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Research Article

Development and Stability of Bioactive Compounds in Carbonated Black Ice Tea Beverage

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Abstract: The aim of present research project was to investigate potential use of black tea extracts to develop carbonated beverage from indigenous raw material. Healthy and nutritious black ice tea beverages were developed with different levels of extracts. Carbonated black ice tea samples were subjected to analyze after one-month interval for total soluble solids, pH, sugars and ascorbic acid content up to period of 90 days. Changes in bioactive compounds in terms of total phenolic contents, theaflavins and DPPH free radical scavenging activity were also investigated. The storage study showed that total soluble solids, titratable acidity and reducing sugars increased whereas decline in pH, ascorbic acid, total phenolic contents, theaflavins, thearubigins and DPPH free radical scavenging activity was observed. Results of sensory evaluation indicated that carbonated black ice tea beverage with 0.2% black tea extract was highly acceptable at 5°C storage conditions. In Pakistan, because of prolonged summer season, there must be an alternate of hot black tea. Iced black tea will be a good option for people who want to take tea in summer too. This black tea needs to be commercialized and will get market attention as it has great potential to get consumers attraction.

Keywords: Black ice tea, carbonated beverages, physicochemical analysis, bioactive compounds, storage behavior.

1. INTRODUCTION

Human health is considered an important issue in recent years. An interest in health, nutrition and fitness has been increased. To meet consumers demand, food companies have rather high expectations in food products for a healthy life style. The growing interest in new functional foods with special characteristics and health promoting properties has led to the development of new functional beverage. It is difficult and challenging task to develop unfermented beverages than semi or fully fermented beverages [1,2,3]. In Japan, barley tea is common drink, it is non-caffeinated, non-tannin drink which has high percentage of β-glucan (polysaccharides) and antioxidant compounds [4,5]. Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants [6]. Phytochemical analysis of plants is commercially important being great interest of pharmaceutical industries for the production of new drugs to cure various diseases [7].

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Second most consumed beverage after water is tea. Abundant quantity of bioactive compounds are present in tea, which contain ability to fight against many diseases such as diabetes, liver diseases, CVDs, cancers and obesity [8,9,10]. This has been proven by many epidemiological. Clinical trials have also revealed the beneficial effects of tea and its bioactive compounds against CVDs [11]. Cardiovascular diseases (CVDs) are critical global public health issues with high morbidity and mortality. Additionally, in vitro and in vivo studies have also shown that bioactive compounds from tea are effective in protecting against many diseases including CVDs [12].

Black tea (*Camellia sinensis* L) utilization has a historic tradition in Pakistani society and people enjoy tea serving. Pakistan is the leading importer of tea after United Kingdom with increasing per capita demand [13]. There are generally three types of teas available in the markets unfermented as green tea, semi-fermented as oolong and fully fermented as black tea [14]. Nearly thirty-eight carotenoids have been estimated in tea leaves and the main pigments are from pheophytin a and b [15]. These coloring compounds exhibit strong antioxidant activities on human health and is of great importance as additives in food and cosmetic industry [16]. Nowadays carbonated beverages are popular among the consumers due to their sensation that is produced on the tongue. Mixtures of cola, sugar vanilla were served as tonics in 1870's for people who were sick; it was also used to add flavours to beverages [17]. Polysaccharides are added in soft drinks in the form of hydrocolloids to capture the CO_2 bubbles, it also adds mouth feel to beverages [18]. Sensory characteristics are very important to measure the quality of the beverage, chemical composition also have equal importance [19]. Keeping in view the above said properties, the objectives of present research project was to develop a nutritious formulation from indigenous resources in a new popular way with comparison to the commercially available carbonated ice tea beverage. The stability of bioactive compounds and sensory attributes were evaluated at 5°C as a function of storage.

2. MATERIALS AND METHODS

2.1 Procurement of Raw Materials

The ingredients and raw materials like sugar, black tea, preservatives, flavor and 1500mL plastic pet bottles were procured from local super market of Faisalabad, Pakistan. This research project was conducted at Food Technology Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan.

2.2 Development of Black Tea Extracts and Carbonated Black Ice Tea Beverages

The black tea extract/infusions were prepared according to the method [20] with some modifications. The basic formulation and procedure for the development of black tea infusions and carbonated beverages were as followed:

a. Extract preparation

Black tea (30g) and water (3000mL) was taken

⇩

Steeped in hot water

⇩

Left it for 5 minutes after boiling of water

⇩

Filtration of the contents with fine muslin cloth to get clear black extract

⇩

Syrup was prepared by heating the extract after adding sugar (5%) and preservatives.

⇩

Filtered again prepared syrup

⇩

The final volume was made exactly 6 liters with the addition of water.

⇩

Acorbic acid (2-3g) and lemon flavor (few drops) was added after cooling of syrup below 50°C then.

⇩

300mL syrup was filled in each 1500mL plastic pet bottle (The remaining volume was made up during carbonation process)

b. Carbonation

Chilled water (5°C) and CO_2 gas was added simultaneously up to the volume 1500mL (Carbonation unit Model: local made, steel top)

⇩

CO_2 gas pressure was maintained at 50lb/in^2

Sodium benzoate (0.1%) was used as preservative to make all treatments. The remaining treatments were developed through similar pattern with incorporated different levels of black tea extract according to the treatment plan (Table 1.). The developed carbonated black ice tea samples were placed at 5°C to study the storage behavior.

2.3 Analysis of Carbonated Black Ice Tea Beverage

The developed carbonated black ice tea beverages were analyzed for various physicochemical tests, bioactive compounds and sensory attributes at 5°C during storage. The analyses were performed in triplicate for quality parameters and five replicates for sensory evaluation. The methods of analysis and their protocols have described below:

Table 1. Treatment plan

Treatments	**Black tea extracts**
T_1	Control, commercially available carbonated black ice tea
T_2	0.2%
T_3	0.4%
T_4	0.6%
T_5	0.8%

2.3.1 Total Soluble Solids (°brix)

Refractometer (Abbe refractometer model: 2WAJ) was used to determine total soluble solids expressed as °brix according to the method explained in AOAC [21].

2.3.2 pH

The pH of the developed carbonated black ice tea beverages was determined with digital pH meter (Hanna, HI 2211, pH/ORP meter, Europe). An amount of 50mL sample was taken in 100mL beaker and pH meter was used to measure pH values according to method described in AOAC (2006).

2.3.3 Titratable Acidity (%)

The acidity of each prepared sample was estimated according to standard method given in AOAC (2006). Took the 10mL sample of carbonated black ice tea and added distilled water up to 100mL, then titrated it with 0.1N NaOH by using phenolphthalein as an indicator, till light pink end point.

2.3.4 Reducing and Non-Reducing Sugars (%)

The reducing and non-reducing sugars of developed carbonated black ice tea beverages were calculated by using the respective method as mentioned in AOAC [21].

2.3.5 Ascorbic Acid (%)

The ascorbic acid contents were determined with the utilization of dye (2, 6-dichlorophenolindophenol) by following the standard method of AOAC [21].

2.3.5 Total Phenolic Contents

The total phenolic contents were measured through folin ciocalteu reagent method [22]. The sample, folin ciocalteu reagent and distilled water with amounts 125µL and 500µL respectively was mixed together and left it at 22ºC for 5 minutes, then added 4.5mL of $NaHCO_3$ (7%) into the prepared mixture. The UV spectrophotometer model; CECIL CE7200 was used to calculate the absorbance of the mixture at 765 nm after 90 minutes. The estimated amounts of total phenolic contents were expressed in gallic acid equivalent (mg/100GEA).

2.3.6 Theaflavins and Thearubigins Content

The theaflavins and thearubigins content were estimated through the method of Angayarkanni [23]. Took the separating funnel in which equal volume of samples and iso-butyl methyl ketone was mixed. Calculated the value “A”, at 380 nm absorbance of the separated organic diluted layer with 9mL ethanol, then diluted the organic phase (10mL) with the addition of 2.5% Na_2HPO_4 (10mL) . Ethanol was used to dilute the separated layer and calculated the absorbance at 380 nm and mentioned as “B”. Similarly “C” was calculated when the absorbance was measured at 380 nm with aqueous phase of butanol in which 9mL ethanol was added.

$$TF\ (\%) = 4.313 \times C$$

$$TR\ (\%) = 13.643 \times (A+C-B)$$

2.3.7 DPPH Free Radical Scavenger Activity (%)

The DPPH radical scavenging activity was estimated. Each 4mL sample was taken and added 1mL DPPH then placed the mixture for 30 minutes at room temperature. The UV spectrophotometer (Model, CECIL CE7200) was used to measure the absorbance of the samples at 520 nm wave length. Measured the inhibition (%) with the formula given below:

$$\text{Reduction of absorbance } (\%) = [(AB - AA) / AB] \times 100$$

AB = absorbance of blank sample (t = 0 minute)

AA = absorbance of tested extract solution (t = 30 minutes)

2.3.8 Sensory Evaluation

The organoleptic evaluation attributes such as color, taste, flavor and overall acceptability of developed carbonated black ice tea beverages were evaluated by a panel composed of students, research and technical staff from the Post-Harvest Research Center, Ayub Agricultural Research Institute, Faisalabad, Pakistan, according to 9-points hedonic scale [24].

2.3.9 Statistical Analysis

The obtained results were analyzed statistically by Completely Randomized Design (CRD) and two-way Analysis of Variance (ANOVA) using software statistx 8.1. Tukey LSD was used to separate means of significant treatments and storage times. Results have been presented as means ± standard deviation and considered to be significantly different when $p < 0.05$ [25].

3. RESULTS AND DISCUSSION

3.1 Total Soluble Solids (°brix)

Changes in total soluble solids are expressed in Fig. 1. Mean values showed significant difference within the treatments of carbonated black ice tea beverages. The total soluble solids did not change considerably during storage. At first day of storage, it was ranged from 7.65 to 11.54 for T_1 to T_5, with $P \leq 0.05$. While at the end of storage period a slight increase was observed (7.69-11.55). This slight increase in total soluble solids is considered as a result of hydrolysis of polysaccharides into monosaccharide.

The same increasing trend of total soluble solids was observed [26], during storage period in different types of carbonated and non-carbonated RTS beverages. These results are in agreement with previous findings [27] who reported no change in the total soluble solids of soft drinks during the storage periods.

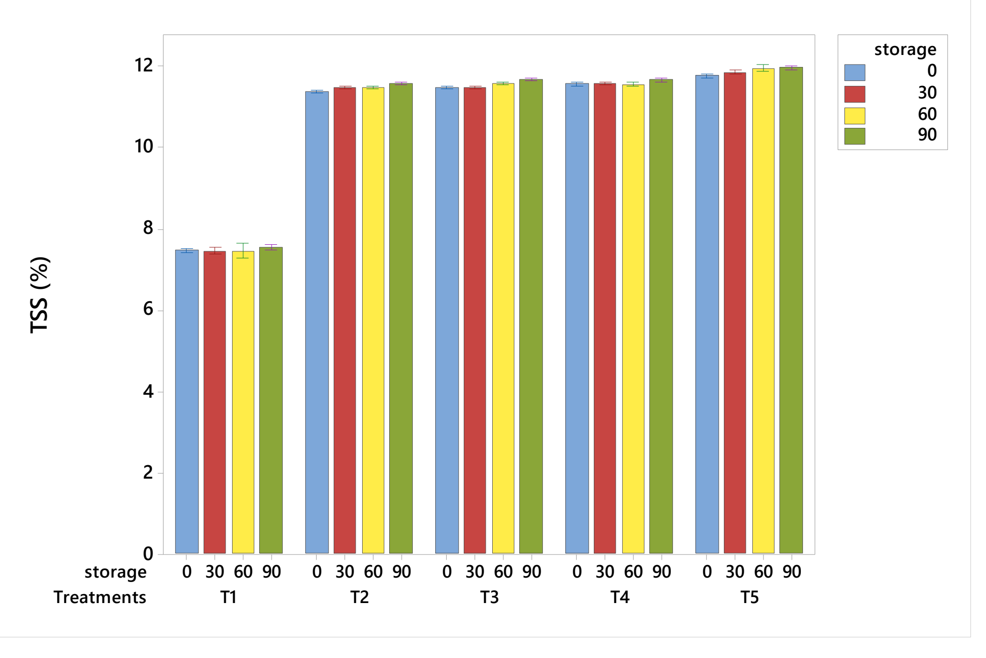

Fig.1. Effect of treatments and storage intervals on total soluble solids (°brix) of carbonated black ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract) (Level of significance $p \leq 0.05$

3.2 Titratable Acidity (%)

Titratable acidity plays a vital role in the shelf life of any type of beverage or fermented drinks and provides unfavorable conditions for the multiplication of microorganisms. It also helps to ensure some chemical changes during processing and storage. Titratable acidity (%) was increased significantly during storage while non-significant increase was observed within the treatments with increase in percentage of black tea extract (Fig. 2). Mean values were ranged from 0.42 to 0.45 for treatments from T_2 to T_5 while T_1 showed highest value for titratable acidity i.e. 1.09 in commercial sample. All the treatments showed gradual increase in titratable acidity with mean values of 1.32, 0.72, 0.73, 0.74 and 0.75 for T_1, T_2, T_3, T_4 and T_5 respectively. Slightly acidic nature of aqueous solution of black tea was also observed by Sharma *et al.* [28]. The same increasing trend of acidity (0.19-0.51) was observed [29] during storage period of different types of non-alcoholic blended beverages. The decrease in pH and increase in acidity during storage might be due to degradation of carbohydrates and sugars by the action of enzymes.

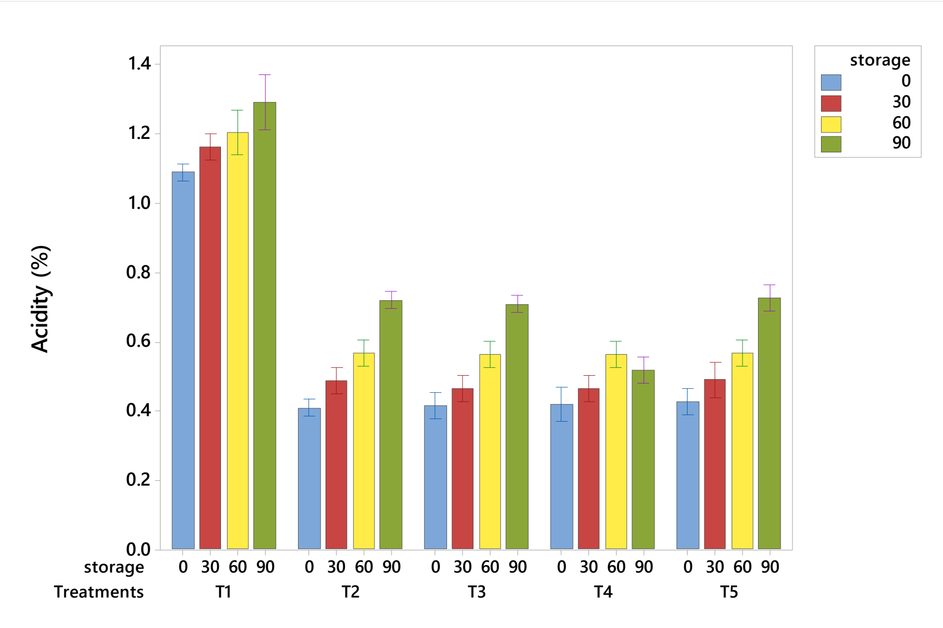

Fig. 2. Effect of treatments and storage intervals on titratable acidity (%) of carbonated black ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract)

3.3 pH

PH is inversely proportional to acidity of any medium. The present study for carbonated ice tea beverage also depicted the same changing pattern of pH parameter. pH values affected by various treatments and storage interval given in Fig. 3 showed non-significant difference within treatments of carbonated black ice tea beverages while varied significantly during storage period. pH values were in the range of 3.45 to 4.26 for T_1 to T_5 at 0 day. With the passage of time, pH showed a declining trend and was decreased to 3.12, 3.82, 3.83, 3.84 and 3.85 for T_1, T_2, T_3, T_4 and T_5 respectively. This decrease may be due to the fermentation process which produces the acids. The same decreasing trend in pH of black ice tea was observed by Akinwale *et al.* [30] in his study for physico-chemical, microbiological changes in blends of medicinal tea. These results are also in accordance with those reported by El-Faki and Eisa, [27] who stated that the pH of various carbonated beverage showed a slight decrease from 3.35-3.12, after 90 days of storage at ambient temperature conditions.

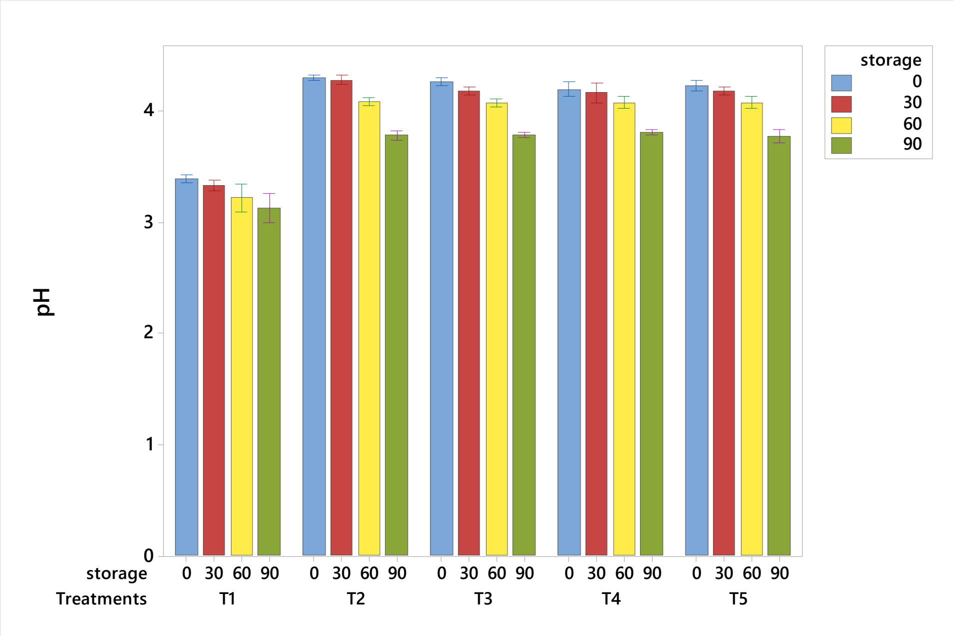

Fig. 3. Effect of treatments and storage intervals on pH of carbonated black ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract) (Level of significance $p \leq 0.05$).

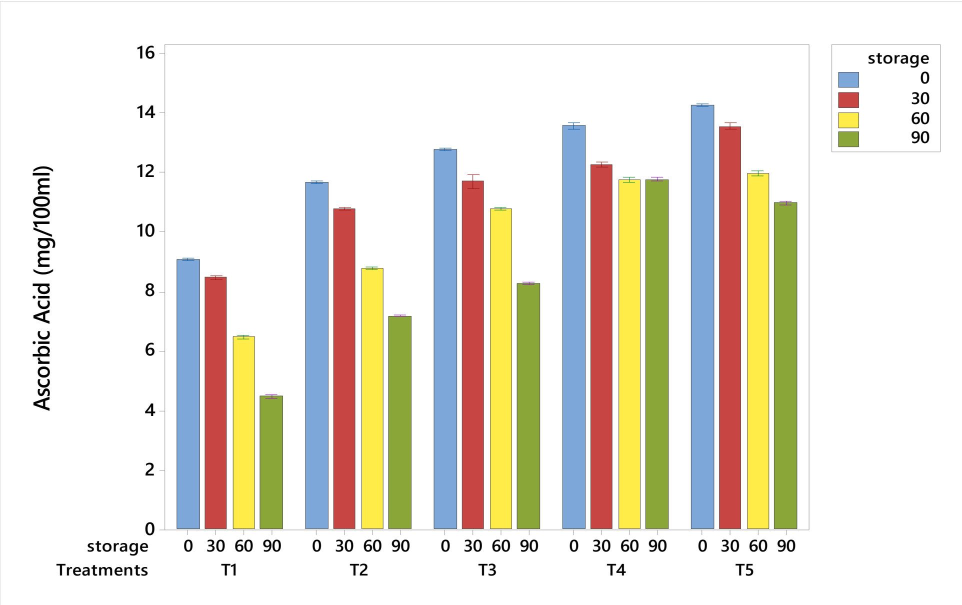

Fig. 4. Effect of treatments and storage intervals on ascorbic acid of carbonated black ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract).

3.4 Ascorbic Acid (mg/100mL)

The ascorbic acid contents significantly decreased within the time interval. The results for ascorbic acid of carbonated black ice tea beverages are depicted in Fig. 4. Ascorbic acid contents decreased from 9.15 to 4.45 for T_1, 11.60 to 7.26 for T_2, 12.73 to 8.35 for T_3, 13.50 to 11.60 for T_4 and 14.44 to 10.79 for T_5 with the passage of time. These present findings are in close agreement with the research studies of Sahota *et al.* [29] who prepared low alcoholic naturally carbonated blended beverage from guava and lemon that was within the range of 25.7 to 8.4 and 27.8 to 2.9.

3.5 Reducing Sugars (%)

The results regarding changes in reducing sugars in black ice tea beverages indicated that different treatments and storage period had significant effect on reducing sugars. Reducing sugars increased from 0.71 to 1.02 % in case of T_1,1.07 to 1.35%, 1.10 to 1.37 %, 1.10 to 1.40 % and 1.08 to 1.48 % in case of T_2, T_3, T_4 and T_5 respectively as shown in Fig. 5. These changes may be attributed to the inversion of sucrose under acidic environment. Similar results were found earlier where reducing sugars increase from 2.13 to 70 percent during six months storage of bottle gourd-basil leaves juice [31].

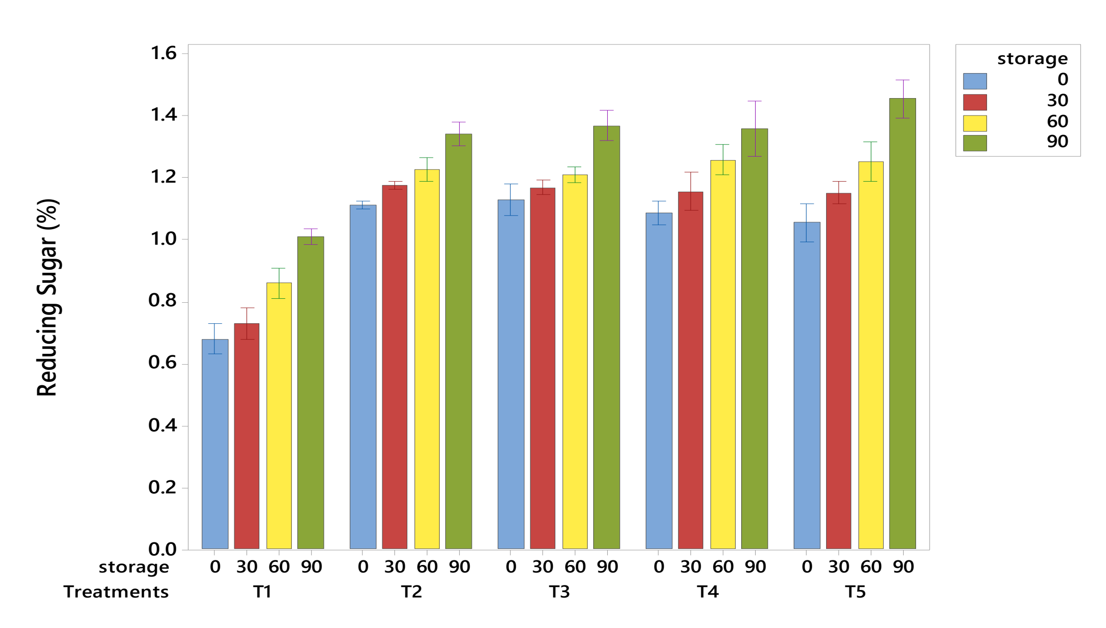

Fig. 5. Effect of treatments and storage intervals on reducing sugars of carbonated black ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract) (Level of significance $p \leq 0.05$)

3.6 Non-Reducing Sugars (%)

Non-reducing sugars showed significant variation in treatments as well as during storage intervals in carbonated black iced tea beverages. Highest non reducing sugar contents were found at 0 day of storage i.e., 6.13 for T_1, 9.55 for T_2, 9.55 for T_3, 9.53 for T_4 and 9.55 for T_5 which reduced to 5.36 in case of T_1, 8.74, 8.73, 8.77, 8.80 in case of T_2, T_3, T_4 and T_5 respectively. The findings of current research are well supported [32] who found that with increase in storage time non-reducing sugars decreased. The results are also in line with the findings of the Chowdhury *et al.* [33] who studied the six month storage effect on the shelf life of mixed juice and found significant decrease in non-reducing sugars due to breakdown of sucrose with the reaction of acids.

3.7 Total Phenolic Contents (mg/100GEA)

Total phenolic contents are included in the volatile flavoring compounds of black tea. Polyphenols are the most important chemical constituent that influences the taste and flavor in tea. Means related to total phenolic contents in carbonated black ice tea beverages are demonstrated in Fig. 7. Data revealed a significant decrease in total phenolic contents from 704.2 to 404.3mg/100GEA in case of T_1, 828.9 to 582.8mg/100GEA in T_2, 1246.3 to 665.3mg/100 GEA in T_3, 1296.9 to 678.3 GEA in T_4 and in case of T_5 they were decreased from 1323.3 to 706.1mg/100 GEA after 90 days of storage. These results are almost similar to earlier study of total phenolic contents of Indian herbal teas, which were ranged from 786.00 to 2789.00mg/100GEA of sample studied [34]. Susanne *et al.* [35] has also observed that these volatile compounds are more stable at high pH hence this decrease in total phenolic compounds are due to the decrease in pH of the black ice tea beverages during storage.

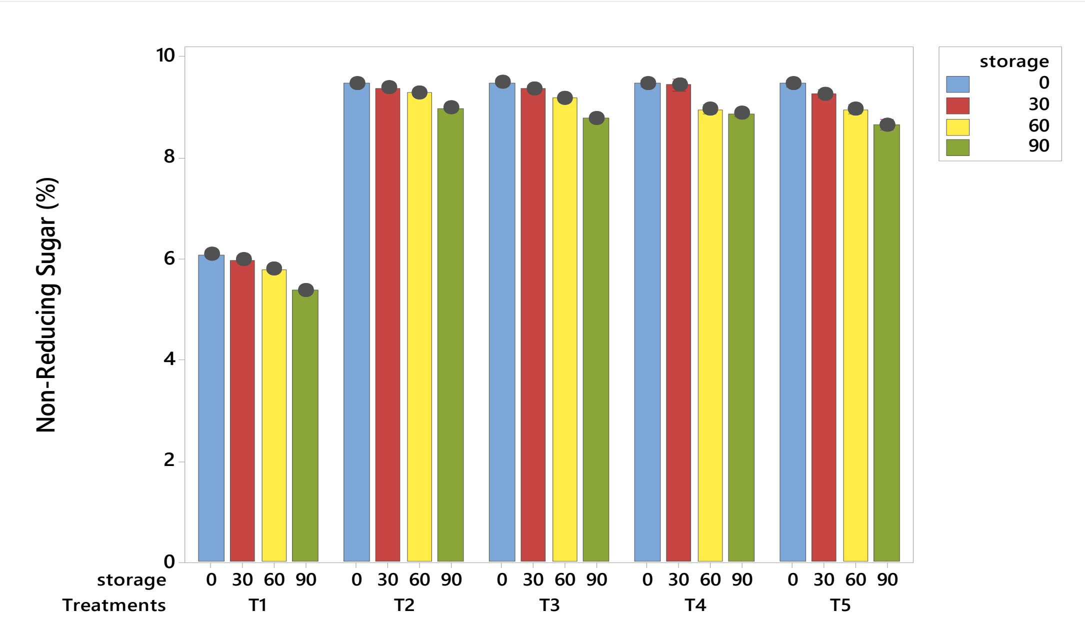

Fig. 6. Effect of treatments and storage intervals on non-reducing sugars of carbonated black ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract)

3.8 Theaflavins (%)

Theaflavins in black ice tea are responsible for the astringent taste of the beverage. Changes in the quantity of theaflavins in the carbonated black ice tea beverages during storage affect the quality of the tea. The statistical analysis showed significant decrease in theaflavins from 1.84% to 1.01% during storage period of 90 days. The mean values of all the treatments were found to be decreased from (1.80-0.70), (1.82-1.10), (1.85-1.15), (1.89-1.20) and (1.90-1.26) in case of T_1, T_2, T_3, T_4, and T_5 respectively (Fig.8). A gradual degradation in the flavoring compounds was also observed [20], who reported a decrease in percent theaflavins in black tea during storage at 4°C analyzed by HPLC and Electrophoresis chromatography.

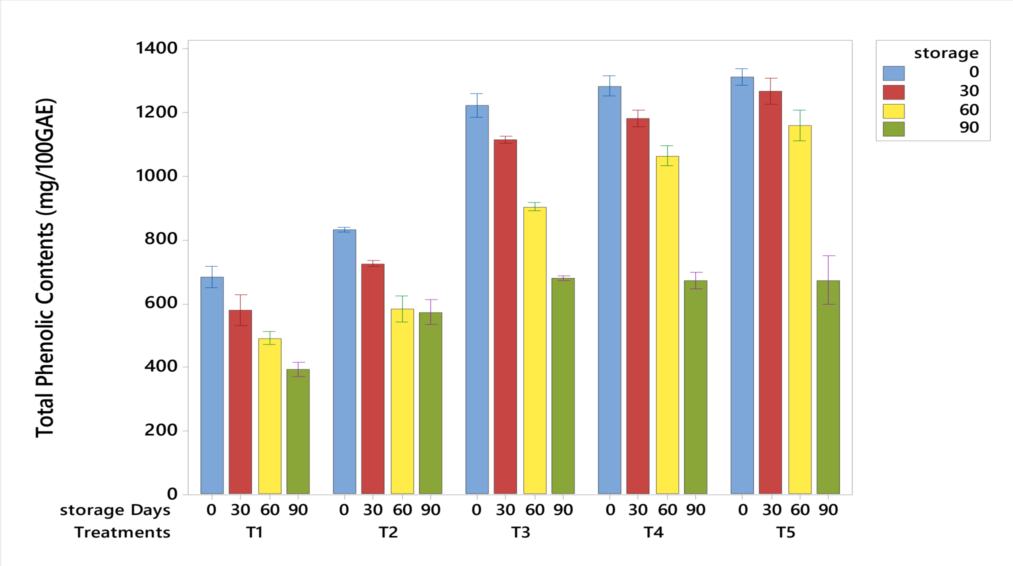

Fig. 7. Effect of treatments and storage intervals on total phenolic contents of carbonated black ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract) (Level of significance $p \leq 0.05$)

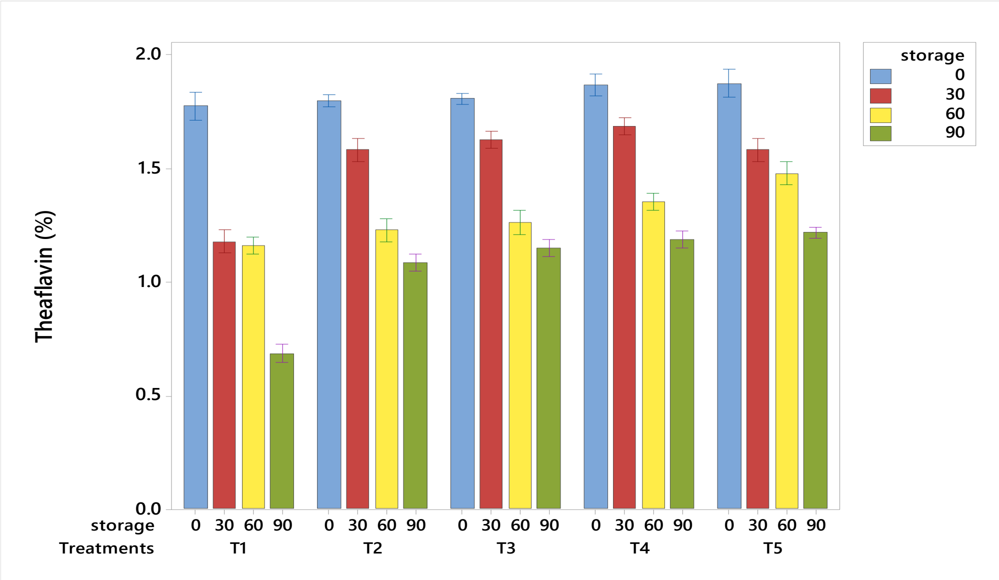

Fig. 8. Effect of treatments and storage intervals on theaflavins of carbonated black ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract)

3.9 Thearubigins (%)

Thearubigins are responsible for body and richness of tea brew. The statistical analysis showed that storage intervals and treatments have a significant effect on thearubigins. Initially the thearubigins were in the range of 10.20 to 12.26. T_1 had the lowest value i.e., 10.20 while highest thearubigins were found in T_5 i.e., 12.26 followed by T_4 (12.20), T_3 (11.34) and T_2 (11.26) as shown in Fig. 9. As storage proceeds the thearubigins contents started to degrade and decreased up to 4.00%, 4.20%, 5.30%, 6.20%, and 6.45 % for T_1,

T_2, T_3, T_4 and T_5 respectively after three months storage period. Thearubigins significantly decreased from 47.39% to 60.0% during storage.

3.10 DPPH (%)

DPPH free radical scavenger activity of carbonated black iced tea beverages were found to be decreased among all the treatments during the storage period of 90 days. Significant difference was observed statistically within the treatments and storage intervals, (Fig. 10) with maximum value of DPPH in T_5 (56.20) as compared to that of T_1 (50.00), commercially available iced tea beverage. At the end of storage period the DPPH free radical scavenging activity values were reduced up to 25.00 for T_5 and 26.00, 25.30, 23.00 and 21.67 for T_4, T_3, T_2 and T_1 respectively. Quan *et al.* [36] was also studied the DPPH free radical scavenging activity in several types of Vietnam commercial green tea, oolong tea and black tea and reported DPPH of tea was attributed to their phenolic contents and ranges up to 288, 155 and 155 mg Vitamin C equivalent in green tea, oolong tea and black tea respectively and it reduced with the passage of time.

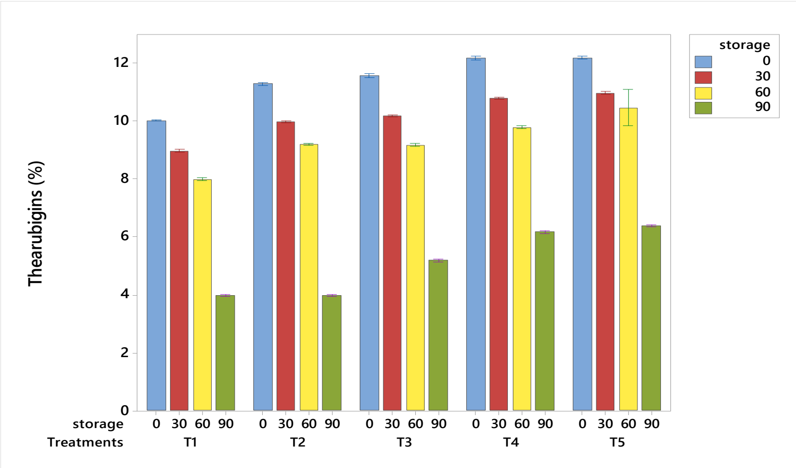

Fig. 9. Effect of treatments and storage on thearubigins of carbonated black ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract) (Level of significance $p \leq 0.05$)

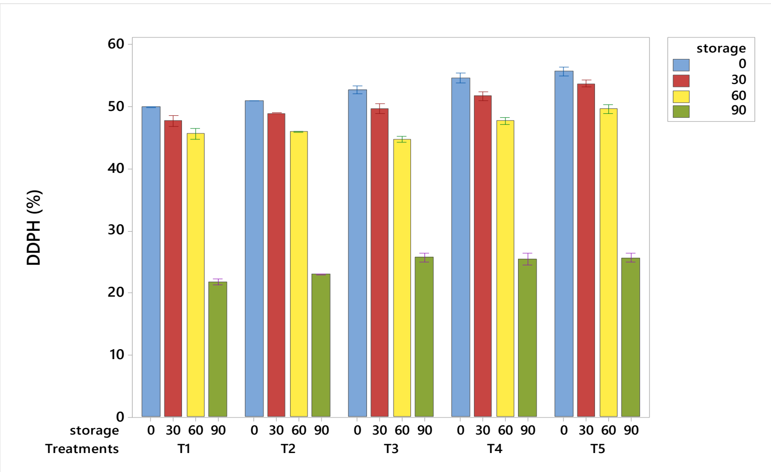

Fig. 10. Effect of treatments and storage on DPPH of carbonated black ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract)

3.11 Sensory evaluation

Carbonated black ice tea beverages were evaluated organoleptically during 90 days of storage. The evaluation was conducted after 30 days interval by 15 students and 10 professional colleagues on the basis of nine point hedonic scale. The statistical results revealed that all sensory characteristics differ significantly ($p \leq 0.05$) with regard to treatments as well as during storage. The organoleptic evaluation of different black ice tea treatments as shown in Fig.11 indicated that quality of ice tea was referred to all the characteristics such as color, taste, flavor and overall acceptability. The treatment T_2 (with 0.2% extract) was the highly ranked for all aspects of organoleptic evaluation. The control treatment was the next best ranked treatment. The least ranked treatment by the panelists was T_5 with 0.8 % extract followed by T_3 and T_4. The scores for taste and flavor were decreased through storage intervals due to degradation of phenolic compounds which are key components for specific tea flavor. Color changes during storage also showed declining trend in all samples that may be attributed to light and storage conditions. Decrease in the scores assigned to flavor of different carbonated ice tea beverages may be attributed to the increase in acidity of beverage. A gradual decrease in flavor may be due to degradation of flavor during storage of product and also due to heat treatment applied during processing steps. Such reasons for decrease in flavor have been reported [37]. Scores for overall acceptability which were cumulative effect of all other sensory parameters showed that during storage period of 90 days there was slight decrease and among all the treatments best scores were observed by T_2 with 0.2% black tea extract. Similar trend in sensorial properties of ice tea was observed [38] as a function of storage.

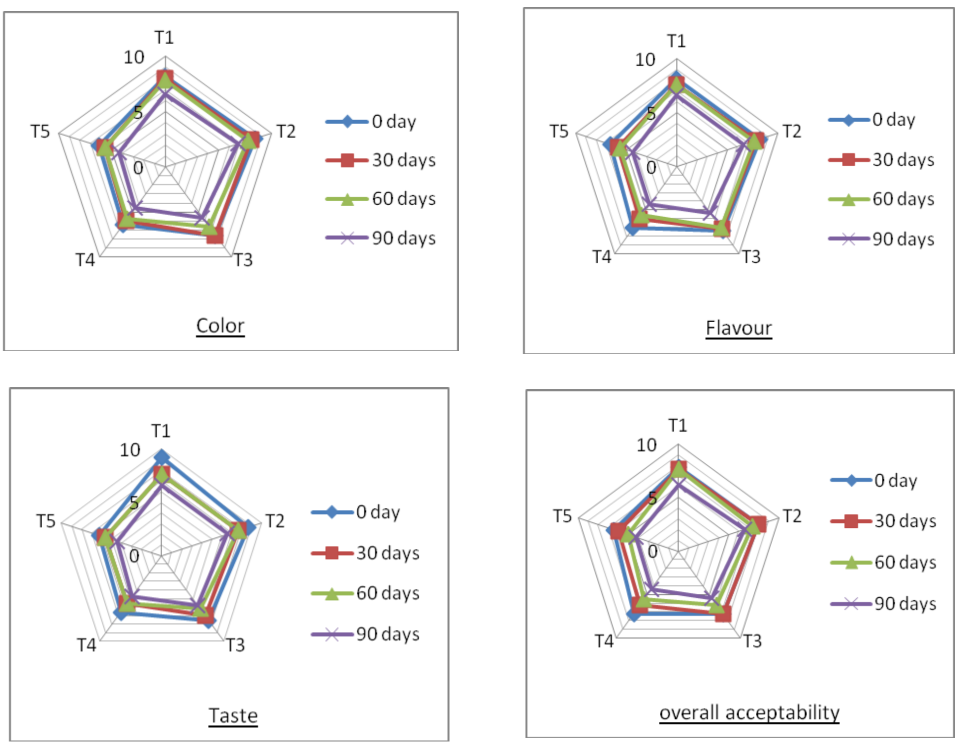

Fig. 11. Effect of storage and treatments on organoleptic attributes of carbonated ice tea beverages (T_1: Commercial, T_2: 0.2% extract, T_3: 0.4 % extract, T_4: 0.6 % extract, T_5: 0.8% extract)

4. CONCLUSION

A novel formulation of carbonated black ice tea beverages was developed. Tea is a good source of bioactive compounds like phenolic contents. All levels of black tea extracts were found to be acceptable for qualitative parameters however, ice tea formulated with 0.2% extract was pleasant for sensory attributes as compared to other treatments including commercial carbonated ice tea sample. Carbonated black ice tea could be introduced into beverage industry by incorporating some functional components and more effective antioxidant properties. It has been concluded from the present research that to maintain the healthy life such functional beverages are suitable for consumption due to the presence of vital bioactive compounds with good quality attributes. Future research would be needed in this regard to explore the more bioactive compounds to estimate the effect of processing on quality parameters and stability during storage.

5. ACKNOWLEDGEMENT

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6. REFERENCES

1. Kim, E.S., Y.R. Liang, J. Jin, Q. F. Sun, J.L. Lu, Y.Y. Du & C. Lin. Impact of heating on chemical compositions of green tea liquor. *Food Chemistry,* 103: 1263–1267 (2007).
2. Wang, L.F., D.M. Kim & C.Y. Lee. Effects of heat processing and storage on flavanols and sensory qualities of green tea beverage. *Journal of agriculture and Food Chemistry*, 48: 4227–4232 (2000).
3. Yamanishi, T., Y. Hara, S. Luo & R.L. Wickremasinghe. Special issue on tea. *Food Reviews International,* 11: 371–546 (1995).
4. Etoh, H., K. Murakami, T. Yogoh, H. Ishikawa, Y. Fukuyama & H. Tanaka. Antioxidative compounds in barley tea. *Bioscience Biotechnology Biochemistry,* 68: 2616-2618 (2004).
5. Tsunagi, K., H. Sugiyama & Y. Shoji. Barley beta-glucan and its physiological function. *Arerugi no Rinsho,* 23: 949-953 (2003).
6. Altemimi, A., N. Lakhssassi, A. Baharlouei, D.G. Watson & D.A. Lightfoot Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*. 6: 1-24 (2017).
7. Kumar, S., A. Yadav, M. Yadav & J.P. Yadav. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of *Aloe vera* (L.) Burm.f. *BMC Res. Notes.* 10: 1-12 (2017a).
8. Xu, X.Y., C.N. Zhao, S.Y. Cao, G.Y. Tang, R.Y. Gan, H.B. Li. Effects and mechanisms of tea for the prevention and management of cancers: An updated review. *Critical Reviews in Food Science and Nutrition.* 1-13 (2019)
9. Grosso, G. A. Micek, J. Godos, A. Pajak, S. Sciacca, F. Galvano & E.L. Giovannucci. Dietary flavonoid and lignan intake and mortality in prospective cohort studies: systematic review and dose-response meta-analysis. *American Journal of Epidemiology*. 185: 1304–1316 (2017).
10. Grosso, G. J. Godos, R. Lamuela-Raventos, S. Ray, A. Micek, A. Pajak, S. Sciacca, N. D'Orazio, D. Del Rio, & F. A. Galvano. Comprehensive meta-analysis on dietary flavonoid and lignan intake and cancer risk: Level of evidence and limitations. *Molecular Nutrition and Food Research.* 61, 1600930 (2017)
11. Samavat, H. A. R. Newman, R. Wang, J. Yuan, A.H. Wu, M.S. Kurzer. Effects of green tea catechin extract on serum lipids in postmenopausal women: a randomized, placebo-controlled clinical trial. *American Journal of Clinical Nutrition.* 104:1671–1682 (2016).
12. Cao, S. Y., C.N. Zhao, R.Y. Gan, X.Y. Xu, X.L. Wei, H. Corke & H.B. Li. Effects and mechanisms of tea and its bioactive compounds for the prevention and treatment of cardiovascular diseases: An updated review. *Antioxidants*. 8: 166 (2019).
13. Latif, A., A.U. Jan, A.F. Chishti, M. Fayaz & F.S. Hamid. Assessing potential of local tea production in Pakistan. *Sarhad Journal of Agriculture*, 24: 339-344 (2008).
14. Arts, I.C.W., P.C. Hollman, E.J.M. Feskens, H.B.B. Mesquita & D. Kromhout. Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen Elderly Study. *American Journal of Clinical Nutrition*, 74: 227–232 (2001).
15. Suzuki, Y. & Shioi, Y. Identification of chlorophylls and carotenoids in major teas by high-performance liquid chromatography with photodiode array detection. *Journal of Agriculture and Food Chemistry,* 51: 5307-5314 (2003).

16. Yanishlieva, N.V., E. Marinova, & J. Pokorny. Natural antioxidants from herb and spices. *European Journal of Lipid Science and Technology*, 108: 776-793 (2006).
17. Gibson, S. Sugar-sweetened soft drinks and obesity: a systematic review of the evidence from observational studies and interventions. *Nutrition Research Reviews*, 21: 134–147 (2008).
18. Descoins, C., M. Mathlouthi, M.L. Moual, & J. Hennequin. Carbonation monitoring of beverage in a laboratory scale unit with on-line measurement of dissolved CO_2. *Food chemistry*, 95, 541-553 (2006).
19. Duffey, P., P. Gordon-Larsen, J.M. Shikany, D. Guilkey, J. Jacobs & B.M. Popkin. Food Price and Diet and Health Outcomes. *Archives of Internal Medicines,* 170: 420–6 (2010).
20. Zhao, W., R. Yang &M. Wang. Cold storage temperature following pulsed electric fields treatment to inactivate sublethally injured microorganisms and extend the shelf life of green tea infusions. *International Journal of Food Microbiology*, 129: 204–208 (2009).
21. AOAC. Official methods of analysis. The Association of Official Analytical Chemists, Inc., 15th ed. Arlington, USA (2006).
22. Singleton, V.L., Orthofer, R. & Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Enzymology*, 299: 152-178 (1999).
23. Angayarkanni, J., M. Palaniswamy, S. Murugesan & K. Swaminathan. Improvement of tea leaves fermentation with *Aspergillus* spp. Pectinase, *Journal of Bioscience and Bioengineering,* 94: 299-301 (2002).
24. Lee, J.Y., H.J. Park, C.Y. Lee & W.Y. Choi. Extending shelf-life of minimally processed apples with edible coatings and antibrowning agents. *Wissenschaft und – Technologie,* 36: 323-329 (2003).
25. Saxena, S., B.B. Mishra, R. Chander & A. Sharma. Shelf stable intermediate moisture pineapple (Ananas comosus) slices using hurdle technology. *LWT-Food Science and Technology,* 42: 1681–1687 (2009).
26. Balaswamy, K., P.P. Rao, A. Nagender & A. Satyanarayana. Preparation of Sour Grape (Vitis Vinifera) Beverages and Evaluation of their Storage Stability. *Journal of Food Processing and Technology,* 2: 3 (2011).
27. El-Faki, A.E. & E.S. Eisa. Physico-Chemical Characteristics of Some Soft Drinks of Sudan During Shelf Life. *Journal of Science and Technology,* 11: 2 (2010).
28. Sharma, O.K., M. Ali & D.K. Yadav. Physico-chemical and phytochemical evaluation of different black tea brands. *Journal of Applied Pharmaceutical Science,* 01: 121-124 (2011).
29. Sahota, P. P., K. Davneet & P. Gulab. Studies on the preparation of low alcoholic naturally carbonated blended beverage from guava and lemon. *International journal of Food Safety,* 12: 165-180 (2010).
30. Akinwale, T.O., S.O. Aroyeun, & C.R. Obatolu. Physico-chemical, microbiological profiles of blends of tea and mistletoe_ a highly medicinal mix. *Journal of Food Technology,* 5: 123-125 (2000).
31. Majumdar, T.K., D.D Wadikar, C.R. Vasudish, K.S. Premavalli & A.S. Bawa. Effect of storage on physico-chemical, microbiological and sensory quality of bottle gourd-basil leaves juice. *American journal of Food Technology,* 6: 226-234 (2011).
32. Singh, P., Shukla, A., R. Singh & Singh, K. Utilization of guava juice by value addition through blended beverages, Acta Hort. (ISHS*). International guava symposium*, 735: 639-645 (2007).
33. Chowdhury, M.G.F., M.N. Islam, M.S. Islam & M.S. Hossain Study on preparation and shelf life of mixed lime juice based on wood apple and papaya. *Journal of Soil Nature,* 2: 50-60 (2008).
34. Vijay, N., S. Niar & P. Kakkar. Decline in antioxidant capacity of Indian Herbal teas during storage and its relation to phenolic contents. *Food Research International*, 39: 176-181 (2006).
35. Susanne, M.H., C.F. Lira, H.W. Lee, A.A. Youssefian, V.L.W Go & D. Heber. Catechin content of 18 teas and a green tea extract supplement correlates with the antioxidant capacity. *Nutrition and Cancer*, 45: 226-235 (2003).
36. Quan, P.T., T.V. Hang, N.H. Ha & B.L. Giang. Total phenolics, total catechins contents and DPPH free radical scavenger activity of several types of Vietnam commercial green tea. *Science and Technology Development,* 10: 5-11 (2007).
37. Pruthi, J.S., J.K. Manna, M.S. Tectia, S.G. Radhakriahna, W.E. Eipeson, S. Saroja & A. Chikkappaji. Studies on the utilization of kinnow and malta orange. *Journal of Food Science and Technology,* 21: 121-127 (1984).
38. Plestenjak, A., M.J. Simcic, M. Hribar, A. Veber, M. Skorja, P. Kordis-Krapez & R. Vidrih. Sensorial properties of ice tea as affected by packing. *Food Technology Biotechnology,* 39: 101-107 (2001).