Production and Evaluation of Probiotic Whole Wheat Flour

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ABSTRACT
This study investigated the production and evaluation of probiotic whole wheat flour using two probiotic cultures, Lactobacillus acidophilus and Lactobacillus casei. The probiotic cultures were added in the lyophilized form, individually and in combination to formulate probiotic whole wheat flour. The developed probiotic whole wheat flour was stored under ambient storage conditions for seventy five days. The changes in colour, smell, pH and titrable acidity of the probiotic whole wheat flour were also monitored for the entire storage period. The data was subjected to JMP statistical software (Version 10, SAS, USA) for analysis of variance (ANOVA). Least significant differences (LSD) were presented at a 95% confidence level (P <0.05). A probiotic viability of >10^9 cfu/g was found in the developed probiotic whole wheat flour. A statistically significant decline was found in the probiotic count after two months of storage. The pH decreased and the titrable acidity increased with the elapse of the storage period.

Key words: Lactobacillus casei, Lactobacillus acidophilus, Probiotic, Probiotic cereal, Whole wheat flour.

INTRODUCTION
Over the past decade, functional foods also called as healthy foods have become increasingly important with positive impact on both world health and international trade. At the same time, economic benefits of functional foods are growing in both developing and industrialized countries (Yang, 2008). Production of functional foods is being recognized as the number one food biotechnology industry as changing trends in population demography, consumer affluence, increased education, life expectancy and improved health care give rise to rapidly emerging diet and health conscious clientele (Dillard and German, 2000). The term functional foods was first introduced in Japan in mid 1980s and refers to the processed food containing ingredients that aid specific bodily functions in addition to being nutritious (Swinbanks and O’Brien, 1993). It is suitable for consumption by special group of people and has the function of regulating human body functions and not used for therapeutic purposes. Later, in July 2005, “The guideline of registration for functional foods” was promulgated by the China State Food and Drug Administration (SFDA) and the definition of a functional food was extended as the following, “health food means that a food, that has special health functions or is able to supply vitamins or minerals. It is suitable for consumption by specific group of people and has the function of regulating human body functions and it will not cause any harm whether acute or sub-acute or chronic” (SFDA, 2005).

Functional food ingredients include probiotics, prebiotics, vitamins and minerals and are found in diverse products such as fermented milk, yoghurt, sports drinks, baby foods, sugar-free confectionery and chewing gum (Khan and Ansari 2007). In the recent years, there has been an upsurge in the research of probiotics, as well as growing commercial interest in the probiotic food concept (Senok et al., 2005). This research has resulted in significant advances in our understanding and ability to characterize specific probiotic organisms. Probiotic foods constitute a sizeable part of the functional food market (Stanon et al., 2001).

Two main genera of gram positive bacteria Lactobacillus and Bifidobacterium are used extensively as probiotics (Holzapfel et al., 2001). Viable lactic acid bacteria of probiotic foods have several scientifically established and clinically proved health effects, such as reduction and prevention of diarrhoeas of different origin, improvement of the intestinal microbial balance by antimicrobial activity, alleviation of lactose intolerance symptoms, prevention of food allergy, enhancement of immune potency and anti-tumor activities (Anderson et al., 2001). A probiotic strain should withstand the manufacturing process without the loss of viability or negative effect on the sensory properties of the food product. The strain and the claimed properties should maintain stability in the food product during processing and also during subsequent storage (Saarelä et al., 2000). A large number of viable organisms are required in order to exert a probiotic effect in the food product. It is postulated that an active probiotic food should contain at least 10^9 cfu/g and the food should be consumed in order to achieve a beneficial effect (Lee and Salminen, 1995).

Probiotic bacteria are used widely in producing foods based on their positive qualities. The common probiotics that have been extensively studied and found in the market are dairy products such as yoghurt and cheese. Latest studies reveal that other novel probiotics such as fruits juices,
cereals and chocolate etc. are better and superior carriers for delivery of probiotics. Hence, there is a need to develop diverse probiotic foods, which can be used as nutrient supplements to promote health.

MATERIALS AND METHODS

Procurement of material
The wheat variety, PBW-343 was purchased from the local market and the grains were ground to obtain whole wheat flour.

Procurement of freeze dried cultures of probiotics
The probiotic cultures *Lactobacillus acidophilus* and *Lactobacillus casei* were obtained in the freeze dried form from the IMTECH, Chandigarh.

Preliminary analysis of the whole wheat flour
The whole wheat flour was subjected to preliminary microbial and physicochemical analysis. The total plate count, yeast and mould count, coliforms and *E.coli* were enumerated by serial dilution method by pour plating using nutrient agar, PDA, VRBA and EMB agar. The colour, smell, pH and titrable acidity of the whole wheat flour was estimated. Estimation of the pH of the whole wheat flour was done using a laboratory pH analyser and the titrable acidity was measured as per the methods of AOAC (1970).

Incorporation of probiotic cultures into the whole wheat flour
The whole wheat flour was sterilised for killing all the undesirable microbial flora present in it. Probiotic wheat flour was developed by the addition of probiotic cultures *Lactobacillus acidophilus* and *Lactobacillus casei* in the freeze dried form (at the rate of 10% of weight of flour) to the wheat flour. The probiotic bacteria were added individually as well as in combination to formulate probiotic wheat flour. This mix was blended aseptically for the uniform distribution of the probiotics and stored at a cool and dry place at ambient temperature. Controls which did not contain any probiotic were also run simultaneously.

Statistical analysis
All the tests were performed in triplicate. The data were subjected to JMP statistical software (Version 10, SAS Institute Inc., Cary, NC, USA, 2012) for analysis of variance (ANOVA). The Student’s t test was applied for pair wise comparisons. Least significant differences (LSD) were presented at a 95 % confidence level (P <0.05).

RESULTS AND DISCUSSION

Probiotic viability in probiotic whole wheat flour
Probioitic whole wheat flour was formulated by the addition of the probiotic cultures *Lactobacillus acidophilus* and *Lactobacillus casei*

Table 1: Probiotic viability in probiotic whole wheat flour.

| Days  | 0 day  | 7th day | 15th day | 30th day | 45th day | 60th day | 75th day |
|-------|--------|---------|----------|----------|----------|----------|----------|
| LA    | 9.777±0.001 | 9.698±0.001 | 9.477±0.001 | 9.001±0.001 | 9.000±0.001 | 8.903±0.001 | 7.598±0.001 |
| LC    | 9.698±0.001 | 9.698±0.001 | 9.602±0.001 | 8.477±0.001 | 8.903±0.001 | 8.778±0.001 | 6.987±0.001 |
| LA'   | 9.845±0.001 | 9.698±0.001 | 9.477±0.001 | 8.601±0.001 | 8.301±0.001 | 8.954±0.001 | 6.874±0.001 |
| LC'   | 9.698±0.001 | 9.602±0.001 | 9.602±0.001 | 8.778±0.001 | 8.477±0.001 | 8.777±0.001 | 6.986±0.001 |

The values represent mean ±SD where n=3 (values followed by different superscripts a, b, c etc. represent significant difference)

LSD-Least significant difference (p<0.05)

**LA-Lactobacillus acidophilus**
**LC-Lactobacillus casei**
**LA’-Lactobacillus acidophilus**
**LC’-Lactobacillus casei**

*Fig 1:* pH of probiotic wheat flour (LA-Lactobacillus acidophilus, LC-Lactobacillus casei, LALC- Lactobacillus acidophilus and Lactobacillus casei present together).
Lactobacillus casei individually and in combination. In freeze-dried form, the probiotic cultures, Lactobacillus acidophilus and Lactobacillus casei were found to be viable throughout the 75 days of storage (Table 1). The probiotic viability was found to be quite good in sets of the whole wheat flour having the individual probiotic as well as the ones having the probiotics in combination. The count of the probiotic bacteria was found to decrease slightly over the storage period and the variations were found to be statistically non-significant till the 60th day of storage. After a storage period of two months, the variations in the probiotic viability of the probiotic cultures was found to be statistically significant. In a similar study conducted by Govind et al. (2012) probiotic viability of freeze-dried synbiotic microcapsules in skim milk powder at ambient storage condition was investigated. Probiotic viable count in synbiotic skim milk powder changed after 60 days of ambient storage condition.

pH studies of probiotic whole wheat flour
The analysis of the pH of the developed probiotic whole wheat flour was done for the entire storage period of 75 days at regular intervals. The pH of the developed probiotic whole wheat flour was found to decrease very slightly over the 75 days of storage (Fig 1). This decline in pH was found to be statistically significant for some of the combinations in the later part of the storage period. This decline in pH may be attributed to the presence of the probiotic lactic acid bacteria cultures.

Titrable acidity of probiotic whole wheat flour
The analysis of the titrable acidity of the developed probiotic whole wheat flour was done for the entire storage period at regular intervals. The titrable acidity of the developed probiotic whole wheat flour was found to increase slightly over the 75 days of storage (Fig 2). This increase in acidity was found to be statistically significant for some of the combinations. The increase in titrable acidity may be attributed to the presence of the probiotic lactic acid bacteria cultures. In a similar study conducted by Govind et al. (2012), probiotic viability of freeze-dried synbiotic microcapsules in skim milk powder at ambient storage condition was investigated. The titrable acidity of the synbiotic skim milk powder increased over the storage period of 60 days at ambient storage condition.

Enumeration of microbial contaminants in probiotic whole wheat flour
Throughout the storage period of seventy five days, there were no microbial contaminants i.e., bacteria, yeasts and molds, E. coli and coliforms in the developed probiotic cereal (whole wheat flour). This indicates the good shelf life of the developed probiotic cereal and also reflects the safety aspect associated with its consumption.

Changes in colour and smell of the probiotic whole wheat flour
There was no change in the colour and smell of the developed probiotic wheat flour over the entire storage period. This indicated the good shelf life of the developed product and the absence of any microbial contaminants.

CONCLUSION
The present investigation aims at targeting the population which is lactose intolerant and allergic to milk based products as the majority of the probiotic products available commercially are dairy based. Through this study it has been found that cereals can serve as very good carriers of probiotics and can be used for the delivery of the live probiotic bacteria to the host while remaining in the desired and recommended dose. Probiotic whole wheat flour was successfully formulated using two probiotic cultures, Lactobacillus acidophilus and Lactobacillus casei in the lyophilized state. The probiotics were added individually and in combination. The probiotics survived throughout the storage period of seventy five days. The variation found in the probiotic viability was found to be statistically non-significant for the initial two months of the storage period. After the elapse of two months of storage the decline in probiotic count was found to be statistically significant. The pH and titrable acidity of the developed probiotic cereal was also monitored for the entire storage period and it was found that the pH decreased and the titrable acidity increased with the passage of the storage period. Such probiotic cereals
can be used for the fortification of baby foods, ready to eat cereals, ice cream mixes, thickening of desserts etc. Such non dairy probiotics will be useful for the individuals who are lactose intolerant and are allergic to milk based products.

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