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Physico-chemical and shelf-life study of low calorie ready to reconstitute (RTR) health beverage containing bioactive constituents

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Abstract
The current investigation was carried to determine the physico-chemical properties of the developed ready to reconstitute (RTR) health beverage powder which contains functional ingredients like whey proteins, tea, essential oil like almond and walnut oil, and a low-calorie sweetener, stevia. Further, the RTR was studied for its shelf-life at room temperature for its acceptability. It was found that, the RTR showed an acidity, pH, bulk density, and solubility index of 0.15% LA, 6.4, 0.59 g/ml and 0.603 cm, respectively whereas moisture content and total solids content was around ~4.35% and ~95.65%, respectively. The shelf-life study based on overall acceptability, FFA value, titratable acidity and microbiological analysis revealed that, the RTR health beverage powder was acceptable up to 90 days of storage period at room temperature (27±1 °C). Hence, including this RTR health beverage powder in diet would provide essential nutrients for the individuals on diet control and are looking for better alternative for regular sugar-sweetened tea beverage.

Keywords: Coffee, essential oil, health beverage, ready to reconstitute, shelf-life, tea, whey proteins

Introduction
Lately it has been observed that there is a tremendous increase in consumer adaptation towards healthy diet with the inclusion of better, new, innovative, and functional food products that would not only meet our convenience but also aids us with health benefits. Further utilization of many functional ingredients in our daily diet has become the latest trend towards better health. One of the major dairy waste, whey from products like cheese, paneer is difficult to discard into environment as they have higher values of biological oxygen demand and pose as an environmental issue for its disposal. Hence, utilization of this waste by-product has increased in various functional products like whey-based beverages, sports drink, fruit flavoured drinks, health beverages etc, addition of whey powders in confectionaries, bakery foods, etc. The whey contains about ~6% TS which includes 45-50% of total milk solids, 70% of milk sugar (lactose), 20% of milk proteins (particularly whey proteins, α- lactalbumin and β- lactoglobulin) and 70-90% of milk minerals and most importantly, almost all the water-soluble vitamins originally present in milk (Ozen and Kilic, 2009) [26]. Apart from nutritional significance, whey proteins are also known for containing many potential bioactive peptides where many clinical studies have proven their positive health impact such as antidiabetic, insulinotropic, antioxidant potential, immunomodulating, antiviral and antimicrobial effects, anticancer and anti-ulcer activity, and protect the cardiovascular system etc.
Sugar-sweetened beverages has proven to aid negative effects on health. Hence, cohort studies have been done to find best natural alternative to table sugar without compromising the taste and any harmful effects. Also, consumers are now looking for products which contain natural added ingredients and one such natural sweetener is stevia. The stevia is known to be a zero-calorie sweetener and tastes 300 times sweeter than sucrose and is safe with an ADI of 4 mg/Kg body weight (JEFCA). The leaves of stevia also contain about 6.2% protein and 5.6% lipid on a dry-weight basis, as well as diterpene, triterpenes, sterols, flavonoids, and other compounds (Kuntz *et al.*, 2010) [40]. Stevia has been used in many low calorie, low fat, or reduced fat products such as in beverages, confectionaries, bakery products etc. in dairy products, stevioside has been used in the manufacture of acidophilus milk and results showed that stevioside was able to impart a pleasant, sweet flavour and displayed good functional properties including good solubility in milk, no inhibitory activity against LAB and storage

stability. Addition of 0.075% of refined stevia extract powder could completely replace sugar in production of flavoured milk (Kostina, 2003, Tandel, 2011, and Ena *et al.,* 2013) [20, 34, 10]. Many studies have shown the therapeutic properties of stevia such as anti-hyperglycaemic, anti-inflammatory, anti-tumour, diuretic, immunomodulatory, antimicrobial, antihypertensive, anticancer, anti-obesity etc. (Chen *et al*., 2005, Aghajanyan *et al.,* 2017, and Lanestosa *et al.,* 2017) [7, 2, 6] Stevia leaf extract is used traditionally to treat diabetes. Ingestion of stevia resulted in decrease in plasma glucose level by acting directly on β-cells to secret insulin without effecting the K+ -ATP channel and cAMP level in islets of pancreas (Ena *et al.,* 2013) [10].

Essential oils rich in unsaturated fats i.e., polyunsaturated fatty acids and monounsaturated fatty acids are very crucial as they provide many positive benefits towards cardio-vascular health. According to American diabetes association (2017), intake of unsaturated fat, especially monounsaturated fatty acid (MUFA) and poly monounsaturated fatty acid (PUFA) has been liberalized in diabetic diets to increase HDL cholesterol and improve glycemic control. Nuts, by virtue of their fat and protein content, may also depress postprandial glycaemia and hence ROS production (Kim *et al.,* 2017) [19]. Many scientific studies have shown the beneficial effect of dietary intake of nuts on blood cholesterol level mainly due to its fatty acid profile. It has been reviewed that consumption of nuts which are high in fibre, unsaturated fats and micronutrients reduces incidence of both fatal and nonfatal ischemic heart diseases (Lovejoy *et al.,* 2002 and Gulati *et al.,* 2017) [23, 13]. Both walnut oil and almond oil are known for the presence of PUFA and MUFA, respectively, which can have a positive impact on people with health ailments and these two oils have evidently proved to provide many health benefits to cardio-health (Saxena *et al.,* 2009, Talukdar *et al.,* 2010, Sayed, 2011, Oh *et al.*, 2015, Gannon, 2015, and Coelho *et al.,* 2016) [29, 33, 9, 25, 11, 8].

Milk-based drinks are among the beverages with the longest history of consumption. Regular consumption of milk by adults occurred only after our ancestors started herding cattle. Milk-based beverages are liquid, processed milk products. They are mixtures of milk or milk powder with water added with additives for example colorants, flavours, acids, functional ingredients, fruit mixes/juices, sugar, and preservatives. The milk-based beverages market is still a niche market. However, there have been many innovations and currently it is one of the fastest growing dairy segments (Paquin, 2009) [27]. Tea and coffee beverage has always been part of our diet and the domestic consumption is rising expeditiously (Bhansal, 2017) [4].

Tea is generally classified into unfermented tea (green tea, white tea), semi-fermented tea (oolong tea) and fully fermented tea (black tea and puerh tea). 100 gm of tea contains sodium (4 mg), potassium (18 mg) and caffeine (11mg). It also contains micronutrient *viz*. boron (B), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn) (Suzuki *et al.,* 2016) along with polyphenols (around 45.82 mg/g dried leaf) like Catechin (~1.42 mg/g) Gallic acid (~2.73 mg/g) Total the aflavins (~3.52 mg/g) Caffeine (~36.61 mg/g Epigallocatechin gallate (~43.48 mg/g) with 5.85 - 6.40 pH (Shii *et al.,* 2011) [30]. Tea is known for its potential antioxidant property. Basu *et al.* (2013) [5] reported that the mechanism involved is due the antioxidant activity of tea components such as catechin and gallic acid by directly acting on reactive oxygen species, scavenging free radicals, chelating metal ions in liver, and by increasing the anti-oxidases such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and inhibiting plasma protein carbonylation induced by hyperglycemia.

Gyreling *et al.* (2014) [12] reviewed that high dietary flavonoid intake has been linked to a reduced risk of CVD, possibly through effects on BP and endothelial function which support a clear link between black tea consumption and a reduced risk of CVD.

Coffee is one of the most widely consumed beverages globally. Different amounts of coffee are habitually consumed to different countries or by people. The consumption of coffee has been associated with several health benefits, including a reduced risk of diabetes and cardiovascular disease. Clinical and epidemiological studies have found the consumption of coffee to be associated with risk reductions for the development of diabetes, Alzheimer's disease, several cancers, liver diseases and cardiovascular disease due to its bioactive constituents, which include phenolic compounds, caffeine, diterpenes, melanoidins, trigonelline, chlorogenic acids, cafestol, and EGCG (Shii *et al.,* 2011, Zhang *et al.,* 2011, Mellbye *et al.,* 2015, Yoshinari and Igarashi, 2015 and Loader *et al.,* 2017) [30, 37, 24, 35, 22].

Hence, an attempt has been made to develop a low calorie ready to reconstitute (RTR) health beverage rich in functional ingredients such as whey, almond oil, walnut oil, tea, coffee decoction with addition of natural sweetener stevia using double toned milk.

Materials and Methods

The raw material such as table sugar, stevia powder, almond and walnut oil of good quality was procured from local market. Fresh cheddar cheese whey was prepared and was used in preparation of ready to reconstitute (RTR) health beverage. The RTR health beverage (powder form) was prepared by admixing double toned milk (1.5% milk fat and 9.0% SNF) and sweet whey in 70:30 ratio. The table sugar was completely replaced by bio-sweetener (stevia), 2% of equal blend of almond, and walnut oil and 15% tea &/or coffee decoction was added. The health beverage was homogenized (I stage-2500 psI & II stage-500 psI), HTST pasteurized and then concentrated to 31% total solids and then spray dried at inlet and outlet temperature of 180-190 °C & 80-90 °C with 1.4 bar air pressure and 570 mmHg vacuum. The obtained spray dried powder was packed in plastic pouches for further studies at room temperature. The control beverage was prepared using 8% table sugar. This research study was carried out in the Students Experimental Dairy Plant of Dairy Science College, KVAFSU, Bengaluru, Karnataka.

Physico-chemical analysis

The pH was measured using a digital pH meter (ElicoPvt. Ltd.) At 25 °C. About 20 ml of representative samples were used. The standard method mentioned in ISI: SP 18 (Part XI) 1981 was followed for estimation of moisture, total solids, lactose (Lane eynon method), fat (Gerber method), protein (Micro-Kjeldhal protein), ash, titratable acidity, bulk density, and solubility index. The free fatty acid (FFA) as % oleic acid and titratable acidity as % lactic acid content of experimental samples were determined by following the procedure of IS SP 18 Part (XI) 1981.

Microbiological analysis
Total bacterial count, coliform count and yeast and mold count was determined by method as per ISI: SP 18 (Part XI) 1981 using SPCA, VRBA and MEA (pH adjusted), respectively.

Sensory analysis
The experimental samples were given to a panel of five judges for sensory evaluation with standard score card of a total of 9 Point Hedonic for overall acceptability. The scores given by panel of judges were then statistically analysed. The samples were code numbered to avoid identification and bias (Pimentel *et al.,* 2016) [28]

Shelf-life study
The experimental samples were evaluated for its shelf life at room temperature (27±1 °C) in transparent plastic pouches. The stored samples were drawn and analysed for sensory, chemical, and microbiological parameters.

Statistical Interpretation
The design of experiment was statistically analysed. Results are the average of three replications which was statistically analysed by subjecting to statistical analysis (R Programme, R-Version, Ri386 3.4.3) for accurate interpretation that helped in discussion (Zar, 2003) [].

Results and Discussion
The physico-chemical composition of RTR health beverage
The various physico-chemical characteristics of dried form of control beverage & RTR health beverage is depicted in Table 1. It can be observed from the results that, the developed RTR health beverage powder (B_1 & B_2) and control were significantly comparable in terms of acidity. Statistically (*P*=.05), the control beverage showed lower pH & solubility index and higher bulk density in comparison with B_1 & B_2 (Table 1). The chemical compositional analysis of experimental samples revealed that B_1 & B_2 samples were significantly different from control in terms of protein, fat, carbohydrate, and ash content. The percent of total solid in all three samples were similar however the fat content was 3.5 times higher in B_1 & B_2 than in control which is due to addition of almond and walnut oil blend in B_1 & B_2 whereas control was prepared using double tone milk.
It was also recorded that, the protein (addition of whey) and ash content (addition of whey, tea & coffee decoction) has increased and total carbohydrate content decreased in B_1 & B_2 which can be directly attributed with the addition of bio-sweetener, stevia than addition of sugar in control. These findings are supported by Takami *et al*. (2016) [32] were spray dried milk powder containing 0.7% cocoa powder and 5% banana concentrate had a moisture content of 2.45%, lactose - 47.6% and fat -15.25%, respectively. Furthermore, this can be significantly correlated with lower glycemic index of the health beverages which is supported by Arora and Suhani (2015) [3] were RTS beverages added with 10 mg stevia showed glycemic index ranging from 25 – 35; which proves that developed RTR health beverages comes under low glycemic food classification.

Table 1: Physico-chemical characteristics and composition of control and RTR health beverage

Parameters	Control	B_1	B_2	CD value (*P*=.05)
Physico-chemical attributes				
Acidity (% LA)	0.14	0.15	0.15	2.15
pH	6.4^b	6.5^b	6.6^a	0.036
Bulk density (g/cm^3)	0.77^a	0.59^b	0.53^b	0.02
Solubility index (cm)	0.546^b	0.603^b	0.676^a	0.046
Chemical Composition (%)				
Total solids	96.41^a	95.65^a	95.80^a	1.22
Moisture	3.55^b	4.35^a	4.20^b	0.32
Fat	8.08^b	28.56^a	28.27^a	0.84
Protein	18.71^b	29.13^a	30.31^a	3.37
Total sugar	65.90^a	31.51^b	30.31^a	1.22
Ash	3.53^b	6.45^a	6.64^a	0.809

(**Note:** All values are average of three trials; Superscripts a & b indicate significance difference at the corresponding critical difference;
Control: Double toned milk with 8% sugar, B_1 and B_2 – RTR health beverage containing tea and coffee decoction, respectively)

Effect of storage on overall acceptability of RTR health beverage at room temperature (27±1 °C)
Statistically no significant difference was observed between the experimental samples till 45 days of storage. A significant difference in the scores for overall acceptability was observed from 45th days till 90th days of storage between control, B_1 and B_2 sample. The sensory scores for control, B_1 and B_2 from 45th day till 90th day ranged between 7.83 to 7.85, 8.41 to 7.90 and 8.45 to 7.95, respectively. However, no significance difference was observed between B1 and B2 during 90 days of storage period which may be due to unacceptable changes in the product as reported by panel of judges such as slightly off & oxidized flavour. Further, another observation noted by judges is that RTR health beverage showed caking defects after 60 days of storage period. These results were comparable with the results obtained by Kumar *et al*. (2010) [21] where they developed Ready-to-Reconstitute functional beverages by utilizing cheddar cheese whey protein hydrolysates and probiotics.

Table 2: The effect of overall acceptability of reconstituted RTR health beverage during 90-days storage period

Sample	Titratable acidity (% Lactic acid)						
	0	**15**	**30**	**45**	**60**	**75**	**90**
Control	8.93	8.66	8.56	7.83^b	7.70^b	7.62^b	7.55^b
B_1	9.00	8.75	8.64	8.41^a	8.16^a	8.11^a	7.90^a
B_2	9.00	8.85	8.77	8.45^a	8.33^a	8.15^a	7.95^a
CD (*P=0.05*)	0.09	0.28	0.12	0.13	0.37	0.45	0.25

Effect of storage on chemical changes in dried RTR health beverages stored at room temperature (27±1 °C)

The chemical analysis of dried RTR health beverage was carried out for a period 90 days and sample were drawn for analysis at an interval of 15 days and the results pertaining to chemical analysis are tabulated (Table 3). The FFA value for RTR health beverage powder ranged from 0.52 to 0.98% oleic acid. However, an increase in the FFA value was observed which could be due to the higher fat content of dried RTR health beverage. Further, the titratable acidity was found to acceptable during the entire storage study. The titratable acidity for control ranged from 0.14 - 0.18% lactic acid while 0.15 - 0.17% lactic acid for B_1 and B_2, respectively during period of 90 days storage (Table 3). These findings were similar to the results of Paez *et al*. (2006) [39] where, when the whole milk powder was stored for period of 15 months, the FFA value increased from 951.68 to 628.92 mg/kg powder, respectively at room temperature. These observations were akin with findings of Cristina *et al*. (2008) [38] were whole milk powder when stored at room temperature did not change acid degree value (FFA value) initially from 1.6 cm^3 NaOH to 100 g fat up to 3 months of storage however a significant decrease in titratable acidity was noted from 0.12 to 0.10% LA after 3 months of storage period.

Table 3: Effect of storage on titratable acidity and free fatty acid value of dried RTR health beverages

Sample	Titratable acidity (% Lactic acid)						
	0	**15**	**30**	**45**	**60**	**75**	**90**
Control	0.14	0.14	0.15^b	0.16	0.16	0.17^a	0.18
B_1	0.15	0.15	0.16^a	0.16	0.16	0.17^b	0.17
B_2	0.15	0.15	0.16^a	0.16	0.16	0.17^b	0.17
CD (*P=0.05*)	0	0	0	0	0	1.14	1.15
Free fatty Acid (% Oleic acid)							
Control	0.61^a	0.64^a	0.66^a	0.70^a	0.76	0.84	0.98
B_1	0.57^b	0.62^a	0.63^b	0.68^b	0.74	0.85	0.95
B_2	0.52^b	0.60^b	0.62^b	0.67^b	0.74	0.84	0.94
CD (*P=0.05*)	0.02	0.03	0.01	0.01	0.03	0.03	0.05

(**Note:** All values are average of three trials; Superscripts a & b indicate significance difference at the corresponding critical difference; Control: Double toned milk with 8% sugar, B_1 and B_2 – Dried RTR health beverage containing tea and coffee decoction, respectively)

Effect of storage on the microbiological quality of dried ready to use health beverages stored at room temperature (27±1 °C)

The results pertaining to the microbiology quality of control and dried RTR health beverage samples with respect to total bacterial count, coliform and yeast and mold counts were enumerated once in 15 days interval for a period of 90 days which were packed in plastic pouches and stored at room temperature (Table 4). The total bacterial count for control, B_1 and B_2 on day of production were 0.69, 65 and 0.60 log_{10} cfu/g stored at room temperature. The counts ranged from 2.68 to 3.52 log_{10} cfu/g from 15^{th} day to 90^{th} day of storage whereas coliform, yeast and molds counts were nil for control, B_1 and B_2 from day 1 till the end of 90 days of storage period. The dried RTR health beverage (B_1 and B_2) showed lower microbial count which could be due to less moisture content resulting in lower water activity and can be correlated with the evident antimicrobial effects of various bioactive ingredients added such as stevia, tea, and coffee.

Table 4: Effect on storage on microbiological quality of RTR health beverage

Sample	Total Bacterial Count (log10 cfu/ml)						
	0	**15**	**30**	**45**	**60**	**75**	**90**
Control	0.69	2.68	2.78	2.81	3.10	3.22	3.37
B_1	0.65	2.82	2.99	3.18	3.16	3.38	3.52
B_2	0.60	2.88	2.92	3.11	3.52	3.36	3.45

(**Note:** All values are average of three trials; samples were stored at room temperature i.e., 27 ±1 °C; Coliforms and yeast and molds counts were found to be nil; Control: Double toned milk with 8% sugar, B_1 and B_2 – Dried RTR health beverage containing tea and coffee decoction, respectively)

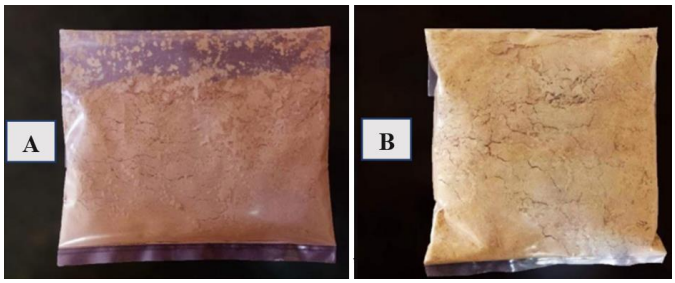

Fig. 1: Experimental sample for storage studies packed in plastic pouches where A) Ready to Reconstitute health beverage containing tea B) Ready to reconstitute health beverage containing coffee

Conclusion

This research findings have shown that the developed RTR health beverage powder has optimal sensory properties along with better functional properties and shelf-life with better consumer acceptability. The product developed can become a potential commercial product in beverage market proven to its low calorie and packed with essential nutrients. However, further studies are required to elucidate its potential health significance, *in-vivo* and *in-vitro* trials to prove its positive impact on health. The shelf-life of the RTR healthy beverage can be improved by using other packaging technologies.

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