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Effect of Storage Temperature on Various Parameters of Extracted Pigment from Roselle (*Hibiscus sabdariffa* L.) Calyces for Edible Colour

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A B S T R A C T

An investigation was carried out to know the effect of storage temperature on various parameters of extracted pigment from roselle (*Hibiscus sabdariffa* L.) calyces for edible colour. For knowing the storage stability of extracted pigment, it was kept for 3 months in both ambient and refrigerated conditions. In storage under ambient and refrigerated condition, a decreasing trend were observed in the anthocyanin content, titratable acidity, total antioxidants in the extracted pigment obtained by different methods of extraction upon storage under both conditions. At 90 days after storage at ambient and refrigerated condition, the anthocyanin content of 802.62 and 1091.92 mg 100 ml^{-1}, titratable acidity 9.73 and 10.18 per cent and total antioxidants of 32.06 mg 100 ml^{-1} and 43.02 mg 100 ml^{-1} were found be highest in treatment ethanol acidified with 1.5 N HCl. An increasing trend was observed in the pH of extracted pigment obtained by different methods of extraction upon storage and it was highest 3.78 and 3.72 was found in treatment of fermentation of calyce (T_5) in under both ambient and refrigerated conditions. Overall from the present investigation, the treatment of ethanol acidified with 1.5 N HCl was found to be the best with highest anthocyanin retention and total antioxidants compare to all other treatments which can used for large scale extraction of biocolour from roselle calyces.

Introduction

Roselle (*Hibiscus sabdariffa* L.) is a multi-use plant, belongs to the family Malvaceae, widely distributed in tropical regions, especially in the Middle Eastern countries and generally considered as a medicinal plant. The calyces, also known as natal sorrel, (Anon., 1999; Mohamed *et al.,* 2012; Plotto, 2004) are potentially a good source of antioxidant agents such as anthocyanins and ascorbic acid. Roselle calyx is a rich source of dietary fiber, vitamins, minerals and bioactive compounds such as organic acids, phytosterols and polyphenols. The phenol content in the plant consist mainly of anthocyanins like delphinidin-3-glucoside, sambubioside and cyanidin- 3- sambubioside contributing to their antioxidant properties. Roselle calyces is frequently used in the production of jelly, jam, juice, wine, syrup, gelatin, pudding, cake, ice cream and as flavouring agent. Its brilliant red colour and unique flavour makes it a valuable food product (Tsai and Ou, 1996; Mohamed *et al.,* 2012). The calyces, stems and leaves are acid in flavour. The juice from the calyx is

claimed to be a health-enhancing drink due to its high content of vitamin C, anthocyanins and other phytochemicals contributing to good antioxidant potents (Mohamad *et al.*, 2002).

Many medicinal applications of the roselle plant have been developed around the world. In China, it is used to treat hypertension, pyrexia and liver damage and also in ayurvedic medicine (Odigie *et al.*, 2003; Mohamed *et al.*, 2012). Of late the sepal extract has been used as an effective treatment against leukemia due to its high content in polyphenols, particularly protocatechuic acid (Tseng *et al.*, 2000). Roselle also has certain therapeutic properties; the reported benefits of taking it internally in the form of herbal tea include soothing colds, clearing blocked nose, clearing mucous, as an astringent, promoting kidney function, aiding digestion, general tonic, diuretic, and helps to reduce fever and said to be a folk remedy for cancer (Anon., 1999; Mohamed *et al.*, 2012).

Food additives are non-nutritive substances added intentionally to food, generally in small quantities, to improve appearance, flavour, texture or storage properties. These include antioxidants, sweeteners, thickeners, preservatives, colours, acidity regulators, emulsifiers, anticaking agents, antibiotics. Among them, colour is one of the major food additive.

Colour is one of the most important quality attribute affecting the consumer's acceptance of food since it gives the first impression of food quality. The global demand for natural dyes world over is about 10,000 tonnes, which is equivalent to one per cent of the world's synthetic dyes consumption and expected to rapidly grow in near future. The recent ban on the use of azo dyes by European Union has also increased the scope for the use of natural dyes (Sivakumar *et al.*, 2011). There is considerable demand for food colourants from natural sources that can serve as alternatives to use of synthetic dyes due to both legislative action and consumer concerns over the use of synthetic additives.

Natural and synthetic food colour market is projected to reach $ 2.3 billion by 2019, growing at a compound annual growth rate of 4.6 per cent currently. The natural food colours market is projected to grow by 2.8 times more than the synthetic food colours market by 2020. At present the global natural food colour market has reached around $ 1.14 billion in terms of value. Region-wise, Western Europe is the largest market in terms of revenue with over 26 per cent of market share. Type-wise, carotenoids is estimated to be the largest segment with over 31 per cent of the total market share followed by anthocyanin with 22 per cent (Anon., 2014).

Currently, anthocyanins in blue and purple corn are being used for the production of naturally coloured blue tortillas. Radish and potato extracts have colour characteristics very similar to those of Allura red (a red synthetic dye used in food applications) and therefore have the potential to be incorporated as food colourants. In this respect, roselle calyces appear to be good and promising source of water soluble red colourants that could be utilized as natural food colourants for application in candies, beverages, bakery products, jams, jellies and other confectionaries (Abou-arab *et al.*, 2011).

The storage of bioactive components from plant materials is affected by different factors, such as the extraction techniques, solvents, time, temperature, solvent-to-plant material ratio and many others. In storage, nutritional components will be changed in refrigeration and ambient condition. So this research is aimed at changes in anthocyanin, pH, titratable acidity and antioxidants in the extracted pigment by using different solvents at ambient and refrigerated conditions.

Materials and Methods

The present investigation was carried out at Department of Postharvest Technology, College of Horticulture, University of Horticultural Sciences campus, GKVK, Bengaluru. The raw material (calyces) required for the experiment were grown in the experimental plot of Plantation, Spices, Medicinal and Aromatic Department, College of Horticulture, University of Horticultural Sciences Campus, GKVK (Post), Bengaluru.

Different solvents, enzymes, fermentation and hot water are used for extraction of anthocyanin from roselle calyces. Solvents used are hydrochloric acid, acetic acid, citric acid.

Anthocyanin was measured through recoding optical density of the filtrate at 535nm using spectrophotometer (Make: SYSTRONICS, Model: UV/VIS Spectrophotometer 117). Directly anthocyanin extract is used for the measurement of pH using pH meter (Make: Trans instruments Model: BO 3001) standardized with buffer solutions of 4.0 and 7.0 according to the method outlined in AOAC (2000).The total titratable acidity of dried rosellecalyces/extract sample was determined by visual titration method (Ranganna, 1986). The total antioxidants present in the roselle powder was estimated using FRAP method given by Benzie and Strain (1996).

Results and Discussion

Anthocyanin content

Significant difference among the treatments for the anthocyanin content present in extracted pigment which are stored in ambient condition represented in table 1, the highest anthocyanin was found in the treatment ethanol acidified with 1.5N HCl (802.62 mg 100 ml^{-1}) after 90 DAS which was decreased from 1638.17 mg $100ml^{-1}$ at time zero (T_0) and the lowest was found in the T_6- Hot water extraction (202.86 mg 100 ml^{-1}) at 90 DAS which was reduced from 372.21 mg 100 ml^{-1} at T_0, this may be probably due to non-enzymatic browning reactions and also the formation of 5-hydroxi methyl furfural and as a result, clarity and quality of colour were lost (Fatemi, 2000; Ruangsri *et al.,* 2008).

The treatment T_6- hot water extraction showed the highest (54.50%) and lowest (27.20%) retention was recorded in the treatment fermentation of calyces. This may be because at higher pH stability of the anthocyanin reduced, hence degradation is faster.

Data presented in the table 2 revealed that after 90 DAS, the maximum and minimum anthocyanin content in treatment ethanol acidified with 1.5 N HCl (T_1) were 1091.92 mg 100 ml^{-1} and 802.62 mg 100 ml^{-1} obtained at refrigerated and ambient condition respectively. Storage temperature, increase in pH, prolonged storage time which had a significant effect on the stability of anthocyanin (Bordignon *et al.*, 2006, Rad and Yavarmanesh, 2006). Same results were obtained by Sharifi and Hassani, (2012) in the study of extraction methods and stability of colour extracted from barberry pigments.

pH and titratable acidity

Changes in pH and titratable acidity of extracted pigment during storage at ambient and refrigerated storage condition were showed in the table 3, 4, 5 and 6. The pH and titratable acidity of the extracted pigment were increased and decreased respectively at ambient and refrigerated storage conditions compare to initial (T_0) towards 90 days of storage and both reading are inversely proportional.

Table.1 Effect of extraction methods on anthocyanin content (mg 100 ml^{-1}) during ambient storage condition

Treatments		T_0	30 DAS	60 DAS	90 DAS	Retention (%)
T_1	Ethanol acidified with 1.5N HCl (85:15)	1638.17	1334.76	1292.43	802.62	48.99
T_2	Ethanol with 2% citric acid	1241.27	920.22	884.94	365.15	29.42
T_3	Ethanol with 2% acetic acid	1311.24	967.26	926.10	441.58	33.68
T_4	Distilled water with 0.2% pectinase	979.60	821.44	741.43	391.02	40.04
T_5	Fermentation of calyce	1093.68	597.41	512.74	297.53	27.20
T_6	Hot water extraction	372.21	281.06	235.78	202.86	54.50
S. Em±		2.15	1.18	2.60	1.72	-
CD at 5%		6.40	3.51	7.72	5.12	-

DAS: Days after storage T_0: Time zero

Table.2 Effect of extraction methods on anthocyanin content (mg 100 ml^{-1}) during refrigerated (4°C) storage condition

Treatments		T_0	30 DAS	60 DAS	90 DAS	Retention (%)
T_1	Ethanol acidified with 1.5N HCl (85:15)	1638.17	1471.18	1380.63	1091.92	66.65
T_2	Ethanol with 2% citric acid	1241.27	1028.42	1009.10	575.65	46.37
T_3	Ethanol with 2% acetic acid	1311.24	1118.38	1063.69	652.09	49.73
T_4	Distilled water with 0.2% pectinase	979.60	957.85	895.52	556.84	56.84
T_5	Fermentation of calyce	1093.68	1004.31	828.49	552.13	50.48
T_6	Hot water extraction	372.21	326.21	275.77	243.43	65.40
S. Em±		2.15	1.45	1.63	2.29	-
CD at 5%		6.40	4.31	4.87	6.82	-

DAS: Days after storage T_0: Time zero

Table.3 Effect of extraction methods on pH during ambient storage condition

Treatments		T_0	30 DAS	60 DAS	90 DAS
T_1	Ethanol acidified with 1.5N HCl (85:15)	1.26	1.51	1.57	1.62
T_2	Ethanol with 2% citric acid	2.31	2.62	2.60	2.62
T_3	Ethanol with 2% acetic acid	2.22	2.51	2.58	2.59
T_4	Distilled water with 0.2% pectinase	2.34	2.39	2.46	2.50
T_5	Fermentation of calyce	3.62	3.66	3.72	3.78
T_6	Hot water extraction	2.39	2.56	2.61	2.67
S.Em±		0.04	0.03	0.01	0.01
CD at 5%		0.12	0.09	0.04	0.02

DAS: Days after storage T_0: Time zero

Table.4 Effect of extraction methods on pH during refrigerated (4°C) storage condition

Treatments		T_0	30DAS	60DAS	90DAS
T_1	Ethanol acidified with 1.5N HCl (85:15)	1.26	1.49	1.56	1.59
T_2	Ethanol with 2% citric acid	2.31	2.47	2.55	2.56
T_3	Ethanol with 2% acetic acid	2.22	2.4	2.51	2.54
T_4	Distilled water with 0.2% pectinase	2.34	2.36	2.41	2.44
T_5	Fermentation of calyce	3.62	3.63	3.66	3.72
T_6	Hot water extraction	2.39	2.53	2.57	2.61
S.Em±		0.03	0.02	0.01	0.01
CD at 5%		0.11	0.08	0.03	0.01

DAS: Days after storage T_0: Time zero

Table.5 Effect of extraction methods on titratable acidity (%) during ambient storage condition

Treatments		T_0	30 DAS	60DAS	90DAS
T_1	Ethanol acidified with 1.5N HCl (85:15)	10.83	10.15	10.09	9.73
T_2	Ethanol with 2% citric acid	8.33	8.07	8.01	7.65
T_3	Ethanol with 2% acetic acid	9.73	9.41	9.19	8.87
T_4	Distilled water with 0.2% pectinase	6.53	6.24	6.12	5.96
T_5	Fermentation of calyce	3.52	3.39	3.33	3.14
T_6	Hot water extraction	2.85	2.79	2.69	2.31
S.Em±		0.16	0.17	0.17	0.19
CD at 5%		0.48	0.50	0.52	0.59

DAS: Days after storage T_0: Time zero

Table.6 Effect of extraction methods on titratable acidity (%) during refrigerated (4°C) storage condition

Treatments		T_0	30DAS	60DAS	90DAS
T_1	Ethanol acidified with 1.5N HCl (85:15)	10.83	10.63	10.44	10.18
T_2	Ethanol with 2% citric acid	8.33	8.20	8.01	7.97
T_3	Ethanol with 2% acetic acid	9.73	9.54	9.35	9.13
T_4	Distilled water with 0.2% pectinase	6.53	6.50	6.37	6.31
T_5	Fermentation of calyce	3.52	3.36	3.30	3.27
T_6	Hot water extraction	2.85	2.81	2.76	2.56
S.Em±		0.16	0.17	0.18	0.18
CD at 5%		0.48	0.50	0.53	0.54

DAS: Days after storage T_0: Time zero

Table.7 Effect of extraction methods on total antioxidants (mg GAE 100 ml^{-1}) during ambient storage condition

Treatments		T_0	30DAS	60DAS	90DAS	Loss (%)
T_1	Ethanol acidified with 1.5N HCl (85:15)	101.05	63.75	40.42	32.06	68.27
T_2	Ethanol with 2% citric acid	84.71	42.76	36.92	27.19	67.90
T_3	Ethanol with 2% acetic acid	89.41	45.89	39.95	28.81	67.78
T_4	Distilled water with 0.2% pectinase	82.55	24.44	21.69	19.31	76.60
T_5	Fermentation of calyce	57.11	24.01	19.82	17.65	69.09
T_6	Hot water extraction	23.72	16.70	14.19	12.62	46.79
S.Em±		0.51	0.59	0.05	0.29	-
CD at 5%		1.51	1.77	0.15	0.87	-

DAS: Days after storage T_0: Time zero

Table.8 Effect of different extraction methods on total antioxidants (mg GAE 100 ml^{-1}) during refrigerated (4°C) storage condition

Treatments		T_0	30DAS	60DAS	90DAS	Loss (%)
T_1	Ethanol acidified with 1.5N HCl (85:15)	101.05	72.89	51.41	39.02	57.42
T_2	Ethanol with 2% citric acid	84.71	50.18	43.52	36.39	57.04
T_3	Ethanol with 2% acetic acid	89.41	57.06	45.22	38.01	57.48
T_4	Distilled water with 0.2% pectinase	82.55	43.04	34.83	33.58	59.32
T_5	Fermentation of calyce	57.11	39.75	32.84	26.24	54.05
T_6	Hot water extraction	23.72	21.35	19.82	16.48	30.52
S Em±		0.51	1.27	0.21	0.35	-
CD 5%		1.51	3.77	0.62	1.06	-

DAS: Days after storage T_0: Time zero

Table.9 Correlation studies on effect of extraction methods on different parameters in relation to anthocyanin content in roselle extract during storage

Parameters		1	2	3	4
1	**Acidity**	1			
2	**pH**	-0.70*	1		
3	**Total antioxidants**	0.60*	-0.42*	1	
4	**Anthocyanin**	0.73*	-0.50*	0.81*	1

* Correlation analysis is significant at 5% level

The lowest pH 1.62 and 1.59 was recorded during 90 DAS in treatment T_1- ethanol acidified with 1.5 N HCl at ambient and refrigerated storage condition respectively. In conformity with above reading, highest acidity of 9.73 and 10.18 per cent was noticed propositionally.

Increase in acidity was may be due to release of organic acids from the extractants and a corresponding decrease in pH was noticed (Tasnim *et al.,* 2010) and also due to acidic hydrolysis of polysaccharides (Bhardwaj and Pandey, 2011). Similar results were obtained by Kilima *et al.,* (2014) in an experiment on influence of storage temperature and time on the physicochemical and bioactive properties of roselle-fruit juice blends. Difference in pH of same treatment at 90 DAS in ambient and refrigerated condition may be attributed to effect of temperature.

Total antioxidants

The changes in the total antioxidants represented in the table 7 and 8 varied significantly among the treatments during storage stability study of extracted anthocyanin pigment from roselle in both ambient and refrigerated conditions.

The antioxidants activity of the roselle extract was found to be decrease across all treatments and in both storage condition. But the magnitude of decreases varied among treatments. Decrease in the antioxidative property of extracts is may be due to degradation in anthocyanin, total phenols, change in the pH composition, which all together was responsible for stability and antioxidant properties of extract. (Tsai *et al.,* 2002, Ho *et al.,* 1992).

The variation in the total antioxidants in the same treatment after storage at ambient and refrigerated condition may be due to slow degradation of anthocyanin content in the refrigerated condition compare to ambient condition which is directly proportional to the antioxidant capacity of extracted pigment (Kilima *et al.,* 2014).

Correlation studies

Correlation studies of effect on various parameters in relation to anthocyanin in roselle extract during storage are presented in the Table 9. Correlation is a measure of association between more than one character and it operates the relationship between dependent and independent characters.

In the present study, both positive and negative correlation among the parameters, the dependent variable was anthocyanin and it was related to many different independent parameters. Anthocyanin exhibited positive and significant association with total antioxidants (0.81), titratable acidity (0.73). While, anthocyanin showed negative and significant correlation pH (0.50). This is in confirmation with the findings of Olaya *et al.,* (2009).

In conclusion, extracted pigment showed very good storage stability in the refrigerated condition compare to ambient condition. By storing the pigment in the refrigerated condition, we can reduce quality loss. From the present investigation, the treatment of ethanol acidified with 1.5 N HCl was found to be the best with highest anthocyanin retention and total antioxidants compare to all other treatments which can used for large scale extraction of biocolour from roselle calyces.

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