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Development of a probiotic honey beverage

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ABSTRACT

The study was carried out with the objective to develop a probiotic honey beverage and ascertain its quality, viable count and shelf life. Lactobacillus acidophilus was used as the probiotic organism ensory characteristics, nutrient profile, chemical composition and shelf life of the product were investigated. The optimization of ingredients or product standardisation is based on the sensory quality evaluation. Fermentation time using 1% inoculum Lactobacillus acidophilus was optimized based on the sensory quality evaluation, nutrient and chemical constituents and viable count. The 25:10:25:40 blend ratio of honey, Aloevera pulp, soyamilk and water fermented for 6 hours give highest scores for overall acceptability and viable count of more than 10^9 cfu/ml. Probiotic beverage was superior to non probiotic beverage in all the aspects such as sensory, nutritional and chemical constituents. The probiotic beverage has a shelf life of fifteen days under refrigerated condition.

I. INTRODUCTION

The relationship between certain foods and health benefits and development of foods that promote health and well-being is one of the key research priorities of food industry (Yoon *et al*., 2004). When food is being cooked or prepared using "scientific intelligence" with or without knowledge of how or why it is being used, the food is called "functional food". Thus, functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates, *etc*., needed for its healthy survival (FAO, 2006).

With an increase in the consumer vegetarianism, there is high demand for the vegetarian probiotic products (Vasudha and Mishra, 2013). The development of new nondairy probiotic food products is very much challenging, as it has to meet the consumer's expectancy for healthy benefits. (Stanton *et al.*, 2001). Lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products (Heenan *et al*, 2004; Yoon *et al*., 2006).Sanz *et al.* (2005) reported that honey could enhance the growth of two probiotic bacteria viz. *Lactobacillus and Bifidobacteria* that is essential for better intestinal health. Soya milk is suitable for the growth of the lactic acid bacteria and the use of fermented soya milk drinks as probiotic as recommended by many researchers (Vasudha and Mishra, 2013).

II. MATERIALS AND METHODS

Material Collection and Preliminary Processing

Honey was procured from local honey growers in Thiruvananthapuram. Aloevera leaves were plucked fresh from the fields maintained for cultivation purposes. Processed soymilk was procured from the sales outlets in Trivandrum.

Aloevera leaves were washed thoroughly with running water to remove dirt and other extraneous matter on the leaves. Leaves were kept to drain out water in a colander. Leaves were kept at low temperature for 2- 3 hours to make the gel solidified. After that each leaves were then carefully peeled off, starting from the both edges first and then the flat side of the leaves. Gel was thus separated from the leaves, washed once again and blended well in a blender to get sooth gel.

Optimization of Ingredients

The different combinations were formulated with different proportions of honey, Aloevera pulp and soya milk using trial and error method.

Sensory Analysis of the formulations

The combination of honey, Aloevera pulp, soya milk and water juice was optimized by preparing a beverage with different levels of ingredients and subjected to sensory evaluation by a trained sensory panels using a five point score card and 9 point hedonic scale for appearance, color, consistency, flavor and overall acceptability. The combinations with highest mean ranks were selected for the further process.

Analytical Methods

The selected combinations quality were ascertained in terms of their TSS (ERMA, make), pH (Digital pH meter), titrable acidity (AOAC, 2005). Reducing sugar and total sugars were determined by the method described by Ranganna (2001). Nutritional constituents were ascertained with respect to their calories, carbohydrates, proteins.

Probiotication Process

The freeze dried culture of Lactobacillus *acidophilus* MTCC 10307 *was* obtained from MTCC. The blends that was rated best after sensory evaluation was selected for acidification with *Lactobacillus acidophilus.* pH required for the growth of organism was optimized, dosage of inoculum was optimized and incubation period and temperature were also optimized. The beverage was evaluated for maximum viable count at different fermentation hours such as 3h, 6h and 9 hours.

The quality of the probiotic beverage with respect to their sensory, chemical and nutritional constituents were ascertained and compared with non probiotic beverage.

For storage stability, the optimized beverage samples were stored at refrigerated (4$\pm$1 °C) and (30$\pm$1 °C). Changes in sensory characteristics, total viable counts, pH, TSS, titratable acidity and total sugars were studied during storage.

Statistical Analysis

Statistical analysis was carried out using ANNOVA, Students t- test and Kruskal wallis.

III. RESULTS AND DISCUSSIONS

Optimization of Ingredients for beverage preparation

In the present study, the product was standardized by blending the different proportions of ingredients in different combinations and dilution with water to attain proper consistency and taste. The intention was to incorporate honey, aloevera pulp and soya milk in an ideal proportion to obtain highest sensory scores and appeal.

Honey, aloevera pulp and soya milk were blended in nine different proportions and evaluated for sensory attributes Out of the nine combinations tried three combinations were adjudged to be the best based on the rank means obtained for sensory qualities. The proportion of honey, aloevera pulp, soya milk and water in the best combination (C_9) was 2.5:1.0:2.5:4.0. The best combination selected was the one having honey and soya milk in equal amount with 10 percent aloevera pulp. The other two combinations namely C_6 and C_8 were with blend of honey and soya milk in the ratio 4.0: 1.5: and 3.0: 2.0 with 10 percent aloevera pulp. Deen and Tiwari (2014) standardized a RTS beverage with best blend containing 75 percent bael pulp and 25 percent aloevera gel.

Sensory evaluation of the combinations tried out.

Table below shows mean ranks obtained for three selected combination based on sensory evaluation.

Honey: Aloevera: Soyamilk: Water	**Appearance**	**Colour**	**Flavour**	**Taste**	**Consistency**	**Overall acceptability**
5.0:2.0:1.5:1.5	39.3	34.9	33.6	31.4	27.4	33.4
5.0:1.5:1.5:2.0	34.0	27.6	30.2	27.8	34.6	31.8
5.0:1.0:1.5:2.5	30.9	38.5	37.1	24.2	29.4	32.2
5.0:1.0:1.0:3.0	36.3	38.5	33.8	31.4	38.2	35.2
4.0:1.0:1.0:4.0	50.8	52.3	47.9	55.1	41.8	55.5
4.0:1.0:1.5:3.5	55.0	55.2	58.4	61.5	58.4	59.0
3.5:1.0:1.5:4.0	36.3	34.5	37.2	35.0	38.2	34.6
3.0:1.0:2.0:4.0	59.2	58.8	62.0	70.1	68.5	66.0
2.5:1.0:2.5:4.0	67.6	69.0	69.2	72.9	75.0	69.5
C.D(0.05)	21.73					

In the present study, the combinations were subjected to sensory evaluation. C_9 was adjudged to be the best combinations because it has got higher acceptance. The mean ranks obtained for the best combination (C_9) for various sensory attributes such as appearance, colour, flavour, taste, consistency and overall acceptability were 67.6, 69.0, 69.2, 72.9, 75.0 and 69.5 respectively. The mean ranks obtained for sensory attributes of the combination C_8 were 59.2, 58.8, 62.0, 70.1, 68,5 and 66.0; while that of C_6 combination were 55.0, 55.2, 58.4, 61.5, 58.4 and 59.0.

Boghani *et al*. (2012) reported that appearance of the blended papaya- Aloevera RTS beverage enhanced with increase in the concentration of *Aloe vera* juice up to a level of 10 percent while further increase in *Aloe vera* juice content reduced the appearance profile.

Proximate Analysis

The analysis of the chemical constituents of the selected beverage was carried out.

Chemical analysis of the selected combinations

Beverage	TSS(^{0}brix)	pH	Titrable acidity (%)	Reducing sugars (g of glucose/100g of juice.)	Total sugars (g of glucose/100 g of juice)
C_9	**23**	**6.5**	0.068	29.40	78.11
C_8	20	5.5	0.074	26.55	73.09
C_6	22	4.4	**0.080**	**32.0**	**93.98**
C.D(0.05)	NS	NS	0.002	1.018	0.592

TSS of the combinations ranged from 20^{0}brix- 23^{0}brix. The pH of the beverage was recorded as 6.5, 5.5 and 4.4 respectively in C9, C_8 and C_6. C_9 combination is having acidity of 0.068%, while acidity of C_8 and C_6 were 0.074% and 0.080% respectively. Singh *et al*. (2014) reported that pH of whey guava beverage ranged from 3.39-4.15. pH and acidity was influenced by the content of the ingredients used.C_6 recorded higher reducing sugar content 32.0 g of glucose/100g of juice) than C_9 (29.40 g of sugar/100 g of juice) and C_8(26.55 g of glucose/100g of juice). C_6 recorded highest sugar content of

93.98 g of glucose/100g of juice followed by C_9 (78.11 g of glucose/ 100 g of juice) and C_8 (73.09 g of glucose/100 g of juice. Reducing sugars and totals sugars of formulated bael and aloevera RTS were found to be 2.30 percent and 10.21percent. (Deen and Tiwari, 2014).

Probiotication Process of the beverage

The selected organism for the probiotication process was *Lactobacillus acidophilus*. After the selection of the culture, optimization of pH for the growth of organism was finalized. Culture was inoculated at varying pH from 4.0- 7.0 and found that growth was adequate with a pH from 6.2- 6.6. In the present study, since the culture depicted good growth at a pH 6.5, the combination C_9 was finally selected and the pH was optimized at 6.5.

Viable count of *Lactobacillus acidophilus* at varying time intervals and doses.

Dosage of inoculums	(x 10^9 cfu/ ml)		
	3 hrs	6 hrs	9 hrs
1.0%	**46**	**85.8**	**64.4**
1.5%	64	115	96
2.0 %	75	135	106

The next step is to decide the dosage of inoculum for the formulation of the beverage. Three doses viz, 1% 1.5% and 2% inoculum was inoculated and viable count was recorded at varying time interval of 3 hours, 6 hours and 9 hours. The maximum viable count obtained by 1% inoculum was 85.8 x 10^9 cfu/ml, 1.5% inoculum attained viable count of 115 x 10^9 cfu/ml and 2% inoculum depicted maximum viable count of 135x 10^9 cfu/ml at 6 hours of incubation. The viable count of *lactobacillus acidophilus* (1%) in beverage at 3hours, 6 hours and 9 hours of incubation were 46x 10^9 cfu/ml, 85.8 x 10^9 cfu/ml and 64.4 x 10^9 cfu/ml. In the present study the dosage was optimized to 1% since the viable count obtained at 6 hours of incubation itself the recommended level of 10^6- 10^9. Zeynab *et al*. (2010) incorporated 1% *Lactobacillus acidophilus* for development of functional symbiotic acidophilus milk. Shukla *et al.*(2013) developed a probiotic beverage based on whey and pineapple juice with *Lactobacillus acidophilus* with a pH of 4.82.

To make the product consumer safe, standardized beverage was sterilized at 100^0C and 80^0C.It was found that sterilization at 100^0C cannot be advised as sensory parameters mainly colour of the beverage altered and become intense besides curdling occurred in the beverage. When product was sterilized with 80^0C, at this temperature also Colour change and appeal was decreased. Thus it was decided to sterilize individual ingredients separately before blending except honey and soya milk.

Thus probiotic honey beverage was formulated with sterilized ingredients, honey, soya milk and 1% *Lactobacillus acidophilus* with 6 hours of incubation and maximum viable count of 85.8 x 10^9 cfu/ml.

Quality assessment of the probiotic beverage

The quality of the probiotic beverage was ascertained with respect to their sensory, chemical and nutritional constituents.

Average score of the sensory attributes of the probiotic beverage with the control.

Beverage	Appearance	Colour	Flavour	Taste	Consistency	Overall acceptability
Probiotic	**4.6**	**4.7**	**4.6**	**4.5**	**4.7**	**4.5**

Beverage						
Non Probiotic Beverage	4.1	4.1	4.1	4.0	4.1	4.0
t value	3.16*	3.26*	3.16*	2.5*	3.26*	3.16*

Significant at 5%- * P<0.05,

Mean score for appearance of probiotic beverage was 4.6 and was significantly different from non probiotic beverage (4.1) and beverage becomes thicker as a result of probiotication. With respect to colour attribute probiotic beverage scored 4. 7 out of 5 and was significantly higher than the control. As indicated in the table Probiotic beverage has recorded an average score of 4.6 and that of non probiotic was 4.1. Probiotic beverage has recorded more acceptances in the taste attribute (4.5) compared to non probiotic beverage (4.0). It was found that the consistency of probiotic beverage was thicker and cloudier than the control and scored 4.7 as against 4.1 in the control (probiotic beverage). It was found that in overall acceptability also probiotic beverage has secured maximum score of 4.5 and significantly differs from the non probiotic (4.0). Shukla (2013) reported that the score obtained for overall acceptability of the whey based probiotic beverage ranged from 8.87-4.99 in hedonic rating.

Chemical Analysis of the probiotic product

Product	TSS(^{0}brix)	pH	Acidity (%)	Reducing Sugars (g%)	Total Sugars (g%)
Probiotic beverage	**24 brix**	**6.6**	**0.083**	**35**	**86.20**
Non Probiotic Beverage	23 brix	6.5	0.076	29.40	78.11
t value	-	-	14*	6.64*	8.61**

TSS of probiotic beverage was 24^0 brix and that of non probiotic beverage was with a TSS of 23^0 brix. pH of probiotic beverage was 6.6, which was slightly higher than the non probiotic beverage which was having pH of 6.5. Probiotic beverage recorded higher acidity of 0.083% than the non probiotic with an acidity of 0.076%. Daneshi *et al*.(2013) found that pH and acidity of milk probiotic carrot juice drink ranged from 5.33- 6.6 and 0.13- 0.31%. Probiotic beverage recorded higher reducing sugar content of 35g/100g while probiotic with reducing sugar content of 29g/100g. Total sugars of the probiotic beverage enhanced and recorded total sugar content of 86.20 g of glucose/100g of juice, while non probiotic beverage recorded 78.11 g of glucose/100 g of juice. It may inferred that chemical constituents were found to enhance by the probiotication process.

Nutrient Analysis of the Probiotic Product

Product	Energy (kcal)	Carbohydrates (g/100g)	Protein (g/100g)	VitaminC (mg/100g)	Iron (mg/100g)	Calcium (mg/100g)
Probiotic beverage	**288**	**72.66**	0.82	**0.072**	**0.59**	**320**
Non probiotic beverage	258	64.66	0.47	0.063	0.25	280
t value	59	5.29*	35*	10.8*	35	39

Energy content of the probiotic beverage was estimated as 288kcal/100g which was slightly higher than the non probiotic beverage of 256kcal/100g. Carbohydrate content of probiotic beverage was estimated as 72.66 g /100 g of sample, which was slightly higher than non probiotic beverage with a carbohydrate content of 64.66 g /100 g. Protein content of probiotic beverage is 0.82g/100g, while non probiotic beverage is 0.47g/100 g. Iron content of the developed beverage was assessed and it is clear that iron content of 0.59 mg/ 100 g of sample which is higher than the non probiotic with an iron content of 0.25 mg/100 g By the probiotication, the calcium content of the beverage increased to 320mg/100 from 280 mg /100g.

Shelf stability of the probiotic beverage

The probiotic beverage was stored in ambient and refrigerated condition and ascertained their quality changes with respect to their sensory, chemical constituents and viable counts. The quality parameters of the beverage stored under ambient were ascertained every day, while qualities of beverage stored under refrigerated condition was ascertained on alternate days.

Beverage stored under ambient condition

Changes in the Sensory quality of the probiotic beverage under ambient condition.

Storage (Period)	Appearance (Mean ranks)	Colour (Mean ranks)	Flavour (Mean ranks)	Taste (Mean ranks)	Consistency (Mean ranks)	Overall Acceptability (Mean ranks)
First day	19.0	20.0	24.60	23.90	22.0	21.5
Second day	16.0	15.35	15.0	16.50	17.0	17.0
Third day	12.0	15.25	8.90	6.10	9.6	8.0
K value	6.84	6.75	22.15	6.76	11.81	16.9
C.D(0.05)	11.49					

Sensory qualities decreased drastically when kept under ambient condition. Percentage decrease for appearance was 36.84, while for colour, flavour, taste and consistency were 24, 64, 74.47, 56. Overall acceptability of the beverage has also altered drastically to 63%. By the end of third day the beverage was not able to relish by the panelist. It may be noted that decrease was more pronounced with respect to flavours and taste followed by appearance and consistency. Junaid *et a*l. (2013) reported that flavoured acidophilus milk was rated about 7.8 in hedonic rating by the panelists, which was decreased to about 6.6 after 6 days of storage.

Chemical analysis of Probiotic Beverage during storage under ambient condition

Days of Storage	TSS(^{0}brix)	pH	Acidity (%)	Total Sugars (g%)
First day	23.0	6.5	0.073	57.7
Second day	24.3	6.4	0.251	39.0
Third day	26.0	6.3	0.323	30.1
C.D (0.05)	0.666	-	0.006	2.515

The TSS of the probiotic beverage stored at ambient condition slightly increased from 23^0- 26.0^0 brix from first to third day.While pH of the developed probiotic beverage decreased from 6.5 – 6.3 from first to third day. Acidity of the probiotic beverage increased considerably from 0.07% - 0.32%. Shukla

(2013) reported that increase in acidity of the beverage was more prominent when stored at ambient temperature where acidity reached 0.89% after 120 hr of storage.The total sugar content of the probiotic beverage also decreased during storage at ambient condition from 57.7 – 30.12 g of glucose/100 g.

Probiotic beverage stored under refrigerated condition

Changes in sensory qualities of the probiotic beverage stored under refrigeration.

Storage period (d)	Appearance	Colour	Flavour	Taste	Consistency	Overall acceptability
Second day	48.5	49.0	50.5	54.0	47.5	49.0
Fourth day	48.5	49.0	50.5	54.0	47.5	49.0
Sixth day	48.5	49.0	50.5	54.0	47.5	49.0
Eighth day	48.5	49.0	50.5	54.0	47.5	49.0
Tenth day	26.1	22.6	31.9	27.7	24.7	22.6
Twelvth day	17.1	17.9	14.5	19.5	20.3	17.9
Fourteenth day	11.3	11.9	7.9	7.7	13.4	11.9
Fifteenth day	11.2	11.5	7.2	6.8	12.3	11.5
K value	56.1*	57.93*	58.47*	58.92 *	48.87*	57.93*
C.D(0.05)	19.69					

Based on the sensory analysis of the probiotic beverage stored under refrigerated condition clearly indicated that till the eighth day of storage all the sensory attributes remained constant. However mean ranks secured for different sensory attributes of beverage viz, appearance, colour, flavour, taste, consistency and overall acceptability found to decline after eight days and their percentage decrease were 76.90, 76.53, 85.74, 87.40, 74.10 and 73.90 by fifteenth day. Sensory attribute of the probiotic beverage reduced by fifteenth day. Daneshi *et al*. (2013) found that milk/ carrot Juice drink inoculated with *L. acidophilus, B. lactis* and *L. plantarum* showed higher sensory acceptability over 20 days storage.

Chemical analysis of probiotic beverage during refrigerated storage

Days of Storage	TSS(^{0} brix)	pH	Acidity(%)	Total sugars(g%)
Second Day	24.0	6.5	0.073	40.10
Fourth Day	27.0	6.5	0.074	28.73
Sixth Day	27.0	6.5	0.083	28.63
Eighth Day	27.0	6.4	0.093	27.07
Tenth Day	27.0	6.3	0.215	27.17
Thirteenth day	28.0	6.3	0.222	26.21
Fourteenth day	31.0	6.2	0.233	25.77
Fifteenth day	31.0	6.2	0.233	25.77
C.D (0.05)	0.855	0.035	0.430	0.308

The chemical analysis of probiotic beverage in refrigerated condition revealed that TSS of the probiotic beverage during storage increased from 24^0 brix to 31^0 brix. pH of the beverage remained stable till 8 days after which it showed declining trend . Acidity of the probiotic beverage ranged from 0.07 to 0.23 during storage of 15 days at refrigerated condition. Shukla (2013) reported that acidity of

the probiotic beverage from whey and pineapple juice increased during the refrigerated storage from 0.546 to 0.89% after 28 days. The total sugar content of beverage at storage in refrigerated condition decreased from 40.0 - 25 g of glucose/100g.

Changes in viable count of Lactobacillus *acidophilus* in the probiotic beverage during storage in the ambient condition.

Storage (d) (30 ± 1^0C)	**Count ($x10^9$cfu/ml)**	**Days of Storage (4 ± 1^0C)**	**Count ($x10^9$cfu/ml)**
First	59.4	Second	21.2
Second	33	Fourth	42.4
Third	21.4	sixth	33.6
		Eighth	38.6
		Tenth	54.4
		Twelveth	76.8
		Fourteenth	94.8
		Fifteenth	94.8

The viable count of the probiotic beverage was assessed during storage at the ambient condition and showed a tremendous decrease in the viable count day by day. The viable count was 74.4 x 10^9 cfu/ml, at incubation day, was decreased to 59.4 x 10^9 cfu/ml after 24 hours and decreased further to 33x 10^9 cfu/ml by 48 hours and then it decreased to 21.4x 10^9 cfu/ml after 72 hours. Junaid *et al.* (2013) reported that viable count of *Lactobacillus acidophilus* in different flavoured milk found to vary from first day to sixth day. At first day viable count of mango, pineapple and strawberry flavoured milk were 2.50x 10^6cfu/ml, 3.60x 10^6 cfu/ml and 2.87x10^6 cfu/ml to 1.50x 10^6 cfu/ml, 3x 10^6 cfu/ml and 2.50x 10^6 cfu/ml after 6 days. However, the total viable count was under acceptable range even after 6 days of storage indicating good keeping quality up to 6 days.

Viable count of *Lactobacillus acidophilus* in beverage stored in refrigerated condition increased till fourth day of storage from 21.2x10^9 cfu/ml to 42.4 x10^9 cfu/ml, while on 6th day it declined to 33.6 x 10^9 cfu/ml and thereafter it increased and on the fifteenth day it reaches maximum viable count of 94.8x 10^9 cfu/ml. Shukla (2013) observed that the initial total viable count of the beverage was 3.8×107 cfu/ ml which decreased to 1.8×10^7 at refrigerated storage.

Cost of the probiotic beverage

Cost of the beverage was computed and found as Rs 15/- per 100 ml and when compared with the commercially available probiotic products cost was comparatively less such products at present available in market were found to be Rs 25- 50/100 ml.

IV. CONCLUSION

Above study has revealed satisfactorily good quality probiotic beverage with therapeutic value prepared by using blend of 25h: 10a:25s:40w with 1 percent inoculum of *Lactobacillus acidophilus* with a shelf life of 15 days at 4 ± 1^0C and 72 hrs at 30 ± 1^0C.

BIBLIOGRAPHY

[1] AOAC. Association official Of Analytical Chemistry,2005.974.24.
[2] Boghani, A. H., Raheem, A and Hashmi, S. I. (2012) Development and storage studies of blended papaya-aloe vera ready to serve (RTS) beverage. *J. Food ProcessTechnol*. 3: 1000185.13-15.
[3] Daneshi, M., Ehsani, M.R., Razavi, H.S., and Labbafi, M. 2013. Effect of storage on refrigerated storage on the probiotic survival and sensory properties of carrot/milk juice mix drink. *Electronic Journal of Biotech nology*. 16 (5): 1-5.
[4] Deen, D.K and Tiwari, B. 2014. Preparation and storage of RTS beverage from bael and aloevera. *An International Quarterly Journal of Life Sciences*. 10(1): 113-116.
[5] FAO/WHO. 2006. Probiotic in foods. Health and nutritional properties and guidelines for evaluation. In FAO *Food and Nutrition*; Pp 85. ISBN 92-5-105513- 0.
[6] Hennan, C. N., Adams, M. C., Hosken, R. W and Fleet, G. H. 2004. Survival and sensory acceptability of probiotic microorganisms in a non- fermented frozen vegetarian dessert. Lebensmittel – Wisenschaftund – Technology. 37:461-466.
[7] Junaid, M., Javeed, M.I., Gulzar, M and Ahmad,N.2013. Development and quality assessment of flavoured acidophilus milk. *The Journal of Animal and Plant Sciences*. 23(5): 1342-1346.
[8] Meilgard, M., Caviled G. V., and Carr, B. T. 2007. Sensory evaluation techniques 4th edition. CRC Press Inc.ISBN 0849338395, 9780849338397
[9] Ranganna, 2001. Handbook and quality control for fruit and Veg.Products,Tata Mc Graw- Hill Publishing company Ltd. New Delhi, India. 112p.
[10] Sanz, M. L., Polemis, N.,Morales V, Corzon, Drakoul arakou A, Gibson G R,Rastall R 2005.A:In vitro investigation into the potential prebiotic activity of honey oligosaccharides. *J Agric Food Chem*, 53:2914-2921.
[11] Shukla, M., Kumar, Y., and Adamassu, S. 2013. Development of probiotic Beverage from Whey and Pineapple juice. *J Food ProcessTechnol*.4: 206. doi:10.4172/2157-7110.1000206.
[12] Singh, R., Farhaan, B and Divya, S. 2014. Development and Quality Evaluation and Shelf Life Studies of Whey Guava Beverage. *International Journal of Current Engineering and Technology*. 4(3): 2171-2175.
[13] Stanton, C., Gardiner, G., Meehan, H., Collins, K., Fitzgerald, G., Lynch, P. B and Ross, R. P. 2001. Market Potential for probiotics. *American journal of clinical Nutrition*. 73 (Suppl.), 476S-483S.
[14] Vasudha, S. and Mishra, H. N. 2013. Agricultural and Food Engineering Department, Indian
[15] Institute of Technology. *International Food Research Journal*. 20(1):7-15.
[16] Yoon, K. Y., Woodams, E. E and Hang, Y. D. 2004. Probiotication of tomato juice by lactic acid bacteria. *Journal of Microbiology*. 42:315-318.
[17] Yoon, K.Y., Woodams, E. E and Hang, Y. D. 2006. Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technology*, 97(12), 1427-1430.
[18] Zeynab, R. A., Pratima,K and Aruna B. R. 2010.Development of acidophilus milk via selected probiotics & prebiotics using artificial neural network. *Adv. Biosci. Biotech*. 1: 224-231.

