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Effect of Ginger (*Zingiber Officinale*) and Aleo Vera (*Aloe Barbadensis Miller*) Extracts on Physicochemical Properties of Grape Juice Under 4℃ and 25℃

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Abstract: This research aimed to investigate the impact of storage period on total phenolic content (TPC), ascorbic acid and nutritional values of functional noncarbonated drink. Drink was prepared with the utilization of herbal plants (*Zingiber Officinale*, *Aloe Barbadensis Miller*) with 8 ml, 6 ml, 4 ml, 2 ml & 2 ml,4 ml, 6 ml, 8 ml in 120ml of grape juice respectively different concentration and stored at 4°C and 25°C for 90 days. Findings of the current study showed presence of *aleo vera* significantly preserved TPC and led to increase in all beverage treatments on both storage conditions, however treatment 4 *(X_4)* possessed the highest values of 3.7 and 3.65 mg GAE/g at 4°C and 25°C respectively. The storage period also increases the total sugar contents significantly and the acidity. Comparing all treatments, a significant increment was found in X_4 with high *aleo vera* concentration. Total sugar and acidity were 5.677 % and 0.456 % in X_4 respectively. Ascorbic acid content tends to decline from 22.22 % to 17.64 % within 15 days of interval at room temperature with the passage of storage time. Current research will provide the platform of herbal ingredients in beverages, profile and impact on health disorders.

Practical application:

Prepared non-carbonated functional drink is helpful to develop the herbal extract base commercialized beverage. Experimental investigation of *aleo vera* contained drink can preserve for a long time with high antioxidant activities.

Keyword: Non-Carbonated Drink, Noncarbonated; *Aleo vera*; Functional beverage; Ascorbic acid, Total phenolic content

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1. Introduction

The functional drinks are important in targeting health issues by reducing root causes with continuous preventive mechanism. Herbal extracts are excellent source of soluble fiber and other antioxidant agents. Recently, the production and consumption of functional products are being attractive in disease prevention and treatment and health beneficial beyond the basic nutritional functions (Younas et al., 2024a). The rise in chronic diseases reduced the consumption of synthetic beverages and

attracts people to produce beverages with good health impacts. Addition of organic compounds in the fruits and vegetables such as flavonoids, anti-oxidants and vitamins is one of the new product development strategies in functional beverage industry to attract customer (Xiong et al., 2022). However, antioxidant rich beverage is one of the innovations in the product development in food industry.

Grapes have a long health-related benefits history and found phenol rich fruits such as anthocyanins which distributed in the skin, stem, leaf and seed of grapes, instead of fruit flesh. High concentration is of total phenolic compounds was reported about 2178.8, 374.6, 23.8, and 351.6 mg/g GAE (Gallic acid equivalent) in seed, skin, flesh, and leaf, respectively (Pastrana-Bonilla et al., 2003). Polyphenols are most important among phytochemicals because of possess many biological activities and health-promoting benefits (Wada et al., 2007). The phenolic compounds mainly include anthocyanins, flavanols, flavonoids, stilbenes (resveratrol) and phenolic acids (Dopico-García et al., 2008).

Aloe vera is a distinctively xerophyte with high flesh proportion covered with spiny leaves. Recently, this herbal plant famous for efficient and prominent role as medicine (Tan & Vanitha, 2004) and higher in water and lower solid matter of 99 % and 1- 0.5 % respectively with 4.5 pH. The mucilaginous jelly from the parenchyma cells of the peeled, spineless leaves of the plant is referred as *Aloe vera* gel. Gel is main part of plant, moist, viscous, colorless fluid, high in all respective medicinal attributes and possesses anthraquinone glycosides, glycoprotein, gamma-lanoline acid, prostaglandins and mucopolysaccharides. These compounds show antibacterial, antifungal and antiviral activity (Shafi et al., 2000). The interest and use of gel has increased dramatically in the field of health care and cosmetics (Devi, 2005). It can be utilized as a valuable ingredient for food application due to its biological activities and functional properties (Pandey et al., 2022). *Aloe vera* gel has a bitter taste which can be unpleasant in raw state and its palatability could be enhanced with addition of some other fruit juices. Ginger scientifically known as *Zingiber ofcinale* belongs to the family Zingiberaceae. Ginger is contraindicated in people suffering from gallstones as the herb promotes the release of bile from of vitamin C content in common fruits this have been inconsistent, and may have blood thinning and cholesterol lowering properties that may make it useful for treating heart disease (Zeeshan et al., 2018)**.** Ginger (*Zingiber officinale*) is a herbaceous aromatic perennial plant

possesses medicinal properties due to its bioactive compounds (Sanwal et al., 2010), antioxidants (Singh & Gupta, 2013) and anti-inflammatory activities (Ali et al., 2008). The aim of the research was to prepared optimal nutraceutical drink. The research was conducted to accomplish the objectives of determine optimized ratio in best functional beverage and determine the physiochemical properties and sensory profile of functional beverage during storage.

2. Materials and Methods

2.1. Experimental Material

The research was conducted in the Department of Food Technology, Pir Mehr Ali Shah Arid Agricultural University Rawalpindi (PMAS), Pakistan. Fully matured and fresh grapes and ginger were purchased from the local market of Rawalpindi and *Aloe vera* was collected from PMAS- Pakistan.

2.2. Preparation of beverage

Fresh grapes, *Aloe Vera* and ginger was used for extraction of juice (Ramachandran & Nagarajan, 2014). Each fruit was cleaned thoroughly washed, blanched and blended to a pulp and the juice was extracted by filtering through muslin cloth and stored at 4°C in refrigerated. The treatments were prepared with different concentration of grapes juice along with *Aloe vera* and ginger extract with syrup as described in treatment Table 1. Moreover, the brief procedure of beverage preparation as all fruits were blended and mixed with 1% strained syrup (sugar, citric acid, water) and 100 pm sodium benzoate added as preservative. The final volume of each concentration of all treatments bottled 200 ml. The filled bottles were pasteurized at 80 ℃ in water batch for 20 min. The pasteurized bottles were allowed to cool at room temperature and divided into two groups for 90 days of storage at 4 ℃ and 25 ℃ for evaluation of physicochemical and microbial analysis. The beverage was taken out after every 15 days of interval with three replications of each parameter.

2.3. Biochemical analysis

pH was measured by using digital pH meter. Total soluble was measured by using digital refract meter. Prism of refractometer was cleaned before and after taking reading. A drop of sample was placed on the prism of refractometer and reading was recorded and expressed in ºBrix. The titratable acidity was measured by using potentiometric titration. 5 ml sample was diluted with 50 ml distilled water and two drops of phenolphthalein was added. Afterward it was titrated against 0.1N NaOH. Ascorbic acid measured with method described by (AOAC, 2000a) with some modification. 10 ml sample of was taken in 100 ml of volumetric flask. Volume was made up to

100 ml by adding oxalic acid solution made with 0.4% concentration and filtered through No.4 Whatman filter paper to get 5 ml extract. Then this filtrate was titrated against 2, 6- dichlorophenolindophenol dye were used up to the end until light pink color was appeared. Following equation was used to calculate ascorbic acid content:

$$\text{Ascirbic acid} = \frac{1 \times R_1 \times V}{\text{R} \times \text{W}\, V_1} \times 100$$

Where *R* and R_1 represent the amount of dye used in ml against reference solution (Standard) and amount of dye in ml used to filtrate against filtrate volume (Sample reading). *V* is filtrate volume mad by adding oxalic acid solution with 0.4% concentration of oxalic acid, V_1 is Juice used in ml against titration, W is juice in ml used.

Total sugar content was determined according to AOAC (2000b) with some modification. 10 ml sample of juice sample was taken in 250 ml flask and diluted with 100 ml distilled water. Then 10 ml solution of potassium oxalate and 25 ml solution of lead acetate were added. Afterward volumetric flask was filled up to 250 ml by using distilled water. After this 5 ml solution of HCl were added in 100 ml flask and 20 ml distilled water were also added and also already prepared filtrate was added. It was allowed to hydrolyze for whole night and next morning it was titrated against NaoH 1N solution. Phenolphthalein was used as indicator to determine the end point and volume were made up to 100 ml by using distilled water. Then titration was done with Fehling solution to determine total sugar content.

2. 4. Total Phenolic Content

The total phenolic content of juice sample measured with Foilin-Ciocalteau's as described by Younas et al., (2024b) were used. 5 ml sample of juice was added in a volumetric flask and 25 ml methanol were added and shacked for 1 hour to homogenize completely. Total phenolic content of juice sample was recorded as gallic acid equivalents (GAE) in mg per g. A standard curve was adopted in duplicate by making standard solution of 1mg/ml gallic acid in 50 % methanol. Foilin-ciocalteau's reagent 500 µL was added along with 100 µL of sample extract and added 6 ml distilled water followed by the addition of 1.5 mL Na_2Co_3 solution after for 5 minutes at ambient temperature. The whole sample was homogenized and allowed to stand for half an hour at 40°C before measuring the absorbance at 765 nm. Blank were also used to double check the readings.

2.5. Sensory Evaluation

Sensory evaluation was carried out by 9 points hedonic scale as described by

Vázquez-Cabral et al. (2014). Sensory evaluation for color, consistency, aroma and taste was carried out. Trained 20 members were randomly selected with age range of 20-35, the samples were coded and served at 15℃ with water and asked the panel to judge product on unbiased basis. Members were guided about product specifications and agreement was approved before sensory analysis by the departmental ethical research committee of Department of Food Technology, Pir Mehr Ali Shah Arid Agricultural University Rawalpindi (PMAS), Pakistan.

2.6. Statistical Analysis

Data was analyzed through analysis of variance (ANOVA) using SPSS V.20. The obtained data of different attributes expressed in mean values ± SD (standard deviation). Tukey's mean test was performed for the mean comparison with confidence level of 95% ($P<0.05$).

3. Results and Discussion

3.1. Physicochemical analysis

The chemical properties of ginger and *aloe vera* extract with grape juice are presented in Table 2. Total Soluble Solids (TSS) mainly sugars are widely used attribute for maturity evaluation and is usually measured in ºBrix. Analysis of variances shows that all treatments were having significant effect during storage at both treatments 4 ℃ and 25 ℃. The TSS contents were significantly increased during storage period of 90 days. This increase in TSS owing to the conversion of polysaccharides into sugar. These finding also attributed the increase in total sugar contents of all treatments (Table 2). All treatments X_1-X_4 observed increased TSS, however X_4 demonstrated maximum increase from 12.520 and 12.303 to12.640 and 12.750 at 4 ℃ and 25 ℃. This highest increase in X_4 due to presence of high concentration of *Aloe Vera* caused acid hydrolysis of starch. The results are in previous study of stored banana herbal beverage (Ramachandran & Nagarajan, 2014), storage of *Aloe* vera (Kausar et al., 2020; Mishra & Sangma, 2017) based beverage and bitter gourd ready to serve beverage (Barwal et al., 2005).

Table 1. of treatments of different concentrations of *Aloe Vera* and ginger juices mixed with grapes Juice

Treatment	Grapes	Ginger	*Aloe Vera*	Sugar Syrup	Water	Total
X_1	120 ml	8 ml	2 ml	33 ml	37 ml	200 ml
X_2	120 ml	6 ml	4 ml	33 ml	37 ml	200 ml
X_3	120 ml	4 ml	6 ml	33 ml	37 ml	200 ml
X_4	120 ml	2 ml	8 ml	33 ml	37 ml	200 ml

The most important measure of juice acidity is pH and has an important role in representative of sourness of juice in taste and on susceptibility to microbial

contamination (Vázquez-Cabral et al., 2014). Storage has significant effect on pH value and decline in pH of all treatments is recorded during storage of 90 days with fifteen days interval shown in Table 2. The maximum decline observed in X_4 (4.323-4.231) and (4.321- 4.235) at 4℃ and 25℃ respectively, due to high concentration of *aloe vera*. This decrease in pH reported in banana and sapota drink and gradual decrease attributed a positive sign of antimicrobial characteristic of product (Ilamaran & Amutha, 2007; Nidhi et al., 2008). With the passage of storage interval there was a significant increase in titratable acidity of treated samples. The mean of titratable acidity of treated samples varied from 0.310- 0.456 and significant increase of all treated samples during every interval of 90 days storage. Highest titratable acidity was seen in X_4 of 0.456 at 25 ℃ and lowest titratable acidity was seen in X_1 and X_2 of 0.340 and 0.359

respectively. The reason behind this phenomenon of increasing of titratable acidity was decrease in pH which automatically increase the titratable acidity of treated samples during storage, due to which the shelf life of treated samples was enhanced. Same trend was reported by Ramachandran and Nagarajan (2014) who studied the polyphenol degradation phenomenon in ginger and amla and protein into amino acids on the process variables of peaches juice that were treated osmotically. There was significant increase in total sugar content of treated sample juices with passage of storage interval. Table 2 shows increase in total sugar content in all treatment with storage intervals. Mean of total sugar content was varied from at 4°C 14.062 to 14.346 % in X_1 while maxim increase determined in X_4 due 13.805 to 15.677 % to high acidity hydrolyze the polysaccharides. Our findings are in agreement with Singh et al. (2017).

3.2. Ascorbic Acid

It can be seen from the Fig. 1 that there was a significant decrease in ascorbic acid

Table 2. Effect of storage period on different physicochemical properties of *Aloe Vera* and ginger juices mixed with grapes juice

Parameters	pH		Acidity		TSS		Total sugar	
	4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C
Storage/day 0								
X_1	4.391	4.390	0.310	0.310	12.200	12.030	14.062	14.000
X_2	4.370	4.371	0.330	0.343	12.150	12.167	14.200	14.200
X_3	4.380	4.380	0.340	0.340	12.303	12.303	14.411	14.400

X_4	4.323	4.321	0.407	0.408	12.520	12.303	13.805	13.800
Storage/day 15								
X_1	4.374	4.381	0.317	0.314	12.180	12.277	14.074	14.200
X_2	4.367	4.367	0.332	0.345	12.150	12.333	14.354	14.600
X_3	4.372	4.375	0.342	0.344	12.310	12.310	14.458	14.600
X_4	4.315	4.320	0.411	0.415	12.510	12.410	13.836	13.900
Storage/day 30								
X_1	4.365	4.350	0.320	0.324	12.130	12.130	14.267	14.400
X_2	4.361	4.361	0.335	0.348	12.170	12.337	14.557	14.900
X_3	4.363	4.358	0.345	0.347	12.320	12.373	14.488	14.700
X_4	4.302	4.313	0.418	0.417	12.500	12.420	14.254	14.572
Storage/day 45								
X_1	4.350	4.320	0.322	0.326	12.230	12.483	14.456	14.600
X_2	4.358	4.358	0.338	0.351	12.250	12.317	15.623	15.100
X_3	4.360	4.353	0.348	0.349	12.327	12.327	14.934	14.800
X_4	4.300	4.307	0.423	0.420	12.520	12.270	15.323	15.300
Storage/day 60								
X_1	4.344	4.317	0.326	0.329	12.220	12.383	14.482	14.900
X_2	4.345	4.337	0.341	0.355	12.300	12.330	15.417	15.300
X_3	4.356	4.332	0.351	0.353	12.330	12.330	14.824	14.700
X_4	4.298	4.281	0.428	0.437	12.550	12.600	15.425	15.500
Storage/day 75								
X_1	4.338	4.312	0.331	0.336	12.260	12.260	14.537	14.150
X_2	4.337	4.330	0.347	0.359	12.320	12.557	15.519	15.500
X_3	4.341	4.318	0.353	0.357	12.320	12.320	14.975	14.900
X_4	4.261	4.257	0.434	0.439	12.570	12.667	15.677	15.800
Storage/day 90								
X_1	4.321	4.308	0.337	0.340	12.300	12.300	14.346	14.190
X_2	4.329	5.327	0.349	0.359	12.250	12.113	15.705	15.700
X_3	5.336	4.308	0.358	0.361	12.350	12.320	15.146	15.000
X_4	4.231	4.235	0.447	0.456	12.640	12.750	14.855	14.900

(vitamin C) content of treated samples during storage. The means of ascorbic acid content during storage were varied from 22.228 to 17.642 at 4 °C in all treatments

during 90 days of storage interval at room temperature. However, maximum decrease in ascorbic acid contents found in X_4. Moreover, ascorbic acid content of treated samples was also decreased at 25 °C during storage of 90 days. The vitamin C tends to deterioration due to heat, light sensitive and oxidized with passage of time. Similar results were also studied by Mishra and Sangma (2017) for ascorbic acid, greater loss of ascorbic acid during storage. It was also suggested that ascorbic acid content decreases as the ripening progresses.

3.3. Total Phenolic Content

The effect of storage period on total phenolic contents of functional beverage is shown in Fig 2. The findings exhibited the significant impact and increased of TPC in beverage of possessing different combination of various functional ingredients with passage of time.

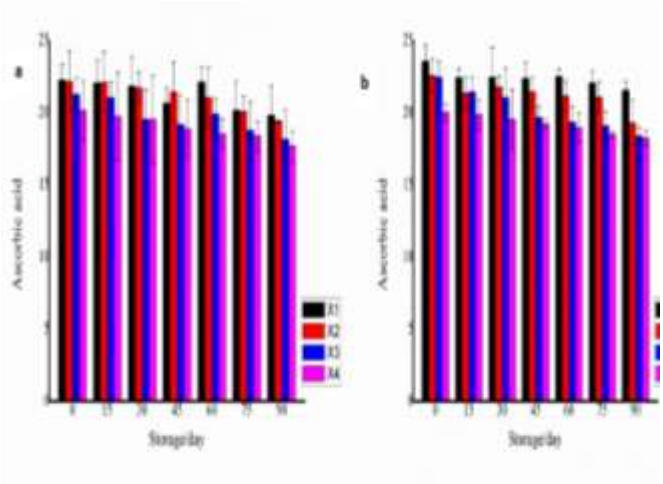

Figure 1. Effect of storage period on ascorbic acid of different concentrations of mixed beverage, (a) 4 ^{O}C and (b) 25 ^{O}C

It can be observed that the value of total phenolic content increased in both storage conditions on 4 °C and 25 °C from 2.59 to 3, 2.48 to 2.56, 2.48 to 2.56, 2.69 to 3.7 in X_1, X_2, X_3 and X_4 respectively. However, the highest increase observed in treatment X_4. Moreover, means of total phenolic contents also increased during storage of 90 days with 15 days interval. This increase in TPC discussed by Ramachandran and Nagarajan (2014) due to breakage of acidic bonds.

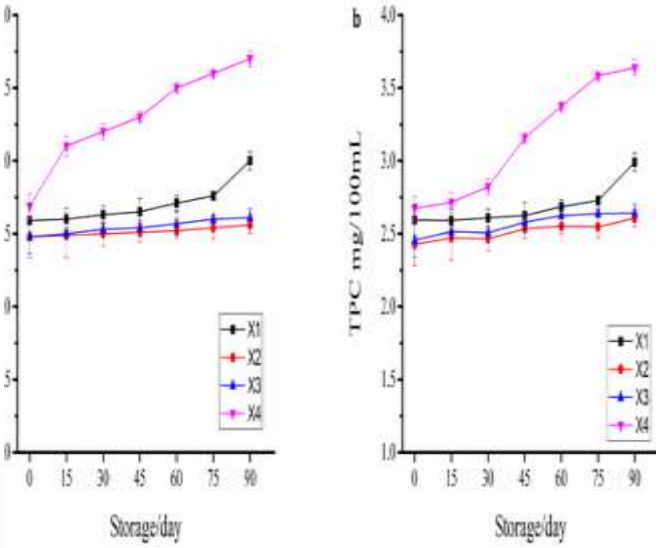

Figure 2. Effect of storage period on Total phenolics of different concentrations of mixed beverage, (a) 4°C and (b) 25°C

3.4. Organoleptic Evaluation

Factors that directly affect the organoleptic content of the juice is storage. Study have proved that polyphenols content of the juice change on storage, the reason is easy oxidation of these polyphenols. Oxidation reactions result in the formation of more or

less polymerized substance, which lead to change in the quality of food, particularly in color and organoleptic characteristics. Organoleptic evaluation of all juices sample was done at first and the last day of three months storage period. Color plays an important role in appearance and

Table 3. Effect of storage period on sensory properties of *Aloe vera* and ginger juices mixed with grapes juice

Parameters	Color		Consistency		Aroma		Taste	
	4ºC	25ºC	4ºC	25ºC	4ºC	25ºC	4ºC	25ºC
Storage/day 0								
X_1	6.79	6.55	6.80	7.45	7.00	7.25	5.00	5.00
X_2	6.56	6.50	7.00	7.65	7.20	7.12	6.74	6.00
X_3	6.34	6.38	6.30	7.15	7.40	7.37	6.21	4.80
X_4	5.27	5.20	6.00	6.52	6.80	6.50	6.20	6.20
Storage/day 15								
X_1	6.53	6.51	7.00	7.32	7.20	6.86	5.20	5.20
X_2	6.71	6.70	7.50	7.50	7.60	6.55	6.30	6.30
X_3	5.81	5.80	6.00	7.30	7.60	6.38	6.35	5.20
X_4	5.59	5.50	6.40	6.07	6.90	6.15	6.10	6.10
Storage/day 30								
X_1	6.49	6.40	8.90	5.30	7.40	6.40	5.10	5.10
X_2	6.75	6.38	7.80	7.80	7.90	6.00	6.40	6.40
X_3	5.93	5.56	5.90	6.50	7.70	6.18	5.72	5.30
X_4	5.42	5.05	6.30	6.33	7.10	5.85	6.00	6.00
Storage/day 45								
X_1	6.30	NA	6.50	NA	7.60	NA	5.30	NA
X_2	6.24	NA	7.60	NA	8.10	NA	6.03	NA
X_3	6.05	NA	5.80	NA	7.80	NA	5.50	NA
X_4	5.00	NA	6.50	NA	7.30	NA	5.90	NA
Storage/day 60								
X_1	6.42	NA	6.30	NA	7.90	NA	5.40	NA
X_2	6.08	NA	7.40	NA	8.30	NA	5.95	NA
X_3	6.20	NA	5.60	NA	7.70	NA	5.48	NA

X_4	5.38	NA	6.40	NA	7.50	NA	5.70	NA
Storage/day 75								
X_1	6.04	NA	6.40	NA	7.15	NA	5.40	NA
X_2	6.14	NA	7.30	NA	8.50	NA	5.75	NA
X_3	6.00	NA	5.30	NA	7.90	NA	5.40	NA
X_4	5.50	NA	6.70	NA	7.80	NA	5.40	NA
Storage/day 90								
X_1	5.86	NA	6.70	NA	7.19	NA	5.50	NA
X_2	6.06	NA	7.10	NA	8.70	NA	5.80	NA
X_3	6.00	NA	5.00	NA	8.00	NA	5.20	NA
X_4	5.32	NA	6.80	NA	7.90	NA	6.00	NA

accountability of fruit juice. Color is perceived as part of total appearance, which is the vital recognition as assessment of quality and organoleptic properties of the juices. The data pertaining to score for the color of different treatment is presented in Table 3. Analysis of variance disclosed a highly significant effect among treatment and storage. At the 0-day X_2 and X_3 were scored highest regarding their color X_1 and X_2 respectively. After three month the last day of storage X_4 is scored at least and maximum scored juice was X_2 following X_3 and X_1. For many products, browning is desirable but in grapes juice browning is undesirable. Browning is the most common quality problem in color. Lose in color was might be due to increase in pH value. Taste majorly depends on polyphenol concentration, acidity and sugar contents in grapes and all over juices. The result pertaining to score for the taste of different treatments is shown in Table 3. On 0-day X_2 treatment maximum score than X_4, X_1, X_3, respectively. After three months of storage taste of X_3 was unlike the most but treatment X_2 and X_4 were liked the most. The results regarding the score for aroma of different treatments. At 0-day treatments X_3 and X_2 was like the most and all had highest score point respectively after three months of storage. Kausar et al. (2020) found the similar trends in sensory parameters during storage of functional beverage. All the results shown that storage at 25°C could not get finding after 30 days while 4°C preserved sensory values upto 90 days.

Conclusion

In this study, functional beverage prepared with medicinal plants and stored for 90 days. The effect of ginger and *aleo vera* on total sugar, pH and acidity as well as impact on total phenolic contents was determined. The storage period showed

maximum increase in total sugar and acidity in X_4 15.677 % and 0.456 at 4℃ and 25℃ respectively due to high concentration of *aleo vera.* There was a decline found in ascorbic acid contents in all treatments in both storage conditions for 90 days. Study showed functional beverage successfully preserved for TPC for longtime in product contains high *aleo vera* contents. Moreover, no observations found in sensory attributes at 25℃ after 30 days while beverage stored at 4℃ preserved color, taste and aroma for 90 days. It can be concluded that storage of product at chilled temperature can preserve nutritional and antioxidant attributes of functional beverages. Our research will provide platform to food processors to produce more non-carbonated and herbal products to improve help health issues.

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