Improving survivability of probiotics (*L. acidophilus*) in Manjari Medika Grape juice

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1. Introduction

In the earlier phase of first decade of 20th century, the children suffering from diarrhoea exhibited low count of stool bacteriacharacterized by a peculiar Y shaped (bifid) morphology that existed abundantly in healthy children and found suitable to restore healthy gut flora (Tissier,1906).The health beneficiary potential of fermented milk products promulgated by Metchnikoff (1907) established the fact that regular consumption of live beneficial bacteria (LAB) in the form of fermented dairy products harmonize superimposing equilibrium of intestinal microflora thereby protecting from orderlyputrefaction.The Greek word probiotic meaning 'for life' first coined in the context of speciality substances as an in-vivo metabolites of organism that stimulate the growth of others (Lilly and Stillwell,1965). FAO/WHO (2001) given the definition of probiotics was emerged out as an institutional confirmation under the flagship notation as'Livemicroorganisms which when administered in adequate amounts confer a health benefit on thehost'.Hypothetical survey of probiotic bacteria critically evaluated for human health benefit by group of scientists, was easily generalized on the basis of commonly used probiotic bacterial (*Lactobacillus* and *Bifidobacterium*) and yeast (*Saccharomyces cerevisiae var. boulardii)* genera (Ranadheera*et al.,* 2017).

Dairy fermented products have been traditionally considered as the best carriers for probiotics; but, nowadays, up to 70% of the world population is affected by lactose-intolerance. Furthermore, the use of milk-based products may also be limited by allergies, cholesterol diseases, dyslipidemia, and vegetarianism; therefore, several raw materials have been extensively investigated to determine if they are suitable substrates to produce novel non-dairy functional foods (Lourens-Hattingh and Viljoen, 2001).Recently, beverages based on fruits, vegetables, cereals, and soybeans have been proposed as new products containing probiotic strains; particularly, fruit juices have been reported as a novel and appropriate medium for probiotic for their content of essential nutrients. Moreover, they are usually referred as healthy foods, designed for young and old people (Luckow*et al.,*2006).

The health benefit of probiotics mainly relies upon their concentration in foods, as well as on their ability to survive to adverse conditions of the gastrointestinal tract (Woods, 1997). Hence, even if the probiotic viability is to be strain-dependent they should be at least 10^7 CFU/mL in the product at the end of the shelf life, which approximately corresponds to 10^9 CFU per portion (Nualkaekul*et al.,*2011). The non-dairy probiotics under vision, though appeared to be customized with existing prebiotics and preferably lacking in desired viable count of microorganisms owing to relative viability reducing phytonutrients (phenolics, organic acids) has tremendous potentials for commercial exploration. (Tripathi and Giri, 2014).

Antioxidant rich Manjari Medika grape (MMG) juice is found to be highly effective againstcolon cancer owing to its matrix of anthocyanins. The juice content in berries is found between 68-74% with average recovery of 71%. This variety is found suitable for juice making. TSS content in juice is recorded 19 to 22 °Brix with acidity of 0.5-0.6 per cent. Due to its teinturier nature, juice of this variety is very dark in colour and contains anthocyanins. One kg grapes contain 4-6 mg anthocyanins which shows richness of anthocyanins in berries of Manjari Medika (Sharma *et al.,*2017).Although MMG juice contains some essentialnutrients, but probiotic survival is suppressed by some factors. An easy way to improve probiotic stability in fruit juice could be the fortification of juice with some prebiotics (dietary fiber, cellulose) or with some ingredients able to exert a protective effect (Rakin *et al.,*2007; Nualkaekul*et al.,* 2011).Juice of Manjari Medika can represent a suitable carrier for probiotics, as they can combine the appearance of healthy and fresh foods, designed for a wide range of consumers, and the healthy benefits from probiotics. Efforts were made to developa probiotic beverage with minimum loss to natural nutrient profile of MMG juice with maximumprobiotic count by adding readily available prebiotic supplements.

2. Materials and methods

2.1 Probiotic culture activation

Strain of *L. acidophilus* was obtained from NDRI Karnal. Strain was activated in 5 mL of De Man, Rogosa and Sharpe (MRS) broth and preserved in cryovials containing 30% glycerol at −20 ºC. The chemicals, glassware, microbiological experimental equipments, packaging material and storage facilities were availed in MIT School of Food Technology, MIT ADT University, Pune, India.

2.2 Grape collection and preparation of juice

Berries of well ripened Manjari Medika grape (MMG) variety was obtained from experimental plots of ICAR National Research Centre for Grapes, Pune, India. After collection grapes were washed with tap water followed by sterile water to remove foreign matters. The grapes were screened to remove diseased, green, old and damaged grapes followed by destemming, extraction of grape juice, filtrationand finally packed in glass bottles and stored under refrigerator at 4°C till further processing (Sharma *et al.,* 2017).

2.3 Inoculation of grape juice with *Lactobacillus acidophilus*

The activated culture of *L.acidophillus* was inoculated at a concentration of 2% in varyingly diluted MMG juice (TSS 10, 15, 20 and 25°Bx) to find out optimum and compatible concentration of natural constituents of grape juice with major focus on innate sugar content. The infusion incubated at 37°C for 24 h. (Mustafa*et al.,* 2016, King and Su, 1993). The probioticated MMG juice samples were then analysed for viable cell count of *L.acidophillus.* The suitable TSS of MMG juice with highest probiotic cell count and acceptable sensory attributes (9-point hedonic cell) was selected as a benchmark to improve cell count to highest possible level by supplementing natural prebiotics(Marhamatizadeh, 2009).

2.4 Improving probiotic cell viability in MMG juice through natural prebiotic supplementation

The 15°Bx MMG juice supporting highest culture cell count after 24 h incubation was used for further study of improving probiotic cell viability in MMG juice through natural prebiotic supplementation. Three different promising prebiotic supplements namely, oatmeal, skim milk powder and inulin were used with concentration ranges (2.5, 5, 7.5 and 10% for oatmeal and skim milk powder and 1,2,3 and 4% inulin). The juice samples were inoculated as mentioned in 2.3. after 24 h samples were tested for viable cell count of *L.acidophillus.*

2.5 Statistical analysis

Statistical procedures as described by (Snedecor and Cochron, 1977) was used to analyse the data for the interpretation of results. Mean, standard deviation and analysis in Microsoft Excel software and variance (ANOVA) was used to describe the results. Differences at $p < 0.05$ were considered as significant.

3. Result and Discussion

3.1 Effect of Juice Dilution on Cell Viability

With juice recovery of 70-72 %, the TSS of fresh MMG juice stands in between 23-25°Bx. It contains exceptionally high amount of anthocyanin (4.0 g / kg) which have antioxidant and anti-cancerous properties(Sharma *et al.,*2017).Higherconcentration of sugar, acid and phenolics affect negatively on survivability of probiotic culture in particular and other microbes too in general.(Corcoran *et al.,* 2005) To perceive the effect of dilution thereby diluting the innate ingredients of antimicrobial behaviour, MMG juice was diluted from its original TSS 25°Bxto 10°Bx with an interval of 5°Bx. The maximum probiotic cell count was obtained at 15°Bx (200 CFU/mL) and lowest at 25°Bx (72CFU/mL) (Fig.1).

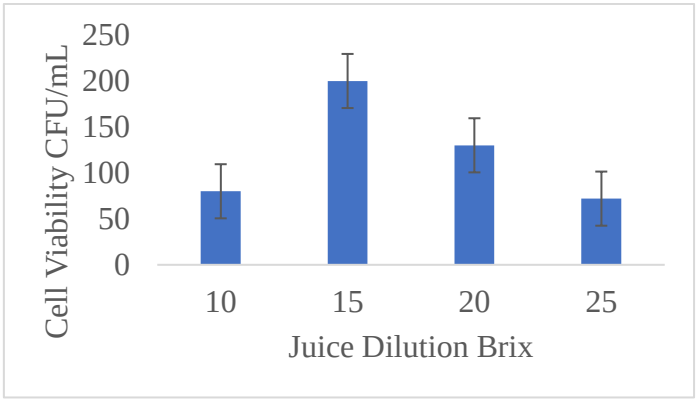

Fig1: Effect of Juice Dilution on the cell viability of*L. acidophilus* in MMG juice

3.2 Effect of Prebiotics and its Concentrations on cell Viability

Jaskari*et al*. (1993) reported cell growth promoting potential of oats due its high β- glucan (soluble dietary fibre). The effect of oat meal on cell viability is depicted in the fig.2 A highest cell viability was recorded at 7.5%oat meal (2000CFU/ml) and the lowest at 2.5 % level(410 CFU/ml). As the concentration of prebiotics was increased the cell viability was observed to be increasing up to 7.5% concentration of prebiotic followed by a gradual decrease (1700 CFU/mL) at 10%.(Robert and Martin, 2014; Baher *et al.,* 2020).

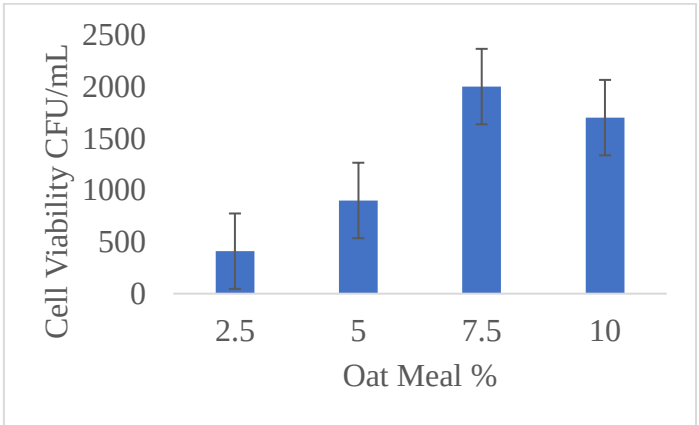

Fig 2: Effect of Oat Meal concentration on the cell viability of *L. acidophilus* in MMG juice.

From the fig. 3 it is clearly observed that among the various concentration selected the highest cell viability of 600 CFU/ml was recorded at SMP 5% and lowest 300 CFU/ml at 2.5% level. Further, increase in the SMP concentration decreased the cell viability to 400 CFU/ml. Presence of lactose, an easy access prebiotic in SMP favoured the growth of probiotic culture. Subsequent decrease in the probiotic viability could be a function of substrate inhibition mechanism (Ryhanen*et al.,* 1996).

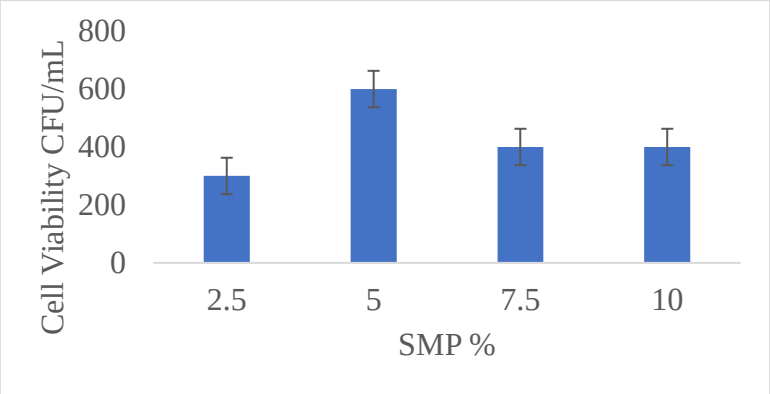

Fig 3: Effect of Skim milk Powder (SMP)concentration on the cell viability of *L. acidophilus* in MMG juice

Inulin at 2% concentration recorded highest viable probiotic count (6x10^6 CFU/mL) among all the three prebiotic supplements. A lowest viable probiotic count of 1.3x10^6 CFU/mL was found at 1% inulin in MMG juice (Fig.4). The inulin provided protective effect by maintaining the residual water levels necessary to preserve cell structures, preventing adverse effects on microorganisms by creating a barrier against environmental factors (Tymczyszyn*et al.,* 2011 and Karimi*et al.,* 2015).

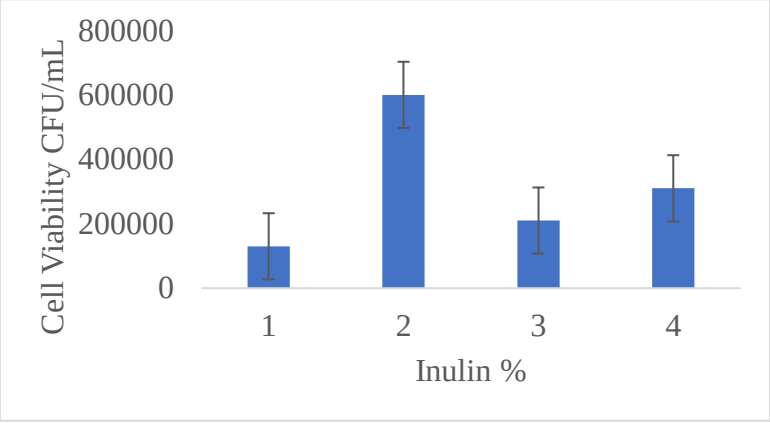

Fig 4: Effect of Inulin concentration on the cell viability of *L. acidophilus* in MMG juice

Inulin provides the conditions for the growth of these bacteria by providing energy and organic acids(De Souza Oliveira *et al*. 2012).

4. Conclusion

Prebiotics helps in maintaining the residual water levels necessary to preserve cell structures, preventing adverse effects on microorganisms by creating a barrier against environmental factors. Inulin provides the conditions for the growth of these bacteria by providing energy and organic acids. Inulin at 2% concentration recorded highest viable probiotic count (6x10^6 CFU/mL) among all the three prebiotic supplements used in this study. Non-dairy

probiotics alternatives with improved nutrient delivery are very much required to overcome the drawbacks of dairy-based probiotics products. These bench mark findings can help to further design fruit juice probiotication experiments to meet the need of hour.

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