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RESEARCH PAPER

Study the Effect of different Packaging Material on Lactic Acid Bacteria in Indian Fermented Foods

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Abstract

Fermented foods are commonly consumed in our daily diets. They not only provide nutritional benefits but can also act as functional foods when optimal level of lactic acid bacteria is achieved which might have probiotic potential. The viability of lactic acid bacteria depends on various intrinsic and extrinsic factors. The packaging material plays an important role in maintaining the shelf life of the product. In the present study various Indian fermented foods were prepared by spontaneous fermentation and effect of packaging material on lactic acid bacteria viability was explored. Products like mix vegetable pickle, raw papaya pickle, mix mango pickle, lemon-ginger and green chilli pickle and kanji was prepared under standardised conditions. Three packaging material; borosil glass bottles, air tight pearlpet bottles and tinted borosil glass bottles were used. As noted from the results that statistically no significant difference was observed. Thus the viability of lactic acid bacteria is not dependent on the material used for packaging unless it is air tight and on the exposure to light till uniform heat is provided.

Keywords: Fermented foods, lactic acid bacteria, pH, Packaging material

Fermented foods have always been part of our daily diets. As defined by FAO (1998) fermentation is a "slow decomposition process of organic substances induced by microorganisms or enzymes, of plant or animal origin". It is essentially the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof, under anaerobic conditions. Lactic acid bacteria (LAB) is a group of bacteria that produce lactic acid as their major end products from carbohydrates through fermentation. Lactic acid bacteria are widely used in food industry as they ferment sugars to lactic acid thereby, lowering the carbohydrate content and pH of food and thus inhibiting the growth of pathogenic microorganisms (Axelsson, 2004; Holzapfel *et al.* 2001). The use of Lactic Acid Bacteria (LAB) in foods and food supplements has a long history and most strains are considered commensal microorganisms with no pathogenic potential.

Earlier safety and shelf-life stability was the main focus while formulating a product, but due to increased awareness among the consumer the demand is now for higher quality with greater convenience. The packaging plays a fundamental role in maintaining the quality and shelf-life of foods. The package is an integrated part of the preservation system and functions as a barrier between the food and the external atmosphere. The package should be designed and developed not only to hold the food product, but also to protect it and add value to it, since its design may directly affect the purchase decision of the consumer (Roberston, 1993).

Further the consumption of probiotic foods has increased over the past years as a result of their benefits for human health. As reported in many studies the packaging material used have an impact on the viability of LAB having probiotic potential.

Thus development of packaging materials that adequately protect and preserve the therapeutic activity of probiotic foods is necessary (Cruz *et al.* 2007). Several aspects of food packaging materials including the type of the packaging materials (Glass and plastic), their thickness, and the application of active/intelligent packaging systems could influence the survival of probiotic bacteria (Korbekandi *et al.* 2011). Dave and Shah (1997) studied the survival of probiotic bacteria in yogurt made in plastic containers and glass bottles. For the samples stored in glass bottles, the counts remained higher than those stored in plastic cups due to low oxygen permeability. Mattila-Sandholm *et al.* (2002) reported that the packaging materials and the storage conditions are important factors for the quality of products containing probiotic microorganisms.

Hisiao *et al.* (2004) study highlighted the effect of the packaging material and the storage temperature on the viability of microencapsulated bifidobacteria. The samples filled in glass bottles; polyester bottles with dessicant and oxygen absorber and polyester bottles without desiccant and oxygen absorber were evaluated. It was reported that the product in glass bottles stored at 4 °C, with a reduction of only 0.15–0.20 log CFU/ g after 42 days storage gave the best results. Similarly, Kasımoğlu *et al.* (2004) investigated the effect of a packaging system using vacuum and brine on Turkish white cheese ripening. The authors observed that vacuum packaged cheese presented best performance in the sensorial evaluation (good flavour and texture) besides a high level of proteolysis.

In Japan, the Fermented Milk and Lactic Beverages Association has specified that there be at least 10^7 CFU/ml of viable *Bifidobacteria* cells in fermented milk drinks. To achieve the desired effects in probiotic yogurts, it is recommended that the minimum counts of viable probiotic bacteria be at least 10^8 CFU/ mL (Lourens-Hattingh and Viljoen, 2001).

Soares *et al.* (2005) highlight the fact that the design and other features of food packaging have gained enormous importance because the packages functions as the first point of contact between the consumer and the product. In the present study the effect of household packaging material used to store the fermented Indian products on the LAB count was studied.

MATERIALS AND METHODS

Formulation of fermented products

Different traditional Indian fermented products were prepared using household techniques under standardized conditions. Good quality raw material was procured from local grocery shops in Delhi, India. Products like mix vegetable pickle, raw papaya pickle, mix mango pickle, lemon-ginger and green chilli pickle and *kanji* was formulated during the study. Mix vegetable pickle was prepared using carrot, turnip and cauliflower with 6% salt concentration, raw papaya pickle was prepared with 5% salt concentration, mix mango pickle was prepared by using raw mango, ginger, lemon, green chilli, cranberry with 6% salt concentration. Lemon-ginger and green chilli pickle was prepared with 8% salt concentration while kanji was prepared using black carrots (*Daucus carota)* with 8% salt concentration. Mustard oil was added in mix vegetable pickle, raw papaya pickle and mix mango pickle. Fermentation and storage of the products was carried out at 37°C.

Variation in packaging material

Three types of packaging material were used during the study:

- **Borosil glass bottles:** The borosil glass bottles used for storing the samples was complying with IS 1388 (Part III). The quantity that could be stored was 250 ml, with 70 × 138(approx OD × height mm) dimensions and GL 45 as thread specification.

- **PET bottles:** PET bottles used were non-toxic, food-grade and re-usable. PearlPET bottle uses virgin PET, PP or LDPE materials which are universally approved by USFDA, EU Directive 2002/72/EC, BIS IS:12252 -1987 and other regulating bodies for Food, Beverage and Water storage.
- **Borosil tinted glass bottles:** The borosil tinted glass bottles specification Complies with IS 1388 (Part I), ISO / DIN 4796 (Part I) with uniform amber colour and 250 ml storage capacity and 70 x 138 (approx OD X height mm) dimensions with GL 45 (thread specification).

Physico-chemical Analysis

All the sample were packaged in the above mentioned material and were analysed periodically for pH, Titratable acidity (TA) (AOAC,1984) and LAB count.

Viable cell enumeration

LAB count was determined on MRS Agar procured from Hi Media (India) (IS:14884). Appropriate dilutions were plated in triplicates. Pour plate method was used for plating and after incubation period of 2 days at 30°C colonies, were counted. Biochemical tests were carried out by picking up the representative colonies and classified by Gram staining and morphological examination. Gram staining of culture, catalase test and Indole test were also conducted (Aneja, 2003).

Statistical analysis

Data were submitted to one-way analysis of variance (ANOVA) with a least significant difference of 0.05 to study the significant difference between various conditions using SPSS version 24. Tukey's multiple comparison method was used to further examine any significant differences between the results.

RESULTS AND DISCUSSION

Mix vegetable pickle packed in three different packaging materials showed a marginal difference. A decline in pH from 6.04 to 3.60 in glass bottle, 6 to 3.59 in plastic bottles and 6.08 to 3.60 in tinted glass bottles was observed. While TA increased from 0.22% to 1.79% in glass bottle, 0.22-1.74% in plastic bottles and 0.22-1.76% in tinted glass bottle. The LAB count was maximum at 7.518 log cfu/g level in glass bottle and with the shelf life of 39 days. While pickle stored in plastic bottle the maximum LAB count observed was 7.523 log cfu/g and shelf life of 39 days. In tinted glass bottles the maximum LAB count was 7.531 log cfu/g and shelf life of 39 days (Fig. 1). Statistically also no significant difference was observed at $p<0.05$ in pH, TA and LAB count of three variations in packaging material. Azeredo *et al.* (2004) consider that the protection provided by the packaging is the factor of greatest importance, since it is directly related with the safety of the consumer. It was also emphasized that the package must be appropriate for each food product to minimize undesirable changes that may affect the product during its shelf life. As seen from the results the packaging material and light do not have an impact on the LAB count of the pickle.

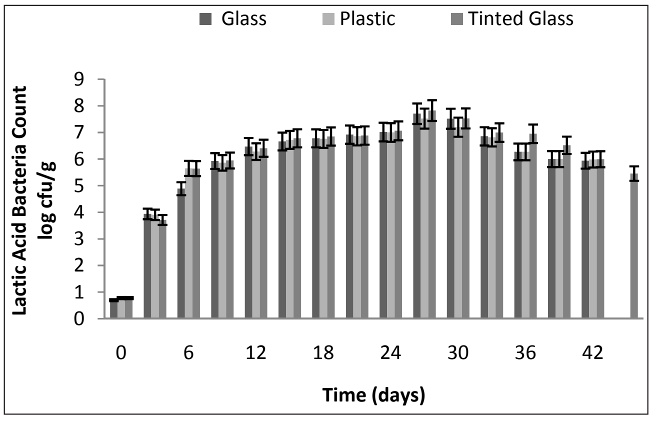

Fig. 1: Changes in LAB count of mix vegetable pickles with varying packaging material

Similar results were observed in raw papaya pickle packed in three different packaging materials as a substantial reduction in pH was observed in all the three samples from 4.62 to 3.54 in glass bottle, 4.64 to 3.59 in plastic bottle and 4.63 to 3.56 in tinted glass bottle. While TA increased marginally from 0.42 to 1.30% in glass bottle, 0.44 -.24% in plastic bottle and 0.44 -1.31% in tinted glass bottle. But the difference between the three samples obtained from three packages in pH and TA was marginal. The LAB count was maximum at 8.303 log cfu/g level in glass bottle and kept the shelf life to 48 days. While in pickle stored in plastic bottle the maximum LAB count observed was 7.820 log cfu/g and shelf life of 44 days. In tinted glass bottles the maximum LAB count was 7.975 log cfu/g and shelf life of 48 days (Fig. 2). Statistically also no significant difference was observed at $p<0.05$. Similar results were obtained by Randheera *et al.* 2014 which highlighted the effect of polypropylene, polyethylene or glass containers, sealed and stored at −20 °C on probiotic microorganism count and it was reported that the viable numbers of all probiotics remained 10^7 to 10^8 cfu g^{-1} up to 52 weeks at −20 °C regardless of the type of packaging.

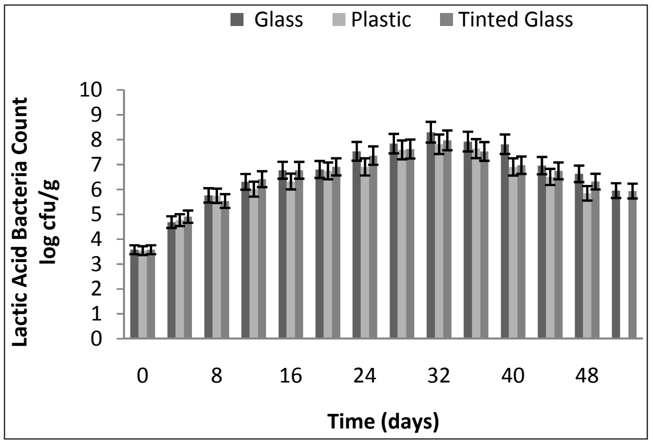

Fig. 2: Changes in LAB count of raw papaya pickles with varying packaging material

In lemon, ginger and green chilli pickle pH declined from 2.91 to 2.72 in glass bottle, 2.91 to 2.70 in plastic bottle and 2.90 to 2.66 in tinted glass bottle and TA increased from 3.28 to 4.05% in glass bottle, 3.28 to 4.08% in plastic bottle and 3.28 to 4.08%. The trend in decline in pH and incline in TA was similar in all the three samples taken from these packages. The LAB count was maximum at 6.880 log cfu/g level in glass bottle and keeping the shelf-life to 18 days (Fig. 3). While in pickle stored in plastic bottle the maximum LAB count observed was 6.924 log cfu/g and shelf life of 18 days. In tinted glass bottles the maximum LAB count was 6.993 log cfu/g with a shelf life of 20 days. Statistically also no significant difference was observed at $p<0.05$. Thus as observed from the results the LAB count is not affected by the packaging material used and there is no influence of light as well.

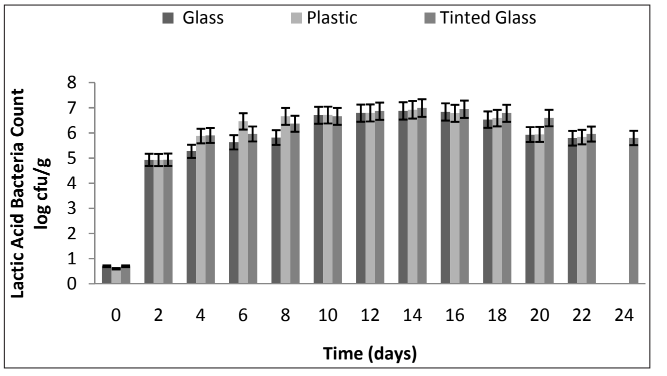

Fig. 3: Changes in LAB count of lemon, ginger and green chilli pickles with varying packaging material

Mix raw mango pickle packed in three different packaging materials showed a decline in pH from 4.03 to 3.20 in glass bottle, 4.05 to 3.25 in plastic bottle and 4.05 to 3.19 in tinted glass bottle. While TA increased from 1.96 to 2.24% in glass bottle, 1.97 to 2.20% in plastic bottle and 1.97 to 2.20 in tinted glass bottle. The maximum LAB count was 7.406 log cfu/g level in glass bottle and keeping the shelf life to 70 days. While in pickle stored in plastic bottle the maximum LAB count observed was 7.287 log cfu/g and shelf life of 65 days. In tinted glass bottles the maximum LAB count was 7.424 log cfu/g and shelf life of 70 days (Fig. 4). Statistically also no significant difference was observed at $p<0.05$.

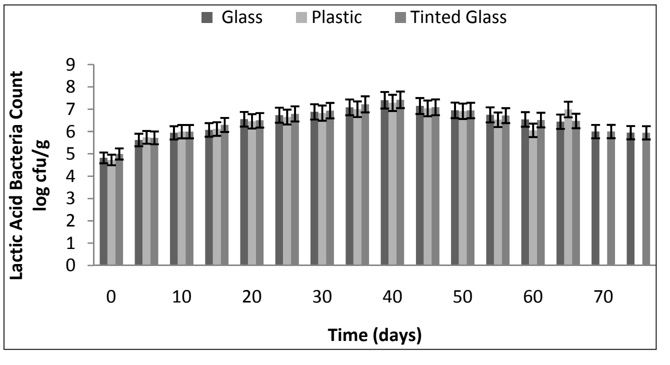

Fig. 4: Changes in LAB count of mix raw mango pickles with varying packaging material

Kanji was also stored in different packaging material and the changes in pH, TA and LAB count was studied. The pH declined significantly from 5.92 to 3.18 in glass bottle, 5.92 to 3.42 in plastic bottle and 5.92 to 3.34 in tinted glass bottle. While TA increased from 0.52 to 0.719% in glass bottle, 0.48 to 0.711% in plastic bottle and 0.50 to 0.708% in tinted glass bottle. However the trends in pH and TA of the three packaging material is quite similar. The LAB count was maximum at 8.478 log cfu/ml level in glass bottle and keeping the shelf life to 28 days. While in pickle stored in plastic bottle the maximum LAB count observed was 8.309 log cfu/ml and shelf life of 26 days. In tinted glass bottles the maximum LAB count was 8.631 log cfu/ml with a shelf life of 30 days (Fig. 5).

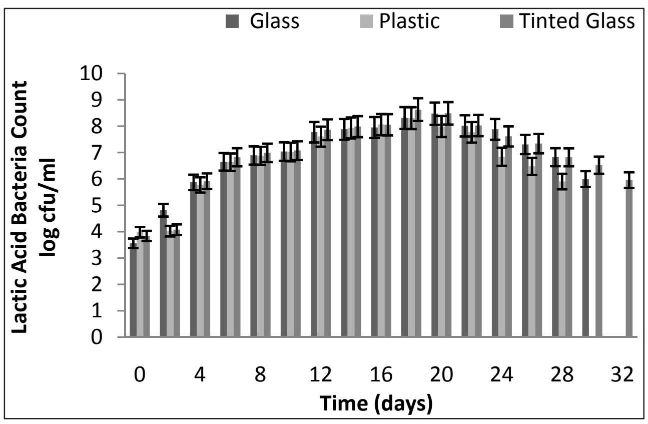

Fig. 5: Changes in LAB count of *kanji* with varying packaging material

Statistically also no significant difference was observed at $p<0.05$. A study was conducted on fermented dairy product which was fermented in three different package types (clay pots, plastic cups and glass bottles) and stored at two different temperatures 29 °C and 4 °C. It was found that the *Bifidobacteria* survived best in the glass bottles, followed by the plastic packages and the clay pots when stored at 29 °C. The results obtained suggest that the use of packaging materials with low oxygen permeability properties is required to obtain a product that produces beneficial effects on the health of the consumer and a shelf life beyond 4 days (Jayamanne and Adams, 2004).

In all the products packaged in three different packaging material a marginal difference in pH, TA and LAB count was observed among the three material used. But shelf life of the raw papaya pickle, lemon ginger and green chilli pickle, mix mango pickle and *kanji* was less in sample stored in plastic containers as the yeast and mold count increased above the optimum level. The growth of molds in food are influenced by multiple variables, such as water activity (a_w), temperature, pH, atmosphere composition, substrate, interaction among species, and time (Astoreca *et al*. 2014).

Further this reason could be supported by theory of Beuchat (1983) which states that 'fungal spoilage of foods occurs more often than bacterial spoilage at a_w 0.61–0.85 not because fungi grow faster at reduced a_w but rather because the competitive effects of the vast majority of bacteria are absent. Higher a_w is generally required for spore formation than for spore germination. The range of a_w permitting germination of spores is greatest at an optimum temperature, but optimum availability of nutrients tends to broaden the range of a_w and temperature at which germination and growth will occur'. Thus PET container was not able to provide protection and may increase the water activity in the product resulting in high yeast and mold growth.

CONCLUSION

Packaging plays an important role in maintaining the shelf-life of the product. All the packaging material used in the study were air tight and thus no significant difference was observed in the trend of lactic acid bacteria count in all the samples. As the lactic acid bacteria are anaerobic thus the condition maintained were suitable for its growth regardless of the type of material used for packaging. These packaging materials are commonly used in our household. Thus as illustrated from the result it could be inferred that viability of lactic acid bacteria was similar in all the product used. Moreover, it could be noted that shelf life of mix vegetable pickle, raw papaya pickle, mix mango pickle was more when compared to lemon-ginger and green chilli pickle and *kanji*. This could be due to addition of mustard oil which cuts the oxygen contact and making the environment anaerobic for better growth of lactic acid bacteria.

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