Progress in Nutrition 2019; Vol. 21, Supplement 1: 435-444 DOI: 10.23751/pn.v21i1-S.6555 

Original article

# Characterization of low calorie ready-to-serve peach beverage using natural sweetener, Stevia (*Stevia rebaudiana* Bertoni)

*Uswa Ahmad[1], Rabia Shabir Ahmad[2], Ali Imran[2], Zarina Mushtaq[2], Syed Makhdoom Hussian[3]*

[1]Department of Food Science, Nutrition & Home Economics, Govt. College University, Faisalabad; [2]Institute of Home and Food Sciences, Faculty of Life Sciences, Govt. College University, Faisalabad, Punjab, 38000, Pakistan - E-mail: rabiaahmad@gcuf.edu.pk; [3]Department of Zoology, Govt. College University, Faisalabad, 38000, Pakistan

**Summary.** Trend of fruits-based beverages has been increased due to their nutritional importance but sucrose used in them resulted in degenerative diseases. *Stevia rebaudiana* Bertoni natural, non-calorie sugar substitute can be used in the preparation of beverages for diabetes and weight maintaining approaches. The present study is focusing at preparation and characterization of peach ready-to-serve beverages with stevioside. Low calorie peach ready-to-serve beverages were prepared with stevioside from stevia aqueous extract at different levels (20, 25, 30 and 35 mg/100 mL) as sugar substitute. Antioxidant, physicochemical and organoleptic properties of functional beverages at 0 day to 60 days storage period were determined. The results depicted that stevioside improved the antioxidant profile of peach ready-to-serve beverages by significantly ($P \leq 0.05$) by increasing total phenolic, total flavonoid contents and DPPH activities from $T_0$ to $T_4$ (18.22±0.14-23.34±0.20 mg GAE/100g), (8.20±0.04-12.30±0.08 mg Catechin/100g) and (12.70±0.22-17.82±0.16 mg Trolox/100g) respectively. Regarding physicochemical characteristics, TSS (3.15±0.11-3.0±0.12 %), reducing (2.01±0.11-1.88±0.10 %), non-reducing (2.96±0.06-3.00±0.09 %) and total sugars (4.97±0.21-4.88±0.02 %) significantly affected among the treatments from $T_0$ to $T_4$. The results of organoleptical attributes significantly decreased with increasing the stevioside. During storage, antioxidant and organoleptic properties significantly decreased from $T_0$ to $T_4$. Physicochemical parameters including pH, non-reducing sugars and color significantly decreased while acidity, TSS, reducing and total sugars increased during storage but remained acceptable by sensory panelists. Conclusively, low calorie peach ready-to-serve beverages could be safe and promising nutracutical drink for the management of obesity, diabetes and its associated complications.

**Key words:** Stevioside, low calorie peach beverage, physicochemical

## Introduction

The consumption of fruit-based beverages has been increased in recent years due to higher consumer awareness about the importance of improving the quality of life and choosing healthy foods to reduce the risk of degenerative diseases (1). But in these beverages large amount of sugar has been used for better taste, color and flavor that resulted in health problems like obesity, diabetes, hyperlipidemia etc. In order to maintain a healthy body weight and avoid the debilitating diseases associated with excessive sugar consumption, the trend to use non-caloric artificial sweeteners like saccharin, sucralose and aspartame in beverages have been increased (2) but these are carcinogenic. However natural non-caloric sweeteners support the effectiveness and safety in human health promotion. Stevia (*Stevia rebaudiana* Bertoni) popularly known as candy leaf, sugar leaf, sweet leaf, honey leaf and is natural, non-caloric sweetener with sweetness several hundred times than sugar (3). Stevia is reported to be safe when used as a sweetener. Stevia leaves have mixture of sweet diterpene

glycosides like stevioside, steviolbioside, rebaudiosides (A, B, C, D, E and F) and dulcoside A (4). Among all sweetening compounds of stevia, stevioside is one of the principal diterpene glycoside having a sweetness of 250 to 300 times that of sucrose (5). Stevia leaf extracts in the diet has been associated with anti-hyperglycemic, hyperlipidemic, insulinotropic, glucagonostatic, hypotensive, anti-carcinogenic, antiviral, anti-microbial, anti-inflammatory, immunostimulatory and chemopreventative responses due to presence of strong nutritional and antioxidant profile (6,7).

Steviosides were reported to be thermo-stable sweeteners of *Stevia rebaudiana* due to high stability at broad pH range (2-10), temperature (< 140 ˚C) and non-fermentable nature (8). Stevia has gained recent attention by numerous food and beverage multinational enterprises. The applicability of steviosides in bakery, soft drinks, beverages and home foods was studied (9). In different food products like grape juice, milk shake, tea, bun, biscuits, fruit custard, jam, besan ladu, wheat ladu and chikki incorporating higher concentrations of stevia (0.25-1.0 %) as sugar substitute were prepared and observed that these food products were remained acceptable during storage (10). Similarly physicochemical and sensory parameters of RTS beverages containing jamun, mango, pineapple, pomegranate and purple grapes fruits with stevia were analyzed during storage for six months. The RTS beverages were well accepted up to four months of storage (8). Due to increasing demand of fruit-based beverages with non-caloric sweeteners. The present work deals with the preparation of low calorie RTS peach beverage and assessed for their stability and acceptability at 0 day interval up to a storage period of 60 days.

## Material and Methods

*Plant material*

Stevia (*Stevia rebaudiana* Bertoni*)* leaves were collected from Ayub Agricultural Research Institute (AARI), Faisalabad. To remove dust, dirt and foreign material on the surface, stevia leaves were properly washed with tape water. After washing, stevia leaves were air-dried under shade at room temperature and finely powdered with the help of grinder **(**MJ-176-NR-3899) (11).

*Stevia aqueous extract preparation*

Steviosides were extracted from the dried ground leaves of stevia plant by using water extraction. The dried ground leaves of stevia were mixed with hot water (65 ˚C) at the ratio of 1:45 (w/v) (12). The mixture was kept at room temperature for 24 h, after properly shaking. It was stirred 2–3 times a day. Then the mixture was filtered through What man No. 1 filter paper after 24 h. Using rotary vacuum evaporator (EYELA N-1110S 115V), the filtrate was evaporated at 40-45˚C (11).

*Purification of Stevia aqueous extract*

Purification of crude stevia aqueous extract containing stevioside was done for the development of low calorie ready-to-serve peach beverages to enhance consumer acceptability. The purification of aqueous extract of stevia was done by the method decribed by (13). The filtered aqueous extract was mixed with 5 % $Ca(OH)_2$ (based on wt. of dried leaves) and heated at 50 $^{o}$C for 0.5-1.0 h up to pH of about 10. The solution precipitated after calcium hydroxide treatment. All undesirable precipitated compounds were removed and supernatant obtained was neutralized with 10 % ferric chloride solution for 10-15 min. After that the resulting solution was passed through Celite (a resin bed) for decolorization and then deionized through Amberlite FPC23H, Amberlite FPA51, and Amberlite FPA98Cl (color removing resins) (Rohm & Haas Co., Germany) at flow rate of 1 mL/sec at 25 $^{o}$C. The solution was spray dried to remove the moisture and the extract powder was extracted in methanol 1:5 (w/v) of powder to solvent ratio with agitation at 20-25 $^{o}$C for 0.5-1.0 h. Then the filtrated precipitate was dried and methanol was evaporated on water bath to remove the methanol and after filtration it was spray dried. For the further purification, the powder was mixed with two volumes of 90 % of ethanol and at 10-12 ˚C with slow agitation for 30 min. The precipitate was separated by filtration and dried using rotary vacuum evaporator (EYELA N-1110S 115V) at 40-45˚C.

*Extraction of Peach pulp*

Peaches were washed under running tap water to remove dust and dirt particles, cut into halves and kernels were removed. Fruit pulp from peaches was

extracted using stainless steel fruit pulper (Model No. PSP 115) diluted to 1:2 ratios and strained through stainless steel sieve.

*Phytochemical properties*

*Phenolic content determination*

The total phenolic contents in stevia aqueous extract and peach pulp were determined by the method described by (14). The absorbance was measured by UV-visible spectrophotometer (Model: Varian AA-240, Victoria, Australia) at 760 nm absorbance level. The total phenolic contents were calculated as mg of gallic acid equivalent per gram of dry matter.

*Flavonoid determination*

Flavonoid contents were measured by aluminum chloride colorimetric assay as mentioned by (15). The absorbance was calculated by UV-visible spectrophotometer (Model: Varian AA-240, Victoria, Australia) at 510 nm. The total flavonoids in stevia extracts and peach pulp were calculated as mg of catechin equivalent per gram of dry matter.

*DPPH radical scavenging activity*

Free radical scavenging capacities of stevia aqueous extract and peach pulp were quantified by using DPPH as described by (16). The absorbance of the stevia aqueous extracts were measured using methanol as a blank, by UV-visible spectrophotometer (Model: Varian AA-240, Victoria, Australia) at 515 nm. The results were calculated as mg 157 of Trolox equivalent per gram of dry matter.

*Physicochemical properties*

Stevia aqueous extract and peach pulp TSS, pH, titratable acidity, reducing sugars, non-reducing sugars and total sugars were determined according to procedures described by AOAC (17).

*Preparation of Low calorie RTS Peach Beverage*

The Peach ready-to-serve beverage was prepared by the addition of stevioside from stevia aqueous extract at different levels i.e. 20 mg/100 mL ($T_1$), 25 mg/100 mL ($T_2$), 30 mg/100 mL ($T_3$), 35 mg/100 mL ($T_4$) as sugar substitute and $T_0$ (control sample with 100 % sucrose) according to the method described by (18) with slight modifications. In order to prepare, control peach ready to serve beverage 25 % peach pulp, sugar syrup and citric acid to adjust the total soluble sugars and acidity up to 10 to 15 % and 0.3 % respectively were used. While the functional peach beverage was prepared by utilizing 25 % peach pulp, 0.3 % citric acid and diluted stevioside at different levels (20, 25, 30 and 35 mg/100 mL). Both types of peach RTS beverages were filtered by sieving through muslin cloth. Then the final products were bottled into hot, sterilized bottles. The filled bottles were pasteurized in boiling water at 100 °C for 15 minutes. The bottles were kept at room temperature and then further studied up to 60 days after diluting it with chilled water at ratios of 1:3 ratios.

*Analysis of low calorie peach ready to serve beverage*

The low calorie peach RTS beverages were analyzed for their antioxidant potency (total phenolic contents, flavonoids and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay) through the methodologies described by (14), (15) and (16) respectively. Physicochemical properties including pH, TSS, reducing sugars, non-reducing sugars, total sugars and acidity was measured by the methods of (17). Both antioxidant and physicochemical properties were studied for storage period of 0 day to 60 days.

*Color*

The color values; $L^*$ (lightness), $a^*$ (–a greenness; +a redness), and $b^*$ (–b blueness; +b yellowness) of peach RTS beverages were estimated with CIE-Lab Color Meter (CIE LAB SPACE, Color Tech-PCM, USA) at storage interval of 0 to 60 days according the method described by (17).

*Organoleptic evaluation*

Functional beverages were assessed at storage interval of 60 days by a trained panel of judges. Various sensory attributes were evaluated like taste, color, flavor, taste, sweetness and overall acceptability of end product according to the method (19).

*Statistical analysis*

Results of replicate experiments were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) (Two-way ANOVA) and least significance

difference (LSD) were carried out on the result data at 95% confidence level using SPSS statistical software package, version 17 (SPSS Inc., Chicago) (20).

## Results

The phytochemical and physicochemical properties of stevia aqueous extract and peach pulp is shown in Table 1. Total phenolics (20.3±0.15 mg GAE/100 g), flavonoids (14.32±0.09 mg Catechin/100 g), and DPPH activity (33.24±0.30 mg Trolox/100 g), in aqueous extract of *Stevia rebaudiana* Bertoni leaves confirmed its strong antioxidant capacity. Physicochemical parameters (pH, acidity, total soluble sugars, reducing sugars, non-reducing sugars and total sugars) of stevia aqueous extract were found to be 5.80±0.002, 0.16±0.004 %, 5.90±0.004 %, 2.09±0.22 %, 3.13±0.43 % and 5.22±0.05 % respectively.

Antioxidant profile (total phenols, flavoniods and DPPH activity) and physicochemical parameters (pH, acidity, total soluble sugars, reducing sugars, non-reducing sugars and total sugars) of peach pulp were recorded as 62.43±0.18 mg GAE/100 g, 22.55±0.02 mg Catechin/100 g, 46.00±0.22 mg Trolox/100 g, 3.32±0.02, 1.04±0.01 %, 9.60±0.04 %, 8.83±0.44 %, 1.17±0.32 % and 10.00±0.07 % respectively (Table 1).

Total phenolic contents, total flavonoids and DPPH (free radical scavenging) activity of low calorie peach RTS (ready-to-serve) beverages are presented in Table 2. Total phenolic contents, total flavonoids and DPPH activity values of peach ready-to-serve beverage increased significantly ($P \leq 0.05$) from $T_0$ to $T_4$ by ranges of (18.22±0.14-23.34±0.20 mg GAE/100g), (8.20±0.04-12.30±0.08 mg Catechin/100g) and (12.70±0.22-17.82±0.16 mg Trolox/100g) respectively. During storage for 0 day to 60 days, the values of total phenolic contents, total flavonoids and free radical scavenging activity of peach ready-to-serve beverage decreased significantly ($P \leq 0.05$) from $T_0$ to $T_4$ (Table 2).

Table 3 shows pH, acidity and TSS of low calorie peach ready-to-serve beverages under ambient condition. pH of low calorie peach ready-to-serve beverages non-significantly decreased from (3.04±0.03-3.03±0.02) and acidity increased from (0.20±0.02-0.21±0.03 %) from $T_0$ to $T_4$. TSS (total soluble solids) of functional beverages significantly ($P \leq 0.05$) decreased from $T_0$ ((3.15±0.11 %) to $T_4$ (3.0±0.12 %) due to decrease level of TSS and total sugars in stevioside. The pH of functional beverages significantly ($P \leq 0.05$) decreased. While acidity and TSS increased from $T_0$-$T_4$ with increasing the storage period (0 to 60 days).

Figures 1, 2 & 3 representing the reducing sugars, non-reducing sugars and total sugars of low calorie peach RTS beverages under room temperature. The results depicted that reducing sugars and total sugars of peach RTS beverages with stevia aqueous extract significantly ($P \leq 0.05$) decreased from $T_0$ to $T_4$ (2.01±0.11-1.88±0.10 %) and (4.97±0.21-4.88±0.02 %) respectively. While the non-reducing sugars significantly increased from $T_0$ (2.96±0.06 %) to $T_4$ (3.00±0.09 %). Significant ($P \leq 0.05$) increasing trend

**Table 1.** Analysis of Stevia aqueous extract and peach pulp

| | | Stevia aqueous extract | Peach pulp |
|---|---|---|---|
| Antioxidants | TPC (mg GAE/100 g) | 20.30±0.15 | 62.43±0.18 |
| | TFC (mg CAE/100 g) | 14.32±0.09 | 22.55±0.02 |
| | DPPH (mg TE/100 g) | 33.24±0.30 | 46±0.22 |
| Physicochemical parameters | pH | 5.80±0.002 | 3.32±0.02 |
| | Acidity (%) | 0.16±0.004 | 1.04±0.01 |
| | TSS (%) | 5.90±0.004 | 9.60±0.04 |
| | Reducing sugars (%) | 2.09±0.22 | 8.83±0.44 |
| | Non-reducing sugars (%) | 3.13±0.43 | 1.17±0.32 |
| | Total sugars (%) | 5.22±0.05 | 10.00±0.07 |

Values are expressed as means ± standard deviation
DPPH= Free radical scavenging activity; TPC= Total phenolic contents; TFC= Total flavonoid contents; TSS= Total soluble sugars

**Table 2.** Antioxidant profile of Low calorie ready-to-serve Peach beverage

| | Treatments | Storage period | | | | |
|---|---|---|---|---|---|---|
| | | 0 day | 15 days | 30 days | 45 days | 60 days |
| TPC (mg GAE/100 g) | $T_0$ | 18.22±0.14Ea | 18.18±0.12Eab | 18.14±0.14Eb | 18.11±0.16Eb | 18.07±0.19Ec |
| | $T_1$ | 19.26±0.13Da | 19.24±0.12Da | 19.20±0.10Db | 19.19±0.14Db | 19.15±0.16Dc |
| | $T_2$ | 20.28±0.09Ca | 20.27±0.07Ca | 20.22±0.08Cab | 20.21±0.09Cb | 20.17±0.05Cc |
| | $T_3$ | 21.32±0.18Ba | 21.30±0.16Ba | 21.26±0.18Bab | 21.23±0.18Bb | 21.20±0.18Bb |
| | $T_4$ | 23.34±0.20Aa | 23.33±0.18Aa | 23.29±0.19Aab | 23.27±0.14Ab | 23.25±0.25Ab |
| TFC (mg CAE/100 g) | $T_0$ | 8.20±0.04Ea | 8.16±0.05Eab | 8.12±0.04Eb | 8.09±0.03Ebc | 8.05±0.02Ec |
| | $T_1$ | 9.24±0.08Da | 9.22±0.09Dab | 9.18±0.10Db | 9.16±0.12Db | 9.12±0.08Dc |
| | $T_2$ | 10.25±0.10Ca | 10.23±0.11Ca | 10.19±0.09Cb | 10.17±0.07Cb | 10.14±0.06Cc |
| | $T_3$ | 11.28±0.05Ba | 11.26±0.04Ba | 11.21±0.03Bb | 11.18±0.02Bb | 11.15±0.05Bc |
| | $T_4$ | 12.30±0.08Aa | 12.28±0.09Aa | 12.24±0.08Ab | 12.22±0.07Ab | 12.19±0.05Ac |
| DPPH (mg Trolox/100 g) | $T_0$ | 12.70±0.22Ea | 12.66±0.24Eb | 12.63±0.20Ebc | 12.60±0.18Ebc | 12.57±0.16Ec |
| | $T_1$ | 13.74±0.25Da | 13.72±0.23Dab | 13.68±0.21Db | 13.66±0.26Db | 13.62±0.25Dc |
| | $T_2$ | 14.77±0.20Ca | 14.75±0.22Ca | 14.71±0.25Cb | 14.68±0.24Cb | 14.64±0.20Cc |
| | $T_3$ | 15.79±0.18Ba | 15.77±0.19Ba | 15.72±0.14Bb | 15.69±0.12Bb | 15.66±0.16Bc |
| | $T_4$ | 17.82±0.16Aa | 17.81±0.18Aa | 17.76±0.17Ab | 17.74±0.09Ab | 17.72±0.19Ac |

Values are mean ± standard error (n=3); Mean followed by different upper case letters in the same columns represent significant difference ($P < 0.05$) treatment wise.; Mean followed by different lower case letters in the same rows represent significant difference ($P < 0.05$) storage wise. $T_0$= 100 % sucrose; $T_1$= 20 mg/100 mL stevioside; $T_2$= 25 mg/100 mL stevioside; $T_3$= 30 mg/100 mL stevioside; $T_4$= 35 mg/ 100 mL stevioside

**Table 3.** Physicochemical parameters of Low calorie ready-to-serve Peach beverage

| Physico-chemical parameters | Treatments | Storage period | | | | |
|---|---|---|---|---|---|---|
| | | 0 day | 15 days | 30 days | 45 days | 60 days |
| pH | $T_0$ | 3.03±0.02a | 3.00±0.01b | 2.95±0.05c | 2.91±0.09c | 2.86±0.07d |
| | $T_1$ | 3.03±0.03a | 3.00±0.02b | 2.96±0.04c | 2.93±0.05c | 2.89±0.06d |
| | $T_2$ | 3.03±0.05a | 3.02±0.05a | 2.97±0.08ab | 2.94±0.09b | 2.90±0.05c |
| | $T_3$ | 3.04±0.02a | 3.03±0.01a | 2.99±0.03ab | 2.96±0.04b | 2.92±0.05c |
| | $T_4$ | 3.04±0.03a | 3.03±0.07a | 3.00±0.08ab | 2.97±0.09b | 2.94±0.05c |
| Acidity (%) | $T_0$ | 0.22±0.03a | 0.27±0.05b | 0.32±0.04ab | 0.36±0.03c | 0.40±0.02d |
| | $T_1$ | 0.21±0.02a | 0.24±0.09b | 0.28±0.10b | 0.32±0.01c | 0.36±0.08c |
| | $T_2$ | 0.20±0.03a | 0.23±0.11b | 0.27±0.09b | 0.30±0.07c | 0.34±0.06c |
| | $T_3$ | 0.20±0.01a | 0.22±0.04a | 0.26±0.03ab | 0.29±0.02b | 0.33±0.05c |
| | $T_4$ | 0.19±0.02a | 0.20±0.09a | 0.24±0.08ab | 0.27±0.07b | 0.31±0.05c |
| TSS (%) | $T_0$ | 3.00 ±0.01C | 3.04±0.08Cbc | 3.10±0.09Cb | 3.14±0.18Cb | 3.20±0.10Ca |
| | $T_1$ | 3.04±0.05B | 3.07±0.05Bb | 3.11±0.11Bab | 3.14±0.10Bab | 3.19±0.02Ba |
| | $T_2$ | 3.07±0.04Bc | 3.10±0.02Bb | 3.14±0.07Bab | 3.17±0.09Bab | 3.21±0.05Ba |
| | $T_3$ | 3.11±0.10ABc | 3.14 ±0.03ABb | 3.18±0.04ABab | 3.21±0.10ABab | 3.24±0.01ABa |
| | $T_4$ | 3.15±0.11Ac | 3.17±0.05Ab | 3.21±0.07Aab | 3.22±0.05Aab | 3.28±0.11Aa |

Values are expressed as mean ± standard error (n=3); Mean followed by different upper case letters in the same column represent significant difference ($P < 0.05$) treatment wise.; Mean followed by different lower case letters in the same row represent significant difference ($P < 0.05$) storage wise. $T_0$= 100 % sucrose; $T_1$= 20 mg/100 mL stevioside; $T_2$= 25 mg/100 mL stevioside; $T_3$= 30 mg/100 mL stevioside; $T_4$= 35 mg/ 100 mL stevioside

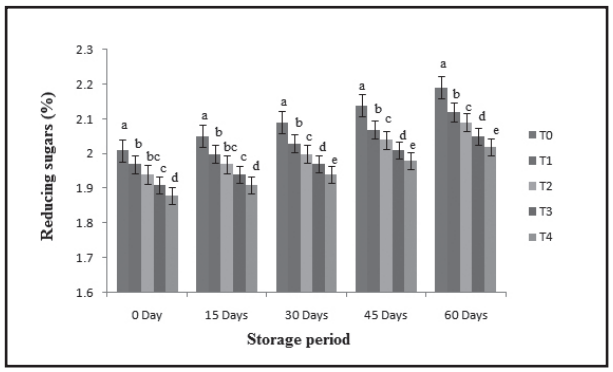


**Figure 1.** Effect of storage on reducing sugars (%) of low calorie ready-to-serve peach beverage
Results are expressed as amount of reducing sugars (%) in low calorie ready-to-serve peach beverage (mean± S.E.M., n=3). The reducing sugars of functional beverages (T1, T2, T3 and T4) with stevioside in different concentrations (20, 25, 30 and 35 mg/100 mL) respectively significantly (P < 0.05) decreased from control (Peach beverage with 100 % sucrose).

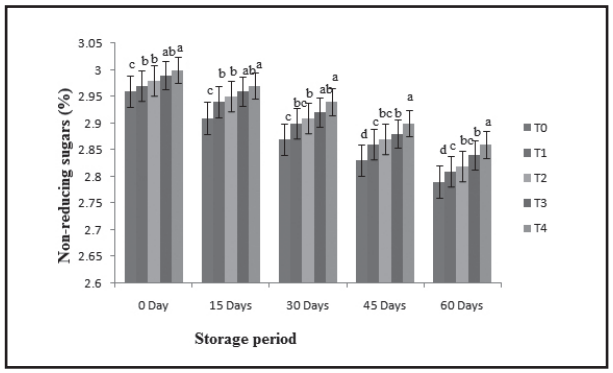


**Figure 2.** Effect of storage on non-reducing sugars (%) of low calorie ready-to-serve peach beverage
Results are expressed as amount of non-reducing sugars (%) in low calorie ready-to-serve peach beverage (mean± S.E.M., n=3). The non-reducing sugars of functional beverages (T1, T2, T3 and T4) with stevioside in different concentrations (20, 25, 30 and 35 mg/100 mL) respectively significantly (P < 0.05) increased from control (Peach beverage with 100 % sucrose).

was observed in reducing sugars and total sugars. While non-reducing sugars of functional beverages significantly (P ≤ 0.05) decreased from $T_0$ to $T_4$ during storage period for 60 days.

The surface color, L* (brightness), a* (redness) and b* (yellowness) values of peach RTS beverages samples were measured and shown in Table 4. Color values of functional beverages depicted that L*, a* and b* values significantly (p < 0.05) decreased from $T_1$ to $T_4$ (77.01±0.15-74.34±0.20), (5.44±0.16-2.13±0.20) and (27.66±0.14-24.34±0.12) respectively. $T_0$ had highest values of L* (78.03±0.10), a* (6.98±0.15) and b* (28.72±0.13). During storage for 60 days, the L*, a* and b* values of low calorie peach RTS beverages significantly decreased from $T_0$-$T_4$ when statistically analyzed (P ≤ 0.05) (Table 4).

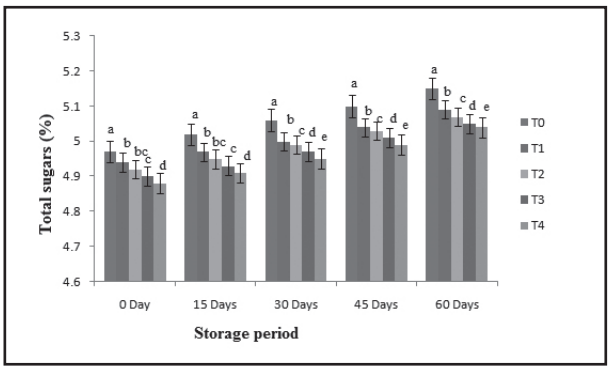


**Figure 3.** Effect of storage on total sugars (%) of low calorie ready-to-serve peach beverage
Results are expressed as amount of total sugars (%) in low calorie ready-to-serve peach beverage (mean± S.E.M., n=3). The total sugars of functional beverages (T1, T2, T3 and T4) with stevioside in different concentrations (20, 25, 30 and 35 mg/100 mL) respectively significantly (P < 0.05) decreased from control (Peach beverage with 100 % sucrose).

Data representing the change in color, flavor, taste, sweetness and overall acceptability scores of low calorie peach RTS beverages are seen in Table 5. The results showed that color, flavor taste and over all acceptability values of peach RTS beverages with stevia aqueous extract significantly (P ≤ 0.05) decreased from $T_0$-$T_4$ (8.20±0.12-7.42±0.20), (7.14±0.18-6.22±0.20) (7.12±0.14-6.20±0.12), (8.00±0.20-7.00±0.21) respectively. While sweetness non-significantly decreased from $T_0$ (7.14±0.18) to $T_4$ (7.12±0.20) (Table 5). The effect of storage for 60 days was recorded as significant (P ≤ 0.05) decrease in the color, flavor, taste, sweetness and over all acceptability scores for low calorie peach RTS beverages with increasing the storage period (Table 5).

## Discussion

Stevia aqueous extract and peach pulp was extracted and analyzed for phytochemical and physicochemical properties. The presence of more phenols and

**Table 4.** Color of Low calorie ready-to-serve Peach beverage

| Color | Treatments | Storage period | | | | |
|---|---|---|---|---|---|---|
| | | 0 day | 15 days | 30 days | 45 days | 60 days |
| L* | $T_0$ | 78.03±0.10Aa | 77.92±0.09Ab | 77.32±0.12Ac | 76.89±0.08Ac | 76.12±0.05Ad |
| | $T_1$ | 77.01±0.15Ba | 77.97±0.07Ba | 77.92±0.11Bab | 77.87±0.09Bc | 77.83±0.13Bd |
| | $T_2$ | 76.22±0.19Ca | 76.18±0.19Ca | 76.13±0.19Cab | 76.08±0.19Cb | 76.04±0.19Cc |
| | $T_3$ | 75.12±0.13Da | 75.08±0.11Da | 75.03±0.10Dab | 75.00±0.09Db | 74.96±0.07Dc |
| | $T_4$ | 74.34±0.20Ea | 74.30±0.20Ea | 74.25±0.20Eab | 74.21±0.20Eb | 74.17±0.20Ec |
| a* | $T_0$ | 6.98±0.15Aa | 6.56±0.12Ab | 6.23±0.06Ac | 5.76±0.04Ad | 5.14±0.07Ae |
| | $T_1$ | 5.44±0.16Ba | 5.40±0.13Ba | 5.35±0.14Bb | 5.30±0.02Bb | 5.26±0.05Bc |
| | $T_2$ | 4.09±0.14Ca | 4.05±0.12Ca | 4.00±0.15Cab | 3.95±0.09Cb | 3.91±0.08Cc |
| | $T_3$ | 3.87±0.19Da | 3.84±0.18Da | 3.79±0.16Dab | 3.75±0.04Db | 3.70±0.02Dc |
| | $T_4$ | 2.13±0.20Ea | 2.09±0.17Ea | 2.02±0.03Eab | 1.98±0.05Eb | 1.94±0.04Ec |
| b* | $T_0$ | 28.72±0.13Aa | 28.22±0.12Ab | 27.88±0.10Ac | 27.67±0.09Ad | 27.09±0.06Ae |
| | $T_1$ | 27.66±0.14Ba | 27.63±0.10Bab | 27.59±0.13Bb | 27.54±0.08Bb | 27.51±0.12Bc |
| | $T_2$ | 26.04±0.18Ca | 26.00±0.15Cab | 25.95±0.19Cb | 25.90±0.0Cb | 26.86±0.19Cc |
| | $T_3$ | 25.21±0.20Da | 25.17±0.18Dab | 25.12±0.13Db | 25.08±0.04Db | 25.03±0.20Dc |
| | $T_4$ | 24.34±0.12Ea | 24.30±0.11Eab | 24.25±0.19Eb | 24.21±0.02Eb | 24.14±0.06Ec |

Values are expressed as mean ± standard error (n=3); Mean followed by different upper case letters in the same column represent significant difference ($P < 0.05$) treatment wise. Mean followed by different lower case letters in the same row represent significant difference ($P < 0.05$) storage wise. $T_0$= 100 % sucrose; $T_1$= 20 mg/100 mL stevioside; $T_2$= 25 mg/100 mL stevioside; $T_3$= 30 mg/100 mL stevioside; $T_4$= 35 mg/ 100 mL stevioside

flavonoids in stevia extract confirmed its strong antioxidant properties (21), (22). Acidity and total soluble solids in dried stevia leaves were low and reducing, non-reducing and total sugars were found to be 5.88 %, 9.77 % and 15.65 % respectively (12), (23). According to previous literature peach also had strong scavenging ability, low pH and high acidity, total soluble solids and total sugars (24, 25).

Stevioside had significant effect on the antioxidant profile of low calorie peach RTS among the treatments and storage time period. During storage the decrease in antioxidants of fruit drinks may be associated with degradation of antioxidants and their conversion to other metabolites due to variation in temperature, pH and oxygen that affected the stability of antioxidants and significantly contributed to the loss of antioxidants (26).

The trend of decrease in antioxidants in peach ready-to-serve drink is therefore supported by the study carried out by (27) and (28) who concluded that antioxidants in black mulberry juice and grape juice may reduce during storage.

Both pH and acidity had non-significant ($P \geq 0.05$) effect treatment wise and during storage significant decreasing trend was observed due to increased values of acidity and TSS of all the treatments during storage due to ascorbic acid degradation in the prepared peach beverages (29). The increased TSS in peach RTS beverages during storage was probably due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides (30).

Malav et al. (31) and Sindumathi et al. (32) reported that in orange-based blended RTS beverages and flavored papaya-pineapple blended RTS beverages, pH decreased due to increase of acidity and TSS with increasing the storage period.

Total sugars significantly affected by increasing the levels of stevioside and storage period. The increase in the reducing sugars and total sugars during storage might be due the hydrolysis process that resulted in gradual inversion of non-reducing sugars to reducing sugar (33).

These results are in agreement with Selvi et al. (34), Malav et al. (31) and Rani et al. (35) who observed a gradual increase in reducing sugar and total sugars con-

**Tabele 5.** Organoleptic evaluation of Low calorie ready-to-serve Peach beverage

| Organoleptic evaluation parameters | Treatments | Storage period | | | | |
|---|---|---|---|---|---|---|
| | | **0 day** | **15 days** | **30 days** | **45 days** | **60 days** |
| Color | $T_0$ | 8.20±0.12Aa | 8.10±0.01Ab | 7.97±0.05Ac | 7.69±0.09Ad | 7.54±0.07Ae |
| | $T_1$ | 8.00±0.15Ba | 7.90±0.02Bb | 7.88±0.04Bc | 7.74±0.05Bcd | 7.69±0.06Bd |
| | $T_2$ | 7.82±0.19Ca | 7.78±0.05Ca | 7.72±0.08Cab | 7.66±0.09Cb | 7.62±0.05Cc |
| | $T_3$ | 7.65±0.13Da | 7.61±0.01Da | 6.56±0.03Dab | 6.51±0.04Db | 6.47±0.05Dc |
| | $T_4$ | 7.42±0.20Ea | 7.38±0.07Ea | 7.33±0.08Eab | 7.29±0.09Eb | 7.24±0.05Ec |
| Flavor | $T_0$ | 7.14±0.18Aa | 7.06±0.05Ab | 6.92±0.04Ac | 6.85±0.03Ad | 6.42±0.02Ac |
| | $T_1$ | 7.00±0.16Ba | 6.90±0.09Bb | 6.84±0.10Bbc | 6.77±0.01Bc | 6.65±0.08Bc |
| | $T_2$ | 6.77±0.14Ca | 6.73±0.11Ca | 6.68±0.09Cb | 6.64±0.07Cbc | 6.39±0.06Cc |
| | $T_3$ | 6.54±0.19Da | 6.50±0.04Da | 6.45±0.03Dab | 6.41±0.02Db | 6.35±0.05Dc |
| | $T_4$ | 6.22±0.20Ea | 6.18±0.03Ea | 6.13±0.08Eab | 6.08±0.07Eb | 6.03±0.05Ec |
| Taste | $T_0$ | 7.12±0.14Aa | 7.05±0.09Ab | 6.95±0.07Ac | 6.77±0.12Ad | 6.54±0.10Ae |
| | $T_1$ | 7.00±0.14Ba | 6.96±0.04Ba | 6.89±0.11Bab | 6.76±0.03Bb | 6.69±0.02Bc |
| | $T_2$ | 6.82±0.18Ca | 6.77±0.01Ca | 6.73±0.06Cab | 6.68±0.09Cb | 6.63±0.05Cc |
| | $T_3$ | 6.60±0.20Da | 6.54±0.03Da | 6.49±0.04Dab | 6.45±0.09Db | 6.38±0.01Dc |
| | $T_4$ | 6.20±0.12Ea | 6.16±0.05Ea | 6.11±0.07Eab | 6.07±0.03Eb | 6.02±0.11Ec |
| Sweetness | $T_0$ | 7.14±0.18a | 7.06±0.05b | 6.92±0.04c | 6.85±0.03d | 6.42±0.02e |
| | $T_1$ | 7.13±0.16a | 7.08±0.09b | 6.94±0.10b | 6.86±0.01bc | 6.44±0.08c |
| | $T_2$ | 7.13±0.14a | 7.09±0.11a | 6.96±0.09b | 6.88±0.07bc | 6.45±0.06c |
| | $T_3$ | 7.12±0.19a | 7.10±0.04a | 6.97±0.03b | 6.90±0.02bc | 6.47±0.05c |
| | $T_4$ | 7.12±0.20a | 7.11±0.03a | 6.99±0.08b | 6.92±0.07bc | 6.50±0.05c |
| Overall acceptability | $T_0$ | 8.00±0.20Aa | 7.90±0.18Ab | 7.76±0.22Ac | 7.64±0.19Ad | 7.43±0.15Ae |
| | $T_1$ | 7.77±0.21Ba | 7.70 ±0.19Bb | 7.65 ±0.13Bb | 7.61 ±0.14Bb | 7.55±0.20Bc |
| | $T_2$ | 7.47±0.22Ca | 7.42±0.21Ca | 7.38±0.18Cab | 7.33±0.17Cb | 7.28±0.23Cc |
| | $T_3$ | 7.22±0.23Da | 7.18±0.18Da | 7.13±0.22Dab | 7.09±0.19Db | 7.04±0.12Dc |
| | $T_4$ | 7.00±0.21Ea | 6.94±0.20Ea | 6.89±0.21Eab | 6.85±0.18Eb | 6.79±0.13Ec |

tent of Guava-Lime-Ginger RTS beverages, guava and aloe blends. While non-reducing sugars decrease due to inversion of non-reducing sugars to reducing sugars.

The color of control (100 % sucrose) beverages was darker due to conversion of the sucrose to fructose and glucose through Millard reaction. Replacement of sucrose with stevioside resulted in development of lighter color due to no sucrose present that required for the development of desirable color (36).

During storage decrease in color of the functional beverages was observed but that change was less than control. Similar results were recorded by Mgaya-Kilima et al. (26) and Elik et al. (37) who found that color of the different fruit-based beverages decreased during the storage but remained acceptable.

The color of control beverages was darker. While flavor and taste was different due to hydrolysis of the sucrose to its component sugar (fructose and glucose) through millard reaction. Replacement of sucrose with stevioside resulted in development of lighter color and different flavor and taste due to no sucrose present that required for the development of desirable color and flavor (36). The sweetness of functional beverages was close to that of control due to presence of stevioside (glycoside in stevia) that resulted in sweet taste (8, 12). Due to decrease of scores of all organoleptic evaluation parameters the overall acceptability of functional beverages also decreased.

Similar outcomes were observed by Bal et al. (29), Giri et al. (38) and Borah et al. (39) who concluded

that color, flavor, taste, sweetness and overall sensorial quality of fruit-based beverages and dairy products showed decreasing trend with storage but remained acceptable.

## Conclusion

The ready-to-serve (RTS) beverages are popular among the consumers but in them cane sugar is used that resulted in health problems. Stevia a natural, non-caloric sweetener had sweetness several hundred times than sugar with no health issues. The stevioside (glycoside in stevia) when used in beverages as sugar substitute then it can be termed as a functional drink due to its health benefits. This study is useful in preparation of low calorie beverages with stevioside that may improve their quality and also prevent against many diseases. According to this study low calorie ready-to-serve peach beverage with 20 mg/100 mL stevioside was most acceptable according to judges scores.

## Acknowledgment

Other than study authors, there were no contributors to this manuscript. UA conceptualized and performed the study; RSA provided the technical assistance, guided in the data collection, ZM and SMH helped to analyze the data and drafting the manuscript. All authors read and approved the final manuscript. No funding or sponsorship was received for this study or publication of this article. The authors declare no conflict of interest.

## References

1. Carbonell-Capella JM, Buniowska M, Maria J, Esteve MJ, Friola A. Effect of Stevia rebaudiana addition on bioaccessibility of bioactive compounds and antioxidant activity of beverages based on exotic fruits mixed with oat following simulated human digestion. Food Chem 2015; 184(1): 122-130.
2. Ogden CL, Carroll MD, Flegal KM. High body mass index for age among US children and adolescents, 2003-2006. JAMA 2008; 299(20): 2401-2405.
3. Megeji NW, Kumar JK, Singh V, Kaul VK, Ahuja PS. Introducing Stevia rebaudiana, a natural zero-calorie sweetener. Curr Sci 2005; 88: 801-804.
4. Starrat, AN, Kirby CW, Pocs R, Brandle JE. Rebaudioside F, A diterpene glycoside from Stevia rebaudiana. Phytochemistry 2002; 59: 367-370.
5. Chatsudthipong V, Muanprasat C. Stevioside and related compounds: therapeutic benefits beyond sweetness. Pharmacol Ther 2009; 121(1): 41-54.
6. Lemus-Mondaca R, Ah-Hen K, Vega-Galvez A, Honores C, Moraga NO. Stevia rebaudiana Leaves: Effect of Drying Process Temperature on Bioactive Components, Antioxidant Capacity and Natural Sweeteners. Plant Foods Hum 2015; Nutr DOI 10.1007/s11130-015-0524-3.
7. Abdo BM. Sweetness equivalence ratio of Stevia (Stevia Rebaudiana Bertoni) with sugar adopted in Ethiopia. IJABBR 2016; 4(1): 58–61.
8. Balaswamy K, Rao PP, Rao GN, Nagender A, Satyanarayana A. Production of Low Calorie Ready-to-Serve Fruit Beverages Using a Natural Sweetener, Stevia (Stevia Rebaudiana L.) FMFI 2014; 3: 59-63.
9. Bharathi N. Uses of sweetening agents found in stevia (Stevia rebaudiana) leaves. Natural Product Radiance 2003; 2: 120-121.
10. Savita, SM, Sheela K, Sunanda S, Shankar AG, Ramakrishna P. Stevia rebaudiana-A functional component for food industry. J Hum Ecol 2004; 15: 261-264.
11. Kujur RS, Singh V, Ram M, Yadava HN, Singh KK, Kumari S, ROY BK. Anti-diabetic activity and phytochemical screening of crude extract of Stevia rebaudiana in alloxan-induced diabetic rats. Pharmacog Res 2010; 2(4): 258–263.
12. Abou-Arab AE, Abou-Arab AA, Abu-Salem MF. Physico-chemical assessment of natural sweeteners steviosides produced from Stevia rebaudiana Bertoni plant. Afri J Food Sci 2010; 4: 269–281.
13. Fuh. Purification of steviosides by membrane and ion exchange process", J Food Sci 1990; 55 (5): 1454-1457.
14. Sakanaka S, Tachibana Y, Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha- cha). Food Chem 2004; 89: 569-575.
15. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agri Food Chem 2002; 50: 3010-3014.
16. Shahidi F, Liyana-Pathirana CM, Wall DS. Antioxidant activity of white and black sesame seeds and their hull fractions. Food Chem 2006; 99:78-483.
17. AOAC (Association of Official Analytical Chemists) Official Methods of Analysis. 18th Edition, Association of Official Analytical Chemists. Washington D.C, USA, 2005.
18. Balaswamy K, Rao PP, Nagender A, Satyanarayana A. Preparation of Sour Grape (Vitis Vinifera) Beverages and Evaluation of their Storage Stability. J Food Process Technol 2011; 2(3): 1-4.
19. Meilgaard MC, Civille GV, Carr BT. Sensory evaluation techniques, 4th ed. C.R.C. Press L.L.C, New York, 2007.
20. Steel, RGD, Torrie JH, Dickeyi D. Principles and Procedures of Statistics. A biometrical approach.3rd ed. McGraw Hill Book Co Inc. New York, 1997.
21. Periche A, Castello ML, Heredia A, Escriche I. Influence of drying method on steviol glycosides and antioxidants in Stevia Rebaudiana leaves. Food Chem 2015; 172(1): 1-6.

22. Lopez V, Perez S, Vinuesa A, Zorzetto C, Abian O. Stevia rebaudiana ethanolic extract exerts better antioxidant properties and antiproliferative effects in tumour cells than its diterpene glycoside stevioside. Food Funct 2016; 7: 2107-2113.
23. Gasmalla MAA, Yang R, Amadou I, Hua X. Nutritional Composition of Stevia rebaudiana Bertoni Leaf: Effect of Drying Method. Trop J Pharma Res 2014; 13 (1): 61-65.
24. Mokrani A, Krisa S, Cluzet S, Costa GD, Temsamani H, Renouf E, Merillon JM, Madani K, Mesnil M, Monvoisin A, Richard T. Phenolic contents and bioactive potential of peach fruit extracts. Food Chem 2016; 202: 212-220.
25. Singh O, Kumar A, Rai R, Kohli K. Quality evaluation of low chill peach cultivars for preparation of ready-to-serve 'Nectar' drink. Asian J Dairy Food Res 2016; 35 (4): 327-330.
26. Mgaya-Kilima B, Remberg SF, Chove BE, Wicklund T. Physiochemical and antioxidant properties of roselle-mango juice blends; effects of packaging material, storage temperature and time. Food Sci Nutri 2015; 3(2):100–109.
27. Jimenez-Sanchez C, Lozano-Sanchez J, Marti N, Saura D, Valero M, Segura-Carretero A, Fernandez-Gutierrez A. Characterization of polyphenols, sugars, and other polar compounds in persimmon juices produced under different technologies and their assessment in terms of compositional variations. Food Chem 2015; 182(1): 282-291.
28. Lopes MLM, Miguel MAL, Fialho E, Valente-Mesquita VL. Grape juice obtained using steam extraction and other small-scale extraction methods: phenolic content, antioxidant capacity and stability during storage. Int J Food Sci Technol 2016; 51(7): 1696–1702.
29. Bal LM, Ahmad T, Senapati AK, Pandit, PS. Evaluation of Quality Attributes during Storage of Guava Nectar cv. Lalit from Different Pulp and TSS Ratio. J Food Process Technol 2014; 5(5):1-5.
30. Sharma A, Singh K. Effect of different treatments on TSS, sugars, viscosity and suspended pulp of lime juice during storage. Haryana J Hort Sci 2005; 33(1-2), 45-46.
31. Malav M, Gupta R, Nagar T. Studies on bio-chemical composition of orange based blended ready-to-serve (RTS) beverages. BBRC 2014; 7(1): 78-83.
32. Sindumathi G, Premalatha MR. Development and storage studies of naturally flavored papaya-pineapple blended ready-to-serve (RTS) beverages. IJSR 2015; 4(2): 856-860.
33. Saravanan K, Godara RK, Goyal RK, Sharma RK. Standardization of recipe for papaya nectar and its storage. Haryana J Hort Sci 2004; 33(3-4): 204-206.
34. Selvi J, Banumathi P, Kanchana S, Ilamaran M. Formulation of therapeutic drink to boon human health (guava-lime-ginger RTS beverage). J Food Sci Res 2013; 4(2): 141-146.
35. Rani TB, Babu JD. Acceptability and storage studies of guava-aloe nectar blends. TAJH 2015; 10(1): 80-85.
36. Hamzah Y, Aluwi NFM, Sembok WZW. Effect of Stevia as a Sweetener Substitution on the Quality of Kuih Baulu. 3th ASEAN Food Conference, 9-11 September 2013, Singapore Meeting Future Food Demands: Security & Sustainability.
37. Elik A, Yanik DK, Maskan M, Gogus F. Influence of three different concentration techniques on evaporation rate, color and phenolics content of blueberry juice. J Food Sci Technol 2016; 53(5): 2389–2395.
38. Giri A, Rao RHG, Ramesh V. Effect of partial replacement of sugar with stevia on the quality of kulfi. J Food Sci Technol 2014; 51(8): 1612–1616.
39. Bohra P, Srinivas, KN. Development of nectar from pummelo (citrus grandis), an underutilized fruit crop, by blending with kokum (Garcinia indica) and mango ginger (Curcuma amada). JASA 2015; 19(1): 70-74.

Correspondence:
Rabia Shabir Ahmad
Institute of Home and Food Sciences, Faculty of Science and Technology, Govt. College University, Faisalabad, Punjab, 38000, Pakistan
Phone+92.313.2200042
Email: rabiaahmad@gcuf.edu.pk