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Screening of Fruit Juice as Probiotic Carrier Food

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Experiment was undertaken to determine the suitability of the fruit juice as probiotic carrier food by incorporating probiotic bacteria in different fruit juices. Pasteurized fruit juices such as apple, banana, grapes, mango, orange, pineapple, pomegranate and tomato were inoculated with 24-h-old probiotic culture (*Lactobacillus acidophilus*) and incubated at 37ºC for 48 h. Changes in the acidity, pH, TSS, total sugars, color and viable cell counts after 48 h of fermentation were analyzed. All the probiotic fruit juices recorded higher percentage of acidity as expressed as lactic acid and lower pH values. The TSS and total sugars were decreased and there were changes in the colour values. The probiotic bacteria used in this study was found capable of rapidly utilizing the juice for cell synthesis and lactic acid production without nutrient supplementation and pH adjustment. The results revealed that apple and pomegranate showed maximum survival of *L.acidophilus* and the numbers reached upto 17.5×10^{7} cfu/ml compared to other probiotic fruit juices. Banana juice showed the lowest survival. Sensory evaluation of probiotic fruit juices was carried out and probiotic apple juice showed higher sensory score of above 8 for different sensory attributes except color compared to other probiotic fruit juices. Based on the changes in the physico chemical properties of fruit juices, survival of probiotic *L.acidophilus* and the sensory evaluation reports, it can be concluded that apple juice was found to be suitable for the production of probiotic apple juice and it could serve as a health beverage for consumers who are allergic to dairy products and old age people.

Key words: Fruit juices, probiotic carrier, pasteurized fruit juices, *Lactobacillus acidophilus*

The word ‘probiotic’, derived from the Greek language, means ‘for life’. Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Anonymous, 2002). The rationale for probiotics is that the body contains miniature ecology of microbes, collectively known as the gut flora. The number of bacterial types can be thrown out of balance by a wide range of circumstances including the use of antibiotics or other drugs, excess alcohol, stress, disease, exposure to toxic substances etc. In cases like these, the bacteria that work well with our bodies may decrease in number, an event which allows harmful competitors to thrive, which is detrimental to our health.

The use of probiotic bacterial cultures stimulate the growth of preferred microorganisms, crowds out potentially harmful bacteria, and reinforces the body’s natural defense mechanisms (Salminen *et al*.,1998). At present it is generally recognized that an optimum ‘balance’ of microbial population in our digestive tract is associated with good nutrition and health (Rybka and Kailasapathy, 1995). The microorganisms primarily associated with this balance are *lactobacilli* and *bifidobacteria.*

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Increasing evidence of health benefits of probiotics indicates that consumption of ‘probiotic’ microorganisms can help to maintain such a favourable microbial profile and results in several therapeutic benefits. Consumption of probiotic bacteria via food products is an ideal way to re-establish the intestinal microflora balance. Adequate numbers of viable cells, namely the ‘therapeutic minimum’ needs to be consumed regularly for transfer of the ‘probiotic’ effect to consumers. Survival of these bacteria during storage, until consumption is therefore an important consideration.

In recent years, consumers have begun to select foods for reasons exceeding basic nutrition and taste. Recognizing the link between diet and health, consumers now demand nutritious functional foods that prevent disease and improve health. In response to this demand, the food industry is promoting the foods fortified with functional ingredients and nutraceuticals. For example, dairy companies have been successful with their manufacture of yogurts and dairy drinks fortified with probiotics but lactose intolerance and the cholesterol content are two drawbacks related to their consumption (Yoon *et al*., 2006) . Most foods containing probiotics are currently dairy based. There is a need to develop technologies for non-dairy probiotic foods. Fruit juice has recently been suggested as a good medium for functional ingredients such as probiotics (Tuorila and Cardello, 2002) because it has a wide consumer appeal and is generally recognized as a healthy product. Furthermore, fruits do not contain any dairy allergens that might prevent usage by certain segments of the population (Luckow and Delahunty, 2004a). The health benefits of probiotic bacteria (*L.acidophilus*) have led to their incorporation into non-dairy foods. Although the addition of probiotics to foods is challenging, the benefits to our society are certainly worth the effort. Keeping the above facts in mind, a research was undertaken by incorporating probiotic bacteria in fruit juices with the following objectives namely to study the physico chemical properties of fruit juices, to incorporate probiotic bacteria in fruit juices, to study the survival of probiotic *L.acidophilus* in probiotic fruit juices and evaluate its organdeptic quality.

Materials and Methods

Raw materials

This study was carried out in the Department of Food and Agricultural Process Engineering, Agricultural Engineering College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2007-2008. Apple (Delhi apple), banana (Poovan), grapes (Bangalore blue), mango (Neelam), orange (Kinnow mandarin), pineapple (Kew), pomegranate (Kabuli) and tomato (Gotya) were purchased from local market and used for this study. All the fruits purchased were at the right stage of maturity and free from spoilage and physical damage. Probiotic bacterium (*Lactobacillus acidophilus* MTCC447) was obtained from the MTCC (Microbial Type Culture Collection), Institute of Microbial Technology, Chandigarh, India.

Probiotic fruit juices preparation

The juices were prepared by using the mixer-grinder (Philips mixer grinder) and strained through the previously washed and dried muslin cloth. The filtered fruit juices were pasteurized at 72$_o$C for 15 s by keeping in a water bath (Genuine Equipment MFRS, Coimbatore) and allowed to cool until it reaches 37ºC at room temperature. The probiotic culture was grown at 30$_o$C for 24 hours in de Man Rogosa Sharpe (MRS) broth (dextrose 20.0 g/L; meat peptone 10.0 g/L; beef extract 10.0 g/L; yeast extract 5.0 g/L; sodium acetate 5.0 g/L; disodium phosphate 2.0 g/L; ammonium citrate 2.0 g/L; tween 80 1.0 g/L; magnesium sulfate 0.1 g/L, manganese sulfate 0.05 g/L) and used as an inoculum (Yoon *et al*., 2006) . One per cent inoculum of probiotic bacterial culture was pipetted out using a micropipette and distributed into fruit juices and mixed thoroughly. The probiotic fruit juices were incubated at 37$_o$C (Prajapati *et al* ., 1986) by keeping it in a BOD incubator (Genuine Equipment MFRS, Coimbatore) for 48 hours for encouraging faster multiplication of probiotic bacteria.

Screening of fruit juices for probiotic enrichment

The fruit juice was selected for the enrichment of probiotic bacteria based on the changes in the physico-chemical properties of fruit juice before and after addition of probiotic bacteria, survivability of inoculated probiotic bacteria and sensory evaluation of probiotic fruit juice.

Analyses of fruit juices

The various physico -chemical analyses *viz*., acidity, pH, total soluble solids, total sugars, and colour were carried out by adopting standard methods. The total sugars (reducing and non-reducing) of the fruit juices were determined by the titration method described by A.O.A.C. (2000). The total soluble solids present in the fruit juices were determined by using ERMA hand refractometer ranging from 0 -32°Brix. Acidity was measured as per the method described by Ranganna (1995). The pH was determined by using a digital pH meter (Systronics µ pH system 361, Ahmedabad, India). The pH meter was standardized with double distilled water of pH 7.0 and buffers at pH 4.0 and 9.2. After standardization, pH of different fruit juices was measured.

Hunter lab color flex meter was used for the measurement of colour of probiotic fruit juice powder. The colour of the probiotic fruit juice powder was measured by using CIELAB scale at 10$_o$ observer at D_{65} illuminant. Before measuring the colour of the samples, the instrument was standardized by placing black and white standard plates. The sample colour was measured by filling the juice in the transparent cup. The deviation of the colour of the samples to standard was also observed and recorded in the computer interface.

Sensory evaluation of probiotic fruit juices

For better acceptability and sustained marketing of the probiotic fruit juice, various sensory parameters such as appearance, colour, flavour, taste and overall acceptability of fresh probiotic fruit juices were considered. Organoleptic evaluation was performed as per the procedure outlined by Ranganna (1979) by a panel of 20 semitrained judges in a 9 point hedonic scale varying from 'like extremely' (rated as 9) to 'dislike extremely' (rated as1).

Enumeration of microbial population

Enumeration of total bacteria, yeast, fungi and the probiotic bacterium *L.acidophilus,* was done by standard plate count method (Allen,1953) with Nutrient agar, Glucose yeast peptone agar medium, Rose bengal agar medium and de Man rogosa sharpe medium respectively. The appropriate quantity of nutrients required for the preparation of each medium were weighed and dissolved one after another in 100 ml of distilled water in a 250 ml conical flask. The pH of the solution was adjusted using 0.1 N NaOH and 0.1 N HCl. Then agar was added and the medium was sterilized in the autoclave at 121^{o}C for 15 min.

Analysis of microbial population in fruit juices

One ml of the juice sample was accurately pipetted using a micropipette into test tube containing 9 ml of sterile distilled water (10^{-1}) and serially diluted until 10^{-6} dilution was reached. One ml aliquots from 10^{-3}, 10^{-4} and 10^{-6} dilutions were transferred to the sterile petriplates for the enumeration of fungi, yeast and bacteria respectively. Plates were duplicated for each dilution. Approximately 15- 20 ml of molten and cooled medium (45°C) for the respective organisms were added into the petri plates and the plates were rotated clockwise and anticlockwise directions on the flat surface to have a uniform distribution of colonies. After the solidification of agar, the plates were inverted and incubated at room temperature for 2-5 days (bacteria-2 days, yeast and fungi-5 days). The colonies were counted after the incubation period and the number of cfu per ml of sample was calculated by applying the formula Number of colony forming units (cfu) per ml of the sample

$$= \frac{\text{Mean number of cfu x Dilution factor}}{\text{Volume of the sample}}$$

Results and Discussion

Screening of fruit juice for probiotic enrichment

Physico chemical properties of fruit juices

Preliminary studies were conducted to screen different fruit juices for probiotic enrichment. The physico chemical properties such as acidity, pH, TSS, total sugars and color were analyzed for fresh, pasteurized and probiotic juices and presented in Table 1. It was observed that probiotic fruit juices recorded the high percentage of acidity after 48 hours of incubation at 37°C compared to fresh and pasteurized fruit juices. This might be due to the fact that the probiotic bacteria, *L.acidophilus* might have produced lactic acid by utilizing the sugars present in the juice during the incubation period. Similar results were reported by Yoon *et al*., (2004) for probiotic cabbage juice. Among the various probiotic fruit juices studied, the percentage of acidity for apple

Table 1. Physico chemical properties of fruit juices

Fruit juice	Acidity(%)	pH	TSS(° Brix)	TS (%)	Hunter lab colorimeter values		
					L	a	b
Apple							
Fresh juice	0.24	4.36	12.0	10.52	31.51	10.57	28.86
Pasteurized juice	0.23	4.44	12.1	10.51	30.80	07.96	28.62
Probiotic juice	0.26	4.04	10.7	9.42	30.79	07.61	25.34
Banana							
Fresh juice	0.23	3.29	10.5	9.12	59.84	07.95	22.79
Pasteurized juice	0.22	3.26	10.6	9.11	59.77	05.74	24.02
Probiotic juice	0.28	3.22	10.0	8.62	59.73	05.56	17.74
Grapes							
Fresh juice	1.40	3.47	11.0	9.57	22.90	22.75	6.48
Pasteurized juice	1.40	3.47	11.0	9.57	22.47	24.28	6.64
Probiotic juice	1.44	3.45	10.2	8.86	22.32	24.20	6.63
Mango							
Fresh juice	0.28	5.05	9.9	8.91	57.70	15.09	67.91
Pasteurized juice	0.27	5.07	9.9	8.91	55.21	13.67	64.10
Probiotic juice	0.36	4.64	9.0	8.11	54.15	13.92	61.33
Orange							
Fresh juice	0.71	4.69	7.0	6.14	59.81	6.82	57.99
Pasteurized juice	0.70	4.71	7.0	6.14	59.32	5.95	57.53
Probiotic juice	0.74	4.61	6.4	5.54	59.52	5.47	56.68
Pineapple							
Fresh juice	0.64	2.49	8.0	6.82	43.31	-4.28	33.53
Pasteurized juice	0.62	2.55	8.1	6.84	42.08	-3.58	29.40
Probiotic juice	0.68	2.40	7.5	6.34	41.32	-3.34	29.30
Pomegranate							
Fresh juice	0.56	4.12	10.0	9.44	57.29	19.92	1.25
Pasteurized juice	0.53	4.40	10.1	9.46	56.26	17.74	2.34
Probiotic juice	0.62	3.58	9.2	8.43	54.49	17.97	2.72
Tomato							
Fresh juice	0.61	3.77	3.4	2.71	38.00	20.43	20.35
Pasteurized juice	0.60	3.79	3.5	2.71	37.71	22.22	23.08
Probiotic juice	0.65	3.45	3.4	2.63	36.85	23.90	24.82

Mature ripe fruits
↓
Washing
↓
Extraction of juice
↓
Filteration of juice (muslin cloth)
↓
Pasteurization (72°C for 15s)
↓
Incorporation of probiotic bacteria (1 % v/v)
↓
Incubation (at 37°C for 48 hours)
↓
Probiotic apple juice

Fig. 1. Process flow chart for the production of probiotic apple juice

was less followed by banana. In all the juices tested, the probiotic fruit juices recorded the lower pH values compared to the fresh and pasteurized fruit juices. This must be due to the increase in percentage of acidity in probiotic fruit juices. The TSS and total sugars of all probiotic fruit juices decreased when compared with the other two. This might be due to the fermentation of juices by the probiotic bacteria which led to decrease in the percentage of total sugars which in turn reduced the TSS of probiotic fruit juices.

There was no significant change in the 'L' values of all the juices irrespective of whether it is fresh/ pasteurized/probiotic fruit juices. Only probiotic pomegranate juice recorded slightly lower 'L' values when compared to all the other juices. Almost all the juices recorded changes in the 'a' values for all the three types namely fresh, pasteurized, and probiotic juices, but it did not had any significant change in the colour of the juice. However, 'b' values differed significantly for apple, banana, mango, pineapple, pomegranate and tomato. There were changes in 'b' values for fresh, pasteurized and probiotic juice respectively.

Microbial analysis of fruit juices

Table 2. Microbial analysis of fruit juices

Fruit juice	Fresh juice			Pasteurized juice		
	Bacteria ($\times10^6$ cfu/ml)	Fungi ($\times10^3$ cfu/ml)	Yeast ($\times10^4$ cfu/ml)	Bacteria ($\times10^6$ cfu/ml)	Fungi ($\times10^3$ cfu/ml)	Yeast ($\times10^4$ cfu/ml)
Apple	10	-	18	4	-	5
Banana	34	2	49	7	1	9
Grapes	29	9	50	4	1	6
Mango	51	-	43	6	-	5
Oranges	17	5	26	5	2	8
Pineapple	19	-	38	6	-	7
Pomegranate	12	-	22	3	-	9
Tomato	25	2	65	7	1	4

The different fruit juices were analyzed for microbial load and presented in Table 2. The data revealed the effect of pasteurization on the reduction of microbial population in the fruit juices. The bacteria, fungi and yeast population were much reduced in pasteurized juices, when compared to fresh juices and no undesirable changes were noticed in the fruit juices after pasteurization for the enrichment of probiotic bacteria.

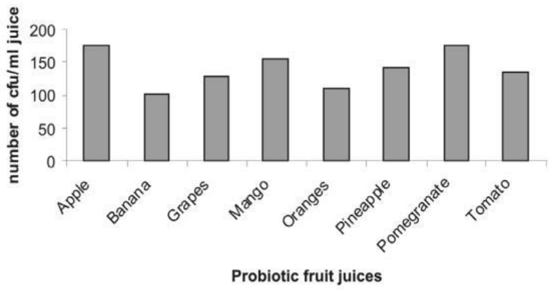

Fig. 2. Survival of *L.acidophilus* (10^6 cfu/ml) in different fruit juices

Survival of probiotic L.acidophilus in different fruit juices

The probiotic bacteria used in this study was found capable of rapidly utilizing the juice for cell synthesis and lactic acid production without nutrient supplementation and pH adjustment. Fig. 2 shows the survival of *L.acidophilus* in different probiotic fruit juices after the incubation period. The data indicated that apple and pomegranate showed maximum survival of *L.acidophilus* (175×10^6 cfu/ml) after 48 hours of incubation at 37°C compared to other fruit juices. The lower percentage of acidity and high sugar content could be there as for the survival. Banana juice showed the lowest survival (102×10^6 cfu/ml). For other juices, the number of colony forming units per ml ranged between 110 and 157×10^6 cfu/ml.

Sensory evaluation of probiotic fruit juices

The sensory score for different probiotic fruit juices is presented in Table 3.

Table 3. Sensory evaluation of probiotic fruit juices

Probiotic juice	Appearance	Color	Flavour	Taste	Overall acceptability
Apple	8.1	7.2	8.3	8.3	8.0
Banana	Spoiled				
Grapes	7.4	8.2	6.7	5.3	6.4
Mango	6.5	7.1	5.4	4.0	4.2
Oranges	6.0	8.0	6.2	6.1	7.0
Pineapple	7.3	8.0	7.1	7.2	7.3
Pomegranate	Spoiled				
Tomato	7.0	8.2	5.2	5.0	5.4

The probiotic fruit juices were subjected to sensory evaluation by a panel of 20 semitrained judges using 9 point hedonic scale. Probiotic apple juice showed higher sensory scores of above 8 for different sensory attributes except color compared to other probiotic fruit juices. Qualitatively, this value was recorded as 'like very much' and for colour it was recorded as 'Like moderately'. Banana and pomegranate juices were spoiled and this might be due to the high initial yeast population that led to the rapid fermentation reaction. Mango juice showed the lowest overall acceptability value of 4.2 and qualitatively this value was recorded as 'Dislike slightly'.

Conclusion

Based on the results of physico chemical properties of fruit juices, survival of probiotic *L.acidophilus* and the sensory evaluation reports, it can be concluded that apple juice can act as a excellent carrier for the production of probiotic fruit juice.

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