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Investigation of physico-chemical, microbiological, and shelf-life of Ready-to-Use (RTU) health beverages for the diabetic population

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Abstract
A research study was carried out to develop a ready-to-use health beverage using various functional ingredients that would benefit diabetic individuals. The health beverage was developed using double toned milk, sweet whey, stevia, a blend of almond and walnut oil, and tea extract and the control beverage was a sugar-sweetened beverage (8% sugar). The analysis revealed that developed health beverage showed higher values for viscosity, titratable acidity, moisture, fat, protein and ash content and lower values for specific gravity, pH, total solids, and total sugar than control beverage prepared. The shelf-life and microbiological analysis showed that the developed health was acceptable up to 15 days of storage at refrigeration temperature.

Keywords: Almond oil, *Camellia sinensis*, shelf-life, stevia, walnut oil, whey

Introduction
India has ranked first as the world's highest milk production past few years and the annual production is approximately 209.6 million tonnes with per capita milk availability of around 406 g per day (BAHFS, 2021) [3]. The consumption of milk and milk-based beverages has always been part of our daily diet irrespective of age as it is considered an important source of nutrition for growth and development. Since, liquid foods such as beverages are an essential part to provide a good source of nutrition which plays crucial roles in the body such as hydration, ample metabolic pathways, etc.

From the viewpoint, milk-based beverages that are widely consumed include milk-based tea, flavoured milk, and fermented milk like buttermilk, lassi, etc. Sweetened beverages like tea or coffee contain high calories due to the presence of sugar and fat and hence need improved formulation or alternatives to reduce the calorie content without compromising its sensory perception and providing better nutrition. Hence, ample research is being carried out to formulate and develop different functional beverages such as low calorie, reduced & low-fat &/or sugar, sugar-free, fat-free, inclusion of functional ingredients etc. However, many challenges are encountered while developing a functional beverage which must be overcome without compromising its health effects, sensory perception, compositional, shelf-life, and consumer preference. So, the addition of appropriate functional ingredients in milk-based health beverages is a challenging task.

Cohort studies have been conducted to include functional ingredients such as milk proteins or its hydrolysate like whey proteins, fruit pulp/extracts, low-calorie sweeteners like stevia, inulin, essential oils, fat replacers &/or substitutes, bioactive components like parts of plant extracts, bioactive ingredients, essential oils like almond oil, walnut oil, cumin oil, thyme oil, oregano oil, food hydrocolloids, probiotics etc. With consumer emphasis on a healthy diet and healthy choices, the demand for speciality products is upsurging but not at the expense of compromising their traditional products. This can however be correlated with lifestyle diseases like obesity-weight gain, diabetes etc (Malik *et al.*, 2006) [31].

India leads the world with the largest number of diabetic subjects, and is called the "Diabetes capital of the world". Diabetes currently affects more than 72 million Indians, which is more than 7.1% of the adult population. Research has demonstrated a clear link between the consumption of sugar-sweetened beverages and increased risk of poor diet quality, higher rates of obesity and diet-related health problems, as well as poor oral health (WHO, 2018) [43]. Furthermore, health-conscious consumers are now looking for a product low in calories as part of a precautionary measure to avoid diabetes.

Acute complications include hypoglycaemia, hyperglycaemia and diabetic coma; chronic complications include immune dysfunction, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, strokes, diabetic cardiomyopathy and many other macrovascular diseases (Tripathi and Srivastava, 2006 and Dabla, 2010) [41, 11]. Hence, emphasising the diet can directly ascertain control over these aforementioned complications.

Globally, tea (*Camellia sinensis*) and tea-based is the most popular beverage consumed and this is mainly attributed to its claimed health significance and as a stimulant. *Camellia sinensis* is one of the most important crops in the agriculture sector where India is the second largest producer in the world after China. Different forms of tea are available such as black tea, green tea, white tea, flavoured tea etc among which black tea is more popularly consumed (~78%) (Brief Guide, 2006 and FAOSTAT, 2008) [6, 13]. 100 gm of tea contains sodium (4 mg), potassium (18 mg) and caffeine (11mg). It also contains micronutrients viz. boron, cobalt, copper, iron, manganese, molybdenum, and zinc (Suzuki *et al.*, 2016) [39] along with polyphenols like catechins and theaflavins (Zaveri, 2006) [45].

The majority of evidence from cellular and animal experiments indicates that tea has positive effects on human health. Many cohort studies have shown that consumption of tea was associated with a reduced risk of Diabetes. Total daily consumption of at least three cups of tea reduced the risk of T2DM by approximately 42% (Dieren *et al.*, 2009) [12]. Basu *et al*. (2013) [5] reported that the mechanism involved is due to 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 the liver, and increasing the anti-oxidases such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and also inhibiting plasma protein carbonylation induced by hyperglycemia. The antidiabetic property of tea components especially catechin, chlorogenic acid, caffeine and theaflavins is through inhibition of α-amylase and α-glucosidase activity which results in reduced absorption and digestion of starch which resulted in decreased plasma levels of glucose, lipid metabolites, and albuminuria (Li *et al.*, 2016; Liu *et al.*, 2016) [29, 50].

Whey contains nearly 50% of milk solids, which represents a heterogeneous pool of protein with a wide range of physicochemical and functional properties. An ample of studies have reviewed that whey protein is excellent both in terms of the nutritional and economic point of view. Studies have shown that whey protein possesses antidiabetic, insulinotropic, antioxidant potential, immunomodulating, protects the cardiovascular system etc. which suggests it may have functional benefits to treat several diseases than other dietary proteins like meat and soya, which may be related to their sulphur amino acids (Keri, 2004) [24]. Recently it has been suggested that in addition to the amount of protein, the quality and its source are important for the reduction of postprandial glycemia and other protein-stimulated metabolic effects. Many functionally and physiologically active peptides are produced from proteins during gastrointestinal digestion such as anti-diabetic peptides, antioxidant peptides, antihypertensive peptides, immunomodulating peptides, anti-appetizing peptides etc., which have a positive impact on the human body. Among all the essential amino acids, leucine is found to be more important for diabetic subjects due to its chemical structure which is more insulinogenic than other essential amino acids as it affects glucose sensing in both insulin-dependent and insulin-independent mechanisms. The potent glucoregulatory effect of whey proteins and bioactive peptides is by directly affects insulin secretion, incretin secretion and /or inhibiting dipeptidyl peptidase IV activity (Comeford and Pasin, 2016) [10]. A study conducted by Gaudel *et al*. (2013) [17] on mice showed that ingestion of whey proteins and their hydrolysates improved the functioning of pancreatic β-cells and hence enhanced insulin secretion. Jakubowicz and Froy (2013) [22] proclaimed the beneficial physiological effects of whey protein on the control of food intake and glucose metabolism through insulinotropic and glucose-lowering properties in healthy and type 2 diabetes subjects via bioactive peptides and amino acids generated during its gastrointestinal digestion.

Almond oil and walnut oil have been studied lately for their health-befitting functional properties. Many studies have proven that these oils are rich in healthy fats i.e., monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). It is seen that diabetic people are more prone to the development of cardiovascular diseases, especially resulting in increased blood cholesterol levels. In order to maintain their serum cholesterol level, they need to include healthy fat such as Monounsaturated fat and polyunsaturated fat in their daily diet. According to the American diabetes association (2017) [2], intake of fat, especially monounsaturated fatty acid (MUFA) and poly monounsaturated fatty acid (PUFA) has been liberalized in diabetic diets to increase HDL cholesterol and improve glycaemic control.

Generally, 100 g of almond contains a total of 49gm of fat out of it contains saturated fat 3.7 g, polyunsaturated fat 12 g, monounsaturated fat 31 g, calcium 26%, iron 20%, magnesium 67%, carbohydrates 7% and 42% of protein (USDA, 2016) [42]. Nuts, by virtue of their fat and protein content, may also depress postprandial glycaemia and hence ROS production (Lovejoy *et al.*, 2002). Almond oil is rich in both MUFA (~69.9%) and PUFA (~17.4) and also rich in vitamin E, A, B1, B6, B2, and D. It contains some amount of phytosterols such as beta-sitosterol, stigmasterol, campesterol, sitostanol, and campestanol, which have been associated with cholesterol-lowering properties (Kim *et al.*, 2017) [25]. Since diabetic people are more prone to an increase in serum cholesterol levels resulting in CVD. Supplementing the diet rich in MUFA and PUFA reduces serum cholesterol levels. Almond oil has proven to lower postprandial glycaemia, insulinaemia and oxidative stress in diabetic individuals. Diet rich in MUFA resulted in improved insulin sensitivity and reduced diabetes risk by reducing the serum LDL/HDL ratio and triglycerols (Jenkin *et al.*, 2006, Li *et al*., 2009, Li *et al*., 2016 and Riserus *et al.*, 2009) [23, 28, 29].

Walnuts contain about 52 - 70% fat, 3.2 - 4.4% water, 12.0-19.6% protein, 61.3 - 73.8% fat, 1.8 - 2.3% ash and 2.2 - 4.5% sugars (Gecgel *et al.*, 2011) [18]. Walnuts are rich in Polyphenols such as ferulic acid, vanillica acid, coumaric acid, syringic acid, myricetin and juglone, iron (16%), Vitamin B6 (25%), Magnesium (39%) and Vitamin C (2%). Walnuts are rich in good fat, is also rich i.e. 100 g of walnut contains approximately 6.5 g of saturated fat, 47 g of polyunsaturated fat and 9 g of monounsaturated fat (Tapsell *et al.*, 2004) [40]. whereas walnut oil contains monounsaturated fatty acids (~17.6%) and polyunsaturated fatty acids (~70.98%). Walnut supplementation, 68 g/day reduced total and low-density lipoprotein cholesterol by 5 and 9% respectively. A reduction in fasting and postprandial glycemia

by 15% was also verified when a higher intake of PUFA (5.2 g/day) was implemented for a shorter period of 8 weeks (Saxenaa *et al.*, 2009 and KOTB EL-SAYED, 2011) [35, 26]. Consumption of walnut was seen to be associated with a lower risk of Type 2 Diabetes in women especially. Incorporation of walnut in the diet in moderate amounts decreases the serum levels of total cholesterol and favourably modifies the lipoprotein profile due to its fatty acid profile (Pan *et al.*, 2013) [33]. Zibaeenezhad *et al*. (2017) [46] reported that consumption of walnut oil (15 ml/day for 90 days) by type 2 hyperlipidemic diabetic subjects resulted in a significant decrease in total cholesterol level from 234.1 ± 33 to 207.7 ± 39.7 mg dl-1 and it improved blood glucose level.

Stevia is one of the popularly known zero-calorie natural sweetener and has zero glycemic index with GRAS status and ADI of 4 mg/Kg body weight. The stevioside powder is composed of reducing sugar (~3.62), non-reducing sugar (~3.08%), total sugar (~6.80), and stevioside (~77.0%) (Arab *et al.*, 2010). In a study conducted on diabetic humans, single acute doses of stevioside (1000 mg) were able to reduce the postprandial glucose level by 18% relative to control (1g corn starch) and appeared to benefit the insulin: glucose ratio in serum by 40% (Ena *et al.*, 2013) [48]. Another investigation was conducted to study the glycemic response of food products prepared using stevia powder in comparison to artificial sweeteners (sucralose) and sugar which served as control, the result showed that recipes prepared showed low glycemic index in normal, obese and diabetic subjects i.e., among RTS beverages (10 mg stevia powder) had 25.68, 21.56 and 34.68 GI, Cake (60 mg stevia powder) had 47.25, 48.12 and 54.75 GI (Arora and Suhani, 2015) [49]. Milani *et al*. (2016) [13] summarised that stevia (non-caloric natural sweeteners) has antioxidant and insulinotropic properties and may add functionality to foods or beverages. Cohort studies have proved that stevia has potent benefits like antihyperglycemic, antihyperlipidemic activity, anti-inflammatory, anti-tumour, diuretic, immunomodulatory, antimicrobial, antihypertensive, anticancer, anti-obesity etc. Stevia has been successfully used as a sugar replacement in ample of food products like milk beverages, tea, coffee, fruit juices, cordials, chocolate, chewing gum, pastries, biscuits and cakes, jams, ice creams, yoghurt, also as a table top sweetener etc. (Kuntz, 2010 and Shrapnel, 2015) [27, 36].

Material and Methods

This research study was carried out in the Students Experimental Dairy Plant of Dairy Science College, KVAFSU, Bengaluru. Fresh milk was obtained from cattle and subjected to cream separation and later standardized to prepared double-toned milk. Other ingredients like whey, stevia, almond oil, walnut oil and tea powder were obtained from the local market. The aqueous extract of tea was prepared by adding 100 ml of demineralized water, and tea powder and brewing for 10 minutes with continuous stirring followed by removing solids matters by filtration. Sweet whey was obtained after cheddar cheese was used in this study. The control beverage was prepared using 8% sugar according to standard procedure as prescribed by Singh, 2006 & 2014 [37, 38, 51] and ready to use health beverage was prepared using double toned milk (1.5% milk fat) and replacing 30% of milk with cheese whey. This was further incorporated with 2% of almond oil & walnut oil (1:1), complete replacement of sugar with stevia, the addition of 15% tea decoction (tea extract) and then both beverage samples were subjected to pasteurization (73 °C/ 15 sec) and packed in glass bottles for shelf life study at 7 ± 1°C.

Physico-chemical and microbiological analysis

The analysis of beverage samples was done according to the method mentioned in ISI: SP 18 (Part XI) 1981 for moisture, total solids, lactose (Lane Eynon method), fat (Gerber method), protein (Micro-Kjeldhal protein), ash, titratable acidity, specific gravity, and viscosity was estimated using U-tube viscometer by taking distilled water as standard liquid. Relative viscosity = (Average time for the flow of product x specific gravity of product Average)/ time for the flow of water at 25 °C. Total bacterial count, coliform count and yeast and mold count were determined during the storage as per the method prescribed in ISI: SP 18 (Part XI) 1981 using SPCA, VRBA and MEA (pH adjusted), respectively. Also, during the shelf-life study, the beverages were subjected to sensory (9-point hedonic scale) and physicochemical analysis.

Statistical analysis

The data interpretation was done using the R Programme (R-Version, Ri386 3.4.3) for accurate interpretation and values were an average of three trials (Zar, 2003) [44].

Result and Discussion

Physico-chemical attributes of the control and developed health beverage

The effect of the addition of whey, stevia, and the mixture of almond oil and walnut oil on the physical properties of health beverage containing tea were studied (Table 1). It was observed that there was a decrease in specific gravity and pH values and an increase in acidity and viscosity of the developed health beverage compared to the control beverage. This could be due to the incorporation of almond oil and walnut oil blend as an increase in fat content increases the viscosity. Also, the developed health beverage showed a slight increase in acidity and decrease in pH compared to the control beverage which could be due to the addition of whey (0.05% LA), tea (pH 6.2) and coffee (pH 5.3) decoction which is acidic in nature. Chakraborty *et al*. (2017) [8] studied physico-chemical characteristics of low-calorie coffee flavour yoghurt incorporated with 5%w/v instant coffee powder and 0.2% w/v aspartame which showed an acidity of 0.86% LA and 4.42 pH with an overall acceptability score of 7.95 with flow index of 0.401.

The Chemical composition of control and developed health beverages are tabulated (Table 2) and it was noted that with an increase in fat, protein and ash content there was a simultaneous decrease in total solids and sugar content of health beverages compared to control was observed. Further, it can be reported that there was a decrease in total solids and total sugar content in developed health beverages than in control beverage, which clearly shows the effect of stevia on the total sugar content due to the replacement of sugar with stevia. The addition of bio sweetener significantly affected total sugar content which forms an important attribute of a case diabetic diet. Also, there was an increase in per cent fat which could be due to the addition of oil blend (2%), protein content was increased due to the contribution of added whey (0.60%) and ash content was increased due to the contribution of whey (0.5%), tea (0.01%) and coffee (0.02%) in developed health beverage which was similarly reported by Chatterjee *et al*. (2015) [9] were 3:2 ratio for concentrated liquid whey and orange juice added with 8% sugar had moisture, total soluble

solids, fat, protein, total sugar and ash of 85.5 ± 0.79, 14.43 ± 0.25, 0.73 ± 0.4, 1.05 ± 0.22, 4.52 ± 0.35 and 0.67 ± 0.06%, respectively.

Table 1: The physical properties of control and developed health beverage

Sample	Specific gravity	Viscosity (cP)	pH	Acidity (% LA)
Control	1.0340[a]	1.54[a]	6.67	0.14
Health beverage	1.0300[b]	1.86[b]	6.61	0.15
CD (*P=.05*)	0	0.009	0.031	3.05

(*Note*: All the values are averages of three trials; Superscripts - a, b indicates significance difference at the corresponding critical difference)

Table 2: The Chemical composition of control and developed health beverage

Sample	Control	Health beverage	CD (*P=.05*)
Moisture	81.35[b]	87.39[a]	0.0
Total Solids	18.30[a]	12.61[b]	0.0
Fat	1.55[b]	3.70[a]	0.03
Protein	3.28[b]	3.51[a]	0.04
Total sugar	12.76[a]	4.51[b]	0.10
Ash	0.71[a]	0.88[b]	0.02

(*Note*: All the values are averages of three trials; Superscripts - a, b indicates significance difference at the corresponding critical difference)

Effect of storage temperature on the shelf life of control and developed health beverage

The effects of storage temperature on health beverage pertaining to sensory attributes for overall acceptability are represented in Table 3. It was observed that with the increase in storage period, the sensory scores were decreasing which can be mainly attributed to changes in its physico-chemical and sensory properties. It was also observed that, as the storage period increased the overall acceptability for control and health beverage decreased till the 9th day of storage from 8.10, and 8.20 to 5.50, and 7.33, respectively. The health beverage was found to be acceptable till the 11th day of storage and after the 15th day, the sensory scores were decreasing significantly and was found to be spoilt after the 17th day. These scores were similar to the flavour scores of whole pasteurized milk (5 out of 5) as reported by Zygoura *et al*. (2004) [47] which decreased to 1.1 with a sour taste on the 7th day of storage

Table 3: Effect of storage on the sensory score of health beverages stored at refrigeration temperature (7±1 ˚C)

Sample	Sensory evaluation (9 Point Hedonic Scale)									
	Overall acceptability (in days)									
	1	3	5	7	9	11	13	15	17	19
Control	8.25	7.95[b]	7.41[b]	6.50[b]	5.50[b]	Spoilt				
Health beverage	8.20	8.20[a]	8.16[a]	7.60[a]	7.33[a]	7.16	6.54	6.00	5.45	Spoilt
CD (*P*=.05)	0.42	0.24	0.36	0.39	0.37	0.28	0.22	0.40	0.42	-

Note: All the values are averages of three trials; Superscripts - a, b indicates significance difference at the corresponding critical difference

Effect of storage on the chemical parameter of beverages at refrigeration temperature (7±1 ˚C)

The control and developed health beverage samples were subjected to a titratable acidity test and free fatty acid and results pertaining to it are tabulated in Table 4. Statically, it was observed that the acidity on day 1 was significantly different among the beverage samples. It could be seen that as the storage period increased, the acidity also increased from a minimum of 0.14 to a maximum of 0.22% LA between the samples. The control beverage showed acidity of 0.14 on day 1 and 0.17 per cent lactic acid on day 9th and the product was spoiled afterwards. The acidity for health beverage increased from initial 0.15 to 0.18 per cent lactic acid on the 15th day, respectively. These findings were in support of Balaswamy *et al*. (2014) [4] were the change in acidity during storage of sterilized RTS beverage added with Jamun, mango, pomegranate, pineapple and purple grape juice containing aqueous stevia extract 4.93 ± 0.12° Brix and 20-25% juice had an acidity of 0.12, 0.21, 0.20, 0.22 and 0.14% acidity on 0th month which increased to 0.14, 0.24, 0.17, 0.20 and 0.12%, respectively on 4th month of storage at room temperature.

Furthermore, it was observed that the FFA value on day 1 was non-significant among the samples. It could be seen that as the storage period increased the FFA value also increased from a minimum value of 0.43 per cent oleic acid to a maximum value of 0.71 per cent oleic acid in control and health beverage. It was observed that there was a non-significant difference in FFA value up to 3 days of storage and a significant change was observed after the 3rd day of the storage period. The control beverage showed an FFA value from an initial 0.46 up to 0.82% OA (11th day). and for health beverage, it increased from the initial value of 0.44 to 0.89 per cent oleic acid till the 15th day and later was found to be unacceptable due to developed acidity and off-flavour. These findings were similar to a shelf-life study of processed milk stored at 6 °C for 17 days where the FFA value increased from 0.9 to 0.25 meq/kg as reported by Fromm and Boor (2004) [16]. Zygoura *et al*. (2004) [47] showed that the FFA value increased from 1.5 equiv/ml to 2.11 equiv/ml during 7 days storage period of whole pasteurised milk. Cappozzo *et al*. (2015) [7] reported that HTST treated milk showed no significant change in FFA value (0.89% oleic acid) during the storage period of 14 days.

Table 4: Effect of storage on titratable acidity and free fatty acid of health beverage at refrigeration temperature (7±1 °C)

Sample	Storage in days (7±1 °C)								
	Titratable acidity (% lactic acid)								
	1	3	5	7	9	11	13	15	17
Control	0.14[b]	0.15	0.16[a]	0.16[a]	0.17[a]	0.20[a]	Spoilt		
Health beverage	0.15[a]	0.15	0.15[b]	0.15[b]	0.15[b]	0.17[b]	0.18	0.18	Spoilt
CD (*P*=.05)	1.52	1.78	0.00	0.00	0.02	0.00	0.00	0.00	-
Free fatty acid (% oleic acid)									
Control	0.46	0.49	0.55[a]	0.68[a]	0.77[a]	0.82[a]	Spoilt		
Health beverage	0.44	0.48	0.52[b]	0.57[a]	0.62[a]	0.65[b]	0.71	0.89	Spoilt
CD (*P*=.05)	0.02	0.03	0.02	0.02	0.03	0.01	0.02	0.02	-

Note: All the values are averages of three trials; Superscripts - a, b indicates significance difference at the corresponding critical difference)

Effect of storage on the microbiological quality of health beverages at refrigeration temperature (7±1 ˚C)

The microbiology quality of control and developed health beverage samples with respect to total bacterial count (TBC), coliform and yeast and mold counts were enumerated once in 2 days. The results pertaining to the same are depicted in Table 5. The TBC increased from 1.60 to 3.95 $\log_{10}$ cfu/ml for control till the 9th day and from 1.54 to 4.34 $\log_{10}$ cfu/ml for health beverage till the 15th day. The coliform counts were found to be nil in both samples. The yeast and mold counts

were nil till the 5th day of storage and then were observed on the 11th day (0.90 log_{10} cfu/ml) for control and for health beverage it was observed after the 15th day which was around 0.47 log_{10} cfu/ml. This could be attributed to the antibacterial and antioxidant properties of the functional ingredients added. Zygoura *et al*. (2004) [47] depicted similar findings where mesophilic count increased from 4.65log cfu/ml to 6.56 log cfu/ml during 7 days storage studies of pasteurized milk. The shelf life of HTST pasteurized milk packed in HDPE bottles had a coliform count of <0.03 MPN/ml and the mesophilic count increased from 3.25 cfu/ml (0th day) to 7.01 cfu/ml (43rd day) when stored at refrigeration temperature (6±2 °C).

Table 5: Effect of storage on the microbiological quality of health beverages stored at refrigeration temperature (7±1 ˚C)

Sample	Microbiological analysis							
	Total Bacterial Count (log_{10} cfu/ml)							
	1	3	5	7	9	11	13	15
Control	1.60	2.69	3.64	3.72	3.95	-		
Health beverage	1.54	2.09	2.47	3.30	3.74	3.90	4.17	4.34

Note: Coliforms were found to be nil; Yeast and mold were nil up to the 9th day for control and health beverage, respectively; All the values are averages of three trials

Conclusion

In the final analysis, the developed health beverage has better composition characters and was stable for a longer period in comparison with the control beverage. The obtained results showed that the developed health beverage had higher protein content and lower sugar content which is mainly attributed to its whey protein and stevia incorporated. From a microbiological and shelf-life point of view, a developed health beverage is acceptable for up to 15 days of storage at refrigeration temperature. It can be concluded that the developed health beverage was found to be better than the control beverage. Instead of using water during milk tea preparation, sweet whey can be used which would not only be a better way of whey waste utilization but also added benefit of whey protein addition. Furthermore, in-depth analysis is required to determine the possible reason behind the longer storage period and better microbial stability along with bio-functional properties like anti-bacterial, antioxidant, *in-vitro* and *in-vivo* studies are required to determine the glycemic response of developed health beverage.

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