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Studies on antioxidant properties of strawberry (*Fragaria ananassa*) mixed *paneer* whey beverage

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Abstract
Paneer whey beverage prepared with strawberry pulp in different proportion of 100:0 (T_1), 98:6 (T_2), 96:8 (T_3) and 94:10 (T_4), respectively. The product obtained was subjected for analyse antioxidant properties. On an average the TEAC 0.543, 1.021, 1.263 and 1.515 µmol/mg protein was found to be for treatment T_1, T_2, T_3 and T_4, respectively. It was revealed that Trolox Equivalent Antioxidant Capacity (TEAC) was increased as level of strawberry pulp increases in *paneer* whey.

Keywords: Antioxidant properties, TEAC, Strawberry, Whey beverage

Introduction
Whey is the watery part of milk that remains after separation of curd / coagulated products that result from acid or proteolytic enzyme mediated coagulation of milk. It is major by-product of dairy industry, during manufacture of products like *paneer*, *channa*, *chakka*, cheese, casein, etc. In the manufacturing of these products, about 10-20 per cent portion of milk is recovered as the desired end product and remaining 80-90 per cent liquid portion is the whey. It is considered to be reliable source of number of high quality and biological active proteins, carbohydrates and minerals. In India, nearly 5 million tones whey is produced of which chhana and *paneer* whey contribute around 80 per cent of total whey (Gupta, 2008) [4] and majority of it is disposed of as a waste. These are two types of whey available, acid whey that is generated as a result of *paneer*, *chhana*, *chakka* and acid casein manufacture and rennet whey, which is produced during cheese manufacture. (Darade and Ghodake, 2012) [1].
The strawberry (*Fragaria ananassa*) is an ancient and significant table fruit in temperate areas. In Maharashtra Nashik region grows strawberry on a considerable basis. It's popular for its cool, refreshing juice as well as its medicinal benefits. Strawberries contain many important dietary components including vitamins, minerals, folate, sugar, polysaccharide and fibre as well as rich source of phytochemical compounds mostly represented by polyphenols. Primarily phosphorus, potassium, calcium and magnesium are abundant in strawberry juice. It's a substantial food item with beneficial components including antioxidants. Strawberry fruit extract is recognized to have antibacterial, antiviral, and antioxidant characteristics that are beneficial to one's health. It also contains a lot of protein, fat, sugar, pectin, calcium, and iron. Several reports have demonstrated various cardiovascular, antiproliferative and neurologic benefits associated with the consumption of strawberries. Moreover several researchers have published lists of total antioxidant capacity (TAC) values of numerous foods, using their own food sources and methodologies. These results have shown that berries are consistently ranked among the top sources of total phenolics and TAC, with levels up to 4 times greater than other fruits, 10 times greater than vegetables, and 40 times greater than cereals. Within the fruit group, strawberries have a greater antioxidant capacity (2 to 11 fold) than apples, peaches, pears, grapes, tomatoes, oranges or kiwifruit. (Giampieri *et al*, 2012) [2].

Materials and Methodology
Collection of Milk
Fresh Buffalo milk was procured from local market of Latur city of Natural Milk Pvt. Ltd. having 6.0 per cent fat and 9 per cent SNF.

Collection of Strawberry
Strawberry was purchased from local market of Latur and pulp was prepared in laboratory.

Ingredients
Sugar: Good quality, clean, crystalline, white cube sugar was purchased from local market of Latur city.

Packaging Material
Glass bottles was used for packaging were purchased from local market of Latur city.

Equipment and accessories
Stainless steel vessels of requisite capacity, gas stove, fruit extractor (mixer grinder), muslin cloth, standard weight balance, thermometer and knives etc. were used for preparation of strawberry *paneer* whey beverage. Before using this material it was properly cleaned and washed with detergent solution. All the precautionary measures were considered during the conduct of trials to avoid contamination.

Chemicals and Reagents
Analytical grade (AR) or guaranteed grade (GR) reagents were used in the chemical analysis of whey beverage.

Treatment combinations (Plan of work)
For preparation of whey beverage by using strawberry (*Fragaria ananassa*) pulp, by adding sugar 8 per cent by weight of *paneer* whey and strawberry pulp as per the treatment combinations on weight basis were as follows:
T_1: 100 Parts of *Paneer* whey + sugar (@ 8 per cent)
T_2: 94 Parts of *Paneer* whey + sugar (@ 8 per cent) + 6 Parts of Strawberry pulp
T_3: 92 Parts of *Paneer* whey + sugar (@ 8 per cent) + 8 Parts of Strawberry pulp
T_4: 90 Parts of *Paneer* whey + sugar (@ 8 per cent) +10 Parts of Strawberry pulp
The different levels were tried and compared with control (T_1).

Preparation of *Paneer* whey Beverage using strawberry pulp
The *paneer* whey beverage using strawberry pulp was prepared by using method of Pandiyan *et al.* (2011) [9] with slight modification done by Kamate (2015) [6].

Procedure for strawberry whey beverage

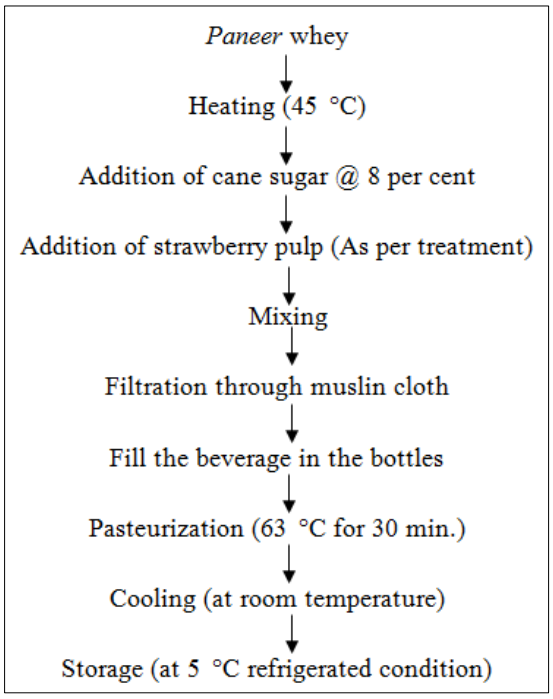

Fig 1: Flow chart for preparation of *Paneer* whey beverage using strawberry pulp (Kamate, 2015) [6]

The *Paneer* whey was heated at 45 °C temperature. Then cane sugar @ 8 per cent was added and maintained in all treatment combinations. Then strawberry pulp was added in the *paneer* whey as per treatment combinations, after adding strawberry pulp mixed properly by stirred it well, there after filtration through muslin cloth was done, Then prepared strawberry whey beverage was filled in glass bottles and bottles were sealed and In-bottle pasteurization was done at 63 °C temperature for 30 minutes. These bottled strawberry whey beverage cooled at room temperature and stored at refrigeration temperature at 5 °C for further use.

Determination of antioxidant activity by ABTS method
The antioxidant activity of strawberry whey beverage samples was determined by ABTS method. The antioxidant activity of strawberry whey beverage was checked for fresh samples at room temperature. ABTS (2, 29-Azinobis (3 ethylene benzothiazoline 6-sulphonic acid) Assay was used by the food industry and agricultural researchers to measure the antioxidant capacities of foods. In this assay, ABTS is converted to its radical cation by addition of potassium per sulphate. This radical cation is blue in colour and absorbs light at 734 nm. The ABTS radical cation is reactive towards most antioxidants including phenolics, thiols and ascorbic acid. During this reaction, the blue ABTS radical cation is converted back to its colourless neutral form Raghavendra *et al.* (2013). The procedure followed during present investigation as per the following flow diagram.

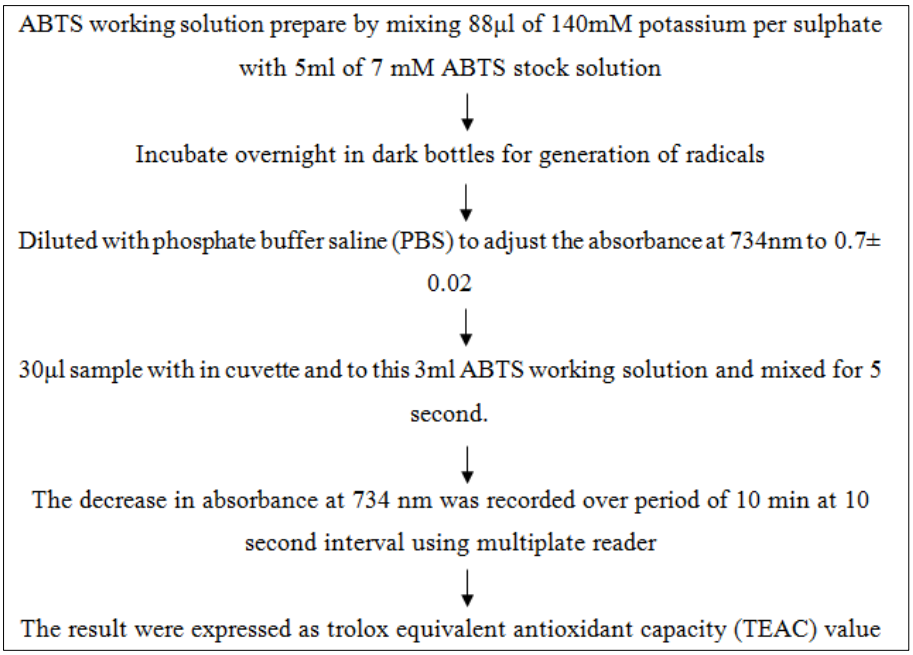

Fig 2: Flow diagram of ABTS method

Reagents

A. Potassium persulphate solution (140 mM)

1.892 gm of potassium persulphate was dissolved in double distilled water and made the volume to 50 ml.

B. ABTS [2, 2'-azinobis (3 ethyl benzothiazoline)-6-sulfonic acid] stock solution

19.2 mg of ABTS (Sigma-Aldrich) was dissolved in 5 ml of double distilled water; added 88 µl of 140 mM potassium persulphate solution and the mixture was kept in an Amber colour bottle in dark for 12-16 hours for production of sufficient free radicals.

C. Phosphate buffered saline (PBS, pH 7.4)

PBS was prepared by dissolving 8.0 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HP0_4 and 0.24 g of KH_2P0_4 in 800 ml distilled water, pH was adjusted to 7.4 with 1N HCl and made the volume to 1 litre with distilled water.

D. ABTS working solution

1 ml of ABTS stock solution was diluted with phosphate buffer saline (approx 1:70) till it give an absorbance of 0.70±0.02, before that absorption spectra of ABTS was analysed and maxima was taken at 734 nm.

E. Trolox solution

12.5 mg of Trolox [6-hydroxy. 2, 5, 7, 8 - tetramethyl chroman-2-carbocyclic acid] (Sigma-Aldrich) was dissolved in 10 ml of ethanol. The resulting solution was 5 mM trolox solution. It was diluted with distilled water to 2000µM concentration.

Preparation of Standard Curve of Trolox against ABTS radicals

3 ml of ABTS working solution was added to cuvette and initial absorbance against buffer blank was recorded at 734 nm using double beam spectrophotometer (SPECORD-200, Analytical zena). 10µl/ml of Trolox solutions (250-2000µM) were added to cuvette using micropipette. The contents were mixed for 30 seconds and change in absorbance at 734 nm was recorded over 10 min. The standard curve was prepared by plotting concentration (µM/1) of Trolx (X-axis) v/s % inhibition.

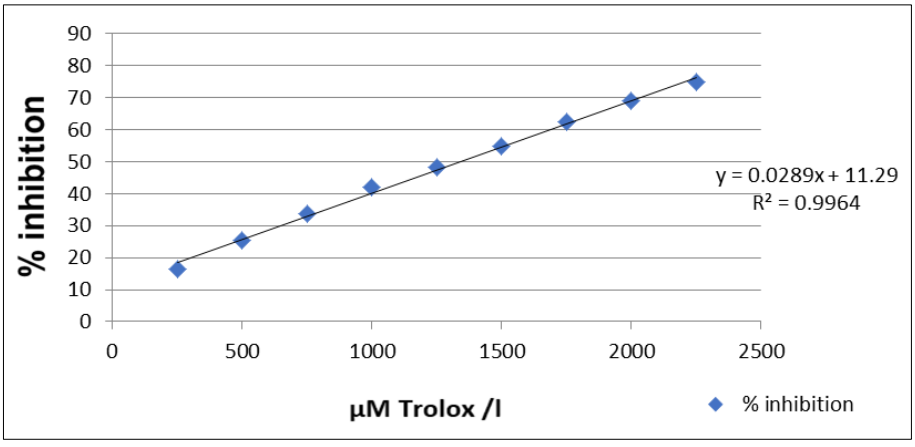

Fig 3: Standard Curve of Trolox against ABTS radicals

Trolox equivalent antioxidant capacity (TEAC)

TEAC is the concentration of trolox with equivalent antioxidant activity to a 1mM concentration of the substance under investigation. 3 ml of ABTS working solution made with PBS (pH 7.4) was added to cuvette (3 ml capacity) and absorbance adjusted to 0.70±0.02 against the buffer. 30µl of sample was added to ABTS working solution as well as in the blank. The contents were mixed for 5 seconds and change in absorbance at 734 nm was recorded over 10 min using SPECORD-200 double beam spectrophotometer (Analytical zena).

Calculation

Based on the per cent Inhibition of absorbance of sample, trolox equivalent was determined from standard curve, using following equation:

$y = 0.0289 X + 11.29$

Where

y is the % inhibition

$= 1(A734\ nm_{control} - A734\ nm_{sample})/A734\ nm_{control}] X 100$

x is the µM concentration of trolox

The results were expressed as trolox equivalent antioxidant capacity (TEAC) values i.e. µ mol of Trolox equivalence/ mg of the protein.

Statistical analysis of data

The data obtained in the present investigation was tabulated. The data was analysed statistically using Completely Randomized Design (CRD) as per Panse and Sukhatme (1985). The significance was evaluated on the basis of critical difference. In all four trials were conducted.

Result and Discussion

Antioxidant properties of strawberry whey beverage

The *paneer* whey, strawberry pulp and developed strawberry whey beverage samples were subjected for antioxidant activity by ABTS method.

Antioxidant activity of *paneer* whey and strawberry pulp

Before evaluation antioxidant activity of strawberry whey beverage the raw materials used to prepared beverage was subjected for its evaluation of antioxidant activity. The antioxidant activity in the *paneer* whey and strawberry pulp was determined. The results obtained are presented in Table 1.

Table 1: Antioxidant activity of *paneer* whey and strawberry pulp TEAC (µmol)/mg protein

Replication Treatment	R_1	R_2	R_3	R_4	Mean TEAC (µmol)/mg protein
Paneer whey	0.543	0.529	0.547	0.553	0.543±0.005
Strawberry pulp	1.792	1.778	1.785	1.782	1.78±0.005

The average antioxidant activity TEAC were observed 0.543 (µmol)/mg protein of *paneer* whey and 1.78 (µmol)/mg protein of strawberry pulp, respectively. It was observed that the antioxidant activity in the strawberry pulp was four times fold more than the *paneer* whey. The strawberry pulp has higher Trolox equivalent antioxidant capacity as it contains higher total phenol content. Panico *et al.* (2009) [10] observed that Maletto strawberry samples showed the highest antioxidant activity, at 53.5µmol Trolox equivalents/gm fresh weight for ORAC assay and 78.24 per cent inhibition for DPPH assay. Tudla strawberries achieved a value of 38.4µmol TE/g free weight by ORAC assay and 41.8 per cent inhibition for DPPH assay. Gore (2016) studied antioxidant property of beetroot *(Beta vulgaris)* mixed paneer whey beverage average antioxidant activity TEAC was observed 0.42 and 2.68 (µmol)/mg protein of *paneer* whey and beetroot extract, respectively. Beetroot extract was five times more than the *paneer* whey. Modi *et al.* (2017) [8] reported that the addition of plant-based antioxidants in dairy food has met acceptance for the retardation of oxidation in dairy products. Also, natural antioxidants used to prolong the shelf life of the products. Purkiewicz and Pietrzak-Fiećko (2021) reported that the highest polyphenol content was found (541.95 mg/100 g) and highest content of β-carotene was identified in homemade orange whey beverage (4.36 mg/100 g).

Antioxidant activity of strawberry *paneer* whey beverage

The antioxidant activity of developed fresh strawberry whey beverage was determined. The results obtained in respect antioxidant activity of are presented in Table 2 and graphical presentation in fig 4.

Table 2: Antioxidant activity of strawberry *paneer* whey beverage TEAC (µmol)/mg protein

Replication Treatment	R_1	R_2	R_3	R_4	Mean TEAC (µmol)/mg protein
T_1	0.543	0.529	0.547	0.553	0.543^d
T_2	1.021	1.010	1.028	1.024	1.021^c
T_3	1.259	1.246	1.270	1.277	1.263^b
T_4	1.515	1.495	1.529	1.522	1.515^a
S. E.±0.006					C. D. at 5% 0.018

The values with different small letters superscripts row wise differ significantly at 5% level of significance.

The average antioxidant activity of Strawberry whey beverage were observed 0.543, 1.021, 1.263 and 1.515 TEAC (µmol)/mg protein for treatments T_1, T_2, T_3 and T_4, respectively. All the treatments were significantly (p<0.05) differed from each other. The highest antioxidant activity was recorded for treatment T_4 i.e. 1.515±0.006 TEAC (µmol)/mg protein) prepared by using 10 per cent strawberry pulp. The lowest antioxidant activity was recorded for control treatment T_1 i.e. 0.543±0.06 TEAC (µmol)/mg protein). It was also revealed that as the level of strawberry in the beverage raised, antioxidant activity of the strawberry *paneer* whey beverage was increased.

This might be due to strawberry is rich source of antioxidant compounds i.e. polyphenolic content, anthocyanin Jaster *et al.* (2017) [5] reported that moreover, the presence of anthocyanins in strawberries is connected to the higher antioxidant activity in this fruit. Correlation coefficient between the antioxidant activity (ABTS) and the anthocyanins content for control yogurt, yogurt 1 and 2 was $R^2 = 0.99$, for both, the first and the 7th days of storage. In the same way, for

DPPH method, the correlation between the antioxidant activity and the anthocyanins content for control yogurt, yogurt 1 and yogurt 2, the values obtained were R^2=0.94 and R^2=0.96 for 1 and 7 days, respectively. For both methods used ABTS and DPPH - the antioxidant activity increased when higher quantities of cryoconcentrate ($p< 0.05$) were added in the yogurt. Khan *et al*. (2019) [7] observed that antioxidant activity of milk and dairy products can be enhanced by phytochemicals supplementation. Singh *et al.* (2012) [14] On addition of 0.5 mg⁄g strawberry polyphenol extract to *dahi*, the TPC in water extract of SBPE *dahi* was observed to be 515.24±1.41 g GAE per g compared with 346.72±2.99 g GAE per g for the control *dahi*. The antioxidant activity of SBPE *dahi* was observed to be nearly sevenfold as compared to control *dahi*. Antioxidant activity of control *dahi* corresponding to 30.50±1.00µg. Gore (2016) [3] studied antioxidant property of beetroot *(Beta vulgaris)* mixed paneer whey beverage and noted TEAC of beetroot whey beverage 0.37, 0.85, 0.96 and 0.98 (µmol)/mg protein for T_1 (100:0), T_2 (85:15), T_3 (80:20) and T_4 (75:25); (*Paneer* whey: beetroot extract), respectively. The highest activity was recorded for i.e. 0.98±0.008 TEAC (µmol)/mg protein and the lowest antioxidant activity was recorded for control T_1 was 0.37±0.008 TEAC (µmol)/mg protein and concluded that as the quantity of beetroot extract in the beverage levels up antioxidant activity of the beverage was increased.

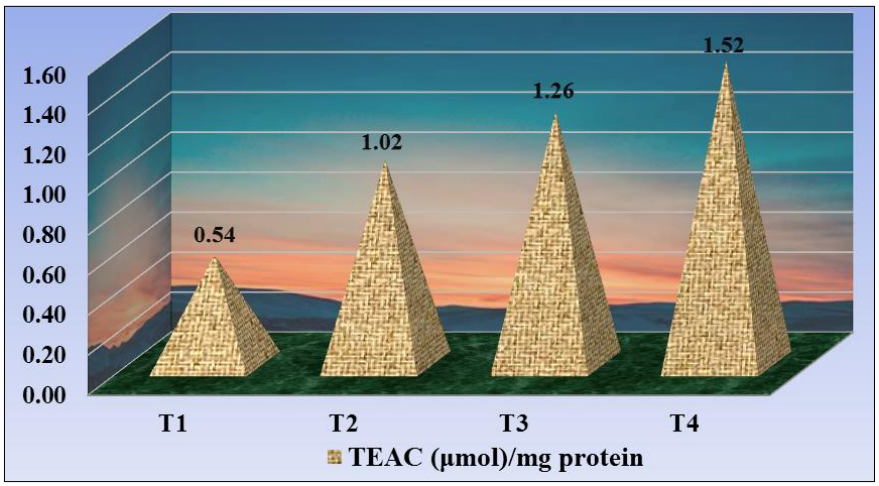

Fig 4: Graphical presentation for Antioxidant activity of Strawberry mixed *paneer* whey beverage

Conclusions

From present investigation it was observed that the strawberry pulp can be used for *paneer* whey beverage on the reason of Antioxidants properties of finished product. It was revealed that Trolox Equivalent Antioxidant Capacity (TEAC) was increased as level of strawberry pulp increases in *paneer* whey.

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