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ORIGINAL ARTICLE

Comparison of cheese and paneer whey for production of a functional pineapple beverage: Nutraceutical properties and Shelf life

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Abstract Whey, a dairy byproduct offers a challenging task in terms of its disposal. Two functional beverages were produced by blending pineapple juice with cheese whey (W_1B) and paneer whey (W_2B) at different concentrations (10 %, 20 % and 30 %). The beverages were compared for physico-chemical, microbial and nutraceutical properties over a period of 60 days. Whey addition significantly improved various physico-chemical parameters of the beverages. Higher protein content in W_1B and a higher mineral content in W_2B without any adverse effects on antioxidant activity was seen because of blending. Whey based beverages showed higher microbial content, sedimentation values and serum separation values than control at all levels of blending. Although, W_1B showed highest protein and microbial count but W_2B showed highest mineral content and improved shelf life due to significantly lower values of serum separation and sedimentation. It could

F. A. Masoodi is the mentor of article.

Research Highlights

- Cheese and paneer whey was blended with pineapple juice and beverages were studied for physico-chemical, antioxidant properties and storage stability for a period of 60 days. Paneer whey based beverages showed consumer acceptability up to 20 % level of addition while in cheese whey addition was acceptable only up to 10 % level of blending. This is an important implication for industrial utilization of paneer whey for beverage production.
- Antioxidant properties were studied using different assays such as DPPH, Reducing power, ABTS, FRAP and Total phenolic content.
- Storage stability was studied by serum separation, sedimentation percentage, color index (ΔE*) and microbial count.
- Both paneer as well as cheese whey based pineapple beverage showed potent antioxidant properties, high protein and mineral content that can impart status of a functional beverage to the blended beverages.
- Microbial analysis showed that Whey addition results in greater survival of microbes during storage.

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be concluded that paneer whey based pineapple beverage enable byproduct utilization with excellent nutrition and nutraceutical quality.

Keywords Whey · Shelf life · Antioxidant properties · Beverage · Nutrition · Microbiology

Introduction

Paneer is a south Asian variety of non-fermented non-renneted, non-melting and unripened cheese obtained by heat and acid coagulation. Paneer processing results in production of large quantities of whey that not only results in loss of valuable nutrients but also raises serious environmental concerns for its disposal due to its high biological oxygen demand. In India alone, it is estimated that about 100 million kg of whey is annually derived as a byproduct, which may cause substantial loss of about 70,000 tons of nutritious whey solids. Whey constitutes 45–50 % of total milk solids, 70 % of milk sugar (lactose), 20 % of milk proteins and 70–90 % of minerals and almost all the water-soluble vitamins originally present in milk. Whey contains all the essential amino acids in excess to FAO standards e.g. isoleucine, lysine, threonine and tryptophan. Whey constituents have indispensable value as human food. Due to its high nutritional profiling whey can be used in beverages, geriatric and athletic foods (Baljeet et al. 2013; Saxena et al. 2015). Whey and its biological components have also been proven effective in treatments of several chronic diseases like diarrhea, bile illness, skin problems, scales in the urinary tract, cancer, hypertension and cardiovascular disorders (Ashoush et al. 2013; Kerasioti et al. 2014). However, whey proteins, because of their colloidal unstability (protein aggregation) seem to pose a major problem in utilization of whey for the production of such beverages (Baccouche et al. 2013). Aggregation of globular proteins can form spheres (microgels) or strands that depend on various factors such as pH, salt, protein composition, etc. (Pan-Xun et al. 2014). Protein aggregation in whey is mainly driven by β-lactoglobulin that imparts undesirable viscosity, appearance or decreases colloidal stability. So utilization of deproteinized/denatured whey results in formation of soluble aggregates (Ryan and Foegeding 2015), which maintain the colloidal stability of beverage. Paneer whey production involves high heat treatment that results in deproteinization of whey. Hence, it is postulated that use of paneer whey instead of cheese whey would enhance the shelf life of whey-based beverages. This research article reports the use of varied concentrations of two different types of whey (cheese and paneer) and their effect on nutritional profiling, shelf life, antioxidant properties and microbial stability of the beverages.

Pineapple is a rich source of polyphenolic compounds (Baljeet et al. 2013). Antioxidants offer numerous health benefits such as anticancerous, antidiabetic and antihypertensive effects (Ahmad et al. 2014, 2015; Gani et al. 2016; Rahmanian et al. 2015). pH of pineapple juice ranges between 3 to 4, which is lower than the isoelectric point of β-lactoglobin and lower pH increases energy barrier for unfolding of the proteins, which hinders the non-covalent (disulphide interactions) bonding (Hoffmann and van Mil 1997). Such non-covalent interactions are significant contributors for whey protein aggregation and hence can lead to colloidal unstability of whey beverages (Ryan et al. 2012). Thus, use of pineapple juice in particular for the production of a whey-based beverage can be a good option for a shelf stable beverage with high nutraceutical potential.

Material and methods

Milk was obtained from local farms of south Kashmir, India. Folin-Coicalteu's, sodium carbonate, Gallic acid, DPPH (1,1-Diphenyl-2-picrylhydrazyl), trichloro-acetic acid, $FeCl_3$, phosphate buffer, potassium ferricyanide, methanolic, ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate)], potassium persulfate, TPTZ (2,4,6-tripyridyl-s-triazine) HCl were procured from Sigma. Rest of the chemicals were procured from HiMedia and all chemicals used were of analytical grade.

Whey preparation

Cow milk procured from local farms was used for whey preparation. Cheese whey (W_1) was prepared according to the method of Gallardo-Escamilla et al. (2007) by adding 0.04 %, *v*/v, calf rennet to milk in a water bath at 35 °C and allowing 45 min for a coagulum to form. The coagulum was then cut manually and heated in the water bath until a temperature of 55 °C was attained. The whey (W_1) obtained was filtered through muslin cloth.

Paneer Whey (W_2) was prepared according to the method described by Baljeet et al. (2013) with some modifications. Milk was heated to 70 °C for 30 min and acidified by adding 1 % citric acid at a concentration of 2.34 g/kg of milk followed by continuous stirring, resulting in complete coagulation of milk protein. Whey (W_2) produced was filtered using muslin cloth.

Production of juice and whey based pineapple beverage

Production of juice and whey based pineapple beverages are shown in flow diagram (Fig. 1). Ripe pineapples were selected. The crown and stem portion were removed and the fruit was washed in tap water. Pineapple was peeled, eyes were removed and fruit was sliced. The prepared slices were pulped

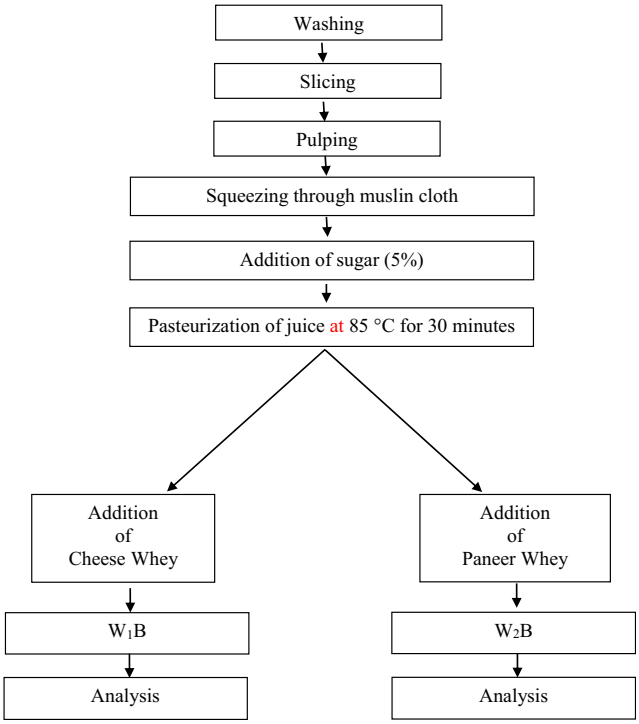

Fig. 1 Process flow chart for preparation of whey based pineapple beverage. W_1B: Cheese whey based beverage. W_2B: Paneer whey based beverage

in a mixer juicer grinder (Sujata, Powermatic plus) and the juice was recovered by filtration through muslin cloth. Sugar was added to the extracted juice at a concentration of 5 % followed by pasteurization at 85 °C for 30 min. Two types of beverages, W_1B and W_2B, were produced by adding different concentrations (10 %, 20 % and 30 %) of W_1 and W_2, respectively with pineapple juice. The beverages were stored in glass bottles at refrigerated temperature until further analysis.

Physico-chemical analysis

Whey based pineapple beverages (W_1B & W_2B) were analyzed for different physico-chemical properties. Protein content was measured by Lowry's method and bovine serum albumin (BSA) was used as standard. Moisture, fat, ascorbic acid, mineral analysis and total ash were determined by standard techniques described in Ranganna (1986). Titratable acidity and color analysis were determined according to the method described by Baljeet et al. (2013) and Eissa et al. (2014), respectively. The pH values of the beverages were determined with conventional electronic pH meter (HANNA) equipped with a penetration electrode model. Total soluble solids were determined with Abbe Mark II digital refractometer and the values were expressed as °Brix.

Antioxidants properties

Control and beverage samples were dried and subjected to solvent extraction using 80 % methanol. Extraction was carried out in triplicates for about 36 h in a shaker at 37 °C with gentle shaking. The extracts were then dried at room temperature. 10 mg of each dried extract was dissolved in 10 ml of 80 % methanol to form sample extract (SE) and stored at −80 °C until further analysis.

Total phenolic content

Total phenolic content was measured using Folin-Coicalteu's reagent according to the method of Singleton and Rossi (1965) with some modifications. Sample extracts (500 µL) each of control, W_1B and W_2B were introduced into test tubes followed by addition of 2.5 ml of Folin–Ciocalteu reagent (diluted 10 times with water) and 2 ml of sodium carbonate (7.5 % *w/v*). The tubes were vortexed and incubated at 50 °C for 5 min. Absorption at 760 nm was measured with a spectrophotometer (UV-visible Spectrophotometer Model U-2900 2JI-0003, Hitachi). The results were expressed as nmol Gallic acid equivalents (nmolGAE)/µg dry mass using a standard gallic acid curve.

DPPH (1,1-Diphenyl-2-picrylhydrazyl)

The DPPH assay was performed according to the method of Nisar et al. (2015) with some modifications. A stock solution was prepared by dissolving 5 mg DPPH with 100 ml methanol. 500 µL of sample extract was reacted with 100 µL DPPH. Each mixture was brought to a total volume of 4 ml using 80 % methanol. The mixture was allowed to react in the dark for 30 min. Absorbance was read at 515 nm, against the blank. The radical scavenging activity was expressed as percent inhibition using the equation

$$\%\text{inhibition} = [(A_c - A_s)/A_c] \times 100$$

Where A_c is the absorbance of control and A_s is the absorbance of sample.

Reducing power

Reducing power was done according to the method described by Baba et al. (2014) with the minor modifications. 500 µL of sample extract was dissolved in the 2.5 ml of the 0.2 M phosphate buffer solution (pH 6.6). It was followed by an addition of 2.5 ml of 10 % potassium ferricyanide. Mixture was incubated at 50 °C for 20 min. 25 ml of 10 % TCA (trichloro-acetic acid) *w/v* was added followed by centrifugation at 3000 rpm for 10 min. 2.5 ml of supernatant were collected to which 2.5 ml of distilled water and 0.5 ml of 0.1 % $FeCl_3$ solution

was added. The absorbance was measured at 700 nm. The percentage reduction was calculated using the formulae:

$$\text{Reduction}\% = \left(A_{(test)}/A_{(blank)} - 1\right) \times 100$$

Where A_{test} = Absorbance of the sample, A_{Blank} = Absorbance of the control.

ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate)]

$ABTS^{\bullet}$ radicle scavenging activity was determined using the method of Baskar et al. (2011) with slight modifications. $ABTS^{\bullet}$ radicles were produced by mixing ABTS (7 mM) and ammonium persulphate (2.45 mM) and stored for 12 h in dark. The $ABTS^{\bullet}$ solution was diluted with methanol in a ratio of 1:60 to obtain an appropriate absorbance. Sample extract (150 µL each) was mixed with 2850 µl of $ABTS^{\bullet}$ solution and allowed to react in dark for six minutes before taking absorbance at 723 nm. The percent inhibition was calculated according to the formula:

$$\%\text{inhibition} = [(A_c - A_s)/A_c] \times 100$$

Where A_c is the absorbance of control and A_s is the absorbance of sample.

FRAP (Ferric Reducing Antioxidant Assay)

The FRAP assay was performed according to Benzie and Strain (1999) with some modifications. The stock solution included 10 ml of acetate buffer (300 mM pH 3.6), 1 ml of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in HCl (40 mM) and 1 ml $FeCl_3.6H_2O$ (20 mM) solution. The FRAP reagent [10 ml of acetate buffer (300 mM pH 3.6), 1 ml of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in HCl (40 mM) and 1 ml $FeCl_3.6H_2O$ (20 mM) solution] once prepared was immediately incubated for 10 min at 37 °C. 3 ml of the reagent were added to 0.1 ml of the different sample extracts. The reaction mixture was incubated for 4 min at room temperature and the change in absorbance at 593 nm was measured using a spectrophotometer. The experiment was conducted at 37 °C under pH 3.6. The value was expressed in µM FRAP/g fresh weight sample.

$$\text{Frap value of sample } (\mu\text{M}) = \left[\text{Change in absorbance of sample from 0–4min} \Big/ \text{Change in absorbance of standard from 0–4min}\right] \times 2$$

Shelf life

Shelf life of beverages was assessed in terms of serum separation, sedimentation value, total color difference (ΔE*) and microbial analysis for a period of 60 days.

Serum separation

Serum separation was carried out according to Baccouche et al. (2013). Samples in 5 ml disposable pipettes sealed at both ends were incubated at 5 °C to assess serum separation under gravity. The pipettes were frequently inspected at various time intervals for 60 days. When sedimentation occurred, a layer of clear supernatant was left at the top and the volume recorded as an indication of instability. The serum separated was expressed as percent volume.

Sedimentation

Sedimentation was carried out according to the method described by Gad et al. (2013). Whey-Based Pineapple beverages were centrifugation at 3000 *g* for 30 min at ambient temperature. Sedimentation was expressed as a percentage of the total fluid weight using the equation,

$$\text{Sediment}\% = \{\text{Sediment formed}/\text{Fluid total weight}\} \times 100$$

Microbiological analysis

Microbial analysis was carried out using the method of Oranusi et al. (2012) with slight modifications. 10 ml of control as well as beverages were aseptically mixed with 90 ml of distilled water and homogenized. Subsequent decimal dilutions were prepared with the same diluents and in all cases duplicate-counting plates were prepared of appropriate dilutions. Total viable count was carried out using pour plate method for a period of 60 days.

Sensory analysis

Beverages prepared by blending of whey and juice in different combination were analyzed by twenty panelists for sensory analysis i.e. Color, appearance, flavor, taste, consistency and overall acceptability by using 9 point hedonic scale. Control was marked as A and samples with 10, 20 and 30 % level of blending with cheese whey were marked as B, C and D

respectively, while samples blended with paneer whey (10 %, 20 % and 30 %) were marked as E, F and G, respectively.

Statistical analysis

Experimental analysis was performed in triplicates. Data was analyzed by SPSS version 16. Significant differences between means were determined by one way and two-way analysis of variance (Ducans multiple range test) at 5 % level of significance.

Results and discussions

Physico chemical properties

Results in Table 1 show that addition of whey significantly affects the physico-chemical properties of whey based pineapple beverage.

Protein content of beverages increased significantly in both W_2B (0.61 % to 0.86 %) and W_1B (0.65 %-1.20 %) by the addition of whey than that of control (0.51 %). This is due to higher protein content of whey in comparison to control. However, protein content of W_1B was higher than that of W_2B in all the blends due to heat denaturation of proteins in W_2B. Denaturation leads to protein aggregation leading to sedimentation that are removed during filtration. Baccouche et al. (2013) in prickly pear beverage obtained similar results. Whey based beverages had significantly higher reducing sugars than control and W_1B showed significantly higher value than W_2B (Table 1). Because of blending, mineral content of both the whey-based beverages increased significantly in comparison to control. Increase in mineral content (Ca, K, Mg,) of W_2B was significantly higher than in W_1B. At 10, 20, and 30 % level of blending, potassium levels showed a percentage increase of 7, 31, and 55 % in W_1B while an increase of 5, 33, and 53 % was seen in W_2B at 10, 20, and 30 % level of blending. Calcium showed a 56 %, 155 %, and 178 % & 103 %, 224 %, and 320 % increase at 10, 20, and 30 % level of blending in W_1B & W_2B, respectively. Sodium levels in W_2B showed higher percentage increase than W_1B at all levels of blending. Chlorine level (Traces in control) also showed a multifold increase in beverages with greater increase in W_2B than in W_1B. However, magnesium level showed a mild but significant decrease with blending in both W_1B and W_2B. Higher calcium and magnesium levels in W_2B than W_1B can be due to higher amounts of these minerals in paneer whey. During rennet coagulation, in cheese preparation calcium stays in the curd as calcium caseinate. Higher sodium and chlorine levels in W_2B signify greater amount of salts in paneer whey. Goyal and Gandhi (2009) reported similar results in paneer and cheese whey. In both W_1B and W_2B, ascorbic acid content decreased with increase

Table 1 Proximate composition of whey based pineapple beverage

		Protein content (%)	Fat content (%)	RS content (%)	Total Sugar content (%)	Ash content (%)	Ascorbic Acid content (%)	Mineral content (mg/100 ml)				
								K	Na	Ca	Mg	Cl
W_1B	0	$0.510^{a} \pm 0.02$	$0.04^{a} \pm 0.01$	$0.87^{a} \pm 0.01$	$2.95^{a} \pm 0.01$	$0.21^{a} \pm 0.01$	$0.72^{a} \pm 0.01$	$44.8^{a} \pm 1.1$	$0.31^{a} \pm 1.03$	$4.21^{a} \pm 0.03$	$11.2^{a} \pm 1.03$	traces
	10	$0.655^{b} \pm 0.03$	$0.02^{a} \pm 0.01$	$1.42^{c} \pm 0.02$	$6.25^{e} \pm 0.01$	$0.22^{a} \pm 0.01$	$0.67^{a} \pm 0.02$	$48.09^{b} \pm 2.1$	$2.73^{b} \pm 1.03$	$6.57^{b} \pm 0.05$	$10.75^{b} \pm 1.03$	$10.92^{a} \pm 2.05$
	20	$0.883^{d} \pm 0.01$	$0.02^{a} \pm 0.01$	$1.56^{d} \pm 0.01$	$10.00^{f} \pm 0.03$	$0.27^{b} \pm 0.01$	$0.65^{a} \pm 0.01$	$58.85^{c} \pm 2.1$	$4.81^{c} \pm 1.05$	$10.15^{c} \pm 0.1$	$10.18^{b} \pm 1.03$	$21.83^{b} \pm 1.5$
	30	$1.209^{e} \pm 0.03$	$0.02^{a} \pm 0.01$	$1.72^{e} \pm 0.03$	$11.11^{g} \pm 0.01$	$0.30^{b} \pm 0.02$	$0.57^{b} \pm 0.04$	$69.61^{d} \pm 2.1$	$7.18^{d} \pm 1.03$	$11.73^{d} \pm 1.1$	$9.38^{c} \pm 1.03$	$32.75^{c} \pm 1.5$
W_2B	10	$0.611^{b} \pm 0.02$	$0.03^{a} \pm 0.01$	$1.00^{b} \pm 0.01$	$3.24^{b} \pm 0.02$	$0.27^{b} \pm 0.01$	$0.69^{a} \pm 0.04$	$47.45^{b} \pm 1.1$	$3.56^{b} \pm 1.05$	$8.57^{c} \pm 0.05$	$10.56^{b} \pm 0.53$	$13.47^{d} \pm 1.03$
	20	$0.726^{c} \pm 0.02$	$0.03^{a} \pm 0.01$	$1.16^{b} \pm 0.02$	$3.87^{c} \pm 0.01$	$0.31^{b} \pm 0.02$	$0.68^{a} \pm 0.03$	$59.85^{c} \pm 1.1$	$6.87^{d} \pm 2.03$	$13.65^{e} \pm 0.1$	$10.43^{b} \pm 0.5$	$25.27^{e} \pm 1.03$
	30	$0.859^{d} \pm 0.01$	$0.02^{a} \pm 0.01$	$1.37^{c} \pm 0.01$	$4.20^{d} \pm 0.04$	$0.37^{c} \pm 0.03$	$0.54^{b} \pm 0.01$	$68.61^{d} \pm 1.1$	$10.25^{e} \pm 1.03$	$17.73^{f} \pm 1.1$	$9.75^{c} \pm 0.53$	$39.15^{f} \pm 1.03$

Mean ± SD. Values followed by different superscript letter in a column are significantly different ($p \leq 0.05$)

W_1B: Cheese whey based beverage. W_2B: Paneer whey based beverage. RS: Reducing sugar

in level of blending with whey (W_1 & W_2), due to decrease in the percentage of pineapple portion in beverages. The total sugar content of both W_1B and W_2B increased significantly with increase in the concentration of whey; however, W_2B values were significantly lower than those found in W_1B. The lesser value of sugars in W_2B might be due to maillard reaction resulting in conversion of reducing sugars into browning compounds. Ascorbic acid content of whey-based beverages was significantly lower than control beverage because of the presence of very low concentration of vitamin C in both cheese as well paneer whey. Gad et al. (2013) showed similar results in whey based mango blending decreased vitamin C content of the beverage due to increase in percentage of whey fraction in the beverage (Table 1).

Whey based beverages showed a higher pH than control but the increase in pH was greater in cheese whey based beverages (Table 2). However, whey based beverages showed a significant difference in pH with different levels of blending due to variation in pH of pineapple juice and cheese & paneer whey. TSS values showed significant variation at all levels of blending due to compositional variation of pineapple, cheese and paneer whey (Table 2). Both cheese and paneer whey blending significantly affected color of whey-based beverages and color values of W_1B and W_2B varied significantly to each other as well as control (Table 2). "*L*" value of both the whey-based beverages increased with addition of whey indicating addition of whey resulted in lighter beverages in comparison to control, however, *L* value showed a greater increase in W_1B than in W_2B. Both the beverages showed significant differences in '*a*' and '*b*' values from control. However, greater shift of '*a*' towards negative values and b towards positive values was seen in W_1B than in W_2B and can be attributed to greater content of riboflavin in cheese whey. Greenish yellow tinge of whey is due to riboflavin that is susceptible to degradation by heat in the pH range of 1.3–6.5 that can further increase by various factors such as presence of mineral nutrients present in the beverage (Sheraz et al. 2014). Hence, higher amounts of riboflavin in cheese whey can be a possible reason for greater color change (−ive values of *a*/+ive values of *b*) due to blending in W_1B than W_2B. These color changes increased with increased blending in W_1B. Thus, lesser color change in W_2B because of blending might be due to decreased amount of riboflavin in paneer whey as result of heating. Heating also may lead to degradation of color pigments of pineapple juice producing conspicuous changes in hue and Chroma of the beverages. Total color difference (ΔE*) of whey based beverages increased significantly than control (Table 2). However, total color difference (ΔE*) also showed greater change with blending in W_1B (65.46–168.038) than W_2B (65.46–105.7). The increase in ΔE* values can be due to the combined effect of maillard condensation, difference in colloidal proteins and destruction of pigments (Sady et al. 2013).

Antioxidant activity

Total phenolic content (TPC)

TPC values of W_1B and W_2B at 10 %, 20 %, 30 % of blending were 6.01, 5.58, 5.27 nmolGAE/μg and 5.95, 5.37, 5.16 nmolGAE/μg, respectively. Control showed higher antioxidant activity than whey-based beverages at higher levels (≥20 %) of blending but no significant difference at lower levels (≤10 %) of blending (Table 3). Decrease in TPC content can be due to lower TPC value of whey in comparison to pineapple. Whey is a poor source of antioxidants in comparison to pineapple. However, no significant difference in phenolic content of W_1B and W_2B was seen with respect to each other. Polyphenol-protein interactions are primarily non-covalent and polyphenols are simply adsorbed on surface of proteins (von Staszewski et al. 2012; Thongkaew et al. 2014). hence, use of paneer whey for beverage production would not affect the determination of TPC content of W_2B and W_1B, although the protein content of beverages varies significantly.

Table 2 Physico-chemical analysis of whey based pineapple beverage

		pH	TSS	Acidity (%)	Color			
					L	a	b	ΔE*
W_1B	0	$3.78^{a} \pm 0.01$	$14.6^{a} \pm 0.01$	$1.61^{a} \pm 0.01$	$63.28^{a} \pm 0.02$	$-4.7^{a} \pm 0.01$	$16.12^{a} \pm 0.03$	$65.46^{a} \pm 0.01$
	10	$4.32^{b} \pm 0.01$	$15.0^{e} \pm 0.01$	$1.48^{b} \pm 0.02$	$65.46^{b} \pm 0.01$	$-7.89^{c} \pm 0.03$	$13.04^{e} \pm 0.01$	$29.22^{e} \pm 0.02$
	20	$4.89^{c} \pm 0.02$	$14.7^{f} \pm 0.01$	$1.40^{c} \pm 0.01$	$67.57^{d} \pm 0.01$	$-10.5^{d} \pm 0.01$	$13.00^{e} \pm 0.02$	$61.78^{a} \pm 0.01$
	30	$5.47^{d} \pm 0.01$	$14.9^{g} \pm 0.02$	$1.32^{e} \pm 0.01$	$71.87^{e} \pm 0.01$	$-13.5^{e} \pm 0.01$	$20.22^{f} \pm 0.01$	$168.04^{f} \pm 0.01$
W_2B	10	$4.25^{b} \pm 0.02$	$13.8^{b} \pm 0.01$	$1.56^{b} \pm 0.01$	$65.38^{b} \pm 0.02$	$-4.6^{a} \pm 0.01$	$11.10^{b} \pm 0.01$	$29.02^{b} \pm 0.01$
	20	$4.75^{c} \pm 0.01$	$13.00^{c} \pm 0.02$	$1.45^{d} \pm 0.01$	$66.11^{b} \pm 0.01$	$-4.9^{a} \pm 0.03$	$13.04^{c} \pm 0.01$	$17.53^{c} \pm 0.02$
	30	$5.21^{e} \pm 0.01$	$11.00^{d} \pm 0.01$	$1.39^{e} \pm 0.02$	$70.32^{c} \pm 0.01$	$-5.52^{b} \pm 0.01$	$8.67^{d} \pm 0.03$	$105.7^{d} \pm 0.01$

Mean ± SD. Values followed by different superscript letter in a column are significantly different ($p \leq 0.05$)

W_1B: Cheese whey based beverage. W_2B: Paneer whey based beverage. ΔE*: Total Color Difference

Antioxidant assays

DPPH radicle scavenging activity of control (57.52 %) was higher than W_1B (56.47–50.74 %) that was higher than W_2B (52.41–44.43 %). DPPH radicle scavenging activity of W_1B and W_2B at 10, 20, 30 % level of blending was 56.47 %, 53.11 %, 50.74 % and 52.41 %, 47.11 %, 44.43 % respectively. Reducing power of control (47.82 %) was significantly higher than that of W_1B (46.12–42.13 %) and W_2B (43.78–35.76). Blending of whey decreased reducing power of the beverages. Reducing power of W_1B and W_2B at 10, 20, 30 % level of blending was 46.12 %, 43.90 % 42.13 % and 43.78 %, 38.33 %, 35.76 % respectively. Similar trend was seen in FRAP and ABTS (Table 3). FRAP value of control (0.402 μM Fe^+/g) was higher than both W_2B (0.368–0.293 μM Fe^+/g) as well as W_1B (0.393–0.351 μM Fe^+/g). At 10, 20, 30 % level of blending, FRAP values of W_1B and W_2B was 0.393, 0.371, 0.351 and 0.368, 0.329, 0.293 μM Fe^+/g, respectively. ABTS scavenging activity of control (56.54 %) was higher than both W_2B (52.38–40.29) as well as W_1B (55.21–49.23 μM Fe^+/g). ABTS radicle scavenging activity of W_1B and W_2B at 10, 20, 30 % level of blending was 55.21, 52.46, 49.23 % and 52.38, 45.76, 40.29, respectively. Antioxidant activity of both whey based beverages varied non-significantly at 10 % levels of blending but varied significantly at 20 and 30 % level of blending with paneer and cheese whey.

All antioxidant assays showed a similar trend. Antioxidant activity of control was significantly higher than whey-based beverages due to higher phenolic content of control, but both the beverages showed potent antioxidant activity (Table 3). Sady et al. (2013) found similar trend in orange-whey beverages. This result was in agreement with the finding of Ashoush et al. (2013) in pomegranate peel and whey powder mixtures of varied proportion. However, antioxidant activity of W_1B was significantly higher than W_2B beverages. Whey proteins are reported to exhibit potential scavenging activity (Ashoush et al. 2013). Since protein content of W_1B is much higher than W_2B, it not only improves the antioxidant activity of W_1B in comparison to W_2B, but may also generate more maillard compounds due to higher protein content of cheese whey that further increase its free radicle scavenging potential. Varied antioxidant activity of W_1B & W_2B can also be because the antioxidant activity of maillard products generated from whey show different antioxidant activity depending upon the type of sugar that is involved in maillard reaction e.g. whey proteins produce browning compounds of highest antioxidant activity by reacting with xylose than any other sugar (Wen-qiong et al. 2013).

Shelf life

Sedimentation value and storage stability

There was a significant difference in sedimentation values between control and whey based beverages (Table 4). Sedimentation values of W_2B (6.68 to 7.64 %) as well as W_1B (10.39 to 12.39 %) were higher than the control (6.11 %) on day 1. Whey proteins and pineapple juice are rich in proline and proanthocyanidins, respectively and both proline and proanthocyanidins are essentially haze forming (Siebert 1999). During storage period of sixty days, W_1B showed greater percent increase in sedimentation value as compared to W_2B, which suggests that paneer whey significantly increased the shelf life of W_2B and can be due to lesser protein content of paneer whey. Sedimentation values increased throughout the sixty days of storage in both the beverages. However, it decreased significantly from day 1 to day 20 that was followed by a significant increase by day 60. Baccouche et al. (2013) reported similar trend in sedimentation percentage in whey based prickly pear beverage. This irregular trend in sedimentation during storage can be because haze formation is a function of protein, polysaccharides, polyphenols and pH (Siebert 1999). With storage, change in the microbial content may also induce changes in factors like pH, reducing sugars, or even polysaccharide content and hence alter sedimentation values. Blending significantly affected serum separation values of both W_2B as well as W_1B. In W_1B,

Table 3 Antioxidant properties of whey based pineapple beverages

		DPPH (% inhibition)	Reducing Power (% inhibition)	TPC (nmolGAE/μg)	FRAP (μM Fe^+/g)	ABTS (%inhibition)
W_1B	0	$57.52^a \pm 1.09$	$47.82^a \pm 0.50$	$6.11^a \pm 0.07$	$0.402^a \pm 0.07$	$56.54^a \pm 0.09$
	10	$56.47^a \pm 1.05$	$46.12^a \pm 0.50$	$6.01^a \pm 0.05$	$0.393^a \pm 0.05$	$55.21^a \pm 0.11$
	20	$53.11^e \pm 1.17$	$43.90^b \pm 0.50$	$5.58^b \pm 0.07$	$0.371^b \pm 0.05$	$52.46^b \pm 0.04$
	30	$50.74^f \pm 1.13$	$42.13^c \pm 0.50$	$5.27^b \pm 0.07$	$0.351^b \pm 0.05$	$49.23^c \pm 0.06$
W_2B	10	$52.41^b \pm 1.08$	$43.78^b \pm 0.50$	$5.95^a \pm 0.05$	$0.368^b \pm 0.07$	$52.38^b \pm 0.03$
	20	$47.11^c \pm 1.05$	$38.33^d \pm 0.50$	$5.37^b \pm 0.05$	$0.329^c \pm 0.07$	$45.76^d \pm 0.07$
	30	$44.43^d \pm 1.17$	$35.76^e \pm 0.50$	$5.15^b \pm 0.05$	$0.293^d \pm 0.07$	$40.29^e \pm 0.05$

Mean ± SD. Values followed by different superscript letter in a column are significantly different ($p \leq 0.05$)

W_1B: Cheese whey based beverage. W_2B: Paneer whey based beverage

an increase in serum separation was seen with increase in blending levels in a linear fashion. However, in W_2B, serum separation increased up to 20 % blending level and decreased significantly at 30 % of blending. A possible explanation for it can be that at 30 % level of blending there is a considerable increase in neutral and anionic polysaccharides that may either solubilize (de Freitas et al. 2003) or inhibit (Luck et al. 1994) protein polyphenol complex, thereby decreasing serum separation. It may also be mentioned here that maximum interaction between protein and pectin (anionic polysaccharide) has been reported at pH of 3.2, which is quite close to the pH of W_2B in our study. Serum separation showed a similar trend as that of sedimentation during a storage period of 60 days (Table 4). However, Thongkaew et al. (2014) reported much higher values for different fruit extracts than found in our study, which can be because of lower pH of pineapple beverages. At lower pH (≈3–4) β-lactoglobulin has a high net positive charge that increases the inter-electrostatic repulsive forces between protein molecules and decreases the amount of aggregate formation and hence precipitation is lowered. This agrees to theoretical assumption made by Nicolai et al. (2011)

Total color difference (ΔE)*

Total color difference of beverages was used as an indicator of physical change that determines their shelf life in terms of consumer acceptability. During storage period, ΔE* of control as well as beverages decreased significantly but greater decrease was seen in whey based beverages. Similar results were obtained in whey-based prickly pear beverages (Baccouche et al. 2013) and various other fruit drinks (Eissa et al. 2014). Decrease in ΔE* is attributed to maillard reaction and whey beverages with higher protein content and lower pH would form more maillard compounds than control. W_1B showed greater decrease in ΔE* than W_2B and control (Table 4). Greater change in ΔE* of W_1B can be due to the formation of additional maillard products due to higher protein content. Since color of beverage is affected by various other factors such as colloidal proteins, interaction between constituents, pigment degradation and maillard products, which are susceptible to changes during storage (Cortés et al. 2008; Sady et al. 2013) and hence would also contribute to difference in ΔE* of beverages during storage.

Microbiological analysis

The beverage samples were analyzed for total plate count (TPC) for 60 days of storage (Table 5). Total plate count in W_1B as well as W_2B did not show any significant difference on day 1. With storage, difference between the microbial content of W_1B and W_2B increased significantly. All beverages had microbial load in the range that was safe for human

Table 4 Storage stability of whey based beverages

		Serum Separation (%)			Sedimentation			ΔE*					
W_1B	D 1	D 20	D 40	D 60	D 1	D 20	D 40	D 60	D 1	D 20	D 40	D 60
0	$8.11^{aA} \pm 0.05$	$18.58^{aB} \pm 0.05$	$22.84^{aC} \pm 0.05$	$25.89^{aC} \pm 0.05$	$6.11^{aD} \pm 0.05$	$6.03^{aE} \pm 0.05$	$6.23^{aF} \pm 0.05$	$6.51^{aG} \pm 0.05$	$65.56^{aH} \pm 0.1$	$63.41^{aI} \pm 0.05$	$60.57^{aJ} \pm 0.04$	$63.34^{aJ} \pm 0.03$
10	$22.93^{eA} \pm 0.05$	$55.88^{eB} \pm 0.05$	$58.59^{eC} \pm 0.05$	$58.66^{eC} \pm 0.05$	$10.39^{eD} \pm 0.07$	$10.23^{eE} \pm 0.07$	$11.84^{eF} \pm 0.09$	$12.69^{eG} \pm 0.07$	$69.73^{dH} \pm 0.1$	$58.87^{eI} \pm 0.05$	$51.53^{bI} \pm 0.06$	$45.25^{cJ} \pm 0.05$
20	$25.78^{fA} \pm 0.05$	$58.58^{fB} \pm 0.05$	$61.23^{fC} \pm 0.05$	$61.38^{fC} \pm 0.05$	$11.23^{fD} \pm 0.03$	$11.13^{gE} \pm 0.09$	$11.91^{fF} \pm 0.03$	$12.72^{fG} \pm 0.03$	$68.41^{eH} \pm 0.1$	$59.19^{eI} \pm 0.06$	$49.15^{eJ} \pm 0.05$	$43.67^{dJ} \pm 0.05$
30	$30.59^{gA} \pm 0.05$	$64.62^{gB} \pm 0.05$	$67.23^{gC} \pm 0.05$	$67.37^{gC} \pm 0.05$	$12.39^{gD} \pm 0.13$	$12.19^{hE} \pm 0.13$	$12.03^{gF} \pm 0.1$	$13.26^{gG} \pm 0.1$	$71.73^{fH} \pm 0.1$	$62.93^{fI} \pm 0.06$	$50.09^{dJ} \pm 0.07$	$41.61^{eJ} \pm 0.06$
W_2B 10	$8.85^{bA} \pm 0.05$	$22.82^{bB} \pm 0.05$	$23.68^{bC} \pm 0.06$	$26.45^{bC} \pm 0.05$	$6.68^{bD} \pm 0.05$	$6.52^{bE} \pm 0.09$	$6.88^{bF} \pm 0.06$	$6.86^{bG} \pm 0.04$	$66.13^{aH} \pm 0.1$	$64.67^{bI} \pm 0.05$	$61.07^{aI} \pm 0.05$	$62.35^{bJ} \pm 0.04$
20	$10.43^{dA} \pm 0.05$	$24.84^{dB} \pm 0.05$	$25.84^{dC} \pm 0.05$	$27.37^{dC} \pm 0.05$	$7.98^{dD} \pm 0.06$	$7.88^{dE} \pm 0.06$	$8.13^{dF} \pm 0.06$	$8.38^{dG} \pm 0.05$	$71.73^{bH} \pm 0.1$	$68.89^{cH} \pm 0.2$	$65.19^{bI} \pm 0.05$	$63.75^{aI} \pm 0.07$
30	$9.24^{cA} \pm 0.05$	$23.56^{cB} \pm 0.05$	$25.28^{cC} \pm 0.05$	$26.84^{cC} \pm 0.05$	$7.64^{cD} \pm 0.06$	$7.11^{cE} \pm 0.06$	$7.65^{cF} \pm 0.07$	$7.25^{cG} \pm 0.05$	$75.99^{cH} \pm 0.1$	$72.21^{dI} \pm 0.04$	$62.51^{cI} \pm 0.07$	$65.52^{bJ} \pm 0.04$

Mean ± SD. Values followed by different superscript letter in a column and in a row are significantly different ($p \leq 0.05$)

W_1B: Cheese whey based beverage. W_2B: Paneer whey based beverage. ΔE*: Total Color Difference

D 1: Day 1, D 20: Day 20, D 40: Day 40, D 60:Day 60

Table 5 Microbiological ($Log_{10}CFU\ g^{-1}$) analysis of whey based pineapple beverage

		Day 1	Day 20	Day 40	Day 60
W_1B	0	$1.45^{a} \pm 0.05$	$2.89^{a} \pm 0.05$	$4.69^{a} \pm 0.05$	$6.72^{a} \pm 0.05$
	10	$1.89^{b} \pm 0.05$	$5.12^{e} \pm 0.05$	$6.72^{e} \pm 0.05$	$8.64^{e} \pm 0.05$
	20	$1.92^{c} \pm 0.05$	$5.62^{f} \pm 0.05$	$7.42^{f} \pm 0.05$	$8.97^{g} \pm 0.05$
	30	$1.93^{d} \pm 0.05$	$5.97^{g} \pm 0.05$	$7.77^{g} \pm 0.05$	$9.56^{h} \pm 0.05$
W_2B	10	$1.49^{a} \pm 0.05$	$3.34^{b} \pm 0.05$	$5.04^{b} \pm 0.05$	$7.42^{b} \pm 0.05$
	20	$1.51^{a} \pm 0.05$	$3.56^{c} \pm 0.05$	$5.26^{c} \pm 0.05$	$7.77^{c} \pm 0.05$
	30	$1.47^{a} \pm 0.05$	$4.03^{d} \pm 0.05$	$5.43^{d} \pm 0.05$	$7.92^{d} \pm 0.05$

Mean ± SD. Values followed by different superscript letter in a column are significantly different ($p \leq 0.05$)

W_1B: Cheese whey based beverage. W_2B: Paneer whey based beverage

consumption up to day 40. Whey hosts health benefiting (probiotic) bacteria such as lactobacillus species, *Saccharomyces boulardii*. Data (Table 5) suggested that cheese whey offered more congenial environment for microbial growth. Whey protein isolates have been reported to offer protection to *lactobacillus plantarum* (Hernández-Rodríguez et al. 2014) and *Saccharomyces boulardii* (Duongthingoc et al. 2014). Increase in blending also resulted in greater microbial content ($W_1B > W_2B >$ control) during storage that also suggests greater survival of microbes with increase in whey content. Since increase in microbial content was more in W_1B than W_2B, it further affirms better survival of probiotics with greater whey protein content of whey based beverages.

Sensory analysis

The results of the 9-point evaluation are given in Table 6. The mean scores of whey based beverages varied in color, taste, texture, consistency, appearance, flavor and overall acceptability. With the exception of flavor, whey beverages scored significantly higher for all the evaluated attributes. Descriptive sensory analysis of freshly produced whey has documented a variety of flavors at low intensities including sweet aromatic, cardboard/wet paper, pasta water and soapy flavors, astringent mouth feel and bitter taste (Russell et al. 2006). Both types of beverages achieved a score for color that was comparable to color score of control. Sample B in W_1B and Sample F in W_2B based beverages received the highest score for the taste attribute among blended beverages. Sample B had lowest concentration (10 %) of cheese whey and further increase in cheese whey concentration lead to decreased acceptability due to increased astringency of cheese whey. However, Sample F (20 % blending with paneer whey) showed higher taste score than rest of the samples and can be due to reduced astringency of paneer whey. Paneer whey has lower proteins and polyphenolic content that are removed by heat treatment during production of paneer whey. Sample E (10 % paneer whey) received highest overall acceptability followed by Sample B (10 % cheese whey) as compared to the rest of the samples. It may be pointed out here that addition of cheese whey (10 %) improved the overall acceptability of beverages higher than that of pure pineapple juice while beverages with paneer whey addition at 10 % and 20 % showed higher overall acceptability than pure pineapple juice. This can be an important breakthrough for industries for utilization of paneer whey for production of whey based pineapple beverages.

Conclusion

It can be concluded that whey offers a good option for the production of functional pineapple beverages. Both paneer as well as cheese whey based beverage showed better protein and mineral content than control and thus can help in countering protein energy malnutrition and mineral deficiency among children in developing countries. Use of paneer whey not only improved protein content of beverages, but showed highest mineral content among all the beverages suggesting use of paneer whey based beverage as an electrolytic drink. Paneer whey not only improved the shelf life and color of pineapple beverages, but also improved overall acceptability than cheese whey. Hence, it can be concluded that use of paneer whey is more suitable than cheese whey in several

Table 6 Sensory analysis of whey based beverage

Parameter	A	B	C	D	E	F	G
Appearance	$7.25^{a} \pm 0.03$	$7.25^{a} \pm 0.05$	$7.16^{b} \pm 0.05$	$7.00^{c} \pm 0.05$	$7.58^{d} \pm 0.07$	$7.32^{e} \pm 0.03$	$7.16^{f} \pm 0.05$
Color	$7.56^{a} \pm 0.05$	$7.58^{a} \pm 0.03$	$7.45^{b} \pm 0.05$	$7.41^{b} \pm 0.05$	$7.75^{b} \pm 0.05$	$7.58^{a} \pm 0.05$	$7.60^{a} \pm 0.05$
Taste	$7.33^{a} \pm 0.04$	$7.41^{b} \pm 0.05$	$7.08^{c} \pm 0.03$	$6.83^{d} \pm 0.03$	$7.25^{a} \pm 0.05$	$7.54^{e} \pm 0.05$	$6.82^{d} \pm 0.03$
Consistency	$6.83^{a} \pm 0.05$	$7.32^{b} \pm 0.03$	$7.08^{c} \pm 0.05$	$7.08^{c} \pm 0.05$	$7.16^{d} \pm 0.04$	$7.28^{e} \pm 0.07$	$7.01^{c} \pm 0.05$
Flavor	$7.25^{a} \pm 0.05$	$7.08^{b} \pm 0.05$	$7.00^{b} \pm 0.05$	$6.58^{c} \pm 0.05$	$7.41^{d} \pm 0.05$	$7.18^{a} \pm 0.05$	$7.08^{b} \pm 0.05$
Overall Acceptability	$7.24^{a} \pm 0.03$	$7.32^{b} \pm 0.05$	$7.11^{a} \pm 0.04$	$6.98^{c} \pm 0.07$	$7.43^{d} \pm 0.05$	$7.38^{b} \pm 0.05$	$7.13^{a} \pm 0.01$

A: control, B: Beverage with 10 % cheese whey, C: Beverage with 20 % cheese whey, D: Beverage with 30 % cheese whey

E: Beverage with 10 % paneer whey, F: Beverage with 20 % paneer whey, G: Beverage with 30 % paneer whey

aspects and its use should be encouraged on industrial level. However further research is needed for improved stabilization of whey based beverages for commercializing paneer whey utilization in beverage industry.

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Compliance with ethical standards

Conflict of interest Authors have no conflict of interest.

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