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Evaluation of anthocyanin content in blended roselle and aloe vera ready to serve during storage

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Abstract

The present study entitled “Evaluation of anthocyanin content in blended roselle and aloe vera Ready To Serve during storage” was conducted in the Laboratory of Vegetable Science of Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during the year 2019-2020. The blended RTS beverage was prepared with different concentrations of roselle and aloe vera juice and was evaluated for anthocyanin at an interval of 15 days upto storage period of 75 days with the help of Spectrophotometer. Roselle extracts have potential use as valuable source of anthocyanin, it attracted attention as a potential source of natural colourants in food applications. Among 11 treatments the maximum anthocyanin content was noted for T₁ having 100 per cent roselle juice and minimum was noted for T₁₁ having 100 per cent aloe vera juice having no anthocyanin content. It is evident from the present findings that anthocyanin content decreased non-significantly with the advancement of storage period. The concentration of maximum anthocyanin in T₁ decreased from (1.21mg/100ml to 1.18mg/100ml) upto 0 to 75 days of storage, while minimum anthocyanin concentration in T₁₁ having 100 per cent aloe vera juice and no roselle followed by T₁₀ which decreased from (0.640mg/100ml to 0.6mg/100ml) with the advancement of storage period.

Keywords: Aloe vera, anthocyanin, *Hibiscus sabdariffa* L., RTS, spectrophotometer

Introduction

Roselle, *Hibiscus sabdariffa* Linn. (2n=4x=72) is a shrub belonging to the family Malvaceae. It is a Hibiscus plant native to India and Malaysia that has been introduced to other parts of the world, including Central America, West Indies and even Africa (Bruke, 1975) [2]. Roselle is a multipurpose plant and is used as a folk remedy in the treatment of abscesses, bilious conditions, cancer, cough, dyspepsia, fever, heart ailments, hypertension and strangury (Duke, 1985) [4]. They are recorded to be antihypertensive, antiseptic, sedative, diuretic, digestive, purgative, emollient, demulcent and astringent (Odigie *et al.*, 2003) [10].

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 Wrostand, 1990) [7]. Anthocyanins the biggest group of water soluble natural pigments, are the glycosides of polyhydroxy and the polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts shown in Fig 1. Colour is considered as one of most vital properties determining the acceptability of foods and drinks and is essential element for their identification. The matured, dried calyces or flower pod is used to prepare tea, wine, cocktail, sauce, jam, marmalade, candy, pickles, and curry due to its distinctive scarlet red color and sweet aroma but sour taste. Roselle is one of the under exploited food crops with the potential for processing by the dietary and food industries. The extract of roselle calyces has a distinctive red colour, good flavour, low sugar and high acidic content. But due to its high acidity and strong astringent taste, it is not consumed fresh. However, roselle calyces have unlimited potential in their processed form in world trade.

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Fig 1: Anthocyanin Structure

Aloe vera (*Aloe barbadensis* Miller.) is a perennial succulent plant belonging to family Liliacea. It was used as naturally occurring medicinal and therapeutic product (Dutt, 2002) [5]. It has also been used as traditional contemporary folk remedy (Volger and Ernst, 1999) [11]. Aloe vera contains aloins, a small quantity of volatile oil, resin, gum, anthraquinone, chrysophanic acid and coumarins. Aloe vera is unpleasant in its raw state and has bitter taste so its palatability can be improved by adding some other fruit juices.

Material and Methods

The present investigation is carried out in the Laboratory of Vegetable Science of Indira Gandhi Krishi Vishwavidyalaya Raipur (C.G.) during the year 2019-2020. The present study is designed to prepare blended roselle and aloe vera RTS and evaluation of anthocyanin during storage period of 75 days with the help of spectrophotometer. A blended RTS is designed in CRD (Completely Randomized Design) with different concentration of roselle and aloe vera having 11 treatments and 3 replications. Different blended concentrations of roselle and aloe vera are T₁ having (100) per cent roselle juice, T₂ (90:10), T₃ (80:20), T₄ (70:30), T₅ (60:40), T₆ (50:50), T₇ (40:60), T₈ (30:70), T₉ (20:80), T₁₀ (10:90), T₁₁ (100) per cent aloe vera juice.

For this investigation roselle calyces were collected from the Dantewada, seeds were planted at the month of June during rainy season and was harvested in mid November onwards when the flower has dropped that time calyces were fully matured and dark pink in colour. Aloe vera was collected from IGKV campus.

Extraction of Roselle juice by Hot Water Extraction Method (HWE)

Fresh and Healthy diseased free mature calyces was selected, peeled and washed with water after that it was ready for the juice extraction. For the preparation of Roselle-Aloe vera blended RTS, the Roselle juice was extracted by using the Hot Water Extraction (HWE) method described by (Wong *et al.* 2003) and was performed by using 1kg dried calyces per 5 litre of distilled boiling water (100 °C) for 20-25 minutes as described by (Aina and Odipe, 2006) [1]. The HWE method was the most effective extraction method resulting in high

anthocyanin and ascorbic acid contents of 43g/l (as delphinidin-3-glucoside) and 234g/kg respectively. Fresh Roselle extract were filtered with a muslin cloth.

Extraction of Aloe Vera Juice

Fresh aloe leaves free from disease and pests of uniform size with no mechanical damage is selected. The conventional hand- filleting method of processing aloe vera was created to prevent contaminating the inner fillet with the yellow sap. In this method, the lower (25mm) portion of the leaf base and tapering point (50-100mm) of the leaf top is removed. Spines along the leaf margin, top and bottom rind of the leaves were removed with sharp knife and leaves were blanched for 10 minutes at 60-65 °C.

Preparation of RTS

According to the treatments the ratio of 10 per cent roselle and aloe vera juice is blended with measured quantity of water and TSS is checked, and then the treatment is boiled for 2-3 minutes and sugar is added to it until TSS reaches to 10 per cent and then sodium benzoate 1gm/l of RTS and citric acid 3gm/l was added as a preservative. At last when the prepared RTS get cooled it is filled in glass bottles and sealed properly with crown corking sealing machine and kept at ambient temperature.

Table 1: Different combinations of blended roselle and aloe vera RTS

Treatments	Proportion of juice in the Blends	
	Roselle juice	Aloe vera juice
T ₁	100 parts	0 parts
T ₂	90 parts	10 parts
T ₃	80 parts	20 parts
T ₄	70 parts	30 parts
T ₅	60 parts	40 parts
T ₆	50 parts	50 parts
T ₇	40 parts	60 parts
T ₈	30 parts	70 parts
T ₉	20 parts	80 parts
T ₁₀	10 parts	90 parts
T ₁₁	0 parts	100 parts

Fig 2: Flowchart of preparation of RTS

Determination of Anthocyanin

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:

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

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

The changes in concentration of anthocyanin during storage in different treatment is observed upto 75 days at an interval of 15 days. Different treatments are individually analyzed. The data were subjected without transformation and statistically analyzed with CRD using OPSTAT.

Results and Discussion

It is apparent from the present finding (Table.2 and Fig.3) that anthocyanin content of the blended RTS showed non-significant decrease with the advancement of the storage period. The concentration of anthocyanin was found to be maximum in treatment T₁ having 100 per cent roselle juice with the advancement of the storage period. Whereas minimum anthocyanin concentration was found in treatment T₁₁ having 100 per cent aloe vera juice followed by T₁₀ having 10 per cent roselle juice and 90 per cent aloe vera juice. The concentration of maximum anthocyanin in T₁ decreased from (1.21mg/100ml to 1.18mg/100ml) from 0 to 75th days of

storage, while minimum anthocyanin content was observed in T₁₁ having 100 per cent aloe vera during entire period of observation followed by T₁₀ where concentration of anthocyanin decreased from (0.640mg/100ml to 0.6mg/100ml) from 0 to 75th day of storage period.

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. Some monomeric anthocyanin may have been converted into polymeric compounds during storage (Iversen 1999; Ochoa *et al.*, 1999) [8, 9]. That may be the explanation for the high losses of total monomeric anthocyanin in the blends.

Table 2: Changes in Anthocyanin content of blended RTS during storage under ambient condition.

Treatments	Anthocyanin (mg/100ml)						
	Storage periods (in days)						
	0	15 th	30 th	45 th	60 th	75 th	Mean
T ₁ (100% Roselle juice + 0% Aloe vera juice)	1.210	1.210	1.200	1.200	1.180	1.180	1.181
T ₂ (90% Roselle juice + 10% Aloe vera juice)	1.180	1.173	1.170	1.170	1.170	1.160	1.170
T ₃ (80% Roselle juice + 20% Aloe vera juice)	1.150	1.140	1.130	1.120	1.120	1.100	1.126
T ₄ (70% Roselle juice + 30% Aloe vera juice)	1.140	1.120	1.100	1.100	1.080	1.020	1.093
T ₅ (60% Roselle juice + 40% Aloe vera juice)	0.960	0.940	0.940	0.920	0.920	0.900	0.93
T ₆ (50% Roselle juice + 50% Aloe vera juice)	0.947	0.940	0.927	0.900	0.900	0.880	0.916
T ₇ (40% Roselle juice + 60% Aloe vera juice)	0.940	0.930	0.920	0.887	0.840	0.833	0.891
T ₈ (30% Roselle juice + 70% Aloe vera juice)	0.910	0.860	0.840	0.840	0.827	0.820	0.849
T ₉ (20% Roselle juice + 80% Aloe vera juice)	0.757	0.740	0.730	0.720	0.700	0.700	0.724
T ₁₀ (10% Roselle juice + 90% Aloe vera juice)	0.640	0.620	0.610	0.610	0.600	0.600	0.613
T ₁₁ (0% Roselle juice + 100% Aloe vera juice)	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mean	0.894	0.879	0.869	0.860	0.848	0.835	0.86
SE (m) ±	0.001	0.002	0.002	0.002	0.002	0.002	—
CV (%)	0.276	0.396	0.401	0.405	0.410	0.416	—
CD at 5%	0.004	0.006	0.006	0.006	0.006	0.006	—

Fig 3: Changes in Anthocyanin content of blended RTS during storage under ambient condition

Conclusion

The present findings confirm that concentration of anthocyanin decreased non- significantly from 0 to 75th day of storage period. The maximum anthocyanin content was noted in T₁ which changes from (1.21 to 1.18 mg/100ml) during storage, whereas minimum anthocyanin content was noted in T₁₁ having no anthocyanin content followed by T₁₀ which changes from (0.640 to 0.6mg/100ml). These results are in agreement that the concentration of anthocyanin decreased from T₁ to T₁₁ due to decrease in concentration of roselle with increase in concentration of aloe vera in each treatment.

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