

Quality attributes of a novel cereal based probiotic product prepared by using food grade lactic acid bacteria

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Nutritionally rich probiotic product was prepared after fermentation of mixed cereals (*Triticum aestivum* L., *Linum usitatissimum* L.) and *Chia* seeds (*Salvia hispanica* L.) by exploiting potential of the selected food grade lactic acid bacteria, viz. *L. brevis* UN (Accession No JX046150) and *L. spicheri* G2 (Accession No JX481912). This novel product was examined through physicochemical, sensorial and microbial evaluation. Physicochemical, nutritional characteristics of the product were determined through physicochemical evaluation. Sensorial evaluation suggested that different combinations of *L. brevis* UN and *L. spicheri* G2 exert significant influence on the sensory quality of the probiotic product. The highest sensory score on 9 point hedonic scale was observed in set A & B. The fermented cereal product was accepted by panelists when it was supplemented with fresh fruits. Microbial evaluation proved that there was no loss in the total number of viable cells in the probiotic product during storage. In addition there was no spoilage in the probiotic product during storage.

Keywords: Probiotic, *Lactobacillus*, Fermentation, Sensorial, Cereal

IPC Int. Cl.⁸: C12P, A21, A23, C12N 1/16-C12N 1/18, A23L 1/00, A23L 2/00, A23B 9/00

The fermented foods have been proclaimed as nutritionally rich since ancient times and are consumed all over the world in diverse forms. These fermented foods have gained a reputation for its beneficial effects on immunity, intestinal health and general well being¹. Modern researchers are just beginning to understand that fermented foods encompass health beneficial microflora which offers clear and calculable health benefits to the human diet. Due to the benefits of the beneficial microflora, commonly known as 'probiotics', have augmented rapidly worldwide and are presently known to be used by consumers². Probiotics refer to live microbial preparation which when administered in adequate amounts confer a health benefit on the host by improving the balance of the intestinal microflora and maintaining the chronic and immunological balanced inflammatory response³. In the 21st century, human health is being promoted by using innovative technologies such as formulation of functional foods in nutraceuticals as probiotics by applying number of the *Lactobacilli* or as the target of prebiotics are a commercial reality and have put the global market for probiotic health foods⁴. The use of

probiotic cultures stimulates the growth of intestinal bacteria, mitigates potentially harmful bacteria, and reinforces the body's natural defense mechanisms. There is a growing scientific evidence to support the concept that the maintenance of healthy gut microflora may provide protection against various gastrointestinal disorders including gastrointestinal infections, inflammatory bowel diseases and even cancer⁵. In the industrialized countries, yogurt, *sauerkraut*, *kefir* and *tempeh* are well known fermented food products that are consumed as a large part of traditional diet, but still there are many other health beneficial fermented food products that are consumed by the rural people. There is a need to develop people's interest towards healthy traditional food products which may contribute to healthy living. Since, lactic acid bacteria have a long and safe history of use in food fermentations and have been best proved for their probiotic potential⁶. There is an upsurge of interest all over the world to use these bacteria in commercial food preparations like infant formula, fruit drinks and sweet milk. Keeping in view the above, the present study was carried out to make a nutritionally rich fermented food preparation with the help of food grade lactic acid bacteria that may contribute to human health.

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Methodology

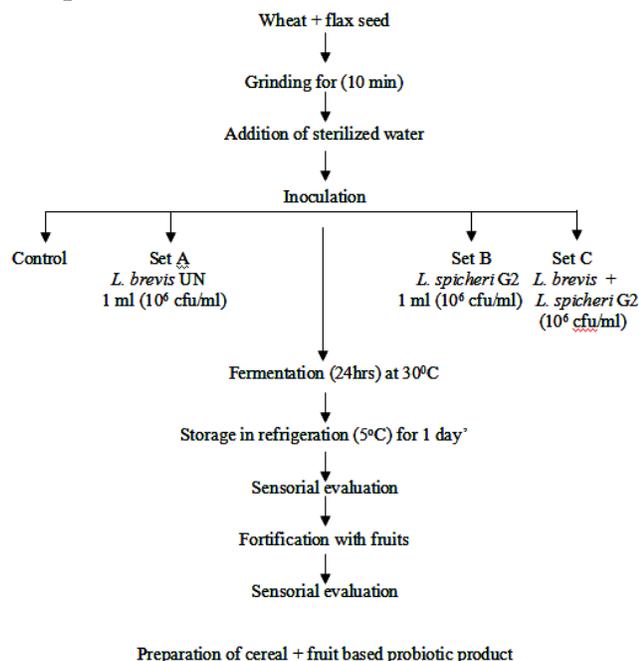
Bacterial cultures

L. brevis UN (JX046150) and *L. spicheri* G2 (JX481912) @ 10^6 CFU/ml were used to prepare cereal based nutritionally rich probiotic product. Each culture was maintained at -20°C on MRS broth containing 40% glycerol (v/v) in deep freezer and the cultures were propagated three times prior to use.

Ingredients used

- | | |
|---|---------------|
| 1. <i>Triticum aestivum</i> L. (Wheat grains) | 150 gm |
| 2. <i>Linum usitatissimum</i> L. (Flax seed) | 50 gm |
| 3. <i>Salvia hispanica</i> L. (Chia seed) | 2 gm |
| 4. Autoclaved distilled water | 100 ml |
| 5. <i>L. brevis</i> UN | 10^6 cfu/ml |
| 6. <i>L. spicheri</i> G2 | 10^6 cfu/ml |
| 7. <i>Punica granatum</i> L. (Pomegranate) | |
| 8. <i>Vitis vinifera</i> L. (Grapes) | |
| 9. <i>Malus pumila</i> Mill. syn. <i>Pyrus malus</i> L. (Apple) | |

Recipes



Physicochemical changes during storage

pH: The pH of each sample was measured in a pH meter.

Total soluble sugars: were determined by placing 1-2 drops of sample on the prism of a hand refractometer, and the results were expressed as $^\circ\text{Brix}$ ⁷.

Acidity in terms of lactic acid: An aliquot of the sample prepared was diluted with recently boiled distilled water. 2-3 drops of 1% phenolphthalein solution was used as an indicator and titration was done with 0.1 N NaOH. Titer value was noted and calculations were done as percent anhydrous lactic acid.

$$\text{Titrateable acidity (\%)} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{volume made up} \times \text{equivalent weight}}{\text{Volume of sample taken} \times \text{Volume of aliquot taken} \times 1000} \times 100$$

Ascorbic acid

Ascorbic acid was determined as described below⁸

Reagents used

1. 3 % metaphosphoric acid (HPO₃): Pellets of HPO₃ were dissolved in distilled water.

2. Ascorbic acid standard: 100 mg of ascorbic acid was weighed and final volume was prepared up to 100 ml with 3 % HPO₃. 10 ml of 3 % HPO₃ was diluted to 100 ml.

3. Dye solution: 50 mg of sodium salt of 2, 6-dichlorophenolindophenol was dissolved in 150 ml of hot distilled water containing 42 mg of sodium bicarbonate. It was cooled and diluted with distilled water to 200 ml and stored in a refrigerator.

4. Standardization of dye: 5 ml of standard ascorbic acid solution and 5 ml of 3 % HPO₃. Were mixed and titrated with the dye to a pink colour that persisted for 15 sec. The dye factor (mg of ascorbic acid per ml of the dye) was determined by following formula:

$$\text{Dye factor} = \frac{0.5}{\text{Titre}}$$

Assay

1 ml of curd sample was diluted with 10 ml of 3 % HPO₃ followed by centrifugation. Aliquot of the HPO₃ of the sample was titrated with the standard dye to a pink end point which persisted for 15 sec. Titration was done rapidly and preliminary determination of the titre was done.

Calculation

The ascorbic acid content of the sample was determined by following method:

$$\text{Mg of ascorbic acid per 100 gm or ml} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of extract taken} \times \text{weight of sample taken}} \times 100$$

Statistical analysis

Data pertaining to the physicochemical attributes of probiotic product was analyzed by completely randomized design (CRD) factorial. Data on sensorial evaluation of probiotic products were analyzed by using randomized block design (RBD)⁹.

Sensorial evaluation

Sensorial evaluation of each sample was done in terms of appearance, texture, flavor and overall acceptability. Nine point hedonic scale was followed for conducting the sensory evaluation of probiotic food products¹⁰. The panel of 7 judges comprising the students of Department of Basic Sciences (Microbiology section) and Department of Food Science and Technology, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan were selected to evaluate the products for sensory parameters such as appearance, texture, flavor and overall acceptability depending upon the type of product. Efforts were made to keep the same panel for sensory evaluation throughout the course of study. The sample was presented to judges and plain water was given to them to rinse their mouth in between the evaluation of samples. No discussion during evaluation was allowed.

Microbial evaluation during storage

The colony count was observed during storage period by standard spread plate method. MRS agar was used to enumerate lactic acid bacteria while nutrient agar, yeast extract agar, PDA were used to enumerate total aerobic mesophilic bacteria including yeast and mold.

Results and discussion

Cereal based product

Cereal based product is the fermented product which is prepared by fermentation of cereals (wheat, flax seed, *chia* seed) by exploiting the potential of selected bacterial isolates (*L. brevis* UN and *L. spicheri* G2). Both the isolates used in the present

investigation were of food grade bacteria. *L. brevis* UN was isolated from Dhulliachar, while *L. spicheri* G2 was isolated from Gundruk¹¹. Raw material was divided into four equal sets of 50 gm each. The first set was left unaltered for the control. *L. brevis* UN was added to the set A, *L. spicheri* G2 was added to set B, combination of *L. brevis* UN and *L. spicheri* G2 was added in set C. All the sets were left for fermentation at 37 °C for 24 hrs. The fermentation was terminated by keeping these sets at 4 °C and all these sets were subjected to evaluation. This product was found rich in antioxidants, dietary fibre, carbohydrates and polyunsaturated fatty acid. Nutritional chart of the cereal based probiotic product is presented in Table 1.

Physicochemical characteristics

In this investigation, already available and bio-available contents were evaluated by measuring pH, titratable acidity, TSS, and antioxidants of the cereal based probiotic product before fermentation and after fermentation. It was observed that cereal blended raw preparation had pH 6.0 which lowered down to 4.30, 4.27, 4.32 after fermentation of 24hrs in all the sets, i.e. set A, set B and set C except in control as presented in Table 2. The decrease in pH is due to microbial metabolic activity. Acidity was estimated in terms of lactic acid. The lactic acid was increased from 0.09 to 0.18 % in set A and set D, respectively. While for set B, it was increased from 0.09 to 0.15 %. As far as TSS was concerned, it increased from 1 to 2% after fermentation in each treatment except in the control. Similarly, there was an increase in antioxidants in all the sets after fermentation. In raw mixture, antioxidants were in the range of 11.40 to 11.42%, which became 47.86 for set B, 46.61 for set C and maximum 55.5% for set D. Our results are in consistent with one of the studies which showed

Table 1—Nutritional chart of cereal based probiotic product

Nutritional facts per 50 gm	
*Lactic acid (%)	0.09
*Antioxidants (%)	46.6 - 55.5
*Total soluble solid (⁰ B)	2 to 3
**Protein (gm)	5.33
**Dietary fiber (gm)	4.81
**Carbohydrate (gm)	13.19
**Mineral (gm)	0.18
**Polyunsaturated fatty acid (gm)	3.8
*Values evaluated in lab	
**Values taken from USDA SR-21	

Table 2—Physicochemical characteristics of cereal based probiotic product during storage

Name of treatment	pH		Titratable acidity in terms of lactic acid		Total soluble solid (⁰ B)		Antioxidants	
	Before fermentation	After fermentation	Before fermentation	After fermentation	Before fermentation	After fermentation	Before fermentation	After fermentation
Control [♦]	6.0	6.1	0.09%	0.09 %	1.0	1.0	11.42	14.20
Set A ^{♦♦}	6.15	4.30	0.09 %	0.18 %	1.0	2.0	11.40	47.86
Set B [^]	6.05	4.27	0.09 %	0.15%	1.0	3.0	11.42	46.61
Set C ^{^^}	6.0	4.32	0.09 %	0.18%	1.0	2.0	11.42	55.51

♦ Without inoculum
♦♦ *L. brevis* UN
[^]*L. spicheri* G2
^{^^} *L. brevis* UN + *L. spicheri* G2

that fermentation increased the antioxidant activity significantly in fermented pomegranate juice¹². It is well documented in several studies that the fermentation process affects nutritional quality of foods including improvement in the nutrient density and increasing the amount and the bioavailability of nutrients. The nutritional impact of fermented foods on the health can be direct or indirect. Food fermentations that raise the protein content or improve the balance of essential amino acids or their availability will have a direct curative effect. Similarly fermentations that increase the content or availability of vitamins, such as thiamine, riboflavin, niacin or folic acid can have profound direct effects on the health of the consumers of such foods¹³.

Sensorial evaluation

Sensorial evaluation of novel cereal based products was assessed by 7 panelists using 9 point sensory hedonic scale. Sensorial evaluation was performed twice, i.e. with fermented cereal based product (Fig. 1a) and with fermented cereal based product which is supplemented with fresh fruits (apple, pomegranate, grapes), salt, and black pepper in to it before presenting it to panelists as shown in (Fig. 1b). The results pertaining to sensorial evaluation of cereal based product (without fruits) are presented (Fig. 2a). The score for this product was in the range of 4.45 - 4.57 which is below than the acceptable level. The low acceptability of this cereal based product was due to its off flavours. Based on low sensorial score of cereal based probiotic product, it was further modified by adding fresh fruits. It was observed from the sensorial evaluation of cereal and fruits (mixed) based probiotic product that the panelists accepted the product. This product had a score in the range of 7.04

to 8.22 as shown (Fig. 2b). Sensorial properties of this novel product were assessed as the sensory evaluation and consumer acceptance is a desirable criteria for a product before launching it in the market. Many authors have evaluated probiotic products through sensory evaluation. In one of the studies it was verified that a probiotic ice cream with acrela pulp did not display a favourable public acceptance¹⁴. On the other hand, probiotic banana yogurts were accepted by 6 panelists when rated on hedonic scale¹⁵.

Microbiological evaluation during storage period

Fig. 3 shows the survival of the probiotic strains *L. brevis* UN and *L. spicheri* G2 during 30 days storage period. The survival of the strains was checked by standard plate count. The initial cell counts in sets A, B, and C were 26, 22.91 and 31.14 log cfu, respectively which increased to 33.81, 35 and 43.6 log cfu for set A, B and C after 15 days. In contrast to all the treatments, initial cell counts for the control (treatment without added inoculum) were 12.48 at 0 hr which increased to 36.12 log cfu/ml on 15th day of storage. After 30th day, cell count for sets A, and set C became log 43, log 37 and log 50, respectively. Hence, it was observed that there was no loss in total number of added viable cells in prepared probiotic product during storage. Simultaneously, it was also noticed that there was no spoilage of the probiotic product during this period.

In one of the studies, authors verified that how probiotic strains resist the manufacturing process in order to be viable in the end product. Specific plate count measured by the authors was $3.2 \times 10^{8 \pm 2.7} \times 10^6$ cfu/ml for *Bifidobacterium* /gm and $2.5 \times 10^{8 \pm 2.3} \times 10^6$ cfu/ml for *Lactobacilli*/g in milk chocolate¹⁶.

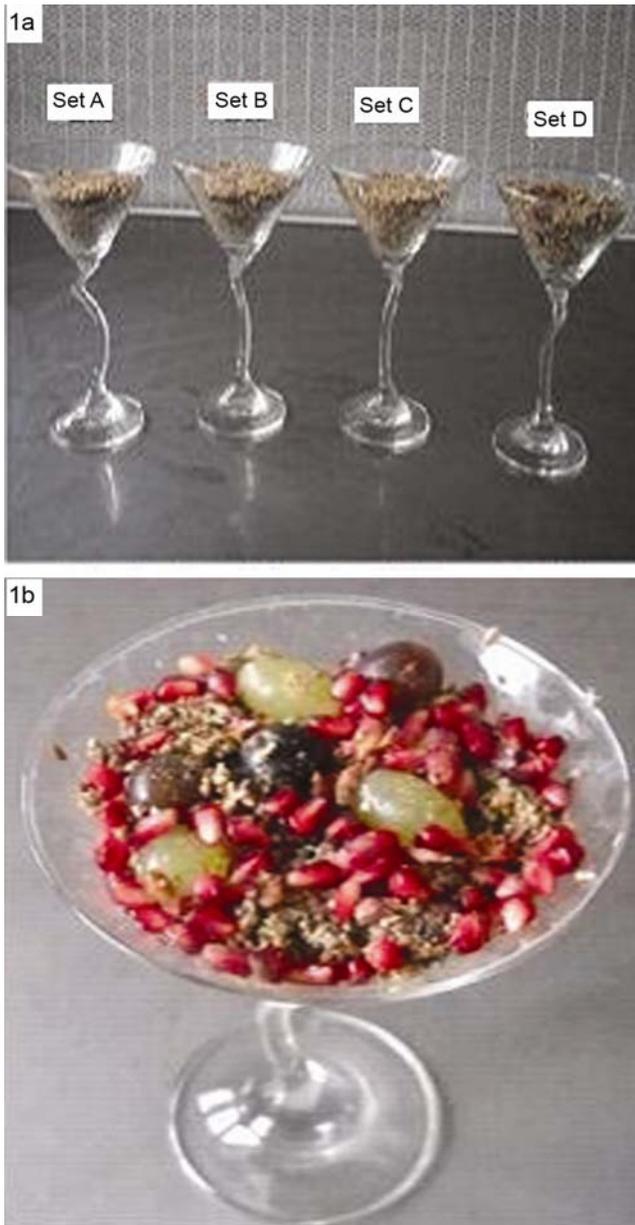


Fig. 1a—Cereal based probiotic product –I; 1b—Cereal and fruits based probiotic product –II

Justification of the study

Day today food habits do not provide the required amount of beneficial bacteria to maintain proper microbial ecology in the host which is an essential criterion for a healthy individual. It has already been proved in many studies by several scientists that probiotics balance the microflora of the intestine. Moreover, World Health Organization has also permitted the use of probiotics in various foods. In the present investigation a novel cereal based probiotic product has been prepared by using mixture of raw

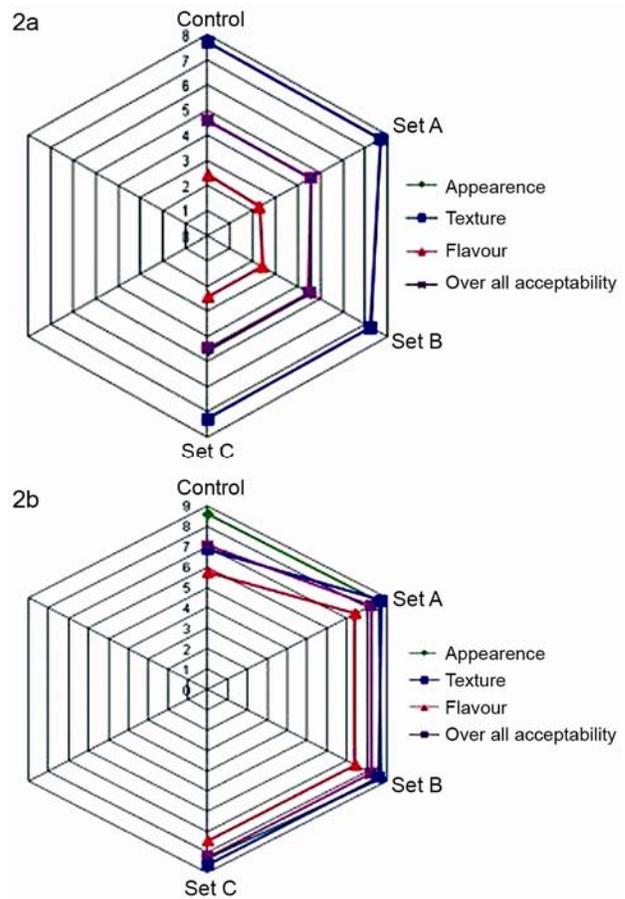


Fig. 2a—Sensorial evaluation of cereal based fermented probiotic product before fortification; 2b—Sensorial evaluation of cereal based fermented probiotic product after fortification

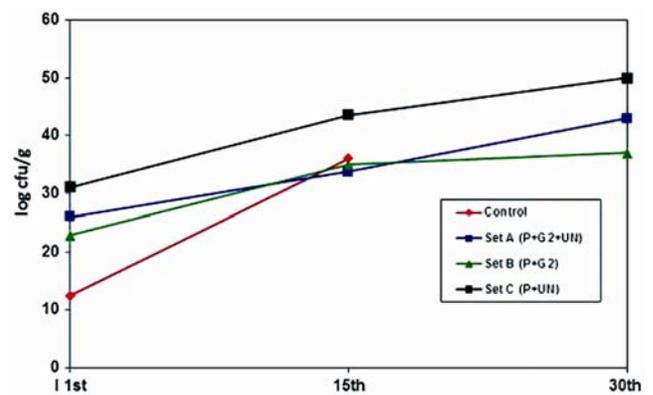


Fig. 3—Microbiological evaluation of cereal based probiotic product after fortification with fruits

materials, viz. *Triticum aestivum* L. (Wheat grains), *Linum usitatissimum* L. (Flax seed), *Salvia hispanica* L. (Chia seeds) which was fermented by using two strains of food grade Lactic acid bacteria

(*Lactobacillus spicheri* G2 and *L. brevis* UN.). Lactic acid bacteria have already been given the status of food grade bacteria. The encouraging results of the sensory evaluation of the present investigation revealed that this novel probiotic cereal based food can be taken up as a breakfast meal that would be advantageous to maintain microflora of the individual.

Traditional significance to society and some constructive recommendations

The consumption of ready to eat processed foods are a first choice of a today's consumer due to growing urbanization, breakdown of large families in to nuclear families and increase in the number of working women. These ready to eat processed foods do not provide complete nutrition to the individual. But if this novel cereal based food product is commercialized it will provide health benefit to the society. Also this novel product can be beneficial for the mountaineers on a perilous mission or soldier on battle front.

Conclusion

Fermentation of the cereal based products is useful because these can be used as nutritive base that can further be fortified with fresh fruits in addition to salt, black pepper and others for enhancing their taste. The observations recorded in this investigation are encouraging because this novel product is a potential functional food with beneficial properties. Therefore, this novel product would be an effective vehicle to deliver probiotic health effects to the society as the fermentation process of such type of cereal blended products can contribute to health benefits.

References

- Joshi VK & Sharma S, Preparation and evaluation of sauces from lactic acid fermented vegetables, *J Food Sci Technol*, 47 (2) (2010) 214-218.
- Saarela M, Mogensen GR, Matto J & Mattila-Sandholm T, Probiotic bacteria: safety, functional and technological properties, *J Biotechnol*, 84 (2000) 197-215.
- Suverna VC & Bobby VU, Probiotic in human health: a current assessment, *Curr Sci*, 88 (11) (2005) 1744-1747.
- Kleerebezem M & Vaughan EE, Probiotic and gut *Lactobacilli* and *Bifidobacteria*: molecular approaches to study diversity and activity, *Ann Rev Microbiol*, 63 (2009) 269-290.
- Sreekumar G & Krishnan S, Isolation and characterization of probiotic *Bacillus subtilis* SK09 from dairy effluent, *Ind J Sci Technol*, 3(8) (2010) 863-866.
- Parvez S, Malik K A, Kang S. Ah & Kim HY, Probiotics and their fermented food products are beneficial for health, *J Appl Microbiol*, 100 (2006) 1171-1185.
- Ranganna S, Handbook of analysis and quality control for fruit and vegetable products, 2nd edn, (Tata McGraw Hill Publishing Company Ltd, New Delhi, India), 1997, 1109
- AOAC, Official methods of analysis of association of official analytical chemists, 16thedn, Vol I & II, Association of Official Analytical Chemists, (Arlington, Virginia, USA), 1995.
- Mahony MO, Sensory evaluation of food: statistical methods and procedures, (Marcel Dekker Inc, New York), 1985,135.
- Khanna P & Dhaliwal YS, Physico-chemical and Sensory evaluation of probiotic dairy products, *CIB Tech Microbiol*, 3(1) (2013) 1-3.
- Gautam N, *Detection and characterization of gene encoding bacteriocin and to study preservative potential of purified bacteriocin*, PhD Thesis, Dr Y S Parmar UHF, Nauni, Solan, HP, India, 2012.
- Mousavi ZE, Mousvi SM, Razavi SH Emam- Djomeh Z & Kiani H, Fermentation of pomegranate juice by probiotic lactic acid bacteria, *World J Microbiol Biotechnol*, 27 (2011) 123-128.
- Karovicova, J & Kohajdova Z, Lactic acid fermented vegetable juices, *Hort Sci (PRAGUE)*, 30(4) (2003) 152-158.
- Trindale F, Bernardi C S, Bodini R B, Balieiro J CC & Almeida E, Sensory acceptability and stability of probiotic microorganisms and vitamin C in fermented acerola (*Malpighiaemarginata* DC.) ice cream, *J Food Sci*, (71) (2006) 492- 495.
- Cakmakci S, Cetin B, Turgut T, Gurses M & Erdogan A, Probiotic properties, sensory qualities and storage stability of probiotic banana yogurt, *Turk J Vet Animal Sci*,36(3) (2012) 231-237.
- Possemiers S, Marzorati M, Verstraete W & Wiele TV, Bacteria and chocolate : A successful combinations for probiotic delivery, *Int J Food Microbiol*, 141, (2010) 97-103.