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Original Article

Utilization of Peach Juice as Substrate for *Lactobacillus casei* to Develop Probiotic Beverage

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ABSTRACT

The probiotic *L. casei* is proved to be very effective against pathogenic microorganisms. Peach fruit is a packed with bundle of nutrients which can be utilized for medicinal purpose i.e. anti-cancerous, anti-diabetic, anti-inflammation, improve vision and to treat cardiovascular diseases. **Objective:** To develop peach based probiotic beverage. **Methods:** Peach pulp were fermented at different temperature using probiotic *Lactobacillus casei* to develop peach based probiotic beverage. The developed beverage was then examined for physicochemical, microbial and sensory characteristics. The obtained data was subjected to the statistical analysis. **Results:** The pH, sugar acid ratio, total soluble solids and total sugars decreased significantly throughout the storage period whereas acidity, total plate count and total probiotic count was increased significantly. Total plate count and probiotic count ranged from 5.27 to 9.83 CFU/mL and 8.29 to 12.68 CFU/mL, respectively. As the sensory properties of developed beverage are concerned; color, taste, flavor, odor and overall acceptability decreased significantly throughout storage period. The T_2 was assigned maximum scores by the panelists for the sensory characteristics. **Conclusions:** It was concluded that peach based probiotic beverage can be developed by using isolated *Lactobacillus casei.*

INTRODUCTION

Probiotics are the live microorganisms which confer positive health effects to the host when taken in appropriate amount. The most frequently used and advantageous probiotics are *Lactobacillus* spices which are known to be safe for the consumers [1]. The addition concentrations of probiotics to the food material should be higher than 10^6 CFU/mL in order to achieve beneficial effect on the host. The most generally used probiotic strains are belonging to genus *Lactobacillus* which includes *L. crispatus, L. casei, L. gallinarum, L. reuteri, L. johnsoni, L. rhamnosus, L. delbruecki* subsp. *bulgaricus, L. paracasei subsp. tolerans, L. paracasei subsp. paracasei, L. sali-varius subsp. salivarius, L. plantarum, L. helveticu, L. gasseri, L. fermentum, L. amylovarus* [2]. Some of the species of *Lactobacillus* have been isolated by natural lactic acid fermentation from vegetables like *L. dellbrueckii, L. paracasei, L. brevis, L. plantarum, L. casei* [3]. The probiotic *Lactobacillus casei* has been significantly utilized in many food products for fermentation because of its several technological properties. *Lactobacillus casei* has numerous qualities due to which it is favored as a probiotic in many different foods and beverages such as cholesterol elimination [4], active against proliferation of cancer cell [5], antimicrobial mode of action against different pathogenic microorganisms and beneficial for human gut [6]. Moreover, *L. casei* exhibit more resistance to higher temperatures as compared to any other *lactobacillus* species [7]. Peach fruit basically belongs to family *Rosaceae* and its botanical name is *Prunus persica* L. Peach was originated from China and was grown about 2000 B.C.

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Later, it was moved to Persia and then Greece about 350 B.C. In Pakistan, among stone fruits it is the second important fruit and Peach is most popular fruit cultivated in K.P. The best places for peach cultivation include Peshawar, Hazara and Malakand divisions are supposed for best growing areas of plum, peer, peach and apricot. Indian blood, Maria desiza, Early grand, Florida King 6-A are popular varieties of peach. In Pakistan, Irrigation systems, seasons and fertile soils are the best natural resources. Agriculture contributes about 21% in GDP and 43.4% work force employs. All over the Pakistan the maximum area is under fruits and vegetables cultivation. In 2005-2006 the total area under fruits cultivation was 0.80 million hectares. In Pakistan, Fruits are cultivated on 8570601 hectares with the production of 70515121 tones. In which the total area is under Peaches cultivation is 15774 hectares with total production of 83670 tones and in Baluchistan fruits cultivation land occupy 254695 hectares and producing 1175737 tones fruits. While in K.P.K, fruits are cultivated on 47364 hectares and its production is about 522412 tones, in which the total area under peach cultivation is 6191 hectares with production of 57834 tones. In Sindh the fruits cultivated on 154865 hectares and its fruit production is 1015416 tones [8].

METHODS

The present investigation was performed at National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan. Yogurt samples were gathered from local market of Faisalabad. In pre-sterilized glass bottles, samples were brought to laboratory of NIFSAT, UAF. Cereal grains were milled by adopting the protocols of (CC, 2000) method No. 26 – 95 by JIQI high speed multifunction grinder (Model zal118dr; China). Completely randomized design (CRD) under factorial arrangement was used (n =4). All glassware (bottle, test tubes, petri-plates, glass flask etc.) used in current research were washed using detergent, soaked in distilled water and dried by air. Sterilization was done by placing glassware in hot air oven for 1 hour at 160 °C following the technique as explained by Wang *et al.*, [9]. Culture media used for the growth of bacteria like De Man Rogosa Sharpe (MRS) agar, Nutrient agar and MRS broth were prepared according to Wang *et al*. *Lactobacillus casei* was isolated from yogurt. Serial dilution was done using test tubes. One gram (yogurt) sample and peptone water (9mL) were added in first test tube and mixing was done by inverting test tubes (three times), It was tagged as 10^{-1} (1 in 10) dilution. Similarly, 1 mL solution from first test tube was poured into 2^{nd} test tube which already had peptone water (9 mL) to get 10^{-2} dilution. New pipette (disposable) was used for each dilution. By repeating the above process, all further serial dilution i-e, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} were also prepared. In each petri plate, the MRS agar was added and was allowed to solidify. 1 mL of serially diluted sample was transferred on MRS agar and circular movement was done to spread the sample on medium. The incubation was done for 48 hours at 37°C after adsorption of material on agar pallets. The streaking of presumed colonies was done on MRS agar to get pure culture. The obtained form of *Lactobacillus casei* was stored at refrigeration temperature in 25% v/v glycerol solution [9]. The bacterial isolates inoculated in MRS broth having pH (3, 5, 6.5, 7 and 9) incubated for 48 hours at 37°C. Media was examined for growth and outcomes were recorded. Growth of bacterial isolates was seen at different temperature. MRS broth was set up to for pure culture inoculation, incubated for 48 hours at different temperature i.e 10°C, 15°C, 37°C and 45°C. The results were recorded [10]. The tolerance of isolated bacteria was observed against different NaCl concentrations (2%, 4%, 6%, 8% and 10%). The MRS broth was prepared and transferred into tubes with different concentration of NaCl and incubated at 37°C for 3 days [11]. Only single bacterial colony was put on a slide by streaking technique and onedrop of 3% H_2O_2 was included to it. No bubbliness of oxygen showed the reaction of the microscopic organisms to catalase test [12]. This test evaluated *Lactobacillus casei* characters for fermentation of sugar. Some colonies were taken from MRS agar and then biochemically examined for raffinose, fructose, trihalose, lactose, ribose, glucose and maltose fermentation by following the technique as explained by Mahmoudi *et al.*, [11]. Stock solution of each sugar having concentration of 10% was prepared and along with new peptone water autoclaved at 121 °C, 15 lbs for 15 mins. The methyl red indicator was also added. Medium (peptone water; was apportioned 15 ml with Durham tubes in inverted style) in the test tubes with 0.1 ml of that sugar solution. Sterilized loop full unadulterated culture added in the medium and then incubated at 37 °C for 48 hrs. Color change in media and gas generation in Durhum tubes were observed. The screening for tolerance of bile salt was determined for *Lactobacillus* in MRS broth containing0.3% bile salt for 24 hours at 37 °C (under aerobic conditions). Culture stocks with turbidity more than 0.5 units at 500nm were labeled as bile tolerant strain of bacteria. These bacterial isolates were taken for exposure to stocks for having high concentrations of 0.5, 1.0 and 2% (w/v) of bile salt. Every strain's survival rate was then denoted as percent of viable cells in the presence of bile salt as compared to the value taken without bile salt [13]. The Peaches were washed using water to remove the contamination (Dust and other adhering residues) from fruit peel. The deteriorated parts of fruit will be removed using sterilized knife and cutting into 5-6 cm slices. The

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slices of peach, sufficient water and *Lactobacillus casei* (9.18 Log CFU/mL) were mixed to get homogenous mixture then shifted to a steel tank (stainless) or glass bottle. Six samples (peach based probiotic beverage) were made and then placed for fermentation process at specific temperatures (15, 20, 25, 30 and 35°C) for 3 to 10 days. Also, a controlled sample will be run which does not contain *Lactobacillus casei*. The quality evaluation of all samples (peach based probiotic beverage) will be done at 0, 7, 14 and 21 days [14]. pH can be defined as negative log of H (hydrogen) ion conc. pH of prepared probiotic juice samples was examined by using pH meter by following the technique as demonstrated by AOAC (2006). Buffer solutions were used to standardize the pH meter. An adequate amount of prepared juice sample was taken in 100 ml beaker and pH was determined by immersing the electrode of pH meter in beaker and reading was recorded. TSS of each sample was determined using refractometer by following the method number 983.17 as described by AOAC (2006). First of all, calibration of instrument was done by using distilled water. A drop of prepared sample (peach based probiotic beverage) was positioned on clean prism of the refractometer; reading was recorded and expressed as °Brix. Titratable acidity of prepared sample was determined by following method described by AOAC (2006). 10 mL beverage sample was poured in flask and with 50 mL distilled water dilution was made, then titration was done against 0.1 N NaOH utilizing 2-3 drops of phenolphthalein indicator till by utilizing 0.1 N NaOH in pH dependent titration process. Total sugars of samples determined by using guidelines of AOAC (2006). 10 ml of prepared sample was taken in 200 ml beaker and equal volume of potassium was added in it and then 25 ml of 25% lead acetate and 100 ml of distilled water was also added. The ratio of sugar and acid of prepared juice sample was determined by applying the formula as described in AOAC (2006) [15]. Ratio of sugar and acid = (Total sugar in probiotic peach beverage %) / (Titratable acidity of probiotic peach beverage %). To determine the total probiotic count, 10 ml of prepared juice sample was added in 90 ml of 0.1% peptone water. The 6-fold serial dilution was developed in 0.1% peptone water for analysis. *Lactobacillus casei* was examined on MRS agar that was incubated for 72 h at 37 °C by following the technique as demonstrated by de Lima *et al.*, [16]. Total bacterial count was determined using plate method. 1 mL of prepared juice sample was added aseptically in petri plates. Then viable bacteria's colonies were calculated and outcomes were presented as CFU/mL of beverage. The prepared juice sample was evaluated on the basis of 9 hedonic scale by special panel of judges at NIFSAT, UAF. The Juice samples were assessed for sensory characteristics including taste, odor, color, flavor and overall acceptance as demonstrated by Bhat *et al.*, [17]. The Obtained data from various parameters was subjected to 2 factorial LSD design statistical analysis by using two factors factorial test was done to determine the level of significance [18].

RESULTS

The probiotic *Lactobacillus casei* were found to have capability to grow and properly ferment the peach beverage. The probiotic *Lactobacillus casei* showed good growth on each beverage and reached $1.12*10^{9}$ CFU/ml at 30 Oc after 48 hours. The variation in pH and acidity of peach based probiotic beverage have been presented in Table 1. The initial value of pH and titratable acidity of peach beverage was detected 4.41 and 0.26. The pH values of fermented beverage are given in Table 3.1. At 0 day, the value of pH in T_0, T_1, T_2, T_3, T_4 and T_5 was observed to be 4.41, 4.22, 4.14, 3.82, 3.71 and 3.65 respectively. At 21 days of storage the values of pH were 3.82, 3.75, 3.60, 3.51, 3.43 and 3.37 for treatments T_0, T_1, T_2, T_3, T_4 and T_5 respectively. In case of titratable acidity, fermented beverage values are given in Table 3.2 ranges between 0.26-0.42 followed by T_0, T_1, T2, T_3, T4, T_5 and 0, 7, 14 and 21 days of storage respectively. Role of lactic acid bacteria increasing the acid content of probiotic beverage, in reducing the pH up to 4.41–3.37 as providing a favorable environment and fermentation progressed for the noticeable growth of yeast. The results of TSS are mentioned in Table 1. There was decreasing trend observed in TSS from 0 to 21 days of storage period. The maximum TSS value (10.30) was observed in T_0 while the minimum value was found in T_5 which was 6.30. The control treatments showed TSS range of 6.30-10.30 while the T_5 showed 7.57, 7.37, 7.16 and 6.30 for days 0, 7, 14 and 21 respectively. The results of total sugars are mentioned in Table 1. There was decreasing trend observed in total sugars from 0 to 21 days of storage period. The maximum total sugars value (19.20) was observed in T_0 while the minimum value was found in T_5 which was 18.60. The control treatments showed total sugars range of 19.20-18.60 while the T_5 showed 18.80, 18,70, 18.60 and 18.60 for days 0, 7, 14 and 21 respectively.

Table 1: Effect of different treatments and storage days on pH, acidity, TSS and total sugars of probiotic beverage

Treatments	Storage (Days)	pH	Titratable acidity	TSS (°Brix)	Total sugars (%)
To	0	4.41	0.26	10.30	19.20
	7	4.17	0.27	10.19	19.20
	14	4.05	0.29	9.50	19.10
	21	3.82	0.30	8.60	18.90
T1	0	4.22	0.26	9.90	19.20
	7	4.09	0.27	9.62	19.20
	14	3.87	0.30	9.61	19.10
	21	3.75	0.30	8.21	18.90

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T2	0	4.14	0.30	9.60	19.10
	7	3.95	0.31	9.28	19.10
	14	3.75	0.31	8.50	18.90
	21	3.60	0.32	7.41	18.70
T3	0	3.82	0.32	9.11	18.90
	7	3.85	0.33	8.31	18.80
	14	3.61	0.33	8.21	18.80
	21	3.51	0.34	7.16	18.70
T4	0	3.71	0.35	8.62	18.90
	7	3.71	0.36	8.31	18.80
	14	3.52	0.37	7.82	18.70
	21	3.42	0.39	6.95	18.60
T5	0	3.65	0.37	7.57	18.80
	7	3.40	0.39	7.37	18.70
	14	3.44	0.40	7.16	18.60
	21	3.37	0.42	6.30	18.60

The results pertaining to the microbial analysis of probiotic beverage are presented in Table 2. The results showed that total plate count were low in controlled sample (T_0) at day 0 as compared to all other treatment. At 0 day of storage period, the value of total plat count was 5.27, 5.68, 6.00, 6.50, 7.00 and 8.00 log CFU/g for all treatments such as, T_0, T_1, T_2, T_3, T_4 and T_5 respectively (Table 2).

Table 2: Effect of different treatments and storage days on TPC (log CFU/g) of probiotic beverage

Treatments	0 Days	7 Days	14 Days	21 Days
T0	5.27	5.38	6.88	5.92
T1	5.68	5.18	7.41	8.00
T2	6.00	5.53	8.00	8.41
T3	6.50	7.57	8.72	8.62
T4	7.00	7.58	9.10	9.20
T5	8.00	8.66	9.40	9.83

On the other side, total probiotic count was seeming to be 8.29, 8.70, 9.00, 9.50, 10.05 and 11.04 log CFU/g at day 0. In both total plat count and total probiotic count, there was significant ($p < 0.05$) differences among all treatment. After 21 days of storage span, the total plat count and total probiotic count were 5.92, 8.00, 8.41, 8.62, 9.20, 9.83 log CFU/g and 9.90, 10.43, 11.00, 11.74, 12.37 and 12.68 respectively (Table 3).

Table 3: Effect of different treatments and storage days on TPC (log CFU/g) of probiotic beverage

Treatments	0 Days	7 Days	14 Days	21 Days
T0	8.29	8.40	9.06	9.90
T1	8.70	9.20	9.11	10.43
T2	9.00	9.54	10.59	11..09
T3	9.50	10.59	11.08	11.74
T4	10.00	10.60	11.60	12.37
T5	11.03	11.68	11.91	12.68

The prepared beverage sample was evaluated on the basis of 9 hedonic scale by special panel of judges at NIFSAT, UAF. The beverage samples were assessed for sensory characteristics including taste, odor, color, flavor and overall acceptance Yet, developed probiotic beverage from 0 days fermented beverage was preferred more in terms of color, flavor, appearance, taste and overall acceptance (Table 4).

Table 4: Effect of different treatments and storage days on sensory properties of probiotic beverage

Treatments	Storage (Days)	Color	Flavor	Taste	Aroma	Overall acceptability
T0	0	8.44±0.03	8.11±0.03	8.11±0.01	8.50±0.10	8.79±0.12
	7	7.28±0.05	7.46±0.01	7.22±0.09	7.41±0.16	8.53±0.03
	14	6.57±0.07	6.27±0.09	6.63±0.02	7.10±0.26	7.86±0.09
	21	6.11±0.01	6.07±0.02	6.29±0.11	6.50±0.32	7.50±0.02
T1	0	8.42±0.04	8.28±0.01	7.98±0.11	8.10±0.05	8.47±0.03
	7	7.17±0.03	7.35±0.01	7.78±0.15	7.21±0.12	7.54±0.01
	14	6.32±0.06	6.27±0.09	6.21±0.02	7.03±0.03	6.61±0.04
	21	6.09±0.07	6.04±0.3	6.18±0.02	6.47±0.14	6.35±0.07
T2	0	8.20±0.01	8.16±0.07	7.84±0.09	8.55±0.12	7.94±0.03
	7	7.00±0.01	7.24±0.01	6.64±0.15	7.90±0.01	7.28±0.02
	14	6.14±0.03	6.80±0.07	6.12±0.02	7.54±0.01	6.61±0.02
	21	6.01±0.02	6.45±0.8	6.01±0.03	6.54±0.05	6.11±0.08
T3	0	8.00±0.02	7.82±0.03	7.90±0.06	8.31±0.03	8.98±0.06
	7	7.81±0.07	7.81±0.07	7.34±0.04	7.70±0.07	7.45±0.06
	14	7.32±0.06	6.73±0.03	6.21±0.09	7.33±0.07	7.33±0.17
	21	6.30±0.01	6.19±0.12	6.10±0.12	6.75±0.21	6.46±0.13
T4	0	8.69±0.04	7.85±0.04	7.71±0.02	8.21±0.02	6.84±0.04
	7	7.43±0.08	6.31±0.31	6.41±0.02	7.72±0.14	7.45±0.11
	14	6.84±0.03	6.88±0.03	6.12±0.07	7.26±0.03	7.26±0.19
	21	6.11±0.04	6.03±0.06	5.99±0.23	6.79±0.22	6.02±0.14
T5	0	8.17±0.03	7.87±0.06	7.73±0.02	8.21±0.02	7.85±0.01
	7	7.32±0.06	7.46±0.17	7.62±0.08	7.33±0.08	7.55±0.01
	14	6.80±0.03	6.47±0.03	6.13±0.02	6.83±0.02	6.40±0.02
	21	6.19±0.06	6.11±0.23	5.84±0.02	6.32±0.02	6.07±0.01

DISCUSSION

The obtained outcomes are similar with the outcomes given by Pakbin *et al.*, they prepared probiotic peach juice by using *Lactobacillus casei* and concluded that probiotic *Lactobacillus casei* have ability to reduce the peach juice's pH [14]. Martinez-Flores *et al.*, revealed the impact of sonication process on physicochemical characteristics of carrot juice and they observed that because of fermentation process the carrot juice's pH decreased [19]. Demir *et al.*, investigated the impact of storage span on carrot juice which was developed through lactic acid fermentation and they concluded that because of fermentation process, acidity of carrot juice was increased [20]. Hammad *et al.*, investigated that after treatment of carrot juice with irradiation method the value of total soluble solids showed decline trend during storage period [21]. Vandresen *et al.*, described temperature's impact on its theological attributes and on carrot juice and concluded that because of heat treatment there was a decline in total sugars of carrot juice. The pH of carrot juice was also reduced because of lactic acid fermentation induced by LAB (lactic acid bacteria) that consume sugars, hence there was a significant decrease in total sugars because of

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storage days and fermentation process [22]. Shi *et al.*, investigated process of microencapsulation of *Lactobacillus* species in set type yogurt and concluded that there was an increase in plate count as storage time increased [23]. Peterska-Ivanovska *et al.*, investigated microencapsulation impact to prepare symbiotic of juice carrot and concluded that in the storage days there was an increase in the total probiotic count [24]. Hussain *et al.*, reported the increase in probiotic count of probiotic yogurt during storage days, the yogurt was fortified with aloevera [25]. Costa *et al.*, also investigated that storage span have significant impact on juice's color. The probiotic carrot juice's color was changed during storage span. In present study the probiotic peach beverage developed from T_2 and T_3 got best scores and β-carotene degraded by oxygen (O_2) present in peach based probiotic beverage, that's why the color of the peach beverage changed during storage span [26]. Pereira *et al.*, investigated the probiotic drink's acceptability with the storage and they concluded that with the passage of time the flavor of probiotic juice was changed negatively. The probiotic beverage's original flavor was developed by the combined effect of fermentation temperatures and lactic acid bacteria. Because of the spoilage microorganisms, developed flavor was reduced by the storage period (21 days) [27]. Luckow and Delahunty concluded that the non-dairy probiotic juices were affected by the byproducts of probiotics. Due to metabolic activity of byproducts of LAB (lactic acid bacteria), the taste of developed beverage was deteriorated [28]. Pereira *et al.*, concluded that during storage days the overall acceptability of the probiotic juices decreased. The decreased value of overall acceptability is because of disturbance in flavor and deterioration in the color during storage period [27].

CONCLUSIONS

The present study was designed to isolate probiotic culture (*Lactobacillus casei)* and prepare peach based probiotic beverage supplemented with isolated probiotic culture in proper amount. The *Lactobacillus casei* was isolated from indigenous source (yoghurt). The bacterial isolates were characterized on the morphological, biochemical and physiological basis. The growth rate of isolates at 30 °C and 37 °C indicates that isolates are thermophile in nature. *Lactobacillus casei* produced acid from fructose, sorbitol, lactose, mannitol, sucrose, maltose, glucose but zero gas production was observed. *Lactobacillus casei* is a gram-positive probiotic bacterial species used in a many fermented food products. The primary concern of probiotification is to improve the value of non-dairy based probiotic foods. In current study, *L. casei* proved to be promising probiotic for preparation of probiotic peach beverage as peach is a nutritious fruit. Therefore, the probiotic peach beverage may be marketed as a value added food product.

Authors Contribution

Conceptualization: SP
Methodology: SP
Formal Analysis: SP, QUA
Writing-review and editing: SP, QUA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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