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# Studies on antioxidant properties of kiwi fruit (*Actinidia deliciosa*) pulp mixed *Chakka* whey beverage

**Ranjit Shivaji Rananavare, Dr. Narendra Sudamrao Kamble, Sachin Namdev Waychal and Mohini Bhimrao Shevale**

**Abstract**
*Chakka* whey beverage prepared with kiwi fruit pulp in different proportion of 100:0 ($T_1$), 95:5 ($T_2$), 90:10 ($T_3$) and 85:15 ($T_4$), respectively. The product obtained was subjected for antioxidant properties analysis. On an average the TEAC 0.422, 0.879, 1.259 and 1.515 µmol/mg protein 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 kiwi fruit pulp increases in *chakka* whey.

**Keywords:** Antioxidant properties, TEAC, kiwi fruit, *Chakka*, whey beverage

## Introduction
Fermented milk products are palatable and economical source having wide range of nutrients. The nutrient composition is similar to that in milk, but concentrations of vitamins are in little lower, with the possible exception of folic acid. Fermented milk products have an image of possessing almost magical health-giving properties. The nutritive value of fermented milk compared with natural milk in which digestion of lactose in relation to the benefits of cultured products for lactose-intolerant individuals (Gurr, 1987) [5].

The main dairy products are condensed milk, milk powder, cheese, and butter. Skim milk and butter milk are by products, yet they are used properly. Whey is produced during the production of cheese, casein, *chhana* and *chakka*. On the other hand, it is not used in the human food chain.

During the making of *shrikhand*, a huge quantity of *chakka* is created, particularly in Maharashtra, Gujarat, and Karnataka. As a result, a large amount of *chakka* whey becomes available, either at the household level or at the plant level, which is completely squandered. As a result, whey can be used to make a variety of beverages. *Chakka* whey includes the majority of the lactose and water-soluble vitamins found in milk, as well as a small amount of fat and protein.

Whey is produced in large quantities as a byproduct of the making of *paneer*, *chhana*, *chakka*, and cheese. Pollution due to whey is a serious problem, thus using whey in the manufacturing of beverages, which contains almost all of the nutrients found in milk except casein and fat, is a highly nutritious and healthful solution to cope with the problem. Whey accounts for 45-50 per cent of total milk solids, 70 per cent of milk sugar (lactose), 20 per cent of milk protein, 70-90 per cent of milk minerals, and nearly all of the water-soluble vitamins found in milk (Chavan *et al*., 2015) [3].

Whey is considered to be reliable source of number of high quality and biological active proteins, carbohydrates and minerals. Whey proteins referred to as “fast protein” for its ability to quickly provide nourishment to muscles. Additionally, whey contains variable amount of lactic acid and non-soluble nitrogen. Whey has protective and curative facet to treat against different diseases such as jaundice, infected lesion of skin, gonorrhea, arthritis, anemia, liver complaints, antioxidative action, anti-carcinogenic activity and act against HIV infection (Darade & Ghodake, 2012) [4].

Kiwi fruit or Chinese gooseberry (*Actinidia deliciosa*) is known as ‘China’s miracle fruit, and ‘The Horticultural Wonder of New Zealand’. From China it is spread to New Zealand where it was recognized as a potential fruit and became a popular backyard vine. In India, the area under this fruit is negligible being a new exotic introduction. With extensive research and development support, it’s commercial cultivation in India has been extended to the mid hills of Himachal Pradesh, Jammu and Kashmir. In North East, it is being cultivated in Arunachal

Pradesh in some sizable area but other states like Sikkim, Meghalaya, and hills of Manipur have vast potential for successful cultivation of kiwi fruits. They are highly convinced about the potential and importance of this fruit crop to become commercial in the North-Eastern states (Singh *et al*., 2008) [13].

Kiwi fruit can be considered as highly nutritional product due to its high level of vitamin C and strong antioxidant capacity due to a wide number of phytonutrients including carotenoids, lutein, phenolics, flavonoids and chlorophyll. Based on these characteristics, kiwi fruit offers benefits for specific health conditions and has a great potential for industrial exploitation. Now days, the kiwi fruit derivatives available in the market are mainly represented by semi processed products addressed to the food industry as ingredients or components for ice-cream, yoghurt, cakes and juice blending (Cassano *et al*., 2006) [2].

## Materials and Methodology

The present investigation on "Studies on Preparation of *Chakka* Whey Beverage by using Kiwi fruit (*Actinidia deliciosa*) Pulp" was carried out at the Department of Animal Husbandry and Dairy Science, College of Agriculture, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

### Materials

### Collection of buffalo milk

Buffalo milk was procured from local market of Latur city, of Natural Milk Pvt. Ltd. having 6 per cent fat and 9 per cent SNF.

### Collection of kiwi fruit pulp

Kiwi fruits were collected from fruit market, Latur. During the course of present study while selecting the kiwi fruit, stage of ripening, size, colour and taste were considered so that there should not be any variation in the quality of pulp from the fruit.

### Equipment and accessories

Stainless steel vessels of requisite capacity, muslin cloth, standard weight balance, thermometer, mixer, Gas stove and glass rod, Whatman no.1 filter paper, Knives etc. were used for preparation of whey beverage. Before using this material, it was properly cleaned and washed with detergent solution. All the precautionary measures were taken during the conduct of trials to avoid contamination.

### Chemicals

Analytical reagents (AR) or guaranteed grade (GR) were used in the chemical analysis.

### Packaging material

Glass bottles were used for packaging the prepared beverage and subsequent cold storage refrigerator.

### Methodology

The following procedure were followed during experiment.

### Preparation of *chakka* whey

### Procedure for preparation of *chakka* Whey

The buffalo milk (6 per cent fat and 9 per cent SNF) was heated at 95 ºC for 15 minute and cooled up to temperature 30 ºC. After cooling the standard culture was added in milk at the rate 2 per cent and incubated at 37 ºC for 10-12 hrs. The curd so obtained was tied in muslin cloth and hanged for drain off the whey for 6-8 hrs. The c*hakka* and whey obtained after draining were weighed.

### Preparation of *chakka* whey beverage blending with kiwi fruit pulp

The *chakka* whey was used as base material for preparation of beverage. This whey was mixed with grinded sugar @ 10 per cent by weight of *chakka* and kiwi fruit pulp as per the treatment was added.

*Chakka* Whey
↓
Heat treatment (80 ºC for 15 min.)
↓
Cooling (at room temperature)
↓
Addition of sugar @ 10%
↓
Addition of kiwi fruit pulp (as per treatments)
↓
Filtration
↓
Sterilization
↓
Cooling and storage at 5 ºC

**Fig 1:** Flow chart for preparation of *chakka* whey beverage by using kiwi fruit pulp (Borkar *et al.* 2020) [1]

The kiwi fruit whey beverage was prepared by using method of Borkar *et al.* (2020) [1] with little modifications. The *chakka* whey was heated at 80 °C temperature for 15 min. The sugar added was at the rate 10 per cent by weight of whey, which was maintained in all treatment combinations. Then kiwi fruit pulp was prepared by grinding kiwi fruit in mixer at rpm 1100 for 3 to 4 min. The kiwi fruit pulp obtained 100 per cent of the total kiwi fruit used. The quantity of pulp depends upon quality and moisture of fruits. Then kiwi fruit pulp was added in *chakka* whey as per treatment combinations and simmering at 70 °C and then strained through two-fold muslin cloth. Thus, prepared kiwi fruit whey beverage was packed by filling in glass bottles and bottle pasteurization was done at 63 °C temperature for 30 min.

### Treatment combinations

For preparation of *chakka* whey beverage by using kiwi fruit (*Actinidia deliciosa*) pulp, by adding sugar 10 per cent by weight of *chakka* whey and kiwi fruit pulp as per the treatment combinations were finalized on weight basis as follows:

$T_1$ - 100 Parts of *chakka* whey.
$T_2$ - 95 Parts of *chakka* whey + 5 Parts of kiwi fruit pulp.
$T_3$ - 90 Parts of *chakka* whey + 10 Parts of kiwi fruit pulp.
$T_4$ - 85 Parts of *chakka* whey + 15 Parts of kiwi fruit pulp.

The different levels were tried and compared with control ($T_1$).

### 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) [11]. The procedure followed during present investigation as per the following flow diagram.

ABTS working solution prepare by mixing 88µl of 140mM potassium per sulphate with 5ml of 7 mM ABTS stock solution

↓

Incubate overnight in dark bottles for generation of radicals

↓

Diluted with phosphate buffer saline (PBS) to adjust the absorbance at 734nm to 0.7± 0.02

↓

30µl sample with in cuvette and to this 3ml ABTS working solution and mixed for 5 second.

↓

The decrease in absorbance at 734 nm was recorded over period of 10 min at 10 second interval using multiplate reader

↓

The result were expressed as trolox equivalent antioxidant capacity (TEAC) value

**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.

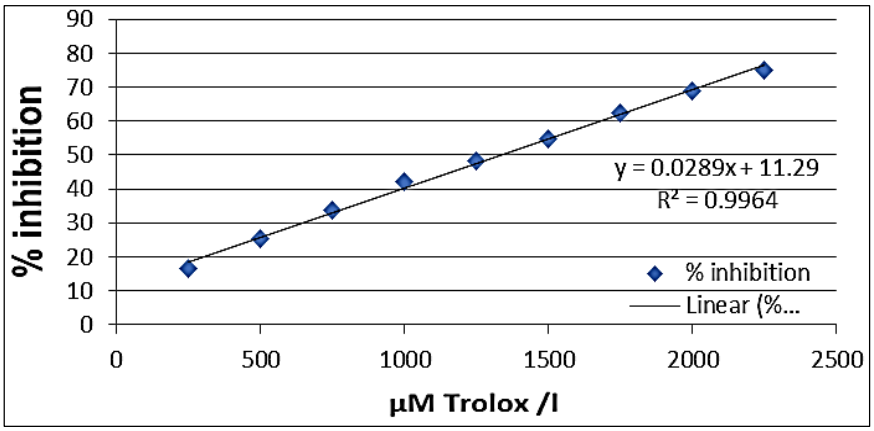


**Fig 3:** Standard Curve of Trolox against ABTS radicals

**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) [9]. The significance was evaluated on the basis of critical difference. In all four trials were conducted.

**Result and Discussion**
**Antioxidant activity of *chakka* whey and kiwi fruit pulp**
The developed kiwi fruit whey beverages samples were subjected for antioxidant activity by ABTS method before evaluation antioxidant activity of whey beverages, kiwi fruit the raw materials used to prepared beverage was subjected for its evaluation of antioxidant activity. The antioxidant activity in the *chakka* whey and kiwi fruit pulp was determined. The average antioxidant activity in TEAC were observed 0.422 (µmol)/mg protein and 1.78 (µmol)/mg protein of *chakka* whey and kiwi fruit pulp, respectively. It was observed that the antioxidant activity in the kiwi fruit pulp was four times more than the *chakka* whey. Antioxidant activity of *chakka* whey and kiwi fruit pulp was presented in table 1.

**Table 1:** Antioxidant activity of *chakka* whey and kiwi fruit pulp TEAC (µmol)/mg protein

| Treatment \ Replication | R1 | R2 | R3 | R4 | Mean TEAC (µmol)/mg protein |
|---|---|---|---|---|---|
| *Chakka* whey | 0.419 | 0.425 | 0.405 | 0.439 | 0.422±0.005 |
| Kiwi fruit pulp | 1.792 | 1.778 | 1.785 | 1.782 | 1.78±0.005 |

The kiwi fruit pulp has higher trolox equivalent antioxidant capacity as it cantain higher phenolic content Pal *et al*. (2015) [8] observed that the DPPH radical scavenging ranged from 16.02 to 64.63 per cent while ABTS radical scavenging inhibition ranged from 16.75 to 66.35 per cent inhibition. Vitamin C (ascorbate) and polyphenols contribute a large portion of the free radical scavenging activities because these are the most effective antioxidants in the fruits and vegetables. Modi *et al*. (2017) [7] reported that the use of plant-based antioxidants in dairy foods has been found to be effective in reducing oxidation in dairy products. Moreover, Purkiewicz & Pietrzak-Fiecko (2021) [10] 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 kiwi fruit whey beverage**
The antioxidant activity of developed fresh kiwi fruit 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 kiwi fruit *chakka* whey beverage TEAC (µ mol)/mg protein

| Treatment \ Replication | R1 | R2 | R3 | R4 | Mean |
|---|---|---|---|---|---|
| T1 | 0.419 | 0.425 | 0.405 | 0.439 | 0.422$^{d}$ |
| T2 | 0.868 | 0.886 | 0.868 | 0.889 | 0.879$^{c}$ |
| T3 | 1.266 | 1.249 | 1.246 | 1.273 | 1.259$^{b}$ |
| T4 | 1.522 | 1.526 | 1.502 | 1.512 | 1.515$^{a}$ |
| S.E. ± 0.006 | | | | C.D. at 5% 0.019 | |

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

The average antioxidant activity of kiwi fruit whey beverage was 0.422, 0.879, 1.259 and 1.515 TEAC (µmol)/mg for treatments $T_1$, $T_2$, $T_3$ and $T_4$ respectively. Table 4.5 indicated that 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 kiwi fruit pulp. The lowest antioxidant activity was recorded for control treatment $T_1$ i.e., 0.422±0.06 TEAC µmol/mg protein). It was also revealed that as the level of kiwi fruit in the beverage increased, antioxidant activity of the kiwi fruit *chakka* whey beverage was also increased. This might be due to kiwi fruit is rich source of antioxidant compounds.
Richardson *et al.* (2018) [12] studied that kiwi fruit are exceptionally high in vitamin C and contain an array of other nutrients, as well as various bioactive components, including a wide range of antioxidants, phytonutrients and enzymes.
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 7$^{th}$ 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) [6] observed that Antioxidant activity of milk and dairy products can be enhanced by phytochemicals supplementation and Singh *et al*. (2012) [14] on addition of 0.5 mg⁄g strawberry polyphenol extract to *dahi*, the TPC in water extract of *dahi* was observed to be 515.24 ± 1.41 g GAE per g compared with 346.72 ± 2.99 g GAE/g for the control *dahi*. Antioxidant activity of control *dahi* corresponding to 30.50 ± 1.00 µg.

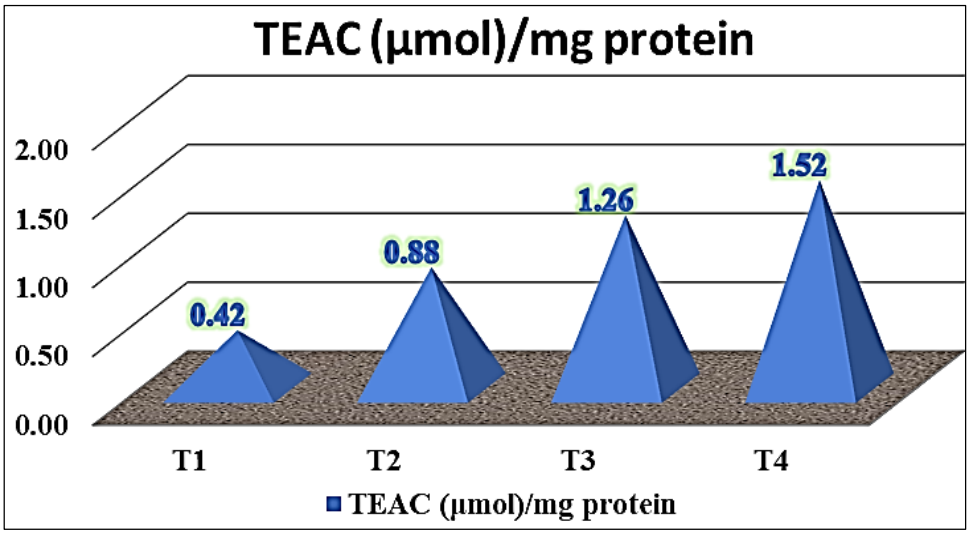


**Fig 4:** Graphical presentation for Antioxidant activity of *chakka* whey beverage by using kiwi fruit pulp

## Conclusions

The developed product's samples were subjected for the evaluation of antioxidant by using ABTS method. The average antioxidant activity of kiwi fruit whey beverage was 0.422, 0.879, 1.259 and 1.515 TEAC µmol/mg for treatments $T_1$, $T_2$, $T_3$ and $T_4$, respectively. All the treatments were significantly ($p$<0.05) differed with 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 kiwi fruit pulp. The lowest antioxidant activity was recorded for control treatment $T_1$ i.e., 0.422 ± 0.06 TEAC µmol/mg protein). It was also revealed that as the level of kiwi fruit in the beverage increases, antioxidant activity of the whey beverage also increased. This might be due to kiwi fruit is rich source of antioxidant compounds i.e., anthocyanin, ascorbate and polyphenolic content. It was revealed that Trolox Equivalent Antioxidant Capacity (TEAC) was increased as level of kiwi fruit pulp increases in *chakka* whey.

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