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DEVELOPMENT AND EVALUATION OF PASSION FRUIT-TOMATO PROBIOTIC DRINK

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ABSTRACT

The growing demand for probiotics has widened the scope for innovation and development of new probiotic products. Passion fruit is as an underutilised fruit crop and a good source of vitamins, like A and C and minerals. Hence, an attempt was made to develop a probiotic drink containing passion fruit and tomato involving *L. acidophilus*. In the study, five treatments along with one control with three replications were standardized. The most acceptable combination (70% Passion fruit + 30 % Tomato) of the drink was pasteurised at 80^{0}C for 20 minutes and allowed to cool. The pasteurised drink was then inoculated with 4ìl *L.acidophilus* and incubated for a period of one hour at 37^{0}C which had availability of 13. 39 log cfu g^{-1}. The probiotic passion fruit based drink along with its control (non-probiotic drink) had TSS content of 12.30 and 13.10 ^{0}Brix, titratable acidity of 2.68 % and 1.67 %, total sugar content of 14.28 and 15.20g 100g^{-1}, reducing sugar content of 3.08 and 4.18 g 100g^{-1}, protein content of 1.37 and 0.61 g 100g^{-1}, carbohydrate content of 13.94 and 14.74100g^{-1}, energy of 61.24 and 61.40 Kcal, ascorbic acid of 10.52mg 100g^{-1} and 13.20 mg 100g^{-1} and total ash 2.07 % and 2.05 %, respectively.

Keywords: Passion fruit, *Lactobacillus acidophilus*, Tomato, Organoleptic evaluation

The deeply entwined relationship between food and health benefits has been a fertilefield for research since the dawn of the scientific age. This in turn has triggered thedevelopment of functional food products. Probiotic food isan example for such type of food which provide various beneficial effects on human body. Probiotics are live microorganisms when administered in adequate amounts confer a health benefit on the host (WHO, 2001). Addition of probiotics to food provides several health benefits such as 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 has anticarcinogenic and antimutagenic effects (El-Deeb *et al*., 2018). The growing demand for probiotics has widened the scope for innovation and development of new probiotic products. According to Krishnakumar and Gordon (2001) the widely used probiotic strains are lactobacilli, bifidobacterium and streptococci. *Lactobacillus acidophilus* is one of the most commonprobiotic bacteria which have beneficial effects on the microbiota of the gastrointestinal tract.

Probiotic products are usually marketed as dairy products. This initiated the development of non-dairy based probiotic products. The presence of vitamins, minerals, antioxidant compounds, dietary fibres and minerals, makes fruits and vegetables idealvehicles for probiotic culture. The incorporation of probiotics to underutilised fruits like passion fruit can improve their acceptability, nutrient profile and market potential.

Yellow passion fruit (*Passiflora edulisflavicarpa*), which is native to tropical America, is considered as an underutilized fruit crop but can be a good source of vitamins, like A and Cand minerals (Kishore *et al*., 2010). Passion fruit stands out not only for its exotic and unique flavour and aroma but also for its amazing nutritional and medicinal properties. Passion fruit contains anti-inflammatory, anticonvulsant, antimicrobial, anticancer, antidiabetic, antihypertensive, antisedative, antioxidant properties and is used in treating conditions such asosteoarthritis, asthma and also act as colon cleanser (Thokchom and Mandal, 2017). Considering these factors, passion fruit can serve as a potential science for the incorporation of

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probiotics. If a probiotic product is developed from this fruit, it would definitely attract consumer attention and improve its economic value.

MATERIAL AND METHODS

For the study, ripepassion fruits (yellow variety) were collected from Cashew Research Station of Kerala Agricultural University. Tomato and all other ingredients needed for the study were procured from the local market. Pure cultures of the probiotic strain *L. acidophilus* MTCC 10307 needed for the study was obtained from Institute of Microbial Technology (IMTECH), Chandigarh.

Standardisation of passion fruit drink

Drink combinations were prepared using ripe passion fruit and tomato (Table 1). For the preparation of passion fruit based drink, the standard procedure of the 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 the 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 80^0C for 20 minutes.

Organoleptic evaluation

Organoleptic evaluation of the drinks were conducted using a score card (9 point hedonic scale) by a panel of 15 judges. A series of acceptability trials were carried out using simple triangle test at the laboratory level to select the panel of judges between the age group of 18-35 years as suggested by Jellinek(1985). Based on the organoleptic qualities the best combination of the drink was selected.

Development of passion fruit based probiotic drink

For the preparation of passion fruit based drink, different combinations of passion fruit juice (50% to 90%) and tomato juice (10% to 50%) were tried. The selected fruit drink (25ml) was pasteurised at 80^0C for 20 minutes and allowed to cool. The pasteurised drink was then inoculated with 4ìl *L. acidophilus* and incubated for a period of one hour at 37^0 C. The 5 probiotic passion fruit based drinks along with their control (non-probiotic drink) were then packed in food grade plastic bottles and stored under refrigerated condition.

Viability of *L. acidophilus* in passion fruit based probiotic drink

The viable count of *L. acidophilus* present in the passion fruit based probiotic drink was enumerated by serial dilution and plate count method as detailed by Agarwal and Hasija (1986). The microbial enumeration was completed by pour plate method using MRS agar and the results are expressed as 10^9 cfu g^{-1}.

Physicochemical qualities of the drinks

The developed probiotic drink along with its control (non-probiotic sample) was assessed for TSS, titratable acidity, reducing sugar and total sugar according to the method of Ranganna (1986). Protein, carbohydrate, energy and ascorbic acid of the drinks were determined according to the standard procedure of Sadasivan and Manickam (1992). Total ash was analysed by the procedure of AOAC (1994).

Statistical analysis

The observations were analysed statistically in completely randomised design (CRD). The scores of organoleptic evaluations were assessed by Kendall's coefficient of concordance and the differences among treatments in nutritional qualities were assessed using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Standardisation of combination of ingredients in the drink

For the preparation ofpassion fruitbased drink, different combinations of passion fruit juice (50% to 90%) and tomato juice (10% to 50%) were tried (Table 1). Blending of two or more juices enable to produce beverages of superior quality with sensory, nutritional and medicinal properties (Bhagwan and Awadhesh, 2014). The mean scoresfor the organoleptic evaluation of passion fruitbased tomato drinks (Table 2), revealed that the treatment which contained 70 percent passion fruit juice and 30 per cent tomato juice (T_3) scored maximum for the organoleptic attributes, with a mean score of 8.88, 8.02, 7.63, 8.81, 7.84 and 7.83 for appearance, colour, flavour, texture, taste and overall acceptability, respectively and thetotal score of this treatment was 49.01 (Table 2). The scores of

Table 1. Proportion of ingredients in the passion fruit drinks

Treatments	Combinations
T_0(Passion fruit) - Control	100%
T_1 (Passion fruit + Tomato)	90% + 10 %
T_2 (Passion fruit + Tomato)	80% + 20 %
T_3 (Passion fruit + Tomato)	70% + 30 %
T_4 (Passion fruit + Tomato)	60% + 40 %
T_5 (Passion fruit + Tomato)	50% + 50 %

Viability of *L. acidophilus* in passion fruit based probiotic drink

L. acidophilus present in the drinks was enumerated and given in Table 3. The viable count of *L. acidophilus* was 13.39 log cfu g^{-1} as against the desired level of 8 log cfu g^{-1} in probiotic foods.

Beverages from fruits, vegetables, cereals etc. are the new probiotic products that serveas a good medium for probiotic organism to survive and are also equally accepted among allage groups (Prado *et al*., 2008). Probiotication of fruit juice is important to provide

Table 2. Mean score and mean rank scores for the organoleptic qualities of passion fruit based drinks

Treatments	Mean score						Total Score
	Appearance	Colour	Flavour	Texture	Taste	Overall Acceptability	
T_0- Control (100% Passion fruit)	8.57 (3.93)	8.48 (4.30)	7.84 (3.07)	8.04 (4.47)	7.82 (4.47)	8.10 (4.13)	48.89
T_1- (90% Passion fruit +10% Tomato)	7.53 (3.40)	7.62 (3.07)	7.57 (3.27)	7.82 (3.37)	7.46 (2.83)	7.60 (2.63)	45.60
T_2- (80% Passion fruit + 20% Tomato)	7.68 (3.27)	7.75 (3.37)	7.68 (3.30)	8.02 (3.40)	7.84 (3.73)	7.79 (3.77)	46.76
T_3- (70% Passion fruit + 30% Tomato)	8.88 (4.60)	8.02 (4.17)	7.63 (4.43)	8.81 (4.27)	7.84 (3.93)	7.83 (3.90)	49.01
T_4- (60% Passion fruit + 40% Tomato)	7.64 (3.10)	7.68 (2.63)	7.53 (2.97)	7.64 (3.20)	7.17 (3.20)	7.53 (2.83)	45.19
T_5 - (50% Passion fruit + 50% Tomato)	7.46 (2.47)	7.34 (2.53)	7.22 (2.97)	7.26 (2.10)	6.77 (2.53)	7.21 (2.47)	43.26
Kendall's W value	0.25	0.34	0.29	0.34	0.36	0.38	

organoleptic evaluations were assessed by Kendall's coefficient of concordance and it was found that there was agreement between the judges.

Earlier, Shaw and Wilson (1988) prepared passion fruit orange blended nectar with sensory acceptance score between 5.1 and 6.8 and also concluded that nectar having high proportion of passion fruit had better acceptance. Deliza *et al*. (2005) reported that, passion fruit juice prepared in the ratio 6:9 (water:juice) and 13g of sugar in 100ml have strong fruity passion fruit aroma, sweet flavour and refreshing mouthfeel.A passion fruit nectar developed by Charan (2016) had total score of 52.1, 50.9 and 47.3, respectively for first, second and third months of storage under ambient condition.

Table 3. Viable cell count of *L. acidophilus* in the drinks

Fruit juice drink	Viable count (log cfu g^{-1})
Non-probiotic drink	Nil
Probiotic drink	13.39

health beneficial products toconsumers who are allergic to milk products. Even though fruit juices are established inmarkets, market for probiotic fruit juices are growing. Fruit juice act as a good medium for growth ofprobiotic organism (Mattila *et al*., 2002) and also to maintain minimum therapeuticlevel 10^9 cfu/g or ml (WHO, 2001).

Babu *et al*. (1992) reported that the growth of *L. acidophilus* was stimulated by addition of tomato juice to skimmed milk and resulted in higher viable counts, shorter generation time and improved sugar utilisation with more acid production and lower pH. Yoon *et al*. (2004) said that the viable cell counts of tomato juice inoculated with*Lactobacillus acidophilus* increased till third week storage and reduced on fourth week ofstorage. The count was 1.4 ± 0.1 x109 during the first week and then increased to 2.4 ± 0.1 x 10^9 during third week. They concluded that the organism rapidly utilised tomato juice for cellsynthesis and also lactic acid production. The initial cell count of *Lactobacillus acidophilus* in tomato juice sample was 2.49x10^8 and after 72hr incubation, the cell counts of *L. acidophilus* increased to 2.95 x 10^8. Reports also say that the organisms utilise tomato juice sugar and increase lactic acidproduction without any additional nutrient addition or pH adjustments (Kaur *et al*., 2016).

Physico-chemical qualities of the drinks

The physico-chemical qualities such asTSS, titrable acidity, total sugar, reducing sugar, protein, carbohydrate, energy, ascorbic acid and total ash in the probiotic and non-probiotic drinks were analysed (Table 4). There was significant reduction in the TSS content of probiotic drink (12.30° Brix) compared to non-probiotic drinks (13.10° Brix). The reduction may be due to the utilisation of sugars for the metabolic activity of the probiotic organism. This metabolic activity convert starch to fermentable simple sugars which is used by probiotic organisms (Adams *et al*., 2008).

It was observed that there was significant increase in titratable acidity of probiotic drinks (2.68) compared to non-probiotic drink (1.67). Titratable acidity increased significantly (Pd"0.05) with increasing fermentation time irrespective of the medium. Similar finding was observed by Shukla (2013), in which, whey-pineapple juice blend gave higher titratable acidity for 5 and 10 hours of fermentation. Sivudu *et al* (2014) concluded that total sugar content of watermelon and tomato probiotic drink with *L. casei* asprobiotic organism was 20.70 ± 4.99 mg/ml and the probiotic culturesutilised sugar in the juice for their growth subsequently reducing the pH of the product.

The probiotic drinks showed a significantly lower content of total sugar and reducing sugar compared to non-probiotic drink. According to Yoon *et al*. (2004), a decrease in sugar and pH and increased acidity intomato juice inoculated and incubated with *Lactobacillus delbrueckii*, *L. acidophilus*, *L.plantarum* and *L. casei*and observed the sugar gets converted into acid in the presence of bacteriaand thus get reduced with time, and the acidity content increase. Fernandes *et al*. (2011) concluded that on pasteurising passion fruit juice there was difference in total sugar and reducing sugar. The pasteurized juice had 9.63 per cent totalsugar and 8.33 per cent reducing sugar.

A higher value of proteincontent was observed in the probiotic drink (1.37 g 100 g^{-1}) than non probiotic control (0.61g 100 g^{-1}). The carbohydrate content was higher in non-probiotic juice compared to probiotic samples. Total energy content was 61.40 Kcal and 61.24 Kcal in non-probiotic and probiotic drinks, respectively. Stanton*et al*. (2005) reported that both genera *Lactobacillus* and *Bifidobacterium*were reported to have high requirements of free aminoacids, peptides, vitamins and fermentable carbohydrates for their growth and development. The reduction in energy content of probiotic drink compared to non-probiotic drink was due to higher carbohydrate and fat content in fresh juice than probiotic juice (Rafiq *et al*., 2016).

Non-probiotic passion fruit and tomato drink combination showed comparatively higher ascorbic content of (13.20 mg 100g^{-1}) than the probiotic drink (10.52 mg 100g^{-1}). 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). The study reported non-significant changes in the total ash of probiotic and non-probioticdrinks. As stated by Jood and Khetarpaul (2005), bacterial culture might increase the bioavailability of various minerals but there need not be any change in the total mineral content in probiotic foods.

CONCLUSION

It can be concluded that good quality probiotic drink can be prepared by using 70 % passion fruit juice and 30 % tomato juice with good acceptability, nutritional qualities and with a viable count of 13.39

Table 4. Physico-chemical qualities of the drinks

Treatments	TSS (° Brix)	Titrable acidity (%)	Total Sugar ($g100g^{-1}$)	Reducing sugar ($g100g^{-1}$)	Protein ($g100g^{-1}$)	Carbohydrate ($g\ 100g^{-1}$)	Energy (Kcal)	Ascorbic acid ($mg100g^{-1}$)	Total ash (%)
Non Probiotic (control)	13.10^{a}	1.67^{b}	15.20^{a}	4.18^{a}	0.61^{b}	14.74^{a}	61.40^{a}	13.20^{a}	2.05
Probiotic	12.30^{b}	2.68^{a}	14.28^{b}	3.08^{b}	1.37^{a}	13.94^{b}	61.24^{b}	10.52^{b}	2.07
CD Value (0.05)	0.0227	0.023	0.161	0.023	0.023	0.023	0.023	0.161	0.023
Significance	S	S	S	S	S	S	S	S	NS

S- Significant, NS- Non Significant

Values with different superscript differ significantly at 5%

DMRT Column wise comparison

log cfu/ ml. The probiotic passion fruit-tomato drink had a TSS content of 12.30 ^{0}Brix, 2.68 percent titratable acidity, 14.28 g 100g^{-1} ^{o}=total sugarcontent, 3.08 g 100g^{-1} reducing sugarcontent, 1.37 g 100g^{-1} proteincontent, 13.94 100g^{-1} carbohydratecontent, 61.24 Kcal energy, 10 mg 100g^{-1} ascorbic acid and a total ash 2.07 percent. Passion fruit can be a suitable substrate for the development of probiotic foods with good nutritional profile.

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