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Gunjeshree Gond
Doctoral Research Scholar, Department of Vegetable Science, CoA, IGKV, Raipur, Chhattisgarh, India

Annu Verma
Professor, Department of Vegetable Science, CoA, IGKV, Raipur, Chhattisgarh, India

Oshin Pali
Doctoral Research Scholar, Department of Vegetable Science, CoA, IGKV, Raipur, Chhattisgarh, India

Corresponding Author:
Gunjeshree Gond
Doctoral Research Scholar, Department of Vegetable Science, CoA, IGKV, Raipur, Chhattisgarh, India

Development and analysis of ascorbic acid and anthocyanin of *Hibiscus sabdariffa* tea blended with spices during storage

Gunjeshree Gond, Annu Verma and Oshin Pali

Abstract
Spiced beverages are natural products with high value due to their natural antioxidant and antimicrobial properties. Hibiscus tea is a caffeine-free herbal tea made from the Roselle, scientifically known as *Hibiscus sabdariffa* L. Hibiscus tea contains high levels of antioxidants, such as flavonoids, which are beneficial to our hearts and bodies. To improve the processing of *Hibiscus sabdariffa* calyces into drinks, the calyces were dried and packaged in tea bags under conditions that preserved desirable contents such as anthocyanins, vitamin C, protein and minerals. The current study was designed to develop Hibiscus tea blended with spices in order to take advantage of the potential health and processing benefits of this crop. A research experiment was conducted at the laboratory of Vegetable Science Department, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). From the research it was found that ascorbic acid content decreased during storage period of 90 days. The data revealed that the maximum ascorbic acid concentration was recorded in T_1 (17.63 mg/100 ml) at 0 day and decreased with storage period, with the value decreasing to (17.59 mg/100 ml) at 90 days. Anthocyanin content show decreasing trend during storage period. T_7 had the highest value (1.30 mg/100 ml) which decreased from (1.30 mg/100 ml to 1.25 mg/100 ml) at 0 to 90 days.

Keywords: Anthocyanin, Hibiscus tea, *Hibiscus sabdariffa* L., physicochemical, roselle, spiced beverage

Introduction
Hibiscus tea is typically made from the dried calyces of the tropical plant *Hibiscus sabdariffa* L., which belongs to the Malvaceae family. *Hibiscus sabdariffa* is also known as 'roselle' or 'red sorrel' in English and 'karkade' in Arabic (Ali *et al.*, 2005) [1]. The calyces (flower's outer parts) are the most commonly used to make hot and cold infusions. *Hibiscus sabdariffa* is also high in organic acids, which give it a distinct tart flavour (Serban, 2015) [11]. Hibiscus extracts have also been discovered to have antioxidant and antimicrobial properties (Jabeur *et al*., 2017) [5]. Red colour of roselle is due to pigment known as "Anthocyanin". The anthocyanin found in roselle calyces have been reported to contain delphinidin-3-sambubioside, cydine-3-sambubioside, delphinidin-3-monoglucoside and cynidin-3-monoglucoside (Hong and Wrostland, 1990) [2]. Vitamins like ascorbic acid, niacin and pyridoxine were also present in appreciable amounts (Puro *et al*., 2014) [10].
As the roselle calyx is prone to decomposition, it must be dried. Drying is probably the oldest and most important method of food preservation practised by humans; it is one of the most important post-harvest operations for biological materials because it has a significant impact on the quality of the dried products by preserving nutritional properties, specifically the ascorbic acid content (Imad 2010 and Miranda *et al*., 2008) [3, 7]. The mode of packaging also has an impact on the shelf life of the drink; proper packaging is a means of preventing product quality loss because improper packaging can cause microbial contamination of the drink. Due to the limitations of the liquid extract, this study aims to develop a suitable process for dry processing and packaging the calyces of *Hibiscus sabdariffa* with spices so that they can be readily prepared fresh at the table to the desired quantity while retaining their nutritional constituents.
Spices are widely used in indigenous medicines, pharmaceuticals, nutraceuticals, aroma, therapy, preservatives, beverages and natural colours, in addition to flavouring and seasoning. Apart from having appetizing properties, spices also have medicinal and therapeutic properties that have a significant impact on human health because they affect many functional processes. In this research spices like black pepper, cardamom, clove, dry ginger, mint and tulsi were

used along with the roselle extract in different concentration into 7 treatments.

Materials and Methods

Hibiscus calyces were collected from fields of local farmer from Dantewada. Other materials like spices and tea bags were collected from local market.

Drying of Hibiscus calyces

Collected roselle calyces were cleaned and washed under running tap water and shade dried for 24 hours then dried in an oven at 70 °C for 3 days and then stored in an air tight container.

Preparation of Hibiscus tea

Dried Hibiscus calyces were crushed into small pieces and then according to the treatments 2 gm of roselle and 1 gm of spices were put into a individual tea bags. Dip the tea bags in hot water to make Hibiscus tea and add sugar accordingly.

Ascorbic acid analysis

Reagents

1. Metaphosphoric acid (HPO_3) solution (3%)

For the preparation of 3 per cent metaphosphoric solution 30 gm of metaphosphoric acid sticks was diluted in 1 litre of distilled water.

2. Dye Solution

50 mg of 2,6-dichlorophenol-indophenol was dissolved in about 150 ml of hot distilled water, containing 42 mg of Sodium bicarbonate and was cooled and diluted to 200 ml with distilled water, solution was stored in a brown bottle in a refrigerator at 3 °C and standardize every day.

3. Standard ascorbic acid solution

100 mg of L-ascorbic acid was weighted properly and dissolved in a small amount of 3 per cent metaphosphoric acid and volume make up to 100 ml with the same solution. 10 ml of this stock solution was diluted to 100 ml with 3 per cent metaphosphoric acid (0.1 mg ascorbic acid/ml).

Standardization of Dye

5 ml of standard ascorbic acid solution and 3 percent metaphosphoric acid each was taken in a volumetric flask and was titrated with dye solution filled in the microburette, until pink colour persists for 10 second. Dye factor was calculated (mg of ascorbic acid/ ml of dye) as follows.

$$\text{Dye Factor} = \frac{0.5}{\text{Titre Value}}$$

Sample preparation and Titration

10 ml of sample was taken and make upto 100 ml with 3 per cent metaphosphoric acid and filtered. 10 ml of filterate was pipet out into a conical flask and was titrated with standard dye till pink red end point appears.

$$\text{Ascorbic Acid (mg/100 ml)} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Volume of filterate taken} \times \text{Volume of sample taken}}$$

Anthocyanin analysis

Reagents - 0.1 N HCL

Preparation of standard

Standard was prepared by taking 10 ml of sample and diluted with 50 ml of distilled water.

Procedure

10 ml of sample was diluted with 50 ml of 0.1 N HCL and was allowed to equilibrate in the dark for 1 hour. Absorbance was recorded at the optical density (O.D.) 510 nm in spectrophotometer. Anthocyanin is determined by the formula:

$$\text{Total O.D/100 ml} = \frac{\text{O.D} \times \text{Volume made up} \times 100}{\text{ml. of juice taken}}$$

$$\text{Total anthocyanin (mg/100 ml)} = \frac{\text{Total O.D/ 100 ml}}{87.3}$$

Statistical analysis

Experiments were repeated in triplicates and the results were expressed as the mean values ± standard deviations. The statistical significance for each experiment was determined using the analysis of variance test (ANOVA). Differences were considered to be significant at $p<0.05$.

Results and Discussions

Ascorbic acid

The results presented in (Table 1) show that the ascorbic acid content decreased slightly throughout the storage period. As it is a dried product, it does not change much during storage. The data revealed that the maximum ascorbic acid concentration was recorded in T_1 (17.63 mg/100 ml) at 0 day and decreased with storage period, with the value decreasing to (17.59 mg/100 ml) at 90 days, followed by T_7, whose value decreased from (17.51 to 17.47 mg/100 ml). T_3 had the lowest value (11.51 mg/100 ml) at 0 day and decreased to (11.46 mg/100 ml) at 90 days, followed by T_6, whose value decreased from (13.63 to 13.57 mg/100 ml).

Ascorbic acid is particularly sensitive to high temperatures, it is possible that ascorbic acid content has decreased, and vitamins are easily lost during food processing and storage. The fact that ascorbic acid is highly susceptible to high temperatures and the vitamin is easily lost during food processing and storage can explain the decrease in ascorbic acid content (Potter and Hotchkiss 1995) [9]. Teotia *et al*., (1997) [13] discovered a similar decrease in ascorbic acid content during storage in muskmelon RTS beverage. Sogi *et al*., (2001) [12] discovered that the ascorbic acid content of kinnow RTS beverage and squash decreased with storage time.

Table 1: Changes in ascorbic acid of Hibiscus tea during storage under ambient condition

Treatments	Ascorbic acid (mg/100 ml)						
	Storage periods (in days)						
	0	15	30	45	60	75	90
T_1 (Roselle + Mint	17.63	17.63	17.61	17.61	17.59	17.59	17.59
T_2 (Roselle + Tulsi)	15.36	15.36	15.34	15.34	15.29	15.29	15.29
T_3 (Roselle + Dry ginger)	11.51	11.51	11.50	11.50	11.46	11.46	11.46
T_4 (Roselle + Cardamom)	14.57	14.57	14.55	44.55	14.53	14.53	14.53
T_5 (Roselle + Blackpepper)	14.32	14.32	14.30	14.30	14.28	14.28	14.28
T_6 (Roselle + Clove)	13.63	13.63	13.61	13.61	13.57	13.57	13.57
T_7 (Roselle)	17.51	17.51	17.49	17.49	17.47	17.47	17.47
Mean	14.93	14.93	14.91	14.91	14.88	14.88	14.88
SE (m) ±	0.120	0.120	0.096	0.096	0.139	0.139	0.139
CD at 5%	0.364	0.364	0.291	0.291	0.422	0.422	0.422

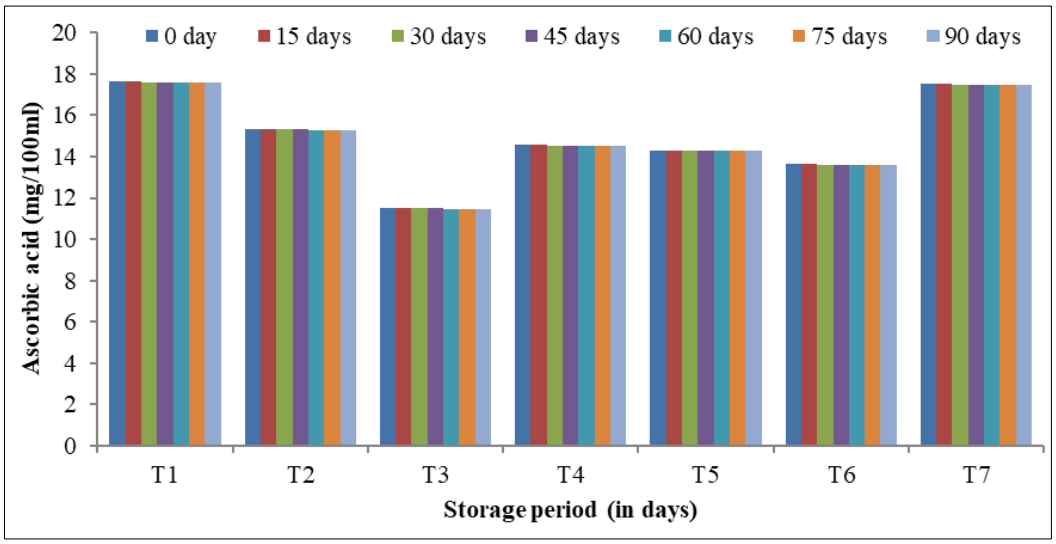

Fig 1: Changes in ascorbic acid of Hibiscus tea during storage under ambient condition

Anthocyanin

According to the current findings, the anthocyanin content of roselle tea presented in (Table 2) decreased over a 90 day storage period at room temperature. The data show that anthocyanin content does not change much between 0 and 30 days, but then it decreases slightly from 45 to 90 days of storage period. T_7 had the highest value (1.30 mg/100 ml), while T_6 had the lowest value (0.61 mg/100 ml). T_7 had the highest anthocyanin content at 90 days, which decreased from (1.30 mg/100 ml to 1.25 mg/100 ml) at 0 to 90 days. Whereas, anthocyanin content was lowest in T_6 which decreased from 0.61 mg/100 ml to 0.48 mg/100 ml from 0 to 90 days of storage.

Anthocyanin is a highly volatile, easily oxidised phenolic compound. It may also degrade during storage due to condensation into brown pigments. Because of the presence of ascorbic acid and the higher pH of the prepared roselle nectar, anthocyanin degradation may have been accelerated. Some monomeric anthocyanin could have been converted into polymeric compounds during storage (Iversen 1999; Ochoa *et al.*, 1999) [4, 8]. This could account for the high total monomeric anthocyanin losses in the beverages. The anthocyanin content decreases with increasing operating temperatures and storage times. Wasker and Khurdiya (1987) [14] discovered a similar pattern in phalsa beverages. Kannan and Thirumaran (2004) [6] found comparable results in jamun products that had been stored for six months.

Table 2: Changes in anthocyanin of Hibiscus tea during storage under ambient condition

Treatments	Anthocyanin (mg/100 ml)						
	Storage periods (in days)						
	0	15	30	45	60	75	90
T_1 (Roselle + Mint	1.28	1.28	1.28	1.25	1.25	1.22	1.22
T_2 (Roselle + Tulsi)	1.19	1.19	1.19	1.17	1.17	1.15	1.15
T_3 (Roselle + Dry ginger)	1.16	1.16	1.16	1.13	1.13	1.10	1.10
T_4 (Roselle + Cardamom)	1.13	1.13	1.13	1.10	1.10	1.07	1.07
T_5 (Roselle + Blackpepper)	0.82	0.82	0.82	0.78	0.78	0.75	0.75
T_6 (Roselle + Clove)	0.61	0.61	0.61	0.58	0.58	0.48	0.48
T_7 (Roselle)	1.30	1.30	1.30	1.28	1.28	1.25	1.25
Mean	1.07	1.07	1.07	1.04	1.04	1.01	1.01
SE (m) ±	0.011	0.011	0.011	0.007	0.007	0.009	0.009
CD at 5%	0.033	0.033	0.033	0.020	0.020	0.028	0.028

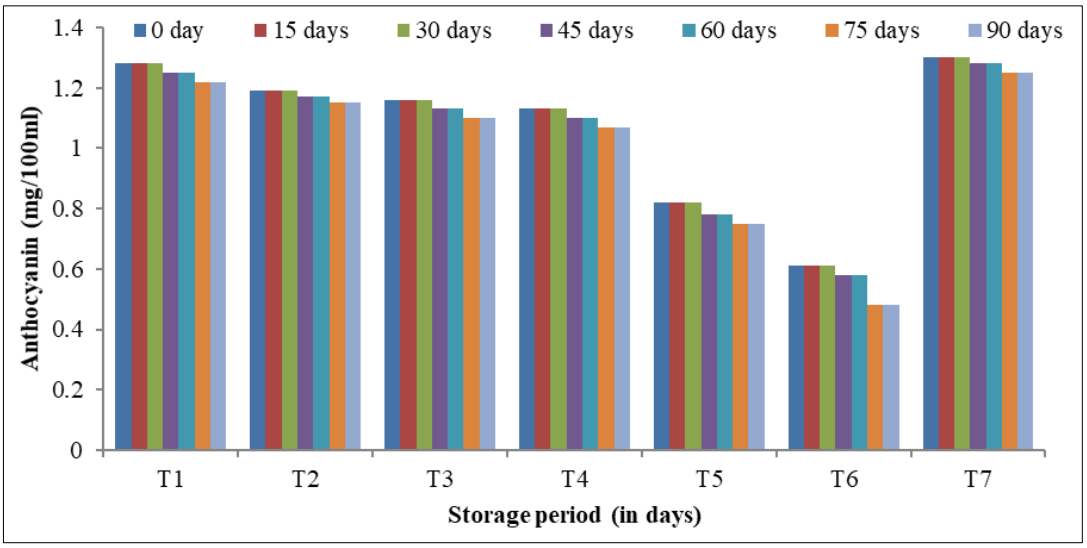

Fig 2: Changes in anthocyanin of Hibiscus tea during storage under ambient condition

Conclusion

From the research findings it is concluded that the concentration of ascorbic acid and anthocyanin decreases during storage period and do not show much changes as Hibiscus tea is a dried product. During processing and subsequent oxidation in storage, the decline in ascorbic acid concentration may be due to thermal degradation because it is very sensitive to heat and pressure treatment, Oxidation and light. It may be due to ascorbic acid conversion to dehydroxy ascorbic acid. Both ascorbic acid and dehydroxy ascorbic acid are highly volatile and unstable forms of vitamin C. Anthocyanin degradation may have been accelerated due to presence of ascorbic acid and higher pH of the prepared roselle-fruit juice blends. It is also known that ascorbic acid interaction with anthocyanin can lead to the degradation of compounds by a condensation reaction. Addition of spices enhances the medicinal and antimicrobial properties of the product.

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