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Loveleen Kaur Sarao
Department of Processing and Food Engineering, College of Agriculture Engineering and Technology, Punjab Agricultural University, Ludhiana, Punjab, India

Maninder Arora
Department of Processing and Food Engineering, College of Agriculture Engineering and Technology, Punjab Agricultural University, Ludhiana, Punjab, India

Corresponding Author:
Loveleen Kaur Sarao
Department of Processing and Food Engineering, College of Agriculture Engineering and Technology, Punjab Agricultural University, Ludhiana, Punjab, India

Development and evaluation of synbiotic refined wheat flour (functional cereal)

Loveleen Kaur Sarao and Maninder Arora

Abstract
This study investigated the development and evaluation of synbiotic refined wheat flour using two probiotic cultures, *Lactobacillus acidophilus* and *Lactobacillus casei* and the addition of the prebiotic ingredient inulin. The probiotic cultures were added in the lyophilized form, individually and in combination to formulate synbiotic refined wheat flour. The prebiotic ingredient inulin was added at four different concentration i.e. 9%, 11%, 13% and 13%. The developed synbiotic refined wheat flour was stored under ambient storage conditions for sixty days. The changes in colour, smell, pH and titrable acidity of the synbiotic refined wheat flour were monitored for the entire storage period. The data were 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 synbiotic refined wheat flour without any statistically significant differences. Hence, the developed synbiotic cereal had a shelf life of two months.

Keywords: synbiotic, probiotic, prebiotic, *Lactobacillus*, inulin, refined wheat flour, functional cereal

Introduction

In the last few years, functional foods also known as healthy foods have become increasingly important with positive impact on both world health and international trade. In both developing and industrialized countries, the economic benefits of functional foods are growing (Yang, 2008) [19]. 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 & German, 2000) [3]. The term functional foods refers to the processed food containing ingredients that aid specific bodily functions in addition to being nutritious (Swinbanks & O' Brien, 1993) [18]. Probiotics, prebiotics, vitamins and minerals are included in functional foods. These are found in diverse products such as fermented milk, yoghurt, sports drinks, baby foods, sugar-free confectionary and chewing gum (Khan & Ansari, 2007) [7].

The definition of probiotics is given as, "a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract" (Mc Farland, 2000) [9]. The two main genera of gram positive bacteria which are extensively used as probiotics are *Lactobacillus* and *Bifidobacterium* (Holzapfel, 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-tumour activities (Anderson *et al.*, 2001) [2]. It is important for the probiotic strain to 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 (Saarela *et al.*, 2000) [12]. 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 10^5 cfu/g and the food should be consumed in order to achieve a beneficial effect (Lee & Salminen, 1995) [8].

A prebiotic is defined as "non absorbable food component that beneficially stimulate one or more of the gut-beneficial microbe groups and thus has a positive effect on human health"

(Gibson & Roberfroid, 1995) [4]. The most commonly used prebiotics are carbohydrate substrates (e.g. dietary fiber) with the ability to promote the components of the normal intestinal micro flora which may provide a health benefit to the host. This group involves certain dietary components resistant to digestive enzymes hydrolysis and that are not absorbed in the upper portion of gastrointestinal tract including the small bowel. These compounds need to get to the large bowel, where the micro biota is located and stimulate the growth of some beneficial microorganisms in the gut (Roberfroid, 2002) [10].

"Synbiotics" is the word coined for the combined administration of specific probiotics with prebiotics to provide definite health benefits by synergistic action. A synbiotic is a product containing probiotic and prebiotic in which the prebiotic compound specifically favours the probiotic compound (Schrezenmeir & de Vrese, 2001) [14].

Probiotic strains are used widely in producing foods based on their positive qualities. Dairy products such as yoghurt and cheese are the common probiotics that have been extensively studied and found in the market. Very less work has been done on synbiotic foods. Hence, there is a need to develop diverse synbiotic foods, which can be used as nutrient supplements to promote health. This paper focuses on the production and evaluation of non-dairy synbiotic product i.e. synbiotic refined wheat flour to target the human population which is lactose intolerant and cannot consume dairy based probiotics and synbiotics.

2. Materials and methods

2.1. Procurement of material

The wheat variety, PBW-343 was purchased from the local market and the grains were finely ground to obtain refined wheat flour (all purpose flour).

2.2. Procurement of probiotic cultures and prebiotic ingredients

The probiotic cultures *Lactobacillus acidophilus* and *Lactobacillus casei* used in this study were obtained in the freeze dried form from the IMTECH, Chandigarh. The prebiotic ingredient inulin, was procured from the HiMedia company.

2.3. Preliminary analysis of the refined wheat flour

The refined 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 refined wheat flour was estimated. Estimation of the pH of the refined wheat flour was done using a laboratory pH analyser and the titrable acidity was measured as per the methods of AOAC (1970) [1].

2.4. Incorporation of the prebiotic ingredient into refined wheat flour

The prebiotic ingredient, inulin was added into the refined wheat flour at four different concentrations i.e. 9%, 11%, 13% and 15%.

2.5. Incorporation of probiotic cultures into refined wheat flour containing prebiotic ingredient (inulin) to develop synbiotic refined wheat flour

The finely milled refined wheat flour was sterilised for killing the entire unwanted microbial flora present in it. Synbiotic refined wheat flour was developed by addition of probiotic cultures *Lactobacillus acidophilus* and *Lactobaillus casei* in the freeze dried from at the rate of 10% of the weight of the refined wheat flour containing the prebiotic ingredient inulin. The probiotic bacteria were added individually as well as in combination to the prebiotic refined wheat flour. This mix was blended aseptically for the uniform distribution of the probiotics and stored at a cool and dry place. Controls which did not contain any probiotic and prebiotic were also run simultaneously.

2.6. Statistical analysis

All the tests were performed in triplicate. The data was subjected to JMP statistical software (Version 10, SAS Institute Inc., Cary, NC, USA, 2012) [11] 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 ($P < 0.05$).

3. Results and discussion

3.1. Probiotic viability in probiotic refined wheat flour

Synbiotic refined wheat flour was developed by the addition of the prebiotic ingredient inulin along with the probiotic cultures *Lactobacillus acidophilus* and *Lactobacillus casei* individually and in combination. The addition of the prebiotic ingredient inulin was done at four different concentrations i.e. 9%, 11%, 13% and 15%. Both the probiotic cultures were found to viable throughout the storage period of two months (Table 1) with a small decline towards the last part of storage period. The variation in the probiotic count of various sets of synbiotic refined wheat flour was found to be statistically non significant. This trend was found in all the combinations of the synbiotic refined wheat flour irrespective of the concentration of the inulin. Govind *et al.* (2012), in a similar study, investigated the probiotic viability of freeze dried synbiotic microcapsules in skim milk powder at ambient storage condition.

3.2 pH studies of probiotic refined wheat flour

The analysis of pH of the developed synbiotic refined wheat flour was done for the entire storage period of two months at regular intervals. The pH of the developed synbiotic refined wheat flour having inulin was found to decrease very slightly over the 60 days of storage as depicted in Fig 1 (a,b,c,d). This decline in pH was found to be statistically significant for some of the combinations in the latter part of the storage period. The decline in pH may have occurred because of the presence of the probiotic lactic acid bacteria cultures.

3.3 Titrable acidity of probiotic refined wheat flour

The analysis of the titrable acidity of the developed synbiotic refined wheat flour was done for the entire storage period at regular intervals. The titrable acidity of the developed synbiotic refined wheat flour having inulin was found to increase slightly over the 60 days of storage as depicted in Fig 2 (a,b c, d). In the last part of the storage period, this increase in acidity was found to be statistically significant for some of the combinations. The decline 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.

3.4 Enumeration of microbial contaminants in probiotic refined wheat flour

Throughout the storage period of two months, there were no microbial contaminants i.e., bacteria, yeasts and molds, *E. coli* and coliforms in the developed probiotic cereal (refined wheat flour). This indicates the good shelf life of the developed probiotic cereal and also reflects the safely aspect associated with its consumption.

3.5. Changes in colour and smell of the probiotic refined wheat flour

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

4. Conclusions

Formulation of synbiotic refined wheat flour was successfully accomplished using two probiotic cultures, *Lactobacillus acidophilus* and *Lactobacillus casei* in the lyophilized state along with the prebiotic ingredient inulin. The probiotic bacterial cultures were added individually and in combination for the development of synbiotic cereal. The probiotics survived throughout the storage period of sixty days. The variation found in the probiotic viability was found to be statistically non significant for the entire storage period. The pH and titrable acidity of the developed synbiotic 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. The parameters studied were almost similar for all the concentrations of the prebiotic ingredients. Therefore for commercial application, even a low amount of the prebiotic ingredient can serve the purpose. Such synbiotic cereals can be employed for the fortification of baby foods, ready to eat cereals, ice cream mixes, thickening of desserts, animal feeds, etc. The individuals who are lactose intolerant and allergic to milk based products and cannot consume dairy based probiotics, can be provided the benefits of the probiotics through non dairy carriers such as these cereals. The addition of the prebiotic ingredient will enhance the survival and proliferation of the probiotic bacteria. Thus, through a single product, both probiotic as well as prebiotic can be delivered successfully to the host.

Table 1: Statistical analysis of probiotic count in synbiotic cereals (refined wheat flour) having inulin as the prebiotic ingredient

Prebiotic↓	**Days→ Probiotic↓**	**0 day**	**7th day**	**15th day**	**30th day**	**45th day**	**60th day**
Inulin-9%	LA	9.906^{a}±0.001	9.826^{a}±0.001	9.643^{a}±0.001	9.333^{a}±0.001	8.966^{a}±0.001	8.793^{a}±0.001
	LC	9.836^{a}±0.001	9.827^{a}±0.001	9.523^{a}±0.001	9.334^{a}±0.001	8.723^{a}±0.001	8.613^{a}±0.001
	LA’	9.956^{a}±0.001	9.826^{a}±0.001	9.743^{a}±0.001	8.513^{a}±0.001	8.053^{a}±0.001	8.863^{a}±0.001
	LC’	9.906^{a}±0.001	9.753^{a}±0.001	9.743^{a}±0.001	8.514^{a}±0.001	8.653^{a}±0.001	8.963^{a}±0.001
	Control	-	-	-	-	-	-
	LSD	0.1908	0.1328	0.2880	0.9641	0.9972	0.4608
Inulin-11%	LA	9.786^{a}±0.001	9.726^{a}±0.001	9.563^{a}±0.001	9.274^{a}±0.001	8.926^{a}±0.001	8.773^{a}±0.001
	LC	9.716^{a}±0.001	9.727^{a}±0.001	9.443^{a}±0.001	9.273^{a}±0.001	8.683^{a}±0.001	8.593^{a}±0.001
	LA’	9.836^{a}±0.001	9.726^{a}±0.001	9.664^{a}±0.001	8.454^{a}±0.001	8.013^{a}±0.001	8.843^{a}±0.001
	LC’	9.786^{a}±0.001	9.653^{a}±0.001	9.663^{a}±0.001	8.453^{a}±0.001	8.614^{a}±0.001	8.943^{a}±0.001
	Control	-	-	-	-	-	-
	LSD	0.1708	0.0828	0.2780	0.9041	0.9572	0.4408
Inulin-13%	LA	9.840^{a}±0.001	9.771^{a}±0.001	9.599^{a}±0.001	9.300^{a}±0.001	8.944^{a}±0.001	8.782^{a}±0.001
	LC	9.770^{a}±0.001	9.772^{a}±0.001	9.479^{a}±0.001	9.301^{a}±0.001	8.701^{a}±0.001	8.602^{a}±0.001
	LA’	9.890^{a}±0.001	9.772^{a}±0.001	9.699^{a}±0.001	8.480^{a}±0.001	8.031^{a}±0.001	8.852^{a}±0.001
	LC’	9.840^{a}±0.001	9.698^{a}±0.001	9.698^{a}±0.001	8.480^{a}±0.001	8.631^{a}±0.001	8.952^{a}±0.001
	Control	-	-	-	-	-	-
	LSD	0.1248	0.0778	0.2440	0.9311	0.9752	0.4498
Inulin-15%	LA	9.852^{a}±0.001	9.781^{a}±0.001	9.607^{a}±0.001	9.306^{a}±0.001	8.948^{a}±0.001	8.784^{a}±0.001
	LC	9.782^{a}±0.001	9.781^{a}±0.001	9.487^{a}±0.001	9.307^{a}±0.001	8.705^{a}±0.001	8.604^{a}±0.001
	LA’	9.902^{a}±0.001	9.781^{a}±0.001	9.707^{a}±0.001	8.486^{a}±0.001	8.035^{a}±0.001	8.854^{a}±0.001
	LC’	9.852^{a}±0.001	9.708^{a}±0.001	9.708^{a}±0.001	8.487^{a}±0.001	8.635^{a}±0.001	8.954^{a}±0.001
	Control	-	-	-	-	-	-
	LSD	0.1368	0.0878	0.2520	0.9371	0.9792	0.4518

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* count in synbiotic cereals containing both *Lactobacillus acidophilus* and *Lactobacillus casei*
LC’--*Lactobacillus casei* count in synbiotic cereals containing both *Lactobacillus acidophilus* and *Lactobacillus casei*

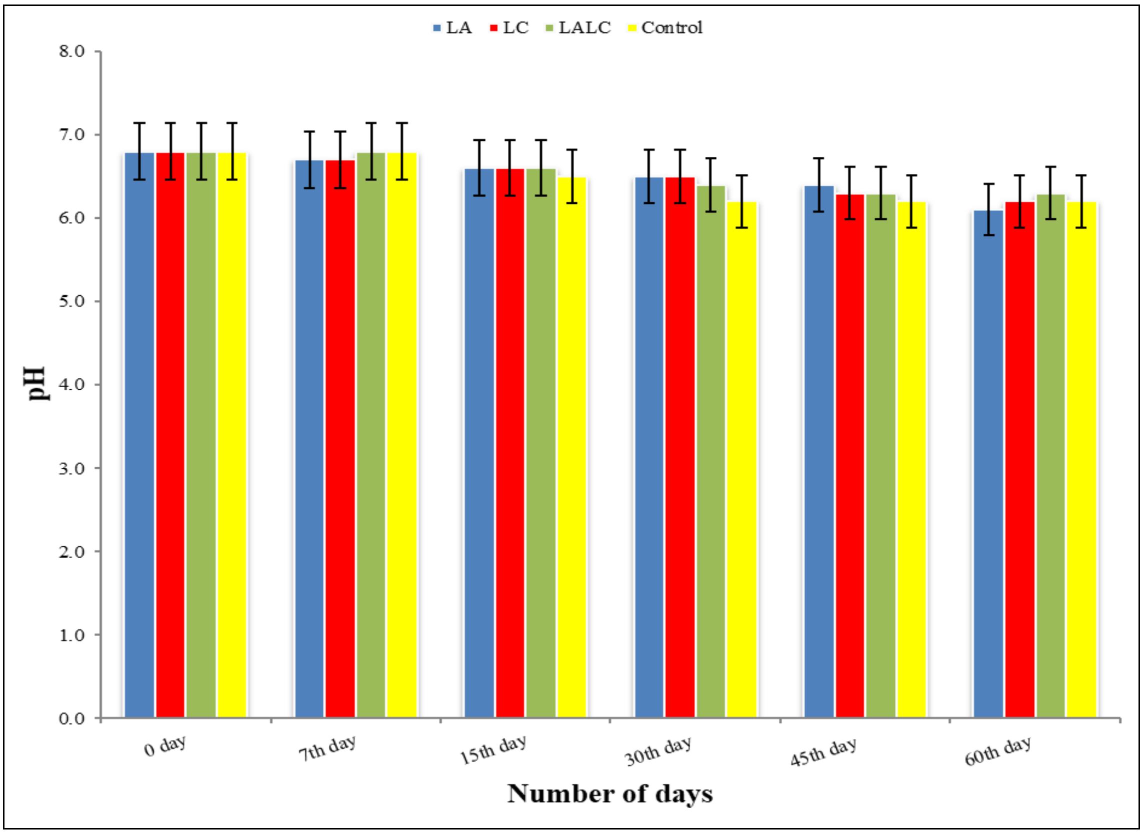

Fig 1a: pH of synbiotic refined wheat flour having 9% Inulin (LA-*Lactobacillus acidophilus*, LC-*Lactobacillus casei*, LALC- *Lactobacillus acidophilus* and *Lactobacillus casei* present together)

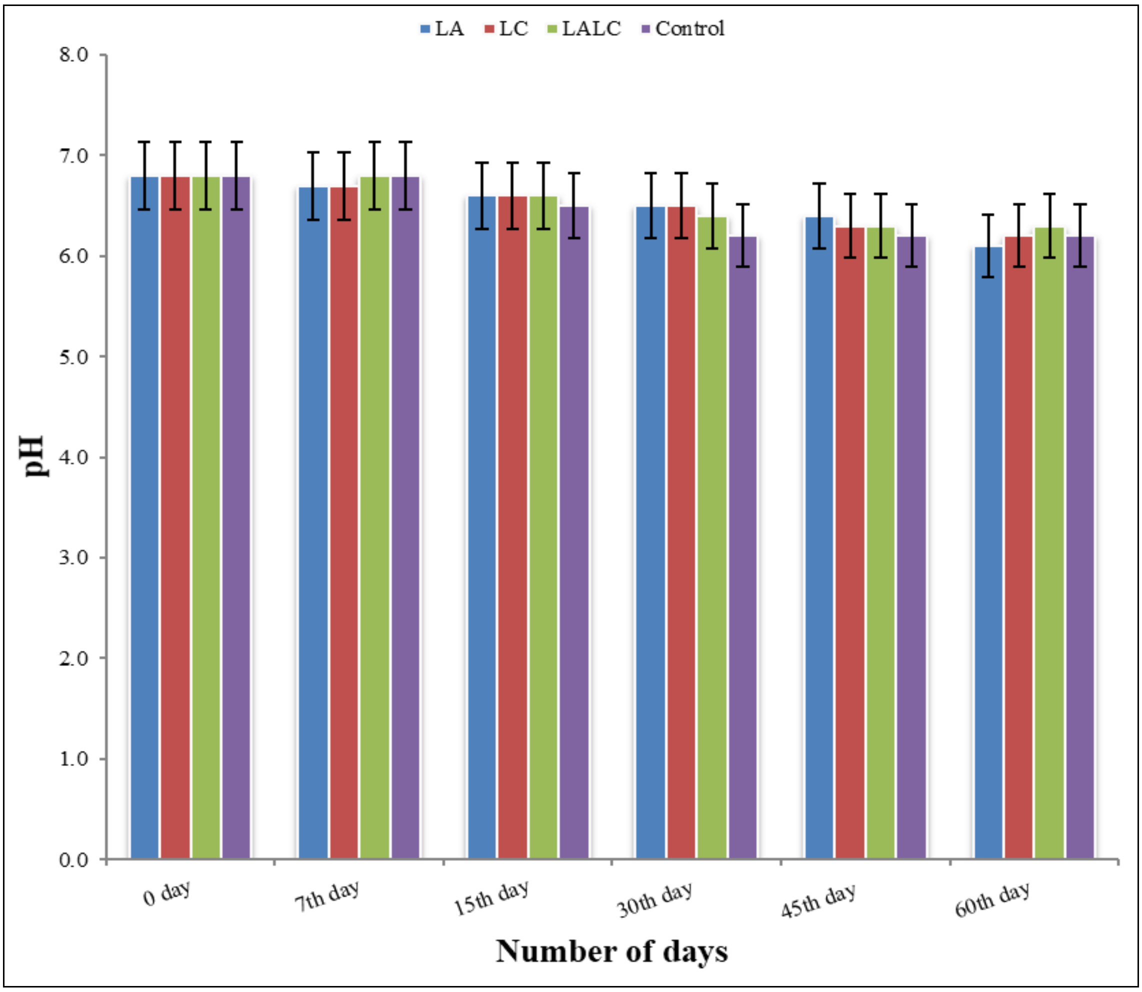

Fig 1b: pH of synbiotic refined wheat flour having 11% Inulin (LA-*Lactobacillus acidophilus*, LC-*Lactobacillus casei*, LALC- *Lactobacillus acidophilus* and *Lactobacillus casei* present together)

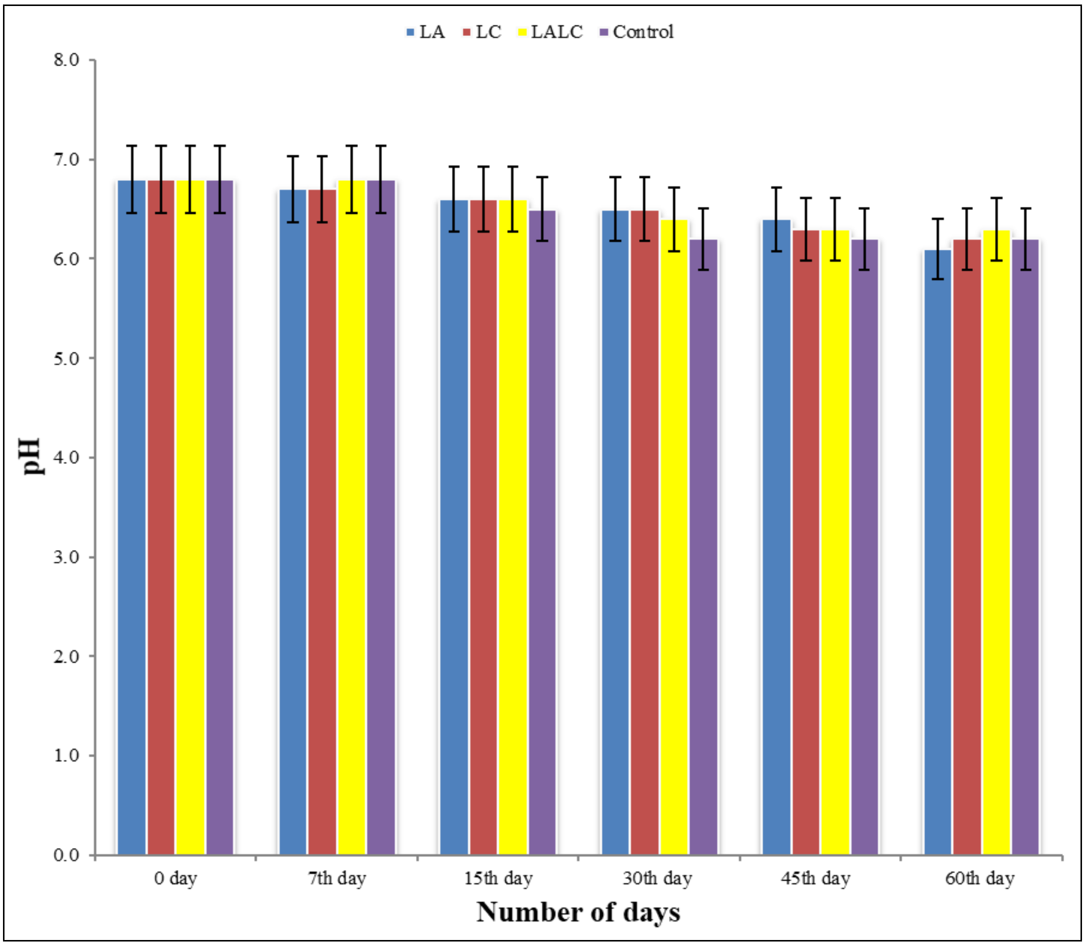

Fig 1c: pH of synbiotic refined wheat flour having 13% Inulin (LA-*Lactobacillus acidophilus*, LC-*Lactobacillus casei*, LALC- *Lactobacillus acidophilus* and *Lactobacillus casei* present together)

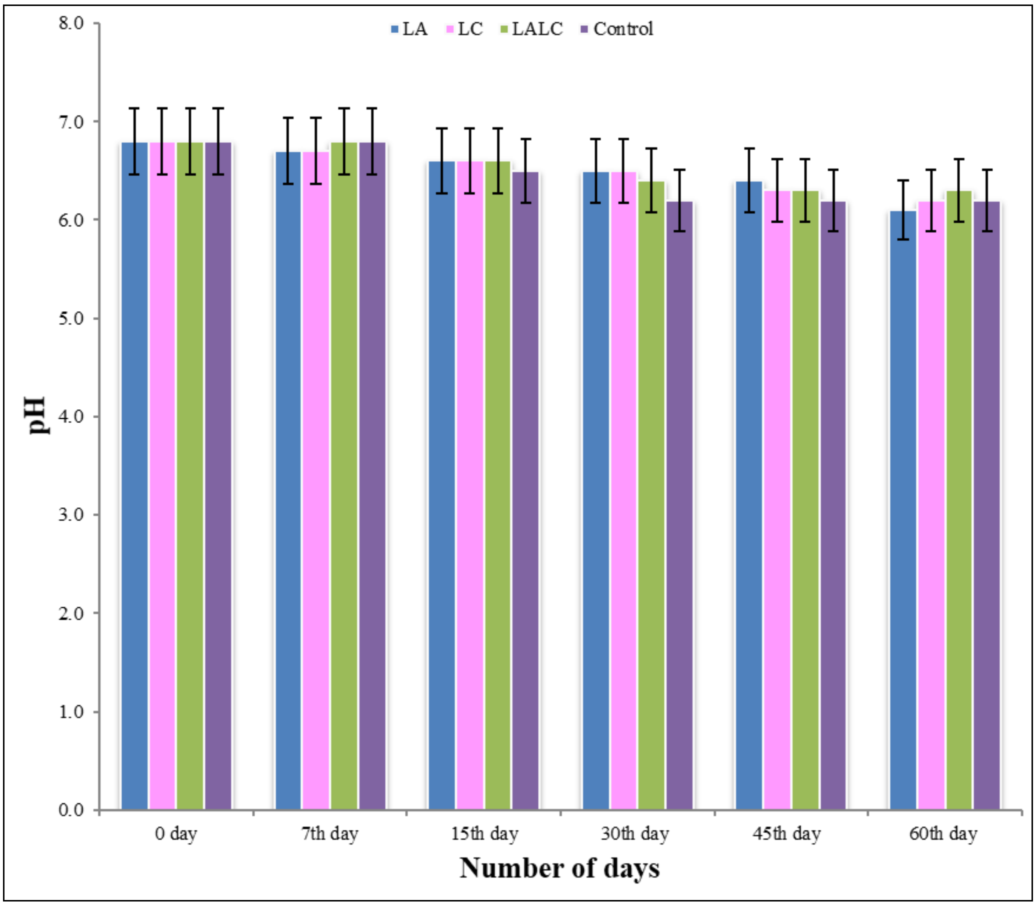

Fig 1d: pH of synbiotic refined wheat flour having 15% Inulin (LA-*Lactobacillus acidophilus*, LC-*Lactobacillus casei*, LALC- *Lactobacillus acidophilus* and *Lactobacillus casei* present together)

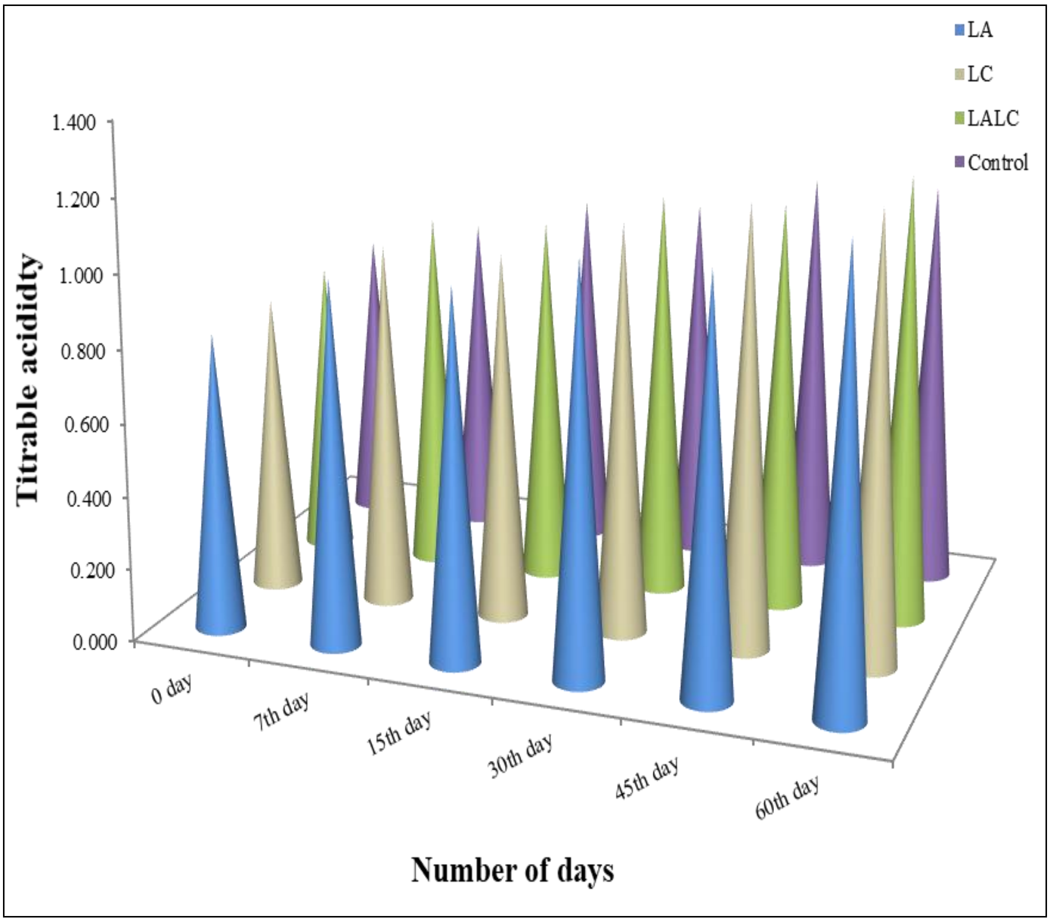

Fig 2a: Titrable acidity of synbiotic refined wheat flour having 9% inulin LA-*Lactobacillus acidophilus*, LC-*Lactobacillus casei*, LALC-*Lactobacillus acidophilus* and *Lactobacillus casei* present together)

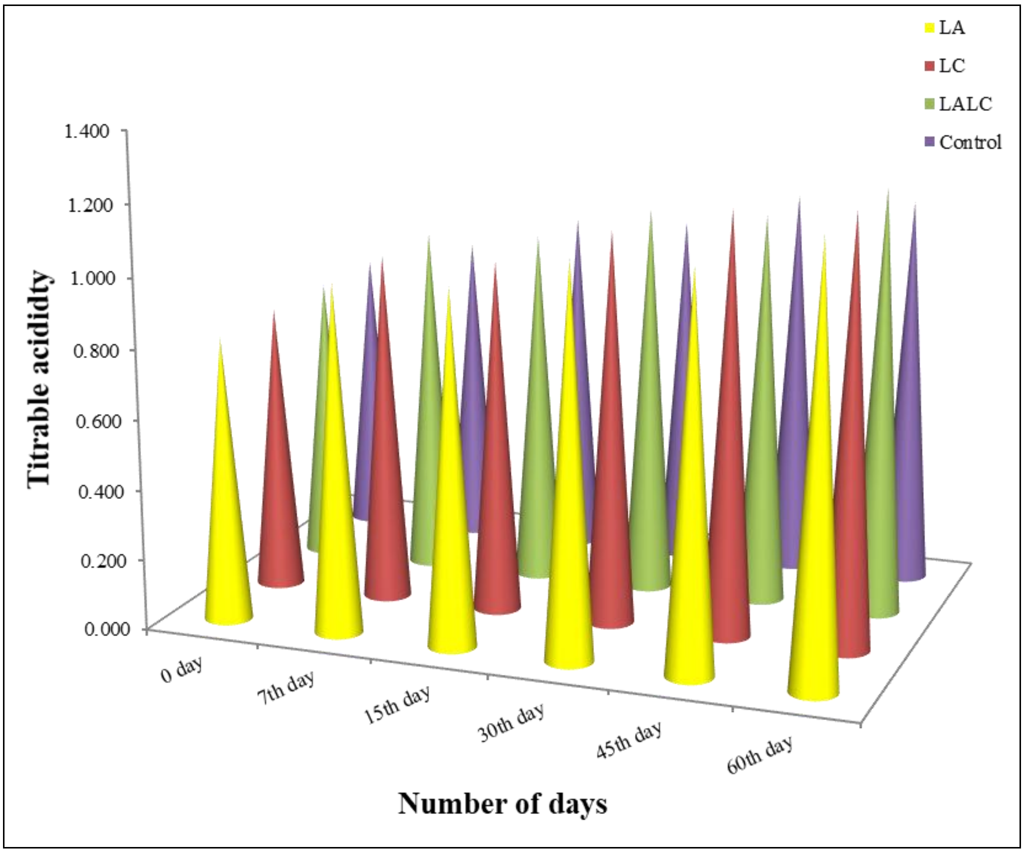

Fig 2b: Titrable acidity of synbiotic refined wheat flour having 11% inulin LA-*Lactobacillus acidophilus*, LC-*Lactobacillus casei*, LALC-*Lactobacillus acidophilus* and *Lactobacillus casei* present together)

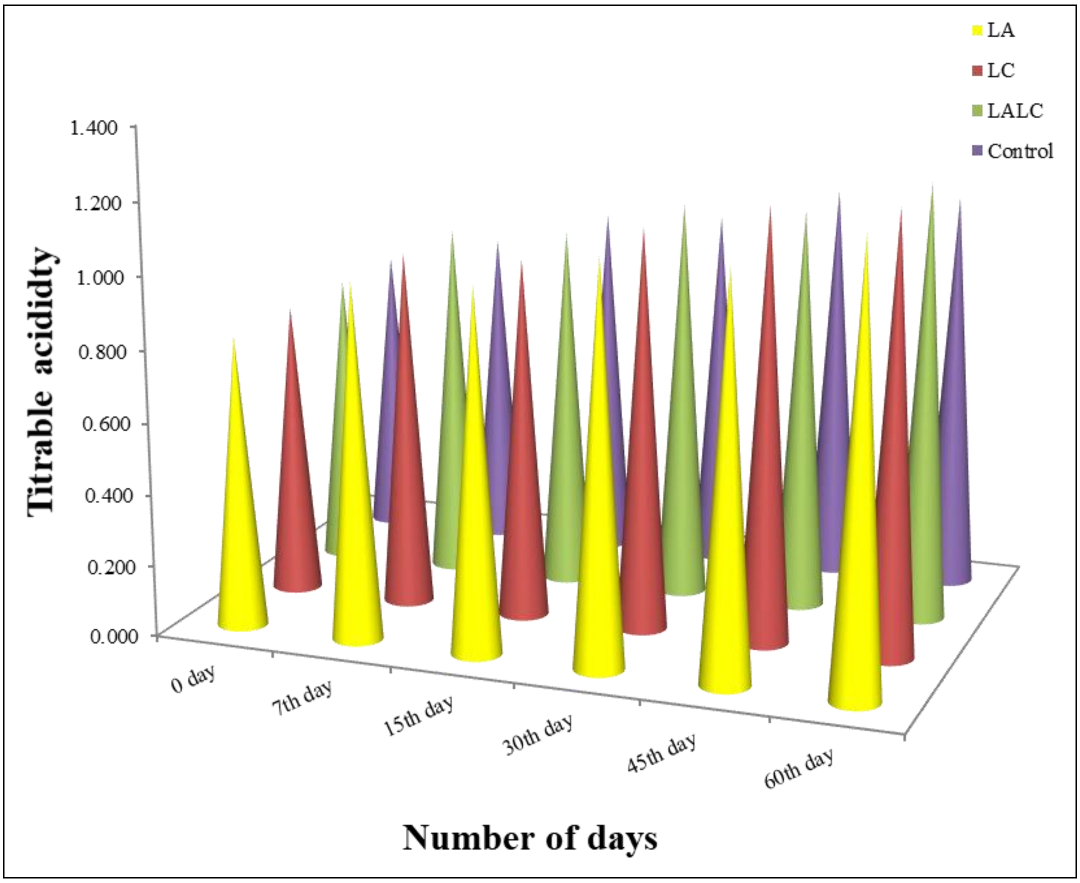

Fig 2c: Titrable acidity of synbiotic refined wheat flour having 13% inulin LA-*Lactobacillus acidophilus*, LC-*Lactobacillus casei*, LALC-*Lactobacillus acidophilus* and *Lactobacillus casei* present together)

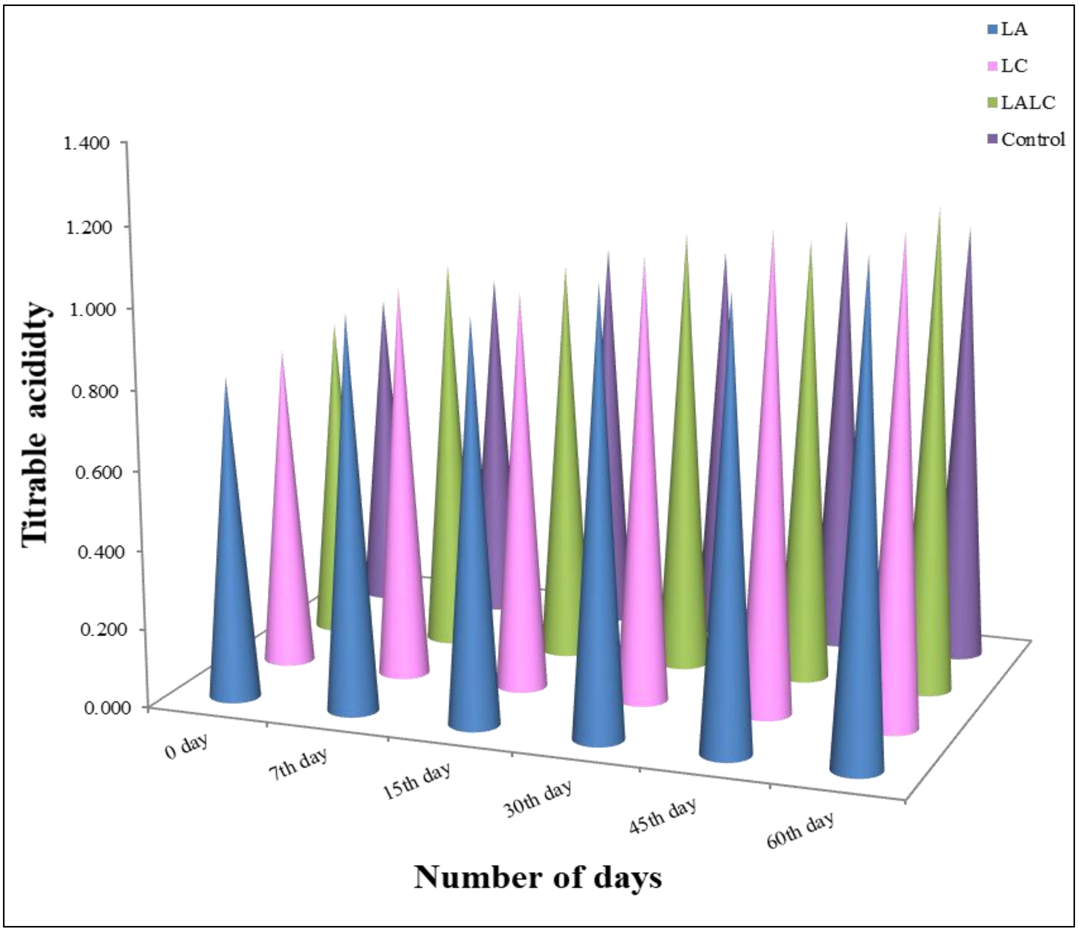

Fig 2d: Titrable acidity of synbiotic refined wheat flour having 15% inulin LA-*Lactobacillus acidophilus*, LC-*Lactobacillus casei*, LALC-*Lactobacillus acidophilus* and *Lactobacillus casei* present together)

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