

# Development of whey based ready to serve (RTS) beverage using *Rhododendron arboretum* extract

Anuradha Dutta, Shweta Suri, Raushan Khan, Nalini Trivedi, Urvashi Sagar and Deepa Tiwari

The aim of the present study was to formulate low cost ready to serve whey based health drink enriched with five ingredients namely, Milk Whey(65%), Buransh (*Rhododendron arboretum*) juice(10%), Honey (25%), Green Tea Extract (Guardian natural extracts- GUARDIAN®) (0.5%), Probiotic culture (Howard HOWARU® Bifido)(2%). The methodology for preparing the RTS beverage comprised of preparing whey, then mixing with *Rhododendron* (Buransh) extract/ juice, honey and green tea extract and pasteurizing the prepared RTS beverage. This step was followed by immediate incorporation of probiotics into the RTS, bottling, capping incubation, and storage under refrigerated conditions. The results of chemical analysis showed that the RTS beverage contains 115.580 calories, 1.28 g protein, 27.75 g carbohydrate, 0.28 g of total fat and 147.44 g calcium per 100 ml. In addition it has a Total Phenolic content of 0.00914 GAE/100g, Flavonoid content of 0.000138mg/100g and DPPH activity of 299.91 mg TE/100g. The RTS beverage has a TSS of 36.6°Brix, pH of 4.65, titrable acidity of 5.4g/l and had insignificant microbial growth on day one. Sensory evaluation of the product revealed that its organoleptic acceptability increased from 43.33% on the first day to 60% on the 7<sup>th</sup> day. Antioxidants from Buransh (*Rhododendron arboretum*) and green tea extract add to the therapeutic value of the RTS Beverage, helping the body to fight against oxidative stress, thereby strengthening the immune system. Unlike white sugar, honey added the benefit of providing antioxidants and a variety of nutrients. The RTS beverage has a shelf-life of 30 days under refrigerated conditions.

**Key Words :** RTS, *Rhododendron arboretum*, Whey, Beverage, Probiotic

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## INTRODUCTION

Ready-to-serve (RTS) beverages offer the advantage of convenience and portability to today's busy consumers. Demand is stronger for nutritious products

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which offer ease of distribution and storage. Whey is a nutrient rich by-product left after cheese making. Whey proteins are an excellent source of  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, bovine serum albumin, caseinomacropptides, immunoglobulins, lactoferrin, and lysozyme which are often associated with health benefits, such as enhanced immunity, anticancer properties, anti-adhesive effect against pathogenic properties, as well as antiviral, antimicrobial (iron binding properties) and anti-hypertensive properties (Gottschalk, 2005; Chavan *et al.*, 2015). There are 2 types of whey, sweet whey and acid whey depending on the method/substances used to coagulate milk. Acidic whey has a pH of 6-7 contains

6.4% total solids, 0.5% protein, 4.4% lactose, 0.6% minerals, and 103mg/100g calcium (USDEC, 2003; Chavan *et al.*, 2015). Whey protein is known for its high biological value as it contains all the 21 amino acids required by humans including the 9 essential amino acids. It is especially rich in leucine and lysine the limiting amino acid of cereals so it can be used to supplement the normal Indian diet.

Ready to serve beverage consisting of acidic whey, Buransh (*Rhododendron arboretum*) extract, honey and probiotic culture can be utilized for treating various diseases Buransh (*Rhododendron arboretum*) is used in various traditional treatments of serious health conditions like heart disease, diarrhea, blood dysentery, fever, inflammation, detoxification, constipation, bronchitis, asthma and tissue regeneration to enhance cell-mediated immunity (Dhar *et al.*, 1968). The flower extract possesses anti-diabetic properties. *Rhododendron arboretum* contains 79.40% moisture, 1.52% crude fat, 2.90% crude fibre, 58% total nitrogen, 1.63% total protein, 12.20% carbohydrates, and 97.70% organic matter. Studies show that the flower offers good amount of phytochemicals of medicinal value including phenols, saponins, xanthoproteins, tannins, flavonoids and coumarins. Three biologically active compounds – Quercetin, Rutin and Coumaric acid have been found in this flower (Saklani and Chandra, 2015). Honey is a potential functional food having antioxidant, anti-inflammatory, and antimicrobial properties (Vallianou *et al.*, 2014). Honey is said to decrease venous blood pressure, which can reduce the preload of the heart and consequently may diminish the congestion in the venous system (Rakha *et al.*, 2008). Phenolic compounds found in honey are known to have antioxidant capacities that can help eliminate or reduce free radicals in the body (Gheldof and Engeseth, 2002). Researches prove its potential to help reduce inflammation (Tonks *et al.*, 2003). Natural honey contains about 200 compounds, including amino acids, vitamins, minerals and enzymes, but it primarily contains sugar and water. Sugar accounts for 95–99% of honey dry matter. The principal carbohydrate constituents of honey are fructose (32.56 to 38.2%) and glucose (28.54 to 31.3 %), which represents 85–95% of total sugars that are readily absorbed in the gastrointestinal tract (Moundoi *et al.*, 2001; Ezz El-Arab *et al.*, 2006). Other sugars include disaccharides such as maltose, sucrose, iso-maltotetraose, nigerose, meli-biose, panose,

maltotriose, melezitose. A few oligosaccharides are also present. Honey contains 4 to 5% fructo-oligosaccharides, which serve as probiotic agents (Chow, 2002; Ezz El-Arab *et al.*, 2006).

## METHODOLOGY

### Procurement of ingredients for preparing RTS beverage:

AmulTaza tetra pack milk was procured from the local market of G.B. Pant University of Agriculture and technology, Pantnagar. Ready-made *Buransh* (*Rhododendron arboretum*) extract/ Juice was used because of unavailability of fresh flowers. The *Buransh* tree flowers only in the spring season. For preparation of RTS beverage, *Buransh* (*Rhododendron arboretum*) extract/ juice was obtained from various commercial sources (*viz.*, Jaunsar fresh *Buransh* juice, *Rhododendron*, by Theo Organics and Kanak fresh *Buransh* juice) All these variations were tested during product development. The best results were obtained by using Kanak fresh *Buransh* squash thus it was selected for incorporation in the final product. Honey was procured from two different sources namely; Dabur and Hill honey. After initial trials, latter was selected for the incorporation in the final product as it is a local produce of Uttarakhand Himalayas. The fourth ingredient of the beverage was the Dupont Guardian natural extracts- GUARDIAN® Green tea extract. The freeze dried and *Bifidobacterium lactis* probiotic strain (Howard premium probiotics HOWARU® bifido) was obtained from Dupont Danisco, Mumbai, Maharashtra.

### Process of whey preparation:

Milk was heated to 82°C in a stainless steel container on a gas stove. 2% citric acid solution was prepared by taking 2 g food grade citric acid in a 100 ml volumetric flask and its volume was made up by distilled water. Citric acid solution was gradually poured into the milk with continuous stirring until casein coagulated and clear whey was obtained which was filtered through a muslin cloth. Citric acid solution was added at a rate of 54ml per liter of milk. The obtained whey was put back on the stove to make it more concentrated; thereby enhancing its physical properties. The whey volume was reduced to half its original amount by this process. On reaching the desired concentration the whey was re- filtered using a double layered muslin cloth in order to remove remaining

sediments. The whey was cooled and refrigerated at 4°C for further use. It was heated to 60°C when used afterwards.

### Probiotic beverage formulation trials:

Different trials were done for formulation of final RTS beverage. After five trials and numerous formulations, the final product was standardized with the following ingredients in the given proportions; (Whey- 65%, Honey- 25%, Burash Juice- 10%, GUARDIAN® Green tea extract- 0.5%), respectively. All the ingredients were mixed together in the above given proportions. For mixing first honey was added to the whey followed by *Buransh* Juice and Green tea extract. All the ingredients were mixed well in a big steel vessel. RTS Beverage was then pasteurized using the Batch or vat pasteurization method with slight modifications. The modification was that a double boiler was used to maintain the required temperature. The Beverage was heated to 154.4 degrees Fahrenheit (63 degrees Celsius) in a large container and held at that temperature for 30 minutes. The next step was rapid cooling that was done by placing the pot in ice cold water (Cazaux, 2009).

Probiotic culture was activated by mixing the required weight of probiotic granules in a small amount of the RTS beverage in a sterilized container. This mixture was then transferred immediately into the rest of the RTS beverage and mixture was stirred together using sterilized ladle. The mixture was filtered once again for proper homogenization. The beverage was now bottled as well as capped and left for 10 hours at room temperature for incubation. The prepared beverage was stored in refrigerated conditions at temperature less than equal to 4°C and used for further analysis.

### Nutritional analysis:

The nutritional parameters of RTS beverage was analyzed using (AOAC, 2000) method.

### Shelf life analysis:

The three parameters taken for estimating the shelf life of RTS beverage were pH, Titrable acidity and Total microbial count. The pH of the beverage was examined using the digital pH meter (Model No. CP 901). Titrable acidity was calculated by using standard method given by Pearson (1991). The total microbial count *viz.*, total plate count on successive period of 0 and 7<sup>th</sup> day was assessed

using method (APHA, 1992).

### Estimation of total soluble solids:

The total soluble solids in beverage was determined by using digital hand Refractometer (Model No. PS042 080910000NP).

### Antioxidant activity of beverage:

The antioxidants present in the RTS beverage was determined. Total phenol content (Singleton *et al.*, 1999), Total flavonoid content (Zhishen *et al.*, 1999) and DPPH scavenging activity (Brand *et al.*, 1995) of beverage was examined.

### Sensory evaluation:

RTS beverage was evaluated for sensory quality by a semi trained panel of fifteen members using Nine point Hedonic scale and Sensory score card method (Amerine *et al.*, 1965).

### Statistical analysis:

Statistical methods described by (Snedecor and Cochran, 1977) were used to examine the data. Mean was used to describe the results of the study.

## OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

### Standardization of RTS beverage:

RTS beverage shown in Fig. 1 was optimized by conducting various trials which were as follows:

In the first trial proposed ingredients were used *i.e.*



Fig. 1 : Image showing whey based RTS beverage enriched with *Rhododendron arboretum*

75% whey prepared from Aanchal Toned polypack milk, 45% Dabur honey, 05% Buransh Juice extract, 1.3% green tea extract and 0.2 % probiotics. Sensory evaluation of the product revealed that The RTS beverage had a very bland flavor and weak consistency (Table 1). In addition the drink exhibited an artificial flavor probably due to the Buransh extract.

In the second trial the percentage of ingredients was changed and sensory evaluation was repeated. Weak consistency and synthetic flavor was still prevailing and sweetness quotient was high. The RTS Beverage left a grainy mouth feel after consumption due to solid remnants present in the whey extract. This problem was overcome by using a double layered muslin cloth.

In the third trial the Aanchal milk was replaced with AmulTaza milk and Jaunsar Fresh Buransh was replaced by Theo Organics *Rhododendron* extract. Colour of prepared RTS beverage was not acceptable, it gave a brownish appearance. Sensory evaluation revealed that the RTS beverage was over sweet.

In the fourth trial, Dabur Honey was replaced by sunflower Hill Honey as the former was found to have more sweetness. In addition Hill Honey is a local produce of Uttarakhand. And Kanak Buransh Juice was selected for further beverage preparation. This time the colour observed was highly acceptable but the product was still

on the sweeter side.

In the fifth trial percentage of Honey was reduced by 5% and this resulted in highly acceptable organoleptic attributes. Thus after five trials and numerous formulations the final RTS beverage was standardized with the following ingredients as shown in the Table 1.

The final RTS beverage was prepared using 65% whey, 10% buransh juice/ extract, 25% Honey and 0.5% green tea extract inoculated with 0.2% Bifido bacterium.

### Nutritional analysis:

The data for nutritional content of RTS beverage is shown in Table 2. The formulated RTS beverage contains good amount of calories (115.80 calories per 100 ml) and calcium (147.44 g calcium per 100 ml) content.

Singh *et al.* (2014) showed protein content of 0.357% in the whey based beverage containing guava pulp. The protein content of present whey based RTS beverage came out to be 1.28% which was higher than that reported by Singh *et al.* (2014). High protein content of present beverage is due to the good protein content of whey. Also whey proteins are of greater value than several other sources of animal proteins (Devraj, 2005).

### Shelf life estimation of RTS beverage:

The data for shelf life evaluation is shown in Table

**Table 1 : Standardization of RTS beverage**

Ingredients	Percentage				
	First Trial	Second Trial	Third Trial	Fourth Trial	Fifth Trial
Whey	75% (Prepared from Anchal Toned polypack milk)	75% (Prepared from Anchal Toned polypack milk)	70% (Prepared from AmulTaza Tetra pack milk)	65% (Prepared from AmulTaza Tetra pack milk)	65% (Prepared from AmulTaza Tetra pack milk)
Honey	45% (Dabur)	40% (Dabur)	35% (Dabur)	30% (Hill Honey)	25% (Hill Honey)
Buransh ( <i>Rhododendron</i> )	05% (Jaunsar Fresh Buransh Juice)	05% (Jaunsar Fresh Buransh Juice)	05% (Theo Organics <i>Rhododendron</i> extract)	5 % (KanakBuransh juice)	10 % (KanakBuransh juice)
Guardian natural extracts- GUARDIAN® Green Tea Extract	1.3%	1.0%	0.8%	0.5%	0.5%
Probiotic culture (Howard Premium probiotic) - HOWARU® Bifido)	0.2%	0.2%	0.2%	0.2%	0.2%

**Table 2 : Nutritional parameters of RTS beverage**

Nutritional information (Per 100 ml)	
Energy (Kcal)	115.80
Protein (g)	1.28
Total Carbohydrates (g)	27.75
Total fat (g)	0.28
Calcium (g)	147.44

3. The RTS beverage was found to have 4.65 pH, 5.4g/L titrable acidity and total plate count was too less to count on day one. On the basis of the results it can be assumed that the shelf life of our beverage is 30 days when stored under refrigerated conditions  $5 \pm 1^\circ\text{C}$ . Once beverage is opened it should be consumed within 24 hours. The shelf life of beverage is 24 hours at room temperature ( $30 \pm 1^\circ\text{C}$ ).

The values obtained in the present study was compared with the study of (Shukla *et al.*, 2013) which showed that the mean value of pH for fermented whey alone ranged from 4.82-3.30 and the mean value of pH obtained in case of whey-pineapple juice blend ranged from 4.36-3.87. Also (Shukla *et al.*, 2013) showed that RTS beverage prepared by 65:35 blend of whey and pineapple juice inoculated with 1 per cent inoculum of *Lactobacillus acidophilus* has a shelf life of 24 d at  $5 \pm 1^\circ\text{C}$  and 48 hrs at  $30 \pm 1^\circ\text{C}$ . These values were found to be comparable to the present study.

#### Total soluble solids ( $^\circ\text{Brix}$ ):

The total soluble solids of RTS beverage was 36.6  $^\circ\text{Brix}$  which was within the parameters given by FSSAI permissible limits for preparing RTS beverage *i.e.* TSS should not be less than 10  $^\circ\text{Brix}$ . A study done by (Singh *et al.*, 2014) showed TSS of 20  $^\circ\text{Brix}$  in a probiotic

beverage prepared by 67.5% whey and 20% guava pulp.

#### Antioxidant activity:

The values for antioxidants present in the RTS beverage is reported in Table 4. The results showed that RTS beverage exhibited good DPPH radical scavenging activity (299.91 mg TE/100g) in the direct refrigerated beverage whereas incubated beverage showed 246.45mg TE/100g of DPPH scavenging activity.

The total phenol content was found to be comparably high in direct refrigerated RTS Beverage *i.e.* 0.009141175mg GAE/ 100g whereas and total flavonoid content was low in direct refrigerated beverage and high in the incubated beverage *i.e.* 0.0001472mg RE/100g.

#### Sensory evaluation of RTS beverage (Nine Point Hedonic Scale):

The results of sensory evaluation (Nine Point Hedonic Scale) are given in the Table 5. The sensory evaluation of the product using the Nine Point Hedonic Scale (Amerine *et al.*, 1965) showed that on Day 1, RTS beverage was liked moderately by 43.33 per cent of consumers whereas 20 per cent of consumers liked the beverage extremely. The results of Day 7 showed increase the organoleptic acceptability of RTS beverage *i.e.* 60 per cent consumers liked the beverage moderately

**Table 3 : Shelf life evaluation of RTS beverage on day 1**

Parameters	Unit	Value
pH	-	4.65
Titrable acidity	g/L	5.4
Total viable count	CFU/ml	Insignificant number of colonies

**Table 4 : Antioxidant activity of RTS beverage**

Antioxidant parameters	Unit	Incubated RTS beverage	Direct refrigerated RTS beverage
TPC (Total Phenolic content)	mg GAE/100g	0.0006909	0.009141175
TFC (Total Flavonoid content)	mg RE/100g	0.0001472	0.0001380
DPPH	mg TE/100g	246.45	299.91

**Table 5 : Sensory evaluation (Nine point hedonic scale)**

Beverage	Liked extremely	Liked very much	Liked moderately	Liked slightly	Neither liked nor disliked	Disliked slightly	Disliked moderately	Disliked very much	Dislike extremely
Day 1	10%	20%	43.33%	23.33%	3.33%	-	-	-	-
Day 7	10%	30%	60%	-	-	-	-	-	-

**Table 6 : Sensory evaluation by using score card method**

Beverage	Taste	Colour	Flavour	Aroma	Mouthfeel	Sweetness	Aftertaste	Overall acceptability
Day 1	7.51	7.38	7.73	7.83	7.7	7.93	7.8	7.83
Day 7	7.85	7.75	7.8	7.85	8.10	7.65	7.8	7.9

and 20 per cent liked extremely. This might be due to activation of the probiotic culture leading to desirable change in the sensory qualities of the beverage. The overall acceptability of this beverage was found to be high.

### Sensory evaluation of RTS beverage using score card method:

The data for sensory evaluation of RTS beverage using Score Card Method is shown in Table 6. The results showed that the overall acceptability of RTS beverage increased from 7.83 on Day 1 to 7.9 on Day 7 of sensory evaluation.

The present RTS beverage was compared with the probiotic beverage containing whey and pineapple juice (Shukla *et al.*, 2013). The study showed that 65:35 blend ratio of whey and pineapple juice fermented for 5 hr showed anticipated results with maximum sensory scores for overall acceptability and a total viable count of more than  $10^6$  cfu.ml<sup>-1</sup>. Another study done by (Singh *et al.*, 2012) showed that a beverage which was prepared using sugarcane juice and curd was preserved and packed in 200 ml glass bottles and kept for different storage periods (0, 5, 15, 20 days) and it was found that beverage containing 4:1 proportion of sugarcane juice: curd was superior after 15 days of storage.

### Conclusion:

The formulated whey based RTS beverage is a nutrient dense product full of bio active compounds that helps to double up as a health drink. The ingredients used for preparation were found to have high nutritional value and functional properties. The prepared RTS beverage contains 65% whey, 10% buransh juice/ extract, 25% Honey and 0.5% green tea extract inoculated with 2% *Bifido* bacterium. The RTS beverage has a shelf life of 30 days under refrigerated conditions  $5 \pm 1^\circ\text{C}$  and 24 hours at room temperature  $30 \pm 1^\circ\text{C}$ . The formulated RTS beverage contains 115.580 calories, 1.28 g protein, 27.75 grams carbohydrate, 0.28 g of total fat and 147.44 g calcium per 100 ml. In addition it has a Total Phenolic content of 0.00914 GAE/100g, Flavonoid content of 0.000138mg/100g and DPPH activity of 299.91 mg TE/100g. The formulated RTS beverage showed a good overall acceptability as a health drink. It can be recommended for athletes and sports persons because of good quantity and quality of whey proteins. It can also

be suggested as a healthy alternative to the empty calorie carbonated beverages. The good antioxidant quality of the beverage makes it an attractive alternative to fight oxidative stress.

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