International Food Research Journal 25(4): 1675-1681 (August 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Formulation and shelf life study of a whey-based functional beverage containing orange juice and probiotic organisms

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Article history

Received: 29 April 2017
Received in revised form: 19 June 2017
Accepted: 20 June 2017

Keywords

Functional whey beverage
Probiotication
Lactobacillus fermentum PH5
Handva batter

Abstract

Looking to the fast growing market potential of functional beverages, the aim of this study was to formulate functional orange-whey based RTS beverage by adding probiotic strain *Lactobacillus fermentum* PH5. Based on a statistical analysis of the sensory evaluation of the drinks, final formula contained a ratio 60F:40W (B2) for orange juice and unprocessed pasteurized whey. The physico-chemical and microbiological evaluations (viability of probiotics) of the formulated blend (B2) and control (C) were carried out at regular intervals (weekly) during the storage period (28 days) under refrigeration storage (7±1°C). The product remained good in terms of overall acceptability and maintained the viability of probiotic bacteria *viz.* 6.10 and 7.25 log CFU/ml for control (A) and B2 (60F:40W), ($p<0.05$), respectively at the end of the storage period.

Introduction

Designing and producing probiotic products have been considered a lot because of both their natural healthful effects (proteins, fibers, vitamins, minerals) and inducing a lot of varieties. Probiotic bacteria are included as healthy and live nutritive ingredients in the fruit based beverage (Saarela *et al.,* 2002). The role of lactic acid bacteria (LAB) in functional fruit-whey based beverages is to assist in preservation of milk by generation of lactic acid and possibly antimicrobial compounds that suppress the putrefactive and other undesirable bacteria in the intestine; production of metabolites that provide organoleptic properties desired by the consumers; improving the nutritional values of food by releasing free amino acids or the synthesis of vitamins besides their provision of special therapeutic or prophylactic properties against cancer, ulcerative colitis, gastritis, enteritis, dyspepsia and hypercholesterolemia (O'Sullivan *et al*., 1992). Therefore, juice fortification with probiotic microorganisms is a challenge and a frontier goal due to their nutritional status besides being delicious (Yadav *et al*., 2010).

Whey or milk plasma is greenish yellow, semi translucent liquid that separates from the curd and one of the highly nutritious by-products obtained from the dairy industry producing cheese, chhanna and paneer. Rather being considered as a waste product, whey is formulated to prepare various value added fruits and vegetables blended products that are rich in nutritional and functional properties as whey proteins has a fresh, natural taste besides good solubility and make light and refreshing beverage (Flores-Andrade *et al*., 2013). It consists of 45-50% total milk solids, 70% of lactose, 20% of milk proteins, 7-9% of milk minerals and almost all the water soluble vitamins like riboflavin, folic acid and cobalamine in significant amount (Dhamsaniya and Varshney 2013). Whey proteins are one of the best quality food proteins in developing value-added functional beverages (Beristain *et al*., 2006) and control biological activities such as appetite suppression, antioxidative action and stimulation of immune system (Rupnar *et al*., 2009). Unprocessed pasteurized whey means whey in its native form, without given any denaturation or other clarification/ filtration treatment.

Oranges (*Citrus sinensis* L.) constitute a significant source of antioxidants (Vitamin C), polyphenol compounds (hydroxyl cinnamic acids, flavones), vitamins and minerals. These compounds exhibit anti-inflammatory, antihypertensive, diuretic, analgesic and anti-hyperlipidemic activities (Klimczak *et al*., 2007). Fruit-whey based drinks are light, refreshing and less acidic than fruit juices alone

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as well as nutritious. Fruit juices have been reported as a novel and appropriate medium for probiotic for their content of essential nutrients. Moreover, they are referred as healthy foods, designed for young and old people (Luckow *et al*., 2009). Thus, current research work was aimed to formulate functional orange-whey based RTS beverage by adding probiotic strain *Lactobacillus fermentum* PH5. In previous work, probiotic strain *L. fermentum* PH5 isolated from batter of traditional Indian cereal based fermented food product Handva has been proven for anti-hypercholesterolemic potential (Thakkar *et al.*, 2015).

Materials and Methods

Material collection and sample preparation

Double tone skim milk 'Taaza' brand having 3.1% fat and 7.9% SNF was obtained from Amul. The milk whey was prepared following the procedure suggested by De (2001). The milk whey thus obtained was filled in a glass bottle and stored in the refrigerator (7±1°C) still further use. Orange juice (Tropicana) was procured from the local market of Ahmedabad, Gujarat. The probiotic strain *L. fermentum* PH5 isolated from Handva batter was activated from stock culture (containing 80% glycerol) which was preserved at -20ºC using (2%) litmus milk (Thakkar *et al*., 2015).

Preparation of formulated probiotic beverages

The fruit juice-orange and whey were mixed at different proportions during formulation of probiotic beverage and based on organoleptic evaluations; 70F:30W (B1), 60F:40W (B2) and 50F:50W (B3) were further chosen for standardization along with 100% fruit juice sample (A) as control. These blends were probioticated at 2% rate by inoculating potent strain *L. fermentum* PH5. Formulated beverages of various combinations were chilled before subjected to sensory evaluation.

Organoleptic evaluation and optimization of potential blend

Prepared beverage was given to 10 trained panel members for evaluating the organoleptic attributes such as flavor and taste, color and appearance, body and texture and overall acceptability of the product through nine points Hedonic scale method (9 and 1 points showing like extremely and dislike extremely). The blend that was rated best after sensory evaluation was selected to formulate probiotic functional beverage with strain *Lactobacillus fermentum* PH5.

Shelf life study

The experimental sample (B2) of probiotic functional beverages, containing blend of orange juice and whey in 60F:40W ratio and control (A) sample (100% orange juice) were prepared; filled in different bottles (200 ml in each) and were stored at 7±1°C for 28 days. Both the samples A and B were taken at weekly intervals including 0 day to evaluate physicochemical, microbiological and sensory quality analysis during storage.

Evaluation of physicochemical parameters (pH and acidity)

The pH of the beverages was determined using the digital pH meter (OakTon pH 700) after homogenizing 10 ml of the fruit juice in 90ml of distilled water. Standard method was used to measure titratable acidity (Ferrati *et al*., 2005). Titratable acidity was expressed as g lactic acid/100g of juice and calculated using the formula:

TA= [M NaOH x ml NaOH x 0.09 x 100] / ml juice sample

Where, TA = Titratable acidity; M NaOH = Molarity of NaOH used; ml NaOH = amount (in ml) of NaOH used; 0.09 = equivalent weight of lactic acid.

Microbial analysis (viability study)

Microbiological quality, specifically in terms of viability of *L. fermentum* PH5 in the samples were periodically analyzed during storage taking 10 ml from the experimental as well as control samples, which were aseptically mixed with 90 ml distilled water and homogenized by shaking. Subsequent decimal dilutions were prepared and plated on MRS (de Man *et al*., 1960) agar to enumerate total numbers of probiotic bacteria after 48hr of incubation at 37°C in anaerobic jar and expressed as colony forming unit (CFU).

Sensory quality profile

Sensory quality was checked using standard Hedonic scale method with trained professionals.

Statistical analysis

The results of three individual experiments were gathered to generate the mean±standard deviation (SD). One way analysis of variance (ANOVA) was used to determine the significance by using Minitab at $p<0.05$.

Results and Discussion

Optimization of ratio of orange juice and whey for beverage preparation

Orange juice is highly refreshing, rich in vitamin C and dietary fibers and almost liked by all age groups. With respect to improve the taste, aroma, palatability and nutritive value and to reduce bitterness, orange juice was blended with highly nutritive component whey.

Preliminary studies were carried out to optimize balanced ratio of orange juice and whey having best quality sensory profile by a panel comprising of 10 judges drawn from faculty members and post graduation students on the sensory sheet based on a 9 point Hedonic scale. The highest score for all attributes was given to the beverage containing orange juice and whey in the ratio of 60F:40W (B2) besides it showed non-significant result compared to control as indicated in Figure 1. From the sensory evaluation it was revealed that the mean scores of this blend (B2) for flavor and taste, color and appearance, body and texture and overall acceptability were 8.15, 7.9, 7.75 and 8.25, respectively as compared to control (A) showing 8.3, 8.1, 8.0 and 7.95. Blend B2 got maximum score in all parameters and differed significantly ($p<0.05$) from B1 and B3 and rated best among others in evaluation. Therefore, the B2 (60:40) blend of orange and whey was chosen for the further course of investigation. One liter of selected B2 (60F:40W) blend was formulated by mixing 600ml of fruit juice (F) with 400 ml of nutritive whey (W); and filled in glass bottles (200 ml each). Same way, 1000ml of orange juice control (A) without whey supplementation was filled (200 ml each); both the samples (A and B2) were stored under refrigerated condition (7±1°C) for 28 days. Probiotication of *L. acidophilus* as functional ingredient in fruit juices (sapodilla, grapes, orange and watermelon) was reported by Anita *et al*. (2013).

Shelf life study

Any attempt to prepare a fruit product is incomplete if its storage stability is not properly studied. Thus, the formulated probiotic beverage was evaluated for the pH, acidity, viable count of probiotic strain and sensory profile on weekly interval.

Evaluation of physicochemical parameters (pH and acidity)

The pH is one of the most important factors affecting the survival of probiotics. Table 1 shows mean values of pH for both the samples 4.34 and 4.41, for orange juice control (A) and orange-whey

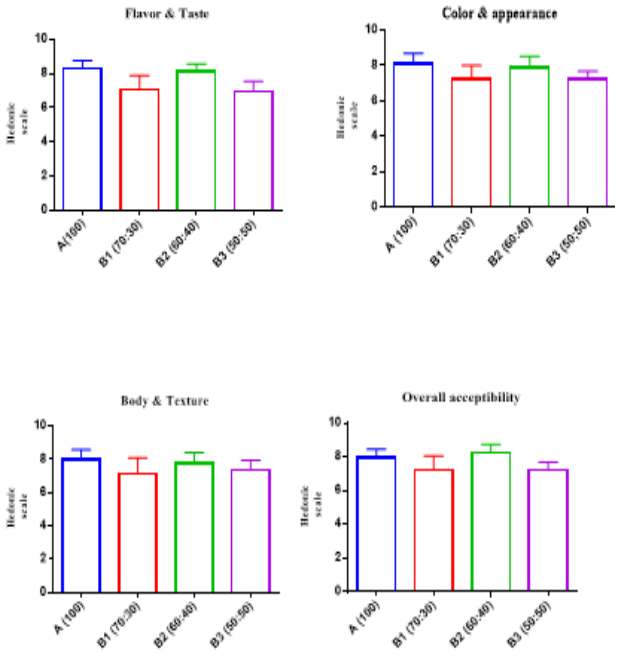

Figure 1. Evaluation of four sensory characteristics using Graphpad Prism 6.0 (Hedonic scale analysis for optimization of Orange: whey blend probiotic juice) for the four beverage formulations

based functional beverage (B2), respectively. Thus, at the end of the storage period, there was non-significant difference between pH for control (A) and orange-whey beverage (B2). Thus, storage period had a significant decreasing effect on the pH of the beverages with a mean values of 4.69 for a freshly prepared beverages and 4.16 for the beverages stored for 28 days ($p<0.05$). We also found a rapid decrease ($p<0.05$) in the pH of both samples during the initial phase (7^{th} day- 4.42) which was of great importance for the quality of product at the end as suggested by Viander *et al.* (2003).

The similar trend of pH was reported by Naik *et al*. (2009) in case of whey based watermelon beverage. Das *et al.* (2015) also found the reduction in the pH values from 4.28 to 3.95 in case of whey-based orange beverage. A decline in pH during storage is observed which may be due to the action of citric acid and ascorbic acid on the sugar and protein component of the product. Production of organic acids and amino acids also lead to an increase in acidity thereby a decrease in pH, whereas protein and dietary fiber could protect probiotics from detrimental effect of pH as reported for mango based beverages (Sikder *et al*., 2001). The lower pH value of the beverage certainly increases the storage stability of the final product by impeding growth of undesirable microorganism including coliforms (Uzeh *et al.*, 2009).

The changes in the titrable acidity during storage are affected by recipe and treatment combinations.

Table 1. Physiochemical profile of the beverages during storage at (7±1°C)

Sample	Parameters	Duration of the study (week) 0	1	2	3	4	P * Mean
A (100)	pH	4.77	4.41	4.24	4.23	4.05	4.34 ^{a}
	Acidity	1.28	1.29	1.37	1.47	1.68	1.42 ^{B}
B2 (60F:40W)	pH	4.61	4.43	4.38	4.37	4.26	4.41 ^{a}
	Acidity	1.39	1.44	1.7	1.84	2.17	1.71 ^{A}
T* Mean	pH	4.69 ^{a}	4.42 ^{b}	4.31 ^{b}	4.30 ^{b}	4.16 ^{c}	
	Acidity	1.36 ^{D}	1.37 ^{D}	1.54 ^{C}	1.66 ^{B}	1.93 ^{A}	

Values expressed are Mean ± S.E.M; * Values with different superscripts differ significantly ($p<0.05$) in each columns and rows

Table 2. Sensory quality (Flavor and Taste) by Hedonic scale during storage period

Sample	Duration of the study (week) 0	1	2	3	4	P * Mean
A (100)	8.21	7.63	7.66	7.53	7.72	7.75 ^{a}
B2 (60F:40W)	7.53	7.94	8.26	8.01	7.75	7.90 ^{a}
T* Mean	7.87 ^{a}	7.79 ^{a}	7.96 ^{a}	7.77 ^{a}	7.74 ^{a}	

Values expressed are Mean ± S.E.M; * Values with different superscripts differ significantly ($p<0.05$) in each columns and rows

In both the samples control (A) and orange based whey functional beverage (B2), initial titrable acidity (in terms of lactic acid) was measured as 1.28 and 1.39% respectively, showed a progressive increase after 28 days of storage under refrigeration. Table 1 shows mean values of titrable acidity 1.42 and 1.71, for orange juice control (A) and orange-whey based functional beverage (B2), respectively ($p<0.05$). From the 7th day (at the end of 1st week), there was a continuous increase in titrable acidity up to 28th day (till the end of storage period, 4th week). The increase in titrable acidity of the formulated beverage (B2) could be due to acidic nature of both orange juice and whey. Lactose and proteins are converted into lactic acid and amino acids leading to increase in acidity and decrease in pH of beverages (Jaspreet *et al*., 2015).

An increase in acidity from 0.28 to 0.34% for mango based and 0.25 to 0.28% for jackfruit based RTS beverage were reported by Krishnaveni *et al.* (2001). Similar findings were also observed in case of mango-based RTS (Sikder *et al*., 2001) and for whey-based watermelon beverage (Naik *et al*., 2009). Hence, pH and acidity are the two important factors co-related to each other. Recently, Kumar (2015) found similar trend of acidity (0.546 to 0.870%) in shelf life study of probiotic beverage using whey and aloe vera juice.

Microbial analysis (viability study)

In order to claim a product to be probiotic, the viability of the used probiotic strain(s) is of primary importance. The orange-whey base functional beverage was evaluated weekly interval during the storage period to confirm viability of potential probiotic strain *L. fermentum* PH5. According to the International standards, the total viable counts in a probiotic product must be at least 6 log CFU/ml at the time of consumption (Korbekandi *et al.*, 2011). The highest viable count for probiotic observed on day 0, the day of production (treatment mean 7.85) was continuously decreased in case of both samples A and B2 till the end of the storage period (treatment mean 5.97). The mean values (during storage) was found to be 6.10 and 7.25 log CFU/ml for control (A) and B2 (60F:40W), ($p<0.05$), respectively. Thus, orange-whey based functional beverage (B2) maintained highest viable count (log CFU/ ml) 7.25 compare to 6.10 of control (A).

However, the total viable count was under acceptable range (>6 log CFU/ ml) even after 28 days of storage under refrigeration indicating good keeping quality up to 4 weeks. On the basis of this result, we could postulate that consumption of formulated product (B2) will provide high nutritive values as whey served as an important component in the production besides having highest probiotic bacterial load in compare to the markedly available fruit juices. Sohail *et al.* (2012) improved the viability of *L. rhamnosus* and *L. acidophilus* in orange juice using a novel microencapsulation method. Anita *et al*. (2013) reported >10^7 encapsulated probiotic *L. acidophilus* bacteria in orange, grapes and watermelon juices. In a recent study, Tootoonchi *et al.* (2015) reported 8.81 log counts of encapsulated *L. acidophilus* in orange juice after 4 weeks of storage at 4°C.

Sensory quality profile

For enhancing the consumption of value added functional probiotic products, the consumer satisfaction must be achieved keeping in view the cost effectiveness and health prospective (Hilde *et al*., 2003). The effect of probiotics on sensory traits of juices relies upon the kind of microorganism and fruit juice, storage temperature and other added compounds (Antonio *et al*., 2015; Patel 2017). The mean sensory score assigned to the beverage samples

Table 6: Viability study of *L. fermentum* PH5 in the beverages during storage at (7±1°C)

Duration of the storage study	Viable count of *L. fermentum* PH5 (log CFU/ml)		Treatment Mean*
	A(Control) Orange juice	B2(60:40) Orange-whey juice	
0 Day	7.27±0.08	8.44±0.13	7.85 ^{a}
7th Day	6.22±0.10	7.71±0.13	6.97 ^{b}
14th Day	5.87±0.06	6.89±0.03	6.38 ^{c}
21st Day	5.68±0.04	6.75±0.04	6.22 ^{c}
28th Day	5.45±0.07	6.48±0.02	5.97 ^{d}
Storage Period Mean*	6.10 ^{b}	7.25 ^{a}	

Values expressed are Mean ± S.E.M; * Values with different superscripts differ significantly ($p<0.05$) in each columns & rows

at different day interval during storage are shown in below tables.

Flavor and taste

Flavor means an overall integrated perception of taste and aroma associated with the product. The score for 'Flavor and Taste' was rated (P mean) as 7.75 and 7.90 for control probiotic orange (A) and probiotic orange-whey functional beverage (B2), respectively as shown in Table 2 ($p>0.05$). During the whole storage period the score for flavor and taste for control probiotic orange (A) decreased from 8.21 to 7.72; while in case of orange-whey based functional beverage (B2), the score was improved from 7.53 to 7.75 during storage as compare to the day of production. At the end of the study, on 4th week treatment mean 7.74 was found to be non-significant with 7.87 of the 0 week.

The flavor and taste of both the beverages remain steady during whole shelf life study (treatment means of every week interval found non-significant); indicated both of them can store for a month in the refrigerator with better quality flavor and taste. Similar results were also reported by Luckow *et al.* (2006) in the blackcurrent juice incorporated with probiotic *L. plantarum* 299v.

Color and appearance

Color serves as a preliminary parameter for the acceptance of any food product and indicates the fitness of the juice for consumption (Gupta *et al.*, 2015). The score for 'Color and Appearance' was rated (Storage period mean) as 8.26 and 7.80 for control probiotic orange (A) and probiotic orange-whey functional beverage (B2), respectively (Table 3) and found non-significant to each other.

At the end of the study, on 4th week treatment mean 7.99 was found to be non-significant with 8.03 of the 0 week (on the day of production). Thus

Table 3. Sensory quality analysis (Color and Appearance) by Hedonic scale during storage period

Sample	Duration of the study (week)					P * Mean
	0	1	2	3	4	
A (100)	8.30	8.31	8.28	8.20	8.21	8.26 ^{a}
B2 (60F:40W)	7.77	7.79	7.86	7.81	7.76	7.80 ^{a}
T* Mean	8.03 ^{a}	8.05 ^{a}	8.07 ^{a}	8.00 ^{a}	7.99 ^{a}	

Values expressed are Mean ± S.E.M; * Values with different superscripts differ significantly ($p<0.05$) in each columns and rows

from the above results, we can postulate that color and appearance of both the beverages remain steady (T×P interaction found non-significant) during whole shelf life study; indicated both of them can store for a month in the refrigerator with improved color quality. The present result was supported by Gupta *et al.* (2015), the color of the orange based blended RTS beverage decreased non-significantly with the advancement of storage period.

Body and texture

As shown in Table 6, formulated probiotic orange-whey based functional juice (B2) maintained its consistency in terms of body and texture, during the entire storage period of 28 days as the score remained steady (7.94) on the day of production and (7.96) at the end of the study (28th day of storage period). Control (A) was scored 8.31 on the day of production but, during storage at the end of 28th day under refrigerated condition, it showed non-significant decrease in the score as 8.17. Thus, storage period had a non-significant effect ($p>0.05$) on the body and texture of the beverages with a mean values of 8.13 for a freshly prepared beverages and 8.07 for the beverages stored for 28 days ($p<0.05$).

The score for 'Body and Texture' was rated (storage period mean) as 8.23 and 7.94 for control probiotic orange (A) and probiotic orange-whey functional beverage (B2), respectively ($p>0.05$) as shown in Table 4. Thus, in terms of texture of the orange juice, both the beverages A and B2 showed fruitful results as T×P interaction also found non-significant; indicated both of them can store for a month in the refrigerator with better quality body and texture.

Overall acceptability

Overall acceptability is based on multiple organoleptic quality parameters i.e. color, flavor, texture etc. and shows the accumulative perception and acceptance by the panelists. The overall

Table 4. Sensory quality (Body and Texture) by Hedonic scale during storage period

Sample	Duration of the study (week)					P * Mean
	0	1	2	3	4	
A (100)	8.31	8.21	8.25	8.19	8.17	8.23 [a]
B2 (60F:40W)	7.94	7.91	7.90	7.97	7.96	7.94 [a]
T* Mean	8.13 [a]	8.06 [a]	8.07 [a]	8.08 [a]	8.07 [a]	

Values expressed are Mean ± S.E.M; * Values with different superscripts differ significantly ($p<0.05$) in each columns and rows

Table 5. Sensory quality (Overall Acceptability) by Hedonic scale during storage period

Sample	Duration of the study (week)					P * Mean
	0	1	2	3	4	
A (100)	8.31	8.16	8.13	7.74	7.90	8.05 [a]
B2 (60F:40W)	7.97	8.01	8.10	7.90	7.83	7.96 [a]
T* Mean	8.14 [a]	8.09 [a]	8.12 [a]	7.82 [a]	7.87 [a]	

Values expressed are Mean ± S.E.M; * Values with different superscripts differ significantly ($p<0.05$) in each columns and rows

acceptability was around 8.05 in control orange beverage (A); while in probiotic orange-whey functional beverage (B2) 7.96 (non-significant) at the end of the storage period. Thus, based on the overall sensory quality evaluation, probiotic orange-whey functional beverage (B2) was considered fruitful in terms of nutritive whey, higher probiotic viable counts and better overall acceptability through sensory quality analysis. The present results are in accordance with findings reported by Jain *et al.* (2003) in orange drinks.

According to a consensus made with the panelists during sensory evaluation, it was determined that the main descriptors that characterized the product were acidity and sweetness, with acidity being the attribute responsible for the sensory difference perceived by the panelists. Even though a slight more acidification was detected by the sensory panels in case of orange-whey based functional beverage (B2) compared to control having probiotic orange juice (A), the agreement was made that the beverage was acceptable for a period of 28 days at 7±1°C. Moreover, looking to the higher nutritious qualities of a prolific combination of orange and whey, the developed probiotic orange-whey based functional beverage (B2) could be recommended for the large scale production at industrial level.

Conclusion

Currently available fruit beverages are generally synthetic flavored, bottled and sold in the market. If this could be substituted with fruit juice and dairy whey, it will be more beneficial to the consumer, dairy industries and beverage manufacturers as well as fruit cultivators. The formulated functional probiotic beverage (60F:40W) had better color, flavor, and overall acceptability plus a potent strain *L. fermentum* PH5 proven for cholesterol lowering profile besides stability profile compare to markedly available orange juices. This functional orange-whey beverage could be stored in the refrigerator via maintaining highest viable count of probiotics for about a month without addition of any preservatives. Looking to the higher nutritious virtues, the developed probiotic orange-whey based functional beverage could be recommended for the large scale production at industrial level.

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