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EFFECT OF FRUIT AND VEGETABLE BASED PROBiotic YOGHURTS AND ITS EFFICACY IN CONTROLLING VARIOUS DISEASE CONDITIONS

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ABSTRACT

Fruit and vegetable pulp are often perceived as healthy foods and they have taste profiles that are appealing to all age groups. Fruits and vegetables contain beneficial compounds such as phytochemical and antioxidants that are important in maintaining human health. They are also rich in essential nutrients including sugars, mineral, vitamin and dietary fibers, making them ideal substrates for probiotics. Therefore, there have been increasing interests in the application of vegetable and fruit pulp as alternative carriers of probiotics. There is a genuine interest in the development of fruit pulp based yoghurt with probiotics because they have taste profiles that are appealing to all age groups and because they are perceived as healthy and refreshing foods. Yoghurt is easily digested, has high nutritional value, and is a rich source of carbohydrate protein, fat, vitamins, calcium, and phosphorus. Because milk protein, fat, and lactose components undergo partial hydrolysis during fermentation, yoghurt is an easily digested product of milk (Sanchez-Segarra et al., 2000). In the present research, physicochemical and sensory properties and microbial quality of fortified probiotic yoghurt with Fruit (Sapota and papaya) and vegetable based products (carrot and beetroot) have been investigated on the first, 3rd, 6th and 9th day of storage in refrigerator. Samples were analyzed for pH, Syneresis, Acidity, Solids Not Fat, total solids, and microbial analysis. The efficacy of these fruit and vegetable products with probiotics in gastrointestinal disease conditions was studied by supplementing these products amongst the risk individuals.

INTRODUCTION

Probiotics are increasingly used as food supplements, due to mounting scientific evidences supporting the concept that the maintenance of a healthy gut micro flora may provide protection against gastrointestinal disorder including infections and inflammatory syndromes of the bowel (Parvez et al. 2006; Nomato 2005; Shanahan 2002, 2004; Madden and Hunter 2002). It has been suggested that fruit juices could serve as suitable media for cultivating probiotic bacteria (Mattila-Sandholm et al. 2002). Fruit juices have an established market sector as functional drink through sale of calcium and vitamin-fortified juices, and they are consumed regularly, which is essential if the full benefits attributed to probiotics are to be experienced (Sheehan et al. 2007). Different studies have been carried out to explore the suitability of fruit juices such as tomato, beet and cabbage juices as raw materials for the production of probiotic drinks. *L. plantarum*, *L. acidophilus* and *L. casei* have been employed as probiotic bacteria cultures. Results have indicated that all the strains are capable of growth in the fruit juices mentioned and as a result, the microbial population increases significantly after 48 h of fermentation. Moreover, *L. plantarum*, *L. acidophilus* and *L. Delbrueckii* have shown to be resistant to the high acidic and low pH conditions during storage periods at 4°C.

However results on *L. casei* have indicated that this strain loses its availability during cold storage (Yoon et al. 2004, 2005, 2006). Enrichment of the fruit juice-based medium with nutritive substances has also been studied. Rakin *et al.* (2007) enriched beet root and carrot juices with the brewer's yeast autolysate before the lactic acid fermentation using *L. acidophilus*. The addition of the autolysate yeast favorably increased the number of lactic acid bacterial cells during the fermentation (Aeschlimann and Stocar 1990) and reduced the time of fermentation. Fermentation of vegetable juices enriched with yeast autolysate caused the amino acid, vitamin and mineral content and antioxidant activity of the final drink to increase (Chae and Joo 2001).

Pomegranate (*Punicagranatum*, Punicaceae) is known to have considerable health-promoting properties with antimicrobial, antiviral, anticancer, antioxidant and antimutagenic effects (Negi et al. 2003). The fresh juice contains 85.4% water and considerable amounts of total soluble solids (TSS), total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid and proteins and has been reported to be a rich source of antioxidants. These antioxidants are more potent, on a molar basis, than many other antioxidants including vitamin C, vitamin E,

coenzyme Q-10 and alpha-lipoic acid (Aviram et al. 2002). The antioxidant level in pomegranate juice was found to be higher than in green tea and red wine (Gil *et al.* 2000).

The aim of this research was to investigate the growth rate and substrate metabolism during the fermentation of fruits (sapota, papaya) and vegetables (beetroot, carrot) via selected probiotic yogurt culture and evaluating their viability in cold storage conditions.

MATERIALS & METHODS

Fat free milk was obtained from the local shopping mall, Tirupati. The yogurt culture including *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus* was obtained from National Collection of Dairy Cultures (NCDC) in the Division of Dairy Microbiology at National Dairy Research Institute, Karnal, Haryana, India. Fresh fruits (Sapota and Papaya) and vegetables (Carrot and Beetroot) were procured from local markets in Tirupati.

SELECTION OF SAMPLES

Fat-free skimmed milk (Milk fat - 0.5g, milk SNF - 9.0% minimum) was used for the preparation of Fruit and vegetable yogurt. Skimmed milk powder was added to increase solidity. 8% of sugar was added to increase the taste. The fat free skimmed milk was heated 85°C for 30 minutes, and then rapidly cooled to 42±1°C. The selected Fruits and Vegetables were first cleaned and chopped by peeling the outer layer, deseeded, blended, extract pulp, heat treated at 80°C for 15 minutes, and cooled to 42±1°C. Milk was taken in twelve cups (150ml) for the experiment. Out of these four cups, one for each type of fruit and vegetable, were added with 1.5% of their corresponding concentration. In the similar way, remaining eight cups were prepared with concentration of 2.5% and 3.5% for each individual fruit and vegetables. Inoculation of each *Streptococcus thermophilus* (1%) and *Lactobacillus delbrueckii* ssp. *Bulgaricus* (1%) served as starter culture into each of the fruit and vegetable mixed milk. The inoculated fruit and vegetable mixed milk was incubated at 42°C for four hours. The yogurt samples were cooled to 25°C by resting in a temperature controlled room at 15°C. Then it was stored at 3-5°C for a period of 72 hours.

QUALITY EVALUATION

The samples were analyzed in triplicate for physical parameters (pH, acidity, syneresis), chemical parameters (Solid not fat and total solids) and microbial analysis (coliforms, yeast and mold). The pH was measured with the pH meter. Acidity was titrated by N/10 NaOH solution and 0.5% phenolphthalein solution as an indicator. Syneresis was measured by the method of Modler et al. (1983). Chemical parameters were determined according to the methods described by AOAC. Coliforms, yeast and mold determination were done according to the standard methods for the examination of dairy product, using Potato Dextrose Agar and Maccon-Key Agar.

SENSORY EVALUATION

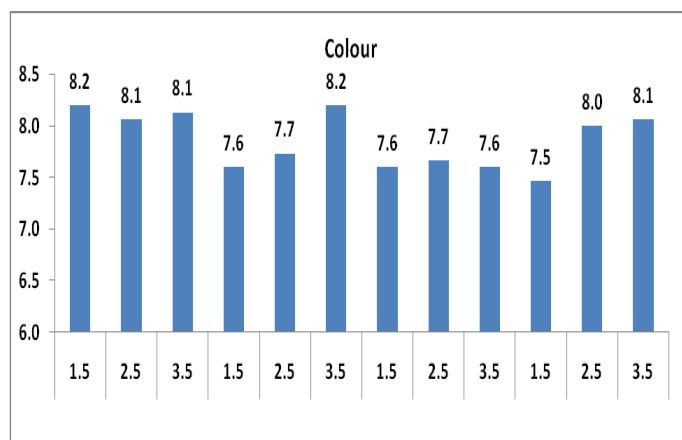
Organoleptic evaluation was done by evaluating the color, appearance, flavor, body and acidity of fruit and vegetable based yogurt sample by a trained panel of 15 members using nine point hedonic scale.

RESULTS AND DISCUSSIONS

According to the 9 – point scoring system with weighted factors like colour, appearance, flavour, body and acidity of produced fruit and vegetable flavoured yoghurts were evaluated

The data was analyzed by using the IBM SPSS Statistics V20. One way and two way analysis of variance (ANNOVA) was adapted to study the attributes (e.g. color, appearance, flavor, body, and acidity). For each of these attributes, Set1 corresponds to the selected fruits & vegetables (i.e. sapota, papaya, beetroot, carrot) and Set2 corresponds to the concentration of pulp added (e.g. 1.5%, 2.5%. 3.5%). This ANNOVA test F-Value and p-value revealed the dominance of any each of the Fruits and Vegetables from Set1, either at 1% or 5% statistical level. Duncan’s Multiple Range test was also carried out to find the significance of the variability for each of the Fruits and Vegetables from Set1. Summary of statistics was carried out to find the Rank of a particular attribute at a particular concentration level from Set2. Further, Paired Sample t-test was carried out for pH and Acidity analysis for a particular Fruit and Vegetable chosen from Set1. This is done at each level of concentration chosen from Set2, while individually evaluating the product before storage and after storage. Subsequently, this test was repeated for each of the chosen type of fruits and vegetables from Set1.

Figure 1: Comparison of sensory scores for colour in fruits and vegetable yogurts prepared



* Significant@5% level, ** Significant@1% level

Summary of Two-way ANOVA on Colour

Fruit	N	Subset	
		1	2
Beetroot	45	7.62	-
papaya	45	7.84	-

Carrot	45	7.84	-
Sapota	45	-	8.13
Sig.	-	0.084	1.000

The results from the above table show that there is difference in color among fruits and vegetables at 1% level. Also there is difference in color amongst chosen concentrations 1.5, 2.5 and 3.5 at 5% level. The results also show that there is a significant difference among fruits at 1% level ($0.001 < 0.01$) and among concentrations at 5% level ($0.028 < 0.05$) by means of colour.

Duncan's Multiple Range Test: Colour

Source	Sum of Squares	df	Mean Square	F-value	p-value
Fruit	5.928	3	1.976	6.014**	0.001
Concentration	2.411	2	1.206	3.669*	0.028
Fruit *	3.989	6	0.665	2.023	0.065
Concentration					
Error	55.200	168	0.329	-	-
Total	67.528	179	-	-	--

From analysis it is clear that color is concerned, among these selected fruits and vegetables. From the results it is evident that Sapota differs significantly (8.13) from other fruits with respect to colour and also identified that there is high rank for colour for Sapota at 1.5 concentration.

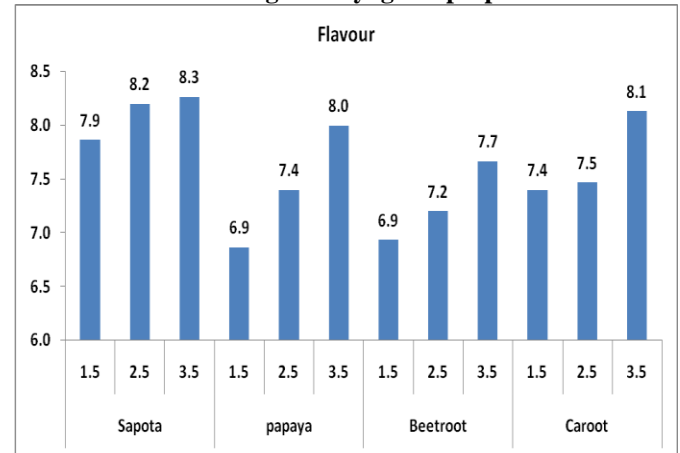
Table1: Comparison of sensory scores for appearance in fruits and vegetable yogurts prepared

Fruit	Concentration	Mean	Std. Deviation	N
Sapota	1.5	8.07	0.704	15
	2.5	8.07	0.704	15
	3.5	8.46	0.516	15
	Total	8.20	0.661	45
papaya	1.5	7.20	0.862	15
	2.5	7.47	0.516	15
	3.5	8.47	0.516	15
	Total	7.71	0.843	45
Beetroot	1.5	7.73	0.458	15
	2.5	7.33	0.488	15
	3.5	8.00	0.000	15
	Total	7.69	0.468	45
Carrot	1.5	7.73	0.458	15
	2.5	7.73	0.458	15
	3.5	8.00	0.000	15
	Total	7.82	0.387	45
Total	1.5	7.68	0.701	60
	2.5	7.65	0.606	60
	3.5	8.23	0.427	60
	Total	7.86	0.644	180

From the analysis we can observe that there is a significant difference among the chosen fruits and vegetables with concentrations at 1% level, so far as the Appearance is concerned. Further from analysis it is evident

that Sapota differs significantly at 8.20 from other fruits and vegetables and it can be identified that there is high rank for appearance for Sapota at both 1.5 and 2.5 concentrations

Figure 2: Comparison of sensory scores for flavour in fruits and vegetable yogurts prepared



From analysis it is observed that there exists a significant difference among the chosen fruits and vegetables with concentrations at 1% level so far as the flavor is concerned. Further it is evident that Sapota differs significantly 8.11 from other fruits and vegetables with respect to flavor. From summary statistics it can be clearly identified that there is high rank for flavor for Sapota at 2.5 concentrations.

Duncan's Multiple Range Test: Flavor

Fruit	N	Subset		
		1	2	3
Beetroot	45	7.27		
papaya	45	7.42	7.42	
Carrot	45		7.67	
Sapota	45			8.11
Sig.		0.299	0.103	1.000

Fruit stirred yogurt is popular among masses and particularly in children who dislike the flavour of plain yogurt. This modification has made the yogurt flavor attractive for them. Addition of fruit makes the yogurt more delicious. The product contains both the nutritive effect of yogurt and refreshing taste of fruit. Fruit stirred yogurt has more sweetness and pleasing flavor (Hursit and Temiz, 1999).

The fruit and vegetable yoghurt samples had a flavour score of 6.9 – 8.3 at various concentrations for different products respectively. The study indicated that the flavour of all the developed yoghurts were highly acceptable. It might be due to the reason that Lactobacillus bulgaricus, Streptococcus thermophilus and imparted good aroma to yoghurts. The aroma compounds that were identified in typical yoghurts were acetaldehyde, acetone, ethyl acetate, butanone, diacetyl and ethanol (Tamime & Robinson, 1999).

Balow *et al.* (1991) reported that while fermenting milk, *Lactobacillus bulgaricus* produces acetaldehyde, which is one of the main yogurt aroma components. C1 and C2 yogurt i.e., *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifido bifidum*, *Bifido longum*, and *Bifido infantis* blend of bacteria, imparted less aroma in C1 and C2 yoghurt.

The statistical analysis shows that there is significant difference among the chosen fruits and vegetables with concentrations at 1% level so far as the Body is concerned. It is evident that Sapota differs significantly 8.18 from other fruits and vegetables with respect to body. From statistics it can be clearly identified that there is high rank for body for Sapota at 2.5 concentrations, as well for papaya at 2.5 concentrations.

Table 2: Comparison of sensory scores for body in fruits and vegetable yogurts prepared

Fruit	Concentration	Mean	Std. Deviation	N
Sapota	1.5	8.00	0.535	15
	2.5	8.13	0.640	15
	3.5	8.40	0.507	15
	Total	8.18	0.576	45
papaya	1.5	7.13	0.640	15
	2.5	7.33	0.488	15
	3.5	7.47	0.640	15
	Total	7.31	0.596	45
Beetroot	1.5	7.67	0.488	15
	2.5	7.47	0.516	15
	3.5	7.73	0.458	15
	Total	7.62	0.490	45
Carrot	1.5	7.73	0.458	15
	2.5	7.73	0.458	15
	3.5	8.07	0.594	15
	Total	7.84	0.520	45
Total	1.5	7.63	0.610	60
	2.5	7.67	0.601	60
	3.5	7.92	0.645	60
	Total	7.74	0.629	180

Dependent Variable: Body

Source	Sum of Squares	df	Mean Square	F-value	p-value
Fruit	18.017	3	6.006	20.619**	0.000
Concentration	2.878	2	1.439	4.940**	0.008
Fruit * Concentration	0.900	6	0.150	0.515	0.796
Error	48.933	168	0.291		
Total	70.728	179			

Duncan's Multiple Range Test: Body

Fruit	N	Subset		
		1	2	3
Papaya	45	7.31		
Beetroot	45		7.62	
Carrot	45		7.84	
Sapota	45			8.18
Sig.		1.000	0.052	1.000

Table 3: Comparison of sensory scores for acidity in fruits and vegetable yogurts prepared

Fruit	Concentration	Mean	Std. Deviation	N
Sapota	1.5	8.00	0.756	15
	2.5	8.40	0.632	15
	3.5	8.27	0.458	15

	Total	8.22	0.636	45
Papaya	1.5	5.60	1.595	15
	2.5	6.53	1.407	15
	3.5	7.07	1.438	15
	Total	6.40	1.572	45
Beetroot	1.5	6.60	1.639	15
	2.5	6.33	1.759	15
	3.5	6.40	1.639	15
	Total	6.44	1.645	45
Carrot	1.5	6.53	1.356	15
	2.5	6.87	1.246	15
	3.5	7.33	1.496	15
	Total	6.91	1.379	45
Total	1.5	6.68	1.600	60
	2.5	7.03	1.529	60
	3.5	7.27	1.471	60
	Total	6.99	1.544	180

Dependent Variable: Acidity

Source	Sum of Squares	df	Mean Square	F-value	p-value
Fruit	97.661	3	32.554	17.865**	0.000
Concentration	10.344	2	5.172	2.838*	0.041
Fruit * Concentration	12.856	6	2.143	1.176	0.322
Error	306.133	168	1.822	-	-
Corrected Total	426.994	179	-	-	-

The statistical analysis suggests that there is significant difference among the chosen fruits and vegetables with concentrations at 1% level and concentrations at 5% level so far as the acidity is concerned.

Further it is evident that Sapota differs significantly 8.22 from other fruits and vegetables with respect to Acidity. From the statistical analysis it can be identified that there is high rank for Acidity for Sapota at 3.5 concentrations.

Table 4: Comparison of PH of sapota before and after storage

Sapota	PH	Mean	N	Std. Deviation	t-value	p-value
1.5	Before	5.14	5	0.114	6.500**	0.003
	After	4.620	5	0.0837		
2.5	Before	5.220	5	0.1304	6.736**	0.003
	After	4.560	5	0.1140		
3.5	Before	5.360	5	0.1140	16.885**	0.000
	After	4.240	5	0.0894		

* Significant @ 5% level, ** Significant @ 1% level

The pH values before and after storage on Sapota was recorded and the results concluded that there is an significant difference between pH values before and after

storage in Sapota at three levels of concentrations at 1% level of significance.

Table 4: Comparison of Acidity variable of sapota before and after storage

Sapota	Acidity	Mean	N	Std. Deviation	t-value	p-value
1.5	Before	3.140	5	0.1140	20.004**	0.000
	After	4.12	5	0.084		
2.5	Before	2.100	5	0.1581	21.664**	0.000
	After	3.860	5	0.1140		
3.5	Before	2.340	5	0.1140	29.000**	0.000
	After	2.92	5	0.084		

The Paired sample t-test has been carried out on Acidity values before and after storage on Sapota and from results it is evident that there is an increase in the Acidity

values before and after storage in Sapota at all the three levels of concentrations at 1% level of significance.

Table 5: Comparison of PH variable of papaya before and after storage

Papaya	PH	Mean	N	Std. Deviation	t-value	p-value
1.5	Before	4.36	5	0.114	2.359	0.078
	After	4.200	5	0.0707		
2.5	Before	4.380	5	0.1304	1.233	0.285
	After	3.460	5	1.7141		
3.5	Before	4.180	5	0.0837	6.324**	0.003
	After	3.980	5	0.0837		

The statistical analysis was done to find the pH values before and after storage on papaya. From the results it is evident that there is marginal decrease in the pH values

before and after storage in papaya with 3.5 concentration level, at 1% level of significance.

Table 5: Comparison of Acidity variable of papaya before and after storage

Papaya	Acidity	Mean	N	Std. Deviation	t-value	p-value
1.5	Before	2.680	5	0.0837	12.55**	0.000
	After	3.32	5	0.084		
2.5	Before	2.640	5	0.1140	6.32**	0.003
	After	3.040	5	0.1140		
3.5	Before	2.560	5	0.1140	11.07**	0.000
	After	3.70	5	0.158		

Paired sample t-test has been carried out on calculated acidity values before and after storage on papaya. From the results it is evident that there is an increase in the

acidity values before and after storage in papaya at all the three levels of concentrations at 1% level of significance.

Table 6: Comparison of PH variable of Beetroot before and after storage

Beetroot	PH	Mean	N	Std. Deviation	t-value	p-value
1.5	Before	4.28	5	0.084	2.449	0.070
	After	4.160	5	0.0548		
2.5	Before	4.300	5	0.1000	5.715**	0.005
	After	4.160	5	0.0548		
3.5	Before	4.180	5	0.0837	3.162*	0.034
	After	4.080	5	0.0837		

The PH values were compared at different concentrations has been carried out before and after storage on Beetroot. From the results it is evident that there is a

decrease in the pH values before and after storage in Beetroot especially with 2.5 concentration level at 1% significance and with 3.5 concentration level at 5% significance.

Table 6: Comparison of Acidity variable of Beetroot before and after storage

Beetroot	Acidity	Mean	N	Std. Deviation	t-value	p-value
1.5	Before	3.440	5	0.1140	6.00**	0.004
	After	4.04	5	0.114		
2.5	Before	3.040	5	0.4930	2.49*	0.047
	After	3.560	5	0.1140		
3.5	Before	2.340	5	0.1140	18.5**	0.000
	After	3.08	5	0.084		

The statistical analysis was done to check the Acidity values before and after storage on Beetroot. From the results it is evident that there is an increase in the acidity

values before and after storage in Beetroot at all the three levels with 1.5 and 3.5 concentrations at 1% significance and with 2.5 concentrations at 5% significance.

Table 6: Comparison of PH variable of Carrots before and after storage

Carrot	PH	Mean	N	Std. Deviation	t-value	p-value
1.5	Before	4.36	5	0.114	6.00**	0.004
	After	4.120	5	0.1304		
2.5	Before	4.380	5	0.0837	2.138	0.099
	After	4.300	5	0.1581		
3.5	Before	4.220	5	0.0837	1.000	0.374
	After	4.200	5	0.0707		

Paired sample t-test has been carried out on pH values before and after storage on Carrot. From the results it evident that there is an decrease in the pH values before and after storage in Carrot especially at 1.5 concentration level with 1% significance

Table 7: Comparison of Acidity variable of carrots before and after storage

Carrot	Acidity	Mean	N	Std. Deviation	t-value	p-value
1.5	Before	3.440	5	0.1140	11.15**	0.000
	After	4.26	5	0.114		
2.5	Before	2.960	5	0.1140	20.84**	0.000
	After	4.520	5	0.0837		
3.5	Before	1.920	5	0.0837	7.01**	0.002
	After	2.60	5	0.158		

The Titratable Acidity values were compared at different concentration levels before and after storage of the carrot yogurt prepared. From the results it evident that there is an increase in the acidity values before and after storage in Carrot at all the three levels. Gueimonde *et.al.* (2003) found similar results in pH and titratable acidity when they studied the quality of plain yogurt stored at 4°C for 44 d

SUMMARY AND CONCLUSIONS

Probiotic lactobacilli are gaining enormous attention because of their established health effects such as anti-diarrheal, anti-pathogenic, anti-diabetic, anti-cholesterol and anti-cancer activities, etc. Fruit juices are also extremely healthy, having a high content of antioxidants, vitamins, minerals, dietary fiber and many other beneficial nutrients, and hence could serve as a good medium for cultivating probiotics.

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