Green tea catechins based functional drink (*Green cool*) improves the antioxidant status of SD rats fed on high cholesterol and sucrose diets

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Abstract: In the recent epoch, functional and nutraceuticals foods are gaining wide range of acceptability from the consumers. In the present research investigation, efforts were directed to exploit the green tea phytochemicals. Functional beverage was prepared with catechins and epigallocatechins gallate (EGCG) added individually @ 550 mg/500mL in respective drink. Prepared drinks were evaluated for their physicochemical analysis. Efficacy trial was also conducted, in which diets consisting of high sucrose and cholesterol were provided to rats with concurrent intake of functional drinks. CIE-Lab Color analysis of functional drinks showed that indices of color tonality were non-significantly affected. However, decreasing trend in pH and increased tendency in acidity of drink was noted. While scores for sensory evaluation remained in acceptable range showing suitability for industrial applications. Results of efficacy trial revealed that functional drinks improved serum antioxidant potential of rats. Thus results paved the way for the development of functional beverages using green tea catechins for vulnerable segments.

Keywords: Green tea, green cool, catechins, color, pH, sensory evaluation, antioxidant, TBARS.

INTRODUCTION

The liaison between nutrition and health with inclination to avoid maladies burgeoned the interests in diet based therapy that further resulted in coinage of terms like functional foods/beverages. The concept of functional food was first promoted in 1984 by Japanese scientists and gained legal status in 1991(Burdock *et al*., 2006). The important functional foods are fruits, vegetables, whole grains, fortified foods and functional beverages. A functional beverage is non-alcoholic drink containing non-traditional constituents like phytochemicals in its formulation. Such drinks are becoming popular among the consumers who are more concerned with their health. Phytochemicals especially phenolics can be utilized as functional ingredient because of their ability to combat various maladies. Major concern in developing a beverage is to make it eye catching from sensory view point (Zulueta *et al*., 2010) as product color is one of fundamental components for consumer acceptance. The CIE-Lab color system (CIE, 1986) is used for color measurement as it provides detailed description of color (De Rosso and Mercadante, 2007). Products containing phytochemicals need careful assessment not only to evaluate consumer suitability but also find their effect on specific fragment of population. Considering the importance, scientists working on development of functional foods include not only physiological functionality but also sensory properties (Quílez *et al*., 2006). Moreover, consumers should be educated regarding the functional foods otherwise they will not be benefited because of unlike taste or sensory characters of the product (Wildman and Kelley, 2007).

Oxidative stress occurs when there is imbalance between pro-oxidant relative to antioxidant and antioxidant defense mechanism. The imbalance may be due to increased production of reactive oxygen species and impaired antioxidant defense after consumption of diets containing high amount of cholesterol and glucose. It is revealed that green tea improves overall anti-oxidative status because of its radical quenching ability (Erba *et al*., 2005).

Considering all, present project was designed to prepare functional drink from active ingredients of green tea (catechins and EGCG). This functional beverage was evaluated for sensory and physical characteristics to check its compatibility for use by human beings. Further efficacy trial was conducted on Sprague Dawley (SD) rats to propose its use for the treatment of oxidative stress and allied disparities.

MATERIALS AND METHODS

Functional drink

Three types of functional drinks (Green Cool) were prepared i.e. T_0 (control drink), T_1 (drink containing catechins) and T_2 (drink containing EGCG). Catechins and EGCG were extracted according to their respective methods. The major ingredients of functional drinks were aspartame, citric acid, sodium benzoate, carboxy methyl cellulose (CMC), food grade color and flavor. After processing of drinks, active ingredients including catechins and EGCG were added individually @ 550 mg/500mL in respective drink.

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Physico-chemical analysis of functional drinks

Functional drinks were analyzed for the following characteristics during two month storage at 0, 15, 30, 45 and 60 days.

Color

The color of prepared functional drinks was estimated using CIE-Lab Color Meter (CIELAB SPACE, Color Tech-PCM, USA). To run the experiment, 5mL of sample was taken and color values as *L** (lightness), *a** (–a greenness, +a redness), and *b** (–b blueness, +b yellowness) were recorded. The data thus obtained was used to calculate chroma (C*) and hue angle (Peng *et al*., 2006).

Chroma (C*) = $[(a^*)^2 + (b^*)^2]^{1/2}$

Hue angle (h) = $\tan^{-1} (b^*/a^*)$

Soluble Solids

Total soluble solids in functional drink samples were estimated by hands refractometer (TAMCO, Model No. 90021, Japan) and results were expressed as percent soluble solids (°Brix).

pH and total acidity

Functional drinks samples were evaluated for pH and total acidity according to methods described in AOAC (2003).

Total catechins

Amount of total catechins in functional drinks during storage period were determined through HPLC. Functional drinks were analyzed for active ingredients (epicatechin EC, epigallocatechingallate EGCG, epigallocatechin EGC, epicatechingallate ECG) by HPLC (PerkinElmer, Series 200, USA) containing C18 column (250 mm x 4.6 mm, 5.0 µm particle size) and an autosampler. Sample amount 10 µL and column temperature 40°C were kept during HPLC analysis. During catechins quantification, mobile phase including solvent A (acetonitrile/acetic acid/water 6:1:193) and solvent B (acetonitrile/acetic acid/water 60:1:139) was used with a flow rate of 1 mL/min. Quantification of individual components was carried out by UV detector at 280 nm, likewise extracted epigallocatechingallate (EGCG) rich fraction was also analyzed through HPLC (Liang *et al*., 2007).

Sensory evaluation

The Functional drinks (T_0, T_1, T_2) were subjected to sensory evaluation by trained taste panel using nine point hedonic scale system (9 = like extremely, 1 = dislike extremely) as described by Meilgaard *et al.* (2007). Sensory evaluation regarding attributes like color, flavor, sweetness, sourness and overall acceptability was performed at 0, 15, 30, 45 and 60 days. Hedonic response was judged in Sensory Evaluation Laboratory of NIFSAT, University of Agriculture, Faisalabad. On the day of evaluation, panelists were seated in separate booths with white fluorescent light and drinks were presented in transparent glasses labeled with random codes. Panelists were provided water and unsalted crackers to neutralize their mouth between samples testing. Samples were presented to the judges randomly and were asked to rate their acceptance by giving score for selected parameters.

Efficacy study

One hundred twenty male Sprague Dawley rats were procured from National Institute of Health (NIH), Islamabad and housed in the Animal Room of National Institute of Food Science and Technology. Four types of studies diets i.e. study I (normal diet), study II (high cholesterol diet), study III (high sucrose diet) and study IV (high cholesterol + high sucrose diet) were conducted (see appendix). Rats were divided in four groups and each group consisted of three sub groups of ten rats each. In each study the respective diet was given to the rat groups with concurrent provision of functional drinks (T_0, T_1 and T_2). At the initiation of study some rats were sacrificed to get baseline values. The temperature (23±2ºC) and relative humidity (55±5%) were maintained throughout the experiment with 12 hours light-dark period.

Antioxidant status

Glutathione content was also estimated after eight weeks in rats by using the protocols of Beutler (1982). Indicator of lipid peroxidation was determined according to the prescribed method (Ohkawa *et al*., 1979) including thiobarbituric acid reactive species (TBARS).

RESULTS

Physico-chemical analysis of functional drinks

The L* values for drinks T_0 (control), T_1 (drink containing catechins) and T_2 (drink containing EGCG) were 97.41±0.72, 97.61±0.79 and 97.45±0.81, respectively (table 1). Two months storage resulted in non-substantial ($P \geq 0.05$) decrease in L* value. Addition of catechins resulted in marked increase in a* value of drinks from -0.13±0.011 in T_0 (control) to -0.60±0.014 in T_1 containing catechins and -1.15±0.10 in T_2 containing EGCG. During storage, values for a* decreased from -0.73±0.06 at 0 day to -0.53±0.04 at the termination of study. It is evident from the table (1) that b* value increased non-significantly as function of active ingredients and storage. Means for chroma value and hue angle showed that addition of active ingredients in drinks resulted in non-significant variations in these parameters. Storage of drinks also led to non-momentous variations for this trait.

Brix for three functional drinks T_0, T_1 and T_2 was 1.71±0.008, 1.73±0.005 and 1.72±0.008, respectively. Storage did not affect the TSS substantially and at 0 day value for brix was 1.71±0.005 whilst at 60^{th} day it was 1.73±0.004 (fig. 1).

Table 1: Effect of treatment and storage on color of functional drinks

Parameters	Storage intervals (days)	Drinks			Means
		T_0	T_1	T_2	
L* value	0	99.48±6.523	99.99±6.557	99.99±4.358	99.9±0.17
	15	98.43±6.147	98.64±2.610	98.61±5.998	98.6±0.07
	30	97.07±1.681	97.21±2.572	97.33±4.243	97.2±0.07
	45	96.74±4.433	96.83±1.677	96.81±4.436	96.8±0.03
	60	95.32±4.368	95.40±2.524	96.43±1.670	95.1±0.35
	Means	97.41±0.72	97.61±0.79	97.45±0.81	
a* value	0	-0.22±0.014c	-0.66±0.043f	-1.33±0.058j	-0.73±0.06d
	15	-0.19±0.012c	-0.63±0.017f	-1.27±0.077i	-0.70±0.04c
	30	-0.12±0.002b	-0.61±0.016ef	-1.15±0.050h	-0.63±0.05b
	45	-0.07±0.003ab	-0.57±0.010de	-1.02±0.047g	-0.55±0.03a
	60	-0.04±0.002a	-0.54±0.014d	-1.02±0.018g	-0.53±0.04a
	Means	-0.13±0.011a	-0.60±0.014b	-1.15±0.10c	
b* value	0	131.97±8.65	134.83±8.84	132.96±5.79	133.25±0.84
	15	132.25±8.25	134.97±3.57	133.13±8.09	133.45±0.80
	30	132.49±2.29	135.52±3.58	133.63±5.82	133.88±0.89
	45	133.15±6.10	135.86±2.35	134.03±6.14	134.34±0.80
	60	133.33±6.11	136.02±3.59	134.43±2.32	133.70±0.78
	Means	132.63±0.26	135.44±0.45	133.09±0.49	
Chroma	0	131.97±8.65	134.83±8.84	132.97±5.79	133.26±0.84
	15	132.25±8.25	134.97±3.57	133.14±8.09	133.45±0.80
	30	132.49±2.29	135.52±3.58	133.63±5.82	133.88±0.89
	45	133.15±6.10	135.86±2.35	134.03±6.14	134.35±0.80
	60	133.33±6.11	136.02±3.59	134.43±2.32	133.70±0.79
	Means	132.64±0.26	135.44±0.45	133.10±0.49	
Hue angle	0	-1.57±0.103	-1.57±0.073	-1.56±0.068	-1.56±0.002
	15	-1.57±0.098	-1.57±0.041	-1.56±0.095	-1.56±0.002
	30	-1.57±0.027	-1.57±0.041	-1.56±0.068	-1.56±0.002
	45	-1.57±0.072	-1.57±0.027	-1.56±0.072	-1.56±0.002
	60	-1.57±0.072	-1.57±0.041	-1.56±0.027	-1.55±0.002
	Means	-1.57±0.001	-1.57±0.001	-1.55±0.001	

Values are expressed as means ± standard deviation. Means carrying same letter in a column do not differ significantly ($P \leq 0.05$)
T_0 = Control (drink without active ingredients) T_1 = Drink containing catechins T_2 = Drink containing EGCG

Drinks T_1,T_2 and T_0 had pH value 4.41±0.062, 4.39±0.061 and 4.46±0.07, respectively. Storage period of 60 days effected pH substantially as there was momentous decrease in the pH of drinks (fig1). Likewise, values for acidity in different treatments affected non-significantly and were 0.172±0.01, 0.170±0.01 and 0.178±0.01 for T_0, T_1 and T_2, respectively. However, acidity increased significantly as function of storage (fig1). The Green Cool was also evaluated for catechins contents and results indicated that during 60 days of storage, catechins contents were reduced from 90.00±1.00 to 83.33±0.58 mg/100mL and 89.00±1.00 to 81.67±1.15 mg/100mL (fig. 1), in catechins and EGCG based functional drinks respectively.

Sensory evaluation

Results for color score presented in table 2 indicated that score at initiation of study was 7.71±0.05 that decreased non-significantly ($P \geq 0.05$) to 7.26±0.14 during 2 months.

It is obvious from table 2 that treatment and storage did not affect the flavor of drinks significantly however, decreasing trend for this trait was recorded with increasing storage. Means for sweetness showed that there existed significant differences due to treatment and storage. Sourness scores revealed that neither treatment nor storage affected this attribute significantly. However, slight decreasing trend in sourness was noted with passage of time. Means for overall acceptability explicated non-significant ($P \leq 0.05$) behavior as feature of treatment and storage.

Glutathione and Thiobarbituric acid reactive substances (TBARS)

Statistical analysis in table 3 showed that functional drinks imparted significant effect on serum glutathione contents and serum thiobarbituric acid reactive substances (TBARS) of rats in the entire efficacy trial. However the effect was non-significant on TBARS in study I.

Table 2: Effect of treatments and storage on sensory evaluation of functional drinks

Parameters	Storage intervals (days)	Drinks			Means
		T_0	T_1	T_2	
Color	0	7.62±0.50	7.80±0.38	7.70±0.50	7.71±0.05
	15	7.51±0.47	7.78±0.45	7.59±0.43	7.63±0.08
	30	7.46±0.45	7.62±0.46	7.42±0.36	7.50±0.06
	45	7.38±0.32	7.53±0.39	7.23±0.30	7.38±0.09
	60	7.29±0.33	7.49±0.32	7.01±0.46	7.26±0.14
	Means	7.45±0.06	7.64±0.09	7.39±0.10	
Flavor	0	7.80±0.51	7.65±0.37	7.70±0.50	7.72±0.044
	15	7.75±0.48	7.59±0.44	7.64±0.43	7.66±0.047
	30	7.58±0.46	7.42±0.45	7.57±0.36	7.52±0.052
	45	7.45±0.32	7.33±0.38	7.49±0.31	7.42±0.048
	60	7.39±0.34	7.28±0.32	7.33±0.48	7.33±0.032
	Means	7.59±0.08	7.45±0.06	7.55±0.05	
Sweetness	0	7.78±0.51	7.35±0.35	7.24±0.47	7.46±0.16a
	15	7.54±0.47	7.22±0.42	7.13±0.40	7.30±0.12ab
	30	7.36±0.45	7.15±0.43	7.05±0.34	7.19±0.09abc
	45	7.22±0.31	6.85±0.36	6.90±0.28	6.99±0.11bc
	60	7.08±0.32	6.65±0.29	6.80±0.44	6.84±0.13c
	Means	7.40±0.12a	7.04±0.08b	7.02±0.05b	
Sourness	0	7.70±0.50	7.57±0.36	7.68±0.50	7.65±0.04
	15	7.63±0.48	7.42±0.43	7.56±0.42	7.54±0.06
	30	7.40±0.45	7.34±0.45	7.44±0.36	7.39±0.03
	45	7.39±0.32	7.14±0.37	7.37±0.30	7.30±0.08
	60	7.25±0.33	7.13±0.31	7.08±0.46	7.15±0.05
	Means	7.47±0.08	7.32±0.07	7.42±0.05	
Overall acceptability	0	7.90±0.52	7.80±0.38	7.60±0.50	7.77±0.09
	15	7.70±0.48	7.63±0.44	7.42±0.42	7.60±0.08
	30	7.70±0.47	7.56±0.46	7.54±0.36	7.58±0.05
	45	7.45±0.32	7.34±0.38	7.33±0.30	7.37±0.04
	60	7.30±0.33	7.20±0.31	7.15±0.47	7.22±0.04
	Means	7.61±0.10	7.51±0.09	7.41±0.09	

Values are expressed as means ± standard deviation. Means carrying same letter in a column do not differ significantly
T_0 = Control (drink without active ingredients), T_1 = Drink containing catechins, T_2 = Drink containing EGCG

DISCUSSION

Physico-chemical analysis of functional drinks

In a study Lu *et al*. (2010) mentioned decrease in L* and b* values for crust and crumb but increase in a* value for crumb when they substituted green tea powder in sponge cake which indicated that added powder resulted in darker color. The variations in color parameters with the current research may be due to the fact that they used green tea powder having coloring matter which imparted darker color to cake moreover, oxidation of polyphenols also participated in darker look. Research work of González-*Molina et al*. (2009) supported the current findings as they recorded non-significant changes in total soluble solids of apple and pomegranate juices and their mixtures during 70 days storage.

During storage, decreased pH and increased acidity might be attributed to break down of citric acid in the prepared drinks. Moreover, acidic nature of aspartame may be contributed to increased acidity (Ahmad *et al*., 2008). Ahmed *et al*. (2008) also documented a decreasing trend in pH and increasing tendency in acidity of mandarin drink during 60 days storage.

Reduction in catechins content of functional drinks are comparable by work of González-Molina *et al*. (2009), they reported 15-20% reduction in total phenolic content of drink prepared from pomegranate and lemon juices during 70 days storage period.

Sensory evaluation

The results of current investigation regarding sensory evaluation of functional drinks are further assured by the work of Lu *et al* (2010), they prepared sponge cake in which green tea was substituted (10, 20 and 30%) with flour and observed non-significant differences in crust and crumb color, sweetness, flavor, texture and overall liking

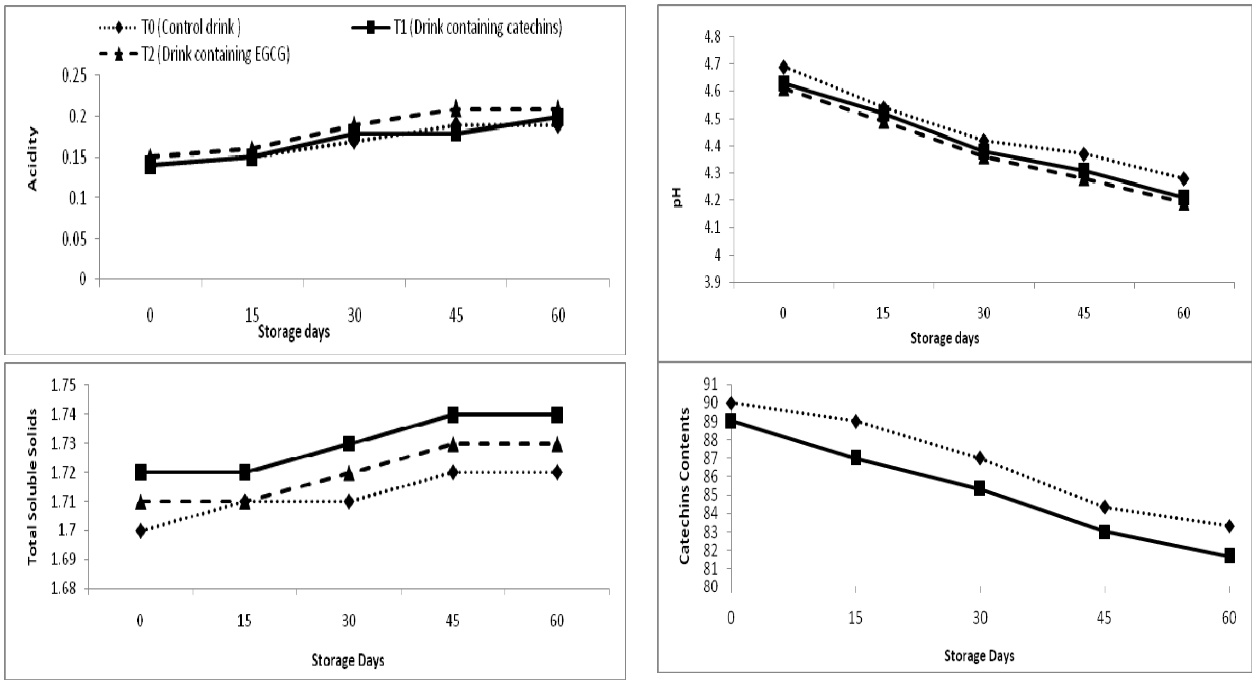

Fig. 1: Effect of treatments and storage on acidity, pH, total soluble solids and catechins contents of functional drinks

Table 3: Effect of treatments and studies on antioxidant status of functional drinks

Parameters	Studies	Drinks		
		T_0	T_1	T_2
Glutathione (mg/L)	Study I	48.41±0.99b	49.78±1.85b	51.84±2.05a
	Study II	36.09±1.31b	41.81±3.46a	42.35±2.87a
	Study III	40.89±1.18b	45.56±2.77a	46.19±3.22a
	Study IV	32.32±2.86b	38.97±1.23a	40.49±2.70a
TBARS (µmol/L)	Study I	7.47±0.32	7.17±0.41	6.95±0.27
	Study II	8.56±0.34a	7.83±0.55ab	7.38±0.69b
	Study III	8.87±0.28a	7.96±0.46b	7.62±0.51b
	Study IV	10.13±0.75a	8.29±0.38b	7.96±0.64b

Values are expressed as means ± standard deviation. Means carrying same letter in a row do not differ significantly
Study I: Normal diet, Study II : High cholesterol diet, Study III: High sucrose diet, Study IV: High cholesterol + high sucrose diet
T_0 : Control drink (without active ingredients), T_1 : Drink containing catechins, T_2 : Drink containing EGCG

Appendix: Composition of experimental diets

Ingredients (%)	Normal diet	High cholesterol diet	High Sucrose diet	High cholesterol + High Sucrose diet
Corn Oil	10	10	10	10
Corn Starch	66	65	26	25
Casein	10	10	10	10
Cellulose	10	10	10	10
Salt mixture	3	3	3	3
Vitamins	1	1	1	1
Cholesterol*	-	1	-	1
Sucrose*	-	-	40	40

* Added in rats modeling in different studies

scores among control and green tea substituted cakes (10 and 20%).

Glutathione and Thiobarbituric acid reactive substances (TBARS)

Earlier, Feillet-Coudray *et al* (2009) indicated that high fat + high sucrose diet uplifts lipid and protein oxidation, resulting in oxidative stress.

In current study increase in GSH concentrations and decrease in TBARS in experimental animals might be due to reason that polyphenols have the ability to take part in vitamin E recycling thus complements the GSH functions (Zhu *et al*., 1999). Moreover, catechins also prevent the oxidative damage through reduction of aspartate aminotransferase and uric acid concentration (Panza *et al*., 2008). Antioxidant activity of catechins is attributed to their structural chemistry as their ortho-trihydroxy group has ability to scavenge free radicals and EGCG is the most important antioxidant due to possession of four dihydroxy groups thus effective against oxidative stress (Yin *et al*., 2008).

CONCLUSION

Conclusively, addition of catechins and EGCG for the preparation of functional drinks does not impart any adverse effect on hedonic response. The added food grade color to the functional drinks mask the tint of active ingredients, thereby no unpleasant response was recorded by the panelist. During sensory evaluation only mild decrease in sweetness was noted however, the values were within acceptable limit during storage. Efficacy study also showed that drinks lead to improvement in antioxidants status of SD rats provided high sucrose and cholesterol diets. Owing to the results of physicochemical analysis and efficacy trial, the prepared functional drinks are suitable for further use in diet therapy for the treatment of various lifestyle-related disorders.

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