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Effect of Maturity Stages and Cultivars on Chemical Constituents of *Hibiscus Sabdariffa* (Roselle) Grown in Sudan.

Rasha Khalid Abbas[1], Fatma Soliman Elsharabasy[2] and Awatif Ahmed Mohammed Siribel[3]

[1]Depratment of Biochemistry, Faculty of Applied and Industerial Science University of Bahri Sudan.
Department of Chemistry, Faculty of Science and Arts in Mukhwa, University of Albaha, 65931 Saudi Arabia.

[2]Department of Chemistry of Natural and Microbial Products, National Research Center, Dokki 12622, Egypt.
Department of Chemistry, Faculty of Science and Humanity studies, Sattam bin Abdul Aziz University, Alkharj City 11942, Saudi Arabia.

[3]Department of Agrotechnology, Medicinal and Aromatic Plants Research Institute [National center for research] Khartoum, Sudan.

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ABSTRACT

The effect of maturity stages (premature, mature and over mature) on performance of four cultivars of *Hibiscus sabdariffa* (Roselle)- (namely : Rahad, Fashir, Kass and Abaid)- were studied by evaluating some of its chemical constituents, organic acids (citric, ascorbic and tartaric), anthocyanin's as (malvidin and pelargonidin), pH and essential amino acids under the semi – arid conditions of Sudan. HPLC and amino acid analyzer were used in this study. It was found that, there was a significant effect of maturity stages on pH, organic acids, essential amino acids and anthocyanin's. Mature stage of four Roselle cultivars gave significantly higher pH. Total percentages of identified organic acids (citric, ascorbic and tartaric acids) were the highest in all cultivars at mature stage, Fashir and Abiad cultivars gave significantly higher organic acid than other one. pre mature stage gave significantly higher amount of the essential amino acids, on the other hand Abiad and Kass cultivarss gave the highest amount of the essential amino acids among all cultivars. Mature and over mature stages in all cultivars gave higher percentage of anthocyanin's as (malvidin and pelargonidin), within cultivars Rahad cultivar has the highest value of anthocyanin, and Abiad cultivar has lowest amount of these pigments.

Introduction

Hibiscus sabdariffa L. (family *Malvaceae*), commonly known as Roselle, red sorrel, or karkadè, is widely grown in Africa, South-East Asia, and some tropical countries of America[1,2,3,4]. *Hibiscus sabdariffa* Roselle is a perennial shrub that grows in tropical and subtropical regions. In Sudan where it is considered as an annual shrub it is grown in rain fed areas in the western states in large scale and it is cultivated in small scale in irrigated areas. Almost any part of the plant can be utilized but it is cultivated mainly for its calyces. The calyces are commonly used to make jelly, juice, jam, wine, syrup, pudding, cake, ice cream, and flavor[5,6,7].The beverages produced by Roselle calyces are called hibiscus tea, Sudan tea, or karkadè[8]. Roselle anthocyanin's can contribute to health benefits as a good source of antioxidants as well as a natural food color[5,7]. *Hibiscus sabdariffa* contain components of high nutritive value such as protein, amino acids, carbohydrate, fats, minerals and organic acids[9]. Karkade is widely used as food to make jellies, jam and beverages. The aqueous extract of *Hibiscus sabdariffa* attenuates hypertension, reverses cardiac hypertrophy and hypercholesterolemia)[8,10,11], alcoholic extract of *Hibiscus sabdariffa* has an anti- hyperammonemic and antioxidant effects on brain tissues[12]. Many studies were carried out to determine the right harvest time. the acids and pigments affect by the maturity stages of the Roselle calyces[13,14,15].

This plant has gained an interest for study because of the high demand in the world market and is expected to replace chemical products in many industries and medicinal uses.

Materials and methods

The experiment was executed at the experimental farm of Medicinal and Aromatic Plants Research Institute at Shambat, Sudan (Latitude 15° 40′ N, Longitude 32 ° 32′ and 360m above sea level) . The climate is semi-arid with low relative humidity and daily mean air temperature ranging from 25 to 40 °C in summer, and 15 to 21 °C in winter Four cultivars of *Hibiscus sabdariffa* namely Rahad, Fashir, Kass and Abiad were cultivated in the Demonstration Farms of Medicinal And Aromatic Plant Research Institute at Shambat (Sudan). Plants were identified in the department of plant taxonomy in the same institute. The calyces of four cultivars of Roselle harvested at three different stages after flowering namely premature 1-2 weeks old, mature 4-5 weeks old and over mature 6-7 weeks old. Were collected, dried and kept in carton bags for extraction.

Tele:
E-mail address: rashakhalid09@gmail.com

Determination of pH values and organic acids

2.5g of each sample were macerated in 25ml hot distilled water at 100oC for organic acids and for pH values[16].

Organic acids:- were determined using HPLC (Shimadzu corporation (Koyoto, Japan) pumps (LC-10 ADVP), Degasser (DGU-14A) UV detector (SPD-10 AVP), system controller (Scl-10 AVP), according to the method described by[17]. Authentic compounds of organic acids were obtained from Chemistry of Natural and Microbial Product Laboratory, National Research Centre, Egypt, (ascorbic acid 3mg, citric acid 5mg and tartaric acid 5.5mg).

Determination of pH

pH value was determined using pH meter Hanna instrument.

Determination of essential amino acids

5gm of the test samples were macerated in 50% alcohol until all pigment was extracted and concentrated under reduced pressure at 40 °C. 10 ml NaCl (10%) was added to the extract, stirred for one hour then 10 ml of trichloroacetic were added and filtrated. The precipitate was collected by centrifugation, washed and dried in desiccators

20 mg of protein were refluxed with 6N HCl (10ml) for 20 hrs. and the acid removed by evaporation under reduced pressure, the residue was dissolved in 10 % isopropanol for amino acids identification [18],using (Eppendorf-Germany Lc 3000) Amino acid analyzer.

Determination of Anthocyanin's

According to the method described by[19]. Methanol with 1ml Hcl was added to 5 gm. of sample until all pigments were extracted. The extract was dried and 20 ml of 6NHcl were added to the dried extract and then reflux for about one hour, after that 20 ml amyl alcohol were added to form two layers, the upper layer (organic layer) was separated and analyzed using HPLC. (Shimadzu corporation (Koyoto, Japan) pumps (LC-10 ADVP), Degasser (DGU-14A) UV detector (SPD-10 AVP), system controller (Scl-10 AVP), according to the method described by [17]. Authentic compound of Anthocyanin's were obtained from the Central laboratory of The National research Centre, Egypt, as (malvidin 5mm and pelargonidin 10mm).

Statistical analysis was done according to Duncan, Multiple Range Test [20]

Result and Discussion

Effect of maturity stages

Table (1) shows that there were significant differences among maturity stages on pH of four cultivars, but there was no significant different in pH values among cultivars. The mature stage gave significantly higher pH

Table1. Effect of maturity stages on pH of four cultivars of *Hibiscus sabdariffa*.

Cultivars	pH			Mean cultivar
	Pre mature	Mature	Over mature	
Rahad	2.400*	2.580	2.220	2.400 a
Fashir	2.600	2.950	2.140	2.563 a
Kass	2.560	2.660	1.860	2.360 a
Abiad	2.530	3.00	1.950	2.493 a
Mean pH	2.523 b	2.798 a	2.043 c	

Note: * Numbers shown are means of three replicates
Means followed by similar letter are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test.

Organic acids were greatly influenced by maturity stages (Table 2). Mature stage gave significantly better organic acid content. In the same table it was found that the citric acid was absent at mature and rare at premature stages. Ascorbic and tartaric acid were gave higher amount this finding corroborates the previous findings of[9,15] who found that the acids and pigments of the Roselle calyces were increased in amount up to the end of the fourth week starting from the date of the flower opening, this result agree with[13] who reported that the total acidity of karkade started to decrease continuously after the fourth week from flowering, the ascorbic acid of the Roselle fruit increased continuously until just before ripening. there was significant different in pH values among different stages[14]

Table 2. Effect of maturity stage (percentage) on organic acids of *Hibiscus sabdariffa* (ug/ml).

Maturity / Organic. Acids	Premature	Mature	Over mature	Mean Organic. acid effect
Citric acid	1.8*	0.0	14.9	5.6 c
Ascorbic acid	38.4	51.9	10.2	33.5 a
Tartaric acid	29.1	21.7	45.2	32 b
Mean maturity	23.1 b	24.5 a	23.4 b	

Note: * Numbers shown are means of three replicates
Means followed by similar letter are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test.

The essential amino acids- (Threonine, Valine, Methionine, Leucine, Isoleucine, Phenylalanine, Histidine, Lysine, and Arginine)- as illustrated in table (3) were significantly affected by maturity stage where, pre mature stage gave significantly higher amount of the essential amino acids, while over mature stage gave the lowest amount. Isoleucine is higher in maturity stages while methionine is lower one.

Table 3. Effect of Maturity stages (percentage) on essential amino acids of *Hibiscus sabdariffa* (ug/ml).

Maturity / Essential Amino acids	Pre mature	Mature	Over mature	Mean Essential Amino-acid effect
Threonine	**62.50***	**57.57**	**38.71**	**52.93 c**
Valine	85.61	56.10	21.65	54.45 c
Methionine	13.84	2.34	7.49	07.90 e
Isoleucine	192.5	140.5	40.62	124.54a
Phenylalanine	55.85	82.00	35.05	57.63 c
Histidine	53.70	55.72	32.90	47.44 c
Lysine	108.5	83.6	24.73	72.28 b
Arginine	35.59	61.39	20.15	39.04 d
Mean maturity time effect	76.01 a	67.40 b	27.66 c	

Note: * Numbers shown are means of three replicates
Means followed by similar letter are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test.

With respect to the Anthocyanin's (Malvidin and Pelargonidin), in table (4) found that they were significantly affected by maturity stages, where mature and over mature stages gave higher amount of these pigment, this finding corroborates the previous study[21].

Table 4. Effect of Maturity stages (percentage) 0n anthocyanin as malvidin and pelarogonidin of the four cultivars of *Hibiscus sabdariffa*.

Maturity / Anthocyanin	Pre mature	Mature	Over mature	Mean Anthocyanin's effect
Malvidin	20.17*	21.92	23.25	21.78 b
Pelargonidin	18.79	25.37	23.97	22.71 a
Mean Maturity effect	19.48 b	23.65 a	23.61a	

Note: * Numbers shown are means of three replicates
Means followed by similar letter are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test

Effect of cultivars

Table 1. shows that, there was no significant difference in the pH values among the four cultivars, while table 5. shows that, Fashir and Abiad cultivars gave significantly higher organic acid, these results were on line with previously reported observations that light colour hibiscus is more acidic than dark colour[22,23] . The essential amino acids, as illustrated in table 6. were significantly affected by cultivar where, Kass cultivar gave significantly higher essential amino acids than the other. this may be attributed to their genetic characteristics. With respect to the Anthocyanin's (Malvidin and Pelargonidin), it was found that they were significantly affected by cultivar, where Rahad gave higher Anthocyanin's while Abiad the lowest one,this finding corroborates the previous finding study[15].

Table 5. Effect of cultivars on organic acids of *Hibiscus sabdariffa* (%).

Cultivars / O. acids	Rahad	Fashir	Kass	Abiad	Mean O. acids effect
Citric acid	7.1	7.3	5.4	1.8	5.4b
Ascorbic acid	21.9	35.9	31.6	42.4	33.00 a
Tartaric acid	38.4	31.1	31.4	27.7	32.15 a
Mean cultivar effect	22.5 b	24.8 a	22.8 b	24.0 a	

Note: * Numbers shown are means of three replicates
Means followed by similar letter are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test.

Table 6. Effect of Cultivars on essential amino acids of *Hibiscus sabdariffa* (ug/ml).

Cultivars Essential Amino acids	Rahad	Fashir	Kass	Abiad	Mean Essential Amino-acid effect
Threonine	57.18	45.26	61.46	46.81	52.68 a
Valine	39.51	51.30	47.83	79.17	54.45 c
Methionine	2,13	2.25	11.64	15.54	7.89e
Leucine	71.60	49.93	78.76	47.30	73.72 d
Isoleucine	130.40	97.63	160.70	109.60	124.58 a
Phenylalanine	56.09	32.00	57.20	85.25	57.64b
Histidine	42.30	38.07	49.67	59.82	47.47 a
Lysine	80.32	44.54	86.01	77.59	72.12 b
Argnine	37.79	59.56	34.09	24.24	38.92 c
Mean cultivar effect	57.48 a	46.73 b	65.26 b	60.59 a	

Note: * Numbers shown are means of three replicates
Means followed by similar letter are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test.

Table 7. Effect of cultivars on Anthocyanines of *Hibiscus sabdariffa* (%).

Cultivars / Anthocyanin's	Rahad	Fashir	Kass	Abiad	Mean Anthocyanin's effect
Malvidin	24.74	23.54	25.92	12.92	21.78 b
Pelargonidin	38.48	10.19	26.80	15.36	22.70 a
Mean cultivar effect	31.61a	16.87 c	26.36 b	14.14 c	

Note: * Numbers shown are means of three replicates
Means followed by similar letter are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test.

Conclusion

This work comes to conclude that *Hibiscus Sabdariffa* L. constituents is highly affected by maturity stages and cultivars, the experiment was carried out to determine the right harvest time.

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