ significantly at the level of $p<0.05$

Table 2: Effect of Different Treatments and Storage Periods on Mean Body and Texture Scores of Dahi

Treatment	Initial day	4th day	8th day	12th day	16th day	20th day
Control	8.6±0.24	8.0^{ab}±0.20	7.8±0.24	6.6^{b}±0.51	6.6^{b}±0.24	6.0^{b}±0.20
T1	8.4±0.24	8.2^{ab}±0.20	7.8±0.20	7.4^{ab}±0.24	7.0^{ab}±0.20	6.2^{b}±0.20
T2	8.6±0.24	8.0^{ab}±0.20	8.0±0.20	7.4^{ab}±0.24	7.0^{ab}±0.20	6.2^{b}±0.20
T3	8.4±0.24	8.4^{a}±0.24	8.0±0.20	8.0^{a}±0.20	7.6^{a}±0.24	7.0^{a}±0.37
T4	8.6±0.20	7.8^{b}±0.20	7.8±0.24	7.8^{a}±0.20	7.4^{a}±0.24	7.0^{a}±0.20

Each mean value is obtained from four replication

^{ab}Values with same superscripts in a column do not differ significantly at the level of $p<0.05$

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Table 3: Effect of Different Treatments and Storage Periods on Mean Overall Acceptability Scores of Dahi

Treatment	Initial day	4th day	8th day	12th day	16th day	20th day
Control	8.6±0.24	7.8^{b}±0.20	7.4^{b}±0.24	7.0^{b}±0.20	6.2^{b}±0.20	5.4^{c}±0.24
T1	8.6±0.24	8.2^{ab}±0.20	7.6^{b}±0.24	7.0^{b}±0.20	6.6^{b}±0.24	6.4^{ab}±0.24
T2	8.8±0.20	8.0^{ab}±0.20	7.8^{ab}±0.20	7.0^{b}±0.20	6.6^{b}±0.24	6.2^{b}±0.20
T3	8.8±0.20	8.4^{a}±0.24	8.4^{a}±0.24	8.0^{a}±0.20	7.4^{a}±0.24	7.2^{a}±0.37
T4	8.6±0.24	8.0^{ab}±0.20	8.0^{ab}±0.20	7.8^{a}±0.24	7.4^{a}±0.24	7.2^{a}±0.20

Each mean value is obtained from four replication

^{abc}Values with same superscripts in a column do not differ significantly at the level of $p<0.05$

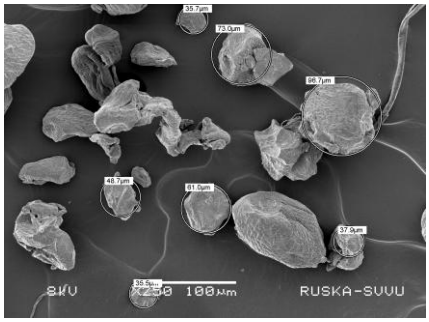

Fig. 1: Scanning Electron Microscopy Showing Varying Sizes of Alginate Micro-Capsule

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