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Process standardization for preparation of paneer whey based iced tea

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Abstract

Whey, a valuable by-product obtained during the manufacturing of dairy products is highly nutritious and whole-some food as it contains milk protein, fat, minerals, and other minor bioactive components. By realizing the functional properties of whey proteins, numerous products are available in market globally. On the other hand, tea is a popular refreshing beverage proven to have antioxidant properties due to the presence of polyphenols. Therefore, current research project was designed and planned to develop 'paneer whey based iced tea' in which water would be replaced with whey. In the first phase, the concentration of whey, tea, citric acid, flavour & intensity of heat treatment were optimized and evaluated for the physicochemical, microbiological and sensory parameters as well as antioxidant activity. Based on the statistical analysis of the sensory evaluation of the drinks, final product contained 100% whey, 1% tea, 8% sugar, 120 mg citric acid and 100 µL lemon juice, respectively. Moreover, the final product contains 0.6% protein and observed 25% antioxidant activity. The newly developed paneer whey based iced-tea is a healthy beverage enriched with polyphenols and further, it may offer consumers to drink whey without changing its original attributes.

Keywords: By-product, beverages, black tea, bioactive peptides, antioxidant activity, polyphenol, whey proteins

1. Introduction

There has been a great interest in creating new horizons for utilization of dairy and food industry by-products throughout the world. Whey is one the most interesting nutritional by-product obtained during the production of milk based products like cheese, paneer, chhanna, chakka and casein (Shah and Patel, 2019) ^[29]. It contains 45-50% of total milk solids, 70% of milk sugars, 20% of milk proteins, 70-90% of milk minerals and almost all water soluble vitamins present in milk. Whey is rich in organic matter; hence it has a very high biological oxygen demand (BOD) and chemical oxygen demand (COD). Whey is also having other issues like as it can lead to corrosiveness and had short shelf life which creates serious difficulty for its disposal (Verma *et al.*, 2024) ^[36]. By realizing the functional properties of whey, food industries target upon utilizing whey as the functional food ingredient. The annual production of whey is more than 160 million tones, with an estimated growth rate of 1-2% yearly (Das *et al.*, 2016, Verma *et al.*, 2024) ^[8, 36]. Whey protein concentrates, whey protein isolates and whey powder are prepared and widely marketed all over the world but all these process incurs sophisticated equipments (Shah and Patel, 2019) ^[29].

Currently, processed whey is used for the manufacturing of a variety of drinks and beverages, such as fermented or non-fermented beverages with or without addition of fruit juices (Babar *et al.*, 2008; Chavan *et al.*, 2015; Siddique *et al.*, 2023) ^[4, 5, 32], pulp (Gupta *et al.*, 2017) ^[11], or flavours; soups (David and Kumar, 2014; Rai *et al.*, 2014) ^[7, 21]; carbonated beverages (Silva e Alves *et al.*, 2018; Patel, 2017) ^[33, 18] probiotic drinks (Kamble *et al.*, 2017; Thakkar *et al.*, 2017) ^[15, 35], dahi (Rathi *et al.*, 2015; Raghavendra *et al.*, 2017; Gandhi *et al.*, 2022) ^[25, 22, 9], confectionary items supplemented with whey such as bread (Kakan *et al.*, 2018) ^[14] and jam & jelly (Wasnik, 2016; Raj *et al.*, 2017) ^[38, 23]. Many of these products have been successfully developed at small scale and have a limited market. Whey have been also utilized for the production of alcoholic beverage (wine, ethanol, and beer), organic acids, butter milk, yoghurt, ice cream, ice candy, coffee drink, cream yoghurt, caramel, and lactose

(Raghavendra *et al.*, 2017; Olivera *et al.*, 2019; Chavan *et al.*, 2020) [22, 17, 6]. With modern developments and technologies, nowadays whey is becoming a part of the human diet.

Tea is well known due to its refreshing taste and antioxidant properties over the globe. Polyphenols are a type of antioxidant found in black tea (*Camellia sinensis* L.) in form of catechins, theaflavins and thearubigins, and may promote overall health. In addition to their antioxidant activities in tea, catechins and theaflavins could have also pharmacological activities, such as the ability to lower glucose, lipid and uric acid (UA) levels (Peluso and Serafini, 2017) [19]. However, the amount of these antioxidants in tea depends upon origin of tea and its processing technology (Akram *et al.*, 2012; Yi *et al.*, 2015) [1, 40]. Black tea is consumed throughout different countries as a second popular beverage after water and ensures that an antioxidant agent available on each day. Hence, our current research project was designed and planned to develop 'whey based iced tea' in which water is replaced with whey.

2. Materials and Methods

Procurement of Raw materials

All the required ingredients including high quality sugar and flavour were purchased from the local market, while raw milk was procured from Dudhsagar Dairy, Mehsana, Gujarat. All the microbiological media were purchased from Hi-Media (Mumbai, India) whereas all the analytical grade chemicals were purchased from Merck (Mumbai, India).

Preparation of Paneer whey, its compositional analysis and paneer whey based iced tea

Paneer whey was prepared as mentioned by Rath *et al.*, (2015) [25] with few modifications. The pre-heat treated milk (90 °C) was acidified at 70 °C by adding citric acid (2%) and continuously stirred till coagulation of milk casein protein as mentioned. Paneer whey was subjected for analysis of the following compositional parameters such; pH, total solids & moisture, ash content, fat, crude protein and carbohydrate. Total solids (TS) were determined gravimetrically according to the procedure described in IS: 1479 (II) (1961) [13]. Fat content and crude protein were determined as per the procedure given by Yasmin *et al.*, (2015) [39], whereas ash content of acid whey was estimated by AOAC International (2019)², Official method 940.26. Total carbohydrates were determined by difference (IS (SP: 18 (part XI), 1981b) [13]. The liquid whey was filtered using a muslin cloth. The detailed process is diagrammatically represented in Figure 1.

Optimization of the level of paneer whey

Three different levels of paneer whey i.e. W1 (50%), W2 (80%), and W3 (100%) were taken into remaining part water. The sugar was added @ 8% (Singh *et al.*, 2011)³⁴. The most acceptable level as determined by sensory evaluation of the product was selected and further experiments were carried out with the optimized concentration of the paneer whey.

Optimization of the heat treatment

Paneer whey was undergone for different heat treatment e.g. 85 °C/1 min, 90 °C/30 sec & 95 °C/no hold. The most acceptable heat treatment as determined based on sensory evaluation of the product was selected to prepare the optimized product.

Optimization of tea and citric acid concentration

Whey based ice tea was prepared by incorporating tea at 0.3%, 0.5%, & 1% levels and citric acid at 80 mg, 100 mg, and 120 mg levels, respectively. The optimum level of both the ingredients was assessed based on sensory evaluation of the product.

Sensory evaluation

All the samples were evaluated for sensory attributes such as color and appearance, body and texture, flavor, and overall acceptability on a nine-point hedonic scale (9 for liking extremely and 1 for disliking extremely) by the panel of discriminative and experienced expert judges. The products were marked randomly and served in 50ml of sample during each trial.

Microbiological analysis

During microbiological analysis, the suitable dilutions were poured with respective agar media in sterile petridishes in duplicates. Product was analyzed for total plate count on standard plate count agar- SPCA (incubated at 30 °C/24-48h in aerobic condition), coliform count on Violet red bile agar-VRBA (incubated at 37 °C/24h in aerobic condition), and yeast & mold count on Potato dextrose agar-PDA (incubated at 28 °C/5day in aerobic condition) in order to judge the hygienic conditions during the product manufacturing process. The colony forming units (cfu) were counted from the respective plates following incubation under specific time- temperature combinations.

Determination of pH

The pH of paneer whey was determined electrometrically with pH meter by the method as described in IS: SP:18, part XI (1981) [12]. The pH meter was first calibrated using standard buffers of pH 4.0 and 9.2 and standardized using buffer of 7.0 pH at 20.0±0.1°C. The pH was determined by electronic pH meter, Welltronix, Model PC 100.

Antioxidant activity of product

The antioxidant activity from newly developed product was measured by DPPH [2, 2 diphenyl-1- picrylhydrazyl] assay as described by Shimada *et al.*, (1992) [31] with some modifications. Briefly, 2 mL of the product supernatant from an appropriate dilution, collected after centrifugation at 10,000 g for 30 min, was taken in a test tube and, mixed with 2mL of freshly prepared 0.008% DPPH in 50% ethanol and incubated in dark for 120 min at 37°C after covering the tube with aluminum foil. The absorbance of the solution was measured at 517 nm against methanol using UV-Visible Spectrophotometer (Shimadzu UV-1800, Japan). The experiment was performed in triplicates and the results were expressed as below; where 'blank' means DPPH solution and PBS without sample.

$$\% \text{ Scavenging activity} = \{ (A_{517 \text{ nm blank}} - A_{517 \text{ nm sample}}) / (A_{517 \text{ nm blank}}) \} \times 100$$

Statistical analysis

All the experiments were carried out in three trials (n=3). All experiments were carried out in triplicate (n=3). The significance was tested at five percent level using ANOVA by SYSTAT (Version 6.0).

Results and Discussion

In a view of healthy & functional beverage, whey based lemon flavor iced tea was formulated. Despite of great nutritional profile of acid whey that lacks in polyphenols content, its incorporation with polyphenols reach black tea would definitely fulfill the aim of current research work by yielding innovative healthy product. Furthermore, the finished product was having 0.6% protein as determined from Kjeldahl method.

Compositional analysis of paneer whey

Freshly prepared whey was determined for chemical composition. The mean value of fat, protein, ash, moisture and lactose demonstrated in Table 1 shows very minute variations between various lots. These values are in line with analysis carried out by Singh *et al.*, (2011) [34], Perisiriyan *et al.*, (2016) [20], Raghavendra *et al.*, (2017) [22] and Shende *et al.*, (2022) [30].

Optimization of the level of paneer whey

Three different levels of paneer whey i.e. W1 (50%), W2 (80%), and W3 (100%) were taken into remaining part water. As it is evident from Figure 2A and Table 2, the score of all the sensory attributes i.e. flavor, body & texture, color & appearance, overall acceptability increase with increase in percentage of whey. Score for body & texture was found non-significant ($p > 0.05$) between 50% and 80% paneer whey concentrate. The low score for treatment W1 followed by W2 indicates weak structural stability of whey when mixed with water. Iced tea prepared with 100% whey shows significantly ($p < 0.05$) higher score of 7.25. This could be correlated with no phase separation which had resulted into smooth consistency of tea at the time of sensory. The observance of rapid phase separation (sedimentation) can be directly correlated with decrease amount of whey proteins; possess water binding properties, in treatment W1 & W2. Flavor score for iced tea prepared using 80% & 100% showed non-significant ($p > 0.05$) difference whereas it was differ significantly ($p < 0.05$) when compared to the lowest whey concentrate (50%). However, the highest flavor score (7.35) was achieved for W3 treatment. The least score of 5.24 obtained for treatment W1 could be attributed to the lower intensity of flavor marked by judges due to less interaction between whey solids and synthetic flavor. Furthermore, phase separation of whey and water had probably masked the flavor. Likewise, the color & appearance and overall acceptability score of treatment W2 and W3 was also did not differ significantly, on the other hand, score differs significantly ($p < 0.05$) for both the parameters for treatment W1. Product made with only whey received maximum score of 7.35 and 7.69 for both color & appearance and overall acceptability, respectively. Certainly, product with only whey found significantly ($p < 0.05$) superior for all sensory aspect. Earlier, many research workers, Singh *et al.*, (2011) [34], Baba *et al.*, (2016) [3], Satpute *et al.*, (2018) [28] have developed beverages with maximum proportion of paneer whey. Baba and co-workers (2016) [3] have prepared both cheese and paneer whey pineapple beverages and reported significantly overall higher score for all sensory attributes as compared to control. They also marked more suitability of paneer whey than cheese whey for beverage formulations.

Optimization of Tea concentration for product

The result of incorporation of tea on sensory attributes is shown in Table 2. Tea prepared with 1% black tea was liked the most by judges as it has received significantly ($p < 0.05$) higher score of 8.01 due to its balanced aroma. Similarly, Perasiriyan *et al.*, (2013) [20] also noticed that overall acceptability score of whey based herbal drink with 1% black tea was > 8.0 . Non-significant difference ($p > 0.05$) for treatment T₁ & T₂ revealed that judges did not perceive any remarkable effect of black tea addition rate on sensory parameters of product. It is very well co-inside with the finding of Perasiriyan and co-workers (2013) [20]. The possible explanation belongs to complexes formed due to interaction of whey protein with available low amount of polyphenols in black tea might be responsible for masking the typical flavor of tea. Incorporation of Tea @ 1% (T₃) had obtained significantly ($p < 0.05$) higher scores for overall acceptability followed by 0.5% (T₁) and 0.3% (T₁). As it is seen from Figure 2B, non-significant difference was observed between treatment T₁ & T₂ as well as treatment T₂ & T₃ for each sensory parameter.

Optimization of heat treatment

The heat treatment results into deproteinization whey or denaturation of whey protein (Keheller *et al.*, 2018; Baba *et al.*, 2016) [16, 3]. It involves physico-chemical reactions between different molecules e.g. lysine and lactose which contributes typical flavor in whey. Heating of whey produces several reversible and irreversible reactions. Heat treatment was found to have a direct impact on acceptability of product (Table 2). Therefore, significant ($p < 0.05$) difference was found between H1 (85°C/1 min) and H3 (95°C/no hold) for all the parameters as illustrated in Figure 2C. The maximum body & texture score (8.22) was obtained with minimally heat treated whey. The results are in line with Singh and co-workers (2011) [34] who reported the highest overall acceptability score for lemon based whey beverage heat treated at 90°C/2 min than heat treated at 5 psi/5 min. The probable reasons are; product encountered with higher percentage of aggregated particles, production of cooked flavor (Kelleher *et al.*, 2018; Singh *et al.*, 2011) [16, 34], visually identified color changes associated with particle size (Kelleher *et al.*, 2018) [16] and slightly bitter taste. Development of cooked flavor is attributed to increase level of various sulphur compounds in whey when heated indirectly (Kelleher *et al.*, 2018) [16]. In case of flavor and color & appearance, score of each product varied significantly ($p < 0.05$). The results are in accordance with Singh *et al.*, (2011) [34]. They also reported the highest sensory scores for lemon based whey beverage heat treated at 90°C/2 min compared to 5 psi/5 min.

Optimization of citric acid

Citric acid is generally used as preservative in variety of food products. It gives the mild sourness upon addition in whey by slightly lowering the pH. Such phenomenon helps in boosting the acceptance of product. The most significant ($p < 0.05$) effect of citric acid incorporation was seen on flavor score of product (Figure 3A). An average overall acceptability score (8.05) for treatment C3 (120 mg) differ significantly ($p < 0.05$) compared to other treatments. The preference for C3 by judges was due to its optimum astringency and tanginess. Singh *et al.*, (2011) [34] have used citric acid solution for adjusting final pH (3.8) of lemon based hydrolyzed whey

beverage probably to enhance the sensory characteristics as well as shelf life of the product.

Optimization of lemon flavor

To make the product more appealing, synthetic lemon flavor were added to the mixture of whey and black tea after cooling it to room temperature (Rivero *et al.*, 2020) [26]. The overall acceptability score was ranging from 6.62 to 8.38 (Table 2). Judges commented that the higher addition rate of lemon flavor was striking sharply with little bitter after taste whereas; flavor with low addition rate was not easily detectable in the product. The synthetic lemon flavor @ 100 µL was found to give optimum aroma in the product. In another study conducted by Singh *et al.*, (2011) [34], the addition rate (0.1-0.2%) of synthetic lemon flavor was optimized through central composite rotatable design matrix. They have also found the optimum level @ 0.1%. An average overall acceptability score (8.05) for treatment C3 (120 mg) differ significantly ($p < 0.05$) compared to other treatments (Figure 3B). The preference for C3 by judges was due to optimum astringency and tanginess.

Changes in pH

The initial pH of whey, i.e. 5.33 ± 0.04 was found in close proximity of earlier research work performed by Raghavendra *et al.*, (2017) [22]. This value remained almost unchanged in all treatment except citric acid. Incorporation of citric acid at 80, 100 & 120 mg in whey further lowers the pH of product to 5.02 ± 0.02 , 4.97 ± 0.01 & 4.91 ± 0.02 , respectively.

Microbiological analysis

Usually heat treatment is designed to kill all pathogenic microbes including few spoilage causing bacterial groups in

order to provide safe and stable product. The microbiological assessment of product revealed absence of total bacterial count, coliform count and Yeast & Mold count indicating the acceptable quality and the good hygienic condition during preparation of product. Rather *et al.*, (2024) [24] also reported no development of colonies for total plate count and yeast & mold count in freshly prepared soy whey fortified pineapple juice beverage. Conversely, Singh and co-workers (2011) [34] found presence of total plate count in freshly prepared beverage which was increased significantly ($p < 0.05$) during 40 days' storage at refrigeration temperature. Moreover, the percentage of whey also greatly affected the count of bacteria. However, product was safe for consumption as the microbial load did not cross the safety level.

Antioxidant activity

The antioxidant activity of fresh product was measured using the most frequent DPPH method. The product showed the antioxidant activity of 25.45 ± 0.80 . The antioxidant value achieved in present study is still considered to be potent while comparing percentage of whey used by Sady *et al.*, (2013) [27] and Baba *et al.*, (2016) [3]. Experiment conducted by earlier group showed antioxidant activity of 10.9 (µmol of TE/mL) for 50% whey based orange beverage whereas latter group obtained a 44% scavenging activity for beverage prepared with 30% paneer whey based pineapple beverage. However, due to poor candidature of whey as an antioxidant and interaction between protein and polyphenolic compounds found responsible for decreased antioxidant activity compared to control (Baba *et al.*, 2016) [3]. Maillard reaction products of whey protein and lactose show less antioxidant activity than products of whey protein and xylose (Wang *et al.*, 2013) [37].

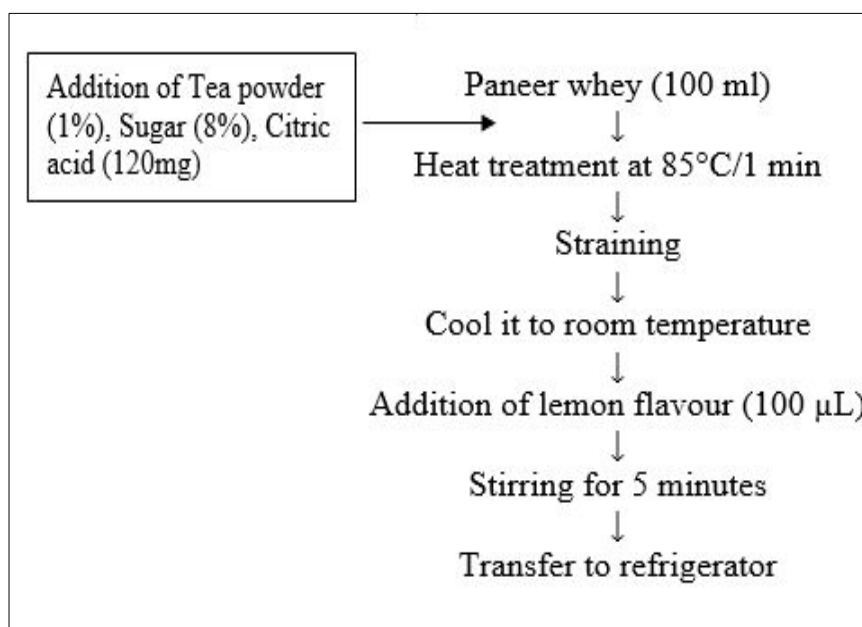


Fig 1: Flow diagram for preparation of paneer whey based iced tea (Modified from Perasiriyana *et al.*, 2013)

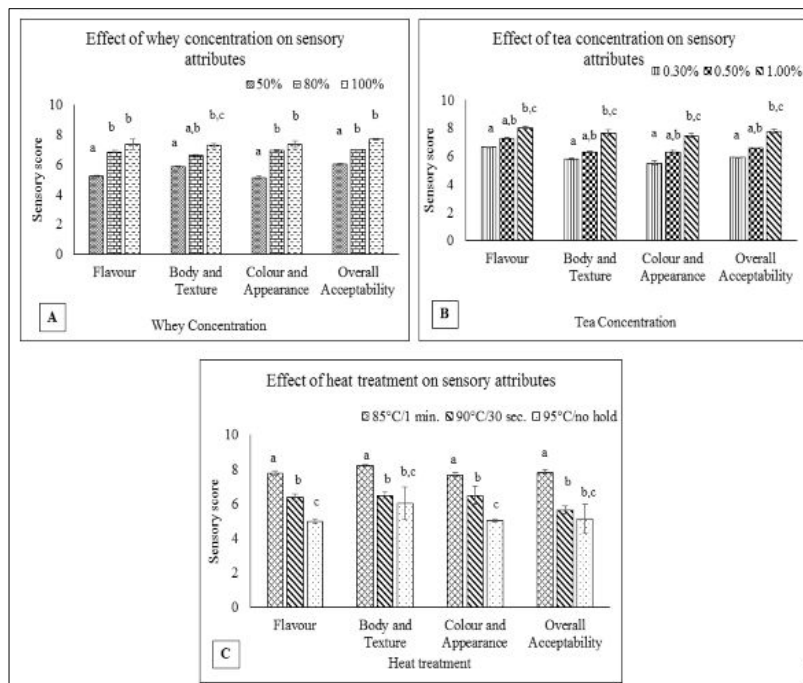


Fig 2: Effect of (A) whey concentration, (B) tea concentration and (C) heat treatment on sensory score of whey based ice tea. Means (n=3) with different small superscripts differs significantly ($P<0.05$).

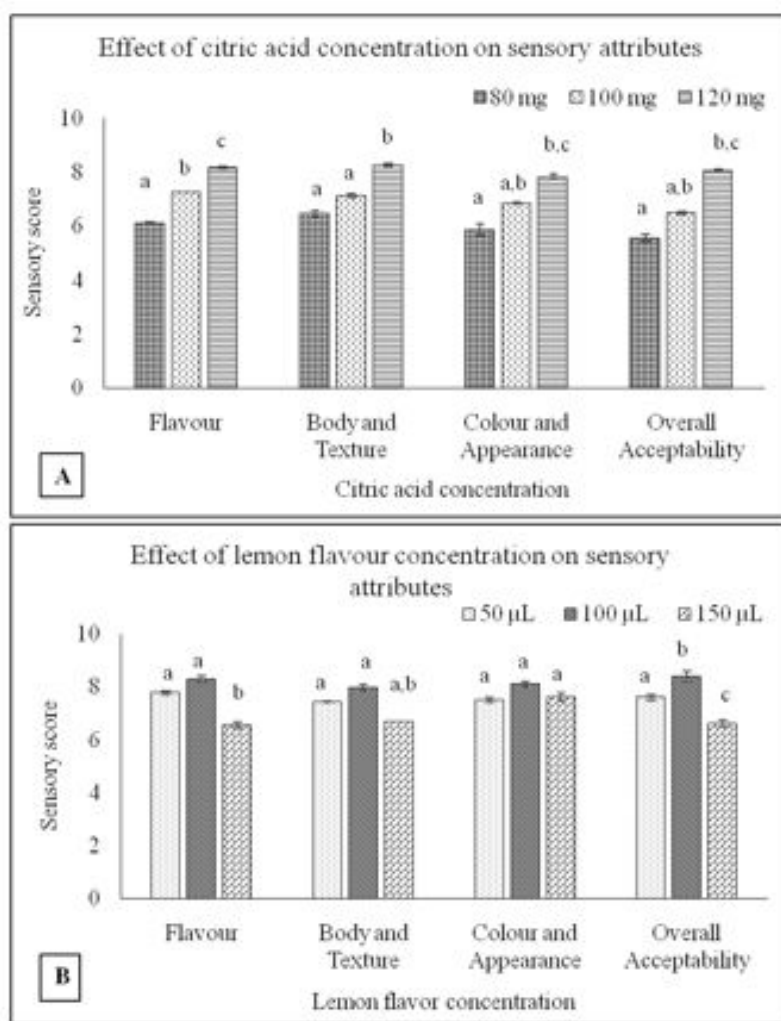


Fig 3: Effect of (A) citric acid concentration and (B) lemon flavor concentration on sensory score of whey based ice tea. Means (n=3) with different small superscripts differs significantly ($P<0.05$).

Table 1: Proximate composition of paneer whey

Sr. No.	Composition	Value (%)
1	Fat	0.35±0.02
2	Protein	0.78±0.03
3	Ash	0.59±0.02
4	Moisture	93.25±0.03
5	Lactose	5.03±0.03

Values are Mean ±SD (n=3).

Table 2: Sensory evaluation of whey based ice tea

Sr. No.	Particulars	Sensory attributes	Treatment		
			50%	80%	100%
			Sensory score		
1	Whey	Flavor	5.24±0.06	6.83±0.09	7.35±0.35
		Body & Texture	5.83±0.07	6.58±0.08	7.26±0.12
		Color & Appearance	5.11±0.06	6.94±0.06	7.35±0.22
		Overall Acceptability	6.05±0.03	6.98±0.03	7.69±0.07
			Treatment		
			0.3%	0.5%	1.0%
			Sensory score		
2	Tea	Flavor	6.63±0.04	7.28±0.05	8.02±0.08
		Body & Texture	5.80±0.04	6.31±0.06	7.62±0.25
		Color & Appearance	5.53±0.16	6.28±0.11	7.46±0.16
		Overall Acceptability	5.90±0.01	6.59±0.03	7.79±0.16
			Treatment		
			85°C/1 min	90°C/30 sec	95°C/no hold
			Sensory score		
3	Heat treatment	Flavor	7.79±0.11	6.38±0.19	4.99±0.13
		Body & Texture	8.22±0.05	6.48±0.17	6.04±0.94
		Color & Appearance	7.69±0.13	6.48±0.53	5.04±0.11
		Overall Acceptability	7.84±0.10	5.65±0.24	5.13±0.87
			Treatment		
		mg	80 mg	100 mg	120 mg
			Sensory score		
4	Citric acid	Flavor	6.10±0.04	7.27±0.01	8.17±0.05
		Body & Texture	6.47±0.12	7.14±0.07	8.28±0.05
		Color & Appearance	5.88±0.21	6.84±0.04	7.83±0.10
		Overall Acceptability	5.56±0.14	6.49±0.06	8.06±0.04
			Treatment		
			50 µL	100 µL	150 µL
			Sensory score		
5	Lemon flavor	Flavor	7.76±0.06	8.28±0.11	6.55±0.13
		Body & Texture	7.43±0.03	7.96±0.12	6.67±0.00
		Color & Appearance	7.50±0.09	8.09±0.11	7.59±0.16
		Overall Acceptability	7.58±0.10	8.39±0.19	6.62±0.14

Values are Mean ±SD (n=3).

Conclusion

The final product was formulated using 100% whey as a base along with tea (1%), sugar (8%), citric acid (120 mg) and lemon flavour (100 µL) by giving the heat treatment at 85°C/1 min. However, the colour of the finished beverage needs to be improved. The antioxidant activity and protein content of the optimized newly developed paneer whey based iced-tea offers a healthy beverage enriched with polyphenols without changing the original attributes of whey. Therefore, newly developed product have bright prospects in the Indian diet. Based on the promising results of Phase-I, the optimized product will be studied for shelf life at refrigeration temperature. In summary, a huge quantity of whey is produced globally but not absorbed in a systematically and economically feasible approach. It is prerequisite to develop appropriate processes for the manufacturing of mass consumption products by utilizing both whey and its ingredients.

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