



Process Optimisation and Quality Evaluation of Passion Fruit and Pineapple based Probiotic Drink

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ABSTRACT

Background: The functions of food has extended from satisfying hunger and providing nutrients to the body, health maintenance, well being and prevention of diseases. Probiotics are such functional food and when they are incorporated to locally available foods, it helps to improve the nutritional profile and therapeutic value. Hence, the study entitled "Process optimisation and quality evaluation of passion fruit and pineapple probiotic drink" was undertaken with the objective of standardising probiotic fruit drink with passion fruit and pineapple and also to evaluate the nutritional and organoleptic qualities of these developed passion fruit and pineapple probiotic drinks.

Methods: In the present study 5 treatments along with one control with 3 replications were standardized. Passion fruit juice (PFJ) was used as the major ingredient and used in varying proportions starting from 50 to 90 per cent with 10 to 50 per cent of pineapple juice (PJ). The best treatment was selected through sensory evaluation along with control by using a score card with nine point hedonic scale. In the selected treatments optimization for the growth of *Lactobacillus acidophilus* with regard to substrate concentration, time of incubation, temperature of incubation and inoculum concentration were done. The probiotic drinks and their respective controls were then analysed for their nutrient composition like TSS, titratable acidity, energy and ascorbic acid.

Result: Among the various treatments, highest scores for organoleptic qualities was observed in treatment having 70 per cent passion fruit juice and 30 per cent pineapple juice (T_3) with a mean score of 8.52 for overall acceptability. The selected juice was optimized for the growth of *Lactobacillus acidophilus* and had a viable count of $13.38 \log \text{cfu ml}^{-1}$. The probiotic and non probiotic drinks had TSS content of 12.8 and 13.5° Brix, titratable acidity of 2.28 and 2.03 per cent, energy of 59.32 and 68.96 Kcal and ascorbic acid of 10 and 12.8 mg 100 g⁻¹ respectively.

Key words: Passion fruit, Pineapple, Probiotics, *L. acidophilus*.

INTRODUCTION

The deeply entwined relationship between food and health benefits has been a fertile field for research since the dawn of the scientific age. This in turn has triggered the development of functional food products. Probiotics are live microbial supplement, which beneficially affect the host by improving the intestinal microbial balance. Addition of probiotics to food provides several health benefits by decreasing the number of pathogenic gastrointestinal microorganisms, reducing the serum cholesterol level, improving the gastrointestinal function, strengthening immune system, protection of proteins and lipids from oxidative damage and anticarcinogenic and antimutagenic effects.

The incorporation of probiotics to underutilised fruits can improve their acceptability and market potential. The chief benefits of non-dairy beverages, specifically fruit juices, are their nutritional values, flavor and refreshing quality (Natt and Katyal, 2021). The diverse food mediums of dairy food carriers are the major limitations for the survival of the probiotics whereas the probiotic strains from non-dairy sources are acceptable. *Lactobacillus* and *Bifidobacterium* are widely used probiotics which generally regarded as safe (Sugandhi, 2018). Passion fruit (*Passiflora edulis* Sims.) contains orange coloured pulpy juice with large number of small, hard, dark brown to black pitted seeds. The juice is

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delicious with good flavour, intense aroma and sweet-acid taste and is well known for its excellent blending quality. The demand for passion fruit juice is increasing not only because of its exotic flavour but also for its high nutritional and medicinal properties (Charan *et al.*, 2017). Yellow passion fruit (*Passiflora edulis flavicarpa*), which is native to tropical America, is considered as an underutilised fruit crop and considered to be a good source of vitamins, mainly A and C and minerals. Considering these factors, passion fruit can act as a potential matrix for the incorporation of probiotics. If a probiotic product is developed from this fruit,

it would definitely attract consumer attention and improve its economic value.

MATERIALS AND METHODS

Ripe passion fruit (yellow type) and pineapple were collected from Cashew Research Station and Pineapple Research Station of Kerala Agricultural University, Thrissur, Kerala. Pure cultures of the probiotic strain *L. acidophilus* MTCC 10307 needed for the study was obtained from Institute of Microbial Technology (IMTECH), Chandigarh. Other ingredients needed for the study were purchased from the local market. The study was conducted at Kerala Agricultural University, Department of Community Science, Vellanikara, Thrissur, during 2018-2020.

Standardisation of passion fruit and pineapple drink

Passion fruit and pineapple drink was standard with following treatments (Table 1).

For the preparation of passion fruit based drink, the standard procedure of FSSAI (2010) was followed. The quantity of ingredients used for preparation of drink was taken by calculating the acidity and TSS of the sample and then adding other ingredients in accurate quantity to maintain FSSAI limits. Juices were strained and measured. Sugar syrup was prepared by heating appropriate amount of sugar in required amount of water. After cooling, measured quantity of juice was mixed with sugar syrup. It was then pasteurized at 80°C for 20 minutes.

Organoleptic evaluation

A series of acceptability trials were carried out using simple triangle test at the laboratory level and selected a panel of fifteen judges between the age group of 18-35 years as suggested by Jellinek (1985). The organoleptic evaluation of the drinks were carried out. The drinks were evaluated organoleptically by the judges using a 9 point hedonic scale. The selected drink was taken for further studies.

Optimisation of condition for growth of *Lactobacillus acidophilus* in passion fruit and pineapple drink

Optimisation is a process by which numeric function is maximized or minimized, while satisfying all the constraints on the variable. Hence, using *L. acidophilus* for fermentation, total viable count in the product was maximized while variables like substrate concentration, time and temperature of incubation and quantity of inoculum were kept at acceptable levels.

Optimisation of substrate concentration (25 ml, 50 ml and 75 ml), time of incubation (1,2 and 3 hrs), temperature of incubation (37°C, 38°C and 39°C) and inoculum concentration (3 µl, 4 µl and 5 µl of *L. acidophilus*) was done

by enumerating for the total number of viable cells of *L. acidophilus* after each section.

Optimisation of substrate concentration

From the selected best combination of passion fruit based drink, 25 ml, 50 ml and 75 ml were measured and was pasteurized at 80°C for 20 minutes and allowed to cool. The pasteurized drink was then inoculated with 4 µl of *L. acidophilus* culture. The samples were incubated at 37°C for 15 hours. After 15 hrs the samples were enumerated for the viable counts of *L. acidophilus*.

The viability of probiotic organism in fruit drinks were assessed using MRS (De Man Rogosa and Sharpe) medium. One ml of the sample was measured and transferred to a test tube containing 9 ml sterile distilled water (10^{-1} dilution). This was then serially diluted upto 10^{-9} dilutions. The microbial enumeration was done by pour plate method using MRS agar and the results are expressed as 10^9 cfu ml $^{-1}$.

Optimisation of time of incubation

The best substrate concentration with maximum number of colonies was taken and pasteurized at 80°C for 20 minutes and allowed to cool. It was then inoculated with 4 µl of *L. acidophilus* culture. The samples were then incubated at 37°C for 1, 2 and 3 hours. After this, the viability of probiotic organism was enumerated.

Optimisation of temperature

The passion fruit drink with optimum substrate concentration was selected, pasteurized and then inoculated with 4 µl of the culture and incubated at varying temperatures of 37°C, 38°C and 39°C for optimum time of growth of the organism. The fruit drinks were kept for incubation and then tested for the viability of the *L. acidophilus*.

Optimisation of population of inoculum concentration

Each fruit drink combinations with best substrate concentration was pasteurized and then inoculated with 3 µl, 4 µl and 5 µl of *L. acidophilus* and kept for incubation at optimum temperature for optimum period of time. Fruit drinks were then enumerated for the total number of viable cells of *L. acidophilus*.

Physico chemical analysis of passion fruit and pineapple drink

The developed probiotic drink along with its control (non probiotic sample) was assessed for TSS and titratable acidity according to the method of Ranganna (1986) and energy and ascorbic acid of the drinks were also determined

Table 1: Combinations of passion fruit juice and pineapple juice.

Combination	Treatments				
	T ₁	T ₂	T ₃	T ₄	T ₅
PFJ + PJ	90% + 10%	80% + 20%	70% + 30%	60% + 40%	50% + 50%

PFJ - Passion fruit juice, PJ- Pineapple juice.

according to the standard procedure of Sadasivan and Manickam (1992).

Statistical analysis

The data obtained were statistically analysed using Kendall's co efficient of concordance, DMRT and independent t test.

RESULTS AND DISCUSSION

Standardisation and organoleptic evaluation of passion fruit and pineapple drink

The passion fruit and pineapple drink were standardised with different proportions of passion fruit juice and pineapple juice. The mean scores and the mean rank scores for organoleptic qualities of passion fruit and pineapple drink are presented in Table 2.

The mean scores and mean rank scores for appearance of passion fruit and pineapple drink varied from 8.40 (T_5) to 8.93 (T_3) and 2.97 to 4.60 respectively. The mean scores for colour varied from 8.00 (T_5) to 8.71 (T_4). The mean scores for taste varied from 7.44 (T_5) to 8.53 (T_3). The highest mean rank score for taste obtained was 4.73 for treatment T_3 . The mean scores for overall acceptability varied from 7.88 (T_5) to 8.52 (T_3) with mean rank scores in the range of 2.47 to 4.90.

Among various treatments, the highest mean scores of 8.93 (appearance), 8.48 (flavour), 8.68 (texture), 8.53 (taste) and 8.52 (overall acceptability) were obtained for T_3 (70% passion fruit juice and 30% pineapple juice). Considering highest scores of organoleptic qualities passion fruit and pineapple drink (T_3 -70% PFJ + 30% PJ) was selected as the best treatment for further studies.

Optimisation of condition for growth of *Lactobacillus acidophilus* in passion fruit and pineapple drink

The selected fruit drink along with control was inoculated

with the probiotic strain *L. acidophilus* at various conditions and the optimum growth conditions were concluded from the results. Variables such as substrate concentration, time of incubation, temperature and inoculum concentration were optimised.

Table 3, represents the viable count of *L. acidophilus* with different variables at 10^9 dilution. 25 ml of the substrate concentration showed maximum growth of the probiotic organism with a colony count of 13.36 log cfu ml⁻¹ comparing to other substrates, 50 ml (13.15 log cfu ml⁻¹) and 75 ml (12.79 log cfu ml⁻¹). The number of colonies in control sample was 13.26, 12.99 and 12.38 log cfu ml⁻¹ in 25, 50 and 75 ml of juice sample respectively. One hour of incubation showed maximum growth (13.36 log cfu/ ml⁻¹) compared to 2 hours (13.14 log cfu ml⁻¹) and 3 hours (12.73 log cfu ml⁻¹) and also that of control sample with viability of 13.25, 12.89 and 12.34 cfu ml⁻¹ for 1, 2 and 3 hours respectively. The best temperature for probiotic growth was 37°C with a colony count of 13.33 log cfu ml⁻¹. Inoculation of 4 µl of culture had maximum growth (13.36 log cfu ml⁻¹) and minimum was 12.50 log cfu ml⁻¹ for 5 µl of inoculation. The number of colonies in control sample was 12.83, 13.27 and 12.44 log cfu ml⁻¹ for 3, 4 and 5 µl of inoculum respectively.

Physico chemical analysis of probiotic and non probiotic drinks

As per Table 4, TSS of the developed probiotic drinks differ significantly with their respective controls. TSS of the probiotic drink was found to be 12.80° Brix where as that of non probiotic drink was 13.50° Brix. Titratable acidity was higher in probiotic drink than non probiotic drink. The control probiotic drink contains 2.03 per cent titratable acidity, whereas the probiotic drink contains 2.28 per cent and there was significant difference in the titratable acidity of probiotic and non probiotic drinks.

Table 2: Mean scores for organoleptic qualities of passion fruit and pineapple drink.

Treatment	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_0	8.57(4.20)	8.48(4.33)	7.88(3.07)	8.04(3.43)	7.82(2.77)	8.10(3.40)	48.89
T_1	8.60(3.40)	8.17(3.07)	7.97(3.27)	8.06(3.53)	7.60(2.83)	8.08(2.63)	48.48
T_2	8.57(3.27)	8.24(3.47)	7.97(3.33)	8.11(3.48)	7.91(3.93)	8.16(3.77)	46.03
T_3	8.93(4.60)	8.02(2.37)	8.48(4.40)	8.68(4.33)	8.53(4.73)	8.52(4.9)	51.16
T_4	8.40(2.97)	8.71(4.20)	7.82(2.93)	7.95(3.27)	7.68(3.20)	8.11(2.83)	48.67
T_5	8.40(2.57)	8.00(2.57)	7.91(3.0)	7.73(2.10)	7.44(2.53)	7.88(2.47)	47.36
Kendalls W value	0.24**	0.37**	0.28**	0.35**	0.43**	0.48**	

Figures in parenthesis indicates mean rank scores.

** -Significant at 5% level.

Table 3: Viable count of passion fruit and pineapple based probiotic drink with *Lactobacillus acidophilus* (log cfu ml⁻¹).

Treatments	Substrate (ml)			Time (hr)			Temperature (°C)			Inoculum (µl)		
	25	50	75	1	2	3	37	38	39	3	4	5
Control (100% PFJ)	13.26	12.99	12.38	13.25	12.89	12.34	13.28	13.19	12.59	12.83	13.27	12.44
T_3 (70% PFJ+30% PJ)	13.36	13.15	12.79	13.36	13.14	12.73	13.33	13.22	12.93	12.86	13.38	12.50

PFJ - Passion fruit juice, PJ - Pineapple juice.

Table 4: Physico chemical analysis of probiotic and non probiotic drinks.

Parameters	Probiotic	Non- probiotic
TSS (°Brix)	12.8 ^b	13.5 ^a
Titrateable acidity (%)	2.28 ^a	2.03 ^b
Energy (Kcal/100g ⁻¹)	59.32 ^b	68.96 ^a
Ascorbic acid (mg 100g ⁻¹)	10 ^b	12.8 ^a

DMRT row wise comparison.

Values with different super script differ significantly at 5% level.

Energy content of the probiotic fruit drink were lower when compared with that of the non probiotic drink. Energy content of non probiotic drink was 68.96 Kcal 100 g⁻¹ whereas that of probiotic drink was 59.32 Kcal 100 g⁻¹. The same situation was observed in the case of ascorbic acid content also. The non probiotic drink contain significantly higher amount of ascorbic acid than the probiotic drink (Table 4).

Organoleptic evaluation of passion fruit and pineapple drink

In the present study, the mean scores and mean rank scores for appearance of passion fruit based pineapple drink varied from 8.40 (T₅) to 8.93 (T₃) and 2.97 to 4.60 respectively. The texture of passion fruit based pineapple drink obtained a mean scores from 7.73 (T₅) to 8.68 (T₃) with mean rank scores in the range of 2.10 to 4.33. The mean scores for texture was the highest in T₃ (8.68). The mean scores for colour varied from 8.00 (T₅) to 8.71 (T₄). The mean scores and mean rank scores for flavour varied from 7.82 (T₄) to 8.48 (T₃) and 2.93 to 4.40 respectively. The mean scores for taste varied from 7.44 (T₅) to 8.53 (T₃). The highest mean rank score for taste obtained was 4.73 for treatment T₃. The mean scores for overall acceptability varied from 7.88 (T₅) to 8.52 (T₃) with mean rank scores in the range of 2.47 to 4.90. This was in accordance with Seale and Sherman (1960) developed blended beverage of passion fruit with orange and pineapple which provided good texture and good flavour to the product and showed good physico chemical and sensory qualities. Shaw and Wilson (1988) prepared passion fruit orange blended nectar concluded that nectar having high proportion of passion fruit have better acceptance. Najumudeen (2015) developed blended fruit syrup of pineapple and passion fruit and reported that product were bright in colour and was highly acceptable by the consumers.

Among various treatments in the present study, the highest mean scores of 8.93 (appearance), 8.48 (flavour), 8.53 (taste), 8.68 (texture) and 8.52 (overall acceptability) were obtained for T₃ (70% passion fruit juice and 30% pineapple juice). Considering highest scores of organoleptic qualities passion fruit based pineapple drink (T₃-70% PFJ + 30% PJ) was selected as the best treatment for further studies.

Optimisation of condition for growth of *Lactobacillus acidophilus* in passion fruit and pineapple drink

Fruit juice act as a good medium for probiotic organism growth and also can maintain a minimum therapeutic level 10⁹ cfu/g or ml (WHO, 2001). According to Manasi *et al.* (2013)

the viability of *Lactobacillus acidophilus* decreased upon refrigerated storage of probiotic pineapple juice. The initial count 3.8×10⁷ cfu ml⁻¹ diminished to 1.8×10⁷ cfu ml⁻¹, however the count doesn't go below the minimum level. During storage at 30±1°C, the count expanded to 9.5×10⁸ (in 48 hrs) and afterwards declined to 2.9×10⁷ cfu ml⁻¹ after 120 hrs. A probiotic beverage with whey and pineapple juice was prepared by Shukla *et al.* (2013) at a ratio of 65:35 and revealed that a good quality probiotic drink can be developed using one per cent of inoculum of *Lactobacillus acidophilus* which can be stored for 24 days at 5±1°C and 48 hrs at 30±1°C. Adebayotayo and Akpeji (2016) developed probiotic pineapple juice, were the juice supported the viability of the organism, lactic acid production, vitamin C production and antagonistic potential of the probiotic bacteria. The lactic acid bacteria were viable throughout the storage (1.05 to 1.10×10⁹ cfu ml⁻¹) and there was no difference in taste, colour, aroma or appearance of the final product after a storage period of four weeks. Gallina *et al.* (2019) developed and characterised probiotic fermented smoothie beverage and concluded that the viable count of the probiotic organism of passion fruit and mango blend after 1, 13 and 30 days of storage was 7, 7.5 and 6.5 log cfu ml⁻¹ respectively at a temperature of 8±2°C. Nguyen *et al.* (2019) investigated that without any supplements *Lactobacillus* and *Bifidobacterium* were able to grow well in pineapple juice and acts itself as a matrix for the propagation of probiotic bacteria. Monteiro *et al.* (2020), suggested that passion fruit pulp act as a good medium for probiotic culture, when fermented at a temperature of 30°C.

According to Shukla *et al.* (2013), who developed whey based probiotic pineapple beverage, did not show much difference in sensory evaluation and also concluded that the main descriptors that characterised the probiotic product were acidity and sweetness. The mean score for overall acceptability of whey-pineapple juice blend was 8.87. Flavour and taste of the product was found to be enhanced due to probiotication. This may be due to the process of fermentation occurred in the beverage

Physico chemical analysis of passion fruit and pineapple drink

During probiotic fermentation, the organism convert glucose to lactic acid. This is responsible for the decrease in pH of the product. During fermentation, the probiotic organism produces lactic acid by hydrolyzing starch. This reduces the TSS content and starch in probiotic samples. This metabolic activity convert starch to fermentable simple sugars which is used by probiotic organisms (Adams *et al.*, 2008). Yan-li (2011) produced wine with combination of pawpaw and passion fruit and the pH was estimated as 4.0. Fernandes *et al.* (2011) concluded that upon pasteurisation of passion fruit juice, there is increase in titrable acidity (3.06 g 100 ml⁻¹) were as the homogenised juice have 2.83 g 100 ml⁻¹. The titrable acidity of wine produced from mixed juice of passion fruit, mango and pineapple was 1.4 per cent after fermentation and TSS was 20° Brix (Nzbuheraheza and Nyiramugwera, 2014).

Lactobacillus spp. is more effective in reducing pH than yeasts and other microbes (Gautam and Sharma, 2014). The reduction may be due to the utilisation of sugars for the metabolic activity of probiotic LAB in the probiotic juice samples. Similar observation was reported by Kumar *et al.* (2011), in fruit juice with *Lactobacillus casei*. There was reduction in TSS content of probiotic pineapple juice formulated by Adebayo Tayo and Akpeji (2016) from 15.28 to 12.68° Brix after storage of 4 weeks.

Stanton *et al.* (2005) reported that both genera *Lactobacillus* and *Bifidobacterium* were reported to have high requirements of free amino acids, peptides, vitamins and fermentable carbohydrates for their growth and development. *Lactobacillus* and *Bifidobacterium* strains grow well in pineapple juice meaning this matrix in itself was a suitable medium for propagation of probiotic bacteria. Pineapple juice acts as a good matrix for probiotic growth without any supplements of nutrients. Nazarudeen (2010) suggested that, increased moisture content reduces the nutritive factors such as fat and carbohydrate, thereby reducing the energy value. Shukla *et al.* (2013) reported that reduction in ascorbic acid content of probiotic drinks may be due to pasteurisation of juice and exposure to light. The ascorbic acid content in RTS drink prepared by blending juices of passion fruit and cashew apple in different ratios such as 25:75, 50:50, 25:75 + ginger drops and 50:50 + ginger drops was 80.26 mg 100 g⁻¹, 79.73 mg 100 g⁻¹, 76.39 mg 100 g⁻¹ and 79.29 mg 100 g⁻¹ respectively (Sobhana *et al.*, 2011).

CONCLUSION

It can be concluded that passion fruit and pineapple are good option for the development of probiotic drinks. This study was targeted to formulate a probiotic passion fruit and pineapple drink using *Lactobacillus acidophilus* as the probiotic bacterial culture. Good quality probiotic drink was prepared by using a 70:30 blend of passion fruit juice and pineapple juice with good acceptability, nutritional qualities with a viable count of 13.38 log cfu/ ml.

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