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Influence of a finger millet-soybean matrix on the viability of probiotic lactic acid bacteria exposed to high temperature

Minelly Rodrigues[1], Subhadra Mandalika[1] and Sahayog N Jamdar[2]
[1]Department of Food, Nutrition and Dietetics, College of Home Science, Nirmala Niketan, 49, New Marine Lines, Mumbai 400020, [2]Food Technology Division, Bhabha Atomic Research Centre, Trombay Mumbai 400085

Abstract:- Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Exposure to heat stress during food product applications can lower their viability. Hence, the influence of a food matrix [fermented finger millet- soybean (FM-SY) blend] prepared using malted, irradiated or enzymatically treated commodities on the survivability of lactic acid bacteria (LAB) under heat stress (40°C-70°C) for 10 min was assessed in the current study. Presence of the LAB in the medium of FM-SY enhanced their survivability under heat stress as compared to free cell suspensions (FCS) ($p<0.05$). The desirable probiotic counts of > 7 log cfu/ ml were maintained ($p>0.05$) up to 55°C and 50°C in case of FM-SY blends and FCS respectively. A progressive reduction in cell counts was observed above these temperatures along with an increase in time. No viable counts were detected in FM-SY blends and FCS at 70°C and 65°C respectively. The survivability did not differ remarkably with the use of different processed blends of FM-SY and the use of FCS of different LAB ($p>0.05$). The results showed that the food matrix of finger-millet soybean had an immense positive impact on the viability of LAB exposed to heat stress. Hence, such foods can be used as protective components for improved survivability of probiotics in product development that involves application of heat.
Keywords: Probiotics, Finger millet, Soybean, Heat stress

1.0 Introduction

Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/ WHO, 2002). Various species of genera *Lactobacillus* have been used as probiotics over the years. Countless benefits to health are provided by the ingestion of these cultures and include conditions such as diarrhoea, lactose intolerance, urinary tract infection, immune disorders, hypercholesterolemia, high blood pressure, cancer, food allergy etc (Daliri and Lee, 2015).

Probiotics must be consumed at a level of 10^7 cfu/ mL for successful colonization in the gut (Rosburg et al, 2010). Heat stress during product formulation can lower the viability of these organisms. A synbiotic product providing a mixture of probiotics and prebiotics may be effective in protecting survivability of these organisms. In recent years, the potential of cereals, millets and legumes in the formulation of probiotic foods has been extensively investigated. They can be utilized as fermentable substrates for growth of probiotic *Lactobacilli* and as prebiotics due to their specific content of non digestible carbohydrates. Further on they may also act as encapsulation materials for probiotics in order to enhance their stability (Charalampopoulus et al, 2002; Yeo and Liong, 2010). Processing of finger millet- soybean using methods such as malting, irradiation or enzymatic processing leads to depolymerisation of macromolecules in the commodity leading to the formation of simpler compounds like glucose and amino nitrogen. These changes improve the amenability of the substrates for fermentation (Rodrigues et al, 2014). Hence, the objective of the current work was to study the influence of fermented finger millet- soybean (FM-SY) blends prepared using malted, irradiated or enzymatically treated commodities on the survivability of LAB exposed to heat stress.

2.0 Materials and methods

Finger millet (*Eleusine coracana*) and soybean (*Glycine max*), procured from the local market were cleaned and stored at 5°C until further use. The microorganisms used in the study were *Lactobacillus rhamnosus* MTCC 1423, *Lactobacillus plantarum* MTCC 2621 and *Lactobacillus fermentum* MTCC 903 (Microbial Type Culture Collection, Chandigarh, Haryana, India).

2.1 Slurry preparation and fermentation

Slurries were prepared as per the procedure developed and documented earlier (Rodrigues et al, 2014). Unprocessed, malted (8h soaked, 24h germinated, 16h dried at 60°C), irradiated (10 kGy) and enzymatically processed [α-amylase (18U/ml) + glucoamylase (1.35U/ml) + alcalase (0.001AU/ml)] finger millet-soybean slurries were inoculated with a mixed culture of LAB and fermented at 37°C for 16 h.

2.2 Preparation of FCS

Stock cultures of LAB were inoculated in MRS broth and incubated at 37°C for 24 h, followed by centrifugation at 6,000 rpm for 10 min at 10°C. The cells were washed with saline (0.85%) and were resuspended to obtain a cell count of ~9 log cfu/ ml saline.

2.3 Temperature tolerance assay

1 ml of fermented slurry or FCS were dispensed into 1.5 ml microfuge tubes and placed in a water bath set to the desired temperature (40°C-70°C). The viable counts were enumerated after 5 min and 10 min heat exposure by pour plate technique using MRS Agar (HiMedia Laboratories, India). The plates were incubated at 37°C for 48 h.

2.4 Statistical analysis

The data was analyzed using SPSS, 16.0. (Chicago, SPSS Inc.). One-way ANOVA was used to compare the differences in mean between the samples. Level of significance was set to 0.05. For multiple comparisons Tukey's post hoc test was used.

3.0 Results and discussion

High viable counts of probiotics are desired in foods at the time of consumption to ensure successful colonization in the gut, after the harsh transit through the upper gastrointestinal tract. Food processing that involves the application of heat may affect survival of these beneficial organisms. Hence, the influence of a finger millet- soybean (FM-SY) blend in protecting the viability of LAB was assessed in the current study.

The cell counts in the slurries upon fermentation were found to be in the range of 8.61- 9.31 log cfu/ ml slurry. A similar count was taken in the FCS for the study. The viable cell counts of LAB in finger millet-soybean slurries and FCS upon exposure to heat stress are shown in figure 1 and 2 respectively. The desirable levels of LAB (> 7 log cfu/ ml) were maintained up to 55°C and 50°C in case of FM-SY blends and FCS respectively ($p>0.05$). Above these temperatures, a progressive reduction in cell counts was observed with an increase in time ($p<0.05$). Even though a decrease was observed, the LAB in the matrix of finger millet soybean slurries were found to survive better than FCS ($p<0.05$). A complete loss in viability in FM-SY blends and FCS was observed at 70°C and 65°C respectively.

High temperature leads to protein denaturation in bacterial cells. Membranes and nucleic acids have been also identified as cellular sites of heat injury (Teixeira et al, 1997). Cereal constituents such as starch and prebiotics can provide a protective physical barrier for probiotics reducing thermal conductivity and enabling survival of cells (Chen et al, 2007; Kailasapathy, 2002).

However, no enhanced effect on survivability at high temperature was offered by the use of the processed forms i.e. malted, irradiated or enzymatically treated blends as compared to unprocessed finger millet soybean blend ($p>0.05$). Furthermore, the response of LAB to heat stress either as individual cultures or a mixed culture was found to be similar ($p>0.05$).

4.0 Conclusions

The study demonstrated that presence of a food matrix is a key factor for protection of viability of LAB exposed to heat stress. Hence, the effectiveness of finger millet-soybean based foods as carriers for these organisms was shown in the current work.

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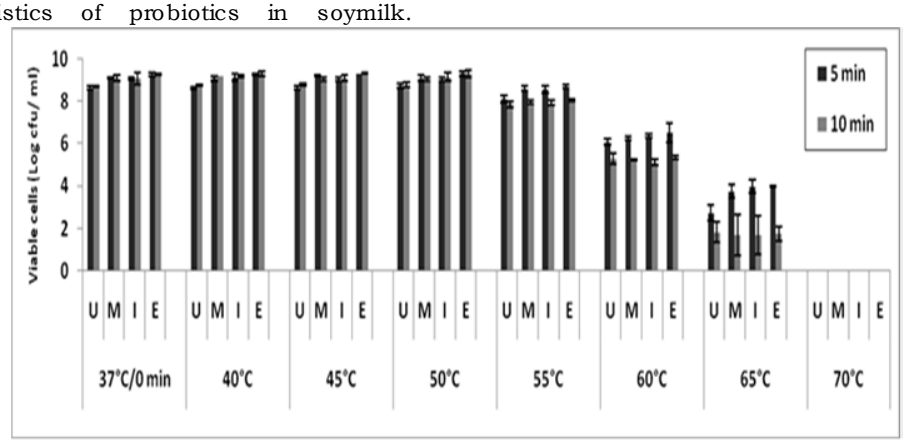

Figure 1: Cell counts of LAB in fermented finger millet- soybean slurries exposed to heat stress (U- unfermented, M- malted, I- irradiated, E- enzymatically processed)

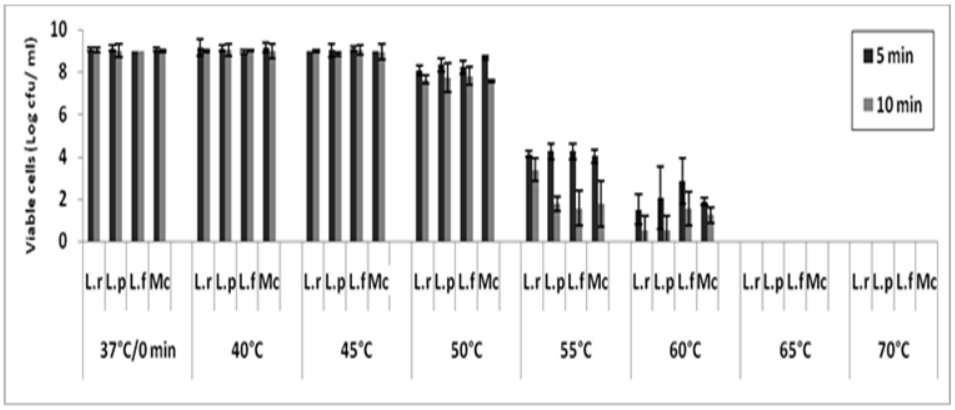

Figure 2: Cell counts in FCS of LAB exposed to heat stress (L.r- *L. rhamnosus*, L.p- *L. plantarum*, L.f- *L. fermentum*, Mc-Mixed culture)

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