A COMPARATIVE STUDY ON FERMENTATION OF ORANGE AND TOMATO JUICE BY LACTOBACILLUS STRAINS

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Abstract

The presence study assesses the feasibility of tomato and orange as a raw substrate for the production of probiotic tomato and orange juice by lactic acid bacteria, (L. acidophilus and L. casei) to expose as a non-dairy probiotic drink. In this study changes in total soluble solids, pH, titrable acidity, total carbohydrate content, lactic acid content, total phenol content, total antioxidant capacity and cell survival properties during varying fermentation period (24 hours, 48 hours and 72 hours) were monitored at 37^0C. The tested strain grew well on the tomato juice than the orange juice. After 48 hour of fermentation L. casei was more active in both juices than L. acidophilus. The lactic acid culture reduces total soluble solids, pH, total carbohydrate and increases titrable acidity, lactic acid, total antioxidant capacity, total phenol and standard plate count.

Key words: Fruit Juices, Lactic acid bacteria, Probitication.

Introduction

With the growing interest in self-care and integrative medicine coupled with health-embracing baby boomer population, recognition of the link between diet and health has never been stronger (Granato et al. 2010). A market for functional food or food that promotes health beyond providing basic nutrition is flourishing. Within the functional food, is the small but rapidly expanding arena of Probiotic- live microbial food supplement that beneficially affect on individual by improving intestinal microbial balance (Zhu et al. 2020). Alteration in intestinal micro flora occurs with antibiotic use, aging, and disease, weakening the immune system. A healthy and vigorous intestinal micro flora, important for robust immune system and a balanced inflammatory response, often requires supplementing the gut. Probiotic, prebiotic and dietary fibers are such supplements that can promote a healthier intestine. They compromise approximately 60-70% of the world functional food market (Tripathi and Giri 2014).

The use of probiotic microorganisms to ferment food is traditional. Fermented product may be part of a daily diet, improving the health and quality of life of consumers. Most of the probiotic bacteria used in commercial products today are members of the genera Lactobacillus and Bifidobacterium (Daly and Davis 1998). Lactose intolerance and cholesterol content are the two major drawbacks related to probiotic foods based on dairy products (Betoret et al. 2012). In addition, the demand for vegetarian probiotic products, in particular in the developed countries, is arising with the increasing number of vegetarian consumers. Therefore, developing new products of non-diary origin rich with probiotics is considered to be a promising field of work (Bernat et al. 2015). Despite the existence of traditional drinks from Africa and the Far East countries made by spontaneous fermentations of cereals with native lactic acid bacteria, commercial products starting with cereals (Proviva– first non-dairy product, Skane Dairy, Sweden) to aromatic plants and fruit-based products have been already manufactured with defined probiotics (Prado et al. 2008). Published data dealing with the fermentation of juices from carrots (Sharma and Mishra 2013; Tamminen et al. 2013), beet root (Czyżowska et al. 2006), cabbage (Yoon et al. 2006), cucumber (Buruleanu et al. 2012) and their mixtures, as well as banana (Tsen et al. 2004), orange (Sheehan et al. 2007) and other tropical fruits (Saw et al. 2011), are found in literature.

In biopreservation technology, lactic acid bacteria are used to lengthen the shelf life and balance the growth and multiplication of internal pathogens in foods (Angiolillo et al. 2014). Lactic acid bacteria yield a wide variety of compounds such as lactate, hydrogen peroxide (H_2O_2) as well as antibiotics, bacteriocins that directly inhibit other microorganisms for their depletion of nutrition (Ruiz et al. 2012). Lactic acid bacteria are also joined with the formation of several foods and thus have typically recognized as safe (GRAS) status granted by the Food and Drug Administration (FDA) (Songisepp et al. 2012). Furthermore, probiotic properties of some lactic acid bacteria provide a number of beneficial effects including regulation of intestinal microbial homeostasis and modulation of local and systemic immune responses (Wells et al. 2008).

India is the second largest producer of fruits after China, with a production of 44.04 million tons of fruits from an area of 3.72 million hectares. Andhra Pradesh is the largest grower of tomato with a production of 2.05 MT. Value additions to tomato and orange through successful processing is a prime importance for a country like India. In view of the above, the present study was planned to evaluate the efficacy of L. acidophilus and L. casei as a probiotic strain to ferment the tomato juice and orange juice.

Materials and Methodology

Lactic Acid Bacteria Cultures

Lactobacilluc casei NCBC 297, Lactobacillus acidophilus NCDC 015, were procured from "National Collection of Dairy Cultures", Dairy Microbiology Division, National Dairy Research Institute, Karnal, India. Both the cultures were grown in Mann Rogasa Sharp (MRS) medium and maintained in 50% glycerol as a pure culture and stored below 0^0C till a further use.

Inoculum preparation

The inoculum were prepared by transferring 100µl of glycerol stock to 100ml of sterile MRS broth (Himedia) containing 0.1 ml of tween 80 (pH 6.5) in a 250 ml conical flask and incubated at 37^0C for 24 hours in a mechanical shaker (New Brunswick Scientific Shaker, USA) at 150 rpm. The strains were thus subcultured twice in fresh MRS broth. Finally cells were harvested in sterile centrifuge tubes by centrifugation at 9000 rpm for 15 minutes. The pellet obtained was resuspended in appropriate amount of sterile distilled water to an optical density of 1 at 600 nm. 5 ml of this prepared inoculum was transferred to 100 ml production media.

Preparation of Tomato Juice and Orange Juice

Fresh fully ripped tomatoes and oranges were obtained from the local market of V.V. Nagar, Gujarat. It was washed under tap water and air dried to remove excess water. Both juices were extracted using a mechanical juicer. The juices were filtered using a cheese cloth to remove any solid particles and heated at 88^{0}C for 2 minutes and then cooled to 25^{0}C. Thermally treated juices were transferred aseptically to sterile flasks for fermentation (Tsen et al. 2008).

Fermentation of Tomato Juice and Orange Juice

About 100 ml of each tomato and orange juices were taken in sterile conical flask. 0.1ml (approximately 1×10^6 cells) of L. acidophilus and L. casei were separately inoculated in to tomato juice and orange juice and incubated at 37^{0}C for three different periods 24hrs, 48hrs and 72hrs. Each sample was kept for incubation in duplicate. Samples were taken during the fermentation process for chemical and microbiological analysis.

Characterization of Fresh and Fermented Tomato and Orange Juice

The total soluble solid content expressed as brix of tomato juice and orange juice was determined using a refractometer (ERMA, INC, Tokyo, Japan). The pH was directly measured using a pH meter (Eutech Instrument, USA). The total titratable acidity (TTA) was determined by the standard AOAC method, and expressed as % lactic acid (Horwitz W. 2000). Lactic acid was estimated by simple colorimetric assay (Miller and Muntz 1938). Total Carbohydrate content was estimated by the phenol-sulfuric acid method (Nielsen S.S. 2010). The numeration of the LAB cells was made by the standard plate count method with MRS agar at 37^{0}C for 48 hrs after a series of proper dilutions and expressed as colony-forming units per milliliter of sample (cfu/mL).

Total Phenolic content

Total phenolic content was determined using Folin–Ciocalteu method (Singleton et al. 1999). Gallic acid was used as a standard and the results are expressed in milligrams of gallic acid equivalent per 100g sample (mg GAE/100g). All the sample of juices was centrifuged for 15 minutes at 5000rpm. Supernatant received was diluted.

Determination of total antioxidant capacity (TAC)

Ferric reducing antioxidant power the ferric reducing antioxidant power (FRAP) assay was carried out by the method of Benzie and Strain (1996). Gallic acid standard was used for the comparison and results are expressed in 44terms of mg GA Equivalent per 100g sample (mg GAE/100g).

Sensory Evaluations

Using a semi trained panel of six judges, sensory evaluation was carried out. A score card was prepared where the product was scored for color, taste and smell for a maximum score of 10 and a minimum score 1 for each characteristic. This was repeated twice for each product. On completion an average of color, taste and smell calculated out of 10 was considered the score for overall acceptability for each product.

Statistical Analysis

All experiments were carried out at least in triplicates and reported as mean±standard deviation of mean using SPSS version 20. The data were statistically analyzed by Duncan's multiple range tests at $p<0.05$ significant levels.

Results and Discussion

Total soluble solids

Total soluble solids is an expression for the combined content of all inorganic and organic substances contained in a liquid which are present in a molecular, ionized or micro-granular (colloidal sol) suspended form. Total soluble solids are differentiated from total suspended solids, in that the latter cannot pass through a sieve of two micrometers and yet are indefinitely suspended in solution. The mean values of total soluble solid (^{0}Brix) of tomato and orange juice are presented in Table 1 shows total soluble solid (^{0}Brix) during tomato and orange juice fermentation by L. acidophilus and L. casei.

The tomato juice containing an initial total soluble solid was 0.65. After 48 hours, the total soluble solid of tomato juice fermented by L. acidophilus at 37^{0}C was significantly ($p<0.05$) reduced to 0.0 ^{0}Brix. The total soluble solid of orange juice was 6.0. The study carried out by Cavalcanti et al. (2008) had reported the total soluble solid was 9.50 % in orange juice. After 24 hour of fermentation of orange juice with L. acidophilus and L. casei, the total soluble solid was reduced significantly ($p<0.05$) by 16.66%. The reduction in total soluble solid might be due to utilization of soluble sugar by lactobacilli bacteria as a carbon source which might be converted to lactic acid.

Table 1: Total soluble solid (^{0} Brix) of Tomato and Orange juice fermented by L.acidophilus and L.casei for varying period of time.

Fermentation period (hrs.)	Tomato juice		Orange juice	
	L.acidophilus	L.casei	L.acidophilus	L.casei
0	0.65 ± 0.07^c	0.65 ± 0.07^b	6.0 ± 0.0^c	6.0 ± 0.0^c
24	0.15 ± 0.07^b	0.10 ± 0.0^a	$5.5 \pm 0.00\ 7^b$	5.5 ± 0.007^b
48	0.0^a	0.0^a	5.0 ± 0.01^a	5.0 ± 0.01^a
72	0.0^a	0.0^a	5.0 ± 0.0^a	5.0 ± 0.007^a
F-value	76.00*	155.66*	6014.06*	6022.66*

- Values are mean ± S.D of two observations.
- Different letters in the same column indicate significant difference ($P \leq 0.05$).
- * indicates significant difference at $P \leq 0.05$. a

Acidity and pH

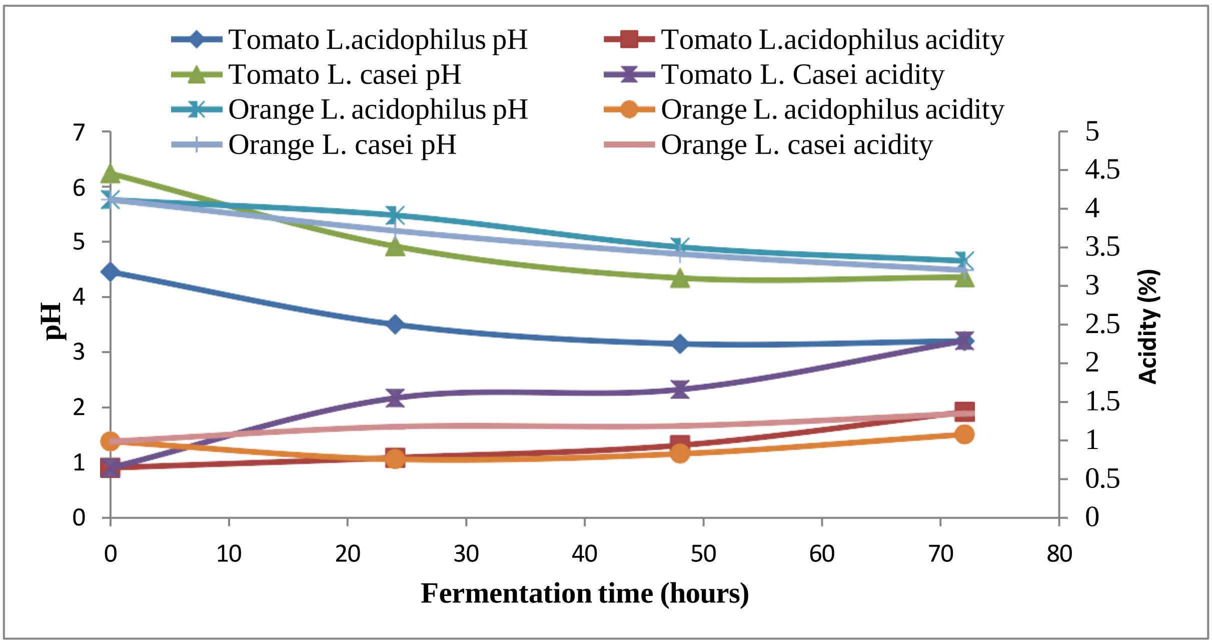

Figure 1: Changes in pH and titratable acidity in Tomato and Orange juice during fermentation.

Figure 1 displays the pH and acidity of fruit juices during fermentation. Tomato juice had an initial pH value of 4.45. After 72 hours of fermentation, the pH was reduced but the changes were non-significant. In the present study, L. casei showed higher reduction in pH compared to L. acidophilus in tomato juice. The initial pH of orange juice was 4.11. The study carried out by Cavalcanti et al. (2008) had reported the pH in orange juice was 4.1. Fermentation of orange juice by L. acidophilus and L. casei showed a significant ($p<0.05$) reduction in pH after 72 hours. Higher reduction (22.14%) was observed in orange juice fermented with L. casei. The reduction in pH of tomato and orange juice during fermentation could be due to the production of organic acid particularly lactic acid and other organic acids, diacetyl, acetylaldehyde and acetoin etc. Similar results are reported by Hossain et al. (2016). The titrable acidity was increased significantly ($p<0.05$) along with fermentation time in both juices. The increased % titrable acidity could be a result of the production lactic acid by bacteria which may have converted sugars to lactic acid.

Total Carbohydrate:

The mean values of total carbohydrate in mg% of tomato and orange juice are presented in figure 2. The total carbohydrate of tomato and orange juice was 884.35 mg% and 1849.50 mg% respectively. The total sugar was decreased significantly ($p<0.05$) along with fermentation time in tomato juice. The total sugar was decreased higher (-56.24 mg %) in tomato juice fermented with L. casei compared to L. acidophilus for during fermentation time. The total carbohydrate was also decreased after 72 hour fermentation in orange juice. As like tomato juice, L. casei showed maximum (-65.6 mg %) decreased in total carbohydrate.

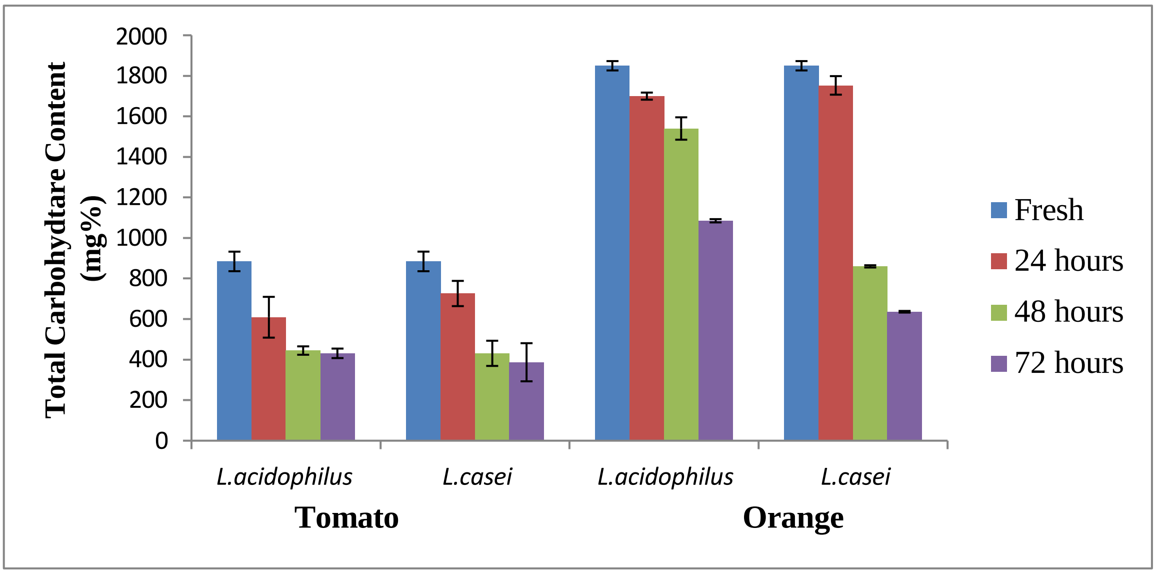

Figure 2: Total Carbohydrate (mg%) of Tomato and orange juice fermented by L.acidophilus and L.casei for varying period of time.

Lactic acid:

Lactic acid is derived by using bacteria such as Streptococcus thermophiles, Lactobacillus acidophilus, or Lactobacillus delbrueckii subsp. Bulgaricus to ferment carbohydrates from nondairy sources which are associated with the pH. The values of lactic acid in mg% of tomato and orange juice are presented in Table 2. After fermentation of tomato and orange juice by both the species (L. acidophilus and L. casei) the lactic acid was significantly ($p<0.05$) increased till 48 hours and there after it was reduced significantly ($p<0.05$).

Total phenol

The mean values of total phenolic content of tomato and orange juice are presented in Table 3. Results show the % change in total phenol during tomato and orange juice fermentation by L. acidophilus and L. casei. The total phenol content of tomato and orange juice was 15.51 mgGAE/100gm 42.27 mgGAE/100gm respectively. Rapisarda et al. (1999), reported 36.10 to 109.0 mg% total phenol in different brand of orange juice. These results are approximately similar with the result of the present study. Ersus and Cam (2007) reported 56.9 mg% of total phenolic content in orange juice. The tomato juice fermented with L. acidophilus and L. casei did not show any major change in total phenol content. In orange juice, the total phenol content was increased significantly ($p<0.05$) after 24 hour of fermentation and was further increased up to 48 hour of fermentation.

Table 2: Lactic acid (mg %) of Tomato and Orange juice fermented by L.acidophilus and L.casei for varying period of time.

Fermentation period (hrs.)	Tomato Juice		Orange Juice	
	L.acidophilus	L.casei	L.acidophilus	L.casei
0	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
24	322.23 ± 64.84^{b}	207.05 ± 62.89^{b}	321.45 ± 6.07^{c}	708.99 ± 18.94^{c}
48	505.36 ± 174.61^{b}	411.0 ± 8.62^{c}	474.23 ± 6.06^{d}	781.28 ± 20.38^{d}
72	240.79 ± 17.78^{a}	197.85 ± 43.81^{b}	163.42 ± 9.0^{b}	120.67 ± 11.0^{b}

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F-value	10.042	37.877*	2151.92*	1425.46*

- Values are mean ± S.D of two observations.
- Different letters in the same column indicate a significant difference ($P \leq 0.05$).
- * indicates significant difference at $P \leq 0.05$.

Table 3: Total phenol (mg %) of Tomato and orange juice fermented by L.acidophilus and L.casei for varying period of time

Fermentation period (hrs.)	Tomato juice		Orange juice	
	L.acidophilus	L.casei	L.acidophilus	L.casei
0	15.51+1.47^{a}	15.51+1.47^{a}	42.27+1.01 ^{b}	42.27+1.01 ^{a}
24	16.55+0.77^{a}	15.50+1.63 ^{a}	45.97+0.10 ^{c}	57.62+0.68 ^{b}
48	18.83+1.58^{a}	16.08+0.72 ^{a}	58.63+0.82 ^{d}	65.85+0.09 ^{c}
72	16.51+1.64^{a}	13.82+1.07 ^{a}	32.06+0.38 ^{a}	44.47+2.41 ^{a}
F-value	1.984	1.171	514.52*	135.27*

- Values are mean + S.D of two observations.
- Different letters in the same column indicate a significant difference ($P< 0.05$).
- * indicates significant difference at $P< 0.05$.

Total antioxidant capacity

There are different methods for the measurement of total antioxidant capacity (TAC) that involve the generation of radical species, where the presence of antioxidants determines the disappearance of radicals. FRAP assay is the only assay that directly measures antioxidants or reductance in a sample. The other methods are more indirect because they measure the inhibition of reactive species. The mean values of total antioxidant capacity of tomato and orange juice fermented by L. acidophilus and L. casei. are presented in the Figure 3.

The tomato juice had TAC value of 8.67mg equivalents of gallic acid for 100 ml juice. After 72 hours, it was reduced significantly ($p<0.05$) by 69.31% and 140.59% by L. acidophilus and L.casei respectively. The initial TAC value of orange juice was 36.0 mg equivalents to gallic acid per 100 ml of juice. Orange juice is an important source of carotenoids and ascorbic acid, a nutrient that besides its vitamin action is valuable for its antioxidant effect. Mahattanatawee et al. (2006) reported second higher TAC for orange juice (6.04 mM/liter Fe^{+2}), after pear juice. They also reported that orange juice had the highest ascorbic acid content (600.1±300.5 mg/kg) and second highest total carotenoid content (4.4±1.9 mg/liter), but had the lowest dry matter (111.1±3.2 g/kg) and total phenol (201.2±34.5 mg/liter) contents. Fermentation of orange juice by L. acidophilus and L. casei showed significant ($p<0.05$) reduction in TAC after 24 and 72 hours. After fermentation increased TAC in tomato juice and orange juice may be due to changes in the structure and polarity of the antioxidants of the fermentation media (Sun et al. 2009).

Standard plate count

The values of colony forming unit of tomato and orange juice are presented in Table 4 shows the change in lactic acid during tomato and orange juice fermentation by L. acidophilus and L. casei. The viable count of L.casei was increased significantly ($p<0.05$)

higher in both juices compare to L. acidophilus. The number of viable cells of lactic acid bacteria and bifidobacter reached almost 10^9 CFU/ml in the first 48 h in the noni juice, but the population tended to decreased to 10^3 CFU/ml during 3 weeks of storage at 48^0C (Wang et al. 2009). Yoon et al. (2004) had reported that the viable cell counts (CFU) reached nearly 1.0 to 9.0×10^9/ml after 72 h fermentation. The viable cell counts of the four lactic acid bacteria in the fermented tomato juice ranged from 10^6 to 10^8 CFU/ml after 4 weeks of cold storage at 4^oC.

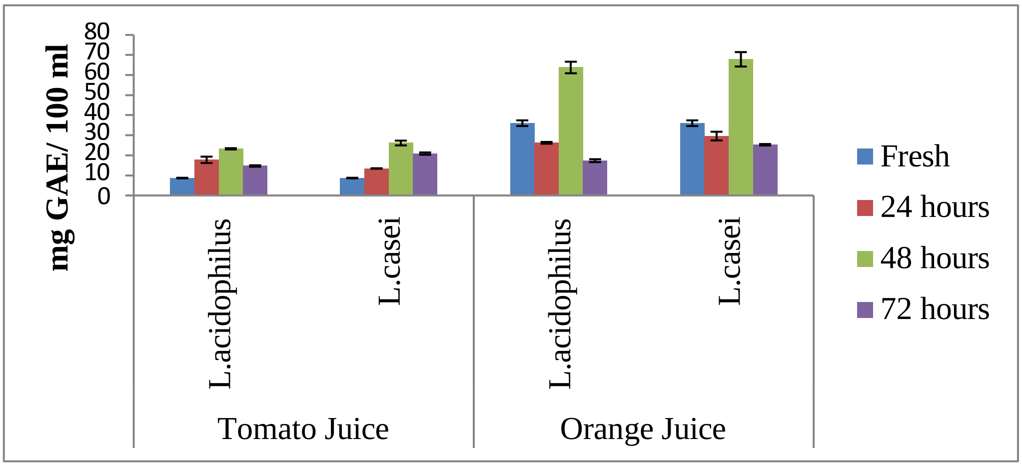

Figure 3: Total antioxidant capacity (mg equivalent to gallic acid/100 ml) of Tomato and orange juice fermented by L. acidophilus and L. casei for varying period of time.

Table 4: Standard plate count of tomato and orange juice fermented by L.acidophilus and L.casei for varying period of time.

Fermentation period (hrs.)	Tomato juice		Orange juice	
	L.acidophilus	L.casei	L.acidophilus	L.casei
0	0.0^a	0.0^a	0.0^a	0.0^a
24	21.30 ± 0.7^a	0.20 ± 0.0^a	0.23 ± 0.009^a	0.23 ± 0.01^a
48	1491 ± 57.98^b	1673.5 ± 222.7^b	10.97 ± 0.48^b	70.70 ± 0.98^b
72	1805 ± 247.48^b	2094.5 ± 106.7^c	15.96 ± 1.17^c	378.78 ± 29.05^c
F-value	112.69*	158.99*	314.74*	308.93*

- Values are mean $\pm$ S.D of two observations.
- Different letters in the same column indicate a significant difference ($P\leq 0.05$).
- * indicates significant difference at $P\leq 0.05$.

Sensory evaluation

The tomato and orange juice were fermented within L. acidophilus and L.casei for varying period of time. The sensory evaluation of fresh as well as fermented tomato and orange juice was carried out by 6 semitrained panel members using composite test. Figure 4 shows the sensory analysis of juices that had been fermented by L. acidophilus and L. casei. According to the sensory results, Fermentation of tomato and orange juice by L. acidophilus and L. casei for varying period of time did not show any significant change on the color. At the end of 72 hour fermentation the color score is remained almost same. Orange juice fermented with L. acidophilus and L.casei for varying period of time showed the significant

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effect on the flavor. The score for flavor was reduced at the end of 72 hour fermentation. The higher reduction was observed in orange juice fermented with L. acidophilus than the L.casei. After fermentation of 48 hours taste of tomato juice was affected. In conclusion all sensory attributes score was better for orange juice than the tomato juice even after fermentation 72 hour.

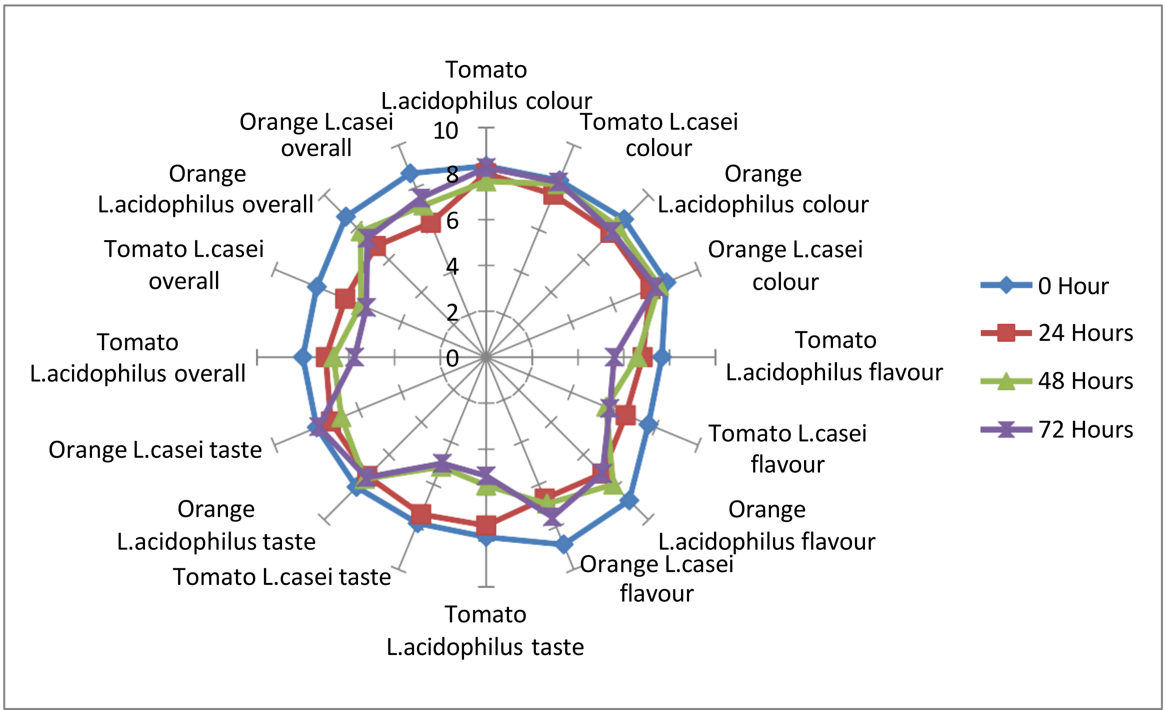

Figure 4: Sensory evaluation of tomato and orange juice fermented by L.acidophilus and L.casei.

Conclusion

Despite the great potential of the use of fruit juice as probiotic carriers, few works have been done in this field considering the fermentation of the juice. Most of reported studies are based on microbial addition of probiotic strains to fruit juices. Maximum reduction in total soluble solids was high in tomato juice after fermentation. The titrable acidity, lactic acid, total phenol and total antioxidant capacity was increased. These results prove the ability of lactic acid bacteria (L. acidophilus and L.casei) to utilize tomato and orange juice in cell synthesis and lactic acid production without external nutrient supplement. From the results obtained on physicochemical, nutraceuticals and microbial analysis it is concluded that both tomato and orange juice could be the substrate for probiotic cultures. From the results of sensory score it was revealed that after fermentation the tomato and orange juice could be improved in their sensory score by addition of sugar and spices to make well acceptable for the consumers. Juices can represent a suitable carrier for probiotics, as they can combine the appearance of healthy and fresh foods, designed for a wide range of consumers, and the healthy benefits from probiotics. There will ultimately increase the use of tomato and orange for processing and adds the value and reduce the wastage.

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