Evaluation and application of prebiotic and probiotic ingredients for development of ready to drink tea beverage

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Abstract Ready-to-drink (RTD) ice tea is a ready prepared tea, produced from green and black tea originating from same plant *Camellia sinensis*. The objective of this study was to determine the effect of prebiotics [galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), and inulin] or synbiotic ingredients (GOS, FOS, inulin, and *Lactobacillus acidophilus*) on the sensory properties and consumer acceptability of RTD. The quality of green tea extract (GTE) and black tea extract (BTE) was improved with pretreatment of cellulase and pectinase enzymes. The combined enzymatic extraction amplified total extractives up to 76% in GTE and 72% in BTE. Total polyphenol was found to be enhanced to 24% in GTE and 19% in BTE. GTE was further selected for development of RTD in two different formats; synbiotic RTD and prebiotic RTD premix and analyzed for sensory attributes (colour, aroma, taste, and acceptability). Synbiotic RTD was also evaluated for stability over a period of 28 days at 4 °C. Synbiotic RTD developed an unpleasant flavor and aroma during the shelf life. Premix format of RTD developed using spray drying was reconstituted and found to be functionally and sensorially acceptable.

Keywords Tea · Prebiotics · Probiotics · Synbiotic · Enzymatic extraction · Premix

Introduction

Tea (*Camellia sinensis*) is one of the most popular beverages worldwide, and its consumption has been claimed to be associated with beneficial health effects (da Silva 2013). Tea is well known good dietary source of biologically active compounds such as polyphenols, polysaccharides, amino acids, and vitamins (Lorenzo and Munekata 2016). Amongst these, polyphenols and polysaccharides are the primary bioactive components. Tea flavanols mainly, epicatechin (EC), epigallocatechin (EGC), EC gallate (ECG), and EGC gallate (EGCG) are a group of natural polyphenols found in green and black tea (Yang et al. 2001). Tea polysaccharides have shown glycosidase inhibitory effects, antioxidant, antibacterial, and immunomodulatory properties (Chen et al. 2009).

Ready-to-drink (RTD) ice tea is a ready prepared tea, produced either from tea extract in the form of reconstituted spray dried powder or as freshly brewed extract. Enzymatic treatment of tea leaves for the production of instant teas offers many benefits for the resulting convenience beverage product. About 45% of the dry weight of tea is due to cell wall material that is composed of cellulose, hemicelluloses, and lignin. Pectic substances are group of polysaccharides closely associated with the cell wall and the intracellular region of higher plants (Ridley et al. 2001). The cell wall components can be degraded by hydrolytic enzymes such as cellulases, pectinases, xylanases, and hemicellulases to enhance the yield of extracted products (Chandini et al. 2011). Carbohydrate hydrolyzing enzymes have been seen to release polyphenols (Barbousse et al. 2009). Plant cell wall polysaccharides are an interesting potential source for the production of oligosaccharides covering a wide range of different structures. Few studies have shown that non digestible

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oligosaccharides, non starch polysaccharides (NSP), resistant starch, and pectic oligosaccharides can act as substrates for the intestinal species of bacteria (Van Laere et al. 2000).

Commercial prebiotics like fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), inulin, and other related carbohydrates are known to beneficially affect host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Nagpal and Kaur 2011). It is known that the growth of probiotic strains are specific to the prebiotic concerned. It is hypothesized that a mixture of prebiotics can influence the intestinal bacteria in a synergistic manner (Bakker-Zierikzee et al. 2005). The combination of prebiotics can be optimized using mixture design studies. The ability of the *Lactobacilli* and *Bifidobacteria* to ferment specific oligosaccharides and polysaccharides can be crucial in the development of synbiotics. Tea phenolics act as metabolic prebiotics and matrix for probiotic strains and exert considerable effects on the intestinal environment by alteration of the intestinal bacterial population (Lacey et al. 2014). Green tea co-encapsulation with probiotics is reported to exert a protective effect of bacteria during exposure to gastrointestinal conditions and refrigerated storage (Vodnar and Socaciu 2012). Prebiotics alone or in combination with probiotic bacteria in the form of synbiotics can have the ability to improve the gastrointestinal health of humans (Tuohy et al. 2003). Obtaining relevant sensory information of prebiotics, probiotics, and synbiotic food contributes to consumer acceptance and is decisive step during the various stages of food product development and commercialization (Cruz et al. 2010). The sensory impact, that prebiotic or probiotic cultures can cause in foods or beverages to which they are added has been little studied. Probiotics create different flavor profiles when compared to the conventional products. Probiotics cultures produce lactic perfumed aroma, and also impart medicinal, artificial, earthy, sour, and salty tastes (Luckow and Delahunty 2004). So far in our knowledge sensory information on tea extract fortified with prebiotics and probiotic culture *L. acidophilus* is not reported.

The present work was undertaken with the objective to enzymatically degrade the tea cell wall polysaccharides using cellulase, pectinase, and their combinations to produce oligosaccharides and further supplementation of the beverage with optimized combination of commercial prebiotics (GOS, FOS, and inulin) to develop ice tea in different formats: ready-to-drink synbiotic ice tea (inoculated with *L. acidophilus*) and prebiotic ice tea premix. Analysis of sensory attributes, colour, aroma, taste, and acceptability was done for synbiotic RTD and compared with instant RTD premix. Impact of storage on sensory characteristics was also studied for synbiotic RTD.

Materials and methods

Materials

Green tea and black tea were purchased from local markets in Matunga, Mumbai. Accellerase 1500 cellulase (2.13 U/ml of enzyme) was obtained from Genecor (India) while SEBMash pectinase (28 U/ml of enzyme) was obtained from Advanced Enzymes (Mumbai, India). The prebiotics (FOS and GOS) were obtained from Tata Chemicals (Mumbai, India). Inulin was procured from Jupiter Food Products Pvt. Ltd., (Etah, Uttar Pradesh). *L. acidophilus* (NCIM 2660) and *E. coli* (NCIM 2065) were procured from NCIM (National collection of industrial microorganisms) (Pune, India). De Man, Rogosa and Sharpe (MRS), Brain Heart Infusion (BHI) and Nutrient Agar (NA) were purchased from Merck (Mumbai, India). Other analytical grade chemicals were purchased from Himedia Laboratories and Sigma Chemicals (Mumbai, India).

Enzymatic extraction and analysis

Enzymatic extraction using cellulase and pectinase were studied both independently and concurrently. The process involved weighing the tea leaves (2 g) and adding optimized concentration of enzymes and 80 ml of water to it. The cellulase concentration of 7.45 U was taken for green tea and for black tea and it was maintained at 6.4 U. The pectinase concentrations of 11.2 U and 14 U were used for green and black tea respectively. The pH was adjusted to 4.5 and reaction mixture was incubated at 50 °C for 30 min at 180 rpm. The extraction was then carried out by refluxing for 30 min at 100 °C with attached air condenser to the flasks. The extracts were then cooled and filtered through Whatman No. 1 filter paper. The tea extract was analyzed for its total solids (Chandini et al. 2011), total polyphenol content by Folin–Ciocalteu method (Anesini et al. 2008) and reducing sugars by Dinitrosalicylic acid (DNSA) protocol. The residual weight was also determined by drying the residue at 105 °C until constant weight.

Sample formulation and analysis

A market sample was analyzed for its total extractives, total carbohydrate content (phenol–sulfuric acid method), reducing sugar content (using DNSA method), Brix and tea solids. This analysis was used as a model for the development of tea based beverages. A loop full of culture maintained on agar slants was inoculated into 50 ml of sterilized respective broths and kept in a shaker incubator overnight at 37 °C, 180 rpm. Some of this overnight grown culture was centrifuged at 5000 rpm for 10 min at 4 °C,

washed with 0.85% saline and OD600 was maintained at 1.0 and used for further inoculation. Tea beverages (50 ml) having 0.14% tea solids and 9% sugar were prepared, autoclaved and then evaluated for their prebiotic activity score (PAS). The assay was carried out by transferring 1% (v/v) of culture of *Lactobacilli* to MRS broth supplemented with 20% (w/v) sample (enzymatically treated tea and non-enzymatically treated) or glucose. The inoculated samples were incubated at 37 °C and enumerated after 0 and 24 h of incubation. For *E. coli*, 1% (v/v) culture inoculation was done in BHI broth with 20% (w/v) sample or glucose and enumerated on NA medium after 0 and 24 h. The PAS was determined using the following equation.

$$\text{Prebiotic activity score} = \left[\frac{(\text{probiotic log } \frac{\text{cfu}}{\text{ml}} \text{ on the prebiotic at 24 h} - \text{probiotic log } \frac{\text{cfu}}{\text{ml}} \text{ on the prebiotic at 0 h})}{(\text{probiotic log } \frac{\text{cfu}}{\text{ml}} \text{ on glucose at 24 h} - \text{probiotic log } \frac{\text{cfu}}{\text{ml}} \text{ on glucose at 0 h})} \right] - \left[\frac{(\text{enteric log } \frac{\text{cfu}}{\text{ml}} \text{ on the prebiotic at 24 h} - \text{enteric log } \frac{\text{cfu}}{\text{ml}} \text{ on the prebiotic at 0 h})}{(\text{enteric log } \frac{\text{cfu}}{\text{ml}} \text{ on glucose at 24 h} - \text{enteric log } \frac{\text{cfu}}{\text{ml}} \text{ on glucose at 0 h})} \right]$$

Using this equation, the PAS of a particular oligosaccharide can be determined relative to any given strain.

Mixture design analysis

The Design expert (ver. 7.0) software was used to determine the optimum proportions of the prebiotics for incorporating in the ice tea samples. A D-optimal design was used with the constraints FOS (A) + GOS (B) + inulin (C) = 9%. The component ranges (%) were as follows: $0 < \text{FOS} < 9$, $0 < \text{GOS} < 9$, $0 < \text{Inulin} < 6$. The constraints of the prebiotics and their ranges were set after extensive preliminary trials. The software designed 16 runs in which 12 runs were different and 4 runs were replicate. For optimization, depending on the influence of each factor; the combination of factors that led to the best responses was determined. Fitting response value was done using the special cubic and cubic models. The predictive dependent i.e. PAS and probiotic viable count (PVC) were analyzed. For determining the probiotic count, the tea based beverages were supplemented with the prebiotic mix (obtained from mixture design studies), sterilized and inoculated with probiotics and enumerated after 48 h. The models were subjected to analysis of variance (ANOVA) to determine their significance ($p < 0.05$).

Storage studies of synbiotic RTD tea

The prebiotic mix optimized in mixture design studies was incorporated in tea extracts and the beverages were autoclaved. Two percent of activated culture was inoculated into the beverages, which were then stored at 4 °C (refrigerator temperature) for 28 days in glass conical flasks. Changes in sensory characteristics (taste, color, aroma, mouthfeel, and overall acceptability), PVC, pH, and titratable acidity (as percent lactic acid) were studied during storage and analyzed every 4 days. Sensory quality evaluation of samples was performed by a trained panel of 15 members on a 9-point hedonic scale where 1 was

‘dislike extremely’ and 9 was ‘like extremely’. Samples were served in plastic cups at chilled temperature. The characteristic traits of tea like its strength, body, astringency etc., were evaluated under the heading of ‘mouthfeel’.

Synthesis and analysis of tea premix

The ice tea feed comprised of the optimized proportion of prebiotics, tea solids (0.14% w/v), citric acid (0.2% w/v) and water was fed into a lab scale spray drier (LSD-48 JISL model mini, India). The operational conditions for the spray drying were as follows: Inlet temperature (170 °C), Outlet temperature (75–85 °C), air velocity (30 psi), aspiration rate (1400 rpm). The feed flow rate was set at 3.5 ml/min (Alasalvar et al. 2013). The prebiotic ice tea mix obtained after spray drying was reconstituted in water, using tea solids (0.14% w/v) and sucralose (0.006% w/v) to maintain its sweetness. It was then analyzed for PAS, PVC, sensory characteristics (taste/flavor, color, aroma, and overall acceptability). The CIE parameters of the samples were determined with a calibrated Hunter colorimeter LabScan XE (Hunterlab, Rostin US). The CIE $L^*a^*b^*$ scale indicating L^* (brightness), a^* (red to green) and b^* (yellow to blue) were measured.

Table 1 Effect of enzymatic treatment on green tea and black tea extracts

Parameters	Control samples (non-enzymatically treated)		Cellulase treated sample (optimized)		Pectinase treated sample (optimized)		Cellulase + pectinase treated samples	
	GTE	BTE	GTE	BTE	GTE	BTE	GTE	BTE
Extractives (g/100 g)	30.03 ± 1.57 ^a	36.14 ± 0.57 ^a	58.66 ± 1.00 ^b	54.43 ± 0.57 ^b	54.65 ± 1.27 ^c	57.88 ± 0.54 ^c	76.41 ± 1.58 ^d (154% increase)	72.25 ± 1.52 ^d (99% increase)
Residual waste (g/100 g)	71.43 ± 0.20 ^a	64.33 ± 1.52 ^a	44.67 ± 0.57 ^c	47.43 ± 0.76 ^b	47.37 ± 0.70 ^b	43.73 ± 0.86 ^c	25.56 ± 1.04 ^d (64% decrease)	30.33 ± 1.22 ^d (53% decrease)
Reducing sugar (g/100 g)	18.67 ± 0.15 ^a	18.93 ± 0.14 ^a	36.05 ± 0.90 ^c	31.18 ± 0.35 ^b	21.33 ± 0.39 ^b	18.38 ± 0.24 ^a	36.36 ± 0.32 ^c (94% increase)	33.83 ± 1.04 ^b (78% increase)
Total polyphenols (g of GAE/100 g)	5.56 ± 0.01 ^a	3.28 ± 0.01 ^a	6.92 ± 0.02 ^c	3.77 ± 0.01 ^b	6.13 ± 0.01 ^b	3.78 ± 0.16 ^{bc}	6.92 ± 0.01 ^c (24% increase)	3.91 ± 0.03 ^c (19% increase)

GTE green tea extract, BTE black tea extract, GAE gallic acid equivalent

Results are mean ± SD of triplicate analyses; data with the same letter between the rows are not significantly different at $p \leq 0.05$. Superscripts of GTE are not comparable with those of BTE

Results and discussion

Enzymatic extraction of tea

The improvement in extractability of total solids was studied with the treatment of cellulase and pectinase, both independently and concurrently. Optimization trials for extraction of tea using enzymes were conducted till a significant increase was found in the total soluble solids. The cellulase and pectinase concentration of 7.45 U and 11.2 U for green tea and 6.4 U and 14 U for black tea, respectively were found to be optimum. In the past, various investigators (Baik et al. 2015; Chandini et al. 2011) have improved the extractable solids yield by using a cocktail of enzymes simultaneously. Thus, to further improve the extractability of total solids and total polyphenols, a combined extraction of green and black tea was attempted using the optimized concentrations of both the enzymes. The combined enzymatic treatment increased total extractives up to 76% in GTE and 72% in BTE (Table 1). Thus the combined treatment had higher extraction efficiency than individual enzyme treatments. As the extractives increased, a simultaneous decrease in weight of residue and an increasing trend in total polyphenols and reducing sugars were noticed in both the teas. The extraction conditions employed in the instant tea manufacture are aimed towards the maximum recovery of solids and are generally harsher compared to the brewing conditions used for tea preparations.

The tea samples under study (both green and black) contained 8.40% of cellulose and 4.71% pectin (pre-determined) and also some amount of hemicellulose, xylanase, and lignin. Cell wall digesting enzymes break down tea cell wall constituents into simpler compounds reducing the structural integrity or increasing the permeability of the cell wall, thus being effective in improving the extractives and also the solubilized bioactive tea polysaccharides. Another reason for the increase in extraction of tea extractives could be the low pH (4.5), leading to high H^+ concentration. Under acidic conditions, the action of H^+ on tea leaves alters leaf structure in such a way that some extra solids are liberated. H^+ may open up parts of the leaf structure that are generally inaccessible and more water soluble components like theaflavins and thearubigins can be released from the leaf (Spiro and Price 1987). Some insoluble substances like polysaccharides, proteins, and pectins can also be released during brewing in acidic conditions. But focusing only on the solids extractability could lead to a poor quality tea, as tea is more valued for its polyphenols. Polyphenols have both hydrophobic aromatic rings, and hydrophilic hydroxyl groups with the ability to bind to polysaccharides and proteins at several sites on the

cell wall (Hanlin et al. 2010). Combined enzyme extraction showed a 24% increase in total polyphenols in GTE and 19% increase in BTE as compared to non-enzymatic treatment. This is more than that showed by Yang-Hee et al. (2013), where the polyphenol extraction for green tea improved by 15% after treatment with Viscozyme (multi-enzyme complex containing carbohydrases). In some cases, as reported by Chamorro et al. (2012), use of carbohydrases may not enhance phenolic compounds due to adsorption of enzymes to lignin or polysaccharides in the wall matrix. In such cases, pretreatments for removing lignin and hemicellulose are required to make the cellulose accessible to enzymatic hydrolysis.

Sample formulation and analysis

The beverages were prepared with compositions in line with that of the market sample and evaluated for their PAS. PAS values followed following order: enzymatically treated green tea (0.08 ± 0.10) > non-enzymatically treated green tea (-0.07 ± 0.02) > enzymatically treated black tea (-0.10 ± 0.05) > non-enzymatically treated black tea (-0.12 ± 0.10). Higher PAS means that the substrate supports good probiotic growth and does not support cell growth of enteric strains. The PAS was higher in enzymatically treated tea as compared to non-enzymatically treated tea. This could be due to the in vitro generation of oligosaccharides after enzymatic treatment. Oligosaccharides obtained from plant cell wall polysaccharides can be fermented by intestinal bacteria, thus having prebiotic potential (Van Laere et al. 2000). Many in vitro animal and human studies show that certain doses of tea polyphenols may modify the gut microbial composition, and while certain bacterial groups can be inhibited, others can thrive in the available niche environment (Cardona et al. 2013). On the contrary, some studies have also pointed out the antimicrobial effects of tea polysaccharides. The inhibitory effect of black tea extracts, as observed, may be attributed to the presence of theaflavins, which are known to possess antimicrobial activities (Ruxton 2008). Therefore, due to more promising results in terms of prebiotic potential, only green tea based beverages were carried forward for further studies (Table 2).

Mixture design analysis

Fitting the model

Both the independent and dependent variables were fitted to linear, quadratic, special cubic, full cubic, and special quadratic models and residuals plots were generated to check the goodness of model fit. For green tea based beverages, the cubic model was found to be most fitted for

PAS and PVC. The models were statistically significant, with satisfactory coefficients of determination (R^2) and the lack of fit showed no significance throughout the model. The polynomial models describing the correlation between the responses (PAS and PVS) and the variables are presented as follows:

$$Y_{PAS} = 0.876 A + 0.223 B - 1.511 C + 0.331 AB + 0.316 AC + 0.265 BC + 0.178 ABC + 0.162 AB(A - B) - 0.018 AC(A - C) - 0.043 BC(B - C) \quad (\text{Model1})$$

with Adjusted R^2 value: 0.8923; Adequate precision = 9.931; p value = 0.0019.

$$Y_{PVC} = 1.089 A + 0.974 B + 2.628 C - 0.048 AB - 0.399 AC - 0.408 BC + 0.074 ABC - 0.016 AB(A - B) + 0.027 AC(A - C) + 0.045 BC(B - C) \quad (\text{Model2})$$

with Adjusted R^2 value: 0.9845; Adequate precision = 29.118; p value < 0.0001.

Where A, B, C are coded values for FOS, GOS and inulin respectively. The value of adjusted R^2 for the regressed model 1 is 0.89. It indicates that the experimental data were in a good agreement with predicted values. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 9.93 indicates an adequate signal. This model can be used to navigate the design space. The obtained model 1 is very significant ($p < 0.0500$ and Model F-value: 14.80) according to ANOVA. The results of t tests and p values show that the linear mixture components AB and AB(A - B) are significant model terms. The interactions between FOS and GOS seemed to have a significant influence on the PAS. The value of adjusted R^2 of the regressed model 2 of 0.98 indicated that the experimental data was in a good agreement with predicted values. The Adequate Precision ratio of 29.11 indicates an adequate signal. This model could be used to navigate the design space. The obtained model 2 was very significant ($p < 0.0500$ and Model F-value: 106.96) according to ANOVA. The results of t tests and p values showed that the linear mixture components AB, AC, BC, ABC, AB(A - B), AC(A - C), BC(B - C) were significant model terms. Table 2 presents the results of mixed design studies for green tea beverages.

Response surface analysis

In the mixture design, the effect of the change of variables on the responses can be observed on the ternary contour

Table 2 Observed and predicted values of prebiotic activity score (PAS) and probiotic viability count (PVC) in green tea based beverage

Runs	Formulations			Prebiotic activity score		Probiotic viable count (log CFU/ml)	
	A (FOS)	B (GOS)	C (inulin)	Observed value	Predicted value	Observed value	Predicted value
1	0.00	3.00	6.00	-1.83 ± 0.08	-1.84	8.95 ± 0.50	8.91
2	4.50	4.50	0.00	-1.64 ± 0.02	-1.68	8.30 ± 1.20	8.84
3	0.00	3.00	6.00	-1.86 ± 0.08	-1.84	8.96 ± 0.49	8.91
4	0.00	9.00	0.00	2.43 ± 0.13	2.03	8.77 ± 0.90	8.77
5	0.00	9.00	0.00	2.45 ± 0.12	2.03	8.78 ± 0.90	8.77
6	6.00	1.50	1.50	-1.15 ± 0.01	-1.15	8.48 ± 1.00	8.48
7	3.00	0.00	6.00	-0.28 ± 0.06	-0.26	10.40 ± 1.17	10.35
8	4.50	4.50	0.00	-1.64 ± 0.10	-1.77	8.30 ± 0.67	8.34
9	0.00	6.00	3.00	-0.19 ± 0.00	-0.19	8.78 ± 0.56	8.82
10	9.00	0.00	0.00	7.37 ± 0.35	7.39	9.80 ± 1.53	9.80
11	1.50	1.50	6.00	4.29 ± 0.21	4.39	9.38 ± 0.78	9.45
12	9.00	0.00	0.00	7.78 ± 0.36	7.81	9.82 ± 1.60	9.80
13	3.00	0.00	6.00	-0.35 ± 0.07	-0.36	10.40 ± 1.12	10.35
14	3.00	3.00	3.00	4.68 ± 0.19	4.82	8.48 ± 1.37	8.50
15	1.50	6.00	1.50	7.72 ± 0.30	7.72	9.90 ± 0.03	9.90
16	6.00	0.00	3.00	5.99 ± 0.02	5.81	8.70 ± 0.92	8.74

Results are mean $\pm$ S.D. of triplicates

maps (Ferreira-Nunes et al. 2018). The PAS and probiotic viability count were well fitted into cubic models with a high determination coefficient (0.95 and 0.99, respectively) in case of green tea based beverages. Figure 1a, b shows the variation in PAS and PVC respectively, when evaluated for different combinations of commercial prebiotics in green tea based beverages. Seeing the overall curvilinear effect and the color scheme, it was deduced that PAS and PVC was enhanced by an enhanced ratio of GOS. In case of green tea based beverages, maximum value of PAS (7.72) was also obtained when the ratio of FOS, GOS and inulin was 1.5, 6 and 1.5, respectively i.e. in the presence of higher concentration of GOS. PVC was observed to be promoted in the presence of FOS and inulin, as can be seen in trial 7 (probiotic viable CFU/ml = 10.4). But the corresponding PAS score was low (-0.27). Even though FOS and inulin promote probiotic cell count, they were seen to be associated with lower values of PAS (when compared to GOS). A low or negative PAS is obtained when the growth of probiotic strain is less on the prebiotic compared with that on glucose and/or has less growth on the prebiotic than the growth of the enteric mixture on the prebiotic carbohydrate. This reflects that the fructose units enhance the survival of *L. acidophilus*, but they also promote the growth of *E. coli*.

Mixture proportion optimization

Three combinations were generated by the software for validation, based on their desirability; the contour plot for desirability function is as shown in Fig. 1c. Confirmatory trials suggested that there was a good degree of fit between the observed values in the experiment and the values predicted by the equation. On the basis of prebiotic optimization obtained from model 1 and 2, the optimized combination of prebiotics was 1.26% (w/v) FOS, 6.75% (w/v) GOS, 0.99% (w/v) inulin, as its desirability is 93%. Experimental value of 7.89 and 10.03 were obtained for PAS and PVC, respectively, for this optimized combination. Thus the preference for a higher concentration of GOS in the prebiotic mix was seen. In many previous studies, GOS as a prebiotic has proved to have an advantage over other substrates (Van Laere et al. 2000). Palframan et al. (2003) used mixed flora samples and showed that the highest prebiotic index scores were generally obtained for GOS compared to FOS and inulin. Rycroft et al. (2001) used various prebiotics as substrates, in pH/temperature controlled anaerobic batch cultures inoculated with fecal homogenates, and showed that all prebiotics to increase the numbers of *Bifidobacteria* but GOS resulted in the largest decrease of clostridial population. This selectivity could be attributed to the ability of strains to utilize GOS and the presence of enzyme β -galactosidase. Carbohydrates have a positive PAS if they are fermented by probiotic strains.

Fig. 1 Evaluation of a combination of prebiotics for its **a** prebiotic activity score, and **b** probiotic viable count. Contour plot **(c)** for desirability function in green tea based beverages

Carbohydrates are selectively fermented by probiotics but not by other intestinal bacteria. The higher the score, the higher the relative growth of the probiotic and/or the lower

the relative growth of the pathogen, which indicates a higher and more selective use of prebiotic in relation to glucose by the probiotic microorganism and/or limited use

of prebiotic in relation to glucose by the pathogenic microorganism.

Storage studies of RTD synbiotic tea

The pH and titratable acidity of the beverage, viable count of probiotic cells and sensory stability of the green ice tea were checked over the storage period of 28 days and are presented in Table 3. The pH of the samples reduced significantly with increase in storage period with the values ranging from 4.85 to 3.92, and the acidity began to increase from 0.13 to 0.21 from green ice tea. The cell count of *L. acidophilus* in green tea based ice tea decreased only slightly from 8.45 to 7.74 log CFU/ml in 4 weeks of storage at 4 °C. This can be because of the optimized mix of prebiotics which acted as substrates for the probiotic strain, and also because of cold storage conditions. The effect of refrigerated storage on the viability of probiotics has been studied by many researchers (Daneshi et al. 2013; Mortazavian et al. 2007). Lactic acid bacteria not only improve health when consumed, but they can also play a protective role against pathogens in the product itself during storage by competing with pathogens for nutrients, producing organic acids and bacteriocins (biopreservation). Biopreservation is the extension of storage life and enhancing of safety of foods using the natural or controlled microflora and/or their antimicrobial products (Alegre et al. 2011).

The scores allocated for taste, color, aroma and overall acceptability showed that during the first week of storage, all the samples (both green and black based ice tea) had maximum sensory acceptability. After 12 days, the sensory properties decreased and the samples started developing an acidic and a spoiled flavor. Of all the parameters, the aroma scores were always the lowest. Phenolic compounds are judged to be the key elements that determine both color

and taste qualities of a tea beverage during processing and storage (Wang et al. 2000). A loss of aroma could be due to the prior autoclaving done to sterilize the beverage before inoculation, which led to certain degradation of the polyphenols and other volatiles (Alasalvar et al. 2013). Even though the viability of probiotics in the drink is more than 10^6 for the storage period, the sensory acceptability of the RTD synbiotic ice tea lasted only for 10–12 days. Therefore, while short-term refrigeration promotes probiotic stability, it is not sufficient enough to preserve synbiotic ice tea for 28 days period.

Synthesis and analysis of tea premix

Instant tea powder is extensively used in large quantity in various tea premixes and saves time. Further, as seen in the previous section, there was loss of the sensory qualities of the ready-to-drink synbiotic tea, due to processing and storage, deeming it to be unsatisfactory after a certain period. Therefore, for this reason, an ice tea premix was attempted, using spray drying process, and then analyzed (Table 4). Stability to processing conditions is crucial if prebiotics are to confer beneficial effects for the host. Even if a prebiotic may get chemically altered, its functional activity should ideally be the same or be enhanced after the processing treatment. The and probiotic counts (log CFU/ml) for RTD green ice tea were 7.86 and 10.03 respectively, whereas for ice tea premix values were 7.63 and 9.98. The values are slightly less in comparison, but they are not significantly different ($p < 0.05$). Thus the functional activity of the prebiotics combination retained after spray drying treatment as well.

It was observed that the taste, mouthfeel and overall acceptability of the premix was not significantly different ($p < 0.05$) from that of the RTD ice tea, though aroma and color seemed to have changed after spray drying. Several

Table 3 Storage stability of ready-to-drink synbiotic green tea based ice tea over a period of 28 days

Storage period (days)	pH	Acidity (% lactic acid)	Probiotic viable cell count (log CFU/ml)	Taste	Color	Aroma	Overall acceptability
0	4.85 ± 0.03 ^a	0.13 ± 0.05 ^a	8.45 ± 0.12 ^a	8.40 ± 0.41 ^a	8.21 ± 0.44 ^a	7.05 ± 0.34 ^a	8.67 ± 0.42 ^a
4	4.81 ± 0.02 ^a	0.14 ± 0.01 ^{ab}	8.41 ± 0.17 ^a	8.13 ± 0.51 ^a	8.02 ± 0.18 ^a	6.80 ± 0.41 ^a	8.33 ± 0.57 ^a
8	4.69 ± 0.11 ^b	0.16 ± 0.00 ^b	8.36 ± 0.10 ^a	7.80 ± 0.22 ^b	7.40 ± 0.55 ^b	6.39 ± 0.27 ^b	7.10 ± 0.22 ^b
12	4.53 ± 0.04 ^c	0.16 ± 0.017 ^{bc}	8.28 ± 0.07 ^{ab}	5.80 ± 0.22 ^c	6.92 ± 0.30 ^b	4.11 ± 0.10 ^c	5.20 ± 1.09 ^c
16	4.21 ± 0.13 ^d	0.18 ± 0.012 ^c	8.10 ± 0.25 ^{ab}	5.20 ± 0.52 ^c	5.57 ± 0.10 ^c	2.00 ± 0.20 ^d	4.40 ± 1.24 ^c
20	4.13 ± 0.05 ^d	0.19 ± 0.01 ^d	7.95 ± 0.19 ^b	3.00 ± 1.41 ^d	4.71 ± 0.44 ^d	1.00 ± 0.00 ^e	3.30 ± 0.74 ^c
24	4.08 ± 0.06 ^{de}	0.20 ± 0.00 ^d	7.83 ± 0.04 ^b	1.00 ± 0.00 ^e	3.40 ± 0.21 ^c	1.00 ± 0.00 ^e	1.60 ± 0.54 ^d
28	3.92 ± 0.03 ^e	0.22 ± 0.01 ^e	7.74 ± 0.35 ^b	1.00 ± 0.00 ^e	2.20 ± 0.44 ^f	1.00 ± 0.00 ^e	1.00 ± 0.00 ^d

Results are mean ± S.D. of triplicates. Significant values are differentiated for rows. Data with the same letter between the rows are not significantly different at $p \leq 0.05$

Table 4 Analysis of ready-to-drink ice tea and spray dried premix (after reconstitution)

Samples	Prebiotic activity score	Probiotic viable cell counts (log CFU/ml)	Taste	Color	Aroma	Mouthfeel	Overall acceptability	L^*	a^*	b^*
Ready-to-drink green ice tea	7.86 ± 0.11 ^a	10.03 ± 0.21 ^a	7.90 ± 1.70 ^a	7.60 ± 0.80 ^a	7.60 ± 0.40 ^a	7.80 ± 0.20 ^a	8.10 ± 1.10 ^a	16.13 ± 0.01 ^a	0.42 ± 0.44 ^a	1.27 ± 0.11 ^b
Green ice tea premix	7.63 ± 0.21 ^a	9.98 ± 0.30 ^a	7.40 ± 1.20 ^a	6.20 ± 1.00 ^b	6.90 ± 0.70 ^b	7.80 ± 1.10 ^a	7.90 ± 1.90 ^a	17.09 ± 0.01 ^b	0.47 ± 0.01 ^a	0.76 ± 0.13 ^a

L^* : brightness; a^* : red to green; b^* : yellow to blue

Data with the same letter between the rows are not significantly different at $p \leq 0.05$

constituents in tea such as volatile compounds and polyphenols, are thermally unstable and may degrade during any thermal process, influencing the flavor and color of the final product (Someswararao and Srivastav 2012). The epistructured catechins can get converted into their non epistructured counterparts or be degraded due to oxidation at high temperatures (Nadeem et al. 2011). The loss in aroma can be avoided by passing the feed through an aroma recovery unit before spray drying. The change in color was further analyzed. The ice tea drinks prepared from premixes are lighter than the RTD samples as their L^* values are higher. A higher L^* indicates higher lightness of the samples. The green tea samples have negative a^* values depicting green color. The color of green tea extract, clear, greenish-yellow, without any trace of red or brown color, has been judged to be very appealing. Both the samples had positive b^* values, indicating yellowness.

Conclusion

This study provides a basis for the evaluation and application of prebiotic and probiotic ingredients for their possible applications in the development of ready to drink tea based beverages with improved product characteristics and consumer acceptability. The optimized formulation of prebiotics using mixture design analysis was found to be 1.26% FOS, 6.74% GOS and 0.98% inulin. Synbiotic RTD had a negative impact on acceptance due to the development of unpleasant taste and odor during storage. The prebiotic ice tea premix developed using spray drying to prevent loss of the sensory qualities was found to be sensorially and functionally acceptable after reconstitution.

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Compliance with ethical standards

Conflict of interest We declare that the work described is approved by all authors and the responsible authorities where the work was carried out.

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